PERSISTENCE OF ^{14}C-LABELED ATRAZINE AND ITS RESIDUES IN A FIELD LYSIMETER SOIL AFTER 22 YEARS.

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Abstract

Twenty-two years after the last application of ring-^{14}C-labeled atrazine at customary rate (1.7 kg ha^{-1}) on an agriculturally used outdoor lysimeter, atrazine is still detectable by means of accelerated solvent extraction and LC-MS/MS analysis. Extractions of the 0-10 cm soil layer yielded 60% of the residual ^{14}C-activity. The extracts contained atrazine (1.0 μg kg^{-1}) and 2-hydroxy-atrazine (42.5 μg kg^{-1}). Extractions of the material of the lowest layer 55-60 cm consisting of fine gravel yielded 93% of residual ^{14}C-activity, of which 3.4 μg kg^{-1} was detected as atrazine and 17.7 μg kg^{-1} was 2-hydroxy-atrazine. The detection of atrazine in the lowest layer was of almost four times higher mass than in the upper soil layer. These findings highlight the fact that atrazine is unexpectedly persistent in soil. The overall persistence of atrazine in the environment might represent a potential risk for successive groundwater contamination by leaching even after 22 years of environmental exposure.

Keywords: atrazine, persistence, leaching, extraction, LC-MS/MS, half-life, bound residues.

Capsule:

Atrazine and its metabolite 2-hydroxy-atrazine are still present in soil after long-term aging.

1. INTRODUCTION

Since its introduction in 1958 the herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropyl-amino)-s-triazine] has been one of the largest selling herbicides worldwide for agricultural and industrial purposes. In the US, atrazine was applied to 68 % of herbicide-treated acreage in 2003 (USDA, 2004) and has been found in most groundwater supplies from agricultural regions in the US (USEPA, 1990). Even years after its prohibition in Germany, where it was banned in 1991, it is still found in groundwater (Tappe et al., 2002). Although atrazine has been the subject of multiple investigations, its long-term environmental behavior is still not clear. Most findings regarding fungal or microbial (Kaufmann and Blake, 1970; Mandelbaum et al., 1993; Assaf and Turco, 1994a) and chemical (Blumhorst and Weber, 1994) degradation are based on laboratory or short-term field experiments which have limited relevance to long-term outdoor trials. The estimated half-life of atrazine from these short-term tests ranges between a few days to about one year (Kruger et al., 1993; Accinelli et al., 2001), depending on application history (Shaner and Henry, 2007), soil depth (Miller at al., 1997), soil moisture content (Kruger et al., 1993), temperature (Dinelli et al., 2000), pH and presence of other nutrients such as nitrogen or carbon

(Abdelhafid et al., 2000; Assaf and Turco, 1994b; Gan et al., 1996; Moorman et al., 2001; Alvey and Crowley, 1995). The environmental behavior of atrazine by addition of organic amendments, like plant residues, or its mineralization during bioremediation, field application and agricultural use has been studied intensively (Alvey and Crowley, 1995; Barriuso and Houot, 1996; Silva et al., 2004). A number of studies have observed a so-called "bound residue" fraction of atrazine in soil (Capriel et al., 1985; Schiavon, 1988; Barriuso et al., 1991; Loiseau and Barriuso, 2002). These bound residues can approach 50 % of the initially applied atrazine, and are mainly located in soil particle size fractions <20 μm (Loiseau and Barriuso, 2002). Even though atrazine forms soil-bound interfaces it is still unclear whether these bound residues are bioavailable or represent a potential risk for future groundwater contamination. Since atrazine was found in bound forms nine years after its application (Capriel et al., 1985), it can be assumed that this chemical compound is not excluded from environmental interaction even after long-term aging under outdoor conditions. Pignatello et al. (1993) suggested that changing environmental dry-wet cycles may cause pulse inputs from resistant herbicide pools to subsurface layers, which might become crucial under changing environmental conditions. Soil organic matter is a key factor in the retention of atrazine by soils and the formation of bound residues (Loiseau and Barriuso, 2002). The objective of this study was to quantify and characterize the atrazine residues still present in the surface soil (0-10 cm) and in the lowest lysimeter increment (55-60 cm) consisting of fine gravel.

The results represent novel information on the long-term environmental persistence of the still widely used herbicide atrazine. It is noteworthy that atrazine as the parent compound is still detectable in soil and deeper layers even after 22 years of environmental exposure. This finding indicates a potential long-term risk for soil and groundwater contamination by atrazine. These findings can be useful for environmental and agricultural assessments and environmental policy decisions concerning pest management.

2. Experimental Section

2.1. **Soil characteristics and atrazine application.** The lysimeter soil was a gleyic cambisol originating from Puch, Fürstenfeldbruck in Bavaria, Germany. Details about soil and atrazine application history and soil characteristics have also been described previously (Jablonowski et al., 2008a,b). The lysimeter was installed in 1979 in connection with a long-term study of corn production in a field plot. Corn was planted annually and the lysimeter-soil treatments under outdoor conditions were in accordance with agricultural practice until the end of the experiment in 2005. The filled plastic lysimeter was 49 cm x 49 cm, and had a depth of 73 cm. Uniformly ^{14}C ring-labeled atrazine was applied in 1983, 1984 and 1985 in three equal portions, totaling 56.2 MBq. The total applied mass of atrazine to the lysimeter soil was 133.3 mg, equivalent to a total application of 5 kg atrazine per hectare. The specific ^{14}C-activity of atrazine was 421.587 kBq mg^{-1}. Since most residual ^{14}C-activity was found in the top soil layer (0-10 cm) and the lowest gravel layer (55-60 cm) (Jablonowski et al., 2008a,b), samples of these depth increments were used for the present investigation. The surface 0-10 cm of the profile contained 1.42 % organic carbon and 0.07 % inorganic carbon (Jablonowski et al., 2008b). A fine gravel layer of 55-60 cm depth was added to facilitate drainage during the long-term lysimeter experiment. Samples of homogenized gravel contained 1.38 % organic carbon and 8.99 % inorganic carbon. The source of the gravel material is unknown.

For the statistical analysis the independent two sample t-test was applied in order to determine the significance of differences between the mean values. Significance values are given in figures and tables as Si in %.

2.2. **Analysis.**

2.2.1. *Quantification of atrazine residues in solid samples.* The gravel samples were sieved (5 mm) dry to separate gravel from intruded soil and clay particles. Penetrated roots were removed by hand. Prior to combustion and extraction, a subsample of 125 g of oven dried (105°C) fine gravel was crushed and homogenized using a Planetary Mill (350 rpm, 45min; Planetary Mill PM 400, Retsch). Calculations of residual ^{14}C-activity and atrazine residues in the lysimeter as a whole were based on estimated soil bulk density of 1.5 g cm^{-3} and gravel of 1.8 g cm^{-3}. For quantification of residual ^{14}C-activity, oven dried and homogenized subsamples of top soil or gravel (nine replicates, each 1-2 g dry-weight) were weighed into porcelain vials for combustion using a Biological Oxidizer OX500 (R.J.Harvey Instrument Corporation). Emerging $^{14}CO_2$ was trapped in Oxysolve C-400 scintillation cocktail (Zinser Analytik). Radioactivity was detected by liquid scintillation counter (LSC) using a 2500 TR, Tri-Carb, Packard Liquid Scintillation Analyzer by internal standard.

2.2.2. *Accelerated solvent extraction of the soil and gravel samples.* An Accelerated Solvent Extraction (ASE) device (ASE 200, Dionex) was used to extract the soils. The ASE-extraction was similar to the extraction method previously described by Gan et al. (Gan et al., 1999). In this study, a methanol-water solution (4:1 v:v) was used for extraction since results showed slightly higher residual ^{14}C-activity in the extracts than when using methanol alone, consistent with previous findings (Huang and Pignatello, 1990). For extraction, triplicates of 10 g freeze dried (Lyovac GT2, Steris) and homogenized soil or 10 g of powdered and homogenized gravel samples were weighed into 11 mL stainless steel ASE cells. The remaining space above the samples was filled with fine, annealed sand (Merck) to reduce the extract volume and to avoid clogging of the ASE steel filter lid. The extraction temperature was 135°C at 100 bar (1500 psi) with a flush volume of 60 % of extraction cell volume. The heat-up time was 5 min, static time 15 min and the total extraction time 15-18 min. Each sample was extracted eight consecutive times under the same ASE conditions to determine extraction efficiency and to recover most of the extractable fraction. To determine ASE-extracted residual ^{14}C-activity, a triplicate of 0.5 mL of each extract sample was mixed with 3.5 mL scintillation cocktail (Instant Scint-Gel Plus^{TM}, Perkin-Elmer) and detection of radioactivity was performed by LSC. An external standard was used for quenching correction.

2.2.3. *LC-MS/MS analysis.* Liquid extracts were analyzed for atrazine and its metabolites as described previously (Jablonowski et al., 2008b), using a Thermo Electron Model TSQ-Quantum 2002 equipped with CTC-HTC-PAL sampler, and HPLC (Agilent) with binary pump and temperature controlled column compartment (Agilent Serie 1100). Atrazine (chemical purity: 97.4 %) and its metabolite 2-hydroxy-atrazine (96.0 %) were purchased from Riedel-de Haën. For the quantification of atrazine and its only detectable metabolite 2-hydroxy-atrazine, deuterated (D_5)-atrazine and (D_5)-2-hydroxy-atrazine (Dr. Ehrenstorfer GmbH, Germany) was used as internal standard with a concentration of 0.01 μg mL^{-1}. One hundred μL of each ASE extract was mixed with 100 μL of D_5 STD standard solution resulting in 0.001 μg 100μL^{-1} of injected sample. MZ Perfect Sil Target ODS-3 was used as the solid phase (2.1 mm $\times$ 125 mm $\times$ 3 μm), and an additional HPLC pre-column (2.1 mm $\times$ 10 mm $\times$ 3 μm) was applied.

Before analysis, a compound separation of the ASE extracts was obtained by HPLC. In accordance with Takáts *et al.* (Takats et al., 2001) a mixture of acetonitrile (Riedel-de Haën, 99.9 % purity) and 0.1 M ammonium acetate solution was used as the gradient HPLC eluent. LC-MS/MS analyses were performed in triplicates in positive electrospray ionization mode (ESI+) and transitions were measured in multiple reaction monitoring (MRM). The total injection volume of each sample was 5 μL. The flow rate was 0.15 mL min^{-1} at 25 °C column temperature. The analytical detection limit for atrazine and 2-hydroxy-atrazine was 0.125 ng mL^{-1} liquid. The method detection limit was 0.188 ng g^{-1} soil extracted.

2.2.4. *Elementary analysis of solid samples prior to and after AS-extraction.* Before analysis, homogenized subsamples were dried for 3 h at 105°C. For elementary (Al, Ca, Fe, K, Mg, Na) analysis of soil and gravel, 50 mg of dried sample was decomposed with a mixture of 0.25 g of lithium-borate for 30 min at 1000°C. The flux was dissolved in 30 mL HCl (5 %; 0.95 M, respectively) and adjusted to a total volume of 50 mL. The analysis was performed using inductively coupled plasma with optical emission spectroscopy (ICP-OES; TJA-IRIS-Intrepid spectrometer, Thermo). Determination of carbon was achieved by radiofrequency heating in flowing oxygen and subsequent infrared absorption by a Leco RC-412 multiphase carbon determinator. For determination of nitrogen a subsample of 2 mg was combusted and analyzed using a Leco TCH 600 nitrogen/oxygen/hydrogen determinator and N_2 was determined by thermal conductivity detection.

3. RESULTS AND DISCUSSION

3.1. **General comments.** After more than 20 years of aging under outdoor conditions, atrazine as the parent compound is still present in the soil (Table 1). Besides atrazine, the metabolite

2-hydroxy-atrazine represents the major identifiable and quantifiable component in the soil extracts; these findings are consistent with the results of Capriel and Haisch (Capriel and Haisch, 1983). As presented in a previous study (Jablonowski et al., 2008b), the residual ^{14}C-activity in the complete lysimeter soil corresponds to 25 % of the total initially applied ^{14}C-atrazine activity. This finding gives important information about the general turnover of pesticide-associated carbon in the soil. Even though a considerable portion of ^{14}C-activity could not be extracted and analyzed it is to be assumed that ^{14}C-activity is associated within the *s*-triazine ring structure. The percentage of residual ^{14}C-activity in the top soil layer (0-10 cm) is equal to 8 % of the total mass of atrazine initially applied to the lysimeter. In the fine gravel layer (55-60 cm) 4 % of the initially applied ^{14}C-activity was detected after separating the gravel from soil particles (Table 1).

The presence of organic carbon sources stimulating atrazine degradation by microbial activity has been previously studied using citrate amendment (Jablonowski et al., 2008a; Silva et al., 2004) and other carbon compounds (Assaf and Turco, 1994b). Neither soil-intruded nutrients, such as plant detritus and root exudates of the annual corn plantations, nor regular fertilizer application, could promote complete atrazine degradation via biological or physico-chemical processes during more than 20 years under environmental influences.

Although a lysimeter study might have limited direct interaction with the surrounding field soil, it does provide relevant data on pesticide behavior in situ, under real environmental conditions. In situ lysimeter studies provide a realistic and comparable system to investigate chemical processes and can often readily be expanded to large-scale calculations.

3.2. **Analysis of ASE extracts.** Table 1 presents the analytical results of the ASE extracts and LSC measurements of oxidized samples. As given in Figure 1, most residual ^{14}C-activity was extracted in the first extraction step. The applied ASE-settings in accordance with Gan et al. (Gan et al., 1999) were highly effective for the extraction of aged atrazine residues from soil and homogenized gravel using a methanol-water solution, as previously suggested (Huang and Pignatello, 1990). In comparison to previous extraction studies using vigorous shaking with water (Jablonowski et al., 2008a) or Soxhlet extraction (data not shown), the ASE yielded a considerably greater amount of aged atrazine residues; approximately 60 % of the total residues in the respective layer in the case of soil and 93 % in the case of homogenized gravel (Table 1). However, the extractable amount of ^{14}C-activity is still measurable after 8 extractions, leveling off at 1.59 % for soil and 0.41 % for gravel.

As shown in Figure 2 A and B, atrazine was detected only in the first extract by means of LC-MS/MS for both soil and gravel. As described previously (Jablonowski et al., 2008b), all the extracts were analyzed for the following metabolites, among the parent compound atrazine: desethyldesisopropyl-2-hydroxy-atrazine, desisopropyl-2-hydroxy-atrazine, desethyldesiso-propyl-atrazine, desethyl-2-hydroxy-atrazine, desethyl-atrazine, desisopropyl-atrazine, and 2-hydroxy-atrazine. The only detectable triazine metabolite was 2-hydroxy-atrazine in each of the eight consecutive ASE extracts. It is noteworthy that the amount of extractable atrazine as the parent compound was approximately four times higher in the lower gravel layer than from the topsoil layer, as given in Table 1: 1.4 % (0.06 % of total applied) versus 0.4 % (0.02 % of total applied). This value equals 3.44 μg kg^{-1} of atrazine in the gravel layer versus 0.99 μg kg^{-1} of atrazine extractable in the topsoil layer. These values show statistically significant differences, at a significance of $Si = 99.95$ % (Table 1). It should be noted that the lowest gravel layer was artificial and did not represent a natural soil layer. The time course of atrazine leaching to the lower gravel layer remains unclear. However, these findings suggest the leaching character and long-term persistence of atrazine as well as its main metabolite 2-hydroxy-atrazine, particularly in lower soil increments. The long-term persistence of atrazine in the gravel layer might also be attributed to reduced microbial and chemico-physical degradation processes. This can be supported by the fact that only half the amount of unspecific residues expressed as atrazine equivalents were found in the lower gravel layer compared to the top-soil layer as given in Table 1: 5.3 mg atrazine equivalents in gravel layer 55-60 cm versus 10.3 mg in soil layer 0-10 cm.

The overall recovery of 2-hydroxy-atrazine in all extracts from soil was 14.9 % of residual ^{14}C-activity (1.15 % of total applied) and 7.22 % of residual ^{14}C-activity (0.29 % of total applied) for gravel. Lerch and Li (Lerch and Li, 2001) found that the content of hydroxy-atrazine in agricultural soil is frequently higher than the chloro-derivatives, with a higher concentration in the top 10 cm layers (Sorenson et al., 1993).

Although the amount of parent herbicide and metabolites appear low when related to the total initially applied atrazine, the absolute amounts need to be considered: atrazine at 0.03 % of total applied atrazine (0-10 cm) and 0.06 % of total applied atrazine (55-60 cm) correspond to 0.11 mg atrazine within the two soil layers of the lysimeter. Di-hydroxy-atrazine amounts to 1.15 % of total applied atrazine (0-10 cm) and 0.29 % (55-60 cm) and totals 1.9 mg. Adjusting these amounts to the soil weights of both layers leads to atrazine concentrations of 0.99 μg kg^{-1} soil (0-10 cm) and 3.44 μg kg^{-1} gravel (55-60 cm), respectively. In the case of 2-hydroxy-atrazine this amounts to 42.5 μg kg^{-1} soil (0-10 cm) and 17.7 μg kg^{-1} gravel (55-60 cm; Table 1 and Figure 2).

3.3. **Analysis of solid samples.** Even after eight consecutive extraction steps, approximately 40 % and 7 % of the ^{14}C-activity could not be extracted from the soil and gravel, respectively. It remains unclear whether the soil-bound residues are the parent compound atrazine or its metabolites. Regardless, the residues are sequestered into soil organic matter compounds or entrapped within nanostructures of other organic soil compounds such as humic acids. The major role of humic substances in the sorption of hydrophobic organic substances has long been known. Abate et al. found an increased adsorption of hydroxy-atrazine and atrazine onto humic acid enriched soil (Abate et al., 2004) that might support this suggestion. Obviously, unspecific ^{14}C-activity might also be part of the soil carbon pool as a result of microbial or chemicophysical degradation processes. Further investigations are in progress to determine the nature of the soil-bound atrazine residues. However, the results of combusted soil and gravel samples after consecutive ASE-extractions gave overall recoveries of about 100 % indicating adequate analytical preparation of the samples and detection of residual ^{14}C-activity by the used methods. It can be estimated that increased extraction efficiency of soil from the top layer by different extraction setups utilizing other solvents, chemical derivatization by silylation (Haider et al., 2000), pH or temperature, might result in a higher quantifiable yield of atrazine. As found previously, up to 50 % of bound residues were associated with the parent compound atrazine and could be released by vigorous extraction (Loiseau and Barriuso, 2002). The fact that atrazine is still detectable provides evidence that soil-bound ^{14}C-activity in the upper soil layer is at least partly associated with the parent compound atrazine. Thus, further continuous leaching into deeper soil horizons must be considered.

As indicated above, the estimated half-life of atrazine ranges between days to months in previous short-term studies; clearly, these data must be reconsidered. Assuming a first-order decay of the parent compound atrazine with an expected environmental half-life of 1 year (approximately the longest half-life reported in the literature, e.g. (Jones et al., 1982)) and a total amount applied of 133.3 mg, the residual atrazine in the lysimeter is calculated to be as little as 0.2 μg in total. In clear contrast to that the amount detected is 110 μg, taking only the extracted atrazine from soil layer 0-10 cm and 55-60 cm into consideration. This is approximately 550 times higher than expected according to the calculation. Estimating the persistence of atrazine or likely other triazine pesticide compounds in soils under environmental conditions by calculating the half-life from short-term experiments is highly problematic. Despite several chemical and biological pathways of atrazine degradation, as well as plant uptake and sequestration over time, the presented environmental long-term persistence is unexpectedly high and is crucial for accurately describing triazine herbicide fate in soils.

3.4. **Elementary analysis of solid samples prior to and after AS-extraction.** Results for elementary analysis are given in Table 2. A considerably higher amount of Al and Fe, known for the adsorption affinity of various organic and inorganic compounds (Sawhney and Singh, 1997; Clausen and Fabricius, 2001), can be found in the surface soil layer. Nevertheless, the residual ^{14}C-activity is more likely associated with the organic carbon fraction, being almost equal in the upper soil and gravel layers. This assumption is in accordance with previous studies suggesting that retention of atrazine is mainly due to soil organic matter (Laird et al., 1994). As shown in Table 2, harsh extraction of soil and gravel samples did not noticeably change the amounts of the analyzed soil elements. It could be observed that the first extracts were slightly clear to yellow. It is likely that some of the extracted ^{14}C-activity was incorporated into humic substances that were subsequently extracted. The minor decrease of organic carbon content after extraction is likely the result of extracted humic substances from the soil; sequential extracts of the soils showed decreasing coloration with extraction number.

As observed, single particles consisting of black porous cinder or intruded root detritus found in the gravel layer might have retained most of the residual ^{14}C-activity and associated atrazine residues due to their high surface area and organic carbon content. Further research concerning this matter is in progress.

3.5. **Environmental significance.** Atrazine is still being applied and is readily detected in water streams and wells in considerable amounts, ranging from 0.12 μg L^{-1} up to 7.0 μg L^{-1} (USDA, 2004; USEPA, 1990).

It is difficult to imagine a site not impacted by agrochemicals worldwide (Nations and Hallberg, 1992; Thurmann and Cromwell, 2000). In earlier studies atrazine was found in fog, air, arctic ice and seawater even at great distance from urban or agricultural areas (Glotfelty et al., 1987; Chernyak et al., 1996). The detection of atrazine in rainwater, and subsequent deposition of the herbicide from the atmosphere has been reported consistently from places such as Canada, the US and Europe (Brun et al., 2008; Goolsby et al., 1997; Sanusi et al., 2000; Bossi et al., 2002).

In addition to parent pesticides, their degradation products or metabolites were also detected in generally higher amounts (Kolpin et al., 1998), representing a potential risk for soil and water contamination. It is questionable whether the presented long-term aged atrazine and/or its residues remaining in the soil, also as "bound residues", are still bioaccessible for exposed organisms. Investigations concerning this matter are being undertaken.

However, previous studies demonstrated the effect of earthworm activity (Gevao et al., 2001) and microbial activity (Khan and Behki, 1990) on the release of bound atrazine residues. These results suggest that even long-term aged or bound atrazine residues can be liberated over time, and may represent a potential hazard to the environment.

Taking soil constituents, groundwater level and application area into consideration, the use of atrazine should be considered carefully due to its long-term persistence and leaching character. Despite the prohibition of atrazine in several developed countries, it is still used prolifically throughout much of the world, potentially representing risks to groundwater supplies.

3.6. **Conclusion.** The results of the current investigation highlight the long-term persistence and environmental behavior of the herbicide atrazine. To date, no comparable results have been published. Therefore, this study provides important and comparable data for the risk assessment of atrazine application areas or atrazine contaminated sites. It is possible that these findings for atrazine presented in this report may be relevant for other persistent chemicals and pesticides as well. Clearly, the calculation of predicted environmental concentrations of persistent chemicals based only on laboratory half-life or short-term field dissipation experiments should therefore be reconsidered. Agricultural soils after being used for many years may contain multiple aged pesticide residues from applications of various pesticides that become stabilized by binding to the soil matrix. This may challenge the environmental risk assessment of the resulting mixture of long-term available pesticide residues in our agricultural soils.

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TABLE 1. Detected atrazine and 2-hydroxy-atrazine by means of LC-MS/MS as percentage of total remaining residues per layer, calculated per kg sample and depth increment. ^{14}C-activity of ASE extracts after eight consecutive extraction steps of 10 g dry soil or gravel using methanol-water solution (4:1, v:v) at 135°C, 1500 psi. ATR atrazine; OH-ATR 2-hydroxy-atrazine; ^{14}C-activity detected by means of LSC (% extractable; % of total applied) and by Oxidizer. Characterization and quantification by means of LC-MS/MS (atrazine concentration detectable; amount of total residual detected as atrazine). (± standard deviation of $n = 3$). Significance (Si) is given for the different concentrations obtained between the soil (0-10 cm) and the gravel layer (55-60 cm) for extracted ATR at *>99.95%; and OH-ATR at **>99.95%; ^{14}C-activity at ***>99.95%.

		% extractable of total residual ^{14}C [%]	% of total applied ^{14}C [%]	concentration [μg kg^{-1}]	amount of total residual [μg layer^{-1}]
Top layer	ATR	0.35	0.03	0.99 ±0.09*	35.56
soil	OH-ATR	14.90	1.15	42.45 ±2.25**	1 528.81
(0-10 cm)	^{14}C	60.37 ±1.86***	7.70	284.90	10 300.00
Bottom layer	ATR	1.40	0.06	3.44 ±0.41*	74.37
gravel	OH-ATR	7.22	0.29	17.69 ±0.53**	382.33
(55-60 cm)	^{14}C	93.09 ±0.75***	4.00	147.00	5 300.00

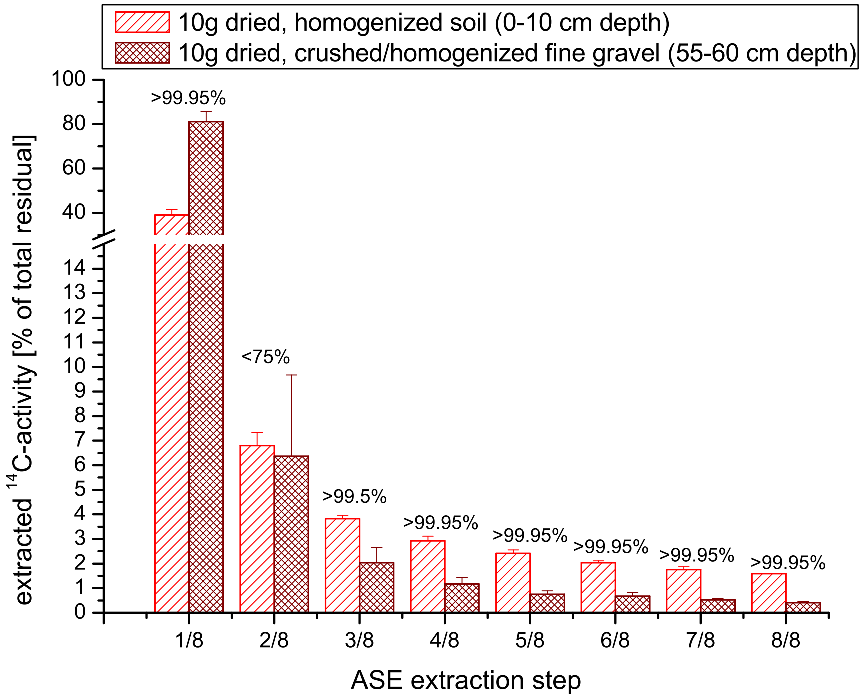

FIGURE 1. Residual ^{14}C-activity in each extract from soil and fine gravel in eight consecutive extraction steps using methanol-water solution (4:1 v:v) by means of accelerated solvent extraction. Standard deviation of $n = 9$. Values above the bars indicate statistical differences in the soil and gravel layer (Si = % of significance).

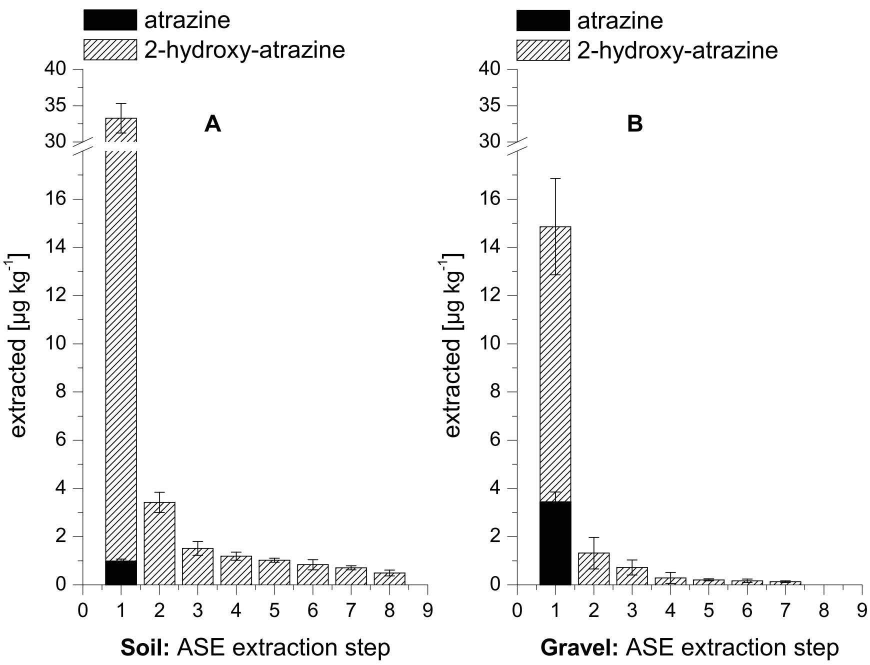

FIGURE 2. Extracted atrazine and 2-hydroxy-atrazine in soil (A) and gravel (B) each triplicates of 10 g sample, after 8 consecutive extraction steps using ASE, quantified by means of LC-MS/MS; calculated per kg sample. Standard deviation of $n = 9$.

TABLE 2. Elements in soil and gravel prior to extraction and after extraction (weight percent). Prior to AS-extraction: ± standard deviation of $n = 3$. After AS-extraction: ± standard deviation of $n = 9$. Mean variation for Al, Ca, Fe, K, Mg, Na, P: for concentration >1%: ± 3%; for concentration <1% and >0.1%: ± 10%, and for concentration <0.1%: ± 20%. Significance (*Si*) is given for the differences within the soil (0-10 cm) and the gravel (55-60 cm) fraction before and after extraction. Significance for soil at: *>99.95% for C_{org}; **<75% for C_{inorg}; and ***>99.5% for N. Significance for gravel at: $^{\diamond}$>99.5% for C_{org}; $^{\diamond\diamond}$>90% for C_{inorg}; and $^{\diamond\diamond\diamond}$>99% for N.

element sample	C_{org} [%]	C_{inorg} [%]	N [%]	Al [%]	Ca [%]	Fe [%]	K [%]	Mg [%]	Na [%]	P [%]
prior to extraction										
soil	1.42 ±0.05*	0.07 ±0.03**	0.19 ±0.01***	4.75	0.44	2.23	1.43	0.49	0.89	0.12
gravel	1.38 ±0.03$^{\diamond}$	8.99 ±0.01$^{\diamond\diamond}$	0.05 ±0.00$^{\diamond\diamond\diamond}$	1.54	20.1	0.99	0.4	4.87	0.2	0.02
after extraction										
soil	1.17 ±0.31*	0.08 ±0.07**	0.16 ±0.01***	4.74	0.41	2.28	1.40	0.49	0.68	0.12
gravel	1.29 ±0.01$^{\diamond}$	8.78 ±0.03$^{\diamond\diamond}$	0.05 ±0.00$^{\diamond\diamond\diamond}$	1.51	19.77	0.94	0.37	4.77	0.19	0.02