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Glyphosate degradation as a soil health indicator for heavy metal polluted soils

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Abstract

Glyphosate is a commonly used herbicide in grassland soils and microorganisms control its degradation. We introduce the concept of using the degradation rate as an indicator for ecosystem health. Testing this concept, we used soils with a long history of heavy metal pollution (Cu, Pb, and Zn). We hypothesized lower degradation rates in metal-polluted compared to less polluted soils. The degradation rates were measured by repeated measurements of the parent compound in spiked soil–water slurries incubated at 20 °C over 21 days. Average rates showed no differences comparing among soils. We observed a positive correlation between glyphosate degradation rates and soil metal pollution. Therefore, we concluded that the expected impact of the metals on the bacteria responsible for the herbicide degradation was not established. We discuss the potential influence on biological degradation rates of soil pH and adsorption and implications using the concept of the soil health indicator.

Keywords: Bacteria; Ecosystem health indicator; Glyphosate degradation; Heavy metal; Copper; Lead; Zinc

1. Introduction

In the Netherlands, some peaty grassland areas received city waste for soil enrichment and reinforcement. As a result, these soils contain elevated metal concentrations, of which lead and zinc are locally exceeding legal intervention values (Bosveld et al., 2000). Risk assessments indicated a serious threat for this soil and legislation demands intervention to clean the soil (Vegter, 1995). Toxicity data of species and processes, obtained in the laboratory, are the basis for estimations of these intervention values. Besides determining the impact on single organisms, current ecotoxicological research seeks indicators for effects at the ecosystem level (Van Straalen, 2002). Degradation of xenobiotic compounds is an important indicator for healthy ecosystems. We hypothesized that the capacity to degrade xenobiotic compounds is reduced because of long-term exposure of bacterial communities to heavy metal pollution. Studies have shown reduced degradation of diesel fuel (Riis et al., 2002) and other organic compounds (Doelman et al., 1994) in soils impacted by heavy metals. In this study, we studied the degradation of the herbicide glyphosate (*N*-phosphonomethyl-glycine). This is often used to control weeds in grasslands but detailed information on degradation rates is lacking.

Glyphosate is the active ingredient of the commonly used herbicide Roundup®. Plants take the herbicides up very easily and then, it exhibits specific growth reduction by interfering upon the metabolism of essential aromatic compounds in the shikimic acid pathway (Cole, 1985). This herbicide is an organophosphonate, characterized by the presence of a stable, covalent carbon to phosphorous (C–P) bond. Very specific bacteria seem to be responsible for degradation (Dick and Quinn, 1995; Forlani et al., 1999). Since few bacteria isolates grow solely on glyphosate, degradation in non-limiting environments is thought to result from co-metabolism (Ternan et al., 1998). Glyphosate can act as a carbon source, but growth stimulation is more apparent when applied in high concentrations (Busse et al., 2001).

We studied the initial degradation rate of glyphosate during an incubation period of 20 days. Previous research

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showed that this is predominantly the rapid initial degradation of the labile fraction of glyphosate (Eberbach, 1998). Soil pH, clay, or organic matter content (Torstensson, 1985) not largely influence this rate, enabling a comparison among different soils in this study.

2. Materials and methods

2.1. Soil sampling

We selected research areas, based on the presence of elevated heavy metal concentrations. Three sample areas were close to each other in an area called Demmerikse polder, Ronde Venen, located southeast from Amsterdam, The Netherlands (52°13′ North 4°56′ East). This region received city waste as fertilizer and soil reinforcement since the 1600s and later, extensive cattle farming was the main form of land use. The area shows a very heterogeneous pollution pattern, thus 150 soil samples were taken earlier to identify spots differing the most in total heavy metal concentrations. We sampled using a soil drill of 3 cm wide and 10 cm deep and discarded the root zone (0–2 cm). Samples were first dried over night at 50 °C, then crushed using a mortar and subsequently analyzed for metal concentrations using a XRF spectrum analyzer (XTAC Analytical, Leiden, The Netherlands). We sampled seven spots in the location containing the lowest concentrations of zinc, lead, and copper (part A) and another seven at the location with the highest concentrations (part B). In adjacent grasslands with more intensive land management, e.g. manure application (part C), six samples were taken. Outside this area, we selected Polder Blokland and Polder Zeevang based on expected lower metal concentrations and comparable land management practices to serve as reference locations. Polder Blokland lies 20 km west of Polder Demmerik while Polder Zeevang is located 38 km north. At all locations, we sampled the top 10 cm soil layer in duplicate, by means of the same soil drill; again first 2 cm discarded. Directly after, samples were stored in the dark at 5 °C. Analyzing duplicate soil samples, we used one for characteristics and metal contents and the other for the degradation experiments.

2.2. Metal analysis

Soil samples were first dried over night at 100 °C and then pulverized using a ball mill. Subsequently, 2 ml demineralized water; 6 ml hydrogen chloride (HCl: 37%, ultrex grade: Baker, Philipsburg, NJ, USA) and 2 ml HNO_3 (65%, ultrex grade, Riedel-de Häen, Seelze, Germany) were added to 1 ± 0.1 g soil. In a microwave oven (CEM Mars 5), we digested this mixture for 60 min at a pressure of 100 psi and a temperature of 150 °C. After cooling, we added 15 ml demineralized water. Metal concentration in the solutions was determined by flame Atomic Absorption Spectrometry (AAS) using a Perkin Elmer 1100B. San Ioaqin soil (NIST, Gaitersburg, MS, USA) served as certified standard reference material to maintain quality control.

To get an estimate of metal exposure to organisms, 0.01 M $CaCl_2$ exchangeable zinc concentrations were determined (Houba et al., 1996). Using fresh soil, we added 20 g to 50 ml 0.01 M $CaCl_2$ in water and rotated this for two hours at 200 rpm. The pH of these samples was determined using a Consort P907. After precipitation of soil particles, we analyzed zinc concentrations using flame AAS.

2.3. Organic matter and clay content

We calculated organic matter contents by determining loss on ignition. For this, porcelain crucibles were previously weighed. We filled these with soil samples, previously dried at 100 °C over night, then weighed them and finally placed them at 450 °C for 6 h and weighed again. For determining the percentage clay, a 'laser particle sizer' A22 was used (Fritsch GmbH, Idar Oberstein, Germany). This method is based on the forward scattering of monochromatic coherent light. We prepared the samples and did the analysis as described in (Konert and Vandenberghe, 1997).

2.4. Glyphosate degradation

Five grams of fresh soil were transferred to a 100 ml Erlenmeyer flask and 20 ml demineralized H_2O was added. We put the flasks in a rotary shaker at 150 rpm and incubated them at 20 °C. The starting concentration was 10 mM glyphosate (*N*-phosphonomethyl-glycine, >96%, purchased from Fluka, Buchs, Germany). Immediately after, we centrifuged 500 µl aliquots at 8000 g. Subsequently; we performed a pre-column derivatization, thus enabling high performance liquid chromatography (HPLC). We added 130 mM *p*-toluene-sulphonyl chloride (>99%, Fluka, Buchs, Germany) in acetonitrile (1:1 v/v, pH 11.3) (Tomita et al., 1991) and injected the extracts on a 4.6× 250 mm Supelcosil ODS column equilibrated with 50 mM di-sodium-hydrogen-phosphate-2-hydrate buffer (99.5%, Riedel-deHaën, Seelze, Germany). While the isocratic elution proceeded at a flow rate of 1 ml/min, we monitored at 240 nm (UV-ABS) using a diode array detector (Gynotek, Munich, Germany). We made a glyphosate standard dilution series and calculated the concentrations using the area under the curve (the lower limit of detection was 0.2 mmol/l). This procedure was repeated 1, 3, 6, 8, 10, 13, 15, 17 and 20 days after the start of the experiment.

2.5. Data handling

We natural log transformed the data, and preformed a linear regression on each individual sample. Assuming first order kinetics, we modeled the starting concentrations (C0) and degradation rate constants (k) from plots of logarithmic concentrations against time using SYSTAT 10 software

Table 1
Average soil characteristics ±SD from the sampled areas in the Netherlands over *N* replicates, total metal concentrations (in bold exceeds Dutch legal intervention values) and 0.01 M CaCl2 exchangeable zinc concentrations (in mg/kg dry soil)

Area		Cu	Pb	Zn	pH[a]	Clay (%)	O.M.[b] (%)
Zeevang (*N*=6)	Total	35±3	90±29	162±31	6.0±0.1	30±4.5	40±3.4
	Exchangeable			2.3±1.1			
Blokland (*N*=6)	Total	67±11	265±59	228±22	5.9±0.4	25±2.2	25±4.2
	Exchangeable			4.6±0.96			
Demmerik A (*N*=6)	Total	115±36	540±205	271±62	6.5±0.5	10±2.3	60±4.6
	Exchangeable			4.6±3.4			
Demmerik B (*N*=7)	Total	134±23	**726±103**	298±35	5.8±0.5	13±0.8	41±0.7
	Exchangeable			7.3±2.7			
Demmerik C (*N*=6)	Total	178±23	**802±89**	397±61	5.5±0.4	13±1.3	42±2.4
	Exchangeable			8.8±2.5			

[a] Measured in 0.01 M $CaCl_2$.
[b] Organic matter (% loss on ignition).

package. From (k), half-lives were expressed as DT_{50} (days at which 50% of the herbicide is degraded). We used SPSS version 10.0 for calculating means for the different polders, based on individual degradation rates and Pearson correlations with soil characteristics, with a significance level of $P<0.05$. At last, we calculated a partial correlation, controlling for soil pH.

3. Results

3.1. Soil analysis

First, we compared the soil characteristics (Table 1). All soils had a high organic matter content, some up to 60% and the clay fraction varied from 10 to 30%. The total lead concentration had the greatest range from 69 to 911 mg/kg DW, while zinc and copper had similar patterns among the different soils, but showed smaller ranges (Cu: 31–207 mg/kg DW, Zn: 134–480). Locally, lead exceeded the intervention value of 750 mg/kg DW, also after correction for the high clay and organic matter contents (Vegter, 1995). Heavy metal concentrations correlated not significantly with organic matter, but in contrast, negatively correlated with the content of clay (Pearson, Cu: −0.65, Pb: −0.62, Zn: −0.50). We found the same for soil pH (Pearson, Cu: −0.38, Pb: −0.47, Zn −0.51). Exchangeable zinc concentrations in 0.01 M CaCl2 solution were around 50 times lower than total metal concentrations and were positively correlated (Pearson coefficients, 0.68, $P<0.01$), making it an estimator of exposure. Exchangeable copper and lead concentrations were below detection limits.

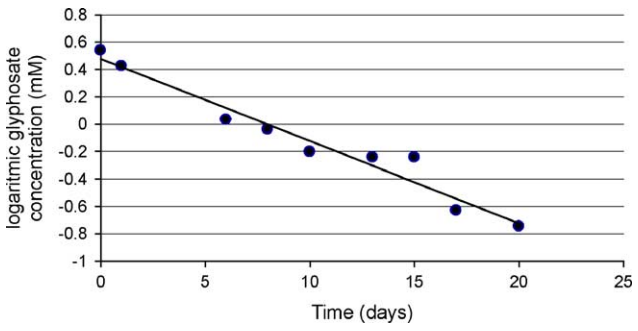

Fig. 1. Representative example of the logarithmic transformed glyphosate concentrations, measured during incubation at 20 °C of a glyphosate–soil suspension, showing the fitted curve.

3.2. Glyphosate degradation

Fig. 1 shows a representative example of the glyphosate concentration measured over time. In total, we selected 31 samples with a significant individual fit using the log-linear regression model (one-way ANOVA, $P<0.01$). We discarded one curve due to technical errors during incubation. The coefficient of determination (r^2) ranged from 0.65 to 0.99. Rapid degradation of glyphosate was comparable in all soils (Table 2) and the derived glyphosate half-lives (DT_{50}) varied from 5 to 23 days. We observed no significant correlations between degradation rates and the soil organic matter or the clay content. On the contrary, we found low, but significant positive correlations with the total metal concentrations (Pearson, $P<0.05$) by performing analyses on all individual observations (Fig. 2, Cu: 0.40, Pb: 0.43, Zn: 0.37). Plotting the exchangeable concentrations showed a higher correlation (0.61) between degradation rates and exchangeable zinc concentrations (Fig. 3). Furthermore, degradation rates positively correlated with soil pH (Pearson, 0.53, $P<0.01$) (Fig. 4). We therefore included a partial correlation (Table 3), which decreased correlation coefficients.

Table 2
Average degradation rates (*k*) and expressed half-lives (DT50) in days of glyphosate in different soils and their range (min–max) over *N* replicates

SOIL	*N*	*k* (day^{-1})±SD	DT_{50}	min–max
Zeevang	6	0.0500±0.009	14.3	12–20
Blokland	6	0.0478±0.018	16.1	9.3–23
Demmerik part A	6	0.0524±0.019	14.3	8.6–23
Demmerik part B	7	0.0557±0.014	13.2	8.7–18
Demmerik part C	6	0.0654±0.010	10.8	8.9–14

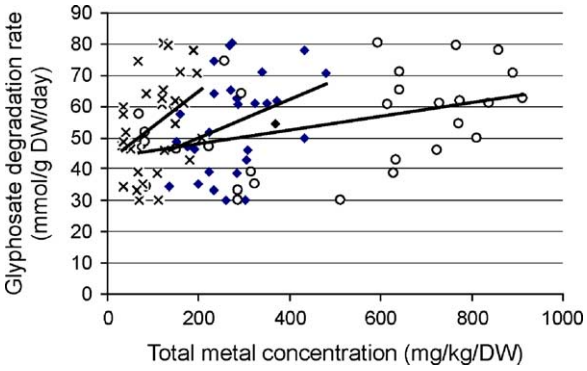

Fig. 2. Glyphosate degradation rates (μmol/g DW/day) plotted against total metal concentrations (× = Cu, ♦ = Zn and ○ = Pb, mg/kg dry soil), all significant correlations: (Pearson, $P < 0.05$, 31 samples).

4. Discussion

4.1. Glyphosate degradation rates

This is the first time degradation rates of glyphosate were determined in relation to existing heavy metal pollution in soils and the use as a soil health indicator. Contrary to our hypothesis, we did not observe reduced rates of degradation in polluted soils. We therefore concluded that these polluted soils degrade glyphosate to at least the same extent as non-polluted soils. This finding is in contrast with earlier studies (Doelman et al., 1994; Riis et al., 2002). From parallel studies on soil from Polder Demmerik (Boivin et al., unpublished), we learned that the presence of heavy metals negatively correlated with bacterial biomass, BIOLOG substrate utilization, and growth rates. Fungi, which might be favored in slightly acidic soils, can contribute to microbial degradation of glyphosate (Krzyśko-łupicka and Orlik, 1997), but we have no specific fungal data available. Our degradation rates seems to be consistent with literature (Cheah et al., 1998; Eberbach, 1998), though comparing these degradation rates of glyphosate is not fully justified because of the higher temperatures (30 and 25 °C

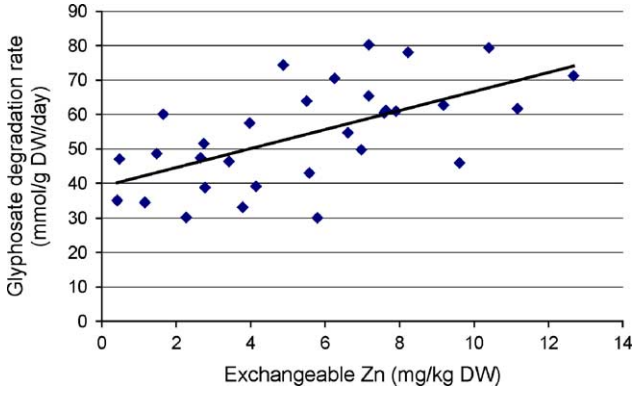

Fig. 3. Glyphosate degradation rate (μmol/g DW/day) plotted against 0.01 M exchangeable zinc (mg/kg DW) in the soil concentration—significant correlation (Pearson, 0.61, $P < 0.01$), 31 samples.

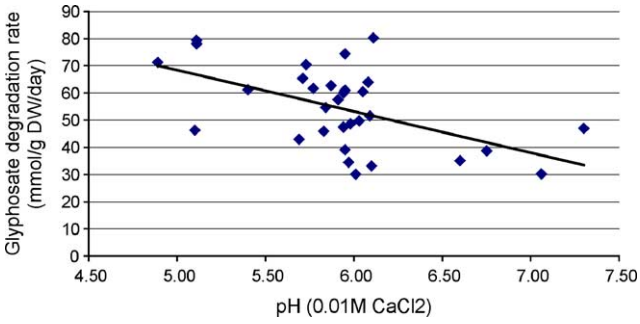

Fig. 4. Glyphosate degradation rates (μmol/g DW/day) plotted against soil pH (as measured in 0.01 M CaCl2 solution)-significant correlation (Pearson, 0.53, $P < 0.01$), 31 samples.

respectively) in these studies, possibly influencing bacterial activity (Heinonen-Tanski, 1989).

4.2. Possible factors determining glyphosate degradation

Although being correlative, the apparent positive effect of the heavy metal pollution on the degradation of this compound is intriguing. Exchangeable metal concentrations in these soils were indicative for lead and copper pollution and these concentrations are one thousand times lower than the total concentrations in these soils. Based on the observed lower bacterial biomass and activity in the parallel study, one would indeed expect a reduced degradation rate, as co-metabolism is the primary mechanism for glyphosate degradation (Ternan et al., 1998). We noted that these methods are based on the biological degradation of aromatic carbon containing compounds, lacking carbon-phosphate bonds, making direct comparisons not fully justified. The degradation of these bonds appear to involve specific bacterial communities as described by (Forlani et al., 1999).

We concluded that the apparent positive effect of heavy metals on glyphosate degradation confounded by pH, as indicated by partial correlation (Table 3). The effect of soil pH on rapid initial degradation rates was thought to be negligible (Torstensson, 1985), which is not the case in this in this study and we paid more attention to this observation. The influence of the soil pH on the bacterial community responsible for this degradation can be of importance. Soil pH can also affect the adsorption of the labile fraction, which seems to be stronger in acidic soils

Table 3
Correlation coefficients between degradation constants (k) and heavy metal concentrations (total and 0.01 M $CaCl_2$ exchangeable) of 31 samples with and without controlling for soil pH (partial correlation)

Correlation k with:	Correlation coefficient	Controlling for pH
Total Zn	0.37*	0.27 NS
Total Pb	0.43*	0.34 NS
Total Cu	0.40*	0.29 NS
Exchangeable Zn	0.61*	0.41*

*Significant ($P < 0.05$), NS: not significant.

(e.g. Eberbach, 1998). Next to this, glyphosate is a metal chelating herbicide (Subramaniam and Hoggard, 1988). Copper–glyphosate complexes showed a higher affinity for soil (Morillo et al., 2000). We did indeed observe that soils with higher exchangeable concentrations showed a higher initial decrease of the nominal glyphosate concentrations. The formation of complexes proceeds rapidly and is not likely to have played a role over the extended time window of our experiments (20 days). Therefore we believe that metal complexation by glyphosate cannot explain the higher apparent degradation rates in metal-polluted soils. Metal–glyphosate complexes might however be transported more efficiently across microbial cell walls than the sole compound, but such a mechanism is not documented. Together, these phenomena are not to be ruled out in assessing degradation rates, while the question remains unclear which is more to occur.

4.3. Conclusion

We concluded that glyphosate degradation is not a sensitive soil health indicator, since this process is complicated due to the chelating behavior of glyphosate. Subsequent soil bound glyphosate exhibits a half-life of up to 22 years (Eberbach, 1998) and investigating these soil–glyphosate complexes is of high interest, since the bulk of the pesticide will remain in this fraction. Next to that, degradation and/or toxicity are not fully understood, especially not for metal polluted soils. Studies on fate and biological degradation in time, using controlled pH and soils polluted artificially with heavy metals give results that are more conclusive. In general, assessments of the purifying function of polluted soils in the light of ecosystem health indicators should consider both short-term and long-term effects.

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