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**Large variation in glyphosate mineralization in 21 different agricultural soils explained by soil properties**

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**Abstract**

Glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) have frequently been detected in surface water and groundwaters. Since adequate glyphosate mineralization in soil may reduce its losses to environment, improved understanding of site specific factors underlying pesticide mineralization in soils is needed. The aim of this study was to investigate the relationship between soil properties and glyphosate mineralization. To establish a sound basis for resilient correlations, the study was conducted with a large number of 21 agricultural soils, differing in a variety of soil parameters, such as soil texture, soil organic matter content, pH, exchangeable ions etc. The mineralization experiments were carried out with 14C labelled glyphosate at a soil water tension of –15 kPa and at a soil density of 1.3 g cm-3 at 20 ± 1 °C for an incubation period of 32 days. The results showed that the mineralization of glyphosate in different agricultural soils varied to a great extent, from 7 to 70% of the amount initially applied. Glyphosate mineralization started immediately after application, the highest mineralization rates were observed within the first 4 days in most of the 21 soils. Multiple regression analysis revealed exchangeable acidity (H+ and Al3+), exchangeable Ca2+ ions and ammonium lactate extractable K to be the key soil parameters governing glyphosate mineralization in the examined soils. A highly significant negative correlation between mineralized glyphosate and NaOH-extractable residues (NaOH-ER) in soils strongly suggests that NaOH-ER could be used as a simple and reliable parameter for evaluating the glyphosate mineralization capacity. The NaOH-ER were composed by glyphosate, unknown 14C-residues, and AMPA (12% - 65%, 3% - 34%, 0% -11 % of applied 14C, respectively). Our results highlighted the influential role of soil exchangeable acidity, which should therefore be considered in pesticide risk assessments and management to limit efficiently the environmental transfers of glyphosate.

**Keywords:** soil properties, exchangeable acidity, aluminium, pesticides, mineralization, non-extractable residues

1. **Introduction**

Glyphosate [*N*-(phosphonomethyl)glycine] based herbicides (GlyBH) are the most widely used commercial formulations of pesticides worldwide (Jensen et al., 2009). GlyBH are applied to agricultural fields before planting the crop and/or pre- or post-harvest, in both conventional and reduced/no-till farming, to control the growth of annual and perennial weeds, both broad-leaved and grasses. Glyphosate was considered in the past to be harmless to the environment and to human health. However, in the recent years this evaluation was been questioned, with some studies associating its use with cancer and endocrine disruption in humans and acute and chronic toxicity to aquatic species. The European Union (EU) recently adopted a renewal of the approval of glyphosate for 5 years after intensive discussion (EFSA, 2015; IARC, 2015; Myers et al., 2016). It is important to note that numerous reviews concerning health effect of GlyBH report conflicting opinions, especially on the long-term effects of glyphosate, and on no-observed-adverse effect levels (NOAEL) (US EPA, 1993; EC, 2002; US EPA, 2009; Antoniou et al., 2012; Mesnage et al., 2015, Niemann et al., 2015; Germany Rapporteur Member State, 2015).

There are also large discrepancies among studies evaluating the environmental fate of glyphosate after its use. Due to its high biodegradability (Landry et al., 2005; Grundmann et al., 2008) and strong adsorption in soil (Mamy et al., 2005), glyphosate has a weak (negligible) leaching potential (Borggaard and Gimsing, 2008; Klier et al., 2008). However, under some environmental conditions, for instance in soils with weak sorption and degradation capacity and in soils with colloidal flow in macropores; glyphosate can also be transported into groundwater (Strange-Hansen et al., 2004; Landry et al., 2005; Borggaard and Gimsing, 2008), thereby threatening drinking water quality. Glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) have frequently been detected in surface and ground water in Norway, Sweden, Denmark and The Netherlands (Sørensen et al., 2006; Adriaanse et al., 2008; Keshteli et al., 2011). Similarly, a recent study by Silva et al. (2017) showed that glyphosate and AMPA were also detected at the highest frequencies in soil samples from the northern regions of the EU, indicating the importance of adapting pesticide use to site specific environmental conditions, which govern the fate of glyphosate, including degradation. Temperature and soil water content have already been reported to affect greatly the half-life of glyphosate and AMPA, ranging from a few days to several months, up to one or two years (EFSA, 2013; Bento et al., 2016).

Studies have also shown a great variation in glyphosate degradation depending on soil properties (Smith and Aubin, 1993; Cheah et al., 1998; Gimsing et al., 2004a; Mamy et al., 2005; Sørensen at al., 2006; Bonfleur et al., 2011), which can be ascribed to soil microbiological and chemical characteristics (Albers et al., 2009; Kim et al., 2011). For instance, correlations have been found between glyphosate mineralization and soil microbial parameters, such as soil microbial biomass (Wiren-Lehr et al., 1997) or population size of *Pseudomonas* spp. bacteria in soil (Gimsing et al., 2004a). It has also been reported that glyphosate degradation is negatively correlated to the adsorption capacity of the soil (Nomura and Hilton, 1977; Moshier and Penner, 1978; Sørensen et al., 2006). Actually, all soil parameters that have been identified as having an impact on glyphosate adsorption; such as pH, clay content, as well as phosphate, copper, iron oxides and aluminium oxides contents (Morillo et al., 2000, de Jonge et al., 1999; Autio et al., 2004; Gimsing et al., 2004b; Mamy and Barriuso, 2005), should have a contrary effect on glyphosate mineralization; in other words strong retention of glyphosate to the soil matrix possibly reduces its availability for the degradation. Several studies have in fact confirmed correlations between increased glyphosate sorption (caused by increased contents of copper, iron and aluminium oxides or decreased pH and phosphate content) and decreased mineralization/degradation (Sprankle et al., 1975; Moshier and Penner, 1978; Morillo et al., 2000; Gimsing et al., 2004a). Unfortunately, despite major scientific efforts, clear patterns between soil properties and glyphosate degradation have not emerged. Comparison between these degradation-correlation studies is difficult due to the high variation of experimental conditions among different studies; in addition, experiments were in general conducted under artificial conditions, especially with regard to soil water content and soil density. In most of these degradation studies, only one, up to few soils were investigated, so it cannot be excluded that the established correlations were found by a chance.

To avoid inconclusive results and to establish a sound basis for resilient correlations, we conducted a glyphosate degradation study with more than twenty different soils under comparable conditions: at identical soil bulk density of 1.3 g cm-3 at temperature of 20 ± 1 °C, and a soil water content at the relevant optimum for pesticide mineralization in all studied soils, which is at a water potential of –15 kPa (Schroll et al., 2006). The agricultural soils used in our study were characterized by (i) large variations in soil texture (sand content 8% − 86%) and organic matter content (1.2% – 4.5%) (ii) fairly diverse soil−water retention curves and (iii) differing pH values (5.0 - 7.1) and exchangeable cations (Table 1). The main aim of this study was to elucidate the impact of soil parameters on glyphosate degradation, thereby explaining the different behaviour of glyphosate and AMPA formation in different soils.

1. **Materials and Methods**
	1. *Soil samples*

The study was conducted with 21 agricultural soils from different sites in Germany and Slovenia, differing in their composition (Table 1, Fig. S1). Selected fields had not been treated with glyphosate before (at least 5 years). All soils were taken on arable fields from the upper Ap horizon (0-30 cm), sieved (< 2 mm) after sampling, homogenized and stored at 4ºC in the dark before use. Prior to the degradation experiments, the soils were moistened to a water potential close to -15 kPa, compacted to a soil density of 1.3 g cm-3 and equilibrated at room temperature (20 ± 1 °C) for 2 weeks.

* 1. *Soil characterisation*

For soil analyses, the samples were air-dried and sieved to 2 mm (ISO 11464, 2006). Soil texture was determined by the pipette method (ISO 11277, 2009). Soil pH was measured in a 1/2.5 (w/v) ratio of soil and 0.01 M CaCl2 suspension (ISO 10390, 2005). Total soil organic carbon (TOC) and total N (TN) were measured by dry combustion (ISO 10694, 1996; ISO 13878, 1987) using an elemental analyser (Elementar vario MAX instrument, Germany). Organic matter was calculated from the content of organic carbon using a conversion factor of 1.724. Ammonium lactate extraction was used for assessing plant available forms of P and K (AL-P and AL-K) (ÖNORM L 1087, 1993). Cation exchange capacity (CEC) was determined as the sum of exchangeable base cations and exchangeable acidity (expressed as H+ and Al3+ cations) (Soil Survey laboratory methods manual, 2014). For exchangeable base cations (Ca2+, Mg2+, K+, Na+) and for exchangeable acidity, extractions with ammonium acetate (1 N NH4OAc buffered at pH 7.0) and BaCl2-Triethanolamine (0.5 N BaCl2 and 0.2 N TEA buffered at pH 8.2) were used, respectively. Copper (Cu2+) was analysed by the CAT-element method (VDLUFA, 1991a). Iron and aluminium (Fe-OX and Al-OX) were determined in acid ammonium oxalate extracts (VDLUFA, 1991b). The water content at a water potential of –15 kPa (pF 2.18) was determined in a sand/kaolin box (Eijkelkamp, Netherlands) (ISO 11274, 1998; Schroll et al., 2006).

*2.3. Glyphosate mineralization experiments*

The mineralization of 14C-labelled glyphosate was studied under the laboratory conditions described below (*2.4.2*). All mineralization experiments were performed in 4 replicates, each with 50 g soil (dry mass). Glyphosate mineralization was followed at regular intervals by determination of evolved 14CO2 trapped in the NaOH solution. At the end of the experiment, 7 g (dry mass) of each soil sample was extracted with NaOH to determine the quantity and quality of the extractable residues (NaOH-ER), as well as to quantify the non-extractable residues (NER) (*bound residues*).

*2.3.1. Pesticide application*

14C-glyphosate [N-(phosphonomethyl)glycine] was labelled on the phosphonomethyl group (PerkinElmer, USA, radiochemical purity > 97%). Non-labelled glyphosate (purity > 98%) and aminomethylphosphonic acid (AMPA, purity > 98%) were purchased from Dr. Ehrenstorfer (Germany). 14C-glyphosate was dissolved in sterilized distilled water and mixed with non-labelled glyphosate, resulting in a specific radioactivity of 1.6 Bq mg-1. This aqueous standard mixture (90 µL) was applied with a Hamilton syringe to an oven dried, pulverized soil aliquot of 3.5 g (dry mass) in a glass beaker and carefully stirred for 1 minute with a spatula. The spiked aliquot was transferred to another glass beaker containing the remaining equilibrated soil (46.5 g dry mass), mixed for another 2 min and transferred to an incubation flask. The total concentration of glyphosate was 10µg g-1 in each set corresponding to a total radioactivity of 83,000 Bq.

*2.3.2. Test system, experimental conditions and sampling*

The incubation system consisted of 500 ml brown glass flasks which were closed with rubber caps. The rubber caps were equipped with an air inlet consisting of a stainless needle with a diameter of 1 mm to prevent anoxic conditions in the incubation flasks. To eliminate CO2 from the ambient air entering the flasks, a 12 ml plastic syringe (Latex FREE, Germany) filled with granular CO2 absorber (soda lime) was connected to the air inlet at the top of the cap. A 25 ml plastic beaker was placed below the cap, containing 0.1 M NaOH solution (10 ml) to trap 14CO2 released from the glyphosate mineralization. The spiked soil samples in the incubators were compacted to a soil density of 1.3 g cm-3, soil water content was individually adjusted to a water potential of -15 kPa of each soil and the soil samples were incubated at 20 ± 1 °C in the dark for 32 days. The soil humidity was controlled weekly. The NaOH solution was exchanged three times per week and, from the collected solution, an aliquot of 2 ml was mixed with 3 ml of scintillation cocktail Ultima Flo AF to determine 14CO2 in a liquid scintillation counter (Tricarb 1900 TR, Packard, Germany).

*2.4.* *Glyphosate, metabolites and bound residues analysis*

Glyphosate and AMPA were determined in soil samples at the end of the mineralization experiment. Soil extraction with NaOH was applied according to Gimsing et al. (2004a): 7 g of soil dry weight was extracted with 28 ml 0.1 M NaOH by shaking on an overhead shaker (Reax 2, Heidolph, Germany) for 17 hours. The supernatant was collected after centrifuging for 10 min at 3020 rcf (Beckman J2-21, Germany) and filtered (No. 589/1, Whatman, Germany). Aliquots of 100 µl of the supernatant were taken and mixed with 5 ml Ultima Gold XR to measure the radioactivity by liquid scintillation counting. Extracts were subsequently concentrated on a Büchi Rotavapor R-114 (Büchi, Switzerland) at 30 °C to around 150 µl. The concentrated samples were filtered through centrifugal filters (modified Nylon 0.2 µm, 500 µl, VWR International GmbH, Germany) in a table centrifuge (Biofuge Pico, Heraeus Instruments, Germany) for 5 minutes at 9070 rcf. Purified samples were stored at -20 °C prior to HPLC analysis.

Twenty µl of each sample (NaOH extract) was injected into an HPLC system consisting of an Auto Sampler AS50 (Dionex, Germany), a gradient pump GP50 (Dionex, Germany), a PRP-X400 column, 7µm, 4.6 x 250 mm (Hamilton, USA), and a Radioflow detector LB 509 (Berthold, Germany). The mobile phase consisted of (A) 5mM KH2PO4 (pH 1.9) and (B) 5mM KOH (Regenerant-RG019, Pickering Laboratories, USA). The gradient program was: 0-20 min: 100 % (A); 21-25 min: 0% (A). The flow velocity: 0.5 ml min-1 Glyphosate in the soil extracts was identified by comparison of its retention time with the respective retention time of the 14C-labelled reference substance. The degradation product AMPA was identified by its retention time only. After each analysis, the column was regenerated with Regenerant-RG019 at a flow velocity of 0.5 ml min-1 for 30 min.

After NaOH extraction, the radioactivity remaining in the soil was considered to be non-extractable residues (*bound residues*). This soil material was intensively mixed and homogenized with 3.5 g diatomaceous earth in a mortar. Four aliquots (between 0.1 and 0.3g) of each soil sample were taken, mixed with some drops of saturated aqueous sugar solution and combusted in an automatic sample-oxidizer 306 (PerkinElmer, USA). 14CO2 from the combustion was trapped in Carbo-Sorb E and mixed with Permaflour E before scintillation counting.

*2.5. Statistical analysis*

All statistical analysis were performed using the R program version 3.3.2 (R Core Team, 2017). A multivariate description of soil data was performed using the unsupervised method of principal component analysis (program prcomp). Relationship between soil parameters and mineralization rates of glyphosate (supervisor) were analysed with the univariate as well as the multivariate methods. Univariate linear model was carried out to describe and test the relation of the supervisor to one soil parameter at a time. With the R-program lm diagnostic plots were produced to check the ANOVA assumptions of normal distribution of residuals and homoscedasticity. For evaluating the potential interaction effects of different soil parameters on glyphosate mineralization, the multivariate supervised methods were applied: the stepwise regression and the best subset regression. In stepwise regression, the parameters with the largest partial correlation with the response variable were successively added to the model until partial correlations were not significant anymore. In best subset regression, all possible variable combinations of size 1,2,3,...7 were included in the regression equation. For each size, the combination with the best fit to the response variable was selected.

1. **Results and discussion**

*3.1. Degradation behaviour of glyphosate*

The degradation behaviour of glyphosate was investigated in a large variety of soils (Table 1). Since mineralization is the only pathway to eliminate a pollutant completely from the environment, the relationship between this key parameter and other degradation processes (extractable residues, bound residues) was elucidated.

*3.1.1. Mineralization of glyphosate*

After 32 days of incubation, 7.6% to 68.7% of the applied 14C-glyphosate was mineralized to 14CO2, depending on the soil properties (Table 2, Fig. 1). It is important to note that in all soils an average of 98% of the totally applied 14C-radioactivity was recovered at the end of the mineralization experiments (Table 2). The lowest mineralization of 14C-glyphosate was identified in Brezje (Br) soil, whereas the highest mineralization of 14C-glyphosate was obtained in Feldkirchen (Fe) and Apace (Ap) soils. Low mineralization of glyphosate was also observed in Zepovci (Ze), Zepovci-Plitvica (ZeP) and Lomanose a (La) soil. In these three soils, less than 30% of the initially applied glyphosate was mineralized after 32 days. In contrast, other soils had a higher mineralization capacity and 14CO2 production after 32 days reached 31.2% - 68.7% of the initially applied glyphosate. A high variability of glyphosate degradation in laboratory experiments was also reported in the studies of Smith and Aubin (1993), Wiren-Lehr et al. (1997), Cheah et al. (1998), Gimsing et al. (2004a) and Bonfleur et al. (2011). In the present study, glyphosate mineralization started immediately after application, with the highest glyphosate mineralization rates within the first 4 days, thereafter mineralization rates decreased over time (Fig. 1, Fig. S2). Mineralization of glyphosate without any lag phase, following a two component first-order kinetic, was already described in previous studies (von Wiren-Lehr et al., 1997; Eberbach, 1998; Gimsing et al., 2004a; Suhadolc et al., 2010). Our results are in accordance with the observation that degradation of glyphosate takes place without a lag phase and it seems to be a common soil property, despite the rate of degradation can be very different from soil to soil (reviewed by Borggaard and Gimsing 2008). It has also been seen as evidence for co-metabolic degradation because the enzymes used in the degradation must be present before the application of glyphosate (Eberbach, 1998; Borggaard and Gimsing 2008).

*3.1.2. Extractable glyphosate residues in soil*

According to the literature, a number of different solvent systems have been used for extraction of glyphosate and its metabolites from soil. Aubin and Smith (1992) found that 0.1 N NaOH yielded the highest glyphosate extraction recovery among the 10 different solvent systems they tested. It is also common to use sequential extraction procedures to distinguish between different glyphosate pools. For example, readily bioavailable glyphosate is determined in soil pore water (Stenrød et al., 2005) or by extraction with NH4Cl (Gimsing et al., 2004a) or CaCl2 (Zablotowicz et al., 2009), followed by a second extraction step with a basic solvent such as KOH or NaOH, to determine the “less bioavailable” glyphosate fraction that is adsorbed to iron and aluminium oxides (Gimsing et al., 2004a). The readily available glyphosate pool is normally very low: Stenrød et al. (2005) found only < 0.2% of the initially applied glyphosate in soil pore water and Gimsing et al. (2004a) detected only 1% - 2% in the NH4Cl extractable fraction. In the present study, total extractable residues were determined by NaOH extraction. Theoretically, the NaOH extract contains readily bioavailable residues as well as less bioavailable residues, but according to the above-mentioned data reported in literature, it can be assumed that the NaOH-ER consisted of a negligibly low amount of readily available residues and a large amount of residues that were presumably adsorbed to the variable-charge surface sites (Borggaard and Gimsing, 2008). NaOH-ER showed big variance between the soils after 32 days of incubation; they ranged from 23.3% to 91.0% (Table 2). Glyphosate was the major component in the NaOH extractable fraction, as compared to unknown metabolites and AMPA (12% - 65%, 3% - 34%, 0% -11 % of applied 14C, respectively) (Table 2). In soils with low mineralization, both the NaOH extractable fraction and glyphosate residues were high. Fig. 2 shows a highly significant negative correlation between mineralized glyphosate and NaOH-ER. The high amount of NaOH extractable residues, mainly glyphosate, in soils with low mineralization, indicates that glyphosate mineralization was hampered in these soils, presumably because of higher adsorbtion to Al- or Fe-oxides (Duke et al., 2012; Gimsing et al., 2004a,b) and therefore not so easily available for mineralization. Probable adsorption to Al- or Fe-oxides is supported by the PCA-biplot of relations between soil parameters (Fig. S3). On the other hand, our results indicate that NaOH extractable AMPA was mineralized quickly after formation, as it contents were very low in all soils (in average of all soils 3.6 % of applied glyphosate, max. 11.3% in ZeP soil) (Table 2).

The highly significant correlation between glyphosate mineralization and NaOH-ER strongly indicates that NaOH-ER can be used as a reliable parameter for evaluation of the glyphosate mineralization capacity of a specific soil. Money- and time-consuming mineralization experiments can thus be avoided.

*3.1.3. Formation of non-extractable 14C-labeled residues in soil*

The amount of bound residues was relatively low, NER varying between 2.5% and 11.4% of the initially applied glyphosate (Table 2). This is consistent with the results of many authors, who found values in a similar range (Smith and Aubin, 1993; Mamy et al., 2005; Weaver et al., 2007; Zablotowicz et al., 2009). However, there are also other sources that reported higher values for NER, from 23.4% (Lancaster et al., 2010) to 57.0% (Getenga and Kengara, 2004). In the present study, we found a significant positive correlation between mineralized glyphosate and NER: high mineralization of glyphosate coincided with a higher formation of non-extractable residues (Fig. 3). Generally, NER can be formed by two different mechanisms: (i) xenobiotic and/ or its metabolites can be bound to the soil matrix and (ii) parts of the pesticide molecule can be incorporated into microbial biomolecules, and after dying of the microbes such molecules will become a part of the soil organic matter (Charney et al., 2004; Grundmann et al., 2011). For most pesticides, soil organic matter plays a key role as a binding site in formation of NER. Mamy et al. (2005) reported that glyphosate NER increased with soil organic carbon content. Lancaster et al. (2010), after the application of 14C-glyphosate to soil, found a considerable portion of the initially applied radioactivity in the soil microbial biomass. Incorporation into the microbial biomass implies growth-linked metabolic degradation, meaning that the degrading microorganisms are able to use the herbicide as a C- and energy source. Such a type of degradation is accompanied by high mineralization rates. Our finding of a positive correlation between mineralization and formation of NER could be a hint that, in the soils with high mineralization, microorganisms are able to use glyphosate for the formation of biomolecules, which can become part of the recalcitrant soil organic matter after cell death. The identified correlation could therefore indicate that in cases of high glyphosate mineralization, NER are of a biogenic origin rather than a chemical-physical binding of the pesticide to the soil matrix.

*3.2. Identification of soil parameters that influence glyphosate mineralization*

As already mentioned above, mineralization is the most important elimination pathway for pesticides from the environment and it is therefore of interest to know by which parameters this process is influenced. It is known from the literature that mineralization of glyphosate varies greatly among soils and in we also found highly differing glyphosate mineralization in the 21 soils under investigation the present study. For a better understanding of the very high variation of glyphosate mineralization in different soils, we tried to identify the soil parameters that govern the glyphosate mineralization process, to be able to explain the different mineralization behaviour of glyphosate in different soils. For that purpose, we analysed the correlations between glyphosate mineralization and a large number of soil parameters. The following parameters were used for calculations with univariate and multiple regression analysis: glyphosate mineralization, sand, silt, clay, pH, organic matter, TOC, TN, C/N, AL-P, AL-K, oxalate extractable Al and Fe, Cu2+, exchangeable acidity (H+ and Al3+), exchangeable base cations (Ca2+, Mg2+, K+, Na+), and CEC.

*3.2.1. Impact of single soil parameters on glyphosate mineralization*

The results of univariate regression analysis revealed no correlation between glyphosate mineralization and most of the investigated soil parameters, except for exchangeable acidity.

A highly significant negative correlation was found for exchangeable acidity and mineralization of glyphosate (Fig. 4). This illustrates that acidic exchangeable H+ and Al3+ cations interfered with the glyphosate mineralization process in the soils. The low mineralization of glyphosate in soils with high exchangeable acidity could be explained either by the formation of strong chemical bonds with the carboxylic or phosphonic acid groups of glyphosate (Vereecken, 2005) resulting in reduced glyphosate bioavailability, or by toxic effects of exchangeable aluminium to soil microorganisms (Kunito et al., 2016). This finding allows a ranking of soils according to their glyphosate mineralization capability by a simple assessment of exchangeable acidity in soils.

In the literature, some correlations between glyphosate degradation and soil parameters have been reported but to best of our knowledge, a negative correlation between glyphosate mineralization and exchangeable acidity has not been shown. On the other hand, correlations have been found that could not be confirmed with the results of the present study, such as correlations between degradation/mineralization and soil copper, iron, phosphate contents and soil pH (Sprankle et al., 1975; Moshier and Penner, 1978; Morillo et al., 2000; Gimsing et al., 2004a). These inconsistent findings between the studies may be due to differences in the experimental approaches, different ranges of examined soil properties, and/or combinations of soil characteristics. For instance, phosphate may have a positive effect, no effect or even a negative effect on glyphosate mineralisation, which presumably depends on different surface sites of the soil (Borggaard and Gimsing, 2008). However, similarly to the results of our study glyphosate degradation rates in soils have not been linked to organic matter or clay content also in other studies (reviewed by Borggaard and Gimsing, 2008; Mamy et al., 2016).

*3.2.2. Interacting effects of different soil parameters on mineralization of glyphosate*

Univariate regression analysis only considers the influence of a single soil parameter; it does not take into account the influence of combined soil parameters. Under natural conditions in soil, the soil parameters will not function separately but will interact with each other and will have a combined effect. To be able to understand which soil parameters interact with one another to regulate the mineralization of glyphosate in soils, multiple regression analysis was conducted with the above-mentioned input parameters (see section 3.2). The mineralized amount of glyphosate after 32 days (Glycum.min) in the different soils was best described by the following model (Eq1., n=21, Adjusted R2 = 0.90, p = 0.25x10-9):

Eq1.

Glycum.min [%] = -0.005 x [Exchangeable acidity] + 1.025 x [Ca2+] + 0.332 x [AL-K] + 56.338

Glycum.min = cumulative glyphosate mineralization after 32 days

[Exchangeable acidity] = exchangeable H+ and Al3+ in soil (mmolc 100 g-1)

[Ca2+] = exchangeable Ca2+ in soil (mmolc 100 g-1)

[AL-K] = Ammonium lactate extractable form of K (mg K2O 100-1g)

Multiple regression analysis revealed exchangeable acidity, exchangeable Ca2+ and the ammonium lactate extractable form of K (AL-K) to be the key soil parameters governing glyphosate mineralization in the examined soils. The negative correlation between glyphosate mineralization and exchangeable acidity that was already shown by univariate regression analysis, was confirmed by the results of multiple regression analysis, thereby strengthening the role of exchangeable acidity as a parameter that reduces the bioavailability of glyphosate in soils, resulting in reduced glyphosate mineralization. In addition to exchangeable acidity, Ca2+ and AL-K were also identified as additional impact factors, which, in contrast to exchangeable acidity, had a positive correlation with glyphosate mineralization. This is not consistent with the study of Caetano et al. (2012), who found that glyphosate formed a metallic complex with Ca2+ in soil, thereby reducing glyphosate bioavailability and glyphosate degradation. We did not find any information in literature on the effect of AL-K on glyphosate availability. The fact that both Ca2+ and AL-K had a positive correlation with glyphosate mineralization in our study can probably be explained by the following hypothesis: Ca2+ and AL-K in this case served as carriers for glyphosate to be transported more efficiently across microbial cell walls than a sole glyphosate compound. This has already been argued for Cu2+-glyphosate complexes in the literature (Kools et al., 2005). However, these mechanisms have not been documented and should be clarified in future. The result of multiple regression analysis indicates that, although some soil parameters did not show any single correlation with glyphosate mineralization, they had a strong influence on mineralization of glyphosate in soils when all soil parameters were taken into account for regression analysis.

1. **Conclusions**

The risk of a long-term, incremental build-up of glyphosate accumulation in soil due to low degradation is driven by highly site-specific factors. Both (i) NaOH extractable glyphosate residues in soils and (ii) soil properties: exchangeable acidity, Ca2+ and AL-K contents were found as good impact parameters for indicating soil potential for glyphosate mineralization.

Interestingly, the present investigation of soils with pH range from 5.0 to 7.1, highlighted the importance of soil exchangeable acidity to suppress glyphosate mineralisation. Since this parameter has not usually been included in pesticide fate models, the risk of glyphosate transfer in the environment may be underestimated, especially in areas with lower pH. Further efforts are therefore needed for understanding if glyphosate mineralization can be enhanced with agricultural measures in soils with low mineralization potential.

To ensure the sustainability of agriculture with respect to GlyBH, as well as other pesticides, the potential risk should be evaluated and reduced before pesticide application, not only through EU and national registration procedures but above all as part of best agricultural practice on the site (*field*) specific level. The results of our study clearly showed immense impact of soil properties on glyphosate mineralization, which could vary from 7% to 70% in a month, large variation between the sites also in glyphosate transfers to the environment may be foreseen.

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**Table 1:** Selected properties of the 21 soils (Ap horizon) used in the study

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil | Soil type(WRB) | Sand0.05-2mm(%) | Silt0.002-0.05mm(%) | Clay<0.002mm(%) | pH(CaCl2) | Organic matter(%) | TN(%) | C/N | AL-P (mg P2O5100g-1) | AL-K(mg K2O 100g-1) |
| Ada-A02 | Ad  | Cambisol | 62.5 | 27.4 | 10.1 | 5.7 | 2.9 | 0.2 | 8 | 34.5 | 43.2 |
| Apace  | Ap | Fluvisol  | 66.4 | 31.2 | 2.4 | 7.0 | 2.6 | 0.2 | 10 | 8.4 | 9.4 |
| Berta-A02 | Be | Cambisol | 46.4 | 39.4 | 14.2 | 5.7 | 2.5 | 0.2 | 9 | 20.7 | 22.6 |
| Brezje | Br | Gleysol | 8.3 | 73.2 | 18.5 | 5.2 | 2.8 | 0.2 | 8 | 10.5 | 21.1 |
| Dunja -A06  | Du | Cambisol | 62.4 | 25.9 | 11.7 | 5.4 | 2.2 | 0.2 | 9 | 48.3 | 24.7 |
| Feldkirchen | Fe | Regosol | 34.8 | 47.0 | 18.2 | 7.0 | 3.4 | 0.3 | 7 | 41.9 | 17.0 |
| Grace -A13 | Gr | Cambisol | 50.3 | 41.3 | 8.4 | 5.4 | 2.6 | 0.2 | 8 | 33.6 | 21.1 |
| Hanna -A15 | Ha | Cambisol | 62.3 | 24.2 | 13.5 | 5.2 | 1.7 | 0.1 | 8 | 26.7 | 7.9 |
| Hohenwart | Ho | Anthrosol | 67.2 | 20.5 | 12.3 | 6.2 | 1.7 | 0.1 | 8 | 37.0 | 16.8 |
| Joy -A19 | Jo | Cambisol | 31.6 | 45.6 | 22.8 | 5.9 | 2.7 | 0.2 | 8 | 56.5 | 24.7 |
| Kelheim | Ke | Cambisol | 76.2 | 15.5 | 8.3 | 6.5 | 1.2 | 0.1 | 7 | 33.8 | 12.2 |
| Konjisce | Ko | Fluvisol | 33.8 | 60.2 | 6.0 | 6.9 | 4.5 | 0.2 | 12 | 10.2 | 4.1 |
| Lomanose a  | La | Stagnosol | 10.3 | 69.6 | 20.1 | 5.8 | 4.3 | 0.3 | 8 | 12.1 | 12.5 |
| Lea -A18  | Le | Cambisol | 18.9 | 66.8 | 14.3 | 5.2 | 1.9 | 0.2 | 7 | 19.7 | 13.2 |
| Lomanose b | Lo | Stagnosol | 21.9 | 60.2 | 17.9 | 5.8 | 1.7 | 0.2 | 7 | 23.5 | 9.6 |
| Neumarkt | Ne | Arenosol | 85.5 | 8.8 | 5.7 | 5.2 | 1.6 | 0.1 | 8 | 31.0 | 8.2 |
| Pearl -A20 | Pe | Cambisol | 29.3 | 51.8 | 18.9 | 5.0 | 2.3 | 0.2 | 8 | 30.7 | 18.7 |
| Scheyern Lys | Sch | Gleysol | 17.2 | 62.6 | 20.2 | 5.5 | 2.7 | 0.2 | 9 | 35.2 | 20.2 |
| Skrinjar | Sk | Fluvisol | 67.5 | 27.0 | 5.5 | 7.1 | 1.6 | 0.1 | 7 | 31.3 | 5.3 |
| Zepovci | Ze | Fluvisol | 41.3 | 43.1 | 15.6 | 5.7 | 2.9 | 0.2 | 8 | 24.1 | 31.7 |
| Zepovci-P | ZeP | Gleysol | 11.8 | 72.2 | 16.0 | 5.2 | 1.9 | 0.2 | 7 | 20.1 | 23.8 |

**Table 1 (continued):**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil  | Water content at-15 kPa (%) | Al-OX(mg 100g-1) | Fe-OX(mg 100g-1) | Cu2+(mg kg-1) | Ca2+ | Mg2+ | K+ | Na+ | H+a | CEC |
| (mmolc 100g-1) |
| Ad  | 21.9  | 63 | 198 | 4 | 8.5 | 0.8 | 1.0 | 0.04 | 5.7 | 16.0 |
| Ap | 20.7  | 62 | 248 | 4 | 11.1 | 2.3 | 0.1 | 0.04 | 1.5 | 15.0 |
| Be | 28.1  | 76 | 265 | 3 | 9.0 | 1.0 | 0.6 | 0.04 | 5.3 | 15.9 |
| Br | 32.7 | 187 | 518 | 2 | 7.2 | 0.9 | 0.6 | 0.07 | 11.1 | 19.8 |
| Du | 17.4 | 80 | 211 | 62 | 7.0 | 0.6 | 0.6 | 0.04 | 5.3 | 13.6 |
| Fe | 28.2 | 139 | 310 | 12 | 26.4 | 2.5 | 0.5 | 0.05 | 3.5 | 32.9 |
| Gr | 21.0 | 106 | 259 | 3 | 8.7 | 0.7 | 0.6 | 0.04 | 7.4 | 17.5 |
| Ha | 18.4 | 83 | 215 | 2 | 7.2 | 0.5 | 0.2 | 0.04 | 5.7 | 13.6 |
| Ho | 22.4 | 75 | 206 | 4 | 5.5 | 1.2 | 0.4 | 0.05 | 3.9 | 11.1 |
| Jo | 31.9 | 101 | 320 | 39 | 13.1 | 1.8 | 0.7 | 0.06 | 6.7 | 22.4 |
| Ke | 12.5 | 44 | 132 | 8 | 5.5 | 1.2 | 0.3 | 0.05 | 2.0 | 9.1 |
| Ko | 34.6 | 88 | 381 | 7 | 10.8 | 4.6 | 0.1 | 0.06 | 3.2 | 18.8 |
| La | 35.8 | 134 | 456 | 4 | 16.4 | 3.6 | 0.3 | 0.06 | 9.2 | 29.6 |
| Le | 28.9 | 107 | 345 | 3 | 6.4 | 0.8 | 0.4 | 0.07 | 6.9 | 14.6 |
| Lo | 25.8 | 72 | 252 | 3 | 9.5 | 1.8 | 0.3 | 0.09 | 5.4 | 17.0 |
| Ne | 12.6 | 88 | 110 | 1 | 2.6 | 0.4 | 0.2 | 0.05 | 4.3 | 7.5 |
| Pe | 28.3 | 125 | 319 | 4 | 6.9 | 0.8 | 0.5 | 0.05 | 8.8 | 17.0 |
| Sch | 30.1 | 102 | 349 | 10 | 9.1 | 1.6 | 0.6 | 0.06 | 7.1 | 18.4 |
| Sk | 19.2  | 57 | 257 | 4 | 10.8 | 0.5 | 0.1 | 0.06 | 1.5 | 13.0 |
| Ze | 24.0 | 165 | 476 | 4 | 7.8 | 0.7 | 0.9 | 0.05 | 10.6 | 20.0 |
| ZeP | 27.4 | 147 | 430 | 2 | 4.4 | 0.5 | 0.7 | 0.10 | 9.4 | 15.1 |

aH+ represents the exchangeable acidity (extractable H+ and Al3+)

**Table 2:** Behaviour of 14C-glyphosate in selected agricultural soils after 32 days of incubation: Cumulative mineralization (Cum. Min.), NaOH extractable residues (NaOH-ER); non-extractable residues (NER), glyphosate, aminomethylphosphonic acid (AMPA) and unknown metabolites are shown.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Soil | Cum. Min (%)c(1) | NaOH-ERa(%)c(2) | NER (%)c(3) | Total 14C-recoveryb(%)c(4) | Composition of NaOH-ER |
| Glyphosate (%)c(5) | AMPA(%)c(6) | Unknown(%)c(7) |
| Ad  | 44.7 (±0.7) | 48.3 (±0.4) | 4.8 (±0.1) | 97.8 (±0.9) | 37.7 | 2.3 | 8.3 |
| Ap | 67.3 (±0.1) | 24.5 (±0.8) | 9.6 (±0.2) | 101.4 (±0.8) | 18.9 | 2.2 | 3.4 |
| Be | 48.9 (±0.4) | 42.7 (±0.7) | 6.3 (±0.5) | 97.9 (±0.5) | 34.4 | 0.0 | 8.3 |
| Br | 7.6 (±0.2) | 91.0 (±0.7) | 2.5 (±0.6) | 101.1 (±0.3) | 88.0 | 0.0 | 3.0 |
| Du | 39.5 (±0.3) | 53.9 (±0.2) | 3.9 (±0.1) | 97.3 (±0.4) | 45.2 | 0.0 | 8.7 |
| Fe | 68.7 (±0.4) | 23.3 (±0.6) | 9.0 (±0.2) | 101.0 (±0.4) | 12.2 | 7.6 | 3.6 |
| Gr | 35.5 (±0.3) | 57.7 (±1.0) | 3.7 (±0.2) | 96.9 (±0.8) | 44.1 | 2.8 | 10.8 |
| Ha | 32.2 (±0.5) | 59.8 (±0.3) | 4.1 (±0.2) | 96.1 (±0.6) | 54.0 | 0.0 | 5.8 |
| Ho | 55.8 (±2.3) | 37.7 (±0.5) | 6.3 (±0.3) | 99.8 (±2.9) | 24.8 | 5.7 | 7.3 |
| Jo | 47.9 (±0.6) | 46.9 (±3.2) | 8.2 (±0.7) | 103.0 (±5.4) | 31.3 | 7.1 | 8.5 |
| Ke | 51.8 (±0.7) | 37.3 (±0.6) | 6.2 (±0.4) | 95.3 (±0.9) | 25.1 | 4.1 | 8.1 |
| Ko | 49.1 (±0.5) | 35.7 (±0.6) | 9.5 (±0.6) | 94.3 (±1.4) | 23.3 | 4.5 | 7.9 |
| La | 25.5 (±0.5) | 64.4 (±3.3) | 6.7 (±0.5) | 96.6 (±3.0) | 45.0 | 8.3 | 11.1 |
| Le | 37.3 (±0.9) | 55.7 (±0.5) | 5.4 (±0.1) | 98.4 (±0.9) | 30.0 | 0.0 | 25.7 |
| Lo | 43.7 (±1.4) | 46.8 (±2.5) | 6.4 (±0.4) | 96.9 (±2.5) | 30.4 | 8.0 | 8.4 |
| Ne | 31.2 (±0.8) | 63.0 (±0.3) | 3.1 (±0.2) | 97.3 (±1.1) | 48.8 | 2.5 | 11.7 |
| Pe | 31.5 (±1.2) | 63.6 (±1.5) | 3.7 (±0.2) | 98.8 (±2.8) | 29.5 | 0.0 | 34.1 |
| Sch | 32.5 (±1.2) | 59.8 (±1.2) | 5.0 (±0.1) | 97.3 (±2.3) | 40.9 | 5.5 | 13.4 |
| Sk | 61.6 (±0.2) | 28.8 (±0.4) | 11.4 (±0.2) | 101.8 (±0.5) | 16.9 | 4.8 | 7.1 |
| Ze | 19.5 (±0.4) | 73.3 (±1.4) | 4.1 (±0.2) | 96.9 (±1.6) | 65.8 | 0.0 | 7.5 |
| ZeP | 18.4 (±0.2) | 78.5 (±4.7) | 2.7 (±0.3) | 99.6 (±5.8) | 55.9 | 11.3 | 11.3 |

a NaOH-ER (2) = (5) + (6) + (7)

bTotal 14C-recovery (4) = (1) + (2) + (3)

c % of applied 14C-glyphosate after 32 days