Journal Pre-proofs

Ubiquity of microbial capacity to degrade metaldehyde in dissimilar agricultural, allotment and garden soils

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Please cite this article as: N. Balashova, S. Wilderspin, C. Cai, B.J. Reid, Ubiquity of microbial capacity to degrade metaldehyde in dissimilar agricultural, allotment and garden soils, *Science of the Total Environment* (2019), doi: https://doi.org/10.1016/j.scitotenv.2019.135412

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3 **Ubiquity of microbial capacity to degrade metaldehyde**

4 **in dissimilar agricultural, allotment and garden soils**

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23 **Abstract**

24 Metaldehyde is a molluscicide used to control slugs and snails. Despite its extensive use,

25 very little is known about the capacity of soil microbial communities to degrade this

26 chemical. This research provides a synopsis of the latent capacity of soil microbial

27 communities, present in agricultural (n = 14), allotment (n = 4) and garden (n = 10) soils, to

28 degrade metaldehyde. Extents of ^{14}C-metaldehyde mineralisation across all soils ranged from

29 17.7 to 60.0 %. Pre-exposure (*in situ,* in the field) to metaldehyde was not observed to

30 consistently increase extents of metaldehyde mineralisation. Where soils were augmented,

31 (*ex situ,* in the laboratory) with metaldehyde (28 mg kg^{-1}), the mineralisation capacity was

32 increased in some, but not all, soils (uplift ranged from +0.10 to +16.9 %). Results indicated

33 that catabolic competence to degrade metaldehyde was evident in both surface (16.7 - 52.8

34 %) and in sub-surface (30.0 - 66.4 %) soil horizons. Collectively, the results suggest that

35 catabolic competence to degrade metaldehyde was ubiquitous across a diverse range of soil

36 environments; that varied in texture (from sand to silty clay loam), pH (6.15 – 8.20) and soil

37 organic matter (SOM) content (1.2 % – 52.1 %). Lighter texture soils, in general, were

38 observed to have higher capacity to mineralise metaldehyde. Weak correlations between

39 catabolic competence and soil pH and soil organic matter content were observed; it was

40 noted that above a SOM threshold of 12 % metaldehyde mineralisation was always > 34 %.

41 It was concluded that the common occurrence of metaldehyde in EU waters is *unlikely* the

42 consequence of low potential for this chemical to be degraded in soil. It is more likely that

43 application regimes (quantities/timings) and meteorological drivers facilitate the transport of

44 metaldehyde from point of application into water resources.

45

47

1. Introduction

48

49 Slugs, snails and other gastropods are significant pests to a range of crops, including

50 agricultural, horticultural and garden plants (Rae *et al*., 2009). Metaldehyde (2,4,6,8-

51 tetramethyl-1,3,5,7-tetraoxcane) is a widely used molluscicide in agriculture and domestic

52 settings globally (including the UK, Europe, the United States and China (EPA, 2011; Gavin

53 et al., 2012; Ma eta., 2012; Zhongguo et al., 2013; EC, 2019)).

54 This pesticide is normally applied to crops in autumn and winter (Environment

55 Agency, 2009). The maximum recommended application rate of metaldehyde in the UK is

56 currently 210 g active substance/ha (from 1st August to 31st December); 700 g active

57 substance/ha is the maximum total dose per calendar year (Metaldehyde Stewardship Group

58 (MSG), 2019). Similar application rates are evident across Europe; allowing a maximum of

59 350 g active substance/ha per single treatment, with up to two treatments per year (EFSA,

60 2010). In the United States the recommended single application rate should not exceed 2240

61 g active substance/ha with a maximum of 6 applications per year (EPA, 2011).

62 Bait pellets release metaldehyde, under moderately moist conditions, for approximately 10

63 days (Puschner, 2006). Metaldehyde is relatively water soluble (190 mg L^{-1};PPDB, 2017)

64 and has as low K_{OW} value (0.12; Hall, 2010). Owing to, i) its physicochemical properties

65 (Table 1 in Supplementary Material), ii) application times that often coincide with wetter

66 periods (when molluscs are more prevalent, compared to dry weather conditions) and, iii) the

67 prevailing wet autumn/winter weather in the UK and northern EU countries, metaldehyde is

68 mobile in the environment. This mobility serves to transfer metaldehyde from soil to both

69 ground- and surface waters. Thus, metaldehyde presence in surface water and groundwater

70 has been reported with high frequency (Busquets et al., 2014; Hillocks, 2012).

71 Kay and Grayson (2014) reported peak concentrations of metaldehyde in the range

72 0.4 – 0.6 µg L^{-1} and highlighted that metaldehyde has been detected above the maximum

3

73   allowable concentration for drinking water of 0.1 µg L^{-1} (Council of the European

74   Communities, 2000) during the October – December periods, when slug pellets are typically

75   applied. These findings agree with metaldehyde concentration trends, observed by Castle et

76   al. (2018), who reported peak concentrations of metaldehyde in the stream water of the River

77   Thames Catchment to vary between 0.1 and 0.35 µg L^{-1} during September – January 2017.

78   The maximum concentration of 5 µg L^{-1} was recorded in November, and no metaldehyde

79   concentrations above 0.1 µg L^{-1} were recorded during the February – August period (Castle

80   et al., 2018). Concentrations up to 1.5 µg L^{-1} were reported in stream water of the same

81   catchment by Lu et al. (2017). Metaldehyde concentrations up to 2.2 µg L^{-1} were reported in

82   a UK chalk aquifer by (Bullock, 2014), with peak concentrations observed in January and

83   February. Metaldehyde presence in the aquatic environment has been reported in other

84   countries. Calumpang et al. (1995) reported maximum metaldehyde concentrations of 1.57

85   mg L^{-1}, in rice paddy water in the Philippines, following application and that concentrations

86   fell to below the detection limit within nine days (Calumpang et al., 1995). Metaldehyde

87   concentrations up to 6.98 µg L^{-1} were observed in run-off water from fish farming ponds in

88   northern France within the Moselle River Basin (Lazartigues et al., 2012).

89   A key factor underpinning metaldehyde fate and mitigating its transport is the latent

90   capacity of soil microbial communities to degrade this pesticide. Yet, literature relating to

91   microorganisms capable of metaldehyde degradation is limited to three studies. Thomas et al.

92   (2013, 2017) reported several metaldehyde-degrading bacterial strains that were isolated

93   from domestic soils (liquid cultures contained 100 mg L^{-1} metaldehyde); *acinetobacter* E1

94   was reported to degraded metaldehyde present in solution at concentrations less than 1 nM

95   (0.16 µg L^{-1}), other *acinetobacter* strains were reported to be unable to degrade the pesticide.

96   A laboratory study (EFSA, 2010), reported metaldehyde to be mineralised (50 -78 %) by soil

97 microbial communities under aerobic conditions; while under anaerobic conditions

98 metaldehyde was observed to be stable.

99 However, to date, no reports have been published that account the capacity (assessed

100 using ^{14}C-respirometry) of dissimilar soils from contrasting settings, to degrade metaldehyde.

101 Thus, this current research sought to establish the level of catabolic competence of soil

102 microbial communities to degrade metaldehyde (i.e. the competence of the microbial

103 community to break down metaldehyde molecules into smaller units that are subsequently

104 oxidised/mineralised to carbon dioxide). The current research considered soils obtained from

105 three contrasting settings: agricultural fields, allotments and gardens (and both surface and

106 sub-surface regimes). The research sought to establish intrinsic metaldehyde mineralisation

107 potential of the microbial community within these soils and the directing influence of

108 metaldehyde augmentation in terms of inducing metaldehyde degradation. Furthermore, it

109 was hypothesised that soil attributes, include texture, SOM and pH would have a shaping

110 influence upon levels of metaldehyde catabolic competence. These original lines of enquiry

111 provide a synopsis of metaldehyde biodegradation in dissimilar soils from contrasting

112 settings.

113

114 **2. Materials and Methods**

115 *2.1. Chemicals*

116 Metaldehyde pellets (1.5% active ingredient) were manufactured by Bayer. ^{14}C-

117 metaldehyde (UL-^{14}C; 5.1 mCi mmol^{-1}) was obtained from American Radiolabeled

118 Chemicals Inc. St Louis, USA. Ultima Gold and Ultima Gold XR liquid scintillation fluids

119 were purchased from Perkin Elmer, UK. Calcium chloride, ethanol, methanol and sodium

120 hydroxide were supplied by Fischer Scientific, UK; and dichloromethane provided by Sigma

121   Aldrich, UK. Mineral Basal Salt (MBS) components (namely: NaCl, $(NH_4)_2SO_4$, KNO_3,

122   KH_2PO_4, K_2HPO_4 and $MgSO_4.7H_2O$) were obtained from BDH, UK.

123

124   *2.2. Soils*

125   Soil was collected from three contrasting settings: agricultural fields, allotments and

126   gardens. Soils were collected in Norfolk and Essex, UK (Table 2). Soil samples (200 g) were

127   collected using a Dutch auger (0-10 cm for top soil; and, 40-50 cm for sub-soil samples);

128   four auger heads were combined to produce a single composite sample at each sampling

129   point and a given location was sampled in triplicate (within 5 m of each other). Between

130   sampling the auger head was thoroughly cleaned (washed with water and tissue, then sprayed

131   with 70% ethanol solution that was allowed to evaporate). Soils were transported to the

132   laboratory and stored (4 °C) in sealed plastic bags, for no more than 2 days, prior to

133   assessment of catabolic competence.

134   Soils were characterised in terms of their: SOM content (mass loss on ignition in a

135   muffle furnace (450 °C) for 12 h; 10 g (n = 3)) (Ghabbour *et al*., 2014); pH (samples

136   (3 g (n = 3) were combined with 30 mL of distilled water in a centrifuge tube, tubes

137   were then shaken (reciprocal shaker (IKA Labortechnik KS501) at 100 r.p.m for 14 h

138   and the soil water pH was measured using an electrode (Jenway) and meter (Mettler

139   Toledo FE20 Five Easy Benchtop pH Meter), and texture (samples of soil were

140   moistened and kneaded into a ball and texture determined following the hand-texture

141   framework of McDonald et al. (1998)). Soil characteristics are listed in Table 2, and

142   its expanded version could be found within the Supplementary Material.

143

144   *2.3. ^{14}C-radiorespirometry assessment of intrinsic and induced catabolic competence*

145   Prior to undertaking the respirometry, soils were transferred to the laboratory

146   incubator for 24 h to bring them back to a temperature of 18 °C. Soil samples (10 g) were

147   added to sterile Duran Schott bottles (250 mL) containing sterile MBS (30 mL) (0.3 g NaCl,

148   0.6 g $(NH_4)_2SO_4$, 0.6 g KNO_3, 0.25 g KH_2PO_4, 0.75 g K_2HPO_4 and 0.15 g $MgSO_4.7H_2O$

149   dissolved in 1 L of deionised water) (Hickman et al. (2008). To each bottle ^{14}C-metaldehyde

150   was added (100 Bq in 100 μL of ethanol). To capture $^{14}CO_2$ generated from the

151   mineralisation of ^{14}C-metaldehyde, a glass scintillation vial (7 mL) containing 1M NaOH (1

152   mL) was suspended (using a stainless-steel clip) from the top of a Teflon^{TM} lined

153   respirometer lid. Bottles were continuously shaken on an orbital shaker (IKA Labortechnik

154   KS501) at 100 r.p.m and the vials were removed and replaced periodically over the 120 h (5

155   d) assay time. Removed vials were wiped with a tissue, and Ultima Gold scintillation fluid (6

156   mL) added. Vials were sealed, shaken and stored in the dark (for a minimum of 24 h) and

157   then analysed by liquid scintillation counting (Perkin-Elmer TriCarb 2900TR liquid

158   scintillation analyser; count time 10 mins). Results were corrected for background radiation

159   using un-spiked respirometers (Reid et al., 2001). The respirometer system was previously

160   validated by Reid et al. (2001), who reported that up to 400 μmol CO_2 could be

161   accommodated in a single trap and a ^{14}C activity balance of 101 ± 8.9 % .

162   In order to assess the inducible capacity of soil microbial communities in response to

163   metaldehyde augmentation the above procedure was repeated with the addition of a

164   metaldehyde pellet to each respirometer bottle. Each pellet had a mass of 0.028 g and a

165   metaldehyde content of 1.5 %. Thus, each respirometer was dosed with the equivalent of 28

166   mg metaldehyde kg^{-1} soil. Sterile respirometers, containing MBS (30ml), were spiked with

167   ^{14}C-metaldehyde to evaluate abiotic degradation and volatilisation of ^{14}C-metaldehyde. All

168   respirometer assays were run in triplicate.

169

170 *2.4. Sample codes*

171   Samples have been coded to indicate: land use regime, Field (F), Allotment (A) and

172 Garden (G); the location qualifier (1-10; see Table 2); if samples were top soil (T) or subsoil

173 (S); if the *in situ* regime had metaldehyde application (p) or no metaldehyde application for

174 at least the last 4 years (n), and; if the *ex situ* laboratory assay was conducted in the presence

175 of a slug pellet (+) or its absence (-). For example, F2Tp+ corresponds to Field 2, a topsoil

176 sample that was exposed to metaldehyde *in situ* and was screened for catabolic competence

177 in the presence of a metaldehyde pellet. In presenting the data, soils have been organised

178 with lighter (sandier) textures presented first and heavier (clay) textures presented last.

179

180 *2.5. Statistics*

181   Significant differences between intrinsic and induced mineralisation levels were

182 established using ANOVA *post hoc* Tukey Tests (SPSS Statistics 22); a significance level of

183 0.05 (95 % level of confidence). Pearson's correlation test was applied to determine linear

184 correlation between mineralisation and pH/SOM values, a significance level of 0.05.

185

186 **3. Results**

187 *3.1. Control flasks and blanks*

188   Abiotic degradation/volatilisation of ^{14}C-metaldehyde was evident at a modest level

189 (7.8 $\pm$ 3.9 %). This value was commensurate with a fugacity (Mackay, 2001) driven pseudo-

190 equilibrium (theoretical value = 9.5 %), where: the respirometer MBS media volume was

191 30ml, the trap volume was 1 ml and the trap was changed three times over the assay period.

192 Background ^{14}C-radiation was negligible (0.06 % of the activity delivered in the respirometer

193 spike).

194

195 *3.2. Agricultural Field Soils (FT, FS)*

196 Intinsic catabloic competence (i.e. in asays with no metaldehyde pellet added (-)) was

197 ubiquitous across all agricultural field soils; mineralisation varied between 17.6 % (FT(p)7

198 and 31.0 % (FT(n)1) (Figure 1).

199 In most instances soils with light texture (FT(n)1, FS(n)2, FS(n)4 – sand, FT(n)2 –

200 loamy sand, FT(n)3 – FT(p)6 – sandy loam, FT(p)7 – silty loam), were observed to have

201 higher intrinsic capacites to mineralise ^{14}C-metaldehyde. Soils with heavier texture (FT(n)8,

202 FT(p)9 and FT(p)10 – loam, FT(p)11 – sandy loam clay, FT(p)12 – silty clay loam) were

203 observed to have lower intrinsic catabloic competence (Figure 1).

204 Similarly, induced (with pellet present (+)) catabloic competence was observed to be

205 higher in lighter textured soils (FT(n)1 – FT(p)7) than in heavier textured soils (FT(n)8 –

206 FT(p)12). This was also the case for the Field Subsoil samples (FS(n)2, FS(n)4 – sandy

207 texture), where an uplift in induced mineralisation was observed (+8.9 % and +0.1 %)

208 (Figure 1). The extent of induced mineralisation in FT (where a pellet was added to the

209 respirometer) varied from 16.5 % (FT(n)8) to 30.3 % (FT(n)3) (Figure 1); this range was

210 almost identical to the intrinsic catabloic competence range, suggesting that catabolism of

211 metaldehyde was operating at its maximum capacity before the pellet was added.

212 With the exception of FT(n)1 (light sandy texture) and FT(n)8 (medium loamy

213 texture), all agricultural soils that were not exposed to metaldehyde *in situ* (n) were observed

214 to show an uplift of catabolic competnce following the addition of a metaldehyde pellet (+)

215 (FS(n)2 – FT(n)5). Lighter FT soil textures included sand (FS(n)2, FS(n)4), loamy sand

216 (FT(n)2), sandy loam (FT(n)3 – FT(n)5). The same outcome was observed for light soils

217 where metaldehyde was used *in situ* (p) (FT(p)6 – sandy loam, FT(p)7 – silty loam).

218 FS(n)2 was the only sample among all agricultural Field soils in which a significant

219 difference between intrinsic and induced mineralisation was observed ($P < 0.05$) (+8.9 %)

9

220   (Figure 1). The maximum level of observed catabolic activity did not exceed 38.9 %

221   (induced mineralisation in FS(n)2 sample) in the Agricultural Field soils (Figure 1).

222

223   *3.3. Allotment Soils (AT, AS)*

224   Intinsic (-) catabloic competence was ubiquitous across all Allotment soils;

225   mineralisation varied between 34.3 % (AT(p)2) and 60.0 % (AS(n)1) (Figure 2). Similar to

226   the Field soils, Allotment soils with lighter texture (sand) exhibited higher intrinsic

227   mineralisation capacities when compared to soils with slightly heavier texture (loamy sand)

228   (Figure 2).

229   Relative difference between intrinsic (-) and induced (+) mineralisation in lighter

230   textured subsoils were also higher, particularly in soil with previous *in situ* metaldehyde

231   application history (p) (AS(p)2) (Figure 2). Sandy Subsoil sample (AS(n)1) with no previous

232   metaldehyde application had the highest metaldehyde mineralisation (both induced and

233   intrinsic).

234   Like Field soils, Allotment soils exhibited elevated mineralisation levels in the

235   presence the of metaldehyde (Figure 2). In the presence of metaldehyde, the extent of

236   mineralisation varied from 35.7 % (AT(p)2) to 66.4 % (AS(n)1) (Figure 2).

237   Only in the case of AS(p)2, intrinsic and induced levels of ^{14}C-metaldehyde

238   mineralisation were significantly different ($P < 0.05$) (a +9.9 % uplift in mineralisation was

239   observed). The maximum level of observed catabolic activity did not exceed 66.4 % (induced

240   mineralisation in AS(n)1 sample) (Figure 2).

241

242   *3.4. Garden soils (GT)*

243   As observed for Field and Allotment soils, competence to degrade metaldehyde in

244   garden soils was ubiquitous across soil types (Figure 3). In the absence of a metaldehyde

245 pellet, the extent of intrinsic metaldehyde mineralisation varied from 28.9 % (GT(n)7) to

246 52.8 % (GT(n)6) (Figure 3).

247 In general, as was the case with Field soils (Figure 3), lighter textures (sandy loam

248 and loamy sand); GT(n)1 through GT(n)6 indicated higher levels of catabolic competence to

249 mineralise metaldehyde when compared to heavier textures (sandy clay loam and sandy clay)

250 (Figure 3).

251 In the presence of metaldehyde all soils showed elevated levels of mineralisation

252 (Figure 3); the extent of mineralisation varied from 39.9 % (GT(n)2) to 53.0 % (GT(n)6).

253 Uplift in mineralisation, in the presence of a metaldehyde pellet (+), was greatest for soils

254 observed to have lower intrinsic catabolic competence; conversely, where soils were

255 observed to already have high catabolic competence only small increases (a few %) in

256 mineralisation were observed following metaldehyde augmentation (e.g. GT(n)1 and GT(n)6;

257 Figure 3).

258 In several instances the augmentation resulted in significant ($P < 0.05$) increases in

259 mineralisation GT(n)3, GT(n)7, GT(n)9 and GT(n)10; +14.4 %, +15.3 %, +12.8 % and +16.9

260 %, respectively). Again, as observed for Field and Allotment soils, the maximum catabolic

261 capacity of 50-55 % appeared to be a ceiling beyond, which catabolic capacity was not

262 exceeded.

263

264 **4. Discussion**

265 The degradation of any pesticide depends upon its physical and chemical

266 characteristics, e.g. aqueous solubility and inherent recalcitrance (Semple et al., 2003) and

267 the physical, chemical and biological properties of the soil (Rao *et al*., 1983), such as pH,

268 redox conditions, matrix attributes, carbon:nitrogen:phosphorus (C:N:P) elemental ratio,

269 temperature, moisture content (Arias-Estévez et al., 2007). Presence/absence/activity of

270 catabolic enzymes in soils affect pesticide degradation directly (Deng et al., 2016) while

271 pesticide bioavailability/bioaccessibility indirectly influence pesticide degradation (Arias-

272 Estévez et al., 2007). Additionally, pesticide transport, biological degradation and chemical

273 transformation processes are affected by application regime (rates/methods and timing), as

274 well as hydrological and weather conditions (Borgesen et al., 2015). Thus, site-specific

275 physical, chemical and biological properties control the fate and transport of pesticides in the

276 environment and determine the variation in spatial distribution of pollutants.

277

278 *4.1. Soil Microbe Response to Chemicals Inputs*

279   The ability of microbial communities to respond to organic compounds (e.g.

280 pesticides) presence/augmentation is well documented for a range of compound classes,

281 including: several semi-volatile hydrocarbon pollutants (Kelsey and Alexander 1997; Reid et

282 al., 2002; Springael and Top, 2004; Hickman et al., 2008), pesticides (Duah-Yentumi and

283 Johnson, 1986; Reid et al., 2005; Bending et al., 2006; Posen et al., 2006; Trinh et al., 2012;

284 Reid et al., 2013) and antibiotics (Islas-spinosa et al., 2012; Bennet et al., 2017). These

285 studies confirm the capacity of microbial communities to respond to organic compound input

286 by becoming more catabolically competent (Reid et al., 2005; Bending et al., 2006; Posen et

287 al., 2006; Reid et al., 2013). For example, Reid et al. (2005) reported soil microbial

288 communities, of initially low catabolic competence, to degrade the herbicide isoproturon,

289 (mineralisation C. 5 %) to increase in their competence following the incubation of soil with

290 a low (0.05 μg kg^{-1}) application of the herbicide (mineralisation increased to C. 40 %). In

291 column studies, Trinh et al. (2012) reported three phases of attenuation/degradation of these

292 herbicides isoproturon and MCPA: an initial sorption phase, followed by an

293 acclimatisation/adaptation phase and a final rapid degradation phase (resulting in complete

294 removal of the herbicides).

295   Several studies on biodegradation of metaldehyde have been reported, for example,

296   Thomas et al. (2013, 2017) isolated and characterised metaldehyde-degrading bacteria in

297   domestic soils. They reported Acinobacter E1 strain to be able to degraded metaldehyde to a

298   concentration below 1 nM. However, to date, the response of soil microbial communities,

299   present in agricultural, allotment and garden soils, to metaldehyde augmentation has not been

300   reported. Thus, our results confirm the potential for soil microbial communities to increase in

301   their competence to degrade metaldehyde following exposure. In keeping with observations

302   for other compounds, metaldehyde catabolic competence was observed to increase

303   significantly, following slug pellet addition (in some cases increasing by a factor of 2).

304   Largest increases in catabolic competence following metaldehyde augmentation were

305   observed for FS(n)2 (+8.9 %), AS(p)2 (+9.9 %), GT(n)3, GT(n)7, GT(n)9 and GT(n)10

306   (+14.4 %, +15.3 %, +12.8 % and + 16.9 %, respectively).

307   In contrast to other pesticides, where low catabolic competence is exhibited in

308   unexposed soils, high levels of intrinsic catabolic competence to degrade metaldehyde were

309   observed (up to 66.0 %). Metaldehyde is a cyclic tetramer of sub-units that can depolymerise,

310   through microbial activity, into acetaldehyde (Castle et al., 2017; Tomlin, 2003). High levels

311   of metaldehyde degradation in the soil environment have been reported in the literature. For

312   example, Bieri (2003) reported fast degradation rates of metaldehyde in agricultural soils in

313   Germany; with, DT_{50} values ranging from 5.3 to 9.9 days. Coloso et al. (1998) reported

314   metaldehyde concentration in pond sediment to rapidly decrease from an initial concentration

315   of 80 mg kg^{-1} to 1 mg kg^{-1} after 15 days. Ma et al. (2012) studied metaldehyde residues in

316   agricultural soils in China and reported metaldehyde residue of up 9 mg kg^{-1} to decrease

317   below 0.3 mg kg^{-1} over 7 days. While Calumpang et al. (1995) reported metaldehyde

318   concentrations in paddy soil to fall from 0.13 mg kg^{-1} to below the analytical detection level

319   within 22 days.

13

320   We suggest the ubiquity of high levels of catabolic competence observed in our

321 research are likely due to the degradation of the simple metaldehyde molecule to

322 acetaldehyde (the primary degradation product), and the subsequent degradation of

323 acetaldehyde to acetate; this being assimilated into Krebs tricarboxylic acid (TCA) Cycle

324 (Tomlin, 2003) and respired as carbon dioxide.

325

326 *4.2. Catabolic competence and its relationship with soil properties*

327   All soil types, drawn from all settings (Field, Allotment and Garden), were observed

328 to exhibit significant levels of catabolic competence. As already highlighted, soil texture had

329 a shaping influence on the extent of ^{14}C-metaldehyde mineralisation; with sandy soils

330 supporting, in general, higher level of catabolic competence. It is widely recognised that soil

331 texture has a substantial influence on the soil environment. It controls soil porosity, and thus,

332 has a directing influence on soil hydrology (Luna et al., 2017) and soil atmosphere (Pagliai et

333 al., 2004). In turn, these drivers exert a shaping influence on soil microbial community

334 structure (Fierer, 2017). Schroll et al. (2006) reported optimum pesticide mineralisation at a

335 soil water potential of −0.015 MPa; pesticide mineralisation was markedly reduced when soil

336 moisture approached soil water holding capacity.

337   Acknowledging the considerable influence soil texture has on soil moisture

338 conditions, it is unsurprising that levels of catabolic competence observed have been

339 influenced by soil texture. We suggest that the higher levels of catabolic competence for

340 metaldehyde, observed in the lighter soil textures, could be linked to a higher redox potential

341 in these more freely drained soils (Voroney and Heck, 2015). These conditions would,

342 putatively, support a more active microbial community with greater capacity to degrade

343 organic substrates (including metaldehyde). In general, pesticide degradation (Fenner et al.,

344 2013), and metaldehyde degradation specifically (EFSA, 2010), have been reported to be

345 faster under aerobic conditions.

346 Beyond its influence on soil, physical, hydrological and biological attributes soil

347 texture also controls pesticide bioavailability (Gavrilescu, 2005). Numerous studies have

348 sustained the general trend that lighter sandy soil textures assist biodegradation by

349 maintaining high pesticide bioavailability and, in contrast, heavier clay textures tend to

350 facilitate greater sorption and entrapment of pesticide (e.g. Reid et al., 2000; Gavrilescu,

351 2005). Thus, heavier clay textures tend to decrease the potential for degradation though

352 stronger sorption. These strong interactions have been reported to preclude the opportunity

353 for pesticides to induce catabolic competence (Reid et al., 2013).

354 In addition to their texture, the dissimilar soils also varied in their SOM content. SOM

355 has been reported to influence the fate, behaviour and biodegradation of pesticides (Hatzinger

356 and Alexander, 1995). However, to date, there have been no reports accounting how soil

357 properties (specifically, SOM and pH) influence the biodegradation of metaldehyde by soil

358 microbial communities. To elucidate any such relationships, SOM and soil pH were

359 correlated with ^{14}C-metaldehyde mineralisation under intrinsic and induced regimes and

360 across all settings (Figure 4).

361 SOM varied (from 1.17 % to 52.14 %) across the dissimilar soil types obtained from

362 contrasting settings (Table 2) and extent of mineralisation in these soils also varied greatly

363 (from 16.51 % to 66.44 %). Considering all soils, ^{14}C-metaldehyde mineralised was observed

364 to increase with increasing SOM for both intrinsic and induced assessment (Figure 4A).

365 While the correlations between mineralisation extent and SOM were not significant ($r = 0.34$,

366 $p = 0.08$; intrinsic and induced mineralisation vs. SOM); the data supports the conclusions

367 that i) beyond a SOM content of 12% metaldehyde mineralisation was consistently > 34%,

368 and, ii) where SOM content was less than 12% metaldehyde mineralisation was observed

15

369   across a very broad range (from 16.5 to 60 %) (Figure 4A). These results suggest that efforts

370   to sustain SOM levels in soil could assist in promoting higher levels of metaldehyde

371   degradation, and thus, reduce the opportunity for metaldehyde to transfer to water resources.

372   In one hand, SOM controls sorption of pesticides in soil (Chiou et al., 1983). Sorption

373   is responsible for retention of pesticides in soil, preventing leaching and decreases pesticide

374   bioavailability (Singh, 2008). While, on the other hand, SOM is the cornerstone of soil food

375   webs, and its amount and quality underpins microbial diversity and its capacity to utilise a

376   broad range of substrates (Neumann et al., 2014). With regards to metaldehyde, as a

377   relatively water soluble compound (190 mg L^{-1}) and as a labile carbon source (Bieri, 2003;

378   EFSA, 2010), we suggest sorption onto SOM is unlikely to be a significant influence on

379   biodegradation. It more likely that SOM has a synergistic influence on metaldehyde

380   biodegradation as it acts as a primer for microbial activity. The higher levels of catabolic

381   competence observed to be synonymous with SOM content of >12% (Figure 4A) support

382   this linkage.

383   Where pH was correlated with mineralisation across all soil types and regimes, no

384   relationship was observed for intrinsic mineralisation ($r = 0.19$, $p = 0.34$) (Figure 4B). A

385   slightly positive correlation was observed between increasing pH and induced mineralisation

386   ($r = 0.44$, $p = 0.02$) (Figure 4B). More useful, perhaps, is the observable distinction between

387   soils of pH lower than 6.9, where mineralisation never exceed 30 %, and soils where pH was

388   greater than 6.9, and mineralisation was more often observed to be greater than 35 % (Figure

389   4B). Thus, while pH influence on pesticide degradation has been reported for other

390   compounds (e.g. atrazine (Houot et al., 2000) and pirimicarb and metsulfuron-methyl (Kah et

391   al., 2007)), it influence upon metaldehyde mineralisation was inconsistent.

392

393   *4.3. Wider Context*

394   The results reported herein highlight soil microbial communities, in dissimilar soils

395   under Agriculture, Allotment and Garden regimes, to all have a considerable latent capacity

396   to degrade metaldehyde (Figures 1-3). Our results suggest that soil microbial communities

397   across these regimes, and present in both top-soil and sub-soil, are well predisposed to

398   degrade metaldehyde. We suggest that it is unlikely that the, at times, high levels of

399   metaldehyde detected in water (Castle et al., 2017; Kay and Grayson, 2014) are due to low

400   degradation capacity in the soil system. It is more likely that runoff and fast leaching of

401   metaldehyde is the main driver underpinning the high incidence and high concentrations of

402   metaldehyde sometimes reported in water resources (Calampung et al., 1995; Coloso et al.,

403   1998; Council of the European Communities, 2000; Bieri, 2003; Hillocks, 2012; Ma et al.,

404   2012; Busquets et al., 2014; Lu et al., 2017).

405   With metaldehyde being applied in autumn and winter, when slug populations are

406   higher due to wet weather (and when young crops are most vulnerable), the opportunity for

407   metaldehyde transport is increased. The situation is further antagonised by metaldehyde

408   having a relatively high aqueous solubility (190 mg L^{-1}). In support of this view there is

409   considerable evidence that pesticides applied to the soil surface can be transported rapidly,

410   bypassing the unsaturated soil zone, to groundwater (Arias-Estévez et al., 2007; Johnson et

411   al., 1995; Lopez-Perez et al., 2006). Indeed, metaldehyde has frequently been detected in

412   groundwater at levels higher than the EU Drinking Water Framework Directive limit (0.1 μg

413   L^{-1}) (EC, 1998); in some cases, concentrations of metaldehyde of up to ten times this limit

414   have been reported (UKWIR, 2013).

415   Given its ubiquity in water resources, metaldehyde has been subject to scrutiny,

416   voluntary initiatives and evolving regulation. Specifically, in the UK the Get Pelletwise

417   campaign of the Metaldehyde Stewardship Group (MSG, formed in 2008), aimed to promote

17

418 sustainable use of metaldehyde by applying principles of Integrated Pest Management and

419 introducing guidelines for metaldehyde application (MSG, 2019). This guidance

420 recommended, the use of the minimum amount of active compound per hectare; that soil

421 conditions, topography and fields proximity to watercourses are factors to be considered in

422 assessing the risk of metaldehyde loss to streams, and; that metaldehyde application is

423 discouraged during heavy rain events and if field drains are flowing (MSG, 2019). However,

424 metaldehyde is still regularly detected at the concentrations above the DWD limit of 0.1 μg

425 L^{-1} (Castle et al., 2017; Lu et al., 2017). Thus, in order to mitigate metaldehyde transfer still

426 further a reduction in the nominal loading of metaldehyde in pellets (e.g. from 3 % to 1.5 %

427 active ingredient) and the development of pellets that afford stronger metaldehyde

428 attenuation might offer further opportunity for improvements.

429 We highlight that soil itself is likely to be a significant reservoir of metaldehyde. With

430 respect to this soil burden, the results reported herein suggest that, there is good prospect

431 that, given time, the indigenous soil microbial communities will degrade this reservoir of

432 metaldehyde. However, further research regarding the levels of microbial catabolic activity,

433 specifically under lower substrate concentrations, should be undertaken.

434

435 **5. Conclusions**

436 Results indicate substantial catabolic competence to degrade metaldehyde in soils

437 with various texture (from sand to silty clay loam), pH (6.15 – 8.20) and organic matter

438 content (1.2 – 52.1 %). Ubiquitous catabolic competence was observed in both topsoil (16.7

439 – 52.8 %) and subsoil horizons (30.0 – 66.4 %). In general, soils with lighter texture (sand,

440 sandy loam and loamy sand; average mineralisation 37.3 %) had higher levels of ^{14}C-

441 metaldehyde mineralisation when compared to soils with heavier texture (sandy clay, sandy

442 clay loam and silty clay loam; average mineralisation 33.3 %). When soils were augmented

443 with metaldehyde (in the laboratory) an increase in mineralisation was observed in some, but

444 not all soils (up to 16.9 % increase in the Garden Soil GT(n)10 (sandy clay)). Overall, pH

445 and organic matter content were weakly correlated with ^{14}C-metaldehyde mineralisation.

446 However, soils with higher SOM (>12%) were, in general, observed to support higher levels

447 of metaldehyde mineralisation. It is suggested that the higher SOM status of these soils

448 exerted a beneficial shaping influence upon soil microbial communities and their capacity to

449 degrade metaldehyde. Collectively, results suggest that the concentrations of metaldehyde

450 (that are at times high), detected in water, are unlikely due to insufficient microbial capacity

451 to degrade this pesticide. It is suggested that application regime (rate and timing), the high

452 mobility of metaldehyde and its loss to the watercourses via runoff and leaching are the

453 driving factors underpinning the ubiquity of metaldehyde in surface and ground water

454 resources. To reduce metaldehyde runoff to watercourses, the application timing should not

455 coincide with wet weather conditions. The use of pellets with reduced concentrations of

456 metaldehyde and development of the pellet products with stronger attenuation capacity could

457 further assist in the effort to reduce metaldehyde transfer to the aquatic environment.

458

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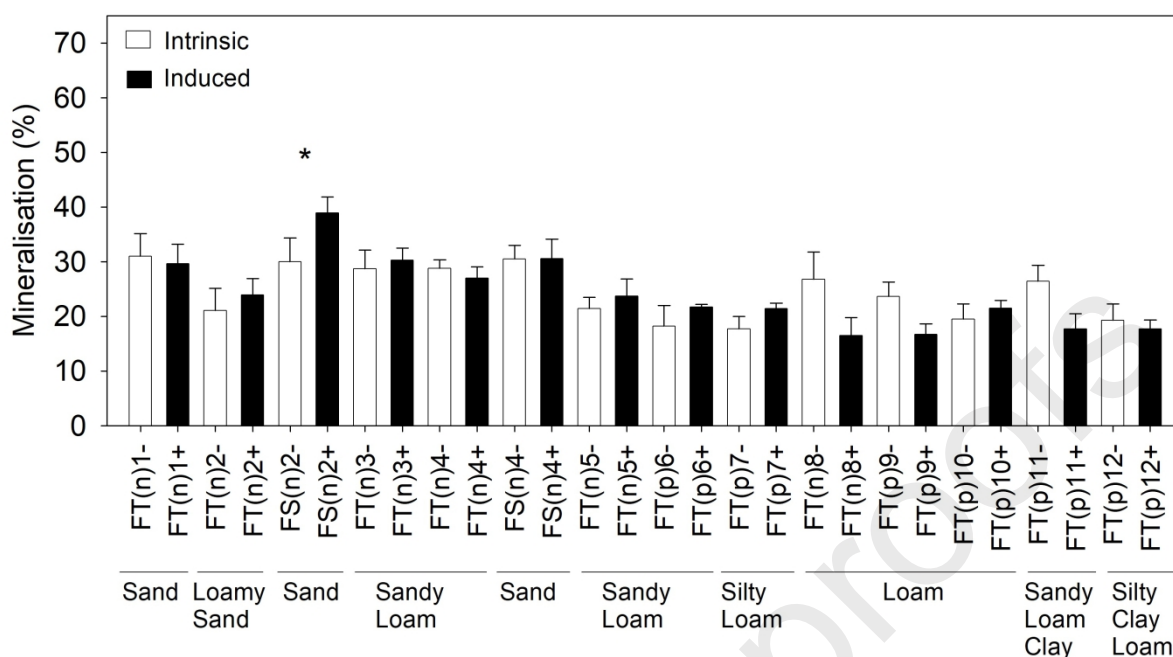European Journal of Soil Science, 54 (2003), pp. 809-818

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Figures

Figure 1. Catabolic competence (^{14}C-metaldehyde mineralisation (%) after 5 days assay time) in Field topsoil (FT(n)1-FT(p)12) and Field subsoil (FS(n)2, FS(n)4): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each texture class. Error bars are standard error of the mean (n = 3). A star indicates significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

Figure 2. Catabolic activity (^{14}C-metaldehyde mineralisation (%) after 5 days assay time) in Allotment soils (AT – Allotment topsoil, AS – Allotment subsoil): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each class. Error bars are standard error of the mean (n = 3). A star indicates significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

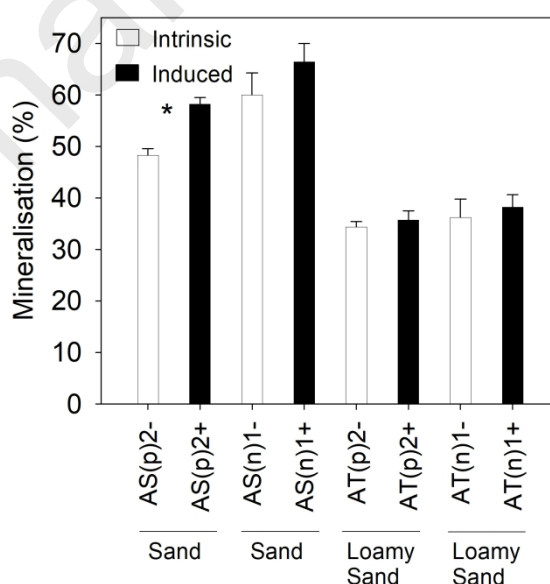**Figure 3.** Catabolic activity (^{14}C-metaldehyde mineralisation (%) after 5 days assay time) in Garden soils (GT(n)1-GT(n)10): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each class. Error bars are standard error of the mean (n = 3). Stars indicate significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

Figure 4. Correlation of intrinsic (black), and induced (white), catabolic activity (% mineralisation) with: OM (A) and pH (B); for, Field soils (O), Allotment soils (□) and Garden soils (△). Errors bars are $\pm$ 1 standard deviation (n = 3). Lines of best fit indicates correlations between intrinsic (solid) and induced (dashed) mineralisation capacity and SOM (A) and pH (B).
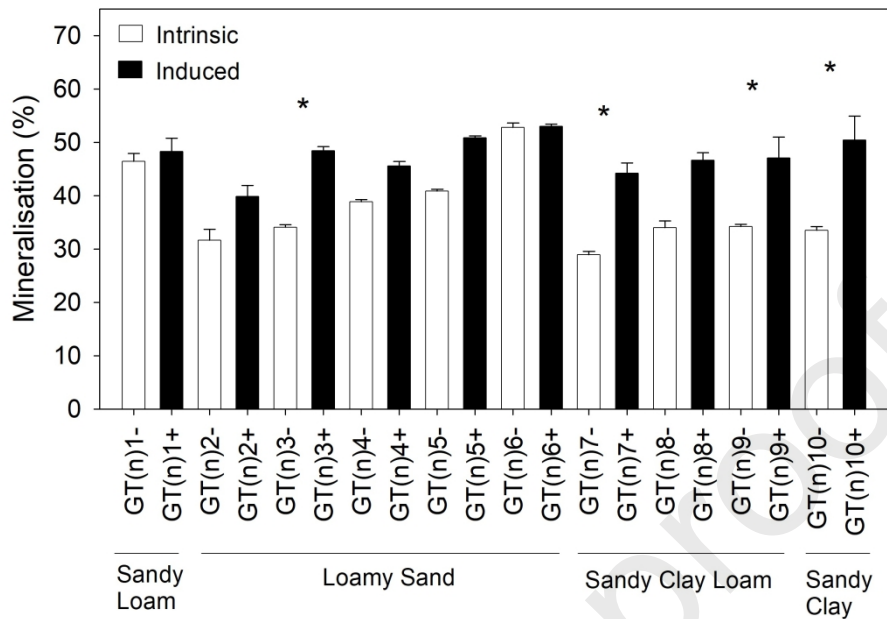
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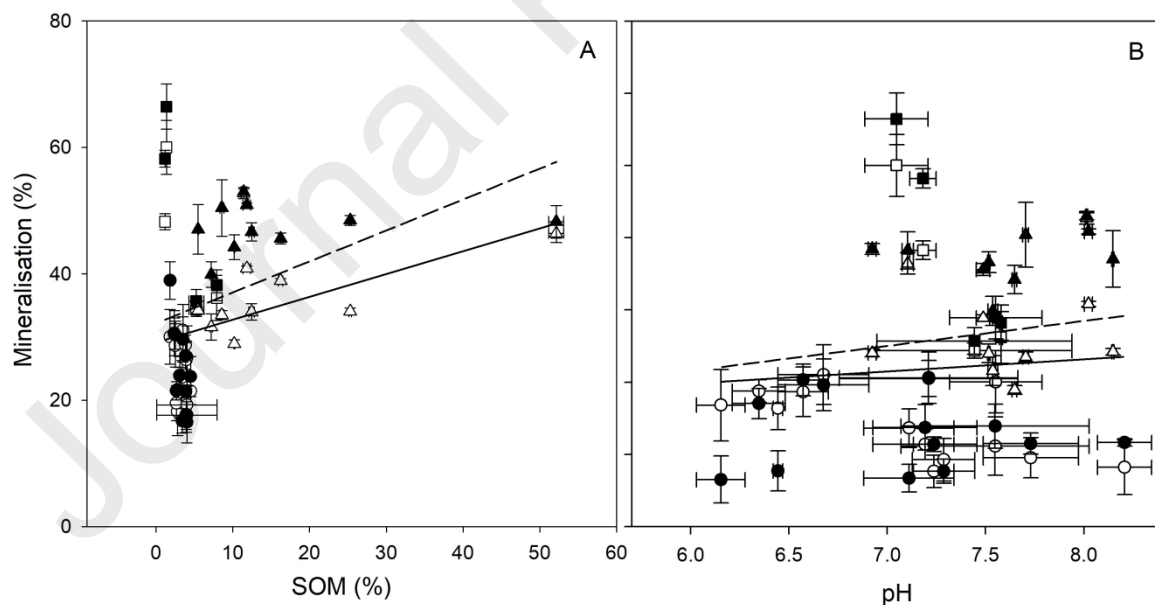

Figure 3. Catabolic activity (^{14}C-metaldehyde mineralisation (%) after 5 days assay time) in Garden soils (GT(n)1-GT(n)10): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each class. Error bars are standard error of the mean (n = 3). Stars indicate significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

Figure 4. Correlation of intrinsic (black), and induced (white), catabolic activity (mineralisation, %) with: OM (A) and pH (B); for, Field soils (O), Allotment soils (□) and Garden soils (△). Errors bars are ± 1 standard deviation (n = 3). Lines of best fit indicates correlations between intrinsic (solid) and induced (dashed) mineralisation capacity and SOM (A) and pH (B).

29

Table 2. Field, Allotment and Garden soil properties.

Soil Code	Setting	Texture	Metaldehyde application	OM (%)	pH
FT(n)1	Field 1	Sand	>4 years ago	3.49 ± 0.1	6.68 ± 0.23
FT(n)2	Field 2	Loamy Sand	>4 years ago	3.08 ± 0.2	7.55 ± 0.5
FS(n)2	Field 2	Sand	>4 years ago	1.82 ± 0.4	7.55 ± 0.2
FT(n)3	Field 3	Sandy Loam	>4 years ago	2.53 ± 0.1	6.57 ± 0.1
FT(n)4	Field 4	Sandy Loam	>4 years ago	3.85 ± 0.03	6.35 ± 0.1
FS(n)4	Field 4	Sand	>4 years ago	2.38 ± 0.1	7.21 ± 0.4
FT(n)5	Field 5	Sandy Loam	>4 years ago	4.52 ± 0.3	7.19 ± 0.3
FT(p)6	Field 6	Sandy Loam	Seasonal (ongoing)	2.79 ± 0.2	8.2 ± 0.1
FT(p)7	Field 7	Silty Loam	Seasonal (ongoing)	3.89 ± 0.1	7.24 ± 0.1
FT(n)8	Field 8	Loam	>4 years ago	4.02 ± 0.3	6.15 ± 0.1
FT(p)9	Field 9	Loam	Seasonal (ongoing)	3.4 ± 0.1	7.11 ± 0.2
FT(p)10	Field 10	Loam	Seasonal (ongoing)	2.67 ± 0.1	7.73 ± 0.2
FT(p)11	Field 11	Sandy Clay Loam	Seasonal (ongoing)	3.96 ± 0.3	6.44 ± 0.02
FT(p)12	Field 12	Silty Clay Loam	Seasonal (ongoing)	4.02 ± 3.9	7.29 ± 0.2
AT(n)1	Allotment 1	Loamy Sand	No previous application	7.91 ± 0.4	7.58 ± 0.01
AS(n)1	Allotment 1	Sand	No previous application	1.36 ± 0.3	7.05 ± 0.2
AT(p)2	Allotment 2	Loamy Sand	Seasonal (ongoing)	5.24 ± 0.1	7.44 ± 0.5
AS(p)2	Allotment 2	Sand	Seasonal (ongoing)	1.17 ± 0.1	7.18 ± 0.1
GT(n)1	Garden 1	Sandy Loam	>6 years ago	52.1 ± 1.0	7.1 ± 0.03
GT(n)2	Garden 2	Loamy Sand	>6 years ago	7.2 ± 0.2	7.54 ± 0.03
GT(n)3	Garden 3	Loamy Sand	>6 years ago	25.3 ± 0.2	6.92 ± 0.02
GT(n)4	Garden 4	Loamy Sand	>6 years ago	16.2 ± 0.3	7.49 ± 0.04
GT(n)5	Garden 5	Loamy Sand	>6 years ago	11.8 ± 0.5	8.02 ± 0.02
GT(n)6	Garden 6	Loamy Sand	>6 years ago	11.4 ± 0.4	8.01 ± 0.01
GT(n)7	Garden 7	Sandy Clay Loam	>6 years ago	10.2 ± 0.1	7.65 ± 0.01
GT(n)8	Garden 8	Sandy Clay Loam	>6 years ago	12.4 ± 0.4	7.52 ± 0.01
GT(n)9	Garden 9	Sandy Clay Loam	>6 years ago	5.5 ± 0.1	8.15 ± 0.01
GT(n)10	Garden 10	Sandy Clay	>6 years ago	8.6 ± 0.3	7.7 ± 0.02

Highlights

- Results indicated ubiquitous catabolic competence to degrade metaldehyde in dissimilar soils

- Metaldehyde catabolic competence was evident in garden, allotment and field soils

- Metaldehyde mineralisation ranged from 17.7 to 60.0 %

- Higher levels of catabolic competence were observed in the lighter soil textures

- Pre-exposure to metaldehyde sometime, but not always, resulted in higher catabolic competence