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Mineralization of Glyphosate in Compost-Amended Soil Under Controlled Conditions

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Glyphosate [N-(phosphonomethyl)glycine] is one of the world's most widely used herbicide. The herbicide is strongly bound to soil particles, and generally regarded as having low potential to contaminate groundwater. However, there is evidence of desorption of glyphosate from soil particles, which would result in glyphosate moving through soil and ground water (IPCS 1994; Piccolo et al. 1994; US EPA 1993). Furthermore, the herbicide is highly soluble in water (11600mg/L) at 25°C and is resistant to hydrolysis in water (Agriculture Canada 1991). Adsorption of glyphosate by clay minerals has been shown to be reduced by the presence of copper, due to the formation of glyphosate –copper complexes. Both soil type and any element in soil capable of forming complexes with glyphosate have been shown to affect the adsorption of glyphosate (Morillo et al. 1997). A study of sandy soils in Western Australia found that adsorption of glyphosate and its metabolite, aminomethyl phosphonic acid (AMPA) increased strongly with iron and aluminium content of the soils, while soil organic matter competed for adsorption sites and inhibited adsorption (Gerritse et al. 1996). Recent studies on four soils, chosen to represent the most widespread soil types in the European Union showed that soils containing higher levels of clay minerals adsorbed more glyphosate and that, the herbicide was extensively mobile in soils which could not bind with glyphosate (Piccolo and Celano 1994).

Whether glyphosate can move through the soil and contaminate both surface and ground water depends entirely on the soil conditions prevailing. Ways of enhancing the biodegradation of glyphosate in soils where it is highly adsorbed should be looked into. The present study was carried out to determine if compost made from organic solid waste could enhance the mineralization of glyphosate and hence, reduce the potential for the glyphosate contaminating surface and ground water in soils with high adsorption capacity.

MATERIALS AND METHODS

Glyphosate-phosphonomethyl-^{14}C-labeled (International Isotopes, Munich) with specific activity of 52mCi/mmol, and radiochemical purity of 99% was determined by TLC (silica gel) using butanol:water:acetic acid (60:25:15) solvent system. The commercially formulated glyphosate of isopropylamine ammonium

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salt (Roundup) with purity of >97% as determined by TLC was purchased from Monsanto Center Africa Inc. The two herbicides were used together in laboratory experiments. Quicksafe A, 2,5-diphenyloxazole (PPO) and 1,4-bis [5-phenyl-2-oxazolyl]-benzene; 2,2'p-Phenylene-bis[5-phenyloxazole] (POPOP) in toluene and Harvey Carbon-14 Cocktail (Zinsser Analytic (UK) Ltd) were used in Liquid Scintillation Counting. All solvents used were re-distilled in all-glass apparatus. The clay soil (organic content (OC) 2.07%, pH 6.08, clay content 60%, sand content 28%, silt 12%, N 0.19%, P 80ppm, Na 0.95%, K 1.85%, Ca 10.5%, Mg 4.95%, Mn 0.51%, Fe 226.96ppm, electrical conductivity (EC) of 0.62μS/cm) and compost (N 1.14%, P 0.72ppm, Cu 140ppm, Mn 2217ppm, Fe 1272ppm and Zn 755ppm) were used. Liquid Scintillation Counter (Tricarb-1000) and Biological Materials Oxidizer (OX-600 model) were used for radio-assaying.

In the incubation experiment, 50g of sieved soil samples in replicas of three were placed in biometer flasks (Bell Co. Glass Inc.) after the air-dried and homogenized soil was sieved though a 2-mm sieve. The soil was conditioned by being moistened to 75% of the field water capacity. The soil samples in biometer flasks were given different treatments before they were incubated at 30°C in the darkness under aerobic conditions. The first set of the soil samples was autoclaved at 121°C for 45 minutes at the pressure of 1.2 bars for three consecutive days. The second set of the soil samples was neither autoclaved nor received compost made from urban solid organic waste. The third set of the soil samples was spiked with compost at concentrations of 1000μg/g, 2500μg/g and 5000μg/g (compost/soil). Glyphosate solution (both labeled and non-labeled) in distilled water was added to the 50g-soil sample in each biometer flask giving an initial herbicide concentration of 100μg/g and initial radioactivity of 2μCi in soil. The soil was thoroughly mixed to ensure uniform distribution of the herbicide in soil. The side arm of each biometer flask was filled with 10ml of 0.1N NaOH to trap the $^{14}CO_2$ gas released during mineralization by soil microorganisms. The inlet of each biometer flask was filled with Ascarite to exclude carbon dioxide from entering the system. The biometer flasks were placed in an incubator to monitor the progress of mineralization. At different time intervals, the 10ml of NaOH solution from the side arm was sampled from which one ml of the solution aliquot was taken and mixed with 5ml of Quicksafe A cocktail in a 20-ml scintillation vial before it was radio-assayed. After every sampling, the side arm was refilled with fresh 0.1N NaOH solution. The experiment was run for 50 days, when maximum rate of $^{14}CO_2$ production was attained. The amount of accumulated $^{14}CO_2$ over the 50-day period was computed from the amount of $^{14}CO_2$ obtained in each sampling.

At the end of the incubation period, soil samples from the biometer flasks were removed and air-dried. A sub-sample of 20g was taken and extracted with 100ml of 0.2M KOH solution by shaking on an orbital shaker for four hours. An aliquot of 1ml of the 0.2M KOH extract was taken and mixed with 5ml of Quick safe A cocktail and radioassayed to quantify extractable residue. The readings from the Liquid Scintillation Counter were corrected by external standardization. The extracted soil samples were air-dried and 1.5g of the soil sample was combusted in a Biological Materials Oxidizer (Packard, USA). The $^{14}CO_2$ released during

combustion of the extracted soil samples was trapped in 15ml of Harvey Carbon-14 cocktail and then radio-assayed to quantify non-extractable residue of glyphosate. 1.5g of soil spiked with known amount of radioactivity was also combusted to determine the efficiency of the Biological Materials Oxidizer. All the readings were corrected according to the efficiency results obtained for the oxidizer. The amounts of the various residues of glyphosate were computed to get the mass balance at the end of the 50-day period of the experiment.

Adsorption studies were also carried out to determine the proportion of the herbicide sorbed to the soil during mineralization in non-amended soil. 4g of homogenized soil samples were weighed and placed in 40ml-centrifuge tubes.10ml of the herbicide solution prepared in 0.01M $CaCl_2$ at different concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0ppm was added to 4g of soil in the centrifuge tubes. The resulting herbicide concentration in soil in each centrifuge tube was determined after adsorption. The centrifuge tubes were shaken on an orbital shaker at 200rpm for 24 hours, when equilibrium was established between the solution and solid phases. Thereafter, the centrifuge tubes were transferred to a centrifuge machine and centrifuged at 3600rpm for 30 minutes. 1ml of the supernatant was taken and mixed with 5ml of the Quick safe A cocktail solution and radio assayed. The difference between the amounts of herbicide in the 0.01M $CaCl_2$ solution before and after shaking was the amount of herbicide adsorbed by soil. At equilibrium, all the 0.01M $CaCl_2$ solution was removed from the centrifuge tube and replaced with a fresh herbicide-free solution of 0.01M $CaCl_2$. Desorption was carried out for 24 hours when the herbicide in the two phases (soil and 0.01M $CaCl_2$ solution) attained equilibrium. The amount of herbicide desorbed from the soil was computed at different concentrations.

To study the effects of natural light on the degradation of glyphosate in soil, [^{14}C] glyphosate herbicide solution was applied to moist soil samples in quartz tubes. Another set of quartz tubes was covered with aluminium foils to shield them from natural light. To 5g of soil in each tube a solution of both non-labeled and labeled [^{14}C] glyphosate in distilled water was applied giving an initial herbicide concentration of 10ppm and radioactivity of 0.25μCi. The tubes were taken to the rooftop of a two-storey building where the average daily temperature was 27°C. At a one-week interval, the soil samples in replicas of three from the quartz tubes were taken and analysed for both extractable and non-extractable residues of glyphosate. The soil from the quartz tube was removed and placed in 40-ml centrifuge tubes and extracted with 20ml of 0.2M KOH by continuously shaking on an orbital shaker for 4 hours. One ml of the extract was mixed with 5 ml of Quick safe A cocktail and radio-assayed.

RESULTS AND DISCUSSION

Figure 1 shows results of mineralization of glyphosate at different concentrations of compost in soil. All the mineralization curves for the soils with different treatments exhibited same patterns. The mineralization curves had only two phases, the initial rapid phase followed by a slow final phase, when the curves attained plateaus. The rapid phase lasted for about 20 days. The initial rapid phase

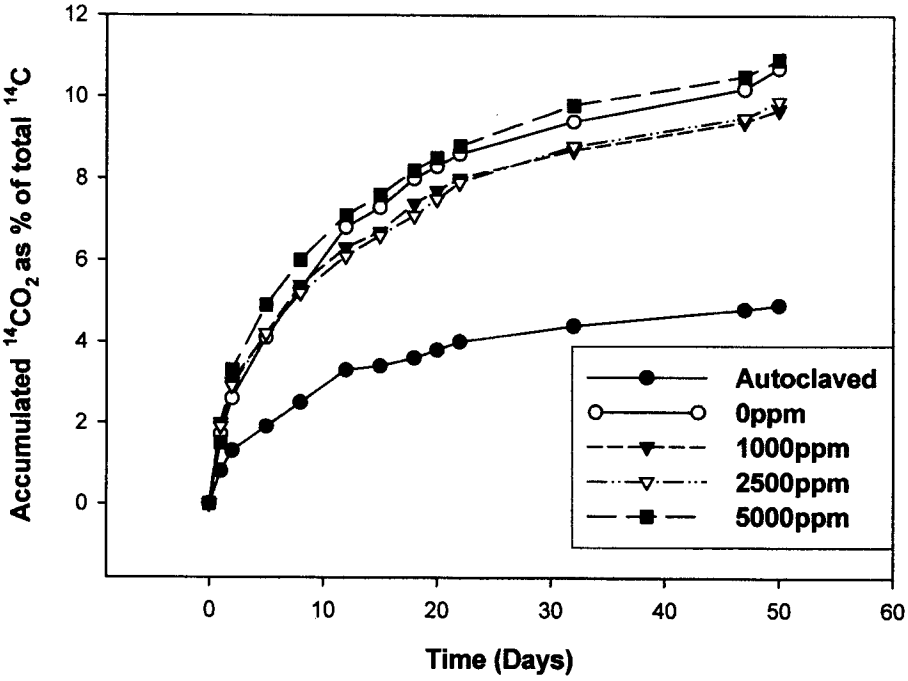

Figure 1. Mineralization of [^{14}C] glyphosate at different compost concentrations in soil.

phase of degradation was attributed to microbial action on the free glyphosate while the slower phase was due to the subsequent attack on the adsorbed glyphosate. Mineralization was suppressed in the autoclaved soil with only 4.9% $^{14}CO_2$ produced. Apparently autoclaving did not completely eliminate microbes but just reduced them. Compost applied to soil at different concentrations did not appear to stimulate the microbial degradation of glyphosate. There was no significant difference between the amounts of $^{14}CO_2$ produced from soil without compost and soils with different concentrations of compost. For instance, after 50 days when the incubation experiment was discontinued only 10.9% of the initial total ^{14}C applied to soil was recovered as $^{14}CO_2$ gas from the soil with compost concentration of 5000ppm while 10.7% was recovered as $^{14}CO_2$ in soil without compost. There was a marginal increase in $^{14}CO_2$ production from 9.7% in soil with compost concentration of 1000ppm to 10.9 ppm in soil with compost concentration of 5000ppm. There was 9.9% of $^{14}CO_2$ produced from soil with compost concentration of 2500ppm. The reduced amount of 4.9% of $^{14}CO_2$ produced from autoclaved soil strongly supports the hypothesis that the $^{14}CO_2$ produced was a result of microbial action on ^{14}C-labeled glyphosate. Absence of lag phase in all the mineralization curves shows that the micro-organisms did not need to adapt to the soil before acting on glyphosate because the herbicide had been used previously in the field from where the soil was taken for this study. The absence of lag phase in the mineralization of glyphosate in soil has also been observed in other studies (Sabine et al.1997; Uan-Boh et al.1998; Haney et al.

2002). The mineralization of glyphosate was very low although the microbes had adapted to conditions in the soil used in this study. The highest mineralization in the 50-day period of incubation was 10.9%, which occurred in soil with compost concentration of 5000ppm. Low mineralization of glyphosate has also been observed in aerobic and anaerobic muck soils where only 14.6 and 9.4% $^{14}CO_2$, respectively, was evolved 60 days after application of the herbicide. This was attributed to the high adsorptive capacity of the herbicide to muck, rendering it inaccessible to microbial metabolism (Uan-Boh et al. 1998). The reason for lack of stimulation of microbes by compost on the mineralization of glyphosate was probably because compost had a high concentration of iron (1272ppm), which increased the adsorption of glyphosate by soil. As a result, adsorption reduced the extent of mineralization of glyphosate. Iron and aluminium in soil have been shown to increase the adsorption of glyphosate by soil (Gerritse et al. 1996). The compost was meant to upset the ratios of carbon, nitrogen and phosphorus elements in soil and therefore, stimulate microbial degradation of glyphosate. Forlani et al. (1999) also observed that there was no increased utilization of glyphosate in conditions where ratios of carbon, nitrogen and phosphorus elements had been increased in soil.

Table 1 below shows the ^{14}C-balance in soil after 50 days of [^{14}C] glyphosate application. The amount of volatile organic ^{14}C-compounds evolved was not quantified because the system used was not capable of doing so. However, other studies showed that the amount of volatile compounds evolved from the mineralization of glyphosate was negligible (Sabine et al. 1997). The results showed that formation of non-extractable residue portion was a biotic degradation process because the lowest amount was formed in the autoclaved soil where the number of microbes was reduced due to autoclaving. It is in the autoclaved soil where the least amount (4.9%) of $^{14}CO_2$ was evolved, which was a biotic process. After 50 days of herbicide application, there was very little extractable residue (<0.5%) while non-extractable residue constituted the highest proportion of glyphosate in soil. This confirmed that glyphosate was highly adsorbed by soil. The mineralization process seemed to have been reduced by the larger proportion of [^{14}C] glyphosate having become non-extractable in the course of incubation. However, the amount of $^{14}CO_2$ evolved during mineralization of glyphosate from soil was not proportionate with the amount of extractable residue in soil. This supports the hypothesis of both non-extractable and extractable residues of [^{14}C] glyphosate having been mineralized to $^{14}CO_2$. This was also inferred from results of adsorption and desorption studies in which an average of 95.7±0.4% of glyphosate was adsorbed by soil from herbicide solution with concentrations ranging from 0.1 to 10ppm. In the desorption studies an average of 1.1±0.2% of the herbicide was desorbed from the adsorbed state in the soil back to solution. The total amount of glyphosate, which was free at any time during adsorption and desorption studies could not exceed 6% of the total herbicide. In photolysis experiment, the non-extractable residue portion of glyphosate, which had accumulated in both exposed and covered soils, decreased later suggesting that even adsorbed glyphosate was mineralized (figure 4). Philip (1998) clearly showed that decomposition of glyphosate was derived from two phases, soluble (labile) and sorbed (non-labile) glyphosate. In studies involving mineralization of

Table 1. ^{14}C-balance in soil after 50 days of [^{14}C] glyphosate application.

Component	Autoclaved soil	compost concentration in soil			
		0ppm	1000ppm	2500ppm	5000ppm
$^{14}CO_2$ evolved(%)	4.9±0.2	10.7±0.7	9.7±0.6	9.9 ±0.4	10.9±0.8
Extract. residue(%)	0.3±0.04	0.3±0.02	0.35±0.05	0.1±0.03	0.15±0.01
Non-extract. residue(%)	47.5±1.7	51.0±2.6	57.0±2.9	57.0 ±3.1	51.0±2.5
Total residues (%)	**52.7±1.94**	**62.0±3.32**	**67.0±3.55**	**65.0 ± 3.43**	**62.0±3.31**

free [^{14}C] glyphosate and of its residues associated with plant material, Sabine et al. (1997) found that soil microflora were able to mineralize both free and fixed glyphosate to same extent. The major difference observed was the lag phase in the mineralization of [^{14}C] glyphosate associated with plant material. Getenga et al. (2000) observed that in the course of mineralization of [^{14}C] malathion, there was an upsurge in $^{14}CO_2$ when the non-extractable residue started decreasing in soil.

Figure 2 shows that the adsorption of glyphosate by soil from solution at different concentrations after equilibrium was linear. The amount of herbicide adsorbed from solution at different concentrations (0.1 to 10ppm) by soil expressed, as percentage of the total initial amount of herbicide in solution was the same irrespective of the concentration of the herbicide solution. On average, 95.7±0.4% of the initial total herbicide in solution was adsorbed by soil irrespective of initial concentration of the herbicide in solution. This behaviour exhibited by glyphosate contrasts sharply with that of atrazine, whose adsorption by soil decreased as concentration of the atrazine in solution increased (*unpublished*). When the data was fitted into the Freundlich adsorption equation, $x/m = K_f C^n_{eq}$, where x/m is the amount of the adsorbed herbicide per unit mass of soil (μg/g), C_{eq} is the equilibrium herbicide solution concentration in solution (μg/ml), K_f and n were determined. Figure 3 represents the linear curve obtained on transforming data on both axes of figure 2 into logarithm form. From the equation of the curve in figure 3, the constants K_f and n were found to be 63 and 1.0387, respectively. The value of n approaches a unit, emphasizing the linearity of the adsorption of glyphosate by soil from solution. Since the concentration range (0.1 to 10ppm) used in the adsorption studies in this experiment was lower than half of the solubility of glyphosate in water (11600μg/ml), the linear relationship between the amount of herbicide adsorbed by soil from solution and the concentration of the herbicide in solution at equilibrium was expected (Brusseau and Rao 1989). The amount of herbicide desorbed back to solution from adsorbed state in soil at equilibrium, was on average 1.1±0.2% of the initial total herbicide in solution. The results showed that once glyphosate was adsorbed by soil it did not readily get desorbed back into solution. The high amount of glyphosate adsorbed could explain why there was little mineralization of glyphosate during mineralization experiments. Sorption experiments conducted on sandy loam with organic content of 3% and coarse sandy soil with organic content of 3.1% yielded K_f values of 78.4 and 59.0,

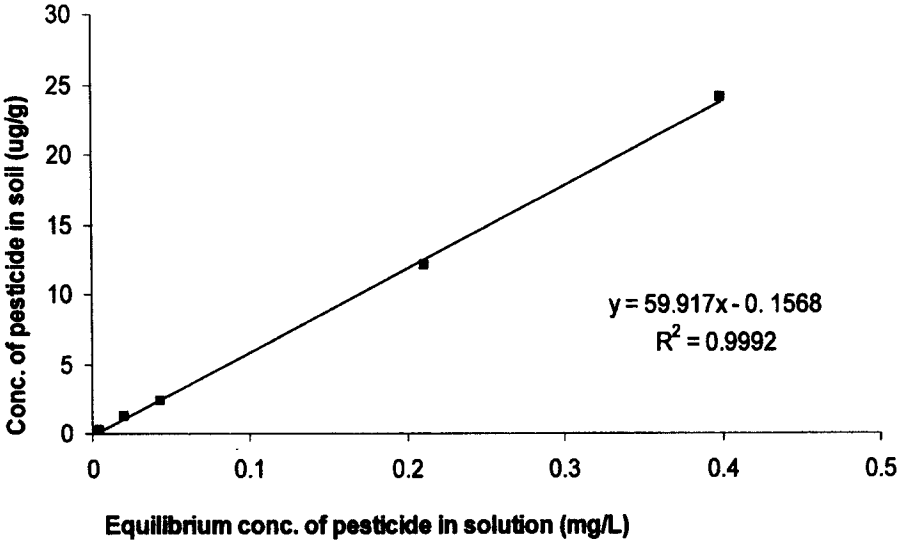

Figure 2. Adsorption curve for glyphosate in non-amended soil

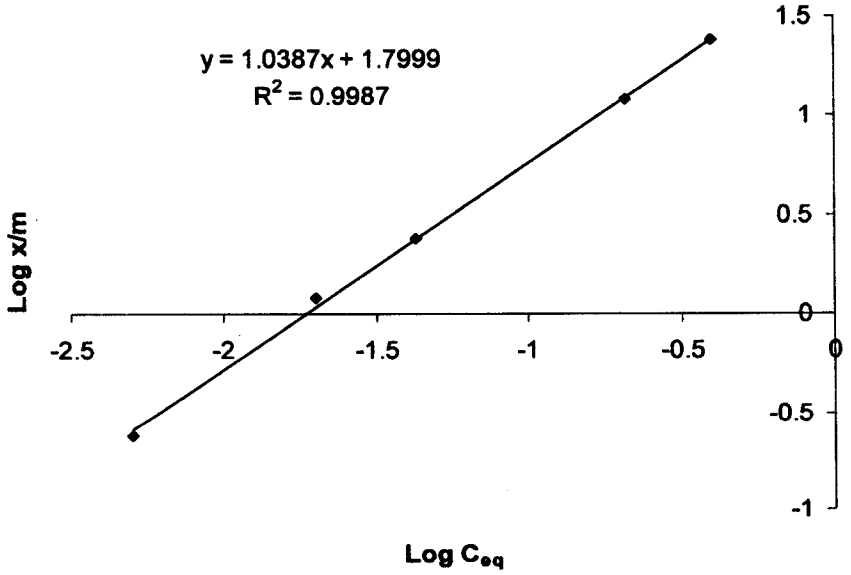

Figure 3. Logarithmic transformation of the data represented in figure 2.

respectively. The corresponding values of n were 0.753 and 0.787, respectively loam, silt loam and sandy loam with carbon contents of 1.56, 1.64 and 1.24% were 76, 56 and 33, respectively (Robert 1987). All these values confirmed that glyphosate is highly adsorbed by soil. Figure 4 shows the effect of natural light on

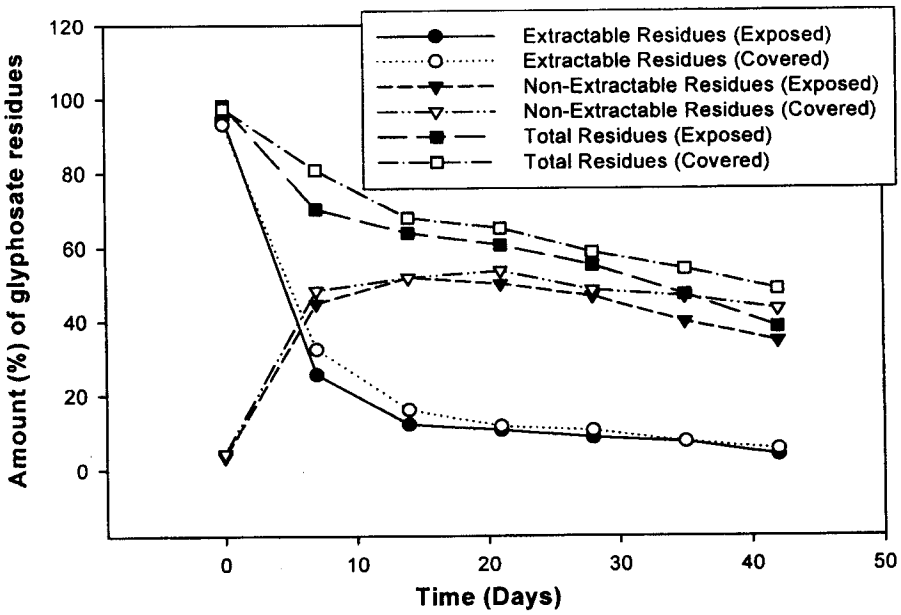

Figure 4. Degradation of glyphosate in soil exposed to natural light and in covered soil.

the rate of degradation of glyphosate. The extractable residue of glyphosate dissipated faster from soil exposed to natural light than from covered soil. After 42 days of herbicide application, there was only 3.8% of the total initial amount of herbicide applied as extractable residue. The corresponding amount of extractable residue of glyphosate in covered soil was 5.4%. Dissipation curves of extractable residue of glyphosate in both soils exhibited same patterns, initial rapid phase followed by slow phase. Non-extractable residue of glyphosate formed in both soils but later decreased faster in the exposed soil than in the covered soil. The non-extractable residue reached the maximum level of 51.7% after 14 days in the exposed soil. In the covered soil the maximum level of 53.5% was attained after 21 days. The non-extractable residue decreased to 34.5% and 43.1% in exposed soil and covered soil, respectively. The dissipation curves for total residues in both soils showed same patterns of dissipation. The dissipation rates of total residues from both soils could be estimated from first order kinetics. This gave half-life periods of dissipation for the total residues from exposed and covered soils of 85.6 and 103.5 days, respectively. The decrease in non-extractable residue, which initially built up in the two soils could be attributed to the mineralization process. The results in figure 4 confirmed the assertion that both non-extractable and extractable forms of glyphosate were mineralized during the mineralization studies in biometer flasks. The lower value of half-life (85.6 days) for glyphosate dissipation from exposed soil than from covered soil is attributed to the photodegradation initiated by natural light. At the ambient temperature of 27°C, the physico-chemical processes also increased the rate of dissipation of glyphosate. The difference between the rates of dissipation in exposed soil and in

covered soil is not very big considering the role played by natural light in the degradation of glyphosate. The presence of soil particles could have intercepted the UV-light responsible for photodegradation of glyphosate thus reducing the impact of photodegradation on glyphosate (Kare and Hakon 1986).

The study showed that compost did not stimulate intense mineralization of glyphosate by microbes. Although the microbes had adapted to the soil conditions in the soil used in the study prior to incubation experiment, the microbes did not mineralize glyphosate satisfactorily due to high adsorption of the herbicide by soil. However, the non-extractable form of glyphosate was mineralized, albeit slowly. In the initial period of glyphosate application, when it was on the surface of the soil, the herbicide was more subject to photochemical decomposition than later when it had been adsorbed by soil.

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