

## Glyphosate Degradation in Malaysian Sandy Loam Soil Amended with Cow Dung or Rice Husk Ash as Influenced by Soil Moisture Content

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### ABSTRACT

This study investigated the effect of cow dung or rice husk ash amendment on glyphosate degradation in a Malaysian sandy loam soil under three moisture regimes. The control and soils amended with 10 tons ha<sup>-1</sup> of either cow dung or rice husk ash were spiked with 5000 µg g<sup>-1</sup> of glyphosate. Water was added accordingly to obtain soil moisture content equivalent to either a submerged condition, field capacity or permanent wilting point. Glyphosate degradation was monitored for 65 days and the data obtained was fitted to first order-double exponential decay model (FODED). The results revealed that glyphosate degradation occurred in two-phases; an initial high degradation rate followed by a slow rate representing degradation of labile and non-labile phases, respectively. The rate constants of the labile phases ranged from 0.0063 to 0.0604 µg day<sup>-1</sup> while those of non-labile phases were from 0.0077 to 0.0732 µg day<sup>-1</sup>. The degradation rate was generally higher in the labile phase. Irrespective of moisture content, the degradation data from the cow dung-amended soil fitted the FODED model best (0.042 ≥ r<sup>2</sup> ≤ 0.909) followed by the rice husk ash-amended soil (0.023 ≥ r<sup>2</sup> ≤ 0.914) and the control (0.030 ≥ r<sup>2</sup> ≤ 0.756). Meanwhile, irrespective of the amendments, soils maintained at field capacity had the highest degradation rate in labile phases (k<sub>1</sub> = 0.0371 – 0.0604 µg day<sup>-1</sup>) while at non-labile phases, soils maintained at permanent wilting point recorded the highest rate (k<sub>2</sub> = 0.0077 – 0.0732 µg day<sup>-1</sup>). The soils maintained at field capacity generally had the lowest glyphosate half-life (11- 42 days) followed by soils at permanent wilting point (9 – 110 days), with the longest half-life being shown by the submerged soils (13-178 days). It can be concluded that the application of cow dung or rice husk ash increased glyphosate degradation in the soil especially when the soil moisture content was maintained at field capacity.

**Key words:** glyphosate, sandy loam, degradation, agricultural waste, soil moisture

### INTRODUCTION

Generally organic manure still remains the most affordable fertilizer supplement for our soils. Meanwhile, drudgery in hand weeding necessitates the application of herbicides for weed control even among the smallholder farmers. The benefits of applying organic manure includes enhancement of soil biological activities and an increase in soil organic matter which result in improved nutrient mobilization and availability, decomposition of toxic substances, increased soil aggregate stability and water retention (Kala *et al.* 2011).

Glyphosate is a common weed killer applied to agricultural land. It is absorbed across the foliage and is trans-located throughout the plant via cuticular wax. It inhibits the shikimic pathway occurring in plants which is responsible for synthesis of aromatic amino acid and consequent plant death (Krüger *et al.* 2013). Glyphosate gets into the soil usually through spray drift and then undergoes adsorption, degradation and leaching processes. Degradation of glyphosate in soil is achieved by different native strains of bacteria and fungi and this occurs through sarcosine and aminomethylphosphonic (AMPA) pathways (Arfarita *et al.* 2013; Sviridov *et al.* 2015). Soil organic matter and moisture are some of the factors affecting pesticide degradation in soil (Shahgholi and Ahangar 2014). An increase in glyphosate mineralisation has been reported in soils as a result of incorporating soybean and corn residues (Rampoldi *et al.* 2008), with a higher mineralisation rate detected in soil applied with fresh residues of soybean. Glyphosate degradation was also found to significantly increase with increasing moisture content in loess soil (Bento *et al.* 2016).

Peninsula Malaysia has a high mean annual rainfall of 2440 mm (Tan 2018) which is spatially distributed across the land resulting in the delineation of the country into eight different rainfall regions (Wong *et al.* 2016). This variation of rainfall is associated with topography and seasonal changes of monsoon winds passing over the South China Sea (Camerlengo and Somchit 2000; Wong 2016). It is evident that this variation of rainfall pattern brings about a fluctuation in soil moisture content with consequent effects on soil biochemical functions. Changes in soil moisture affects microbial colony development and their eco-physiological activities and enzyme action (Borowik and Wyszowska 2016). The present study therefore mimics these fluctuations in soil moisture and their effect on glyphosate degradation in soil subsequent to application of two different agricultural residues. Thus, the objective of this study was to evaluate the influence of added organic residues and variations in soil moisture content on glyphosate degradation.

## **MATERIALS AND METHODS**

### *Sampling of Soil, Cow Dung and Rice Husk Ash*

The soil sampling site was Sementa Hulu (Lat. 3.841663 °N, Long. 101.947251 °E), Raub district, Pahang Malaysia. Surface soils (0-20 cm) were collected from different locations in the sampling site and later bulked to one composite sample. Cow dung was collected from the animal section of the experimental farm, Faculty of Agriculture, Universiti Putra Malaysia, while rice husk ash was obtained from BERNAS Rice Mill Selangor, Malaysia (Lat. 3°40'32.4"N, Long. 100°59'42.5"E). All the samples were properly dried at the drying room, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. The soils and cow dung were ground using laboratory pestle and mortar followed by sieving of the soils with a 2-mm sieve while cow dung and rice husk ash were sieved at 1 mm. The sieved soils and organic materials were stored in a clean container before the degradation study. As the soil had been previously characterized (Garba *et al.* 2019b) and shown to contain 74.17 % sand, 5.83 % silt and 20 % clay, it was classified as sandy loam. Further, the soil had pH of 6.73, EC of 0.024 ds m<sup>-1</sup>, CEC of 12.67 cmol<sub>(+)</sub> kg<sup>-1</sup> and N, P and K to be 0.16%, 0.70% and 0.33%, respectively. Our previous article (Garba *et al.* 2019a) had reported both the physical and chemical properties of cow dung and rice husk ash. Cow dung was reported to have a pH of 8.14, EC of 0.22 ds m<sup>-1</sup>, CEC of 34.50 cmol<sub>(+)</sub> kg<sup>-1</sup> and N, P and K of 2.53%, 0.22% and 0.92%, respectively. Meanwhile rice husk ash was shown to have a pH of 9.95, EC of 0.33 ds m<sup>-1</sup>, CEC of 10.20 cmol<sub>(+)</sub> kg<sup>-1</sup> with P and K of 0.17% and 0.19% respectively with N not being detected in rice husk ash.

### Glyphosate Degradation Study

Three were carried out in triplicate. The mixture of soils and organic materials was homogenised through sets of 250 g of the 2-mm sieved soil were weighed into plastic containers (11 cm x 9 cm) to which was added 1.15 g of either cow dung or rice husk ash to give an equivalent rate of 10 tons ha<sup>-1</sup>. Equal amounts of soils without cow dung or rice husk ash served as control. All treatments repeated stirring with a stainless steel rod. Both control and amended soils were added with a millipore<sup>®</sup> of water appropriate for obtaining moisture content of submerged condition, field capacity and permanent wilting point, representing very high, optimal and very low moisture levels. All the treatments were spiked with 25 mL of 200 mg L<sup>-1</sup> of standard glyphosate solution and arranged in completely randomized design on a laboratory bench at 23<sup>0</sup>C. The soils were weighed regularly to adjust the moisture content. The residual concentration of glyphosate was measured at days 1, 3, 13, 23, 34, 44, 55 and 65. On each of these days, 5g of soil from each treatment was weighed into centrifuge tubes to which was added 20 mL 0.01 M KH<sub>2</sub>PO<sub>4</sub> solution and shaken for 2 h on a rotary shaker at 100 rpm. This was followed by centrifugation at 10,000 rpm for 10 min and filtering of the supernatant with 0.45µm syringe filter. Later, 1 mL of the filtrate was derivatized and analysed for glyphosate using HPLC-UV as described in Garba *et al.* (2018).

### Data Analysis

In order to investigate the kinetics of glyphosate degradation from the studied soils, the residual concentration data obtained from the day intervals were fitted to first-order double exponential decay model (FODED) as reported by Sarmah and Close :

$$X(t) = X_{1(t)} + X_{2(t)} \quad (1)$$

$$= X_{sol} \exp^{-k_1 t} + X_{sorb} \exp^{-k_2 t} \quad (2)$$

where  $X_{sol}$  and  $X_{sorb}$  are concentrations in solution and sorbed phases,  $t$  is the time (days),  $k_1$  and  $k_2$  are degradation rate constants at solution and sorbed phase, respectively. The half-life ( $t_{1/2}$ ) of glyphosate from the studied soil was calculated using the equation below;

$$t_{1/2} = \frac{\ln 2}{k} \quad (3)$$

The mean values of glyphosate residual concentration from both control and amended soils at different moisture regimes were subjected to analysis of variance at 0.05 confidence level using SAS 9.4. The significant means were compared using Tukey's HSD test.

## RESULTS AND DISCUSSION

Glyphosate degradation rate from control and treatment soils during the incubation period (*Figure 1*) shows two phases: initial and final phase with a high rate of glyphosate degradation in the final phase. This could be attributed to glyphosate adsorption by this soil. However, as weaker bonds exist between glyphosate and adsorption sites of the soil, it may likely desorb into soil solution and undergo degradation. Glyphosate adsorbed to soil colloids through H bonding (Piccolo and Celano 1994) and porous diffusion (Herath *et al.* 2016), all of which are weaker bonds and could result in its desorption and bioavailability. The initial phase is ascribed to microbial degradation of free glyphosate available in soil solutions while the final phase is due to microbial attacks on adsorbed glyphosate.

Degradation and adsorption are interrelated processes that determine the fate of glyphosate in soils. Our adsorption study found the studied soils to have 86 % glyphosate adsorption efficiency, and a higher adsorption efficiency of 87 % and 90 % with the application of cow dung and rice husk ash, respectively.

Similarly results from the desorption study found that glyphosate desorbed from both the control and amended soils, with the desorption efficiency for control, soil + cow dung and soil + rice husk ash being, 14 %, 10 % and 8 %, respectively. Therefore, the initial phase of glyphosate degradation observed in the present study can be said to be responsible for the decay of the small amount of the instant desorbed glyphosate molecules. However, the larger molecules adsorbed by the soil tend to be released over time into the soil solution, hence serving as the compounds of the final phase of degradation. It can therefore be postulated that as the pool of glyphosate in the labile phase depletes due to degradation, changes in the soil condition will lead to its replenishment from the sorbed phase. Microbial mineralization is the predominant mechanism of glyphosate transformation in soils which is non-specific and occurs through co-metabolic process (Accinelli *et al.* 2005; Sprankle *et al.* 1975a) mediated by both native and introduced strains of bacteria and fungi (Sprankle *et al.* 1975b) with the resultant products being inorganic phosphorus, CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup>. The AMPA pathway is more commonly reported for glyphosate degradation compared to that of sarcosine (Franz *et al.* 1997; Giesy *et al.* 2000). This pathway involves glyphosate molecules being attacked by oxidoreductase (*Figure 2*) leading to the cleavage of glyphosate C-N bond yielding to AMPA and glyoxylate. Being an energy substrate, glyoxylate will be mineralized in the Krebs cycle yielding CO<sub>2</sub> as end products while AMPA will be exported to extracellular spaces which will further be transformed to methylamine and inorganic P through cleaving of its C-P bonds by C-P lyase enzymes ( Sviridov *et al.* 2015; Giesy *et al.* 2000; Franz *et al.* 1997)

This result is in conformity of with the study of Eberbach(1998) who studied glyphosate degradation in four Victorian soils: clay, loamy sand and two silt clay loam soils. This study reported two sources of glyphosate in these soils: labile and non-labile phases. Over the first 40 days of the study, degradation of glyphosate was from labile and non-labile phases while after the first 40 days, glyphosate was only from the non-labile phase.

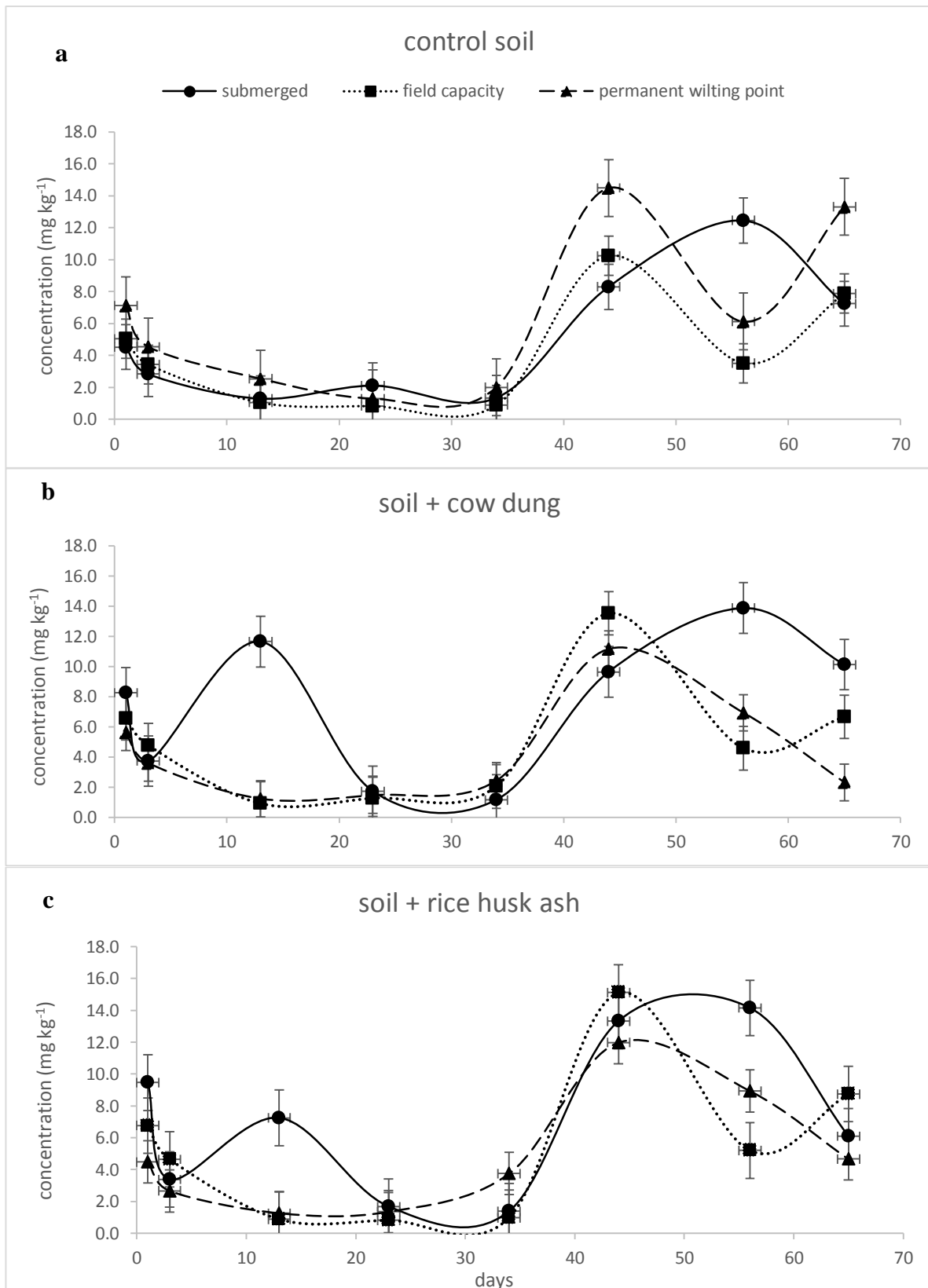


Figure 1. Rate of glyphosate degradation in (a.) Control, (b.) Soil amended with cow dung and (c.) Soil amended with rice husk ash under different moisture regimes

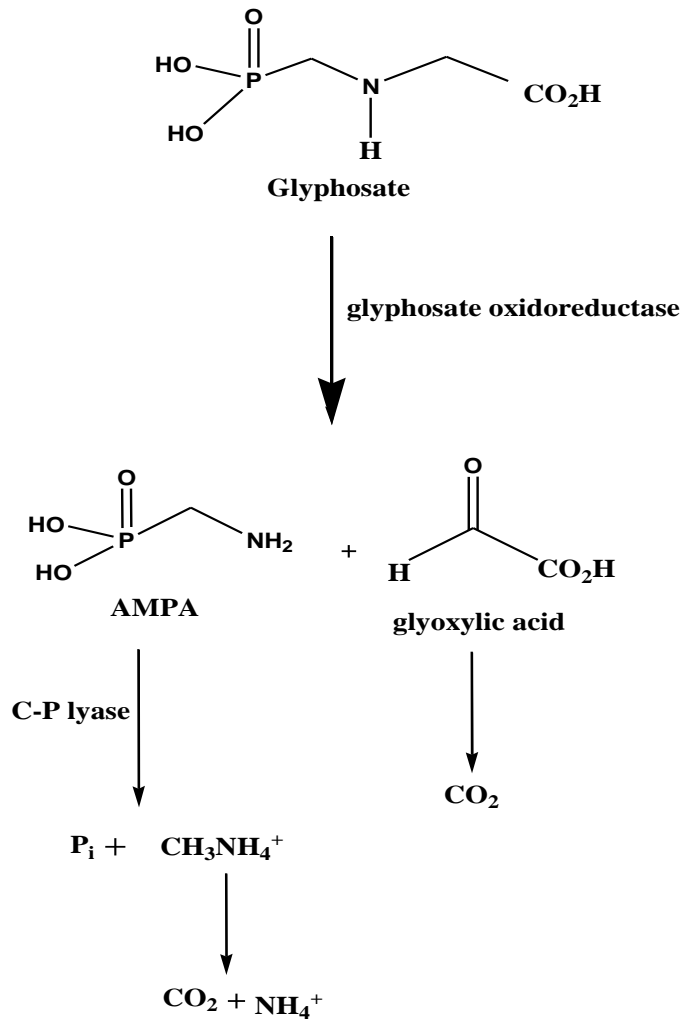


Figure 2. The AMPA pathway of glyphosate degradation in soil.

A study of glyphosate degradation for 60 days using <sup>14</sup>C-glyphosate on Malaysian soils showed slow and rapid glyphosate degradation from muck and sandy loam soil, respectively (Cheah *et al.* 1998). The authors reported <sup>14</sup>CO<sub>2</sub> evolution of 14.61% and 9.4% for aerobic and anaerobic muck soils 60 days after treatment, and attributed this to high adsorption capacity of the muck soil. Meanwhile a rapid degradation in the non-sterilized aerobic sandy loam (89.97%) was attributed to microbial processes. Also, the initial rapid degradation rate observed was followed by a slower rate which commenced approximately 30 days after treatment indicating microbial action on the free glyphosate and subsequent attack on the adsorbed glyphosate, respectively.

Table 1 shows the rate constant, R<sup>2</sup> and calculated half-lives of the degradation of glyphosate from different moisture regimes in the studied soils. The best fit data to FODED model (R<sup>2</sup> = 0.914) was the non-labile glyphosate degradation at moisture condition of permanent wilting point in rice husk ash-amended soil while the poorly fit data was that of the non-labile phase of rice husk ash-amended soil (R<sup>2</sup> = 0.023). The calculated half-life from the different moisture levels at both labile and non-labile phases show that at condition of field capacity, glyphosate had a half-life range of 11-42 days in both control and amended soils. The submerged moisture regime in control, cow dung or rice husk ash amended soils had glyphosate half-life of 25, 13 and 14 days, respectively, at labile phase while the half-life at the non-labile phase was 161, 178 and 20 days respectively. Meanwhile, at permanent wilting point, the half-life for glyphosate degradation in the labile phase for control and soil amended with cow dung and rice husk ash was 17, 28 and 110 days, respectively while at the non-labile phase, it was 90, 9 and 16 days, respectively.

TABLE 1  
Rate constant describing glyphosate degradation, R<sup>2</sup> and calculated half-lives in control and amended soils at different moisture regimes

Soil	Moisture regimes	Phase	k (µg day <sup>-1</sup> )	R <sup>2</sup>	Half-life (day)
Control	Submerged	Labile	0.0282	0.557	25
		Non-labile	0.0043	0.026	161
	Field capacity	Labile	0.0526	0.756	13
		Non-labile	0.0167	0.099	42
	Permanent wilting point	Labile	0.0404	0.698	17
		Non-labile	0.0077	0.030	90
Soil + cow dung	Submerged	Labile	0.0547	0.586	13
		Non-labile	0.0039	0.042	178
	Field capacity	Labile	0.0371	0.371	19
		Non-labile	0.0368	0.498	19
	Permanent wilting point	Labile	0.0252	0.303	28
		Non-labile	0.0732	0.909	9
Soil + rice husk ash	Submerged	Labile	0.0501	0.660	14
		Non-labile	0.0349	0.614	20
	Field capacity	Labile	0.0604	0.671	11
		Non-labile	0.0295	0.338	23
	Permanent wilting point	Labile	0.0063	0.023	110
		Non-labile	1 0.0438	0.914	16

The overall results from both soils show that the condition of field capacity generally had low half-life for glyphosate degradation with narrow variation between labile and non-labile phases but the half-life varied widely between labile and non-labile phases in submerged and permanent wilting points. This suggests strong binding forces retain glyphosate molecules at permanent wilting point but with increasing moisture, the water molecules weaken the forces and make available the glyphosate in soil solution, hence its rapid degradation. However, as anaerobic conditions prevail at submerged conditions, it leads to a low rate of oxygen diffusion and consequently retards degradation (Topp *et al.* 1997). Moisture greatly affects soil microbial community and activity as well as enzyme function. Dry soils have a higher water potential which decreases its soil microbial function (Geisseler *et al.* 2011) but increasing moisture causes a decrease in soil water potential and increasing microbial activity. Meanwhile, at higher moisture levels as in flooded rice paddies, the decrease in soil oxygen diffusion rate will also lower the soil redox potential and consequently lead to low microbial activity (Wolińska and Bennicelli 2010).

Our study results therefore demonstrated that when glyphosate is applied to these soils in flooded conditions or in a very low moisture regimes, it will be retained for a longer period before degradation. However, in a condition of optimum soil moisture, timely glyphosate degradation will be achieved thereby remediating its toxicity effect. Our study results concur with a study by Kanissery *et al.* (2015) where three different types of glyphosate-spiked Mollisols of Illinois were incubated under aerobic and anaerobic conditions. The authors reported lower half-life values of glyphosate for aerobic conditions compared to anaerobic conditions. Similarly, a more rapid glyphosate degradation was reported under optimum soil water conditions by Aslam *et al.* (2015). The results of an earlier study by Rueppel *et al.* (1977) also found increased glyphosate degradation in soil under aerobic conditions compared to anaerobic conditions.

Table 2 shows an increase in glyphosate residual concentration compared to control under the moisture regimes in the order of permanent wilting point > submerged > field capacity but the trends in soils with cow dung and rice husk ash was in the order of submerged > field capacity > permanent wilting point. Compared to control, application of cow dung or rice husk ash increased glyphosate residue at field capacity and in submerged conditions but at permanent wilting point, there was a decrease in glyphosate residue. This indicates that application of cow dung or rice husk ash limit glyphosate degradation with increasing moisture in the studied soils which might be due to changes in mineralogical and surface properties of the soils affecting the bioavailability and degradation of this herbicide. Schroll *et al.* (2006) observed an increase in glyphosate degradation when comparing air dry condition to very low moisture content on loamy soils; however, mineralization was found to be considerably reduced as the moisture approached water holding capacity of the soils. Moisture controls redox condition and solubility of organic matter in the soil. Increasing moisture will lead to reduction of Fe from the oxide mineral (Kanissery *et al.* 2015) and this results in glyphosate bioavailability through its release into the soil solution. Likewise, a fluctuation in soil moisture alters microbial activity and solubility of soil organic matter which controls the release of glyphosate adsorbed to soil organic colloids. Glyphosate is generally considered to be easily degraded in soil, but its persistence varies widely, with its half-life ranging between 1 and 197 (Bento *et al.* 2016). Soil microbes and adsorption greatly affect glyphosate persistence in soils (Erban *et al.* 2018). As glyphosate serves as C, N and P source to soil bacteria and fungi, it is easily degraded by different strains of these organisms. However, due to its effect on the shikimic pathway in both plants and in these microbes, the presence of glyphosate in soil can affect growth and function of the microbes and its consequent persistence. Of equal importance, glyphosate complexes divalent cations in soil,



binds soil organic matter and strongly adsorbs to oxides of Fe and Al. Therefore application of this residue increases the content of organic matter and enhances glyphosate persistence. The mode of action of glyphosate is inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, resulting in the depletion of essential aromatic amino acids needed for plant and microbial growth (Haney *et al.* 2000). Consequently, glyphosate inhibits nitrogen fixation, growth of mycorrhizal fungi and is toxic to earthworms (Giesy *et al.* 2000).

Table 2  
Glyphosate residual concentration among treatments in studied soils at different moisture regimes

Moisture regime	Soil (mg kg <sup>-1</sup> )		
	Control	Soil + cow dung	Soil + rice husk ash
Permanent wilting point	6.434 <sup>abc</sup> ±1.03	4.335 <sup>c</sup> ±0.67	4.890 <sup>bc</sup> ±0.79
Field capacity	4.115 <sup>c</sup> ±0.70	5.040 <sup>bc</sup> ±0.81	5.394 <sup>abc</sup> ±0.98
Submerged	5.018 <sup>bc</sup> ±0.82	7.516 <sup>a</sup> ±1.15	7.092 <sup>ab</sup> ±1.05

Means with the same letter are not statistically different at  $p > 0.05$ . ( $n = 3$ ,  $\pm SE$ )

## CONCLUSION

Our study results confirmed the role of moisture and added residue on glyphosate solubility, bioavailability and subsequent degradation. Water acts as a solvent for herbicide dilution, movements and diffusion besides being essential for microbial functioning. On the other hand, application of cow dung and rice husk ash increased C, nutrient and organic matter content of the studied soils, thereby, stimulating microbial activity and subsequent glyphosate degradation. Therefore, it can be suggested that rate of glyphosate degradation is generally controlled by fluctuating soil moisture level. Depending on soil organic matter content, glyphosate degradation tends to increase with increasing soil moisture content. However, in conditions of very high moisture as in rice paddies, the low rate of oxygen diffusion results in an anaerobic condition which can retard glyphosate degradation.

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