Metabolic and cometabolic degradation of herbicides in the fine material of railway ballast

Harald Cederlund [a *], Elisabet Börjesson [a], Karin Önneby [a] and John Stenström [a]

[a] *Swedish University of Agricultural Sciences (SLU), Department of Microbiology, Box 7025, SE-75007 Uppsala, Sweden.*

** Contact information to corresponding author:*
telephone +46 18 673284
fax +46 18 673393
e-mail Harald.Cederlund@mikrob.slu.se
Department of Microbiology, Box 7025, SE-75007 Uppsala, Sweden.

Abstract

Microbial degradation of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) and mineralization of 4-chloro-2-methylphenoxyacetic acid (MCPA) were studied in soil samples taken from the ballast layers of three Swedish railway embankments. The degradation of diuron followed first-order kinetics and half-lives ranged between 122-365 days. The half-lives correlated strongly with microbial biomass estimated by substrate-induced respiration (SIR; $R = -0.85$; $p<0.05$) and with the amount of organic matter measured as loss on ignition ($R = -0.87$; $p<0.05$). Accumulation of the metabolites DCPMU and DCPU was observed in all samples and these were only detectably degraded in the sample with the highest SIR. Addition of ground lucerne straw to the ballast samples stimulated microbial activity and led to increased formation of metabolites, but further transformation of DCPMU and DCPU was not enhanced. Mineralization of MCPA followed growth-linked kinetics and the time for 50% mineralization was 44.5 ± 7.1 days in samples of previously untreated ballast. In samples of ballast that had been previously treated with the herbicide formulation MCPA 750, the time for 50% mineralization was reduced to 13.7 ±11.3 days. The number of MCPA degraders, quantified using an MPN technique, was clearly increased but highly variable. An average yield of 0.18 cells pg^{-1} of MCPA was estimated from the kinetic data. The yield estimates correlated with the amount of nitrogen in the ballast, indicating that mineralization of MCPA was nitrogen-limited in the railway embankments studied. This has practical implications for weed control using herbicides on railways.

Keywords: metabolic and cometabolic degradation; mineralization; MCPA; diuron; railway ballast

1. Introduction

Herbicides are used on railways in order to maintain the quality of the track and a safe working environment for railway personnel (Torstensson, 2001). Due to the coarse texture and low organic matter content of railway embankments, there is much concern that application of herbicides to railways may lead to groundwater contamination. Several studies have investigated the leaching of pesticides from railway tracks and, with some exceptions, most of them indicate that the leaching potential is considerable and that concentrations in the groundwater beneath the track may significantly exceed the EU limit for drinking water of 0.1 $\mu g\ l^{-1}$ if the application rate is too high (Torstensson, 1983; 1985; Lode and Meyer, 1999; Schmidt et al., 1999; Börjesson et al., 2004; Ramwell et al., 2004; Torstensson and Börjesson, 2004; Torstensson et al., 2005).

While leaching can be substantial from coarse-textured environments such as railways, research indicates that the microbial degradation might be accelerated in these kinds of soils because of the high availability of herbicides to microorganisms (Hassink et al., 1994; Klein, 2002; Strange-Hansen et al., 2004). However, microbial degradation of herbicides is also related to the amount and activity of microorganisms (Anderson, 1984; Torstensson and Stenström, 1986; Voos and Groffman, 1997; Jones and Ananyeva, 2001), and these are both known to be of very limited magnitude on railways (Smith et al., 1981; Cederlund and Stenström, 2004).

If there is concern about herbicide degradation in such a situation, with a high availability of the compound and a low number of degrading microorganisms, it would be relevant to try to identify an herbicide that is used as a substrate for microbial growth. Such an herbicide would potentially induce exponential microbial proliferation in the field and, hence, also give an exponentially increasing degradation rate. On the other hand, the degradation rate of an active ingredient that does not support microbial growth would depend on its concentration and on the initial number of degrading microorganisms and their activity.

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is a substituted phenylurea herbicide used for total weed control in non-crop areas (Tomlin, 2003). In Sweden, the use of diuron was prohibited in 1993 by the Swedish Chemicals Inspectorate but globally it is still one of the main herbicides used for weed control on railways tracks. The long residual activity of diuron is one of the main reasons for its popularity, but is also problematic for environmental reasons. Field studies confirm that diuron can be very persistent and also that it is highly prone to leaching when used on railways (Torstensson, 1983, 1985; Torstensson et al., 2002; Skark et al., 2004). The ability of microorganisms to use diuron as a carbon source for growth does not appear to be widespread. Microbial degradation of diuron in the field is usually very slow and follows first order kinetics (Hill et al., 1955; Sørensen et al., 2003).

MCPA [4-chloro-2-methylphenoxyacetic acid] is an arylalkanoic acid widely used for post-emergence control of broad-leaved weeds (Tomlin, 2003). Degradation of MCPA usually follows growth-associated kinetics, indicating that MCPA is used as a substrate for microbial growth (Audus, 1951). Hence, the dissipation of MCPA in agricultural soils is often rapid, at

least after the first time of its use (Kirkland and Fryer, 1966; Torstensson et al., 1975).

The objectives of this study were to characterize microbial degradation of the herbicides diuron and MCPA in fine materials of railway ballast, to investigate the relationship between microbial biomass and activity and degradation rates and to determine the extent to which adaptation of the railway microflora occurs in response to repeated applications of MCPA.

2. Materials and methods

2.1 Chemicals

Certified standards of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (diuron, 97,7% purity), 1-(3,4-dichlorophenyl)-3-methyl urea (DCPMU, 99.5% purity), 1-(3,4-dichlorophenyl) urea (DCPU, 99.7% purity), 3,4-dichloroaniline (DCA, 99.0% purity) and 4-chloro-2-methylphenoxyacetic acid (MCPA, 99.0% purity) were purchased from Dr Ehrensdorfer GmbH (Augsburg, Germany), while 4-chloro-2-methylphenoxy-[1-^{14}C]-acetic acid (^{14}C-MCPA, 57 mCi mmol^{-1}) was purchased from Amersham Biosciences. Formulations used for the field treatments were BASF MCPA 750 (MCPA dimethylamine salt, 750 g a.i. l^{-1}) from BASF AB and Roundup Bio (glyphosate isopropylamine salt, 360 g a.i.l^{-1}) from Monsanto. All other chemicals and solvents used for the study were of analytical grade.

2.2 Diuron degradation study

Soil was sampled in 2002 from the surface layer of two railway tracks between Mora-Älvdalen (61º00'N, 14º28'E; denoted 'Mora' in the text) and Nässjö-Vetlanda (57º28'N, 15º03'E; denoted 'Vetlanda' in the text). Both tracks were regularly treated with diuron until 1992, but since then only glyphosate and imazapyr have been used. All samples were sieved (< 4mm) and stored at 4ºC. The sampling sites have been previously described by Cederlund and Stenström (2004) and some basic characteristics of these soils are presented in Table 1. Samples were pooled on the basis of their SIR-values so that soils (900 g wet wt.) of 'high' (A), 'intermediate' (B) and 'low' (C) microbial biomass were obtained for each track. A fraction of each soil (100 g) was contaminated with diuron (12 mg) dissolved in acetone (5 ml) (procedure adopted from Brinch et al. 2002). When the acetone had evaporated the contaminated fraction was mixed thoroughly with the rest of the soil to obtain an approximate diuron concentration of 15 µg g^{-1} dry wt. (a realistic in-field concentration). The samples were incubated in aerated plastic jars in the dark at 20ºC for 460 days. The water content was adjusted to and kept at 60% of the water holding capacity (WHC) of the soils throughout the experiment by the addition of deionised water. Duplicate samples (10 g) for HPLC analysis were taken regularly to monitor the concentration of diuron and its metabolites. The SIR and basal respiration were measured on duplicate samples (40 g) on days 8, 194 and 393 of the incubation.

An additional experiment was carried out in which the microbial activity of the soil was stimulated by the addition of ground lucerne straw (5 mg g^{-1} dry wt soil). The soil was incubated as described above and concentrations of diuron and metabolites were monitored for 239 days. The SIR and basal respiration were measured on days 10, 44, 91 and 187.

2.3 Analysis of diuron

The soil samples (10 g) were extracted for 60 min with methanol (25 ml). The samples were then centrifuged at 1500 rev min^{-1} for 10 min (Sorvall T6000D, Lambda Polynom AB, Sweden) and filtered (OOH Whatman; 11cm) before HPLC analysis. The HPLC analyses were performed using an UV detector G1314A, a pump G1311A and an autoinjector G1329A (Agilent Technologies AB; 1100 Series; Sweden) equipped with a Zorbax SB-C18 column (12.5 x 4.6 mm, 5 µm; ChromTech AB, Sundbyberg, Sweden). The eluent was a gradient of acetonitrile-water, 10-100% for 11 minutes, followed by 2 minutes equilibration at 100% acetonitrile, and the flow rate was 1 ml min^{-1}. The samples were detected at 254 nm and the injection volume was 25 µl. Under these conditions the retention times for DCPU, DCPMU, diuron and DCA were 7.39, 8.05, 8.61 and 9.09 minutes, respectively. Calibration curves for diuron were obtained by plotting peak areas against concentrations for diluted standards (0.005 -5.0 µg ml^{-1}). A linear relationship between chromatographic peak area and amount of chemical was found over this concentration range, with R^2 >0.9999 in each case.

2.4 MCPA mineralization study

Soil was sampled in 2005 from a field study site situated on the railway track between Borås and Varberg (57º15'N, 12º 20'E). All samples were sieved (< 4mm) and stored at 4ºC until used. The site is divided into randomly distributed 25 m plots spread over a one km stretch and soil samples were taken from the surface layer (from directly underneath or from within the lower macadam ballast layer) from four replicate plots of each of the following treatments: Treatment 0 = unsprayed; treatment 1 = sprayed the previous year with a mixture of glyphosate and MCPA (commercial formulation Roundup Bio 3 l ha^{-1} + BASF MCPA 750 2 l ha^{-1}); and treatment 2 = sprayed with the same mixture for two consecutive years prior to sampling. The SIR and basal respiration were determined in each sample, as were the amounts of NO_3-, NH_4+ extracted in KCl, and P, K and Mg soluble in ammonium lactate/acetic acid solution (Svensk Standard SS 028310). Some basic characteristics of the soil are given in Table 1.

Triplicate soil samples (20 g wet wt.) from each plot were weighed into small plastic jars. Unlabelled and ^{14}C-labelled MCPA, dissolved in deionised water, was mixed into each jar for 30 s with a spatula to obtain a final radioactivity and total MCPA concentration of about 970 disintegrations sec^{-1} jar^{-1} and 4 µg g^{-1} dry wt. respectively. The soil moisture content was adjusted to 60% of the WHC. The plastic jars were each installed into airtight glass jars together with two scintillation vials containing NaOH (0.2 M; 4 ml) to trap carbon dioxide. The glass jars were incubated in the dark at 20ºC and the base traps were changed regularly; one was used for determination of the release of radioactivity from the soil through mineralization of MCPA (^{14}C-CO_2) and the other was used for assessment of the basal respiration (total-CO_2). The amount of ^{14}C in the base traps was measured on an LS 6000TA liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA) after the NaOH had been mixed with 4 ml of Insta-gel Plus emulsifying cocktail (Chemical Instruments AB, Lidingö, Sweden) and incubated in the dark overnight. The ^{14}C liberated was corrected for the background radiation in controls without soil. The total amount of CO_2 that had been released from the soil was determined by titration. Aliquots of NaOH (2 ml) were mixed with $BaCl_2$ (0.1M; 8 ml) and titrated with HCl (0.1 M) using the Titralab 850 endpoint titration workstation (Radiometer Analytical, Copenhagen, Denmark).

An additional experiment was performed in order to allow the percentage values obtained from the scintillation measurements (e.g. % mineralised) to be converted into actual MCPA concentrations (related to the degraded amount rather than the amount mineralised). In order

to achieve this, 20 glass jars containing soil from one plot were incubated under the same conditions as described above. The base traps were changed regularly and the amounts of ^{14}C in the base traps were determined as described above. On each occasion of base trap change, incubation of two glass jars was terminated and the contents analysed by GC-MS, resulting in a time series consisting of a total of 10 simultaneous measurements of mineralization and concentration values. Calculations are outlined in section 2.7.

2.5 Analysis of MCPA by GC-MS

The soil samples (10 g) were extracted for 24 hours with 25 ml of acetone:water:acetic acid (80:19:1), centrifuged at 1500 rev min^{-1} for 15 min (Sorvall T6000D, Lambda Polynom AB, Sweden;) and re-extracted for one hour with 10 ml of the solvent. The extracts were diluted with water (500 ml) and one portion of each sample (70 ml) was cleaned through 200 mg ENV+ columns (Isolute®, International Sorbent Technology, Sorbent AB, Frölunda, Sweden.), eluted with 2 x 2 ml of methanol with 5% NH_3 and evaporated under nitrogen gas at 40°C. The samples were derivatized with Na_2HPO_4 (8 ml; 0.05 M; pH 8), pentafluorobenzyl bromide in dichloromethane (2 ml; 0.1%) and tetrabutylammonium sulphate (150 µl; 0.15M), shaken for 30 minutes and centrifuged at 3000 rev min^{-1} for 2 min. One ml of the dichloromethane phase was transferred into a 2 ml glass vial, evaporated under nitrogen gas and dissolved in cyclohexane (0.5 ml) before GC-MS analysis. The analyses were performed using a Hewlett Packard model 6890 gas chromatograph equipped with a HP-5MS capillary column, 30 m × 0.251 mm I.D. (0.25 µm film thickness) (ChromTech AB, Sundbyberg, Sweden). The oven temperature was programmed to run at 80°C for 2 min and then to increase by 12°C min^{-1} to a final temperature of 280°C. Under these conditions the retention time for MCPA was 14.02 minutes. The retention time data were complemented with mass spectral (MS) data obtained from a Hewlett Packard model 5973 Mass Selective Detector. Calibration curves for MCPA were obtained by plotting peak areas against concentrations for diluted standards (0.01 -1.0 µg ml^{-1}). A linear relationship between chromatographic peak area and amount of chemical was found over this concentration range with $R^2 > 0.994$.

2.8 Enumeration of MCPA-degraders

A method adapted from Lehmicke et al.(1979) was used for enumeration of MCPA degraders. Duplicate soil samples (10 g wet wt.) from each plot (see section 2.3), were weighed into sterile Duran flasks and were shaken in phosphate buffered saline (PBS; 0.1 M; 100 ml) for 30 min at 200 rev min^{-1}. The extracts were diluted in PBS (0.1 M) down to 10^{-7} and five replicates of 0.1 ml from each dilution were inoculated into sterile 2 ml glass vials together with 0.9 ml of growth medium. The growth medium contained the following salts: KH_2PO_4 (2.27 g l^{-1}), $Na_2HPO_4.2H_2O$ (2.97 g l^{-1}), NaCl (1g l^{-1}), KNO_3 (0.66 g l^{-1}), $(NH_4)_2SO_4$ (0.41 g l^{-1}), $MgSO_4.7H_2O$ (0.5 g l^{-1}), $CaCl_2.2H_2O$ (0.01 g l^{-1}), $MnSO4.7H_2O$ (25 mg l^{-1}) and $FeSO_4.7H_2O$ (25 mg l^{-1}). Sterile filtered MCPA (total concentration 4 mg l^{-1}), ^{14}C-labelled and unlabelled, was added as the only carbon source. The final radioactivity was approximately 10 sec^{-1} $vial^{-1}$. The glass vials were installed standing inside scintillation vials containing NaOH (0.2 M; 4 ml) and the scintillation vials were then sealed and incubated in the dark at 20ºC for at least four weeks. At the end of the incubation, the glass vials were removed and the radioactivity of the NaOH was determined on the scintillation counter. Scintillation vials with a measured radioactivity of more than 5 sec^{-1} were classified as positive in the assay. A most probable number (MPN) table (Cochran, 1950) was used to estimate the number of MCPA degraders in the original soil sample.

2.7 Measurement of substrate-induced respiration and basal respiration

Two replicates of soil (40 g wet wt.) were weighed into 250 ml respirometric jars. The jars were installed inside a respirometer (Nordgren, 1988), and the accumulation of CO_2 trapped in KOH solution (0.2 M; 10 ml) was determined automatically twice every hour for each jar by measurement of the conductivity. The soil samples were incubated until a constant basal respiration rate could be established (after about 6 days) at a constant temperature of 22ºC and with a moisture content adjusted to 60% of WHC. A SIR substrate, consisting of glucose (150 mg), $(NH_4)_2SO_4$ (22.6 mg), KH_2PO_4 (7 mg) and talcum powder (200 mg), was then mixed into each jar (Stenström et al., 2001). Empty jars were incubated as controls.

The basal respiration rate (BR) was calculated by linear regression of accumulated CO_2 data versus time. The instantaneous rate of CO_2 formation after addition of the SIR substrate (SIR) was calculated using non-linear regression of accumulated data. The SIR was divided into the CO_2 production rate by active, exponentially growing (r) and dormant, non-growing (K) microorganisms as described by Stenström et al. (2001).

2.8 Calculations

2.8.1 Calculation of half lives for diuron

The half-lives of diuron were obtained by plotting the natural logarithm of the remaining concentration against time, fitting a straight line to the data and calculating the half life as: $t½ = \frac{\ln 2}{k}$, where k is the slope of the regression line. The coefficient of determination (R^2) for the regression lines ranged between 0.78-0.97 for the different samples, indicating that the assumption of first order kinetics was a reasonable one. For calculations of half-lives for the sum of diuron and its detected metabolites, the sums of the remaining molar amounts were used.

2.8.2 Calculation of qN_0, μ and time for 50 % mineralization of MCPA

The mineralization rates in all samples initially followed logarithmic kinetics (i.e. displayed an exponentially increasing mineralization rate) and then, after reaching an inflection point, they gradually levelled off to a slow and constant rate. The logarithmic phase of the mineralization curves could be approximated by the equation:

$$p = p_0 + \frac{qN_0}{\mu}(e^{\mu t} - 1) \qquad (1)$$

where p is the liberated amount of ^{14}C (expressed as a % of the amount added), p_0 accounts for impurities and losses of ^{14}C, q (% cell^{-1} h^{-1}) is the specific activity of the MCPA degraders, N_0 (number of cells) is the initial amount of growing MCPA degraders, μ (h^{-1}) is their specific growth rate and t (h) is the time. The parameter values for p_0, qN_0 and μ were obtained by non-linear regression of the measured mineralization data using Sigma plot 2000 (v.6.00, SPSS Inc.).

In two samples (1d and 2d), the mineralization was so rapid that only three data points were obtained from within the logarithmic phase. In these cases, the average p_0 obtained from the other soil samples was inserted into equation 1 in order to solve it. In one sample (1c), mineralization was so rapid that in two out of three replicates only two data points fell within

the logarithmic phase. In this case, p was plotted against $\frac{(e^{\mu t}-1)}{\mu}$ for replicates two and three after μ had been estimated from replicate one, and qN_0 was obtained as the slope of the resulting linear regression line.

The time for 50 % mineralization was calculated by solving equation 1 for p = 50%.

2.8.3 Conversion of mineralization data to concentrations

In an additional experiment, both mineralization and degradation were measured in the same soil sample (see section 2.3). For mineralization data, *qN0* and μ were obtained as described above and essentially the same calculation was made for degradation data but according to the equation: $c = c_0 - \frac{qN_0}{\mu}(e^{\mu t}-1)$, where c (µg g^{-1} dry wt) is the remaining MCPA at time t, c_0 is the initial concentration and the specific activity q now has the unit pg MCPA $cell^{-1}$ h^{-1}. The ratio between the two qN_0 values was used as a conversion factor for the mineralization data obtained in the main study.

2.8.4 Calculation of q, N_0 and yield

The numbers of MCPA degraders counted (see section 2.6) were plotted against the qN_0 values obtained from equation 1. A regression line was fitted to the plotted data (R^2 = 0.74; the intercept was forced through the origin of the coordinate system) and the slope of the line was used to calculate estimates of the specific activity q (pg $cell^{-1}$ h^{-1}). The yield Y (cells pg^{-1}) could then be calculated as: $Y = \frac{\mu}{q}$.

2.9 Statistical analyses

Effects of diuron contamination on microbial parameters were tested for significance using a Wilcoxon matched pairs test. Differences between field treatments in the MCPA study were verified using the Mann-Whitney U-test. Correlations and the significance of correlations were computed using the product moment and partial correlation routine in Statistica (v. 7.1, Statsoft Inc.).

3. Results

3.1 Degradation of diuron

Diuron degradation followed first order kinetics, with half-lives ranging between 122-365 days. Half-lives were clearly longer in ballast samples obtained from the embankment in Vetlanda than in ballast samples from Mora. The half-lives were significantly negatively correlated with loss on ignition and with SIR (Figure 1). Microbial degradation of diuron gave the demethylated metabolites DCPMU and DCPU in all samples. In the sample with the highest SIR (Mora A), these metabolites began to disappear during the incubation period whereas in all other samples there was a net accumulation throughout the study (Figure 2). The metabolite DCA was detected in trace concentrations in the samples Vetlanda B and C only.

The half-lives for the sum of diuron and its metabolites ranged between 151-693 days in the different samples (Figure 1). The correlations to loss on ignition and SIR were stronger than for diuron alone and there was also a significant correlation to the basal respiration rate (Figure 1).

The SIR and basal respiration were significantly higher in the contaminated soils compared to the uncontaminated controls when measured soon after contamination with diuron (day 8). However, no significant effects of the contamination or the contamination procedure were seen on days 194 and 393. Both SIR and basal respiration declined gradually and substantially during the study as a result of the long incubation. Values of SIR and basal respiration are presented in Table 2. The active part of the SIR response (r) ranged between 0.34-55.3 ng CO_2-C g^{-1} dry wt h^{-1} in the different samples at the beginning of the study and remained low throughout the incubation.

Lucerne addition caused an almost 10-fold increase in SIR compared to the control at the beginning of the incubation (day 10), from 0.31 ±0.17 to 2.95 ±0.05 µg CO_2-C g^{-1} dry wt h^{-1}, and a similar increase in the basal respiration rate from 0.21 ±0.01 to 2.75 ±0.24 µg CO_2-C g^{-1} dry wt h^{-1}. The active fraction of the SIR response (r) was also increased from 9 to 81% of the SIR. The SIR rate and the basal respiration rates rapidly declined and were comparable with those of the non-amended soil after 44 days of incubation, whereas the active fraction of SIR (r) remained high throughout the study. The diuron degradation in the lucerne treatment was clearly stimulated. However, only the first demethylation steps appeared to be stimulated, as can be deduced from Figure 3. Degradation followed first-order kinetics until day 77 after which it was virtually arrested, making it unfit for calculation of a half life after this time.

3.2 Mineralization of MCPA

Previous treatment of the railway embankment with BASF MCPA 750 drastically reduced the time required for 50% mineralization of MCPA from 44.5 ±7.1 days to 13.7 ±11.3 days ($p<0.05$; Figure 4). The number of MCPA degraders was also clearly affected by the spraying. There was no significant difference between plots with one or two subsequent years of herbicide application prior to sampling. In previously untreated ballast, the number of degraders was below the quantification limit of the MPN assay (< 20 cells g^{-1} soil) in all samples, whereas in previously treated soil the number of degraders was higher, albeit highly variable, ranging between 20–140 000 MCPA degraders g^{-1} soil. Both the yield (Y) and the specific growth rate (μ) were significantly higher in previously treated soil than in untreated soil ($p<0.05$) but were unaffected by the number of previous herbicide applications. Values of SIR, r and basal respiration are presented in Table 2, and the counted number of MCPA degraders and microbial parameters derived from the mineralization curves (μ, N_0 and Y) are presented in Table 3. The specific growth rates estimated from mineralization and degradation data were almost identical (μ_{min} = 0.49 h^{-1} and μ_{deg} = 0.48 h^{-1}) giving confidence to the conversion procedure that was used to calculate q and Y (see section 2.8.4).

The time for 50% mineralization correlated negatively with pH ($R= -0.69$; $p<0.05$), basal respiration ($R= -0.6$; $p<0.05$) and to Y and μ of the MCPA-degraders ($R = -0.77$; $p<0.05$). In addition, Y and μ correlated significantly with the amount of nitrogen present in the railway ($R = 0.63$, 0.73 and 0.73 to NH_4+, NO_3- and total-N respectively; $p<0.05$).

The relationship between mineralization and degradation of MCPA is shown in Figure 5. The inflection point of the mineralization curve (where there is a switch from logarithmic to slower mineralization kinetics) corresponded to when the MCPA had been almost completely degraded.

4. Discussion

4.1. Half lives of diuron

The half-lives for diuron determined in this study fall within the fairly wide range of half-lives that have been reported from other types of soils. Torstensson (1983; 1985) carried out studies of diuron degradation on railways and in samples of railway ballast and reported half-lives *in vitro* of between 25-136 days. However, estimations from several field sites suggested that half-lives could range between 1-3.8 years in railway embankments. Presumably, both dry conditions and low temperatures could limit the microbial degradation of herbicides on railways during large parts of the year. Low numbers of microorganisms would be another restraining factor for degradation as indicated by the strong correlation between half-lives and SIR rates, and although the present study was carried out on ballast samples of what was denoted as having comparably 'high', 'intermediate' and 'low' SIR rates (A, B, C in Table 2.) it should be emphasised that the typical SIR rate from a railway embankment is low. Median SIR values determined from the surface layer of the centre of the track of the two embankments studied were 0.29 and 0.16 μg CO_2-C g^{-1} dry wt h^{-1} in Mora and Vetlanda respectively when measured in 2002, corresponding almost exactly to the SIR rate of the 'low'-activity samples Mora C and Vetlanda C. It therefore seems likely that the slowest degradation rates reported here are the most representative for the railway embankments studied.

4.2. Metabolites of diuron transformation

The major metabolites of diuron found in soil are DCPMU, DCPU and DCA (Gooddy et al., 2002). The non-target toxicity of these degradation products has been evaluated and appears to be considerably higher than that of the parent molecule (Tixier et al., 2000; 2001; 2002), and the importance of evaluating the dissipation of metabolites when assessing the environmental impact of diuron has been emphasised by Giacomazzi and Cochet (2004). In the current study, the half-lives were almost doubled when they were calculated based on the sum of diuron and its measured metabolites (Figure 1). There was a net accumulation of demethylated metabolites in all samples except the sample with the highest SIR (Mora A; Figure 2), where the concurrent degradation rate of the metabolites outweighed their production rate.

4.3. Kinetics of diuron degradation

Degradation of diuron followed first-order kinetics, indicating that it proceeds through co-metabolism, i.e. without supporting growth of the degrading microorganisms. Adsorption and desorption of diuron in soils are mainly a function of the organic matter content (Liu et al., 1970), or, when organic matter is low, of the specific surface area or cation exchange capacity of the soil (Mustafa and Gamar, 1972). Since neither organic carbon nor clay content of the ballast samples studied was high, it appears unlikely that diuron availability would have been rate-limiting.

A few bacteria capable of rapid diuron degradation, belonging to the genera *Arthrobacter* (Cullington and Walker, 1999; Widehem et al., 2002) and *Sphingomonas* (Sørensen et al., 2001) have been isolated from agricultural fields. However, as on railways, degradation of diuron and structurally similar phenylureas is generally slow in agricultural soils and can be described by the first-order rate law (Hill et al., 1955; Madhun and Freed, 1987), even though accelerated degradation has been observed in some cases (Cox et al., 1996; Sørensen et al., 2003; Walker and Austin, 2004). However, the spatial heterogeneity is large and rapid growth-linked degradation along with slow co-metabolic degradation rates have been observed from within the same fields for both isoproturon and linuron herbicides (Bending et al., 2001; Rasmussen et al., 2005). The reason for this large heterogeneity does not appear to be the lack of degrading microorganisms in the field, but rather variation in soil properties that control microbial growth (Bending et al., 2003; Rasmussen et al., 2005). However, studies of the functional microbial diversity of railways reveal low catabolic versatility of microbial communities in railway ballast, suggesting that many functionally important microorganisms could be lacking (Cederlund et al., unpublished data), and it is possible that the railway samples studied here were essentially devoid of organisms capable of rapid diuron metabolism. The fact that the metabolite DCA was only detected at trace concentrations in two of the samples also supports this assertion, since DCA is the major metabolite produced in soil by the diuron-degraders characterized to date (Cullington and Walker, 1999; Widehem et al., 2002).

4.4. Relationship between organic matter and diuron degradation

There was a negative correlation between loss on ignition and the half-life of diuron (Figure 1). This could indicate that degradation is limited by lack of nutrients in the embankment. However, considering the cross-correlation between loss on ignition, SIR and basal respiration, it is difficult to infer whether a low content of organic matter restrains diuron degradation *per se* or rather the size or activity of the microbial community as a whole. Whatever the reasons for this relationship, it is tempting to extrapolate the regression line from Figure 1 to get an idea of what content of organic matter would be required to obtain a more acceptable half-life for diuron in railway ballast. A half-life for diuron of about 50 days would require a loss on ignition of 2.36%, which is somewhat higher than the highest percentages found when the two railways were investigated in 2002 (Cederlund and Stenström, 2004). Additions of straw and NH_4NH_3 to soil have been reported to enhance degradation of diuron by stimulating microbial activity (Berger, 1999) and the same was true for the addition of ground lucerne straw to railway ballast samples in the present study (Figure 3). However, although degradation of diuron was significantly enhanced, lucerne addition stimulated only the first demethylation steps but did not stimulate further diuron transformation. Applications of organic matter or fertilization of railways could possibly enhance diuron degradation, but since the aim of herbicide use is to prevent plant growth and accumulation of organic matter, these measures are not very feasible alternatives.

4.5. Enhanced mineralization of MCPA

The time required for 50% mineralization of MCPA was clearly lowered in soil from plots that were previously treated with MCPA (treatments 1 and 2) compared to previously untreated plots (treatment 0; Figure 4) This was expected since enhanced degradation of MCPA following treatment has been repeatedly observed in soil and on agricultural fields (Audus, 1951; Kirkland and Fryer, 1966; Torstensson et al., 1975; Helweg, 1987; Smith et al.,

1989; Smith and Aubin, 1991a). The mineralization of MCPA followed typical growth-linked kinetics, displaying sigmoid-shaped curves of ^{14}C-CO_2 release, and the differences in times required for mineralization were mainly due to differences in the length of the 'lag phase'. This in turn reflects differences in the initial population size (N_0) and specific growth rate (μ) of MCPA degraders between plots (Table 3). There was a significant negative correlation between pH of the soil and the time for 50% mineralization. The low pH would have decreased the water solubility of MCPA but it is also possible that the activity of microorganisms that degrade MCPA was affected directly. The results are in good agreement with those of Torstensson (1974), who investigated the effects of pH on the degradation of MCPA and reported reduced degradation at pH values below 5.5.

4.6. Numbers of MCPA degraders

In unamended ballast samples, the number of MCPA degraders was below the limit of detection of the MPN assay, reflecting the overall low SIR that is found on railways and the lack of natural selection for microorganisms with MCPA degrading capabilities. The numbers were significantly higher in MCPA-treated soil but the variability was very high. This could reflect uneven application of MCPA in the field trials or simply variation in the initial number of MCPA degraders. It is likely that MCPA degrading communities are not evenly distributed in the ballast but that variability is similar to what has been observed for 2,4-D degraders in agricultural soil, where degradation is spatially structured on a centimetre to microhabitat scale (Gonod et al., 2003; 2005). Since the railway was sampled approximately one year after the latest herbicide application, differences in numbers could also reflect resistance of the degrading organisms to predation and counter-selection mechanisms. Smith et al. (1994) demonstrated that an MCPA-degrading bacterial strain that was added to a field site contributed to enhanced degradation for at least 615 days after its application. However, when studying the loss of enhanced degradation of MCPA in a field site that had been repeatedly treated with MCPA, no significant differences in numbers of MCPA degraders were detected 204 days after the latest MCPA application (Smith and Aubin, 1994). In fact, although degradation of MCPA and 2,4-D is generally found to be enhanced following treatment, contrasting results have been found from studies that have attempted to enumerate degraders of phenoxyalkanoic acids in previously treated and untreated soils, possibly because soils were sampled at different times after cessation of herbicide treatments. Smith and Aubin (1991b) reported mean values of 127 and 193 2,4-D degrading organisms g^{-1} dry wt. soil in two untreated soils and 710 and 254 800 cells g^{-1} dry wt. soil for two treated soils. However, several studies have not detected any significant differences between the numbers of MCPA or 2,4-D degraders in treated and untreated soils (Torstensson et al., 1975; Holben et al., 1992).

4.7. Specific activity (q), specific growth rate (μ) and yield (Y)

A mean value of 1.432 pg MCPA $cell^{-1}$ h^{-1} for q was calculated from the relationship between MCPA degraders counted and qN_0 obtained by fitting mineralization data to Equation 1. This value was used for calculation of Y according to $Y = \mu/q$. Mai et al. (2001) reported a yield for the mineralization of MCPA by *Stenotrophomonas maltophilia* of $3.9*10^9$ cells $(mg\ C)^{-1}$ when grown in liquid culture, which corresponds to 2.1 cells $(pg\ MCPA)^{-1}$, and Stenström (1989) reported yield values for the degradation of 2,4-D by *Alcaligenes euthrophus* in liquid media of between 0.86-1.58 cells $(pg\ 2,4\text{-}D)^{-1}$. These values are about 2-70 times higher than those reported in the current study (Table 3). Lower yield values than theoretically possible for microbial growth on MCPA in soil have been observed by others (Bælum et al., 2006) and

could reflect factors such as predation by protozoa, lower availability of substrates and nutrient limitation. In fact, the specific growth rate and yield of MCPA degraders correlated with the nitrogen content of the ballast samples and it is thus evident that the formation of microbial cells from MCPA is nitrogen-limited on the railway. Hence, this could also be a contributing factor to the large variability in numbers that was observed in treatments 1 and 2.

A theoretical value for the number of MCPA degraders that could be formed following an application of MCPA to a railway can be estimated from the calculated yield values and the dose applied. An application rate of 2 l ha^{-1} of MCPA 750 with 750 g a.i. l^{-1} would give an applied amount of approx. 0.15 g MCPA per m^2. If we assume an even spread of MCPA in the top 10 cm of the railway embankment and a bulk density of 2 g cm^{-3} we would get a concentration of 0.75 µg MCPA g^{-1} dry wt. This value is consistent with concentrations determined in soil sampled from the top layer of the railway directly after spraying (data not shown). If we use the yield estimated from the degradation experiments (Table 3; average 0.18 cells pg^{-1} MCPA), we get a theoretical value of 135,000 MCPA degraders g^{-1} of soil. This value is similar to what was found in the sample from plot B3 that had the highest counted number in the MPN assay, but much higher than the values from the other plots.

4.8. Comparison between mineralization and degradation of MCPA

The experiment where MCPA degradation (remaining concentration) was assessed at the same time as mineralization (^{14}C -CO_2 release) revealed that the inflection point of the mineralization curve coincided in time with an almost complete disappearance of MCPA in the soil (Figure 5). Bergström and Stenström (1998) described the kinetics of 2,4-D degradation in soil and observed that an inflection point coincided in time with when there was no more water-extractable 2,4-D in the sample. However, in their study, the total concentration of 2,4-D was then still around 4 µg g^{-1} in the soil, indicating that the inflection point corresponded to a switch from growth-linked to desorption-controlled degradation kinetics. This is also consistent with the findings of Jensen et al. (2004), who observed an inverse relationship between sorption strength and mineralization rate of MCPA in soil, revealing that availability of MCPA can control degradation. Considering the above, our results suggest that almost the entire amount of MCPA in the railway ballast will be dissolved in the soil water phase and available for microbial degradation.

5. Conclusions

In order to minimize the environmental impact of weed control on railway tracks, it is important to consider the degradation kinetics of the herbicides. The results from this study illustrate the principal differences between cometabolically (diuron) and metabolically (MCPA) degraded herbicides. The degradation of the former type is dependent on the overall microbial activity of the ballast, whereas the degradation rate of the latter is successively increased because of microbial growth. Thus, the difference in persistence between these two types is likely to be more pronounced on railways, where overall microbial activity is very low, and hence co-metabolism proceeds much more slowly than in agricultural soils which are biologically more active.

Microbial biomass and activity of Swedish railways appear to be too low to sustain an environmentally acceptable rate of diuron degradation. The use of diuron on railway tracks is therefore associated with a high risk of groundwater contamination. Amendments with straw

or fertilizers to stimulate the microbial activity could potentially enhance diuron degradation but are not suitable alternatives for railways. If leaching is of concern a long-term strategy might be to incorporate an adsorptive layer in the construction of new railway embankments. Such a layer could potentially consist of burnt crop residues, which have been shown to be 400-2500 times more effective than soil at adsorbing diuron (Yang and Sheng, 2003).

Considering only mineralization kinetics, MCPA would appear to be a more suitable choice than diuron for weed control on railways. A sensible strategy could be to use a rather low dose when MCPA is applied the first time to allow for adaptation of the railway microflora. However, MCPA is known for its high mobility in soils and considering the high variability in the times required for 50% mineralization of previously treated ballast and the fact that the growth of microorganisms that degrade MCPA was nitrogen-limited, it is not certain that degradation will proceed fast enough everywhere on the tracks to guarantee that MCPA does not leach to groundwater.

Acknowledgements
The authors would like to thank Carl Westberg for help with establishment of the field experimental site. This work was funded by the Swedish Railway Administration.

References

Anderson, J.P.E. 1984. Herbicide degradation rate in soil: influence of microbial biomass. Soil Biology & Biochemistry 16, 483-489.

Audus, L.J. 1951. The biological detoxication of hormone herbicides in soil. Plant and Soil 3, 170-192.

Bælum, J., Henriksen, T., Hansen, H.C.B., Jacobsen, C.S. 2006. Degradation of 4-chloro-2-methylphenoxyacetic acid in top- and subsoil is quantitatively linked to class III *tdfA* gene. Applied and Environmental Microbiology 72, 1476-1486.

Bending, G.D., Shaw, E., Walker, A. 2001. Spatial heterogenity in the metabolism and dynamics of isoproturon degrading microbial communities in soil. Biology and Fertility of Soils 33, 484-489.

Bending, G.D., Lincoln, S.D., Sørensen, S.R., Morgan, J.A.W., Aamand, J., Walker, A. 2003. In-field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interactions between degradative *Sphingomonas* spp. and soil pH. Applied and Environmental Microbiology 69, 827-834.

Berger, B.M. 1999. Factors influencing transformation rates and formation of products of phenylurea herbicides in soil. Journal of Agricultural and Food Chemistry 47, 3389-3396.

Bergström, L., Stenström, J. 1998. Environmental fate of chemicals in soil. Ambio 27, 16-23.

Börjesson, E., Torstensson, L., Stenström, J. 2004. The fate of imazapyr in a Swedish railway embankment. Pest Management Science 60, 544-549.

Brinch, U.C., Ekelund, F., Jacobsen, C.S. 2002. Method for spiking soil samples with organic compounds. Applied and Environmental Microbiology 68, 1808-1816.

Cederlund, H., Stenström, J. 2004. Microbial biomass and activity on railway track and embankments. Pest Management Science 60, 550-555.

Cochran, W.G. 1950. Estimation of bacterial densities by means of the most probable number. Biometrics 6, 105-116.

Cox, L., Walker, A., Welch, S.J. 1996. Evidence for the accelerated degradation of isoproturon in soils. Pesticide Science 48, 253-260.
Cullington, J.E., Walker, A. 1999. Rapid biodegradation of diuron and other phenylurea herbicides by a soil bacterium. Soil Biology & Biochemistry 31, 677-686.
Giacomazzi, S., Cochet, N. 2004. Environmental impact of diuron transformation: a review. Chemosphere 56, 1021-1032.
Gonod, L.V., Chenu, C., Soulas, G. 2003. Spatial variability of 2,4-dichlorophenoxyacetic acid (2,4-D) mineralisation potential at a millimetre scale in soil. Soil Biology & Biochemistry 35, 373-382.
Gonod, L.V., Chadoeuf, J., Chenu, C. 2005. Spatial distribution of microbial 2,4-dichlorophenoxy acetic acid mineralization from field to microhabitat scale. Soil Science Society of America Journal 70, 64-71.
Gooddy, D.C., Chilton, P.J., Harrison, I. 2002. A field study to assess the degradation and transport of diuron and its metabolites in a calcareous soil. The Science of the Total Environment 297, 67-83.
Hassink, J., Klein, A., Kördel, W., Klein, W. 1994. Behaviour of herbicides in non-cultivated soils. Chemosphere 28, 285-295.
Helweg, A. 1987. Degradation and adsorption of ^{14}C-MCPA in soil - influence of concentration, temperature and moisture content on degradation. Weed Research 27, 287-296.
Hill, G.D., McGahen, J.W., Baker, H.M., Finnerty, D.W., Bingman, C.W. 1955. The fate of substituted urea herbicides in agricultural soils. Agronomy Journal 47, 93-104.
Holben, W.E., Schroeter, B.M., Calabrese, V.G.M., Olsen, R.H., Kukor, J.K., Biederbeck, V.O., Smith, A.E., Tiedje, J.M. 1992. Gene probe analysis of soil microbial populations selected by amendment with 2,4-dichlorophenoxyacetic acid. Applied and Environmental Microbiology 58, 3941-3948.
Jensen, P.H., Hansen, H.C.B., Rasmussen, J., Jacobsen, O.S. 2004. Sorption-controlled degradation kinetics of MCPA in soil. Environmental Science and Technology 38, 6662-6668.
Jones, W.J., Ananyeva, N.D. 2001. Correlations between pesticide transformation rate and microbial respiration activity in soil of different ecosystems. Biology and Fertility of Soils 33, 477-483.
Kirkland, K., Fryer, J.D. 1966. Pre-treatment of soil with MCPA as a factor affecting persistence of a subsequent application. In: Proceedings 8th British Weed Control 2, 616-621.
Klein, M. 2002. Die Berechnung der Versickerungsneigung von Pflanzenschutzmitteln in der Gleiskörperumgebung mit PELMO. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz Sonderheft XVIII, 405-412.
Lehmicke, L.G., Williams, R.T., Crawford, R.L. 1979. ^{14}C-most-probable-number method for enumeration of active heterotrophic microorganisms in natural waters. Applied and Environmental Microbiology 38, 644-649.
Liu, L.C., Cibes-Viadé, H., Koo, F.K.S. 1970. Adsorption of ametryne and diuron by soils. Weed Science 18, 470-474.
Lode, O., Meyer, K.F. 1999. Utvaskning av plantevernmidler - Proevefelt ved Olaloekka bru - Gardermobanen. 8/99. The Norwegian Crop Research Institute, Ås.
Madhun, Y.A., Freed, V.H. 1987. Degradation of the herbicides bromacil, diuron and chlortoluron in soil. Chemosphere 16, 1003-1011.
Mai, P., Jacobsen, O.S., Aamand, J. 2001. Mineralization and co-metabolic degradation of phenoxyalkanoic acid herbicides by a pure bacterial culture isolated from an aquifer. Applied Microbiology and Biotechnology 56, 486-490.

Mustafa, M.A., Gamar, Y. 1972. Adsorption and desorption of diuron as a function of soil properties. Soil Science Society of America. Proceedings. 36, 561-564.
Nordgren, A. 1988. Apparatus for the continuous, long-term monitoring of soil respiration in large numbers of samples. Soil Biology & Biochemistry 20, 955-957.
Ramwell, C.T., Heather, A.I.J., Shepherd, A.J. 2004. Herbicide loss following application to a railway. Pest Management Science 60, 556-564.
Rasmussen, J., Aamand, J., Rosenberg, P., Jacobsen, O.S., Soerensen, S.R. 2005. Spatial variability in the mineralisation of the phenylurea herbicide linuron within a Danish agricultural field: multivariate correlation to simple soil parameters. Pest Management Science 61, 829-837.
Schmidt, B., Siegesmund, B., Ehses, H., Zietz, E., Mersch, H. 1999. Risk of groundwater pollution from weed control on railway tracks. In: The XI Symposium Pesticide Chemistry, Cremona, Italy. 11-15 September. La Goliardica Pavese s.r.l.; Pavia; Italy 591-597.
Skark, C., Zullei-Seibert, N., Willme, U., Gatzemann, U., Schlett, C. 2004. Contribution of non-agricultural pesticides to pesticide load in surface water. Pest Management Science 60, 525-530.
Smith, A.D., Hughes, R.K., Wood, B.J.B. 1981. The microbiology of railway tracks in three urban locations. Environmental Pollution. 26, 193-201.
Smith, A.E., Aubin, A.J. 1991a. Effects of long-term 2,4-D and MCPA field applications on the soil breakdown of 2,4-D, MCPA, Mecoprop and 2,4,5-T. Journal of Environmental Quality 20, 436-438.
Smith, A.E., Aubin, A.J. 1991b. Metabolites of [^{14}C]-2,4-dichlorophenoxyacetic acid in Saskatchewan soils. Journal of Agricultural and Food Chemistry 39, 2019-2021.
Smith, A.E., Aubin, A.J. 1994. Loss of enhanced biodegradation of 2,4-D and MCPA in a field soil following cessation of repeated herbicide application. Bulletin of Environmental Toxicology 53, 7-11.
Smith, A.E., Aubin, A.J., Biederbeck, V.O. 1989. Effects of long-term 2,4-D and MCPA field applications on soil residues and their rates of breakdown. Journal of Environmental Quality 18, 299-302.
Smith, A.E., Mortensen, K., Aubin, A.J., Molloy, M.M. 1994. Degradation of MCPA, 2,4-D, and other phenoxyalkanoic acid herbicides using an isolated soil bacterium. Journal of Agricultural and Food Chemistry 42, 401-405.
Sørensen, S.R., Ronen, Z., Aamand, J. 2001. Isolation from agricultural soil and characterization of a *Sphingomonas* sp. able to mineralize the phenylurea herbicide isoproturon. Applied and Environmental Microbiology 67, 5403-5409.
Sørensen, S.R., Bending, G.D., Jacobsen, C.S., Walker, A., Aamand, J. 2003. Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. FEMS Microbiology Ecology 1524, 1-11.
Stenström, J. 1989. Kinetics of decomposition of 2,4-dichlorophenoxyacetic acid by *Algaligenes euthrophus* JMP134 and in soil. Toxicity Assessment 4, 405-424.
Stenström, J., Svensson, K., Johansson, M. 2001. Reversible transition between active and dormant microbial states in soil. FEMS Microbiology Ecology 36, 93-104.
Strange-Hansen, R., Holm, P.E., Jacobsen, O.S., Jacobsen, C.S. 2004. Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel. Pest Management Science 60, 570-578.
Tixier, C., Bogaerts, P., Sancelme, M., Bonnemoy, F., Twagilimana, L., Cuer, A., Bohatier, J., Veschambre, H. 2000. Fungal biodegradation of a phenylurea herbicide, diuron: structure and toxicity of metabolites. Pest Management Science 56, 455-462.

Tixier, C., Sancelme, M., Cuer, A., Veschambre, H. 2001. Degradation products of a phenylurea herbicide, diuron: synthesis, ecotoxicity and biotransformation. Environmental Toxicology and Chemistry 20, 1381-1389.
Tixier, C., Sancelme, M., Aït-Aïssa, S., Widehem, P., Bonnemoy, F., Cuer, A., Truffaut, N., Veschambre, H. 2002. Biotransformation of phenylurea herbicides by a soil bacterial strain, *Arthrobacter* sp. N2: structure, ecotoxicity and fate of diuron metabolite with soil fungi. Chemospere 46, 519-526.
Tomlin, C.D.S. 2003. The Pesticide Manual. The British Crop Protection Council, Alton, Hampshire, UK. 347-348.
Torstensson, L. 1974. Degradation of 2,4-D and MCPA in soils of low pH. Environmental Quality and Safety. Supplement Volume 3, 262-265.
Torstensson, L. 1983. Undersökning av diurons rörlighet och nedbrytning i banvallar SNV PM 1764. Swedish Environmental Protection Agency, Stockholm.
Torstensson, L. 1985. Komletterande undersökningar av diurons rörlighet och nedbrytning i banvallar SNV PM 2001. Swedish Environmental Protection Agency, Stockholm.
Torstensson, L. 2001. Use of herbicides on railway tracks in Sweden. Pesticide Outlook 12, 16-21.
Torstensson, L., Stenström, J. 1986. "Basic" respiration rate as a tool for prediction of pesticide persistence in soil. Toxicity Assessment: An International Quarterly 1, 57-72.
Torstensson, L., Börjesson, E. 2004. Use of imazapyr against *Equisetum arvense* on Swedish railway tracks. Pest Management Science 60, 565-569.
Torstensson, L., Stark, J., Göransson, B. 1975. The effect of repeated applications of 2,4-D and MCPA on their breakdown in soil. Weed Research 15, 159-164.
Torstensson, L., Cederlund, H., Börjesson, E., Stenström, J. 2002. Environmental problems with the use of diuron on Swedish railways. Pesticide Outlook 13, 108-111.
Torstensson, L., Börjesson, E., Stenström, J. 2005. Efficiacy and fate of glyphosate on Swedish railway embankments. Pest Management Science 61, 881-886.
Voos, G., Groffman, P.M. 1997. Relationships between microbial biomass and dissipation of 2,4-D and dicamba in soil. Biology and Fertility of Soils 24, 106-110.
Walker, A., Austin, C.R. 2004. Effect of recent cropping history and herbicide use on the degradation rates of isoproturon in soils. Weed Research 44, 5-11.
Widehem, P., Aït-Aïssa, S., Tixier, C., Sancelme, M., Veschambre, H., Truffaut, N. 2002. Isolation, characterization and diuron transformation capacities of a bacterial strain *Arthrobacter* sp. N2. Chemosphere 46, 527-534.
Yang, Y., Sheng, G. 2003. Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. Environmental Science and Technology 37, 3635-3639.

Table 1. Soil characteristics of the three railway embankments studied

Soil/treatment	pH	Loss on ignition[a] (%)	NH_4+ (mg/kg)	NO_3- (mg/kg)	Total N (mg/kg)	P[b] (mg/kg)	K[b] (mg/kg)	Mg[b] (mg/kg)
Mora A	6.23	1.92	ND	ND	ND	ND	ND	ND
Mora B	6.29	1.20	ND	ND	ND	ND	ND	ND
Mora C	6.46	0.77	ND	ND	ND	ND	ND	ND
Vetlanda A	5.78	1.25	ND	ND	ND	ND	ND	ND
Vetlanda B	6.33	0.72	ND	ND	ND	ND	ND	ND
Vetlanda C	6.30	0.51	ND	ND	ND	ND	ND	ND
Varberg 0a	5.17	0.69	0.06	0.56	0.62	12.9	5.01	2.67
Varberg 0b	5.65	0.61	0.02	0.35	0.37	8.70	4.92	6.14
Varberg 0c	6.08	0.98	0.06	1.16	1.22	13.9	14.9	16.3
Varberg 0d	5.44	0.85	0.06	0.77	0.83	16.2	10.4	5.79
Varberg 1a	6.20	1.20	0.09	3.27	3.36	15.9	19.3	25.9
Varberg 1b	5.30	0.73	0.03	0.74	0.77	11.1	1.26	5.17
Varberg 1c	5.94	1.05	0.05	1.84	1.89	11.2	14.0	16.9
Varberg 1d	5.62	0.84	0.11	3.28	3.38	13.4	8.78	10.3
Varberg 2a	5.16	0.80	0.04	0.20	0.24	13.5	15.6	6.45
Varberg 2b	5.27	0.80	0.04	0.56	0.60	15.1	9.31	4.70
Varberg 2c	4.92	0.88	0.06	0.69	0.75	15.1	15.3	7.80
Varberg 2d	5.42	0.72	0.10	2.26	2.36	12.2	9.27	4.15

Varberg 0-2 refer to treatments 0 = previously untreated with MCPA; 1 = sprayed one year prior to sampling with a mixture of BASF MCPA 750 and Roundup Bio; and 2 = sprayed for two consecutive years prior to sampling with the same mixture. a-d refer to different plots of the same treatment.

[a] Loss on ignition was measured as the weight loss from dried soil (105ºC) after 5h of incineration at 550ºC.

[b] P, K and Mg measured by extraction with ammonium lactate and acetate (Svensk Standard, 1993).

Table 2. Microbial respiratory activity from the three railway embankments studied

Soil/treatment	*r* (ng CO_2-C g^{-1} h^{-1})	Basal respiration (µg CO_2-C g^{-1} h^{-1})	SIR (µg CO_2-C g^{-1} h^{-1})
Mora A[a]	35.0 ± 10.7	0.320 ±0.016	1.021 ±0.282
Mora B[a]	30.1 ±4.24	0.139 ±0.008	0.487 ±0.113
Mora C[a]	9.06 ±3.90	0.070 ±0.017	0.288 ±0.045
Vetlanda A[a]	55.3 ±10.7	0.205 ±0.002	0.539 ±0.112
Vetlanda B[a]	0.34 ±0.41	0.065 ±0.040	0.241 ±0.046
Vetlanda C[a]	0.43 ±0.26	0.041 ±0.008	0.159 ±0.019
Varberg 0a	4.42 ±3.97	0.021 ±0.006	0.121 ±0.112
Varberg 0b	4.75 ±0.86	0.017 ±0.000	0.202 ±0.089
Varberg 0c	2.56 ±1.26	0.007 ±0.007	0.077 ±0.035
Varberg 0d	3.17 ±1.40	0.050 ±0.000	0.273 ±0.152
Varberg 1a	26.1 ±14.7	0.126 ±0.003	0.499 ±0.152
Varberg 1b	0.59 ±0.61	0.024 ±0.014	0.185 ±0.040
Varberg 1c	45.3 ±1.92	0.079 ±0.003	0.428 ±0.121
Varberg 1d	3.43 ±1.92	0.052 ±0.005	0.238 ±0.081
Varberg 2a	2.13 ±0.03	0.010 ±0.003	0.067 ±0.077
Varberg 2b	1.18 ±1.14	0.012 ±0.001	0.072 ±0.101
Varberg 2c	10.1 ±0.80	0.060 ±0.005	0.239 ±0.063
Varberg 2d	0.68 ±0.55	0.022 ±0.000	0.058 ±0.017

Varberg 0-2 refer to treatments 0 = previously untreated with MCPA; 1 = sprayed on year prior to sampling with a mixture of BASF MCPA 750 and Roundup Bio; and 2 = sprayed for two consecutive years prior to sampling with the same mixture. a-d refer to different plots of the same treatment.

[a] Values determined after contamination with diuron are shown.

Table 3. Number of MCPA degraders, specific activity of MCPA-degrading microorganisms and the yield

Soil/treatment	N_0 counted (g^{-1})	N_0 calculated (g^{-1})	μ (h^{-1})	Yield[b] (cells pg^{-1})
Varberg 0a	<20	ND	0.039 ± 0.017	0.027
Varberg 0b	<20	ND	0.059 ±0.029	0.041
Varberg 0c	<20	ND	0.176 ±0.110	0.123
Varberg 0d	<20	ND	0.114 ±0.011	0.080
Varberg 1a	130	2333	0.467 ±0.105	0.326
Varberg 1b	2050	1943	0.219 ±0.011	0.153
Varberg 1c	140000	22934	0.277[a]	0.193
Varberg 1d	2935	913	0.729 ±0.051	0.509
Varberg 2a	150	179	0.180 ±0.014	0.126
Varberg 2b	20	103	0.166 ±0.044	0.116
Varberg 2c	125	1534	0.278 ±0.055	0.194
Varberg 2d	7450	6661	0.414 ±0.077	0.289

Varberg 0-2 refer to treatments 0 = previously untreated with MCPA; 1 = sprayed on year prior to sampling with a mixture of BASF MCPA 750 and Roundup Bio; and 2 = sprayed for two consecutive years prior to sampling with the same mixture. a-d refer to different plots of the same treatment.

[a] μ only obtained from one replicate (see Materials and Methods section 2.8.2)

[b] Calculated as μ/q using the value 1.432 pg $cell^{-1}$ h^{-1} for q (see section 4.8)

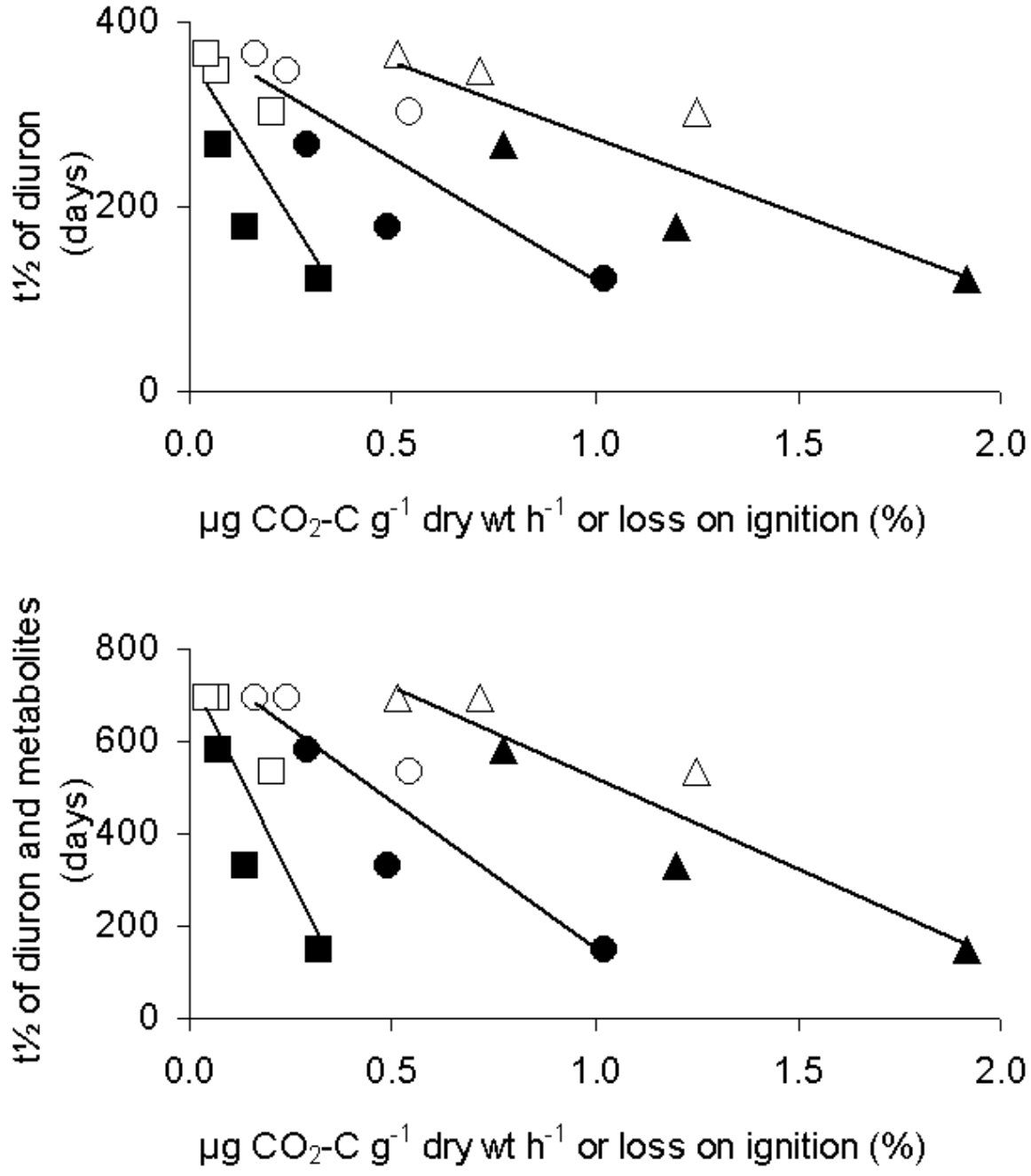

Figure 1. Linear regression between (a) t½ of diuron and loss on ignition in ballast samples from the embankments at Vetlanda (Δ) and Mora (▲) (R= -0.87; $p<0.05$), SIR at Vetlanda (○) and Mora (●) (R=-0.85; $p<0.05$) and basal respiration at Vetlanda (□) and Mora (■) respectively (R=-0.77; non significant). Linear regression between (b) t½ of the sum of diuron and its detected metabolites and loss on ignition in ballast samples from the embankments at Vetlanda (Δ) and Mora (▲) (R= -0.93; $p<0.05$), SIR at Vetlanda (○) and Mora (●) (R=-0.92; $p<0.05$) and basal respiration at Vetlanda (□) and Mora (■) respectively (R=-0.87; $p<0.05$).

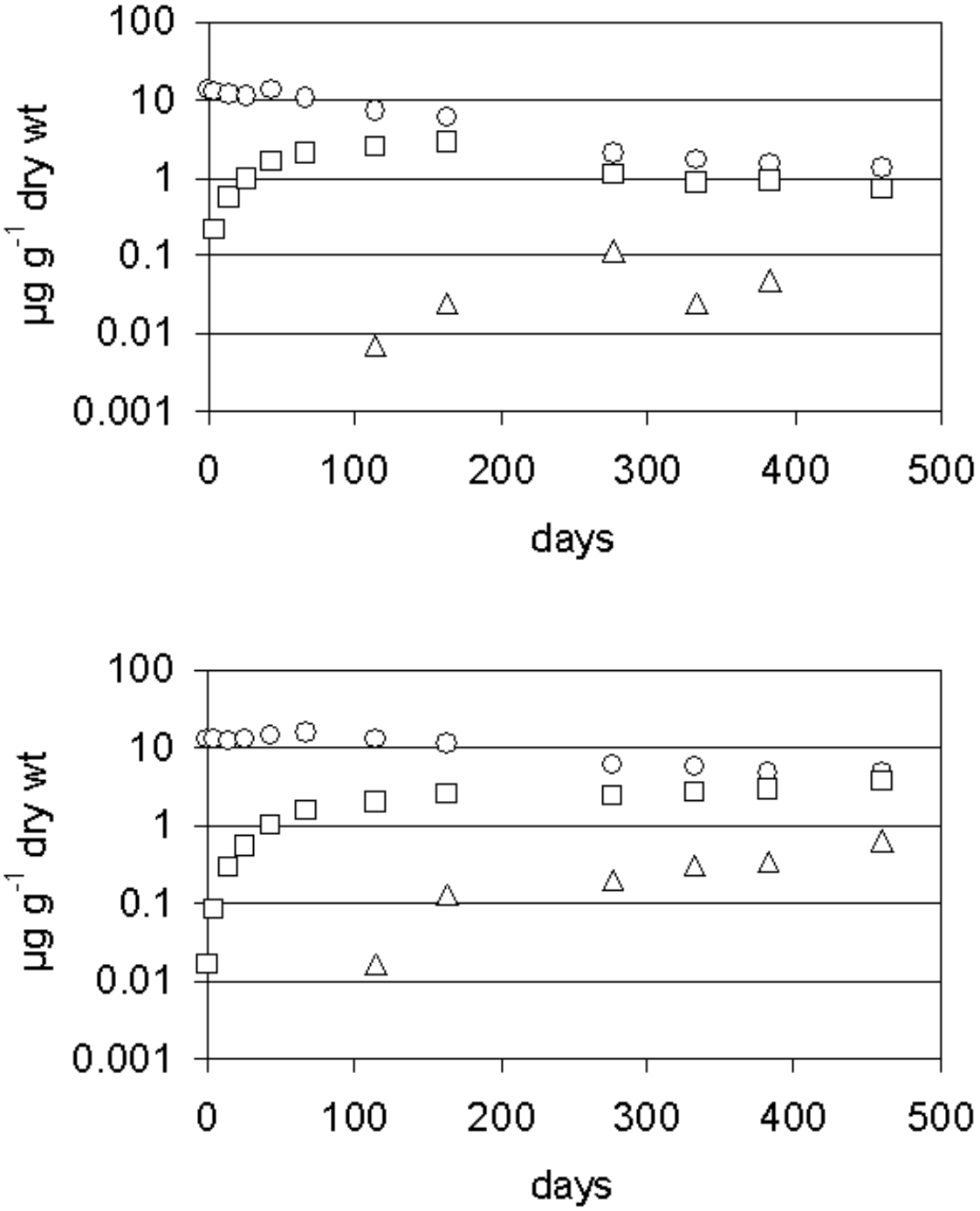

Figure 2. a). Degradation of diuron (○) and formation of the metabolites DCPMU (□) and DCPU (Δ) in the sample of railway ballast from the embankment at Mora with the highest SIR (Mora A). b). Degradation of diuron in the samples with lower SIR (here Mora C is shown as a typical example).

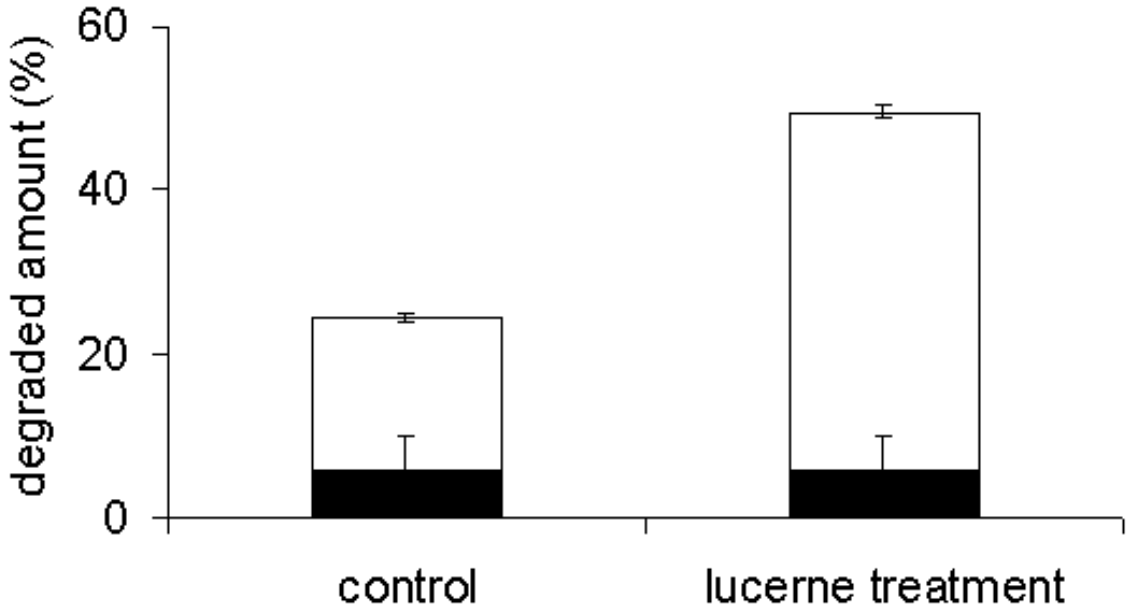

Figure 3. Stimulation of diuron degradation in a sample of railway ballast by the addition of ground lucerne straw. Degraded amount after 239 days of incubation divided into the demethylated fraction; DCPMU and DCPU (□) and the no longer detectable, possibly mineralised, fraction (■).

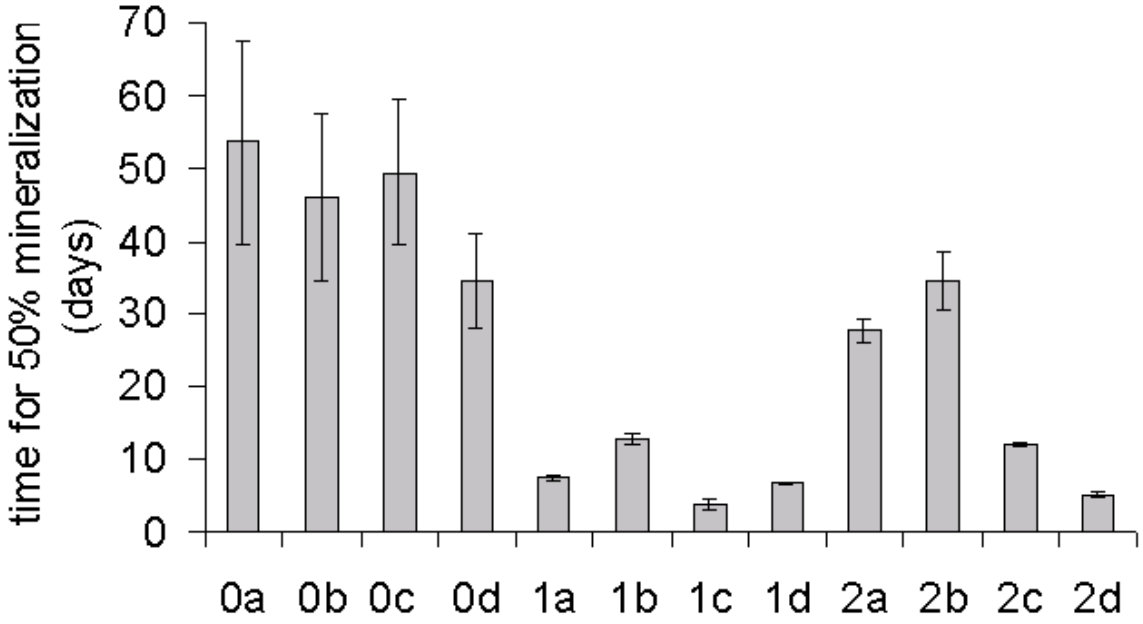

Figure 4. Time for 50% mineralization of MCPA in samples of railway ballast from four different plots of treatments 0 = previously untreated; 1 = sprayed one year prior to sampling with a mixture of BASF MCPA 750 and Roundup Bio; and 2 = sprayed for two consecutive years prior to sampling with the same mixture (n = 3 from each plot).

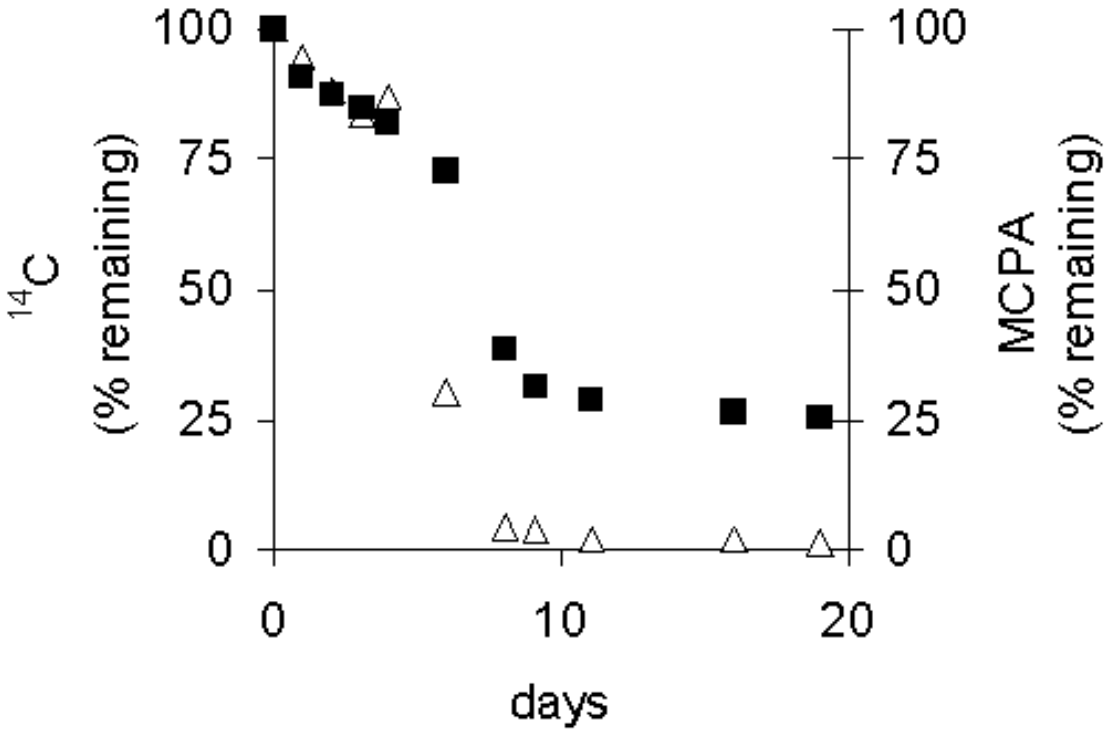

Figure 5. Comparison between mineralization (■) and degradation (Δ) of MCPA in the same sample of railway ballast.