Degradation of Pesticides in Biobeds

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Abstract

Pesticides are used to protect crops from unwanted pests thereby increasing food quality and quantity. However, one side-effect of using pesticides is their ability to pollute surface and groundwaters through diffuse (non-localized) or localized (point) sources. Biobeds were introduced in Sweden in 1993 as a means to protect the environment from point source pollution by pesticides arising from farm activities such as filling of sprayers and sprayer rinsate. A biobed is a hole in the ground into which a mixture of straw, compost and topsoil (2:1:1 by volume) is added and cover with a grass layer. The biobed mix creates a favourable environment for containment and microbial degradation of applied pesticides. The objective of this study is to investigate the relationship between active ingredient breakdown and carbon dioxide emission in both topsoil and biobed mix after pesticide application. Results indicate a five-fold reduction in the half-life of 2,4-D in the biobed mix compared to topsoil. Rapid degradation of some sulphonylurea herbicides occurred in the biobed mix despite their known persistence in soil. There was a correlation between active ingredient breakdown and carbon dioxide emission.

Introduction

The contamination of surface and groundwaters by pesticides is a threat to both humans and the environment. A study conducted across the Northern Great Plains of Canada and the United States found 2 insecticides and 27 herbicides from 15 reservoirs sampled (Donald et al., 2007). This is a major concern particularly in the Canadian Prairies, where there are over 100,000 farm dugout (ponds) use by rural residents as potable and household water, livestock watering and irrigation (Cessna and Elliott, 2004). Biobeds offer an alternative for the treatment of waste pesticide arising from internal and external sprayer cleaning. The biobed composition is a major factor in its functioning. The soil acts as a source of pesticide-degrading microorganisms, while compost offers adsorption sites, maintains aerobic conditions and regulates the moisture inside the biobed. Straw serves as the main carbon source for lignin-degrading microorganisms, which are believed to produce ligninolytic enzymes capable of degrading a broad spectrum of pesticides (Spanoghe et al., 2009, Castillo et al., 1997). The grass layer helps to regulate the moisture of the biobed by creating an upward transport of water and can also produce root exudates (for example peroxidases) to support co-metabolic processes, especially in the upper part of the biobed where most of the pesticides are retained and degraded (Castillo et al., 2008b). The use of the term pesticide in this report refers to synthetic

organic plant protection products such as herbicides, insecticides, fungicides, rodenticides, etc.

Materials and methods

Degradation studies

Formulated herbicides containing the active ingredients of 2,4-dichlorophenoxy-acetic acid (2,4-D amine salt), metsulfuron, tribenuron and thifensulfuron were used in these studies. In the first experiment, 2,4-D was applied in single and multiple applications. For single application experiment, 18.2 mg active ingredient (a.i) and 35 g dry weight (DW) of both biobed mix and topsoil was used. In the multiple application experiment, both biobed mix and topsoil (50g DW) were spiked five times with 26 mg a.i per application. During the incubation period (66 days) a total of 130 mg a.i 2,4-D was applied to the biobed mix and topsoil. After the initial application of 2,4-D, subsequent applications were based on the CO_2 level in the biobed mix. Once the CO_2 level returned to the established baseline, more active ingredient of 2,4-D was added. In the second experiment, 18.2 mg a.i of each of the four herbicides (2,4-D, metsulfuron, tribenuron and thifensulfuron) was used to spike 35g DW of biobed mix. In experiments 1 and 2, 1 L Manson jars with a 25 mL beaker glued to the inside wall were used as bioreactors. The beaker served as a reservoir for 10 mL of 0.2 M potassium hydroxide (KOH) to trap carbon dioxide produced during incubation. Every second day, the KOH was removed and replaced. 1 mL of 1 M of barium chloride was added to the exposed KOH to precipitate the carbonate and a drop of phenolphthalein indicator added. The mixture was then titrated with 0.1 M hydrochloric acid (HCl) and the end-point determined by colour change from orange to colourless. The daily CO_2 production was calculated using equation 1.

$$\mu g\ CO_2\ \text{evolved/g sample/day} = (((B-V)*N*E)*1000\mu g/mg)/35g\ \text{sample/2 days.} \quad \text{…...eq.1}$$

Where:
B = Titre (mL) required to reach blank endpoint
V = Titre (mL) required to reach treatment endpoint
N = Normality of the HCl titrant
E = 22 (equivalent weight of CO_2)

Moisture in the biobed mix or topsoil was maintained at 70% water holding capacity (WHC). In the single application experiment, bioreactors were incubated at 20^0C in the dark; in the multiple application experiment, bioreactors were incubated at 15^0C in the dark. In the single application experiment, destructive sampling was carried out at 1, 3, 5 10 and 28 days after 2,4-D addition for biobed mix while for soil, sampling was at 10 and 28 days. Sampling of soil at 10 and 28 days was as a result of a previous study (data not shown) that indicated no active ingredient degradation in soil within the first 10 days of 2,4-D addition. Samples were immediately frozen at -20^0C after each sampling period prior to extraction. In multiple application experiment, destructive sampling was done at the end of the incubation period (66 days). Herbicide residues were extracted from

topsoil and biobed mix (35g dw single application and 50g dw multiple applications) by shaking for 1h with extraction solvent (water and acetonitrile, 21:79 v/v) and analysed using liquid chromatography/ mass spectrometry (LC/MSMS).

Results

Generally, the biobed mix emitted more carbon dioxide as compared to topsoil for both single and multiple applications (Figures 1 and 2). There was a lag in CO_2 emission after the initial application in the biobed mix in both the single and multiple applications. This lag phase could be attributed to acclimatization or selection of microorganisms capable of degrading 2,4-D. However, subsequent applications (multiple applications) to the same substrate saw an immediate release of CO_2 (Figure 2) suggesting a build-up of 2,4-D degrading microorganisms after the initial application. In topsoil there as a gradual increase in CO_2 emission, however, a sharp increase was only noticed after the fourth application again suggesting slow but gradual build up of 2,4-D degraders (Figure 2).

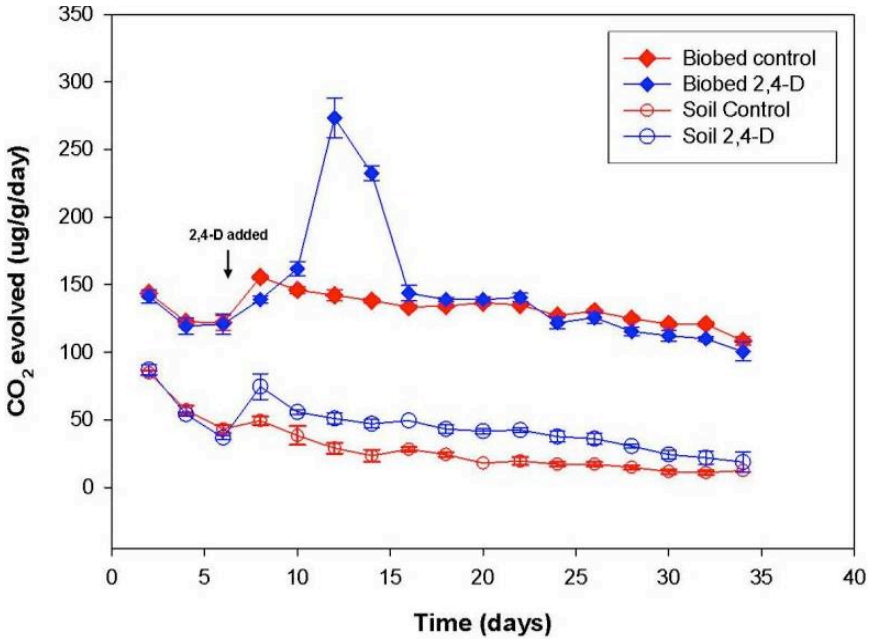

Figure 1. Carbon dioxide evolved from biobed mix and topsoil after a single application of 2,4-D (Error bars are ± standard deviation of the mean of 4 replicates).

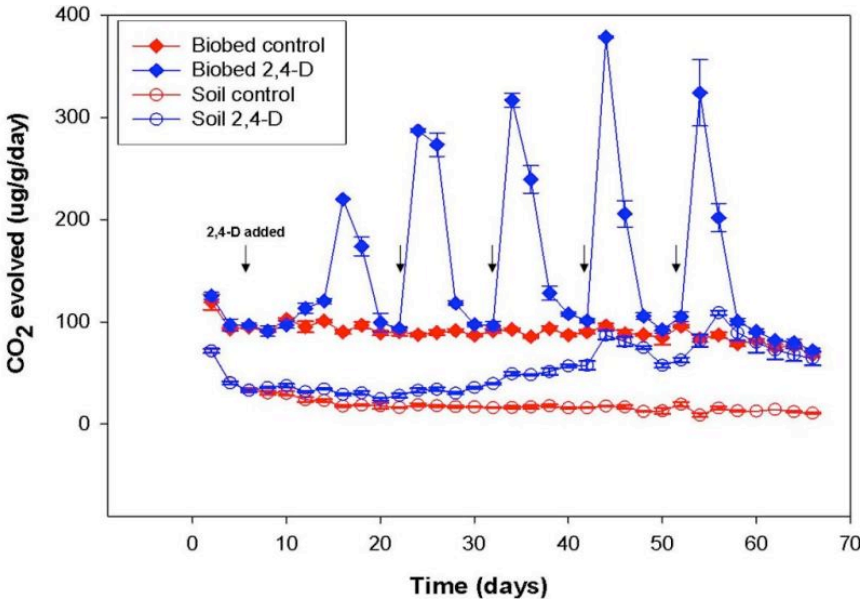

Figure 2.Carbon dioxide evolved from biobed mix and topsoil after multiple applications of 2,4-D. Arrows in the figure indicate the addition of 2,4-D (Error bars are ± standard deviation of the mean of with 4 replicates).

Analysis of herbicide residues indicated no degradation of 2,4-D in the biobed mix from day 1 to 3 after application (Figure 3). This was not surprising as the amount of daily CO_2 produced during this period was close to the control. On the fifth day after 2,4-D application, there was a surge in CO_2 produced (Figure 1). Residue analysis confirmed some breakdown of the active ingredient (Figure 3). When the CO_2 level returned to the baseline (10 days) after application, more than 99% of applied active ingredient of 2,4-D was degraded (Figure 3). In topsoil, there was about 10% and 30% degradation at 10 and 28 days, respectively after 2,4-D addition (Figure 4).
For the multiple application experiment, at the end of the incubation period, more than 99.95% of applied active ingredient was degraded in the biobed mix, while only 30% was degraded in topsoil (Table 1).

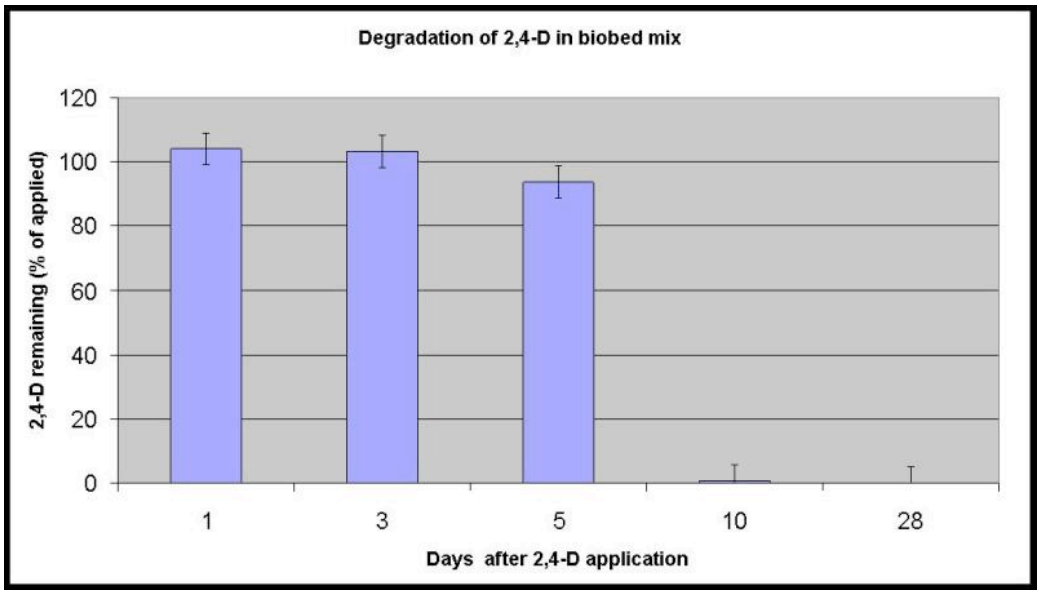

Figure 3. Percentage of 2,4-D degraded in a biobed mix single application (Error bars are ± standard deviation of the mean of 4 replicates).

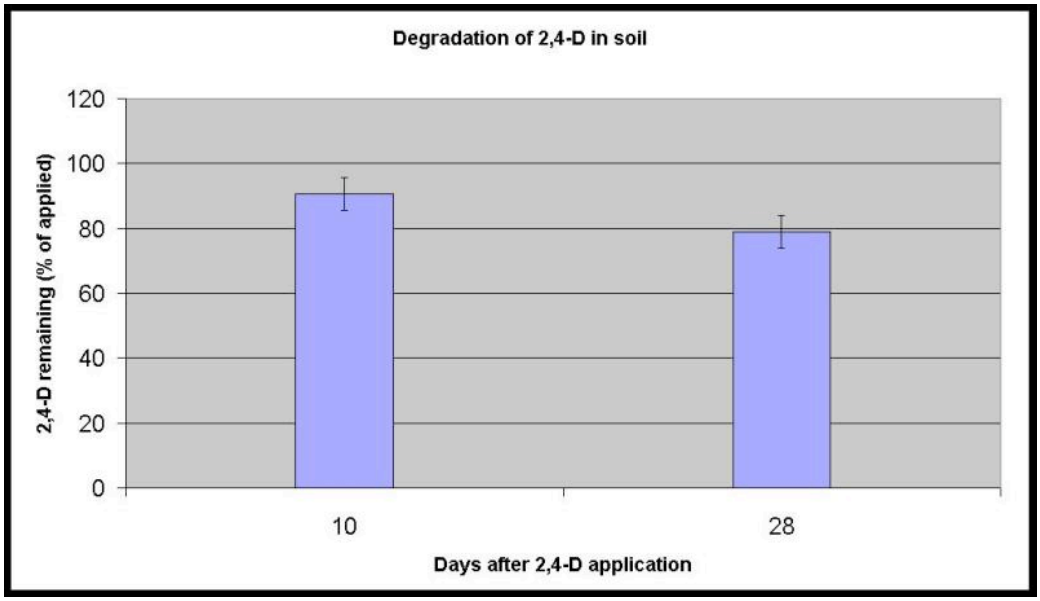

Figure 4. Percentage of 2,4-D degraded in topsoil single application, (Error bars are ± standard deviation of the mean of 4 replicates).

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Table 1. Percentage applied of 2,4-D remaining in the biobed mix and topsoil multiple applications.

Substrate	2,4-D remaining (% applied)
Biobed mix	**0.03**
Soil	**69.70**

In the second experiment, the sulfonylurea herbicides (tribenuron, thifensulfuron and metsufuron) and 2,4-D were monitored for carbon dioxide emission (Figure 5). A similar trend was again noticed with 2,4-D having a CO_2 lag phase (Figure 5). The sulfonylurea herbicides, did not exhibit a lag phase for CO_2 emission (Figure 5). For metsulfuron, there was little CO_2 produced, resulting in about 15% degradation. Metsulfuron is degraded in soil by both microbial organisms and chemical hydrolysis (Bossi et al., 1999, Huang et al., 2007). At low pH chemical hydrolysis dominates while at neutral pH microbial degradation occurs (Bossi et al., 1999, Ismail and Azlizan, 2002). Microbial degradation constitutes only a minor pathway in the degradation of metsulfuron. Hence, the minimal degradation of metsulfuron was expected as the biobed mix was at a pH 7.46 which will favoured microbial degradation.

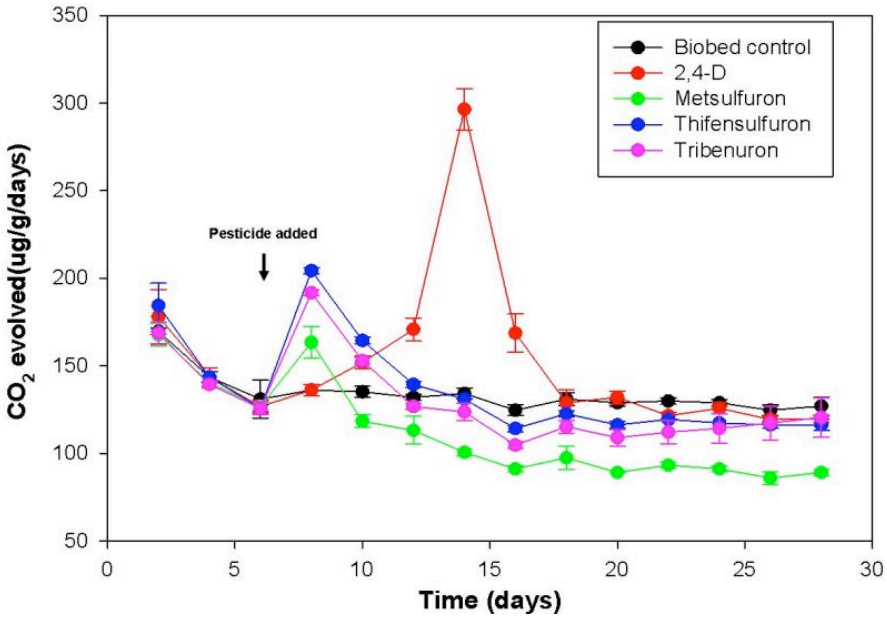

Figure 5. Carbon dioxide evolved from the various herbicides studied during 28 days of incubation at 20^0C. (Error bars are ± standard deviation of the mean of 3 replicates).

Residue data (Figure 6) indicates rapid degradation of 2,4-D, thifensulfuron and tribenuron in a biobed mix after 22 days of incubation. This indicates the biobed mix contains a broad spectrum of microorganisms capable of degrading a wild range of pesticides.

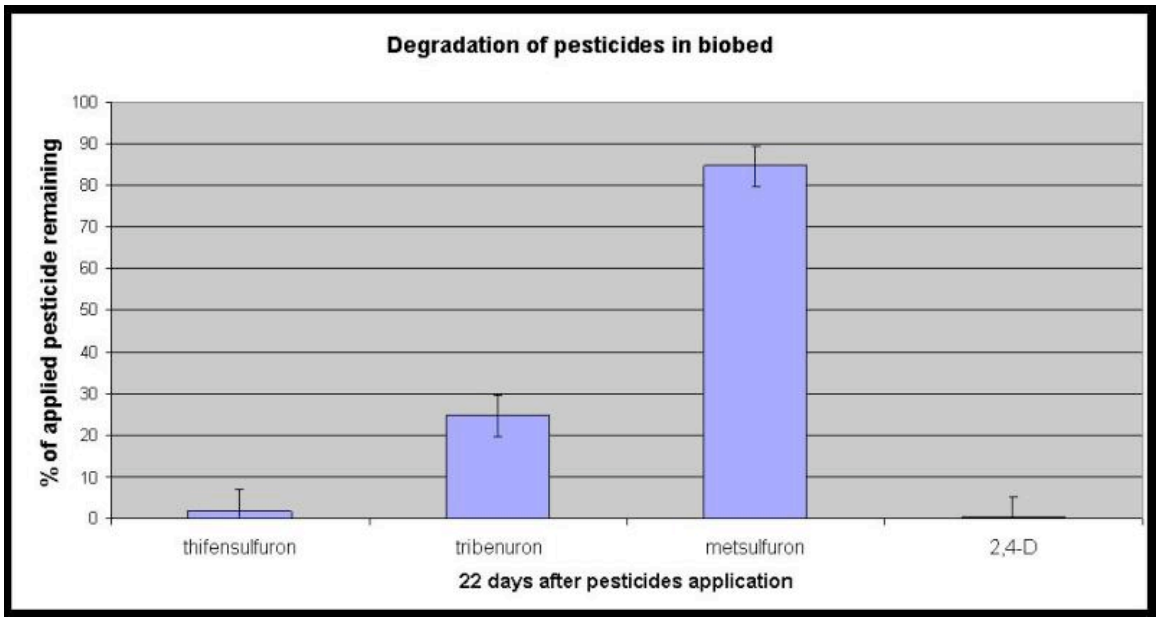

Figure 6 Percentages of applied herbicides remaining after 22 days of incubation at 20^0C. (Error bars are ± standard deviation of the mean of 3 replicates).

Conclusion

More than 99% of applied 2,4-D was degraded within 10 days of application to the biobed mix. Only about 10% of applied 2,4-D was degraded in soil over the same period. The biobed mix degraded tribenuron (75.2%), thifensulfuron (98.08%) and to some extend metsulfuron (15.37%) known to be persistent in soil. Carbon dioxide may be used as an indictor of active ingredient breakdown. Biobeds may be suitable on-farm installation to retain and degrade pesticide spills arising from point sources thereby protecting the environment from contamination.

References:

Bossi, R., Seiden, P., Andersen, S.M., Jacobsen, C.S., and Streibig, J.C. 1999. Analysis of methsulfuron-methyl in soil by liquid chromatography/tandem mass spectrometry. application to a field dissipation study. J.Agric. Food Chem. 47, 4462-4488.

Castillo, M. d. P., and L. Torstensson. 2007. Effect of composition, moisture and temperature on the degradation of pesticides. J. Agric. Food Chem. *55*, 5725–5733

Castillo, M. d. P., Torstensson, L., and Stenstrom, J. 2008b**.** Biobeds for Environmental Protection from Pesticides Uses – A Review. J. Agric. Food Chem. 56, 6206–6219

Cessna, A.J., and Elliott, J.A. 2004. Seasonal variation of herbicide concentration in prairies from farm dugouts. J. Environ. Qual. 33, 302-315

Donald, D.B., Cessna, A. J., Sverko, Ed., and Glozier, N. E. 2007. Pesticides in surface drinking-water supplies of the northern great plains. Environ Health Perspect 115, 1183-1191.

Huang, X., He, J., Sun, J., Pan, J., Sun, X., and Li, S. 2007. Isolation and characterization of a metsulfuron-methyl degrading baaterium *Methylopila* sp. S113. International Biodeterioration & Biodegradation 60, 152-158.

Ismail, B.S., and Azlizan, B.A. 2002. Persistence and bioactivity of metsulfuron-methyl in soils. J. Environ. Sci. Health B37(4), 345-353.

Spanoghe, P., Maes, A., and Steurbaut, W. 2009. Limitation of point source pesticide pollution: Results of bioremediation system. Comm. Appl. Biol. Sci. Ghent University.