Appl Microbiol Biotechnol (1995) 43:1128–1135

ORIGINAL PAPER

M. Kästner · S. Lotter · J. Heerenklage
M. Breuer-Jammali · R. Stegmann · B. Mahro

Fate of ^{14}C-labeled anthracene and hexadecane in compost-manured soil

Received: 7 November 1994/Received revision: 27 January 1995/Accepted: 30 January 1995

Abstract Experiments were carried out to evaluate the impact of the addition of ripe compost on the degradation of two ^{14}C-labeled hydrocarbon model compounds (anthracene and hexadecane) in soil. The addition of mature compost (20% dry wt./dry wt.) stimulated significantly the disappearance of the extractable fraction of both compounds. With compost, 23% of the labeled anthracene was transformed into $^{14}CO_2$ and 42% was fixed to the soil matrix irreversibly. In the unsupplemented control reactor more than 88% of the original anthracene could be recovered by either of two applied organic extraction procedures. The formation of non-extractable bound residues was less significant with [^{14}C] hexadecane since only 21% of the labeled carbon had become non-extractable after 103 days. The results presented show that compost could stimulate the depletion of hydrocarbons by either mineralization or the formation of unextractable bound residues (humification). The latter process might be a significant route of depletion in soil especially, for those hydrocarbons that are mineralized only slowly. The meaning of this finding for the assessment of soil bioremediation is discussed.

Introduction

The relevant authorities and companies of many industrialized countries are currently making many efforts to establish efficient and reasonable techniques for the remediation of soil sites that are contaminated with different sorts and amounts of hydrocarbons. The remediation of soils being contaminated with polycyclic aromatic hydrocarbons (PAH) are of particular concern since these toxic and cancerogenic compounds (Yang and Silverman 1988) represent a major type of soil contaminant at many sites (for example as tar oil contaminations on former sleeper impregnation or coal gasification plant sites).

Various soil remediation techniques like incineration, soil washing or biological soil treatment have been applied in the past but the biological degradation of hydrocarbon-contaminated soils is considered to be the most favorable technique as far as the costs are concerned (Franzius et al. 1989). A very promising strategy in this context is to boost the remediation of the contaminated soil with cheap biomass waste products like bark chips, straw or composted yard waste. This technique has been applied in practical bioremediation treatments (for example by Umweltschutz Nord Co. in Germany; Jandel 1991), and it could also be shown with controlled soil bioreactors that the addition of a particular amount of ripe compost to soil contaminated with either mineral oil or PAH can in fact induce a rapid turnover and depletion of these hydrocarbons in soil (Kästner et al. 1992; Stegmann et al. 1991).

However, the reason for the beneficial impact of compost supplementation on the hydrocarbon and PAH depletion in soil is not well understood yet. One possible reason could be that the addition of the coarse plant debris or waste material to the soil facilitates the aeration of small soil pores, by that means improving the microbial degradation activities. On the other hand, it is particularly puzzling in that context that the high organic carbon input due to the compost addition should theoretically also increase the sorptive capacity of the soil, thereby decreasing the concentration of hydrophobic hydrocarbons like PAH in the aqueous phase. The high affinity of the organic matrix of the soil

M. Kästner[1] · M. Breuer-Jammali · B. Mahro (✉)
Section Biotechnology II, Technical University Hamburg-Harburg (TUHH), Denickestr. 15, D-21071 Hamburg, Germany.
Fax: + 49-40-7718-2127

S. Lotter[1] · J. Heerenklage · R. Stegmann
Section Waste Treatment, Technical University Hamburg-Harburg (TUHH), Harburger Schloßstr. 37, D-21071 Hamburg, Germany

[1] These authors contributed equally to the presented work and should therefore both be considered as first authors

for the adsorption of hydrophobic compounds has been known for a long time (Hassett and Banwart 1989; Means et al. 1980) and it was also shown recently that an increase of the organic content of the soil (including coal particles) correlated with a decrease of microbial PAH degradation (Weissenfels et al. 1992). The observed rapid depletion of hydrocarbons in a highly sorptive environment like compost-supplemented soil requires therefore a very careful assessment. One major concern in that context is that the apparent rapid disappearance of the original hydrocarbon compounds is due to analytical failures, like, for example, the application of insufficient extraction procedures (Eschenbach et al. 1994) or the application of analytical protocols that do not look for metabolites being formed in the course of the degradation process (Püttmann 1988). Both failures would erroneously indicate complete degradation of the original hydrocarbons. To follow the whereabouts of partially degraded xenobiotics in soil is especially important with PAH since these metabolites may contain an even higher risk potential than the original compounds (Cerniglia et al. 1985; Wislocki and Lu 1988).

The scope of the present work was therefore to estimate the actual fate of hydrocarbon xenobiotics in compost-supplemented soil bioreactors in more detail. Two ^{14}C-labeled hydrocarbons, namely [^{14}C]anthracene as an aromatic and [^{14}C]hexadecane as an aliphatic hydrocarbon, were chosen as model compounds. The use of labeled hydrocarbons in closed soil bioreactors allowed us to monitor and quantify all putative depletion pathways in soil (organic extract, gas phase, solid soil phase and biomass) separately and should therefore help to distinguish the relative share of the two major xenobiotic disappearance pathways presumed to function in soil, namely microbial degradation and adsorption. To simulate a more genuine type of soil contamination, both hydrocarbons were, in addition, dissolved in unlabeled diesel oil and mixed into the soil in this form.

Materials and methods

Experimental set-up

The evaluation of carbon transfer from the ^{14}C-labeled contaminants was carried out in closed soil bioreactors (Stegmann et al. 1991) incubated at room temperature (21° ± 2°C). The process scheme of the reactors is shown in Fig. 1. The soil columns in the glass reactors (3 l volume) were aerated by moistened air with a flow rate of 2 l/h. The exhaust gas of each reactor was led through four sorption vessels, which were connected in series. Each of them was analyzed separately. Volatile organic compounds were absorbed in the first two gas-washing bottles containing ethyleneglycol monoethyl ether (first vessel 100 ml, second vessel 50 ml). $^{14}CO_2$ was collected in two additional vessels with NaOH solution (2 mol/l; third vessel 400 ml, fourth vessel 200 ml). The major part (between 95% and 97%) of both gas-phase fractions (^{14}C-labeled volatile organics and $^{14}CO_2$) was collected in the first and the third absorption vessel respectively. The exhaust gas was also led through a filter

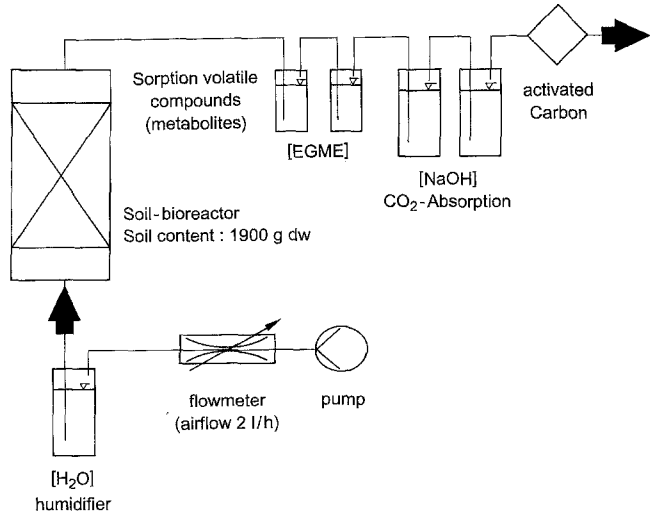

Fig. 1 Process scheme of the bench-scale soil bioreactors; *EGME* ethyleneglycol monoethyl ether

with activated carbon. No radioactivity higher than background levels was found in samples from this filter. Soil samples (5 g wet weight) were taken from the reactor through lateral sampling ports at three different heights in the reactors (5, 15, and 30 cm) and were mixed before analysis. Preliminary experiments had shown that this sampling procedure allowed representative sampling of non-saturated soil material and caused no changes in the activity of these reactors (total soil volume reduction less than 10%).

Soil preparation

A slightly loamy sand from an Ah-horizon of a Luvisol (parabrown soil) collected near Hamburg (Germany) was used as soil material (grain size less than 2 mm; composition: clay 6.4%, silt 15.3%, sand 77.6%; organic carbon content 2.5%; Berghausen and Goetz 1993). The compost material was obtained from the Harburger composting plant (Hamburg, Germany). The compost of this plant was made from yard waste including grass clippings, leaves and other landscaping materials and vegetable kitchen waste. Mature compost (grain size less than 4 mm; 6 months old) was used as soil supplement. The homogenization of the soil/compost mixtures and the addition of water and contaminants were carried out in a stainless-steel pastry-blending machine, type: KM 250 ST (Kenwood Ltd., Hampshire, UK). The water contents of the soil mixtures were adjusted with distilled water to 60% of the maximum water-holding capacity. The respiration activity of the soil was found to be at its maximum at this water content (Stegmann et al. 1991). Before the soils were added to the reactors they were contaminated with a contaminant mixture made from diesel fuel and the labeled model compounds. To achieve more realistic concentrations of soil contamination, non-radioactive anthracene or hexadecane was included in the respective contaminant mixtures. The final concentrations are given in Table 1. The diesel fuel contained approximately 32% aliphatic (C < 16) and 26% aromatic hydrocarbons consisting of 4% tricyclic aromatic compounds and was obtained from Shell AG (Hamburg, Germany). The soil/compost mixture of reactor C was supplemented with diesel fuel containing both, non-radioactive and [1-^{14}C]hexadecane (185 MBq/mmol in toluene; radiochemical purity > 99%; Sigma Chemie GmbH, Deisenhofen, Germany). The diesel fuel (50 g) was mixed with 5 g hexadecane and 0.5 ml [^{14}C]hexadecane solution (462.5 kBq). While the weighed soil was continuously mixed (see Table 1), 15.9 g of this solution was dropped into the soil (approximately 1000 drops of 20 µl) by means of

Table 1 Parameters of the soil/compost bioreactors A, B, and C

Parameter	A	B	C
Soil[a] (g dry wt.)	1927	1592	1592
Compost (g dry wt.)	0	398	398
Total weight sample (g)	2278	2539	2522
Dry matter (%)	86	78	79
Diesel fuel (g/kg dry wt.)	10.1	8.4	7.3
Anthracene (g/kg dry wt.)	0.20	0.17	—
Hexadecane (g/kg dry wt.)	—	—	0.72
^{14}C activity (kBq/kg dry wt.)	124.3[b]	102.4[b]	66.7[c]

[a] The soil weight differences are due to different densities of the soil and soil/compost fillings in the given 3-l reactor volume
[b] [9 – ^{14}C]anthracene
[c] [1 – ^{14}C]hexadecane

a syringe with a cannula diameter of 0.7 mm. The soil/compost mixture of reactor B was mixed with diesel fuel supplemented with non-radioactive and [9-^{14}C] anthracene (559 MBq/mmol in ethanol; radiochemical purity > 99%; Amersham-Buchler, Braunschweig, Germany). To solubilize the anthracene, 3 g was dissolved in 127.8 g dichloromethane. The solution was mixed with 150 g diesel fuel and 1 ml [^{14}C]anthracene stock (1850 kBq). This mixture (31.1 g) was dropped into the soil as described above. Dichloromethane was allowed to evaporate by mixing the soil for 1 h in the blending machine. The soil of reactor A was contaminated with 37.5 g diesel fuel/anthracene mixture (leading to a similar contamination quantity compared to the Ah-soil-material of reactor B) but the reactor was operated without addition of compost.

Analytical procedures

The ^{14}C activity was determined by a β-scintillation counter (type TRI Carb 1950; Packard Instruments, Groningen, The Netherlands). The counting efficiency was 88%. The quenching of the scintillation samples was determined for each of the two different scintillator gels (PF40 and ICGII, Packard Instruments, Groningen, The Netherlands) with both the four different sampling media (NaOH, Carbo-sorb, ethyl acetate and ethyleneglycol monomethyl ether and the extracts from two different soil materials (soil and soil/compost). Except where specified otherwise, all measurements of reactor samples were performed as triplicate analyses. The determination of the total amount of ^{14}C in the soil was conducted by combustion of three subsamples of 0.5 g (wet weight) in a combustion unit (type Coulomat D 702; Ströhlein Instruments GmbH, Kaarst, Germany). The system was standardized by use of ^{14}C standards (code CFR 101, Amersham International Ltd., Braunschweig, Germany). The detection efficiency ranged between 92% and 97%. The standard deviation of all combustion analyses was less than 10%.

The extraction of the soil samples was carried out in two different ways. The basic extraction procedure involved the use of organic solvents. The soil (3 g, wet weight) and 3 ml ethyl acetate were added to a Hungate tube (Bellco International, Feltham, UK). The tubes were closed and extracted for 30 min in an ultrasonic bath (type RK 1028, HF-power: 5000/1000 W, 35 Hz, Bendelein, Berlin, Germany). After sonification, the solvent was separated from the soil particles by centrifugation (4000 rpm, 15 min; Beckmann 6 PRK, Beckmann Instruments, Frankfurt/M., Germany) and analyzed in the scintillation counter. Preliminary experiments had shown that 95%–98% of the added compounds could be recovered from the soils by this procedure. A more rigorous extraction technique was applied in order to extract additional ^{14}C-labeled components or metabolites that were still bound to the soil matrix after extraction with organic solvents. The details of this method (a humic acid extraction by alkaline hydrolysis in methanolic solution) have been described recently (Eschenbach et al. 1994). All extractions were carried out three times. The standard deviations of the analysis data obtained were below 4%.

The anthracene concentrations of the soil extracts were quantified by HPLC with UV detection (analytical pump LC 410, Kontron GmbH, Hamburg, Germany; UV spectrometry detector SPD-2A, Shimadzu, Düsseldorf, Germany). The components of the soil extracts (20 µl injection volume) were separated isocratically on a reversed-phase RP-18 column (5 mm, Merck, Darmstadt, Germany) with a mobile phase of a methanol/water mixture (70/30 v/v; flow: 1 ml/min). Anthracene was detected spectrometrically at 254 nm. The analytical method applied allowed anthracene concentrations in soil to be measured without interference of the diesel fuel or humic compost substances.

The ^{14}C content in the biomass was estimated by use of the fumigation/incubation method of Jenkinson and Powlson (1976). These analyses of the soils were conducted after 86 days of incubation, because one of the basic premises of the method is that the soil does not contain easily degradable carbon sources other than biomass.

Results

Overall mass-balance experiments

The first experiments were carried out to evaluate the fate of the ^{14}C label of anthracene in soil with or without compost supplementation. The experiments were run over a period of 103 days. The respective data for extraction, soil combustion and mineralization are given in the Figs. 2, 3. Volatile [^{14}C] hydrocarbons could only be detected in the gas phase of the tested reactors in amounts less than 0.1% and are therefore not considered further in the following discussion. It can be seen in Fig. 2 that the unsupplemented soil batch did not release any anthracene-derived carbon dioxide, but that about 88.7% of the original radioactivity could be recovered by organic extraction at the end of incubation. A small portion of radioactivity could be assigned to a fraction of anthracene-derived residues in soil that were not extractable by ethyl acetate. HPLC analysis of the liquid extracts showed that

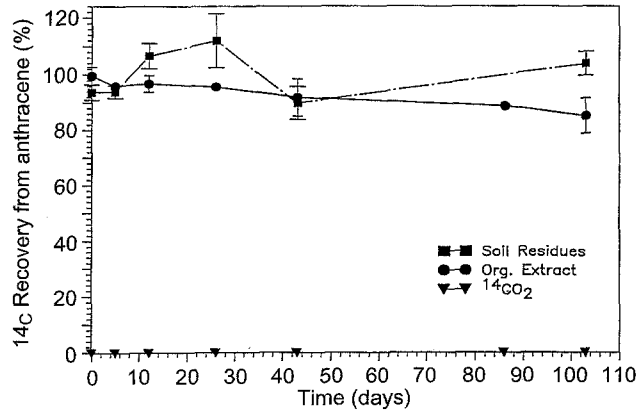

Fig. 2 Mass flow of ^{14}C-labeled carbon from anthracene in an unsupplemented soil reactor (reactor A; Luvisol soil material from an Ah horizon). Indication of standard deviation is only given if more than 10%

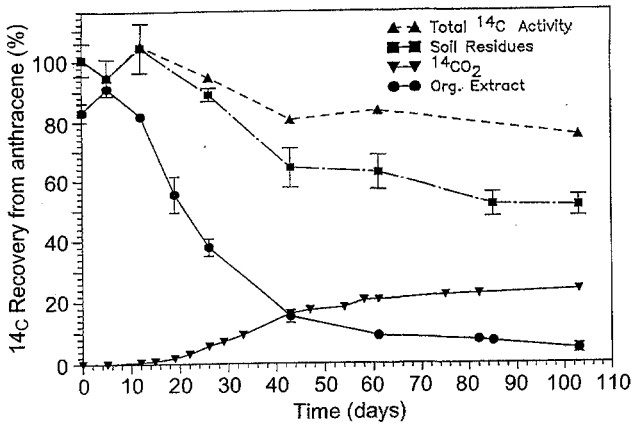

Fig. 3 Mass flow of ^{14}C-labeled carbon from anthracene in a compost-supplemented soil reactor (reactor B; Luvisol soil material from an Ah horizon). Indication of standard deviation is only given if more than 10%

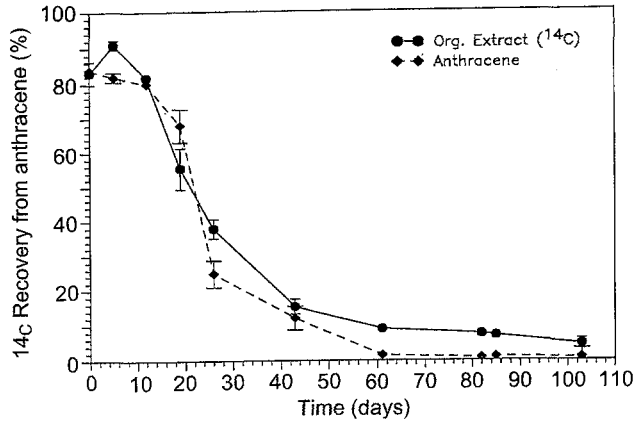

Fig. 4 Comparison of the percentage of identified anthracene and total ^{14}C activity in soil extracts from compost-manured soil (extracts from reactor B)

they contained mainly the original anthracene contaminant (82.5% of the total radioactivity).

The results were completely different if the anthracene-contaminated soil was supplemented with compost (reactor B). The ^{14}C activity in the soil extracts dropped from 80% to 90% at day 12 to a residual ^{14}C activity in the soil of 4.9% after 103 days (Fig. 3). The decrease of the extractable amount of anthracene started after a 12-day-long lag phase and continued almost exponentially up to the 43rd day. This decrease correlated with a detectable rate of $^{14}CO_2$ formation, indicating the start of the anthracene mineralization. While only 0.6% of the original [^{14}C]anthracene had been mineralized during the first 12 days, the $^{14}CO_2$ evolution increased after that time to a final value of 23.8% of the initial radioactivity. The total amount of $^{14}CO_2$ might have been even higher if one assumes that the radioactivity that was missing in the overall mass balance (dotted line in Fig. 3) was probably mainly due to a $^{14}CO_2$ leakage from the gaseous phase. The measurement of the amount of ^{14}C activity that was obtainable by soil combustion revealed that the recovery was significantly higher by this method than by organic extraction (Fig. 3). The total residual soil radioactivity after 103 days amounted to 51.3% of the initially added ^{14}C activity, while the extractable radioactivity was, as given above, only 4.9%. The active phase in the formation of unextractable ^{14}C-labeled soil residues correlated very well with the activity profile of the mineralization process. Both carbon-transformation processes were obviously due to biological activities in the compost since comparable transformation processes could be observed neither in control reactor A (Fig. 2) nor in a sterile control batch (data not shown).

To figure out whether the soil extracts of the compost-supplemented bioreactor B contained a considerable amount of PAH metabolites, we determined the ^{14}C activity that was not represented by original

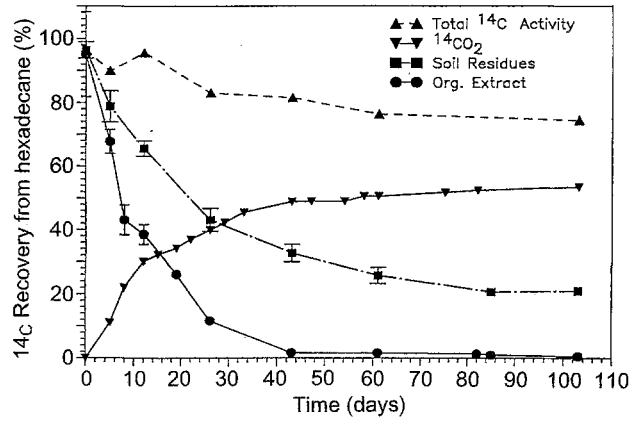

Fig. 5 Mass flow of ^{14}C-labeled carbon from hexadecane in a compost-supplemented soil reactor (reactor C; Luvisol soil material from an Ah horizon). Indication of standard deviation is only given if more than 10%

anthracene (Fig. 4). The use of ethyl acetate as organic solvent should especially help to facilitate the extraction of the presumably more polar metabolites (Heitkamp et al. 1988; Sutherland et al. 1990). It was found that, in the course of the incubation, the amount of extractable anthracene was mostly slightly lower than the extractable amount of radioactivity (0% to 13% relative to the added [^{14}C]anthracene). The amount of extractable metabolites amounted to 3.8% while the proportion of the original anthracene that was extractable came to 1.1% at the end of the experiment. No significant accumulation of metabolites occurred during the time of incubation (Fig. 4).

The experiments with anthracene showed that the addition of compost resulted in a significant formation of unextractable ^{14}C-labeled soil residues. To find out whether these residues were also formed with non-aromatic hydrocarbons, the experiments were complemented by an examination of the fate of [^{14}C]hexadecane in a soil/compost bioreactor (reactor C). It can

be seen in Fig. 5 that the amount of extractable [^{14}C]hexadecane decreased exponentially without any lag phase right after the inoculation of the soil reactor, resulting in a residual concentration of 1.6% of the initial value after 42 days (Fig. 5). The decline of the extractable [^{14}C]hexadecane concentration correlated with the evolution of $^{14}CO_2$, indicating that the mineralization also started right after the beginning of the experiment. The total hexadecane mineralization amounted to 53.6% at the end of the experiment (Fig. 5). The formation of ^{14}C-labeled soil residues was also observed in the hexadecane-spiked soil, though to a much lesser extent that in the anthracene reactors. Soil combustion showed 21% of the initial [^{14}C]hexadecane radioactivity remaining after 103 days while only 0.5% of the activity could be extracted by organic solvents at this time.

Analysis of the soil residues

The considerable amount of ^{14}C-labeled residues in both compost-supplemented soils made it necessary to characterize the carbon traps in the soil in more detail. These experiments were done only once during the incubation period, on the 86th day.

One possible carbon trap in soil that we looked at was that part of the xenobiotic carbon which might have been incorporated into microbial biomass. Surprisingly, ^{14}C incorporated into the biomass was detected only in minor amounts in samples of the hexadecane reactor C (1.9% of the total residual activity). No ^{14}C incorporation into biomass was detected in the soils of the anthracene reactors A and B (Fig. 6). It could therefore be concluded that – at least after 86 days – the incorporation of radiolabeled carbon into biomass did not obviously represent a significant part of the residual carbon radioactivity detected in the soil.

The second putative carbon-flow pathway that was checked was the elimination of PAH by strong adsorption to the humic matrix. This fraction of soil-bound carbon can be efficiently monitored by a humic acid extraction (Eschenbach et al. 1994). The ^{14}C activities recoverable by humic acid extraction of the soils are presented in Fig. 6. The total ^{14}C recovery of each reactor is given as the sum of combustion and mineralization (left-hand bars), while the ^{14}C activities of the different soil compartments (organic extraction, humic acid extraction, biomass, and non-extractable, bound residues) are summarized in the right-hand bars.

The amount of radioactivity recoverable by humic acid extraction from reactor A (no compost addition) amounted to 3.2% of the total radioactivity compared to 88.7% extractable by organic extraction only. The relative contribution of humic acid extraction was, in this case, therefore not very significant. Though the absolute amounts were not very high, the relative contribution of ^{14}C recovery by humic acid extraction was higher in samples with compost addition. The humic acid extraction could recover about one-third of the total extractable radioactivity from reactor B (i.e. 3.1% of the initial anthracene radioactivity) while the initial activity extractable by ethyl acetate amounted to 7.1%. The corresponding extraction efficiencies in reactor C were 2.3% and 1% respectively. The ^{14}C activity that remained unextractable by either method and was not represented by biomass was, therefore, assessed as "bound residues". Thus after 86 days of incubation, the non-extractable, bound residues of the soils amounted – if calculated relative to the ^{14}C activity added to the soil – to 8.7% in reactor A, 41.7% in reactor B, and 15.5% in reactor C. If one relates the amount of unextractable radioactivity of the total ^{14}C activity detected by combustion (not the mineralized compounds), it can be seen that in the compost-supplemented soil the relative share of the bound

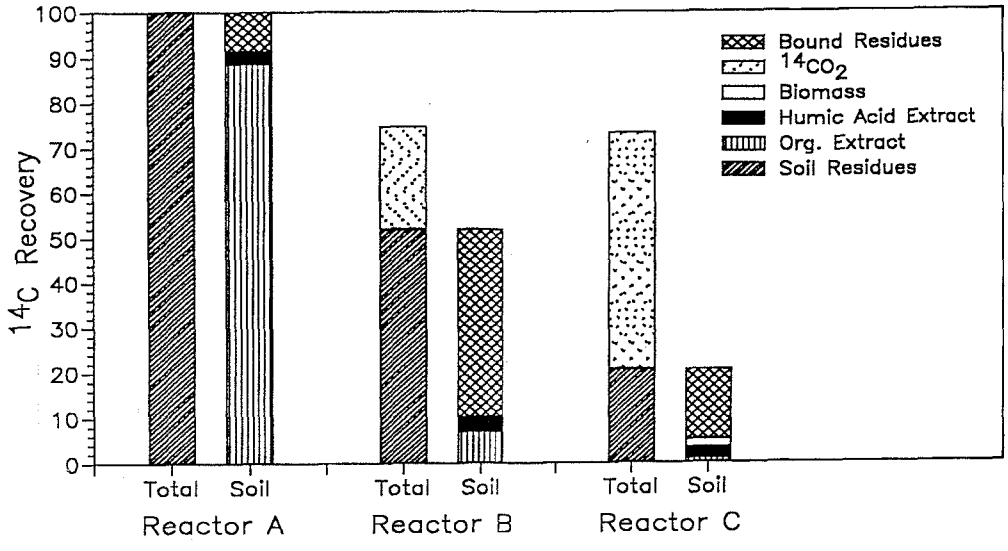

Fig. 6 Comparison of the relative share of different soil compartments (*right-hand bar*) as trap for ^{14}C-labeled carbon from anthracene and hexadecane. *Left-hand bar* the total ^{14}C recovery, i.e. including the soil residues and the carbon flow in the gaseous phase. *Reactor A* anthracene, no other supplements; *Reactor B* soil/compost mixture contaminated with anthracene; *Reactor C* soil/compost mixture contaminated with hexadecane

residue fraction was about 80% for both hydrocarbons. The addition of mature compost to a contaminated soil therefore stimulated both an increased microbial mineralization and an increased formation of non-extractable, bound residues in the solid soil material.

Discussion

The experiments presented support recent findings (Kästner et al. 1992; Stegmann et al. 1991) that the implementation of compost in hydrocarbon-contaminated soils can significantly stimulate the disappearance of the extractable fraction of aliphatic and aromatic hydrocarbons from the soil (Figs. 1, 3, 5). Considerable amounts of the respective carbon pools were transformed into CO_2, indicating that the addition of compost may support or boost cometabolic or mineralizing activities in the contaminated soil efficiently. The relative contribution of this pathway to the overall depletion process differed with the compound, since the aliphatic structure (hexadecane) could be mineralized on a larger scale than the aromatic hydrocarbon anthracene (Figs. 3, 5).

However it had been found already by Stegmann and coworkers, using non-radioactive mass-balancing methods, that another considerable part of the original xenobiotic carbon did not occur in the measurable carbon pools (CO_2, organic extract and biomass; Lotter et al. 1992, 1993) at all. This raised the question of the whereabouts of the missing xenobiotic carbon. Theoretically, the observed balance gap could be due either to leakage from the gas phase, to adsorption to surfaces of the bioreactor or tubing, to strong physical adsorption processes in the soil or – as is hypothesized by Mahro and Kästner (1993) – to the biogenic formation of bound residues. Both the fact that a significant fraction of the xenobiotic carbon pool remained unextractable even by an intensified humic acid extraction procedure and the fact that this binding could be observed neither in the control reactor A nor in sterile control vessels indicate that the balance gap observed by Stegmann and others is at least partially due to a compost-mediated, biogenic stimulation of the binding of the parent hydrocarbon or its oxidized metabolite to the soil matrix.

First of all it should be noted in that context that the formation of bound residues is not equivalent to the strong adsorption of PAH described by Weissenfels et al. (1992). The sorptive binding of PAH described by these authors was still reversible by (organic) extraction which, however, was not the case in the experiments described here. The ^{14}C that is recoverable by humic acid extraction should also not be considered as "bound residue", since it is not known yet whether this technique releases (covalently) bound xenobiotic residues from the soil matrix or whether it only helps to set free those xenobiotics that have been physically trapped in molecular-sieve or cavity-like structures of the humic macromolecule (Eschenbach et al. 1994). In the following, therefore, we consider as "bound residue carbon" only that carbon which could not be extracted by any of the extraction methods applied.

We assume that the compost-mediated biogenic binding of PAH in soil – and probably also the binding of other hydrocarbons like partially oxidized aliphatics – is at least to some extent due to processes similar to those described for enzyme-mediated pesticide binding in soils (Bollag et al. 1992; Bollag and Loll 1983; Führ 1987; Senesi 1992; Shannon and Bartha 1988). At first sight hydrocarbons do not fulfill the criteria for oxidative soil coupling processes since these reactions – as described for pesticides (Bollag et al. 1992; Senesi 1992) – require the participation of compounds with reactive functional groups (e.g. NH_2 or OH). However, it has been shown in many laboratories that both bacteria and fungi may form and excrete partially oxidized, phenolic, carboxylic or quinoid PAH intermediates in liquid culture (Heitkamp et al. 1988; Sutherland et al. 1990; Weissenfels et al. 1991). It was shown that at least small amounts of such oxidized PAH metabolites may also be found in soil (Richnow et al. 1994; Schnöder et al. 1994). The strongest support for the hypothesis of an oxidative coupling of PAH metabolites in soil comes from recent findings that show that (a) PAH metabolites can be recovered from the macromolecular humic matrix by selective chemical degradation techniques and that (b) parts of these PAH metabolites are covalently linked to the humic matrix by ester bonding (Richnow et al. 1994). We assume that actinomycetes and fungi in particular – the predominant microorganisms in compost supplements – may trigger the presumed oxidative coupling activities, since these groups of microorganisms are well known for their exoenzymatic oxidative degradation potential (Sutherland et al. 1990; Bumpus 1989; Fritsche et al. 1994). Further experiments are necessary, however, to evaluate this hypothesis in more detail.

The third biogenic depletion pathway for xenobiotic carbon in soil, namely the formation of biomass, seems to play only a minor role in the compost-manured soil (Fig. 6). The formation of biomass might be of more importance as a mass-balance factor, however, during the early incubation phase, as was found by Lotter and others (Lotter et al. 1992). It also remains to be investigated more closely why both of the tested compounds can be used more efficiently for biomass formation if they are supplied to microorganisms on culture plates or in aqueous environments rather than in a compost-manured soil (Kästner et al. 1994; Michaelsen et al. 1992).

The data presented suggest that compost rather than pure enzymes or chemicals (Berry and Boyd 1985; Bollag 1992; Shannon and Bartha 1988) should be used as a cheap and powerful method of soil remediation and detoxification. Compost may stimulate both the

turnover of the hydrocarbon xenobiotic in carbon dioxide as well as its long-term binding and detoxification. It remains to be investigated in more detail, however, whether bound residues derived from soil contaminants like PAH or tar oil are stable in the long run and to what extent the xenobiotic can become involved in the natural turnover process of the humic substances. This question has already been investigated to some extent for pesticides (Calderbank 1989; Dec and Bollag 1988) and it was found that the annual turnover of humic bound residues may range between 2% and 8% (Haider and Martin 1988; Hsu and Bartha 1976).

The topic nevertheless needs some reconsideration, since the application of pesticides to agricultural soils does not lead to xenobiotic concentrations comparable to those being observed in many contaminated soil sites, nor have pesticides been applied in complex contaminant mixtures, like tar oil, for example. In addition, pesticides being released in the field are mostly optimized for long-term degradation, which is not necessarily the case with hazardous waste xenobiotics.

Acknowledgements We thank Professor Volker Kasche for his continued support and helpful discussions, Professor Dr. Ulrich Förstner for providing the isotope laboratory, and Dr. Gerth for experimental advice on the isotope handling. The investigations were sponsored by a grant of the DFG for the Sonderforschungsbereich (SFB) 188 "Reinigung kontaminierter Böden", projects B1 and B3.

References

Berghausen M. Goetz D (1993) Change of soil physical values through oil contaminations. In: Arendt F, Annokkee GJ, Bosman R, Brink WJ van den (eds) Contaminated soils '93. Kluwer, Doordrecht, pp 907–910

Berry DF, Boyd SA (1985) Decontamination of soil through enhanced formation of bound residues. Environ Sci Technol 19:1132–1133

Bollag JM (1992) Decontaminating soil with enzymes. Environ Sci Technol 26:1876–1881

Bollag JM, Loll MJ (1983) Incorporation of xenobiotics into soil humus. Experientia 9:1221–1231

Bollag JM, Myers CJ, Minard RD (1992) Biological and chemical interactions of pesticides with soil organic matter. The science of the total environment 123/124:205–217

Bumpus JA (1989) Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl Environ Microbiol 55:154–158

Calderbank A (1989) The occurrence and significance of bound pesticide residues. Rev Environ Contam Toxicol 108:71–103

Cerniglia CE, White GL, Heflich RH (1985) Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons. Arch Microbiol 143:105–110

Dec J, Bollag JM (1988) Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid. Soil Sci Am J 52:1366–1371

Eschenbach A, Kästner M, Bierl R, Schaefer G, Mahro B (1994) Evaluation of a new and more effective method to extract polycyclic aromatic hydrocarbons from soil samples. Chemosphere 28:683–692

Franzius V, Stegmann R, Wolf K (1989) Handbuch der Altlastensanierung; RV Decker's Verlag G. Schenk, Heidelberg

Fritsche W Günther T, Hofrichter M, Sack U (1994) Metabolismus von polycyclischen aromatischen Kohlenwasserstoffen durch Pilze verschiedener ökologischer Gruppen. In: Sonder forschungs bereich 193 d. TU Berlin (ed) Schriftenreihe Biologische Abwasserreinigung, TU Berlin, Berlin, vol. 4: pp 167–182

Führ F (1987) Non-extractable pesticide residues in soil. In: Greenhalgh R, Poberts TR (eds) Pesticide science and biotechnology. Blackwell, Oxford, pp 381–389

Haider KM, Martin JP (1988) Mineralization of ^{14}C-labeled humic acids and of humic-acid bound ^{14}C-xenobiotics by *Phanerochaete chrysosporium*. Soil Biol Biochem 20:425–429

Hassett JJ, Banwart BL (1989) The sorption of nonpolar organics by soils and sediments. In: Sawhney BL, Brown K (eds) Reactions and movement of organic chemicals in soils, SSSA and ASA, Madison, Wis, pp 31–44

Heitkamp MA, Freeman JP, Miller DW, Cerniglia CE (1988) Pyrene degradation by a *Mycobacterium* sp.: identification of ring oxidation and ring fission products. Appl Environ Microbiol 54:2556–2565

Hsu TS, Bartha RJ (1976) Hydrolysable and nonhydrolysable 3,4-dichloroaniline humus complexes and their respective rate of biodegradation. J Agric Food Chem 24:118–122

Jandel AS (1991) Ölunfälle schnell beheben. Umwelt 21:358–359

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol Biochem 8:209–213

Kästner M, Schaefer G, Breuer-Jammali M, Mahro B (1992) Comparison of the microbial degradation potential for PAH in liquid medium, soil and soil compost. In: Kreysa G, Driesel AJ (eds) Dechema Biotechnol Conf 5:1043–1046

Kästner M, Breuer-Jammali M, Mahro B (1994) Enumeration and characterization of the soil microflora from hydrocarbon-cantaminated soil sites able to mineralize polycyclic aromatic hydrocarbons. Appl Microbiol Biotechnol 41:267–273

Lotter S, Heerenklage J, Stegmann R (1992) Carbon balance and modelling of the oil degradation in soil bioreactors. In: Klein J (ed) Preprints International Symposium: Soil decontamination using biological processes. Dechema, Frankfurt, pp 219–227

Lotter S, Brumm A, Bundt J, Heerenklage J, Paschke A, Steinhart H, Stegmann R (1993) Carbon balance of a PAH-contaminated soil during biodegradation as a result of the addition of compost. In: Arendt F, Annokkee GJ, Bosman R, Brink WJ van den (eds) Contaminated soils '93. Kluwer Doordrecht, pp 1235–1245

Mahro B, Kästner M (1993) Der mikrobielle Abbau polyzyklischer aromatischer Kohlenwasserstoffe (PAK) in Böden und Sedimenten: Mineralisierung, Metabolitenbildung und Entstehung gebundener Rückstände. Bioengineering 9:50–58

Means JC, Wood SG, Hassett JJ, Banwart WL (1980) Sorption of polynuclear hydrocarbons by sediments and soils. Environ Sci Technol 14:1524–1528

Michaelsen M, Hulsch R, Höppner T, Berthe-Corti L (1992) Hexadecane mineralization in oxygen-controlled sediment seawater cultivations with autochthonous microorganisms. Appl Environ Microbiol 58:3072–3077

Püttmann W (1988) Kriterien zur Beurteilung von Sanierungsverfahren auf mikrobiologischer Basis. In: Rosenkranz D, Einsele G, Harreß HM (eds) Bodenschutz. Erich Schmidt, Berlin, Lfg V/90: 1–25

Richnow HH, Seifert R, Hefter J, Kästner M, Mahro B, Michaelis W (1994) Metabolites of xenobiotica and mineral oil constituents linked to macromolecular organic matter in polluted environments. Org Geochem 22:671–681

Schnöder F, Mittelstaedt W, Führ F (1994) Das Verhalten von Benzo(*a*) pyren in einer Parabraunerde – Lysimeter -und Laborabbaustudien. In: TU Berlin (ed) Schriftenreihe Biologische Abwasserreinigung, vol 4. pp 217–230

Senesi N (1992) Binding mechanisms of pesticides to soil humic substances. The science of the total environment 123/124:63–76

Shannon MJR, Bartha R (1988) Immobilization of leachable toxic soil pollutants by using oxidative enzymes. Appl Environ Microbiol 54:1719–1723

Stegmann R, Lotter S, Heerenklage J (1991) Biological treatment of oil-contaminated soils in bioreactors. In: Hinchee RE, Olfenbuttel RF (eds) On site bioreclamation: processes for xeniobiotic and hydrocarbon treatment. Butterworth-Heinemann, Boston, pp 188–208

Sutherland JB, Freeman JP, Selby AL, Fu PP, Miller DW, Cerniglia CE (1990) Stereoselective formation of a K-region dihydrodiol from phenanthrene by *Streptomyces flavovirens*. Arch Microbiol 154:260–266

Weißenfels WD, Beyer M, Klein J, Rehm HJ (1991) Microbial metabolism of fluoranthene: isolation and identification of ring fission products. Appl Microbiol Biotechnol 34:528–535

Weißenfels WD, Klewer HJ, Langhoff J (1992) Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. Appl Microbiol Biotechnol 36:689–696

Wislocki PG, Lu AYH (1988) Carcinogenity and mutagenity of proximate and ultimate carcinogens of polycyclic aromatic hydrocarbons. In: Yang SK, Silverman BD (eds) Polycyclic aromatic hydrocarbon carcinogenesis: structure activity relationships, vol 1 CRC, Boca Raton, Fla, pp 1–30

Yang SK, Silverman BD (1988) Polycyclic aromatic hydrocarbon carcinogenesis: structure activity relationships. CRC, Boca Raton, Fla, vol. 1 and 2