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Fate of pendimethalin in soil and characterization of non-extractable residues (NER)

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Abstract

One important route of degradation of herbicide pendimethalin (PM) in soil leads to formation of non-extractable residues (NER). To investigate NER nature (irreversibly, chemically bound or strongly sorbed and entrapped) residues of ^{14}C-labelled PM in soil were investigated after conventional extraction with organic solvents by silylation. After 400 days of incubation, 32.0% of applied radioactivity (AR) was transformed into NER, 39.9% AR remained extractable. Mineralization reached 26.2% AR. Additionally, ^{14}C-PM was incubated in soil amended with compost for 217 days to investigate the influence of organic amendments on NER formation. NER amounted to 37.8% AR, with 57.9% AR remaining extractable. Mineralization was negligible (1.4% AR). For all sampling times only low amounts of radioactivity were entrapped (< 5% AR). PM was present only in trace amounts (ca. 0.4% AR), other released residues consisted of undefined fractions (sum ≈2% AR). Silylation overall resulted in release of higher amounts of radioactivity (19% AR). Addition of compost led to an increase in potential entrapment and sorption sites for PM, forming higher amounts of strongly sorbed, entrapped residues. Furthermore, potential release of non-extractable PM residues was investigated by incubation of solvent-extracted and fresh soil for

additional 3 months. NER were partly mineralized (7% AR) and 20% became extractable with organic solvents. However, no PM or any known metabolites were found.
It can be concluded that no parent PM was found and NER of PM in soil are mainly formed by covalent binding to organic matrix with only low potential of remobilization under natural conditions.

Keywords: 14C-labelled; environmental fate of pesticides; organic amendments

1. Introduction

All pesticides deliberately added to the environment during farming are degraded to metabolites, become mineralized by microbial degradation, and form non-extractable residues NER in solid matrices like soil. The fate of chemicals in soil is determined by soil organic carbon content, texture, ion exchange properties, and pH as well as substance properties like the lipophilicity, adsorptivity, presence of functional groups prone for covalent binding to humic matter and (bio-)degradability (Barriuso et al., 2008). Here, we particularly focus on the characterization of the herbicide pendimethalin (PM) derived NER in soil. The substance readily sorbs to soil colloids (Aktar et al., 2008) due to its high K_{ow} and low water solubility (Table 1); thus mobility and the risk of leaching to ground and surface water are generally considered to be low (Petersen et al., 2003; Alister et al., 2009; EFSA, 2016). The strong sorption of PM to soil is also indicated by its high Freundlich adsorption coefficient K_{FOC} (Pedersen et al., 1995; Weber, 1990).
The degradation of PM in soil was reviewed in EFSA (2016) and Vighi et al. (2017). Kulshrestha and Singh (1992) found two metabolites, namely 6-amino-pendimenthalin and 3,4-dimethyl-2,6-dinitroaniline, which were also detected in culture studies of two soil fungi (Singh and Kulshrestha, 1991). The authors suggested that 6-amino-pendimenthalin resulted from the reduction of one of the two nitro-groups and 3,4-dimethyl-2,6-dinitroaniline from N-dealkylation. In the peer review of PM (EFSA, 2016) yet another metabolite was reported to be formed in an aerobic soil study, i.e. 2-methyl-3,5-dinitro-4-(pentan-3-ylamino)benzoic acid (here denominated as 4-carboxyl-pendimethalin). 4-carboxyl-pendimenthalin and 6-amino-pendimethalin were used in the present investigation as reference substances to identify these possibly formed metabolites (see Figure 1).
The examination and speciation of NER is more elaborate compared to that of the analysis of the extractable portion. NER of pesticides in soil have been traditionally valued either as a safe sink for such residues (Bollag et al., 1992; Gevao et al., 2000), or as being immobilized in soil as a long-term storage system. Soil may then act as a slow-release system acting as a source with potential of

remobilization of residues over long periods (Kästner et al., 2018; Schäffer et al., 2018). Kästner et al. (2014) classified three operational types of NER: Type I NER represent strongly adsorbed, sequestered residues, which in fact may be slowly released during the degradation of humic matter, type II NER are formed from metabolites covalently bound to the soil matrix with negligible release potential, while type III NER refer to biogenic residues consisting of biomolecules (amino acids, phospholipids etc.) derived from complete microbial metabolism of the parent compound and subsequent anabolism to biomass. Type III NER are thus of no environmental concern.

Organic amendments such as biochar, straw, farmyard manure or compost are frequently used in agriculture to maintain soil fertility and to increase microbial activity (Hoitink and Boehm, 1999; Tu et al., 2006; Lehmann et al., 2011; Harter et al., 2014; Tejada and Benítez, 2014). Such amendments, therefore, also affect the fate of pesticides as pointed out by e.g. Barriuso et al. (1997). In line with this, Felsot and Dzantor (1995) and Johnson et al. (1997) demonstrated that pesticides may be subjected to faster degradation in amended soils. On the other hand, organic amendment may add sorption sites for the xenobiotic compound and may enhance the formation of NER. In addition, microbial degradation activity may also be altered by the presence of easily degradable compounds and thus may influence the degradation of hardly degradable pesticides.

Therefore, the aim of the present study was to test these effects, i.e. enhanced/retarded degradation and/or enhanced NER formation of PM after compost addition. Incubation studies with ^{14}C-labelled PM were performed both in unamended and compost-amended soil. An additional aim was to distinguish the various types of NER formed. The main focus was laid on the mechanisms responsible for the association of the residues with the soil matrix, i.e., whether NER are strongly sorbed and entrapped or covalently bound as this is unknown for PM. Extracted soil containing only NER was thus subjected to a chemical derivatization procedure (silylation) to identify the binding mechanism as well as incubated with fresh soil to investigate if PM-derived residues in the soil may become bioavailable and mineralized.

2. Material and Methods

2.1. Chemicals

Solvents used were of HPLC grade (methanol, HPLC gradient grade, Merck, Darmstadt; acetonitrile, Ultra gradient HPLC grade J.T. Baker, Deventer NL). PM as unlabeled analytical standard (chemical purity > 99%) as well as ^{14}C-labeled substance (14C-U ring label) was provided by BASF, Germany (radioanalytical purity: 99.7%, specific radioactivity: 10.9 MBq/mg). PM (Figure 1) belongs to the

class of dinitroaniline herbicides. Its chemical and physical characteristics are summarized in Table 1.

2.2. Soil and soil-compost properties

The standard soil used was LUFA 2.2 (Landwirtschaftliche Untersuchungsanstalt Speyer, Germany), a loamy fine sand, stored for max. 2 weeks at 4 °C in the dark and acclimatized to 20±2°C for 14 days before the experiments were performed at a soil moisture of 50% maximum water holding capacity. The plant litter compost was derived from a local supplier (gabco Kompostierung GmbH, Aachen, Germany). For the soil compost mixture, 20% (w/w) of compost were added to the soil. The parameters of the soil alone and the soil-compost mixture are summarized in Table 2.

2.3. Incubation of pendimethalin in soil and soil-compost mixtures

Incubation experiments were performed (referring to OECD test 307) with PM in unamended soil or in soil amended with 20 weight-% of compost. ^{14}C-PM was applied at a concentration of 5 mg kg^{-1} soil or soil-compost mixture, corresponding to 5.25 kBq kg^{-1} soil and 5.36 kBq kg^{-1} soil compost mixtures. 100 g soil samples and 50 g soil-compost samples, respectively, were placed in closed glass bottles (1000 mL and 500 mL volume, respectively). Incubation was performed in triplicates at 20 °C ± 2 °C in the dark, water content was adjusted to 50% of WHC_{max} every two weeks. Formation of $^{14}CO_2$ was quantified with devices consisting of traps with 20 g soda lime inside of the vessels, exchanged every four weeks, and Liquid Scintillation Counting (LSC, HIDEX, sample mixed with scintillation cocktail LumaSafe™ plus or Ultima Gold, both Perkin Elmer, Rodgau, Germany). Volatile organics were trapped with about 5 cm^3 paraffin-coated glass wool (Merck KGaA, Germany) and also quantified by LSC. Incubation times for soil alone were 58, 204 and 400 days, and for compost amended soil 50, 117 and 217 days.

2.4. Extraction procedures and total NER

The soil was extracted under gentle shaking (160 rpm) for 30 minutes with the following three solvent mixtures: A: MeOH + 2% HCl, B: MeOH:H_2O, 7:3 (v/v) and C: MeOH:H_2O, 1:1 (v/v). Each extraction step was performed twice before moving to the next solvent. This extraction method provided a complete extraction (=100%) of residues directly after application. The amount of residues remaining non-extractable was quantified by combustion of aliquots of the extracted and homogenized soil with an Oxidizer OX501 (Harvey Instruments/Zinsser Analytic) and LSC.

2.5. Chromatographic analyses (TLC and HPLC), radioanalysis

Extracts were analyzed for parent compound and its degradation products by radioanalytical thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC).

HPLC was conducted on an Agilent 1100 system, equipped with DAD detector (279 nm) and RAMONA radioanalytical detector (Raytest, Straubenhardt, Germany). The analyses were conducted with a C18 HD column (250x4.6 mm, 5 µm particle size, Macherey Nagel, Düren, Germany) at 25 °C. Initial conditions were 75% solvent A (MilliQ water with 0.1% formic acid), and 25% solvent B (acetonitrile with 0.1% formic acid). This ratio was held for 5 minutes. Amount of B increased in 4 minutes to 65%, was held for 7 minutes and afterwards increased to 95% in 4 minutes. This was held for 7 minutes. Amount of B was decreased to 25% within 6 minutes and was held for another 7 minutes till the end of the run. The flow rate was 1 ml min^{-1}.

TLC analyses were performed with a Radioactivity-Scanner (Rita StarFor, Raytest, Berlin, Germany). TLC plates (SIL G-25 UV 254, Macherey-Nagel) were developed first in a mixture of toluene and methanol, 9:1, v:v + 1% acetic acid. After drying, the plate was developed in the same direction using a second solvent mixture consisting of toluene and MeOH 7:3, v:v + 1% acetic acid.

Reference substances were the parent PM and the metabolites 4-carboxyl-pendimenthalin and M455H001 (see Figure 1).

2.6. Silylation of NER

For determination of the binding mode of the radiolabeled NER to soil, selected samples were silylated according to Berns *et al.* (2005). In order to differentiate type-I and type-II NER (see introduction), solvent-extracted soil or soil-compost mixture were silylated to release potentially sequestered PM-derived residues. When subjected to silylation, protons of functional groups of soil organic matter (COOH, OH, …) are exchanged by trimethyl silyl groups. Thus, hydrogen bridge bonds cannot be formed anymore and, thereby, organic matter disperses into smaller fragments, releasing physically entrapped contaminants. In contrast, this procedure keeps the compounds covalently bound to the soil organic matter after silylation (Haider et al., 1992, 1993 and 2000; Dec et al., 1997). For silylation, aliquots of extracted soil were dried by lyophilization and weighed into a Schlenk flask. Silylation reaction requires complete water and oxygen exclusion, thus the reaction flasks and the sample were kept under protective gas (Argon). All solvents used (chloroform, acetone) were dried over molecular sieve before use.

Thirty ml of dried chloroform, 1.5 g NaOH pellets and 5 ml of trimethylchlorosilane (TMCS, Sigma Aldrich) were added to the sample (500 mg NER containing soil) and stirred for 2 h. Afterwards 1.5 g NaOH pellets and 5 ml TMCS were added and the mixture was stirred overnight. The supernatant was collected and the soil was washed three times with 10 ml acetone each. Finally, the

soil was stirred with 30 ml chloroform. After centrifugation (2,800 x g, 5 min, 5 °C), all supernatants were combined and were evaporated to dryness (<40 mbar to remove eventually formed hexamethylsilane) and the residue re-dissolved in chloroform. The extract was evaluated by radioanalytical TLC and radioanalytical HPLC (see chapter 2.5). To check the recovery, the remaining soil was combusted and the residual radioactivity determined.

2.7. Chromatographic evaluation of silylated samples

The chromatographic behavior (TLC) of the silylated samples was compared to the silylated and untreated reference substances PM, 4-carboxyl-pendimenthalin and M455H001. The solvent system used for development of TLC plates (same as described in 2.5) was toluene/MeOH 9:1 (v:v) + 1 vol-% acetic acid. Developed plates were evaluated with a TLC scanner (Rita StarFor, Raytest, Berlin, Germany) and a BioImager (Fuji, Straubenhardt, Germany).

Samples were analyzed by HPLC with the aforementioned (section 2.5) method and system.

2.8. Remobilization of NER by addition of fresh soil

In order to investigate whether NER are bioavailable for microorganisms as indicated by mineralization, thoroughly extracted soil, containing only NER, was mixed with fresh soil. Such extracted soil, previously incubated with PM for 58 days, was incubated with fresh soil for three months (30 weight-% "NER soil" in fresh soil, 30 g NER soil mixed with 80 g fresh soil, on dry weight basis, 2 replicates). The same procedure was performed on extracted soil previously incubated for 400 days with PM (10 weight-% NER soil in fresh soil, 10 g NER soil + 90 g fresh soil, on dry weight basis, 2 replicates). During incubation with fresh soil, evolving $^{14}CO_2$ was trapped in soda lime and the amount of radioactivity in the trapping agent determined every 4 weeks and at the final day of incubation. At the end of incubation, soil was extracted two times with MeOH + 2 vol-% HCl, then two times with MeOH:H_2O, 7:3 (v/v), and finally two times with MeOH:H_2O, 1:1 (v/v), to check whether parts of the NER became extractable after incubation with fresh soil.

2.9. Statistical analyses

Calculations (derivation of mean values and standard deviations) were made using Microsoft EXCEL (Version 2016). Derivation of DT_{50}-values was accomplished using the software CAKE (Computer Aided Kinetic Evaluation, Version 3.2, Tessella, a free web-tool, generating fits for assessing degradation kinetics).

3. Results and Discussion

3.1. Distribution of radioactivity

PM was incubated in soil to determine its fate (turnover mass balance), in particular the amounts of extractable residues (ER), non-extractable residues (NER), as well as those mineralized ones. One study was conducted with soil (LUFA 2.2) alone, another study with addition of compost to the soil (20 dry weight-%). In Table 3, the distribution of the applied radioactivity (AR) is summarized.

Recovery of radioactivity was always >97% AR in samples with soil alone and >90% AR in compost-amended soil. Volatile residues other than $^{14}CO_2$ never exceeded 0.02% AR and are therefore not included in Table 3.

In both scenarios, soil alone and soil amended with compost, the amounts of NER increased and ER decreased during incubation time. For soil alone, mineralization accounted for 11.1±3.7% AR after 204 days and 26.2±2.2% AR after 400 days (end of study). In soil amended with compost, mineralization was rather negligible reaching an amount of only 1.4±0.03% AR after 217 days (end of study). Shorter incubation time in the compost amended soil (217 days *vs* 400 days in the "soil only" experiment) was considered due to the considerably lower mineralization rate in the amended soil. Thus compost supplementation obviously decreased microbial degradation activity against PM.

TLC analysis (results also displayed in Table 3) of the extracts of incubated soil (without amendment) revealed the presence of the parent compound PM at 31.0±10.6% AR (52.0±9.6% of the ER) after 204 days of incubation. This amount decreased to 20.4% AR (50.4±8.2% of the ER) after 400 days of incubation. The amount identified as ^{14}C-PM incubated in the soil-compost mixture decreased from 68.2% AR (92.7±0.3% of ER) after 50 days to 47.9% AR (82.73±0.7% ER) after 217 days (study end). Results were confirmed by radioanalytical HPLC. With these values, a DT_{50} was estimated for both scenarios, although not enough data points were available for a reliable calculation (OECD 307) since the focus of the study was the investigation of NER. Thus, DT_{50} estimates should be considered only as rough indicators. For incubation in soil alone, a DT_{50} of 140 days was estimated (single first order kinetics, r^2=0.98), which is in the range reported by other authors in different soils (Vighi *et al.*, 2017). In soil amended with compost in the present study, a DT_{50} of 210 days (hockey stick kinetics, r^2=0.9914) was estimated.

Complete mineralization of ^{14}C-PM to $^{14}CO_2$ was much higher in soil alone than in compost amended soil. 11.1% AR were mineralized in soil alone compared to ten times less (1.4% AR) in compost amended soil after 204 and 217 days, respectively.

Our results confirm the findings by Barriuso *et al.* (1997) that for some pesticides, including PM, compost addition reduces the mineralization, presumably due to increased sorption to the matrix, also observed for triasulfuron (Said-Pullicino et al., 2004) and glyphosate (Getenga and Kengara,

2004). Others, however, reported increased microbial activities after soil amendment and faster degradation of pesticides, for instance atrazine (Getenga, 2003), methyl isothiocyanate (Dungan et al., 2003), and glyphosate (Alexa et al., 2009).

NER-formation was higher in soil amended with compost compared to soil alone at comparable incubation times. 28.3% of NER were found for ^{14}C-PM incubated in soil alone for 204 days while 38.2% AR of NER were formed in soil amended with compost after 217 days of incubation. This argues for an enhanced immobilization, and hence a lower bioavailability of PM in soil amended with compost. Organic waste (like compost) addition to soil increases the amount of organic substances in the soil and may increase the amount of voids for sequestration and the number of potential sorption sites for various pesticides, thus reducing their accessibility to microorganisms (Senesi et al., 2001; Barker and Bryson, 2002; Wanner et al., 2005). The microbial degradation of PM was obviously lower in the presence of compost because of strong binding and sequestration in soil organic matter. Moorman et al. (2001) investigated the influence of organic amendment to soil on the fate of several herbicides, including the herbicide atrazine. They found enhanced degradation of atrazine upon soil amendment. The authors suggested that atrazine is mainly used as an N source and only its side chain carbon is readily used by microorganisms. Therefore, for atrazine degradation the addition of compost provides other carbon substrates which are used for biomass production. Although PM may also provide an N source in the present study, other carbon sources did not improve microbial degradation. Thus we can conclude, based on the remaining high amounts of parent PM in the compost extracts, that the effect of compost amendment on dissipation of PM is mainly due to sequestration in the added organic matter and not, as in the case of atrazine, an effect on its biodegradability.

3.2. Characteristics of non-extractable residues

For NER from ^{14}C-PM incubated in unamended soil, the release by means of silylation amounted to 1.8±0.4% (58-day samples), 3.4% (204-day samples) and 3.1±0.8% (400-day samples) of AR (from thoroughly extracted soil that was previously incubated with PM for 58 days and 204 days, respectively). Given in % of NER, 15.5±2.5% (58-day samples), 13.5% (204-day samples) and 9.6±2.2% (400-day samples) of NER could be released. Over time, the absolute NER amount that could be released by silylation increased because of the higher formation of NER in later degradation phases. However, the relative amount of tightly bound NER, which cannot be released by silylation, increased with incubation time.

For ^{14}C-PM incubated in soil amended with compost, much larger amounts of NER were released by silylation, for instance in the 217-day sample more than 50% of the NER were released,

corresponding to 20% of AR, respectively (Figure 2). In comparison, in soil not amended with compost the NER release by silylation was less than 5% of AR throughout the incubation. With regard to these results, it is concluded that a higher percentage of the PM derived residues in soil amended with compost are just sequestered, whereas in unamended soil, a large percentage of PM residues is covalently bound.

Extracts derived by silylation of thoroughly extracted unamended soil containing only NER after 400 days were analyzed for parent compound and known degradation products by radioanalytical HPLC (Figure 3). In this sample, 2.5% of the AR (mean of 5 repetitions) had been released by the silylation procedure.

About a third of the injected radioactivity (32.3%, corresponding to about 0.8% of AR) eluted early (after 3 minutes) indicating polar compounds of unknown structure or compounds associated with co-eluting organic matter. 20.1 and 24.0% of the injected radioactivity (peak area percentages, corresponding to 0.5% and 0.6% AR, respectively) eluted after 12.5 and 14.5 minutes, respectively. The latter peak may originate from metabolite 4-carboxyl-PM as retention times were similar, although a mass spectrometric verification failed due to the low amount extracted. After 17.5 minutes, a minor peak of unknown identity eluted with 4.3% of injected radioactivity (0.1% of AR). After 22.5 minutes 17.8% of the injected radioactivity (0.4% of AR) eluted at the same retention time as PM (identification by mass spectrometry failed due to the low concentration). The radioactivity eluting slightly later than PM (1.5% of injected radioactivity, 0.04% AR, 24 minutes) was not identified. Similarly, in TLC analyses (data not shown), a major portion of the radioactivity remained at the starting point of the TLC plate, indicating that the residues were associated to macromolecular soil organic matrix components.

We conclude that only low amounts of NER from PM formed in unamended soil are released by silylation over time because the major part obviously is covalently bound. In addition, the major part of the released radioactivity, which represents sequestered residues in soil, was found to contain unknown metabolites of PM rather than the parent compound. We could not detect metabolite 6-amino-pendimenthalin nor other reduced amino-groups containing metabolites in the silylation extracts which is known to be preferentially formed under reductive conditions. The absence might be explained either by not being formed at all or, if formed, by rapid reaction with soil organic matter by cross-coupling which is a typical reaction for derivatives with free amino-groups leading to covalent binding (Achtnich et al., 1999; Bollag, 1992; Dawel et al., 1997; Matthies et al., 2016). Such residues would represent type II NER (Kästner et al. 2014; Schäffer et al., 2018). The high amounts of covalently bound residues of PM may be explained by the fact, that after partial reduction of a nitro-group of PM in soil, which even under aerobic conditions provide anaerobicity

296 in restricted zones and in aggregates (Tiedje et al., 1984; Maier, 2019), the formed amino-group
297 readily reacts with humic and fulvic acids. Such covalently bound residues will not be extracted with
298 organic solvent mixtures.
299
300 ## 3.3. Effect of compost addition
301 The amount of NER formed in compost amended soil was significantly higher than in unamended
302 soil and mineralization of PM decreased significantly. By means of silylation, almost half of the
303 radioactivity in the compost amended soil was released, representing type I NER. The higher
304 amount of type I NER compared to the NER in bare soil results presumably from the increased
305 amount of voids from the added organic matter and consequently more entrapment and adsorption
306 sites. At the same time bioavailability of such protected residues was lower as suggested by the low
307 mineralization rate observed.
308 In the compost amended soil, PM forms up to 37.8% NER about half of which was released by
309 silylation, suggesting a significant contribution of sequestered residues (equivalent to about 19%
310 AR). Due to the observed low biodegradation of PM under such conditions, the presence of retained
311 parent PM in the released residues cannot be excluded.
312
313 ## 3.4. Bioavailability of NER after addition of fresh soil
314 Soil previously incubated with PM for 58 or 400 days was thoroughly extracted and thus contained
315 only NER. The extracted samples were incubated with fresh soil in order to assess the bioavailability
316 of the non-extractable residues. The results were normalized to 100% of the former NER for further
317 calculations.
318 After incubation with fresh soil for three months, 4.7% (58-day samples) and 7.1% (400-day
319 samples) of the former NER were mineralized, 21% (58-day samples) and 9.8% (400-day samples)
320 of former NER became extractable using the same extraction solvents as for the other incubation
321 experiments. Thus, 74.4% (58-day samples) or 83.2% (400-day samples) of the former NER
322 remained non-extractable.
323 TLC analysis of the extracts revealed that the parent PM was absent. The radioactivity of the extract
324 remained at the starting point of the TLC plate, indicating either very polar metabolites or residues
325 associated to co-extracted soil organic matter fractions. Due to the low amounts of radioactivity
326 extracted, no further analysis of the extract could be conducted. In conclusion, NER of PM in soil
327 seem to have a low bioavailability and the fractions that can be extracted after remobilization
328 experiments – here performed by mixing with fresh soil - do not contain the parent substance.

4. Conclusion

In general, low amounts of PM were mineralized and low amounts of NER were formed from PM in soil under the conditions applied. NER of PM in soil were mainly covalently bound while in compost amended soil about half of NER were present in the sequestered form. However, a certain amount of bioNER can be expected but were not determined in this study.

After incubation of thoroughly extracted, thus only NER containing soil with fresh soil, only low amounts of the NER were mineralized and became extractable whereas the dominant portion of NER remained non-extractable. Thus, the bioavailability of PM-derived NER is low.

Since NER of PM in soil are predominantly covalently bound, only minor amounts may be released under natural conditions. Since parent PM was not detected, these NER are formed mainly by metabolites, presumably 6-amino-pendimethalin or other reduced, amino-groups containing metabolites, rather than of the parent substance. The addition of compost as organic amendment to soil resulted in even less mineralization and larger amounts of NER due to enhanced sequestration of PM residues. These NER had a lower bioavailability and higher contribution of sequestered type I NER compared to those found in the unamended soil. Independent of the organic matter amendment, the risk of release from NER of PM and its metabolites is therefore considered low.

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6. Literature

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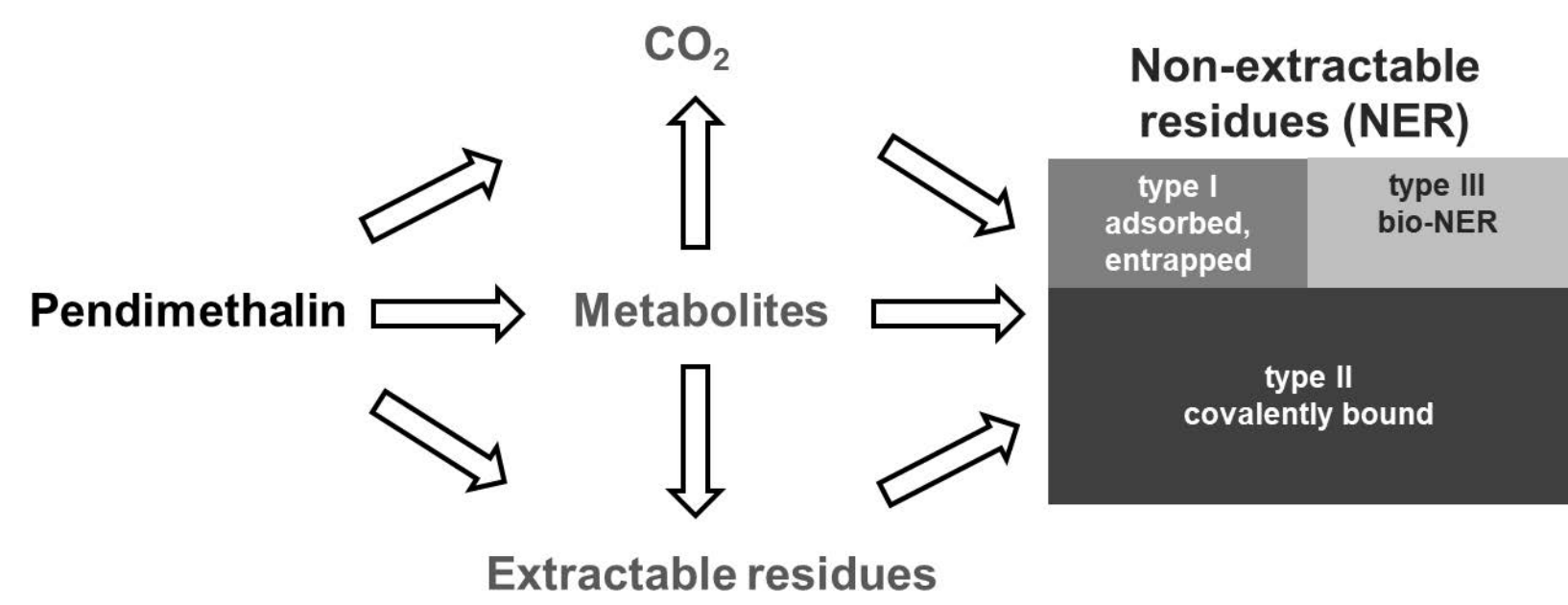
CO_2
Non-extractable residues (NER)
type I adsorbed, entrapped
type III bio-NER
Pendimethalin
Metabolites
type II covalently bound
Extractable residues

- Incubation of PM in soil led to 32% NER and 26% mineralization (after 400 days)
- Compost addition increased formation of NER (38% after 217 days) and decreased mineralization (1%)
- Nature of NER was investigated by silylation experiments: low amounts of RA were entrapped (< 5% AR)
- Pendimethalin was present only in trace amounts (ca. 0.4% AR)
- Addition of compost increased potential entrapment, sorption sites for PM and amounts of strongly sorbed, entrapped residues

Table 1 Physical and chemical properties of pendimethalin (EFSA, 2016).

Parameter	Value
Chemical name	N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
Vapor pressure	3.34 mPa (25 °C)
Solubility in water	0.33 mg/L at 20 °C
log Kow	5.4 (20 °C, pH 6.5)
Molecular mass	281.31 Da
Freundlich adsorption coefficient, K_{FOC} (L/kg)	8,900–27,600
CAS number	40487-42-1
Dissociation constant	pKa 2.8
Henry coefficient (kPa m^3 mol^{-1})	1.27 x 10^{-3} (25 °C)

Table 2 Parameters of the standard soil and the soil amended with compost. Data on soil was provided by Agricultural Investigation and Research Institute (LUFA) Rhineland-Palatinate (provider of the soil).

Parameter	Bare soil	Compost amended soil
Total organic carbon (TOC)	1.40% (dry combustion)	24.7% (LOI * factor 0.58)
pH ($CaCl_2$)	6.1	6.3
Effective cation exchange capacity	4.8 cmol kg^{-1}	n.d. [a]
Max. water holding capacity (MWHC)	31.4 g /100 g dry soil	57.8 g/100 g dry soil
Microbial biomass	73.6 mg C/100 g dry soil	n.d.

n.d.: not determined; LOI=loss of ignition (soil sample was combusted at 500 °C, remains were determined as inorganic residues of the soil; the factor of 0.58 was applied to assess the amount of organic carbon lost by combustion (see e.g. Nelson and Sommers, 1982).

Table 3 Distribution of radioactivity after incubation of 5 mg kg^{-1} ^{14}C-PM in soil and soil-compost in the fractions extractable residues (ER), non-extractable residues (NER) and cumulative mineralization. Percentages refer to the radioactivity applied to the soil (AR) or to the extracted radioactivity (% ER).

Study	Incubation [days]	ER [% AR]	PM (% AR)	PM (% ER)[a]	NER [% AR]	cumulative CO_2 [% AR]	Recovery [%]
unamended soil	58	87.8±0.1	75.5±0.5	86.0±0.6	10.5±1.9	1.7±0.05	100.0±1.9
	204	58.5±9.0	31.0±10.6	52.0±9.6	28.3±3.0	11.1±3.7	97.9±5.2
	400[b]	39.9±7.1	20.4±6.9	50.4±8.2	32.0±0.4	26.2±2.2	100.0±4.1
soil + compost	50	73.6±4.2	68.2±3.8	92.7±0.3	16.4±1.0	0.4±0.03	90.4±4.3
	117	72.4±4.4	63.5±4.5	87.7±1.0	19.3±0.2	0.8±0.04	92.5±4.6
	217[b]	57.9±2.5	47.9±1.7	82.7±0.7	37.8±5.8	1. 4±0.03	97.1±8.3

[a] PM percentages in ER are calculated from corresponding TLC analyses.

[b] Means of samples of 400 days (soil study) and 217 days (soil with compost) of incubation were derived from duplicates instead of triplicates.

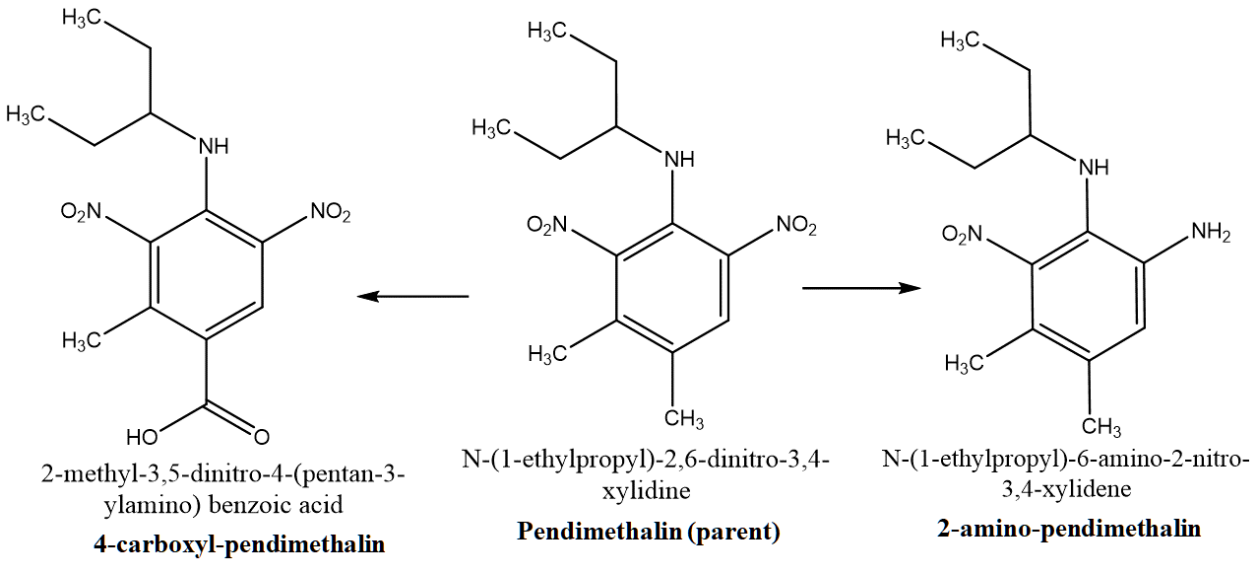

Figure 1 Metabolisation of PM in soil: Oxidation of the methyl group yields 4-carboxyl-pendimenthalin (2-methyl-3,5-dinitro-4-(pentan-3-ylamino) benzoic acid, whereas reduction of one nitrogroup yields 6-amino-pendimethalin (4,5-dimethyl-3-nitro-N^2-(pentan-3-yl)-1,2-diamine). PM and these two metabolites were used as reference substances in this study.

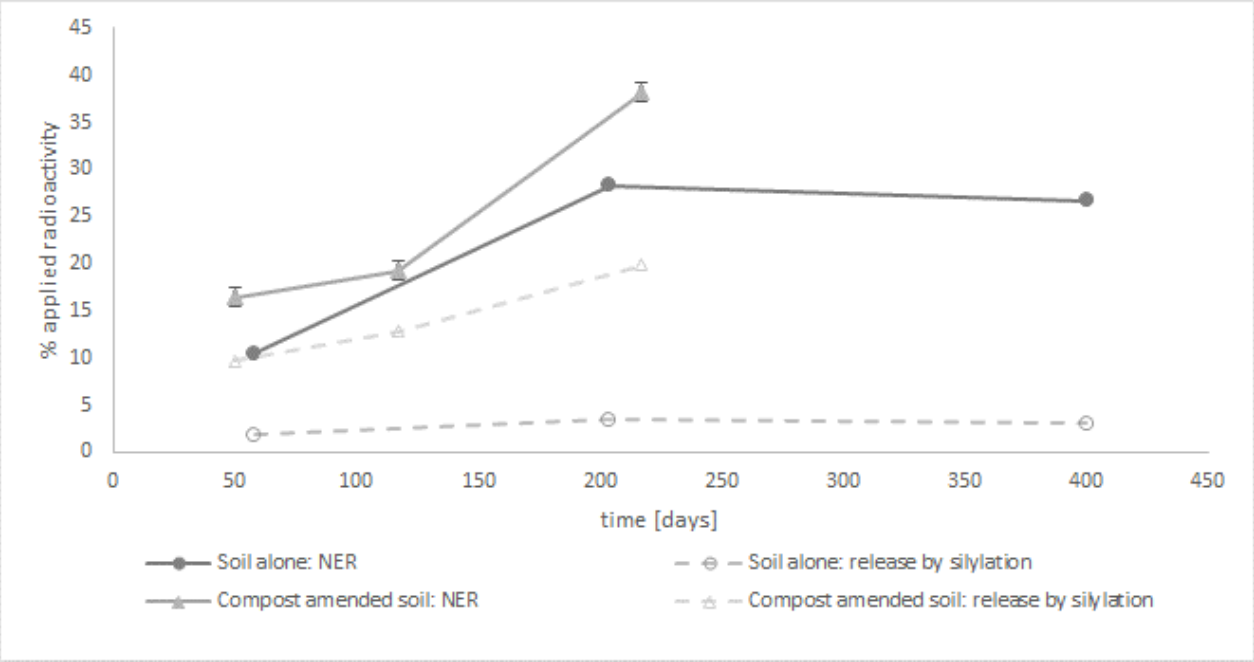

Figure 2 Development of NER after incubation of soil (with and without compost amendment) with PM and the amounts of released radioactivity after silylation of thoroughly extracted soil. Data are given in % of the applied radioactivity.

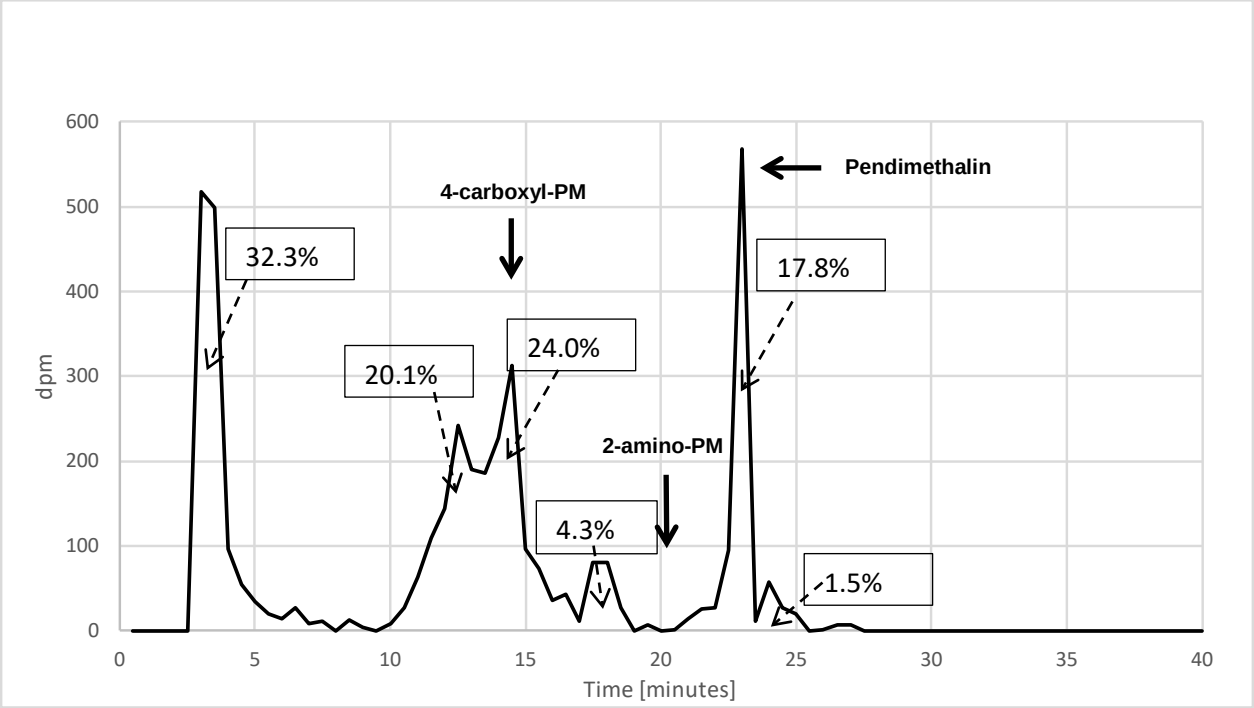

Figure 3 HPLC-chromatogram of a silylated soil sample containing only NER (5 mg PM kg^{-1} soil; thoroughly extracted after incubation for 400 days, end of incubation time). By silylation, 2.5% of the AR (mean of 5 repetitions) were released. The chromatogram was reconstructed from LSC counting of fractionated column eluate (30 s fractions). Reference substances PM, 4-carboxyl-PM and 6-amino-PM had retention times of 22.5, 14.5 and 20 minutes (percentages given in the chromatogram refer to f the summed peak areas).