グリホサートカリウム塩

要旨及び評価結果

（環境動態）

検索期間：2020 年 7 月 1 日～2020 年 12 月 31 日

評価対象：適合性区分 a に該当する文献

シンジェンタジャパン株式会社

1. Information on the study

Data point:	CA 7.1.4.2
Report author	Albers C. N. *et al.*
Report year	2020
Report title	Leaching of herbicidal residues from gravel surfaces - A lysimeter-based study comparing gravels with agricultural topsoil.
Document No	Environmental pollution (2020), Vol. 266, Art. No. 115225
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes (Relevant, Category A acc. EFSA GD 2092, Point 5.4.1) / Reliable with restrictions

2. Full summary of the study according to OECD format

The approval of herbicides such as glyphosate for use on paved and unpaved gravel surfaces in the European Union is based on their behaviour and fate in agricultural soils. However, this might be very different from their fate in gravel surfaces. Therefore, an outdoor study with 21 small lysimeters containing different gravel types and a sandy arable topsoil as control was conducted. The lysimeters were sprayed with a commercial product for gardening, containing diflufenican and glyphosate. The concentrations of glyphosate and its relevant degradation product AMPA in the outlet was followed for 19 months. Glyphosate and AMPA did not leach from any of the mini-lysimeters. It can be concluded, that the different properties of gravel compared to those of agricultural soils may lead to different herbicide leaching patterns but also that the leaching depends on the type of gravel and highly on the type of herbicide.

Materials and methods

Gravels and arable soil

The first gravel type included in the study is sold under the tradename Slotsgrus® and is a mixture of different size classes. This gravel is used as the only gravel course or as a surface course on gravel paths, driveways etc. but also as a carrying layer under top layers of tiles and rock chippings. It contains particles up to 11 mm with ~10 % fine particles (<0.063 mm), 50 % sand-sized and ~40 % gravel-sized particles (>2 mm). For this gravel type, the term "pathway gravel" is used. The organic matter content is ~0.3 %, pH (H_2O) is 8.4, pH (0.01 M $CaCl_2$) is 7.7, amorphous Fe-oxides is 814 mg/kg and amorphous Al-oxides is 364 mg/kg. Amorphous metal oxides were extracted with ammonium oxalate solution and quantified on an ICP-MS.

The second gravel type of the study is typically used below tiles, asphalt and rock chippings in gardens and roads either as a single layer or as the lowest layer with a surface course on top, which could be pathway gravel or another type of finer gravel. It contains particles from clay size up to 63 mm, the particles being a mixture of silicates, granites, flint and calcite with ~4 % fine particles (<0.063 mm) and ~60 % gravel sized particles (>2 mm). For this gravel type, the term "basecourse gravel" is used. The organic matter content is ~0.6%, pH (H_2O) is 9.0, pH (0.01 M $CaCl_2$) is 8.4, amorphous Fe-oxides is 202 mg/kg and amorphous Al-oxides is 49 mg/kg.

The rock chippings used on top of the basecourse gravel were Norwegian diabas with a nominal size distribution from 11 to 16 mm. The chippings were washed in tap water before being packed into the lysimeters to remove fine particles. For this material, the term "rock chippings" is used. The organic matter content is ~0.1%.

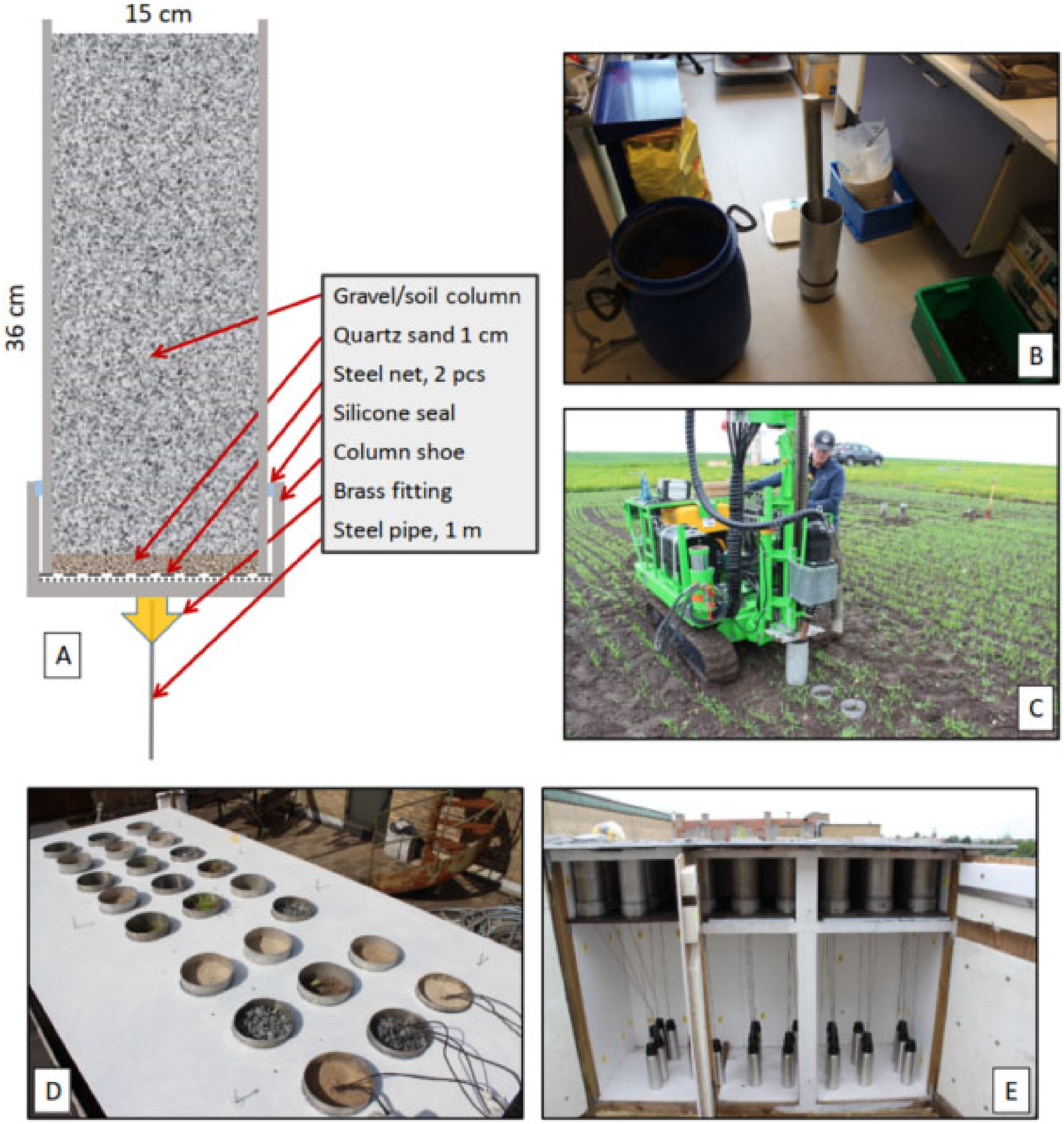
The model agricultural soil is an arable soil from a Late Weichselian outwash plain in south-western Denmark. It is very sandy (mainly coarse sand) and contains ~10 % fine particles (<0.063 mm). The organic matter content is ~3 %, pH (H_2O) is 6.0, pH (0.01 M $CaCl_2$) is 5.4, amorphous Fe-oxides is 1598 mg/kg and amorphous Al-oxides is 961 mg/kg.

Lysimeter construction and installation

The lysimeters were constructed in a way, that the leachate is in contact with no other material than austenitic stainless steel to exclude adsorption of the test substance. The lysimeters consisted of a 40 cm long stainless steel tube (inner diameter 15 cm) inserted into a "column shoe" with a 1 m stainless steel tube (inner diameter 2 mm) hanging in the middle (Figure 1A). To avoid clogging of the steel tube, two steel nets with different mesh sizes (1 mm and 80 mm) plus 12 mm fine quartz sand (0.2-0.3 mm) were placed in the column shoe. Leachate was collected in 1 L stainless steel bottles placed in an insulated cabinet heated to 2°C during winter and cooled to 10°C during summer. The lysimeters containing gravel were packed in the laboratory by compacting gently with a heavy steel rod for each 5 cm of gravel (Figure 1B). The gravel used for packing had a moisture of 6 weight-% (basecourse gravel) or 7 weight-% (pathway gravel) suitable for packing to the prescribed optimal packing density of 2.0 g/cm^3, which was ensured by weighing.

Each lysimeter was packed with either 36 cm basecourse gravel, 36 cm pathway gravel or 26 cm basecourse gravel plus10 cm rock chippings on top. Four replicates were prepared for each gravel type/combination. In addition, one more lysimeter of each gravel type/combination was prepared with temperature and soil moisture-sensors installed, where measurements were taken every hour. Four lysimeters containing arable soil were made by pushing the lysimeter tube 34-36 cm into the soil using the weight of a drilling rig (Figure 1C) and then excavated out of the soil to ensure as little disturbance as possible. The lower 12 mm of each soil core was replaced with fine quartz sand before fitting it into the column shoe. The soil was sampled into the lysimeter tubes in May 2017 and kept outside with no direct sunlight for ten weeks before being installed at the same date as the packed gravel lysimeters. In addition, one empty lysimeter was installed as a negative control, so in total 20 lysimeters of which 16 would receive herbicides.

Figure 1 **Principal sketch of a lysimeter (a), packing gravel lysimeters in the lab (b), sampling soil lysimeters (c), overview of lysimeter setup (d) and a look into the lysimeter housing (e). The insulated housing was heated to 2°C during winter and cooled to 10°C during summer**



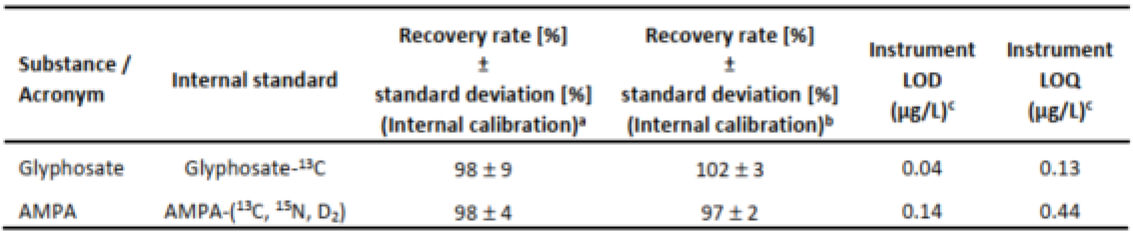
After a month of equilibration, a bromide tracer study was conducted before applying the herbicides. Each lysimeter received 88 mL of a 500 mg/L Br-solution (dissolved as KBr in tap water diluted three times with Milli-Q water) corresponding to 5 mm of precipitation. Bromide in the leachate was analysed by ion chromatography after every larger rain event. After 8 weeks, when bromide concentrations were very low in all leachates, leachate was collected for analysis of background herbicide concentrations and the lysimeters were then sprayed with a commercial herbicide product for use in private gardens (Keeper L®). The Keeper L diluted solution was prepared in Milli-Q water added 25 % tap water and dosed according to the manufacturer's instructions (75 mg/m^2 of glyphosate) so that each lysimeter received 1.8 mL Keeper L diluted solution. The solution was distributed evenly across the gravel/soil surface using a pipette with a 2 mL glass tip. The Keeper L spraying solution should contain 750 mg/L glyphosate, which was confirmed by LC-MS/MS analysis. None of the degradation products were detected in the spraying solution.

Precipitation and air temperature were measured directly by the lysimeters to get the most relevant input for the leaching experiment. Precipitation was measured with a rain gauge with a 0.2 mm resolution at accuracy: ±2 %. Data were measured on an hourly basis. Further, shielded air temperature and temperature at the base of the lysimeters were measured hourly.

Sampling and analysis of pesticide residues

Leachate for pesticide analysis was collected after spraying in October 2017 until May 2019. Leachate was sampled in dry periods whenever leachate was present, and in wet periods before the bottles were full. The amount of leachate was quantified. The leachates equal the net precipitation, which in case of no vegetation equals precipitation minus evaporation. Replicate samples were collected at30 time points within the monitoring period. Sub-samples of 3 mL were stored at 5 °C until analysis for a maximum of two weeks. Prior to spray application, leachate from all lysimeters was collected and analysed, but none of the pesticides or their degradation products were detected. Glyphosate and AMPA were directly analysed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLCMS/MS). Before analysis, samples were vortex shaken for 30 s for homogenization and further centrifuged at 2500 RFC for 10 min to avoid particles being injected in the HPLC. One mL of the supernatant was transferred to a HPLC vial to which internal standard solution of glyphosate was added. Finally, 10 mL of the water samples were injected into the HPLC system. Compound concentration was calculated by linear regression of standard calibration (1/x weighting). The peak areas were corrected by isotope-labelled internal standards. For quality control, procedural blanks were analysed within every experimental sequence to determine instrumental noise and possible contaminations during the sample treatment. Good linearity for the different compounds was obtained ($n \geq 12$; $R^2 > 0.98$). Method limit of detection was 0.04 µg/L for glyphosate and 0.14 µg/L for AMPA. Detailed results of the methods performance are provided in Table 1.

Table 1 **Retention time as well as optimized MS-conditions for the analysis of glyphosate and AMPA. For each compound, two MRM transitions were used as quantifiers**



Substance / Acronym	Internal standard	Recovery rate [%] ± standard deviation [%] (Internal calibration)[a]	Recovery rate [%] ± standard deviation [%] (Internal calibration)[b]	Instrument LOD (µg/L)[c]	Instrument LOQ (µg/L)[c]
Glyphosate	Glyphosate-^{13}C	98 ± 9	102 ± 3	0.04	0.13
AMPA	AMPA-(^{13}C, ^{15}N, D_2)	98 ± 4	97 ± 2	0.14	0.44

[a] recovery function tested for a spiking level of 0.1 µg/L in three different water samples: Roskilde Fjord, leachate from basecourse gravel + rock chippings lysimeter and leachate from basecourse gravel lysimeter (duplicate injections; 3 water samples; n=6)

[b] recovery function tested for a range of concentrations (0.4, 4 and 40 µg/L) using leachate from the basecourse gravel, pathway gravel and sandy soil lysimeters (duplicate injections; 3 water samples; 3 concentrations; n=9)

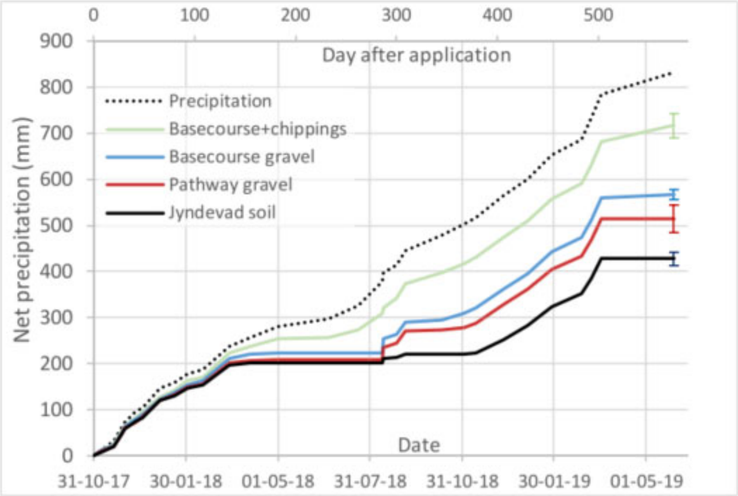
[c] calculated as MeOH blanks average concentration + 3x or 10x MeOH concentration standard deviation (n=3) and cross-validated against S/N.

Results

Tracer study and leachate data

The bromide tracer study showed no differences between replicate gravel lysimeters indicating that these were identically packed. Some differences in active pore volumes were observed between different gravel packings. The peak bromide concentrations were seen after ~30 mm leachate for basecourse gravel plus chippings, ~40 mm leachate for basecourse gravel and ~50 mm leachate for pathway gravel. The intact arable soil showed peak concentrations at ~50 mm leachate indicating a similar active pore volume as that in pathway gravel, but with a slightly higher variation between replicate lysimeters. This variation probably reflects the natural heterogeneity that exists even in a sandy arable soil that is tilled regularly. The net precipitation (leachate) over the complete experimental period was very similar among replicate lysimeters, but differed markedly between the different types of gravel/soil (Figure 2).

Figure 2 **Net precipitation (cumulated mean) in the different lysimeters (n = 4) compared to precipitation (n = 1) from the time of pesticide application until end of experiment. Error bars on the total net precipitation are standard deviation**



Also the periods in which leachate was formed was dependent on the type of gravel/soil. The highest evaporation was observed from the arable soil, probably due to capillary forces drawing moisture to the surface, which would not occur in the gravel due to coarser structure and low organic matter. The arable soil therefore had a net precipitation of only half the gross precipitation and with no leachate during the late spring and summer months. Pathway gravel and basecourse gravel had quite similar leachate patterns concerning both timing and amount ending up with 516-567 mm net precipitation over the whole period compared to 834 mm gross precipitation. The lysimeters with chippings on top had very little evaporation ending up with an average total net precipitation of 717 mm. The lysimeters with chippings on top were the only ones showing significant leachate during late spring and summer. It should be noted that in 2018 this period was very warm and dry (Figure 3). Temperature was more or less the same in all types of gravel lysimeters and fluctuated in the same ways (Figure 4). Daily average temperatures in the lysimeters reached up to ~25 °C during summer and down to ~0 °C during winter and the average temperature from October 2017 to May 2019 was ~9.5 °C at both levels in all lysimeters, very close to the average air temperature during that period (9.6 °C). Fluctuations in soil moisture were also synchronal between different gravel types but with larger fluctuations in the pathway gravel (Figure 4).

Figure 3 **Precipitation and air temperature by the lysimeter**

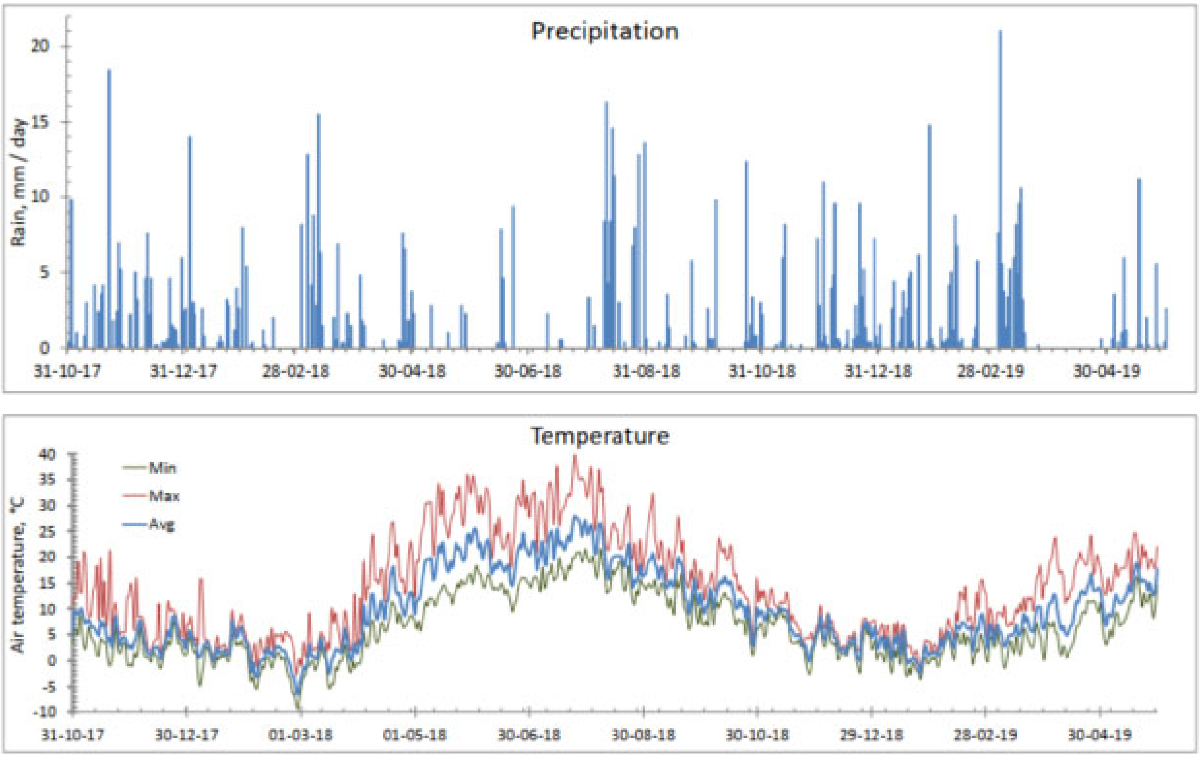
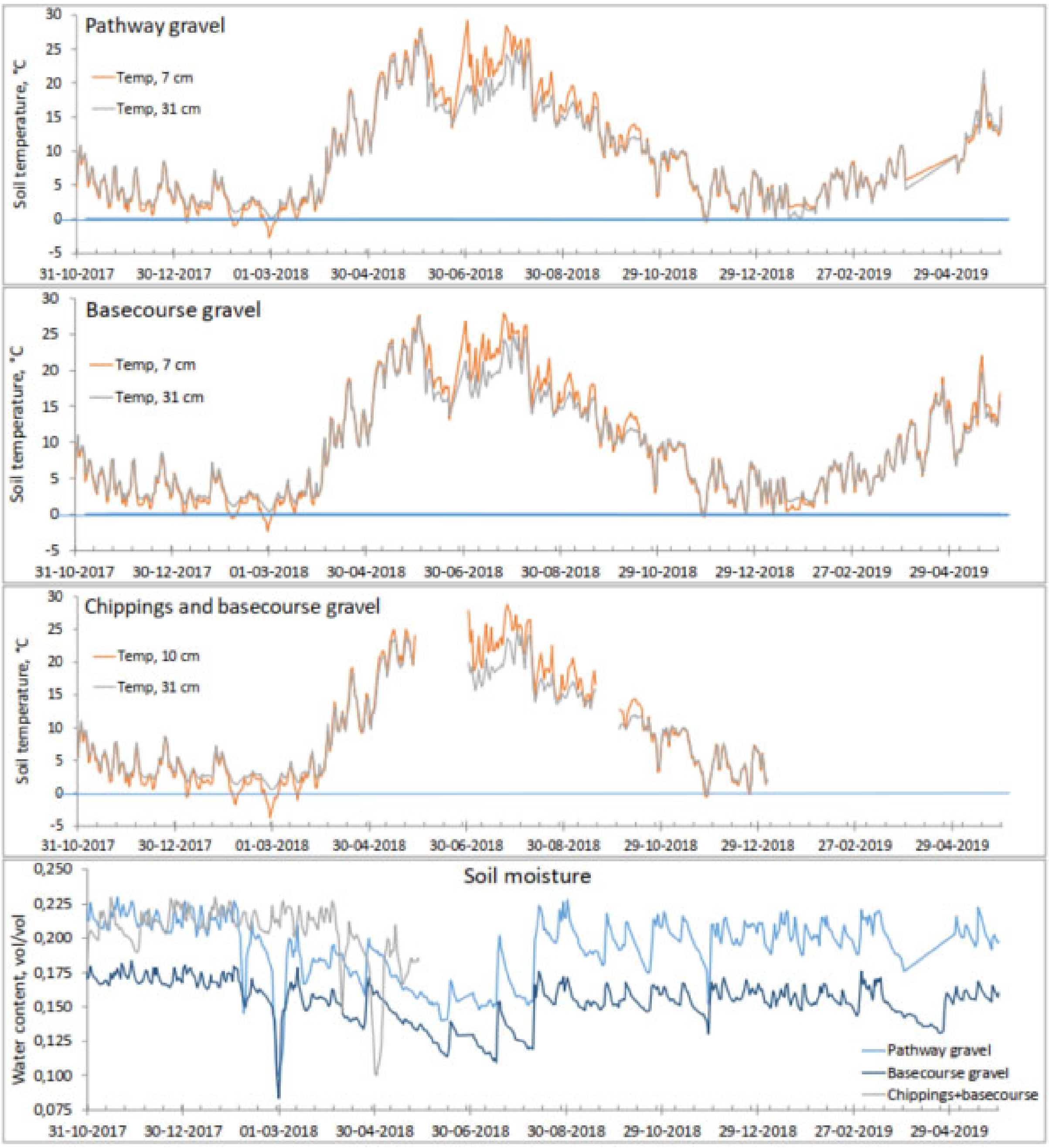


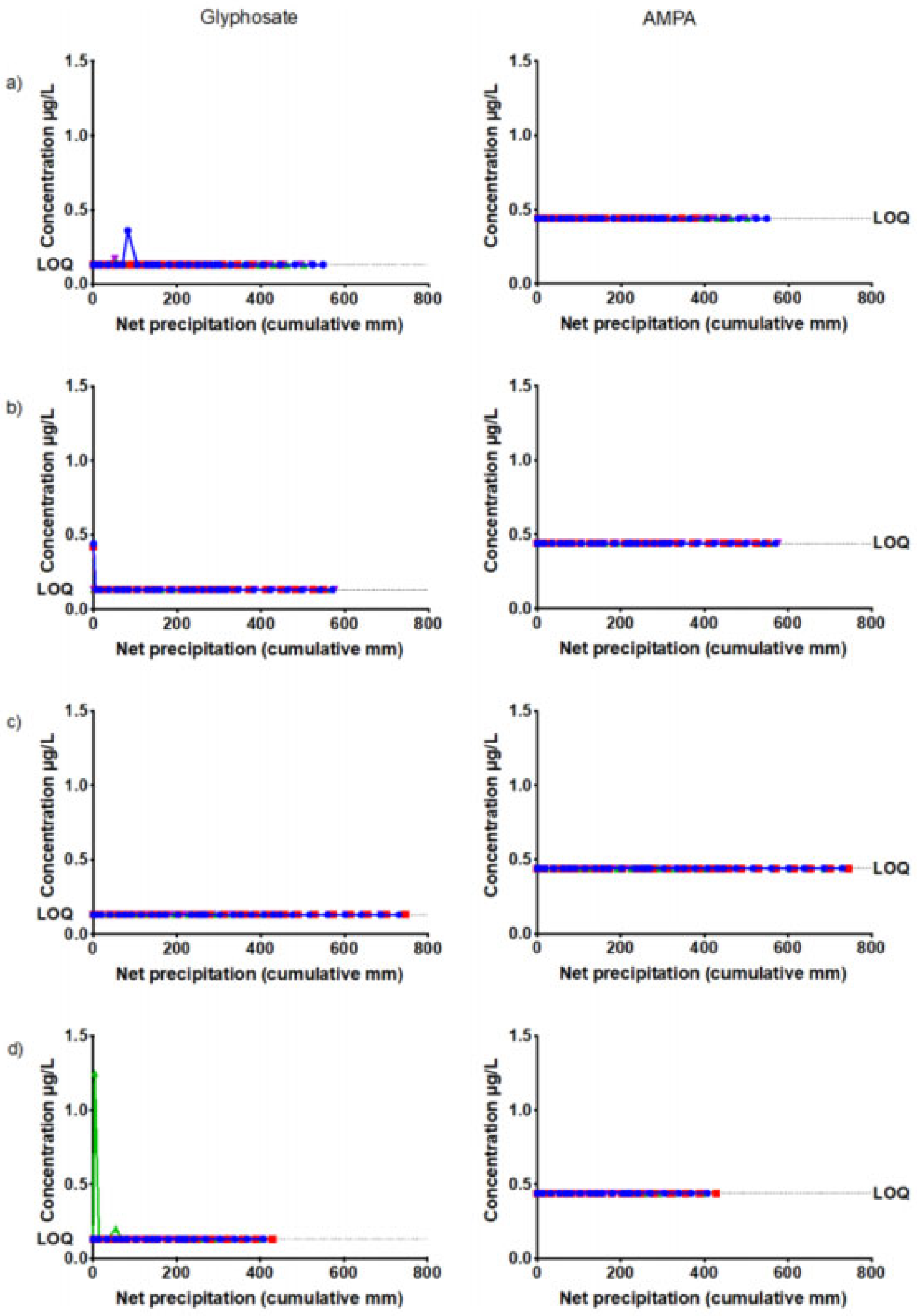
Figure 4 **Daily averages of soil temperature (at 7 or 10 and 31 cm depth) and soil moisture (15-20 cm depth) for gravel lysimeters from the day of pesticide application until end of experiment. Sudden decreases in moisture during winter is not due to desiccation but is caused by freezing of the pore water. The TDR-probe broke down in the Chippings+bascourse-lysimeter, so the measurements stops in May 2018 and for this lysimeter, temperature readings stopped in January 2019**



Leaching of glyphosate

After the pesticide application in October 2017, the concentration of glyphosate as well as of its known degradation product AMPA were followed for 19 months (Figure 5). Except for a few non-systematic glyphosate detections, glyphosate and AMPA were never measured in concentrations above the limit of quantification (0.13 and 0.44 µg/L, respectively).

Figure 5 **Glyphosate and AMPA leaching as a function of cumulated net precipitation in individual lysimeters a) pathway gravel, b) basecourse gravel, c) basecourse gravel + chippings, d) arable soil. Different colours represent each of the replicates (n=4) for each lysimeter type**



Conclusion

Agricultural soils are all in all poor proxies for the behaviour of pesticides in gravel surfaces and potentially other hard and paved surfaces. If pesticides are planned to be used in non-agricultural context new guidelines for their assessment should be developed to protect groundwater. For relatively strong adsorbing pesticides such as glyphosate, the degradation products can be more relevant than the parent compounds with respect to leaching to lower soil layers, also in gravel where generally a low adsorption is expected, although in case of AMPA not indicated due to high adsorption of AMPA itself. Different properties of gravel compared to those of agricultural soils may lead to very different herbicide leaching patterns, but also the leaching depends highly on the type of gravel and type of herbicide.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes mini-lysimeter experiments with glyphosate in agricultural soil as well as in gravel used for construction of gravel paths or driveways. The experiments and results are well described. The LOQ value of 0.13 µg/L for glyphosate is above the current regulatory limit concentration. Glyphosate was not applied as active substance only, but as a product with another strong sorbing active (diflufenican). Therefore, the article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5
Report author	Papagiannaki D. *et al.*
Report year	2020
Report title	Effect of UV-A, UV-B and UV-C irradiation of glyphosate on photolysis and mitigation of aquatic toxicity.
Document No	Scientific Reports (2020), Vol. 10, No. 1, Article No. 20247
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes (Relevant, Category A acc. EFSA GD 2092, Point 5.4.1) / Reliable with regard to photolysis experiments

This summary includes only information related to photolysis. With regards to aquatic toxicity, the publication was considered not relevant.

2. Full summary of the study according to OECD format

The active herbicide ingredient glyphosate [N-(phosphonomethyl)glycine] is frequently detected as a contaminant in groundwater and surface waters. This study investigated effects of UV-A (365 nm), UV-B (302 nm) and UV-C (254 nm) irradiation of glyphosate in water on photolysis. High-resolution mass spectrometry analyses of samples after UV-C irradiation showed > 90 % decreases in glyphosate concentrations and the presence of multiple transformation products. The study suggests that UV mediated indirect photolysis can decrease concentrations of glyphosate.

Materials and methods

Chemicals

Stock solutions of glyphosate were prepared in autoclaved distilled deionized water from a 40 % wt/vol *N*-(phosphonomethyl)glycine, monoisopropylamine salt solution (CAS 38641-94-0), and from *N*-(phosphonomethyl)glycine (CAS 1071-83-6). Stock solutions of commercial brands of glyphosate-based herbicides were prepared in autoclaved distilled deionized water from Roundup Ready to Use containing 7.2 % wt/vol glyphosate, Roundup Garden containing 12 % wt/vol glyphosate, Gallup Super 360 containing 36 % wt/vol glyphosate, and Glyfonova 450 Plus containing 45 % wt/vol glyphosate. According to the product datasheets, these formulations also contain water and various surfactants and formulating agents. All stock solutions of glyphosate and glyphosate-based herbicides were stored in the dark at 5 °C.

Drinking water samples

Drinking water samples were collected at Aalborg, Aarhus, Skagen and Sønderborg municipalities (Denmark). Water samples were also collected at the influent (raw water) and effluent (treated water) of Elsted drinking water treatment plant (Denmark). All water samples originated from groundwater but with regional differences in organic and inorganic constituents according to the national Danish well database on water quality.

UV irradiation of glyphosate in aqueous samples.

Glyphosate and glyphosate-based herbicides dissolved in distilled deionized water or drinking water were exposed to different doses of UV-A, UV-B or UV-C irradiation. Stock solutions with 50 and 100 mg/L glyphosate were exposed to UV irradiation at 22 °C in 10 mm 3.5 mL quartz cuvettes. Dilute glyphosate concentrations were prepared in UV bottom-transparent 96 well microplates to examine the effects of glyphosate concentrations between 0.18 and 100 mg/L on the outcome of UV irradiation. The UV microplates with different glyphosate concentrations were exposed from the top or bottom using similar UV doses as the quartz cuvettes with uniform glyphosate concentrations. The 96-well UV microplates were placed on a cooling plate to avoid heating and maintain the temperature at around 22 °C to minimize evaporation from the small sample volumes used in these plates (100 µL). In all UV experiments, parallel control samples without UV exposure were covered with aluminium foil and stored in the dark for later analysis together with UV exposed samples.

UV irradiation experiments were carried out using a 4 W UVP UVGL-25 lamp equipped with separate UV-A (365 nm) and UV-C (254 nm) tubes, and an 8 W UVP 3UV lamp equipped with separate tubes for UV-A (365 nm), UV-B (302 nm) and UV-C (254 nm). The two UV lamps gave comparable results in experiments for comparable UV doses, and the 8 W lamp with greater intensity was selected to shorten exposure times. Irradiation intensity was measured using an Extech SDL470 Light meter equipped with UV-AB and UV-C sensors. The irradiation intensity at 15 cm distance from the UVP 3UV lamp was 970 $\mu W/cm^2/s$ for UV-A, 1900 $\mu W/cm^2/s$ for UV-B, and 327 $\mu W/cm^2/s$ for UV-C. UV doses (J/cm^2) were calculated from the measured UV irradiation intensity ($\mu W/cm^2/s$) and the exposure time (s). No detectable UV-C irradiation was measured from the UV-A and UV-B lamps and vice versa. UV exposure doses (J/cm^2) were controlled by changing exposure times and distances to the UV lamps. For example, an exposure UV dose of 20 J/cm^2 was obtained using the same exposure time but different distances to the UV lamp (20 cm for UV-A, 35 cm for UV-B, and for 5 cm for UV-C). The presence of active oxygen species in aqueous samples after UV irradiation was confirmed by the addition of different oxygen radical probes. Superoxide radical ($\cdot O_2^-$), was detected by measuring chemiluminescence after post-treatment addition of 1 mM luminol. Hydroxyl radical ($\cdot OH$) was detected by measuring fluorescence after pre-treatment addition of 1 mM coumarin, terephthalic acid or benzoic acid. Chemiluminescence and fluorescence originating from the oxygen radical probes after reaction with active oxygen species were quantified using a Victor X2 Multilabel Plate Reader.

Analysis of glyphosate phototransformation products

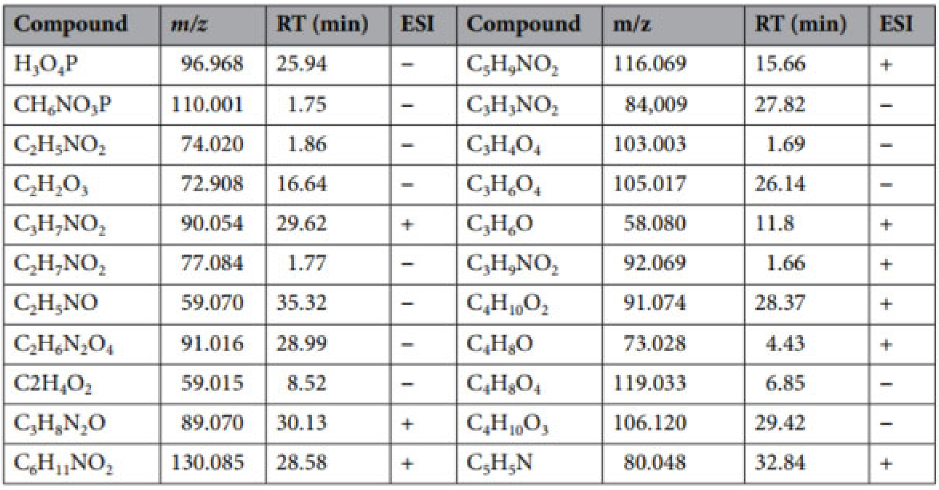
Liquid chromatography-high resolution mass spectrometry analysis (LC-HRMS) of water samples with glyphosate was performed before and after UV exposure to identify transformation products. Analyses were carried out using an Ultimate 3000 High-Pressure Liquid Chromatography coupled through an ESI source to an LTQ-Orbitrap mass spectrometer. Chromatographic separation was achieved using a reversed-phase C18 column by injecting a 10 µL sample volume at a mobile phase consisted of a mixture of 0.1 mM Formic Acid (eluent A) and Acetonitrile (eluent B). The gradient profile started with 5 % B, increased up to 100 % B in 40 min and to 100 % A in 10 min. Samples were ionized in both positive and negative ionization modes. The LC effluent was delivered to the ESI ion source using nitrogen as sheath and auxiliary gas with the following parameters: sheath gas 34 arbitrary unit (arb), auxiliary gas 15 arb, capillary voltage 4.48 kV, and capillary temperature of 270 °C. Full mass spectra were acquired in positive ion mode with a resolution of 30.000. Data analysis was performed using the MZmine 2.53 for peak alignment, peak grouping, background noise and retention time correction, and the METLIN database was used to identify the transformation products.

Results

Identification of glyphosate phototransformation products

Liquid chromatography high-resolution mass spectrometry analysis (LC-HRMS) of water samples with glyphosate was performed before and after UV-C irradiation to identify transformation products after UV doses of 20 J/cm^2 and 70 J/cm^2 (Table 1). The analysis was carried out as a semi-targeted analysis focusing on likely glyphosate transformation products using the METLIN database. The LC-HRMS analysis confirmed that aqueous concentrations of glyphosate decreased by 96 % after UV-C irradiation and that degradation had occurred. More than 20 glyphosate ($C_3H_8NO_5P$) transformation products were observed after UV-C irradiation (Table 1) including main products such as sarcosine ($C_3H_7NO_2$), glycine ($C_2H_5NO_2$), glyoxylic acid ($C_2H_2O_3$), aminomethylphosphonic acid (CH_6NO_3P; AMPA), acetic acid (CH_4O_2) and phosphoric acid (H_3PO_4). CO_2 was also a potential transformation product but was not targeted in the analysis due to a high background concentration of CO_2/HCO_3^- in the water samples. Increases in AMPA and glycine concentrations were observed after UV-C irradiation at 70 J/cm^2, whereas sarcosine concentrations decreased. Phosphoric acid was detected at both high and low UV-C doses whereas glyoxylic and acetic acid were only observed after UV-C irradiation at 70 J/cm^2. Glyphosate concentration decreased in samples irradiated with 20 J/cm^2 and was no longer detectable in samples treated with 70 J/cm^2.

Table 1 **Identified transformation products by LC-HRMS analysis in negative ESI (-) and positive ESI(+) modes after UV-C irradiation of aqueous glyphosate**



Compound	*m/z*	RT (min)	ESI	Compound	m/z	RT (min)	ESI
H_3O_4P	96.968	25.94	–	$C_5H_9NO_2$	116.069	15.66	+
CH_6NO_3P	110.001	1.75	–	$C_3H_3NO_2$	84,009	27.82	–
$C_2H_5NO_2$	74.020	1.86	–	$C_3H_4O_4$	103.003	1.69	–
$C_2H_2O_3$	72.908	16.64	–	$C_3H_6O_4$	105.017	26.14	–
$C_3H_7NO_2$	90.054	29.62	+	C_3H_6O	58.080	11.8	+
$C_2H_7NO_2$	77.084	1.77	–	$C_3H_9NO_2$	92.069	1.66	+
C_2H_5NO	59.070	35.32	–	$C_4H_{10}O_2$	91.074	28.37	+
$C_2H_6N_2O_4$	91.016	28.99	–	C_4H_8O	73.028	4.43	+
$C2H_4O_2$	59.015	8.52	–	$C_4H_8O_4$	119.033	6.85	–
$C_3H_8N_2O$	89.070	30.13	+	$C_4H_{10}O_3$	106.120	29.42	–
$C_6H_{11}NO_2$	130.085	28.58	+	C_5H_5N	80.048	32.84	+

Conclusion

This study investigated effects of UV-A, UV-B and UV-C irradiation of glyphosate on photolysis. The results suggested that UV-C and to some extend UV-B mediated indirect photolysis of glyphosate in water could attenuate concentrations of this pesticide. UV-C mediated formation of less bioactive glyphosate transformation products may subsequently facilitate environmental biodegradation. The results of our study can be relevant for further developments of UV mediated treatment processes for aquatic contaminants.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes experiments on the effects of UV-A, UV-B and UV-C irradiation on glyphosate photolysis. Experiments on aquatic toxicity were neglected in this evaluation as not found relevant. The photolysis experiments and results are well described. Therefore, the article is considered reliable with regard to the photolysis experiments.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.2, CA 7.1.3, CA 7.5
Report author	Tauchnitz N. *et al.*
Report year	2020
Report title	Assessment of pesticide inputs into surface waters by agricultural and urban sources - A case study in the Querne/Weida catchment, central Germany.
Document No	Environmental pollution (2020), Vol. 267, Art No. 115186
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes (Relevant, Category A acc. EFSA GD 2092, Point 5.4.1) / Degradation experiments: not reliable Adsorption experiments: not reliable Monitoring data: reliable

2. Full summary of the study according to OECD format

Relevant pesticide substances were measured in surface waters in agricultural and urban surroundings and in soil samples within the catchment area of the Querne/Weida catchment. Pesticides application data from farmers were analysed. Additionally, batch tests were performed to determine sorption and degradation of relevant pesticides for site-specific soil properties. Frequency of detection, number of pesticides and maximum concentrations were much higher in the surface water samples in mainly urban surroundings compared to those in agricultural surrounding. Glyphosate and AMPA contributed to the highest concentrations in surface water samples (max. 58 mg L^{-1}) and soil samples (max. 0.19 mg kg^{-1}). In most cases, pesticide detections in surface water and soil were not consistent with application data from farmers, indicating that urban sources may affect water quality in the catchment area substantially. However, it was observed that pesticide substances remain in the soil over a long time supported by sorption on the soil matrix. Therefore, delayed inputs into surface waters could be suspected. For the implementation of reduction measures, both urban and agricultural sources should be considered.

Materials and methods

Test site

The study was conducted in the Querne/Weida catchment located in Saxony-Anhalt (central Germany). The entire catchment area covers 247 km^2. The selected sub-catchment comprises 150 km^2 with the largest portion (97%) used as arable land. The main agricultural crops during the study period (2015-2016) were winter wheat (36 %), silage corn (16 %), winter barley and winter oilseed rape (13 %), sugar beets (6 %) and grain corn (4 %). Several towns are located within the catchment, with Querfurt being the largest (11,562 inhabitants). In the catchment, 92 % of inhabitants were connected to two central water run-off wastewater treatment plants (WWTPs) and 8 % to small decentralized WWTPs, which release their water to groundwater, to a septic tank or directly to surface waters.

The climate of the study site is subcontinental and characterized by low precipitation amounts with a long-term (1981-2010) precipitation level of 550 mm and maximum precipitation falling during the summer months. The long-term average annual air temperature was 9.0 °C. The geology consists of Mesozoic rocks (shell limestone, mottled sandstone) which are largely covered by a loess layer. Main soil types at the study site are Chernozems and Luvisols.

Precipitation and discharge

Precipitation amounts were obtained from the German Weather Service station Lodersleben (elev. 204 m, 51°23N, 11°37E). Discharge amounts were registered at two gauge stations in the Kriebuschbach and Weida surface waters by continuously measurement of water levels and periodic measurement of flow velocities. Discharge amounts were calculated by the rating curve method (stage-discharge relation). Discharge data from the two gauge stations were stored at 15 min intervals by the data logger of an automatic water sampler.

Water and soil sampling

Grab water samples were collected monthly from July 2015 to June 2016 at 9 sampling sites in different surface waters. At two sampling sites, one with a mainly agricultural surrounding (SP_{agr}) and one with a mainly urban surrounding (SP_{urb}), water samples were taken with daily resolution and pooled to one composite weekly sample from June 2015 to July 2016. For this purpose, automatic water samplers equipped with polytetrafluoroethylene (PTFE)-tubes and polypropylene bottles were installed in the surface waters. Composite samples were taken at weekly frequencies (7 aliquots every 24 h). Water samples were transferred to glass bottles and stored in the dark at 4 °C until analyses. Topsoil samples (0-30 cm) were taken at 25 different sampling sites in the vicinity of surface waters, in areas which were assessed to be vulnerable for erosion. In addition, soil samples were taken at five sampling sites at permanent soil observation plots up to a depth of 5 m by the core drilling method (drill diameters of 105 mm).

Analysis of water and soil samples

Water samples were measured unfiltered after suspended particles had deposited on the bottom of the glass bottles. The samples were analysed for 53 pesticide substances, amongst them glyphosate and its degradation product aminomethylphosphonic acid (AMPA). Glyphosate and AMPA were analysed by HPLC-MS according to DIN 38407-22 (2001), modified with pre-column derivatization. The limit of determination (LOD) 10 ng L^{-1} for glyphosate and AMPA).

The soil was air-dried and passed through a 2 mm sieve. For analyses of glyphosate and AMPA, 5 g soil was extracted with 10 mL of 0.6 M potassium hydroxide. The sample was shaken for 30 min and centrifuged for 30 min at 5000 rpm. A portion of 600 mL of 32 % hydrochloric acid was added to precipitate humic acids for 15 min. Four mL of the sample were diluted with 6 mL water and filtered through a 500 mg Chromabond®C18 ec filter. The filter was previously conditioned with 5 mL methanol and 1 mL water. Samples were analysed with LC coupled to a triple quadrupole MS (LC-MS/MS). The limit of detection was 0.025 and LOQ was 0.05 mg kg^{-1} soil dry matter (DM).

Pesticide application data

For the analyses of pesticide application in the catchment area, farmers were contacted and asked to provide their pesticide application data for a retroactive period of 6 years (2011-2016). Farmers managing arable land adjacent to surface waters were primarily considered. The following information was requested: date of pesticide application, treated crop, indication of treatment, name of applied pesticide and amount (l ha^1, g ha^1). The amount of active ingredients of each pesticide was searched in the BVL (2020) database. Data from 7 farms encompassing a total of 9573 ha arable land (547 fields), corresponding to 66 % of the total farmland in the catchment area was obtained. Application data were tested for improper use by random checks with respect to application amounts, application date and indications. Pesticide detections in surface waters were compared to the application data of farmland directly adjacent to the considered surface waters at a radius of approx. 3 km around the sampling point. Pesticide detections in the topsoil samples were compared directly to the application data from the respective field (if available). For the depth profiles at permanent soil observation plots, application data from farmers for a retroactive period of 10 years (2007-2016) were available for analysis. For all pesticide detections in water and soil samples, the days between last application and detection were calculated, if application data were available.

Sorption and degradation laboratory study

For the laboratory study, homogenized soil samples from depth profiles (sorption study: 0-1 m, degradation study: 2-4 m) with the following soil properties were used: sandy silt, pH 7.8, 0.12 % of OC DM for degradation studies and clayey silt, pH 7.3, 1.29 % of OC DM for the sorption studies.

Sorption studies were performed by carrying out two classic batch test series (A & B). For each batch series, a solution consisting of synthetic rainwater (distilled water with 1.64 mg L^{-1} $KHCO^3$, 6.86 mg L^{-1} $NaNO_3$ and 40.04 mg L^{-1} $CaSO_4 * 2 H_2O$) spiked with the pesticide substances (glyphosate A: 170 & B: 400) were prepared. For this purpose, pesticide substances were added to the synthetic rainwater, stirred at 40 °C for 12 h and then filtered. For the different approaches, soil dry matters of 226 g, 452 g, 903 g, 1355 g and 1806 g along with corresponding process water amounts to achieve liquid-solid ratios of 8.5, 4.0, 1.7, 1.0 and 0.6 were filled in 2 L glass bottles. As reference for blank subtraction, process water without added soil was used. The batches were shaken overhead for 22 h at 10 °C in the dark and left for 2 h for sedimentation. Then samples were centrifuged for 20 min at 4000 rpm. Supernatants were decanted and filtered and analysed as described above. The difference between the initial pesticide concentration in solution and the concentration after equilibration was assumed to equal the amount of pesticides sorbed to the soil. The isotherm models Henry and Freundlich have been applied to determine the sorption coefficients (K_d, K_{Fr}).

For examination of the microbial degradation, static batch tests (soil saturation extracts, method embedded in LfULG, 2004) were prepared under anaerobic conditions. Analogous to the sorption study, the homogenous soil samples were mixed with synthetic rainwater, which was spiked with the pesticide substances at a concentration of 200 mg L^{-1} (solution stirred at 40 °C for 12 h and then pressure filtered). The produced suspensions with a liquid-solid ratio of 0.3:1 were incubated in the dark in airtight 1 L glass jars at 10 °C. For the analyses, pore water was extracted by a stainless-steel pressure-filtration-cell equipped with a 0.45 mm filter up to 2 bar with N_2. The first sampling of pore water was conducted 1 day after preparation of soil saturation extracts, sufficient time for equilibration between liquid and solid phase. Pore water samples were analysed for glyphosate and AMPA as described above every 1-3 months (time wise increasing sampling intervals) in a time span of up to 333 days. Pesticide degradation was described using first-order kinetics. Correlations between discharge amounts and pesticide concentrations and regression coefficients for the sorption studies were calculated.

Results

Precipitation and discharge

Yearly precipitation during the study period amounted to 479 mm (2015) and 441 mm (2016) whereas the most precipitation fell during the summer months (May to September) with amounts of 287 and 246 mm in 2015 and 2016, respectively. One heavy rain event with 30 mm d^{-1} occurred on 5 July 2015. Average registered discharge in 2015 at the Kriebuschbach gauge station was 0.053 m^3 s^{-1} and in 2016, it was 0.054 m^3 s^{-1}. The high discharge was associated with several heavy rain events in February 2016. Recorded mean discharge at the gauge station Weida in 2015 and 2016 was 0.28 and 0.22 m^3 s^{-1}, respectively. Accumulated for the weekly intervals highest discharge was registered in August 2015. Compared to the long-term mean (2006-2016) of 0.091 m^3 s^{-1} (Kriebuschbach) and 0.308 m^3 s^{-1} (1966-2016, Weida) measured discharge in the study period was markedly lower.

Pesticide application and detection in water and soil

Analyses of pesticide use data in the catchment area showed an application of 112 different pesticide substances during the period of 2011-2016, whereas the number of applied pesticide substances ranged between 29 and 70 per farm and 1 to 58 per field. Types of applied pesticides were mainly herbicides (53 %) and fungicides (26 %) e.g. glyphosate. The proportion of applied insecticides, growth regulators and rodenticides was smaller with 10 %, 10 % and 1 %, respectively. Indications of incorrect uses of pesticides by farmers (e.g. exceedances of allowed pesticide amounts) were not noticeable.

Grab water samples

In the monthly grab water samples, numerous pesticide substances were detected, ranging from 6 to maximum 35 different substances. At five sampling sites, pesticide substances were found in all analysed water samples. The lowest number of detections was observed at sampling site Kriebuschbach, which is located next to a forest. The highest number of pesticides detected, was observed at sampling sites in the vicinity of urban areas. One of the most frequently detected substances was glyphosate and its metabolite AMPA. The pesticide glyphosate and AMPA contributed to the highest concentrations in the water samples with maximum values of 58.0 µg L^{-1}, and 5.4 µg L^{-1}, respectively. (see Figure 3).

Weekly composite water samples

In the composite water samples, in mainly agricultural surroundings (SP_{agr}), nine different pesticide substances were detected during the study period overall (Table 1). The most frequently detected substance was glyphosate (9 % of water samples), followed by AMPA with 7 % frequency of detection, respectively. Glyphosate and AMPA also contributed to the highest concentrations of maximum 0.20 and 0.21 µg L^{-1} and on average of 0.021 and 0.025 µg L^{-1}, respectively (Table 1). At SP_{agr}, positive detections were found in 26 % of analysed water samples. In contrast to SP_{agr}, at the sampling point in a mainly urban surrounding (SP_{urb}), higher concentrations of pesticide substances and a higher frequency of detection was observed (Table 2, see Figure 2). Here, 24 different substances were present and 90 % of analysed water samples showed positive detections. One of the most frequently determined substance was glyphosate and its metabolite AMPA with 63 % and 39 % frequency of detection, respectively. A total of 11 of the detected substances are known to be used for dual (agricultural and urban) or solely urban purposes. The highest mean concentration was measured for glyphosate (0.15 mg L^{-1}) and AMPA was measured at 0.04 mg L^{-1}. The highest concentration of glyphosate was 0.9 mg L^{-1}. Pesticide substances were found throughout the whole course of the year (Figure 2). Total pesticide concentrations in the water samples were positive correlated with discharge amounts ($r = 0.57$, $p < 0.05$, $n = 59$).

Figure 1 **Total pesticide concentration, number of positive findings and discharge at the gauge station Kriebuschbach in mainly agricultural surroundings (SP_{urb})**

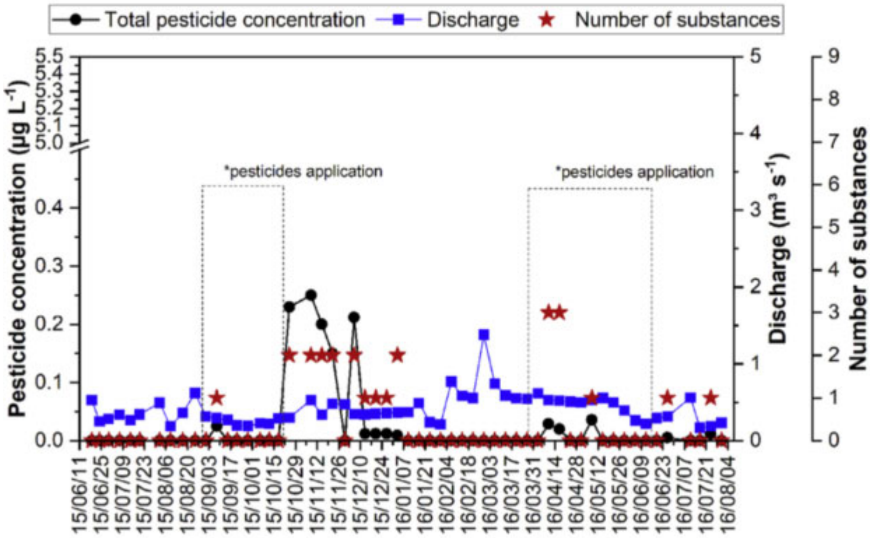


Figure 2 Total pesticide concentration, number of positive findings and discharge at the gauge station Weida in mainly urban surroundings (SP_{urb})

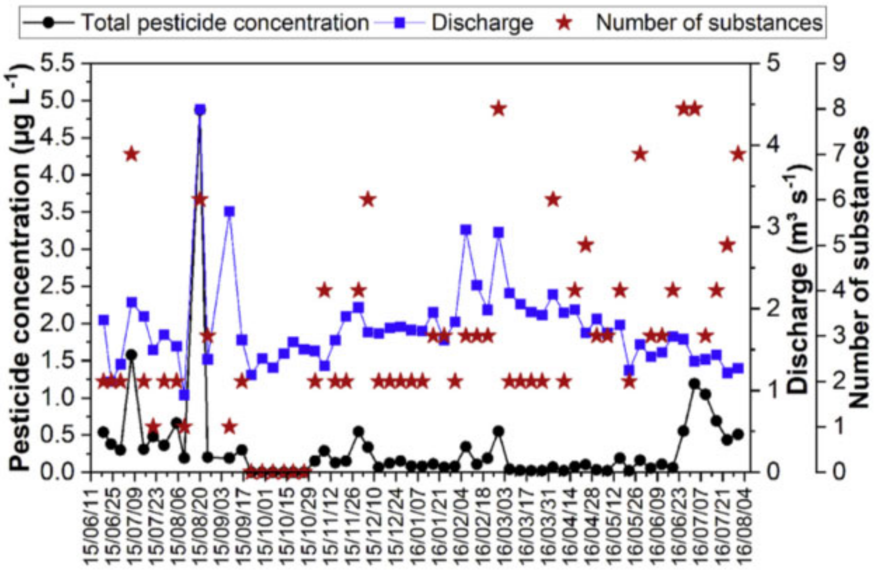


Figure 3 Number of positive detections and maximal concentrations of pesticides in surface water samples (grab water) in the catchment area

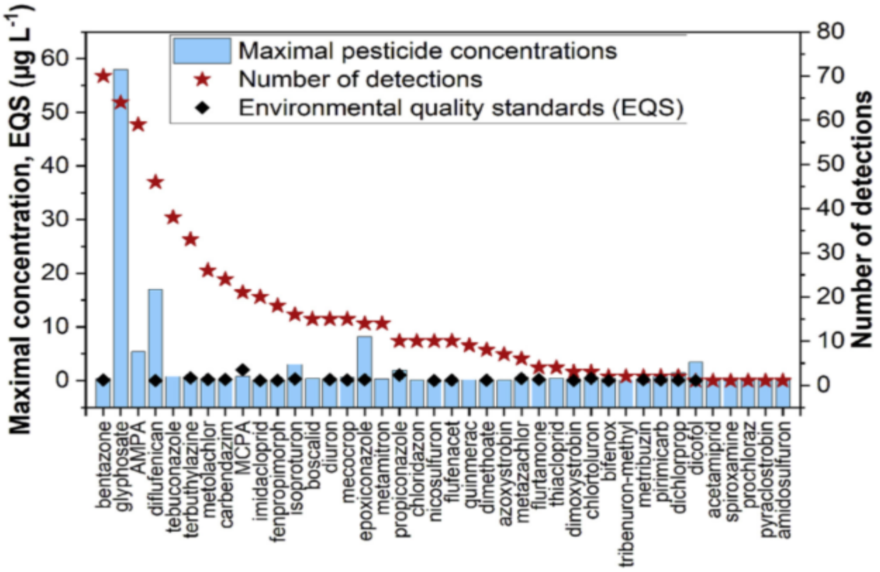
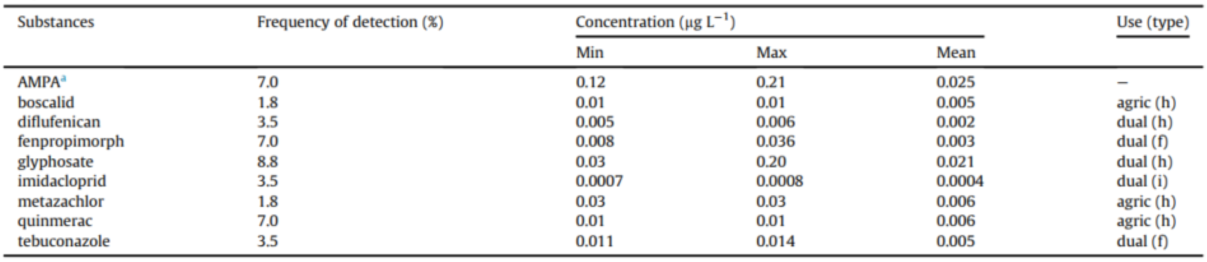


Table 1 **Minimum, maximum, mean concentrations and frequency of detection of pesticide substances in the weekly composite water samples with mainly agricultural surrounding (SP_{agr}) in the study period of 15/06/19 to 16/07/31 (n = 59), pesticide types and their use for agricultural (agric) or dual (urban and agricultural) purposes**

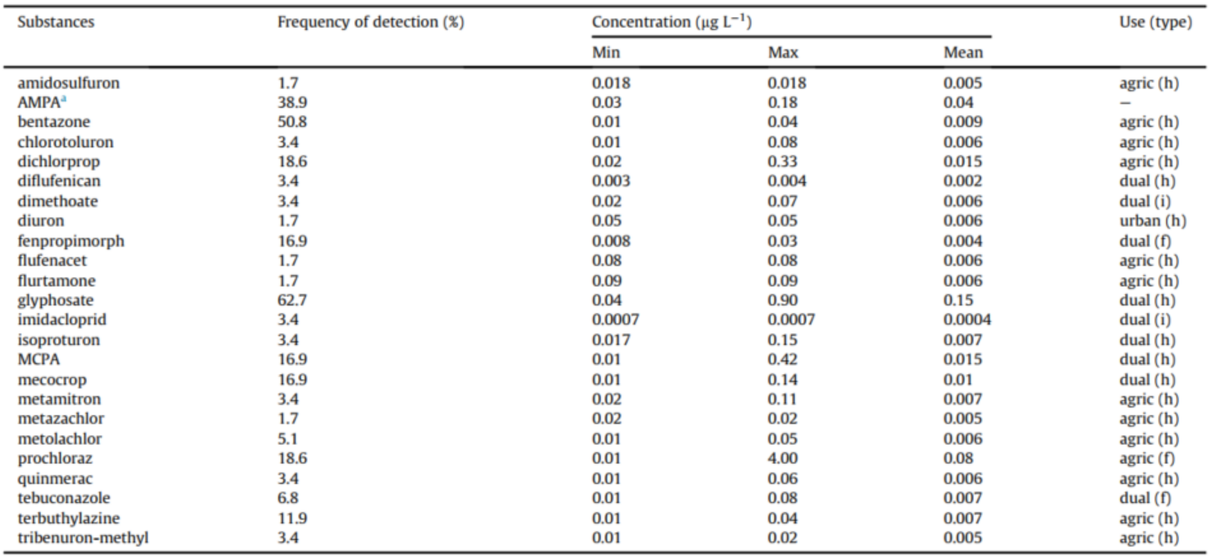


Substances	Frequency of detection (%)	Concentration (μg L^{-1})			Use (type)
		Min	Max	Mean	
AMPA[a]	7.0	0.12	0.21	0.025	–
boscalid	1.8	0.01	0.01	0.005	agric (h)
diflufenican	3.5	0.005	0.006	0.002	dual (h)
fenpropimorph	7.0	0.008	0.036	0.003	dual (f)
glyphosate	8.8	0.03	0.20	0.021	dual (h)
imidacloprid	3.5	0.0007	0.0008	0.0004	dual (i)
metazachlor	1.8	0.03	0.03	0.006	agric (h)
quinmerac	7.0	0.01	0.01	0.006	agric (h)
tebuconazole	3.5	0.011	0.014	0.005	dual (f)

h: herbicide, f: fungicide, i: insecticide

[a] Aminomethylphosphonic acid: metabolite of glyphosate

Table 2 **Minimum, maximum, mean concentrations and frequency of detection of pesticide substances in the weekly composite water samples with mainly urban surrounding (SP_{urb}) in the study period of 15/06/19 to 16/07/31 (n = 59), pesticide types and their use for agricultural (agric), urban or dual (urban and agricultural) purposes**



Substances	Frequency of detection (%)	Concentration (μg L^{-1})			Use (type)
		Min	Max	Mean	
amidosulfuron	1.7	0.018	0.018	0.005	agric (h)
AMPA[a]	38.9	0.03	0.18	0.04	–
bentazone	50.8	0.01	0.04	0.009	agric (h)
chlorotoluron	3.4	0.01	0.08	0.006	agric (h)
dichlorprop	18.6	0.02	0.33	0.015	agric (h)
diflufenican	3.4	0.003	0.004	0.002	dual (h)
dimethoate	3.4	0.02	0.07	0.006	dual (i)
diuron	1.7	0.05	0.05	0.006	urban (h)
fenpropimorph	16.9	0.008	0.03	0.004	dual (f)
flufenacet	1.7	0.08	0.08	0.006	agric (h)
flurtamone	1.7	0.09	0.09	0.006	agric (h)
glyphosate	62.7	0.04	0.90	0.15	dual (h)
imidacloprid	3.4	0.0007	0.0007	0.0004	dual (i)
isoproturon	3.4	0.017	0.15	0.007	dual (h)
MCPA	16.9	0.01	0.42	0.015	dual (h)
mecocrop	16.9	0.01	0.14	0.01	dual (h)
metamitron	3.4	0.02	0.11	0.007	agric (h)
metazachlor	1.7	0.02	0.02	0.005	agric (h)
metolachlor	5.1	0.01	0.05	0.006	agric (h)
prochloraz	18.6	0.01	4.00	0.08	agric (f)
quinmerac	3.4	0.01	0.06	0.006	agric (h)
tebuconazole	6.8	0.01	0.08	0.007	dual (f)
terbuthylazine	11.9	0.01	0.04	0.007	agric (h)
tribenuron-methyl	3.4	0.01	0.02	0.005	agric (h)

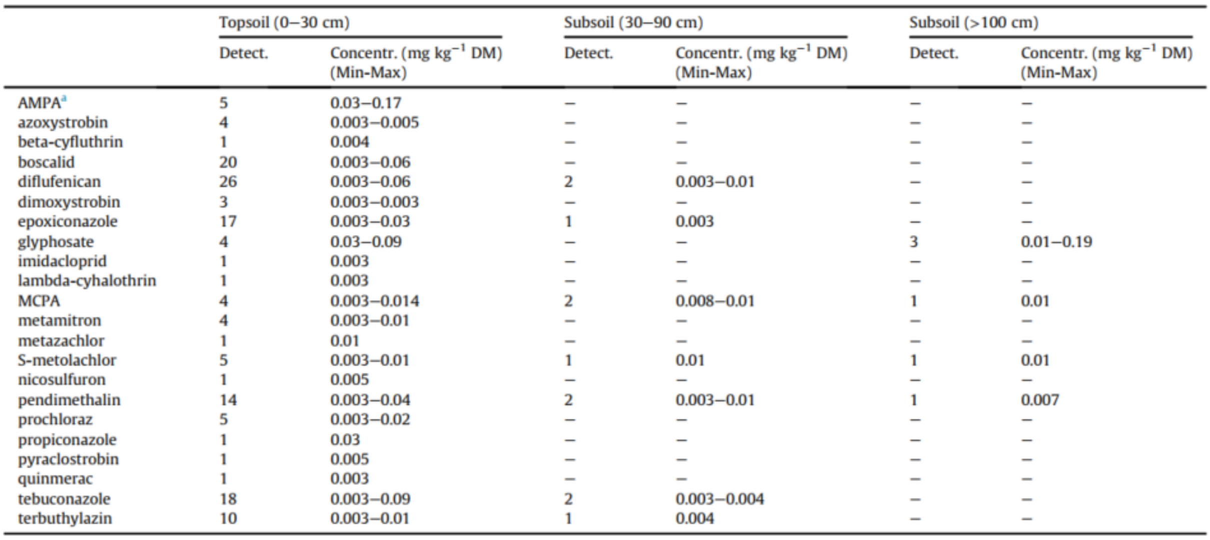
h: herbicide, f: fungicide, i: insecticide

[a] Aminomethylphosphonic acid: metabolite of glyphosate

Soil samples

The soil of the catchment area was characterised by pH values in the range of 7.3-7.9 and organic carbon (OC) contents of 0.07-0.8 % depending on the soil depth. In the analysed soil profiles, 22 different substances were found (Table 3). Concentrations ranged from 0.003 to maximum 0.19 mg kg^{-1} dry matter (DM). Glyphosate and its metabolite AMPA contributed to the highest concentrations of 0.19 and 0.17 mg kg^{-1} DM, respectively but were only detected in 13 % (glyphosate) and 17 % (AMPA) of the considered topsoil samples. Most detected substances were only present in the topsoil, however some amounts of glyphosate were also measured in the subsoil (>30 cm depth) at concentrations between 0.01 and 0.19 mg kg^{-1} DM.

Table 3 **Detected pesticide substances in topsoil (n = 30) and subsoil (n = 5), sampling date: 15/11/01, number of detections (Detect.) and minimal (Min) and maximal (Max) concentrations (Concentr.)**



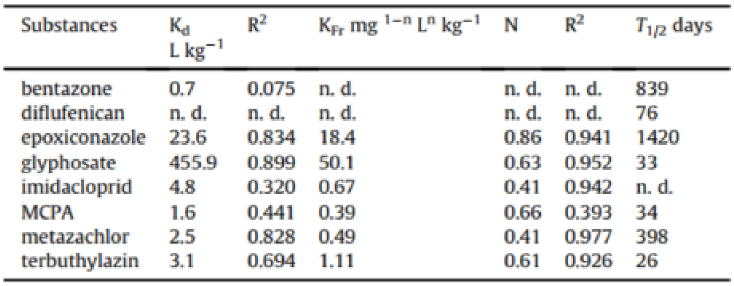
	Topsoil (0–30 cm)		Subsoil (30–90 cm)		Subsoil (>100 cm)	
	Detect.	Concentr. (mg kg^{-1} DM) (Min-Max)	Detect.	Concentr. (mg kg^{-1} DM) (Min-Max)	Detect.	Concentr. (mg kg^{-1} DM) (Min-Max)
AMPA[a]	5	0.03–0.17	–	–	–	–
azoxystrobin	4	0.003–0.005	–	–	–	–
beta-cyfluthrin	1	0.004	–	–	–	–
boscalid	20	0.003–0.06	–	–	–	–
diflufenican	26	0.003–0.06	2	0.003–0.01	–	–
dimoxystrobin	3	0.003–0.003	–	–	–	–
epoxiconazole	17	0.003–0.03	1	0.003	–	–
glyphosate	4	0.03–0.09	–	–	3	0.01–0.19
imidacloprid	1	0.003	–	–	–	–
lambda-cyhalothrin	1	0.003	–	–	–	–
MCPA	4	0.003–0.014	2	0.008–0.01	1	0.01
metamitron	4	0.003–0.01	–	–	–	–
metazachlor	1	0.01	–	–	–	–
S-metolachlor	5	0.003–0.01	1	0.01	1	0.01
nicosulfuron	1	0.005	–	–	–	–
pendimethalin	14	0.003–0.04	2	0.003–0.01	1	0.007
prochloraz	5	0.003–0.02	–	–	–	–
propiconazole	1	0.03	–	–	–	–
pyraclostrobin	1	0.005	–	–	–	–
quinmerac	1	0.003	–	–	–	–
tebuconazole	18	0.003–0.09	2	0.003–0.004	–	–
terbuthylazin	10	0.003–0.01	1	0.004	–	–

[a] Aminomethylphosphonic acid: metabolite of glyphosate; DM: dry matter

Pesticide sorption and degradation

Calculated non-linear Freundlich isotherm coefficients (K_{FR}) provided the best fit of the sorption data with coefficients of determination (R^2) in the range of 0.393-0.977 (on average: 0.855, Table 4). The K_{FR} isotherm coefficients covered a wide range, from 0.39 to 50.1 mg^{1-n} L^n kg^{-1} (Table 4), whereby the highest K^{FR} value was observed for glyphosate. The calculated $T_{1/2}$ values ranged from 26 to 1420 days (Table 4) with a half-life of 33 days for glyphosate.

Table 4 **Henry (K_d) and Freundlich (K_{FR}) sorption coefficients and half-lives ($T_{1/2}$) of selected pesticide substances (anaerobic degradation)**



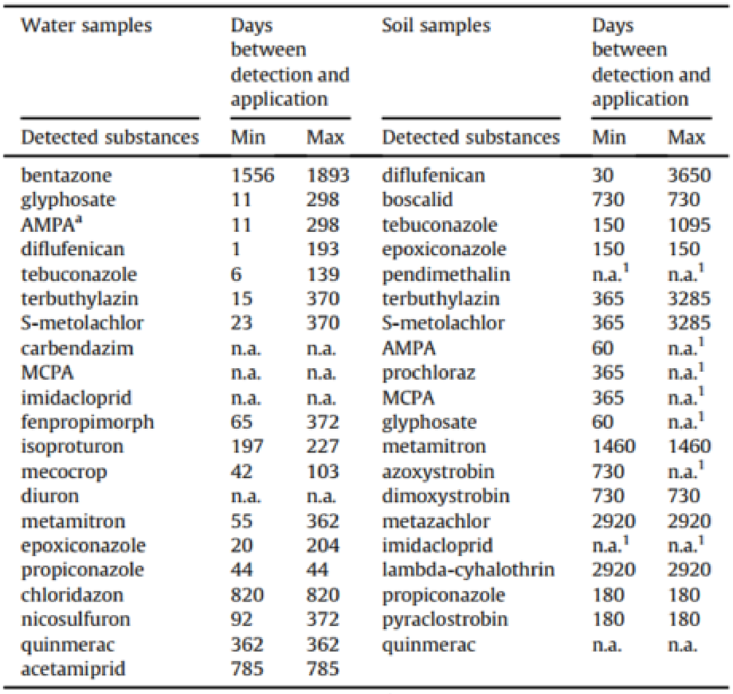
Substances	K_d L kg^{-1}	R^2	K_{Fr} mg $^{1-n}$ L^n kg^{-1}	N	R^2	$T_{1/2}$ days
bentazone	0.7	0.075	n. d.	n. d.	n. d.	839
diflufenican	n. d.	n. d.	n. d.	n. d.	n. d.	76
epoxiconazole	23.6	0.834	18.4	0.86	0.941	1420
glyphosate	455.9	0.899	50.1	0.63	0.952	33
imidacloprid	4.8	0.320	0.67	0.41	0.942	n. d.
MCPA	1.6	0.441	0.39	0.66	0.393	34
metazachlor	2.5	0.828	0.49	0.41	0.977	398
terbuthylazin	3.1	0.694	1.11	0.61	0.926	26

n.d.: not determinable due to no significant decrease in concentration (degradation studies), too low solution concentrations or concentration changes (sorption studies).

Detected pesticide substances related to the application time

Numerous positive detections of up to 35 different substances were found in the studied surface waters. Considering the pesticide application data from farmers, it can be shown that pesticide detections in water and soil samples were not consistent with their application. It was observed that pesticide substances remain in the soil over a long time supported by sorption on the soil matrix. Therefore, delayed inputs into surface waters could be suspected.

Table 5 **Detected pesticide substances in water and soil samples (listed after frequency of detection) related to the application time (for soil samples only analyses of 5 soil depth profiles)**



Water samples	Days between detection and application		Soil samples	Days between detection and application	
Detected substances	Min	Max	Detected substances	Min	Max
bentazone	1556	1893	diflufenican	30	3650
glyphosate	11	298	boscalid	730	730
AMPA[a]	11	298	tebuconazole	150	1095
diflufenican	1	193	epoxiconazole	150	150
tebuconazole	6	139	pendimethalin	n.a.[1]	n.a.[1]
terbuthylazin	15	370	terbuthylazin	365	3285
S-metolachlor	23	370	S-metolachlor	365	3285
carbendazim	n.a.	n.a.	AMPA	60	n.a.[1]
MCPA	n.a.	n.a.	prochloraz	365	n.a.[1]
imidacloprid	n.a.	n.a.	MCPA	365	n.a.[1]
fenpropimorph	65	372	glyphosate	60	n.a.[1]
isoproturon	197	227	metamitron	1460	1460
mecocrop	42	103	azoxystrobin	730	n.a.[1]
diuron	n.a.	n.a.	dimoxystrobin	730	730
metamitron	55	362	metazachlor	2920	2920
epoxiconazole	20	204	imidacloprid	n.a.[1]	n.a.[1]
propiconazole	44	44	lambda-cyhalothrin	2920	2920
chloridazon	820	820	propiconazole	180	180
nicosulfuron	92	372	pyraclostrobin	180	180
quinmerac	362	362	quinmerac	n.a.	n.a.
acetamiprid	785	785			

n.a.: no application in the last 6 years, n.a.[1]: no application in the last 10 years

[a] Aminomethylphosphonic acid: metabolite of glyphosate

Conclusion

Numerous pesticide substances were detected in the water and soil samples within the considered catchment area, where they might substantially contribute to environmental pollution. Analyses of pesticide application data from farmers showed in most cases that pesticide detections in water and soil samples were not consistent with their application. Analyses of soil samples and the accompanying batch tests showed that primarily strong sorbing substances might accumulate in the soil over long periods, exhibiting strong temporal delay compared to their application. This persistence presumably resulted from a decreased microbial availability and thus delayed degradation of the pesticides. It can be assumed by the present study that both agricultural and urban sources affect the water quality in the catchment area substantially. Urban inputs, however, led to the highest pesticide concentrations and the highest number of detected pesticide substances over the entire course of the year.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes pesticide analyses, amongst them glyphosate, in surface waters and in soil samples within a German catchment area. Additionally, batch adsorption and anaerobic soil degradation experiments were conducted.

For the analyses in surface waters and soil samples, methods and results are well described and conclusive. The respective results are therefore considered reliable.

Maximum concentrations found in composite weekly water samples were 0.90 µg/L for glyphosate and 0.21 µg/L for AMPA. In soil, maximum concentrations comprised of 0.09 mg/kg for glyphosate and 0.17 mg/kg for AMPA.

For the batch adsorption experiment, the methods are well described, however the experimental design is not in agreement with the relevant guideline (OECD 106), e.g. due to use of synthetic rainwater instead of $CaCl_2$ solution, insufficient information on the test concentrations and a temperature of 10 °C. From the information provided, it cannot be concluded on the representativeness of the soils used. Further, besides the final adsorption parameters, no detailed results are reported. Thus, no conclusion can be made on the quality of the results. The adsorption results are therefore considered not reliable.

For the anaerobic degradation experiment, the methods are well described, however the experimental design is not in agreement with the relevant guideline (OECD 307), e.g. due to use of synthetic rainwater, insufficient information on the test concentrations, use of slurry of a liquid-solid ratio of 0.3:1, air-tight incubation and analysis of pore water, only. From the information provided, it cannot be concluded on the representativeness of the soils used. Further, besides the final half-live, no detailed results are reported. Thus, no conclusion can be made on the quality of the results. The results from the anaerobic degradation experiment are therefore considered not reliable.

Assessment and conclusion by RMS: