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Author(s): M. Sonia Rodríguez Cruz, Julie E. Jones and Gary D.

Bending

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1 **Spatial variability in the biodegradation rate of the herbicide bentazone with soil**
2 **depth assessed using contrasting incubation methods**

3 M.Sonia. Rodriguez-Cruz*[†], Julie E. Jones, Gary D. Bending

4 *Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK*

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6 * Corresponding author. Tel: +34 923 219606; fax: +34 923 219609.

7 *E-mail address: sorocruz@usal.es (M.S. Rodriguez-Cruz).*

8 [†] *Current address: Instituto de Recursos Naturales y Agrobiologia, CSIC, Apdo 257,*
9 *37071 Salamanca, Spain.*

10

11 **Abstract**

12 Vertical and horizontal spatial variability in the biodegradation of the herbicide
13 bentazone was compared in sandy-loam soil from an agricultural field using sieved
14 soil, which represents the method most widely used to investigate biodegradation
15 rates, and intact soil cores. An initial experiment compared degradation at 5 depths
16 between 0 and 80 cm using sieved soil. Degradation was shown to follow first order
17 kinetics, and DT50 declined progressively with soil depth from 56 days at 0-10 cm to
18 520 days at 70-80 cm. DT50 was significantly correlated with organic matter, pH and
19 dehydrogenase. In a subsequent experiment, degradation rate was compared after 127
20 days in sieved soil and intact cores from 0-10 and 50-60 cm depth from 10 locations
21 across a 160 x 90 m portion of the field. Method of incubation significantly affected
22 mean dissipation rate, although there were relatively small differences in the amount
23 of pesticide remaining in intact cores and sieved soil, ranging between 4.6 and 10.6 %.
24 Spatial variability in degradation rate was higher in soil from 0-10 cm depth relative

1 to that from 50-60 cm depth in both sieved soil and intact core assessments. Patterns
2 of spatial variability measured using cores and sieved soil were similar at 50-60 cm,
3 but not at 0-10 cm depth. This could reflect loss of environmental context following
4 processing of sieved soil. In particular, moisture content, which was controlled in
5 sieved soil, was found to be variable in cores, and was significantly correlated with
6 degradation rate in intact topsoil cores from 0-10 cm depth.

7

8 *Keywords:* Biodegradation, spatial variability, bentazone, intact core, sieved soil, soil
9 depth

10

11 **1. Introduction**

12 Management of environmental contamination by pesticides requires
13 understanding of pesticide sorption and degradation processes, which determine
14 pesticide motility through the soil profile. However, studies on pesticide
15 biodegradation have typically been conducted in topsoils and much less information is
16 available on pesticide transformation processes in subsoils. Generally, pesticide
17 degradation rates decline with soil depth (Stenrod et al., 2006; Rodriguez Cruz et al.,
18 2006), although exceptions have been reported (Di et al., 1998; Karpouzas et al.,
19 2001). Within the topsoil, there can be significant spatial variability in the
20 biodegradation rates of pesticides, even within fields where the soil appears to be
21 uniform (Bending et al., 2006). Similarly, there can be significant spatial variability of
22 degradation rates within the subsoil, with some reports suggesting that spatial
23 variability in degradation processes may increase with depth, reflecting greater spatial
24 variability in the distribution of microbial communities (Rodriguez Cruz et al., 2006).

1 Studies of pesticide fate in soil are usually conducted using samples which have
2 been air-dried, sieved and homogenised following collection from the field, and which
3 are maintained under controlled moisture and temperature conditions during
4 experimentation in the laboratory (Beulke et al., 2005). However, the sampling and
5 handling of soil in this way may irreversibly alter the physical, chemical and
6 biological properties of the soil relative to field conditions (Topp et al., 1994; Beulke
7 et al., 2005) and may influence both pesticide availability and microbial activity,
8 thereby affecting pesticide degradation (Beulke et al., 2005). This is especially
9 important to consider for the subsoil, which is never disturbed by tillage (Fomsgaard
10 et al., 1998).

11 The sieving and air-drying of soil could lead to increased pesticide-soil contact
12 which could potentially have a range of counteracting affects on biodegradation. One
13 possible effect of sieving could be to enhance biodegradation by increasing
14 homogeneity of distribution of pesticide through the soil, and thereby increasing
15 contact between the pesticide and degrader microorganisms (Parkin et al., 1991; Topp
16 et al., 1994; Beulke et al., 2005). On the other hand, enhanced distribution of pesticide
17 within the soil matrix has the potential to increase contact of pesticide with sites at
18 which sorption can occur, thereby decreasing pesticide availability and increasing the
19 formation of bound residues (Lechon et al., 1997; Beulke et al., 2005). Furthermore,
20 the sieving process and subsequent rewetting of dried soil typically result in short
21 term enhanced microbial activity, while drying can release inorganic ions, such as
22 manganese which may be toxic to microorganisms (Angle et al., 1995).

23 Soil cores have the advantage of maintaining undisturbed the soil macrostructure
24 (Parkin et al., 1991; Angle et al., 1995). It has been suggested that degradation of
25 pesticides in soil cores is more representative of field conditions (Parkin et al., 1991;

1 Lechon et al., 1997). However, in undisturbed cores, the distribution of pesticides
2 could be localised and therefore restricted, and this coupled with uneven distribution
3 of microbes within cores, particularly in sub soil (Nunan et al., 2001) could reduce
4 pesticide bioavailability (Parkin et al., 1991; Genod et al., 2003). Several studies have
5 investigated the influence of laboratory incubation method on pesticide degradation
6 rate in soil, with some indicating differences in degradation rate between intact cores
7 and sieved soil (McDonald et al., 2006; Bending et al., 2007) but others finding no
8 difference (Topp et al., 1994).

9 Bentazone is a commonly used herbicide which poses environmental concerns
10 because of its high mobility, persistence and susceptibility to leaching from soil to
11 groundwater (Thorstensen and Lode, 2001; Boesten and Van der Pas, 2000; Li et al.,
12 2003). It is applied at a high rate in agriculture and frequently occurs as contaminant
13 of groundwater and surface water (Helweg et al., 2002; Lagana et al., 2002 Dousset et
14 al., 2004). In general, bentazone degradation in soil follows first order kinetics
15 (Thorstensen and Lode, 2001), suggesting cometabolic degradation, without growth
16 of organisms involved (Piutti et al., 2002). Soil organic matter content and pH are the
17 soil properties that have been identified as having the greatest influence on the
18 degradation of bentazone (Boivin et al., 2004). Contact time and soil type have a
19 significant effect on bentazone availability (Boivin et al., 2004). It has been observed
20 that the rate constant for bentazone dissipation was highest in the topsoil and
21 decreased with depth (Leistra et al., 2001).

22 The objective of this study was to compare spatial variability in the
23 biodegradation of bentazone both vertically and horizontally within an agricultural
24 field. In particular, the spatial variability in degradation rate between locations was
25 determined using both sieved soil and intact cores, to determine how representative

1 spatial variability measurements made using sieved soil were relative to samples in
2 which the physical and microbiological characteristics of the soil were retained.

3

4 **2. Material and methods**

5 *2.1. Pesticide and pesticide treatment history*

6 Bentazone (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide) is a
7 moderately mobile compound, which shows low sorption to soil ($K_{oc} < 100$) and high
8 solubility in water (570 mg l^{-1}). Commercial formulation of bentazone, Basagran
9 (87% w/w), was supplied by BASF plc (Ludwigshafen, Germany). Analytical grade
10 bentazone was supplied by Chem Service Inc. (West Chester, USA). Sampling
11 occurred in Long Close field on the farm at Warwick HRI, Wellesbourne,
12 Warwickshire, UK. The soil is a sandy loam of the Wick series (Whitfield, 1974).
13 There had been no application of bentazone over at least the previous 10 years.

14 *2.2. Soil collection*

15 *2.2.1 Vertical variability in degradation rate using sieved soil*

16 Soil was collected from five depths at three sampling locations in the field. Three pits
17 separated by 60 m were excavated to 1 m depth using a mechanical digger, in
18 February 2003. One side of each pit was further excavated using a surface sterilised
19 trowel, so that the face was free of loose soil. Soil was collected from 0-10, 20-30, 40-
20 50, 60-70 and 70-80 cm depth using two methods. From each depth soil was collected
21 using a trowel and placed into a polythene bag. The trowel was surface sterilised with
22 ethanol between the collection of each soil sample. Soil was spread onto clean
23 polythene bags and left on the bench overnight to reduce moisture content, before
24 being passed through surface sterilised sieves ($< 3 \text{ mm}$).

1 2.2.2 *Vertical and horizontal spatial variability in degradation using intact cores and*
2 *sieved soil*

3 Soil was collected from two depths at ten different sampling locations in Long
4 Close field, in April 2003. Ten pits were excavated in the field at intervals of 80 m (N-
5 S) and 60 m (E-W). Samples of disturbed soil and undisturbed soil cores were
6 collected from 0-15 cm depth and 50-60 cm depth. Sieved soil was collected and
7 processed by methods described above. In order to maintain the physical and
8 microbiological integrity of the soil, further samples were taken using 10 x 5 cm pre-
9 sterilised stainless steel cores. Two cores were obtained at 0-15 and 50-60 cm depth
10 from each of the 10 sampling locations by hammering the core horizontally into soil.
11 Following removal, the top and bottom of each core was sealed with parafilm.

12 2.3. *Analysis of soil characteristics*

13 In the pre-sieved soil, total organic matter, microbial biomass-N, dehydrogenase
14 activity, pH, clay, sand and silt content were measured, as described in Bending et al.
15 (2006).

16 2.4. *Pesticide application*

17 For pre-sieved soil, commercial bentazone formulation was dissolved in distilled
18 water and added to 300 g fw portions of soil from each location to provide 5 mg
19 pesticide kg⁻¹ soil, and further water was added to bring the water holding capacity to
20 40% (-33 kPa). Each soil was mixed thoroughly by hand, and then further mixed by
21 passing through a <3 mm sieve five times. Each soil was transferred to a sterile
22 polypropylene container which was loosely capped and incubated at 15°C in the dark.
23 Moisture content was maintained by the addition of sterile distilled water as necessary
24 (usually once each week).

1 In the case of the intact cores, four 250 µl aliquots of the commercial formulation
2 of bentazone in water were injected centrally at distances of 2 cm apart down the core
3 to give a final concentration within the core equivalent to 5 mg kg⁻¹ soil. The soil
4 cores were sealed base and top with parafilm and incubated vertically at 15°C in the
5 dark.

6 *2.5. Pesticide extraction and analysis*

7 The pre-sieved soils were sampled at regular intervals over a 3-month period, with
8 extraction and HPLC as described by Rodriguez-Cruz et al. (2006). For the second
9 experiment, the cores were sampled after 127 days incubation. Soil was pushed from
10 the cores, mixed by hand and sieved (<3 mm) five times. Sub-samples (10 g) of each
11 soil were dried in an oven at 110°C overnight to determine soil moisture content.
12 Pesticide was extracted from each soil sample and analysed using the procedures
13 described above.

14 Using pre-sieved soil, sorption of bentazone was determined using a batch mixing
15 method, and adsorption distribution coefficients (K_d) measured as described by
16 Rodríguez-Cruz et al. (2006).

17 *2.6. Statistical analysis*

18 All statistical analyses were performed using GenStat software (7th edition, VSN
19 International Ltd.) The exponential and linear models were found to provide best fit to
20 the degradation kinetics, and were used to obtain time to 50% degradation (DT₅₀)
21 values. Analysis of variance was used to determine the significance of differences in
22 pesticide degradation between soil depth and method (sieved soils or soil cores). For
23 the study of vertical and horizontal spatial variability in degradation using intact cores
24 and sieved soil, variability within samples was estimated by replicating the

1 measurement of the cores for each treatment combination measured. Due to the fact
2 that the design was unbalanced a general linear model was used for analysis. An
3 accumulated analysis of variance was calculated with the replication of cores giving
4 an estimate of the underlying (within sample) variability for both the core and sieved
5 samples. Predictions were then made for the treatment combinations to give the
6 estimated means and standard errors for each of the treatment combinations tested. %
7 coefficient of variation in the study of vertical and horizontal spatial variability in
8 degradation using intact cores and sieved soil was determined following angular
9 transformation to normalise the variance.

10

11 **3. Results and discussion**

12 *3.1 Vertical variability in degradation rate using sieved soil*

13 In pre-sieved top-soil, bentazone degradation showed an exponential decrease in
14 concentration over time (Fig 1). This is typical of co-metabolic activity, where
15 pesticide degradation does not result in proliferation of degrader organisms. In sub-
16 soil, there was a very slow degradation of bentazone with time and degradation was
17 fitted to a linear model. Degradation rate declined progressively down the soil profile.
18 In topsoil, DT₅₀ was 56 and 65 days at 0-10 and 20-30 cm depth, respectively, while
19 in sub-soil DT₅₀ value increased from 178 days at 40-50 cm depth to 515 days at 70-
20 80 cm depth (Table 1). Similarly, DT₅₀ values for bentazone degradation in topsoils
21 range from 2 to 15 weeks in previous studies (Huber and Otto, 1994), and degradation
22 rate has been shown to decline with depth (Leistra et al. 2001). In the current study
23 the proportional decline in degradation rate was greater than that of biomass or
24 dehydrogenase, so that the ratio of biomass or dehydrogenase to DT₅₀ increased
25 significantly ($p < 0.001$) with soil depth, in the case of biomass, ranging from 0.85 at 0-

1 10 cm depth, to 27.0 at 70-80 cm (data not shown). This suggests that the specific
2 degraders responsible for bentazone transformation formed a decreasing proportion of
3 the biomass, or reduced their relative activity, as depth increases. Bentazone showed
4 limited sorption, and there was no significant change in sorption with soil depth
5 (Table 1).

6 Bentazone DT₅₀ was significantly correlated with soil biomass ($r = -0.701$, $p <$
7 0.01), dehydrogenase activity ($r = -0.595$, $p < 0.05$), pH ($r = 0.597$, $p < 0.05$), OM
8 content ($r = -0.744$, $p < 0.01$) and Kd (-0.676 , $p < 0.05$). Similarly, von Götze and
9 Richter (1999) observed that soil biomass, organic carbon and pH value had the
10 greatest influence on bentazone degradation behaviour in soil. Bentazone Kd was
11 significantly correlated with OM content ($r = 0.561$, $p < 0.05$) and pH ($r = -0.704$,
12 $p < 0.01$) confirming work by Li et al. (2003) and Thorstensen et al. (2001).

13

14 *3.2. Vertical and horizontal spatial variability in degradation using intact cores and* 15 *sieved soil*

16 In topsoil, significantly less ($p < 0.05$) bentazone remained in intact cores relative
17 to sieved soil, with 6.6 and 17.2 % remaining respectively (Table 2), suggesting more
18 rapid degradation in intact cores relative to sieved soil. In the subsoil, the percentages
19 of extractable bentazone from the soil cores were not significantly different between
20 sieved soil and intact cores, with a mean of 64.7% and 60.1% remaining respectively
21 after 127 days (Table 2).

22 Several studies in which pesticide degradation was associated with growth-linked
23 catabolism have found slower degradation rates in intact cores relative to sieved soil
24 (Parkin et al., 1991, Bending and Rodriguez-Cruz, 2007), while in common with our
25 data, McDonald et al. (2006) investigating a cometabolised compound, found faster

1 degradation in cores relative to sieved soil. A number of factors could account for
2 differences in degradation rate between intact cores and sieved soil. In particular,
3 distribution of pesticide within the soil will differ, reflecting a less homogenous
4 distribution in cores, with concentrations higher at points of injection (Parkin et al.,
5 1991). Such differences could have different effects on communities involved in
6 growth-linked and cometabolic degradation, particularly if distribution of pesticide is
7 associated with the speed at which growth-linked catabolising communities adapt the
8 potential to use the pesticide as an energy source (Bending and Rodriguez-Cruz,
9 2007). Furthermore the capacity of degraders to spread through soil is likely to be
10 more rapid in sieved soil relative to intact cores (Angle et al., 1995).

11 Moisture was maintained at a constant level (40 % WHC, equivalent to 15 %) in
12 the sieved soil, but in the cores mean moisture content was variable, and averaged
13 13.3 % (coefficient of variation 13.1) in topsoil and 16.7 (coefficient of variation
14 17.6) in sub soil (data not shown). Degradation rate was significantly correlated with
15 moisture content in the top soil cores ($r = 0.718$, $p < 0.001$), but not in subsoil cores or
16 in the sieved soil, suggesting that moisture content was an important determinant of
17 degradation rate, and since the cores are likely to closely mimic field conditions
18 (Parkin et al., 1991; Angle et al., 1995), the spatial variability of degradation rate
19 within the field.

20 When top- and sub-soil samples were combined, degradation of bentazone in
21 cores and sieved soil after 127 days was significantly correlated ($r = 0.87$, $p < 0.001$).
22 However, within the top-soil samples amounts of bentazone remaining in sieved soil
23 and cores were not significantly correlated ($r = 0.23$). This was in contrast to the sub-
24 soil samples, in which degradation in cores and sieved soil were significantly
25 correlated ($r = 0.65$, $P < 0.05$). In sub-soil, % CV of sieved soil and cores were similar,

1 at 7.2 and 6.8 respectively. However in the top-soil, there was substantially higher
2 variability within cores relative to sieved soil, with % CV of 29.5 and 15.1
3 respectively. These differences could be attributed to differences in variability
4 between the cores and sieved soil, particularly reflecting moisture content differences,
5 as discussed above. The data suggests that moisture content was variable within the
6 field, and as shown by analysis of degradation rates in intact cores, was a key factor
7 likely to control degradation *in situ*. Processing soil prior to conducting degradation
8 assessment resulted in loss of moisture differences between locations, so that
9 variability in degradation rates determined using sieved soil underestimated likely
10 variability in degradation rates *in situ*

11

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17

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Table 1

Change in key soil properties and bentzone degradation and sorption with soil depth. DT50 and Kd measurements represent data obtained using sieved soil. % bentazone remaining in sieved soil and cores reflects 20 day time point following application. Data represents mean of 3 sampling locations at each depth.

Soil depth (cm)	OM (%) ^a	pH ^a	Biomass (mg C kg ⁻¹ soil) ^a	Dehydrogenase (µg TPF ^b g ⁻¹ soil) ^{a, b}	DT ₅₀ (days)	Kd (ml g ⁻¹)
0 - 10	2.62	7.06	65.5	43.1	56	0.15
20 - 30	2.33	7.13	64.5	22.9	65	0.11
40 - 50	1.98	7.79	36.4	10.7	178	0.10
60 - 70	1.66	7.98	21.5	7.48	306	0.09
70 - 80	1.35	8.09	19.1	6.60	515	0.06
LSD (P>0.05)	0.29	0.45	16.0	14.7	178	0.07
Significance of effect of depth ^c	***	**	***	***	***	NS

^a From Bending and Rodriguez Cruz (2007)

^b Triphenyl formazan

^c NS, not significant; ** significant, P<0.01; *** significant, P<0.001

Table 2 Key soil characteristics and % bentazone remaining after 127 days incubation in sieved soil and intact cores. Data represents mean of 10 sampling locations at each soil depth over a 160 x 90 m sampling area.

Soil depth (cm)	OM (%) ^a	pH ^a	Biomass (mg C kg ⁻¹ soil) ^a	Dehydrogenase (µg TPF ^b g ⁻¹ soil)	% Bentazone remaining in sieved soil ^b	% Bentazone remaining in cores ^b
0 - 10	2.71	6.71	44.5	43.2	17.2 (23.7)	6.63 (12.5)
50 - 60	1.43	7.30	23.4	3.87	60.1 (51.4)	64.7 (54.21)
LSD P>0.05	0.47	0.53	16.3	11.9		3.0
Significance of effect of depth ^c	***	***	***	***	***	***

^a Triphenyl formazan

^b data in brackets gives angular transformed data, to which LSD relates, for comparison between sieved soil and cores, and for the effect of depth.

^c *** significant, P<0.001

Figure 1 Degradation of bentazone in sieved soil from different depths

Data represent average of three replicate sampling locations at each depth

Bars represent +/- standard error of the mean

