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Fate and Stability of Nonextractable Residues of [^{14}C]PAH in Contaminated Soils under Environmental Stress Conditions

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It has been recognized during recent years that polycyclic aromatic hydrocarbons (PAH) may form nonextractable residues in soil and that this process may be stimulated by microbial activities. To use that process intentionally for soil bioremediation, one must ensure that the formed nonextractable PAH residues will not be released from the soil on the long run. The long-term stability of four different nonextractable [^{14}C]PAH residues ([^{14}C]naphthalene, [^{14}C]anthracene, [^{14}C]pyrene, and [^{14}C]benzo[*a*]pyrene) was therefore monitored under different ecological stress conditions. It was found that a considerable fraction of the total [^{14}C]PAH residues could be released as $^{14}CO_2$ from the soil being partly due to a biogenic reduction of the nonextractable ^{14}C residue fraction. The turnover of this fraction was comparable to the natural turnover rate for humic substances. Neither the addition of humus-degrading microorganisms nor a mechanical stress treatment of the soil structure by freezing and thawing led to a mobilization of the nonextractable [^{14}C]PAH residues. However, a significant mobilization of the nonextractable ^{14}C activity occurred when EDTA was added to the soil. The metal−organic soil complexes were destabilized by this complexing agent and released ^{14}C activity that was attached to colloidal or dissolved organic matter.

Introduction

It is generally assumed that the bioremediation of PAH-contaminated sites is mainly due to the activity of microorganisms that are able to degrade polycyclic aromatic hydrocarbons (PAH) in soil (*1, 2*). It was frequently overseen in the past, however, that the apparent analytical depletion of the extractable amount of PAH in soil may not only be due to biodegradation but also partly due to a strong adsorption or binding of the PAH to the soil matrix (*3*). These adsorbed or bound substances can be recovered from soil to some part by more rigid extraction procedures (*3*). However, it was also shown by the use of ^{14}C-labeled PAH that another relevant part may become completely nonextractable (*4, 5*).

Although adsorption is considered in general as a reversible process (*6, 7*), there is also some evidence that adsorbed substances tend to become more resistant to extraction and degradation the longer they are in the soil (*8*). The entrapment of the pollutants in macromolecular humus substances has received more attention recently. It is believed that the entrapment involves slow partitioning of the hydrophobic compounds into organic matter or slow diffusion into micropores where their further availability is hindered (*9, 10*). The covalent binding of xenobiotics to organic matter may result in an even more persistent association with the soil matrix. Since the original PAH do not possess any coupling groups, PAH may only become susceptible to oxidative coupling if reactive metabolites are produced during degradation. These partially oxidized PAH metabolites may then become covalently bound to the soil organic matter (*11*). This has meanwhile partly been proven by the identification of covalent ester bonds between different PAH metabolites and humic polymers (*6, 12*).

The fact that parts of the PAH in soil were not extractable even by organic solvents suggested the use of the PAH immobilization intentionally as a remediation measure (*11, 13, 14*). Especially organic supplements such as compost or bark chips were proposed as suitable substrates to stimulate that process since they might support the humification by both an increase in the number of organic binding sites and by an increase in the number of microorganisms thought to be involved in extracellular PAH humification (i.e., especially fungi or streptomycetes; *11*). However, the intentional use of PAH immobilization as a bioremediation strategy also makes it necessary to gain more knowlewdge on the fate and long-term stability of the formed nonextractable PAH residues. In particular, it must be evaluated whether the PAH residues remain stable also under any presumable environmental "worst case" conditions. Such "worst case" tests are the subject of this work, which represents to our knowledge the first report in which the fate of nonextractable residues of PAH under environmental stress conditions is investigated systematically. The experiments were carried out with nonextractable [^{14}C]PAH residues that had been produced in preceding long-term bioremediation experiments (*15*). These soil samples were now treated again by either (i) biological (effect of humic decomposing pure cultures and organic supplements), (ii) physical (change of temperature), or (iii) chemical (disruption of metal−organic complexes) means.

Experimental Section

The soil samples contained nonextractable residues that had been formed from either [^{14}C]naphthalene, [^{14}C]anthracene, [^{14}C]pyrene, or [^{14}C]benzo[*a*]pyrene as parent compounds in former bioremediation experiments. The effect of each remobilization test was determined in comparison to an untreated control by analyzing the amounts of the ^{14}C activity in the different distinguishable ^{14}C fractions: (i) the mineralized $^{14}CO_2$, (ii) the extractable ^{14}C activity, and (iii) the nonextractable residues. The overall recovery of the respective fractionated ^{14}C activities after the remobilization treatment amounted in all experiments to 100 ± 9% of the total initial ^{14}C activity (A_i).

Production of Nonextractable ^{14}C Residues. Soil samples from a contaminated site near Hamburg, FRG, had been spiked with [^{14}C]PAH at 925 KBq (kg of soil dry wt)$^{-1}$ and incubated with the white rot fungus *Pleurotus ostreatus* for several months. The following [^{14}C]PAH had been used: [1-^{14}C]naphthalene (25.16×10^7 Bq mmol^{-1}, > 98% purity; Sigma Chemie GmbH, FRG), [U-side ring-^{14}C]anthracene (41.44×10^7 Bq mmol^{-1}, 93% purity), [4,5,9,10-^{14}C]pyrene

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TABLE 1. Characteristics of Soil Samples with Nonextractable [^{14}C]PAH Residues

soil samples	soil[a]	[^{14}C]PAH	previous incubation (days)	nonextractable residues (% of total ^{14}C activity)
AL-Nap	A	[^{14}C]naphthalene	174	78
AL-Ant	A	[^{14}C]anthracene	174	71
AL-Pyr	A	[^{14}C]pyrene	174	62
AL-Bap	A	[^{14}C]benzo[*a*]pyrene	174	57
BL-Ant	B	[^{14}C]anthracene	167	80
BL-Pyr	B	[^{14}C]pyrene	167	66
BS-Pyr	B	[^{14}C]pyrene	40	56

[a] Soil A, loamy sand, C_{org}, 4.6 wt %; pH, 7.5; 100 mg of EPA-PAH (kg of soil)$^{-1}$. Soil B, clayey loam, C_{org}, 3.4 wt %; pH, 7.2; 600−650 mg of EPA-PAH (kg of soil).$^{-1}$

(20.7×10^8 Bq mmol^{-1}, 95% purity), and [7,10-^{14}C]benzo-[*a*]pyrene (21.8×10^8 Bq mmol^{-1}, 97% purity; the latter three from Amersham Buchler, Braunschweig, FRG). During this preincubation, the [^{14}C]PAH were mineralized to various degrees, and simultaneously stable fractions of nonextractable residues were been formed (for results, see ref *15*). The amounts of nonextractable residues ranged from 56% to 80% of the total ^{14}C activity in the soil (Table 1). The remainig overall ^{14}C activity at the end of the preincubation is identical with the initial ^{14}C activity (A_i) of the remobilization experiments being presented here.

Biological Treatments. The white rot fungus *P. ostreatus* (grown on a pasteurized straw material) and the pure straw material were supplied from Preussag Noell (Darmstadt, FRG). This material was added to the soil material in a ratio of 1:4 dry wt (*P. ostreatus*/straw material:soil). The white rot fungus *Trametes versicolor* (DSM 3086) and the actinomycete *Rhodococcus erythropolis* (DSM 1069) were supplied from the German Culture Collection (DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, FRG). To inoculate the white rot fungus *T. versicolor*, it was grown on sterilized rolled oats. After 7 days of incubation at 28 °C, the culture was harvested, and *T. versicolor* was mixed with soil samples in a ratio of 1:10 (dry wt *T. versicolor*/oat medium:soil). *R. erythropolis* was cultivated on a culture medium with veratric acid as the sole carbon source (culture medium according to ref *16*). The culture was harvested at the mid-log phase and washed three times in sterilized water before further use. The soil was inoculated with 2×10^7 cells (g of soil dry wt)$^{-1}$. In another experimental setup, pure glucose and starch (6.5 mg of glucose and 6.5 mg of starch (g dry wt of soil)$^{-1}$) were added to the soil as a source of carbon to induce the degradation of the soil organic matter by a "priming effect" (*17*). The forest litter was collected in a beech forest (*Fagus sylvatica* L.) in the south of Hamburg (FRG). It consisted of decomposed and partly decomposed leaf mold from Oe and Oa horizons. The material was freed from roots and small branches and sieved to a particle size of less than 8 mm. The compost, made of green waste such as grass clippings, leaves, etc. and ripened for 6 months, was obtained from the Stuttgart Composting Plant (Stuttgart, FRG). The bark chips were supplied by a commercial recycling plant in Steinkirch (FRG). The shredded bark (>90% from spruce) had been stored at a pit for 4 weeks before sampling. All organic supplements, sieved to a grain size of less than 8 mm, were added to the soil in a ratio of 1:5 dry wt (supplements:soil).

To balance the ^{14}C activity, the experiments were conducted in closed bioreactor systems consisting of a glass vessel (750 mL volume) with an oxygen reservoir (Tecobag, Tesseraux Spezialverpackungen, Bürstadt, FRG) and with a vessel containing 1 M NaOH to trap the carbon dioxide. The NaOH was changed weekly to measure the mineralization ($^{14}CO_2$). During the incubation at a temperature of 20 ± 3 °C, the soil samples were kept moist at 55−60% of the maximum water-holding capacity. At selected time intervals, soil samples were taken to analyze the total ^{14}C activity as well as the extractable and the nonextractable ^{14}C activity of the soil.

Physical Treatments. The impact of temperature stress (e.g., frost damage of the soil aggregate structure) on the release of ^{14}C activity was investigated by 24 h freezing/thawing cycles with temperatures of −20 °C and +40 °C over two periods of 4 weeks each. After the first and the second period, the soil was incubated for 4 weeks at a constant temperature of +20 °C, allowing the microorganims to mineralize the ^{14}C activity eventually released. The ^{14}C activity was balanced over the total incubation period of 4 months. The mineralization was measured as described above, and soil samples were taken at zero time and at the end of each incubation period. These experiments were run in duplicate in comparison to an untreated control that was incubated uniformly at +20 °C. In the same way, experiments were conducted with drying (oven drying at 70 °C) and rewetting the soil samples. The water contents were adjusted every 24 h to <10% and >100% of the maximal water holding capacity of the soil.

Chemical Treatments. The stability of metal−organic complexes was tested by EDTA as a complexing agent. Soil samples (2 g dry wt) were extracted for 90 min on a shaker with 12 mL EDTA solutions (Na_2−EDTA, Titriplex III, Merck, FRG) at the concentrations 0.01, 0.025, or 0.05 mol L^{-1}, adjusted to pH 4.6. The EDTA solution and the soil were separated by centrifugation (3000*g* for 20 min and 16.000*g* for 5 min). The soil pellet was successively extracted by methanol/water, by acetone, and an by alkyline hydolysis as described below. To check whether the ^{14}C activity in the EDTA solutions was related to soil organic matter molecules, the EDTA solution was acidified with concentrated HCl (pH < 1) or with saturated aluminum sulfate solution thus inducing precipitation of dissolved or colloidal organic carbon molecules. After the precipitation the solutions were centrifugated at 16.000*g* for 5 min, and the ^{14}C activity in the supernatant was analyzed. The EDTA soution was extracted by a liquid/liquid partitioning with hexane (twice with 15 mL) in a glass separation funnel (50 mL volume).

Analytical Procedures. The ^{14}C radioactivity was analyzed in all liquids by β-scintillation counting (Tri-CARB 1600TR, Packard Instruments Company, The Netherlands). The total ^{14}C carbon in the solids was determined by the combustion of 1−1.5 g of soil material at 800−900 °C in a combustion unit (I-05/RP and D-02 GTE, Ströhlein Instruments, FRG). The coefficient of variation of this analysis was less than 6%. The amount of the extractable ^{14}C activity was determined by a consecutive two-step extraction procedure consisting of an acetone extraction and an alkaline hydrolysis as described by Eschenbach et al. (*3*), if not noted otherwise. The extraction was done in triplicate; the results given are means. The coefficient of variation of the analyzed data was less than 7%.

In some cases, the part of the ^{14}C activity that was extractable by a mixture of methanol/water was analyzed because this mild extraction is assumed to be more suitable to extract only bioavailable parts of the contamination (*9*). The samples (3 g of soil with 3 mL of solvent) were extracted for 30 min in a ultrasonic bath. The nonextractable ^{14}C residues could be determined by combustion of the remaining soil pellet. Since the analytical variation was less than 6%, the nonextractable ^{14}C residue was generally calculated as the difference between the total and the extractable ^{14}C activity.

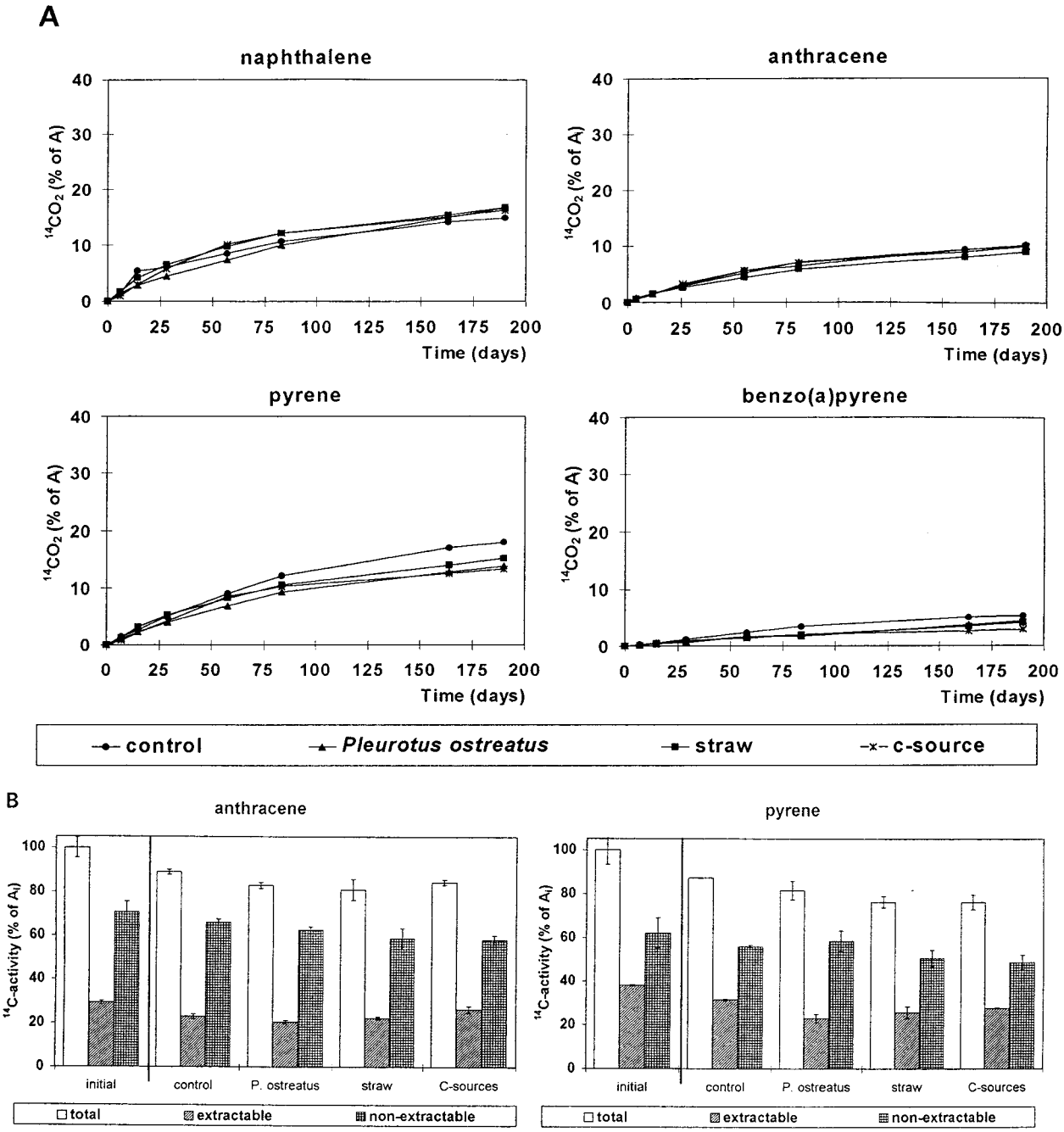

FIGURE 1. (A) Evolution of $^{14}CO_2$ from soil samples with nonextractable residues of [^{14}C]naphthalene (sample: AL-Nap), [^{14}C]anthracene (sample: AL-Ant), [^{14}C]pyrene (sample: AL-Pyr), and [^{14}C]benzo[*a*]pyrene (sample: AL-BaP) in an unsupplemented control or after adding *P. ostreatus*, wheat straw, or a carbon source (glucose and starch) during the incubation of 190 days. (B) Fractionation of the ^{14}C activity (total, extractable, and nonextractable) within the soil samples at zero time (initial) and at the end of the incubation (190 days). The extractable activity is the sum of the solvent-extractable part and that of the alkaline hydrolysis. Means and standard deviations based on three analysis.

Results and Discussion

Impact of Humus-Interacting Biological Supplements. The first group of biological supplements being tested in relation to its impact on the release and transformation of nonextractable PAH residues comprised the white rot fungi *Pleurotus ostreatus* and *Trametes versicolor* and the bacterium *Rhodococcus erythropolis*. All organisms were chosen as supplements due to their known ligninolytic and humus degrading activities (*16, 18*), the white rot fungi in addition due to their ability to metabolize PAH (*19*). Since *P. ostreatus* had been precultivated on fermented straw, it was also necessary to study the particular impact of that cosubstrate separately.

The first experiments with *P.ostreatus* showed that considerable fractions of the total residual [^{14}C]PAH activity could be transformed into $^{14}CO_2$ during the 190 days of incubation (Figure 1A). The total amount of mineralized ^{14}C activity ranged from 9−10% (anthracene), 15−17% (naphthalene), to 14−18% (pyrene) of the initial total ^{14}C activity. The lowest degradation rate was detected with [^{14}C]benzo[*a*]pyrene ($^{14}CO_2$: from 4 to 5%). In comparison to the unsupplemented control, neither the ligninolytic white rot fungus *P. ostreatus* nor straw had a particularly stimulating effect on the mineralization, though the fungus grew well in the soil (checked microscopically for formation of the typical basidiomycetes mycelium). The release of $^{14}CO_2$ led cor-

respondingly to a moderate decrease of the total ^{14}C activity in the soil samples during the 190 days of incubation (Figure 1B). The reduction of the different ^{14}C fractions within the soil (extractable, nonextractable) differed slightly, but a particular supplement effect could not be detected. The nonextractable residue fraction of [^{14}C]pyrene was reduced, for example, by 6 percentage points in the control sample while it was reduced by 4 percentage points in the soil sample supplemented with *P. ostreatus*. A distinct increase of the extractable fraction was not observed in any of the experiments, including the experiments with [^{14}C]naphthalene and [^{14}C]benzo[*a*]pyrene residues.

The experiments carried out with the humus-decomposing microorganisms *T. versicolor* and *R. erythropolis* gave very similar results. The final mineralization rate observed after the incubation period of 210 days accounted for 19% of the initial ^{14}C activity with *R. erythropolis* and for 16% with *T. versicolor*. The $^{14}CO_2$ release in the control was on the same order of magnitude (14%). The corresponding relative decrease of the nonextractable residue fraction amounted to 5 percentage points in the control sample, 7 percentage points in the sample with *R. erythropolis*, and 2 percentage points in the soil sample that had been inoculated with *T. versicolor*. None of the tested humus-decomposing microorganisms were therefore able to increase the intrinsic degradation of nonextractable [^{14}C]PAH residues significantly.

In addition to the experiments with the presumable humus-degrading microorganisms, it was also tested whether excess amounts of carbon sources such as glucose and starch could eventually trigger the release of the nonextractable ^{14}C activity indirectly by inducing an enhanced biogenic humus decomposition. Such a decomposition may occur due to the need to complement the excess amounts of carbon by the mobilization of an extra N source for microbial growth ("priming effect"; *17*). The results of these experiments (i.e., "C-source" in Figure 1) indicated that no discernible differences of the nonextractable ^{14}C fraction between the soil samples with an addition of pure C sources and the unsupplemented control was detectable. The extractable ^{14}C fraction was never higher than it had been at the beginning of the incubation. Considerable parts of the nonextractable ^{14}C fraction were obviously mineralized by the autochthonous soil microflora (supplemented and unsupplemented soil differed negligibly by less than 4 percentage points each; Figure 1).

The last series of experiments with putative humus-interacting biological supplements was carried out with compost, forest litter, and bark chips since these supplements are frequently added to soil for melioriation purposes and since they might carry undefined humus-interacting microorganisms into the soil. The overall results of these experiments were similar to those obtained with the humus-degrading microorganisms. The mineralization in the supplemented soil samples was with all supplements about as high as that one in the untreated control (Figure 2A), though the soil, containing 80% of nonextractable ^{14}C activity from [^{14}C]anthracene, was incubated with each of the supplements for 210 days. The $^{14}CO_2$ released during the incubation period ranged from 14% (untreated control) to 17% (bark chips supplemented soil) of the initial ^{14}C activity. This mineralization occurred again at the expense of the other two fractions: the nonextractable ^{14}C residues decreased by 5−13 percentage points, while the extractable ^{14}C activity decreased by 7−10 percentage points (Figure 2B). The fate of the nonextractable residues was therefore not significantly influenced by these organic supplements, although the supplements stimulated the general soil bioactivity (determined as total CO_2 production, O_2 consumption and decrease of the total carbon content in supplemented soils; data not shown). The microbial activities were also not inhibited by

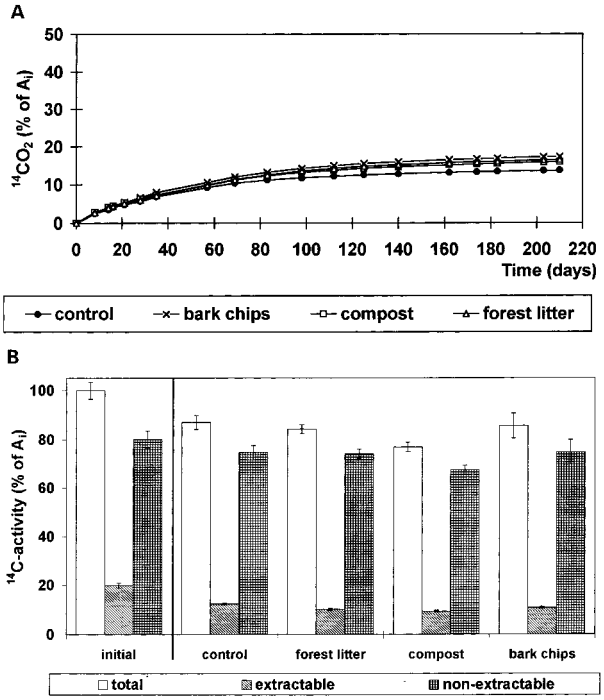

FIGURE 2. Influence of organic supplements such as bark chips, compost, or forest litter on the fate of nonextractable [^{14}C]anthracene residues (sample: BL-Ant). (A) Evolution of $^{14}CO_2$ during the incubation of 210 days. (B) Fractionation of the ^{14}C activity (total, extractable, and nonextractable) within the soil samples at zero time (initial) and at the end of the incubation (210 days). The extractable activity is the sum of the solvent-extractable part and that of the alkaline hydrolysis. Means and standard deviations based on three analysis.

the residual PAH contents. Similar poor effects of biological supplementation on the pollutant residue turnover were found by McRae, who had investigated the impact of supplements such as wheat straw, mung bean, or cassava leaves on the mineralization of nonextractable [^{14}C]fenitrothion residues in soil (*20*).

Summarizing the experiments detailing the impact of biological supplements on the turnover of the fractions of the extractable and nonextractable ^{14}C residues, the first conclusion is that neither the addition of biological supplements nor the addition of pure carbon sources such as glucose led to an increase of the extractable and therefore potentially mobile [^{14}C]PAH residues. This conclusion can also stand up to a long-term extrapolation since—despite small difference among the different soils—the transformation activity in the soil (as based on the mineralization activity) approached in all cases similar levels of a continuous but very low $^{14}CO_2$ release within the first 100 days of incubation. It is therefore very unlikely that longer incubation periods would result in a more pronounced release of ^{14}C activity, especially since the experiments were already run under conditions that favored the degradation of the ^{14}C residues (optimized water contents, temperature, etc.). This means that although the microorganisms used for inoculation in our experiments were shown to be able to degrade artificial soil organic matter complexes in liquid culture experiments (*16*, *21*), they were obviously less effective when they were added to the soil directly.

A second important conclusion from the presented data is the proof that formerly nonextractable PAH residues become degraded to $^{14}CO_2$. This turnover of nonextractable residues could be compared, based on the model that the nonextractable [^{14}C]PAH residues are covalently bound or trapped within the humic matrix (*6*, *22*−*24*), to humus

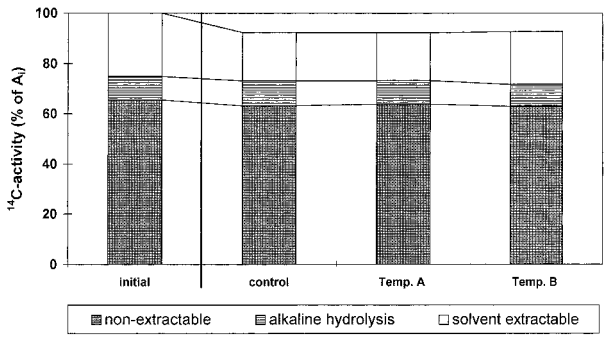

FIGURE 3. Influence of freezing/thawing cycles on the fate of the ^{14}C activity in a soil sample with nonextractable [^{14}C]pyrene residues (sample: BL-Pyr). Fractionation of the ^{14}C activity (extractable by a solvent and by alkaline hydolysis and nonextractable) of the treated soil samples (temp A and B) and the control sample at zero time (initial) and at the end of the total incubation (4 months).

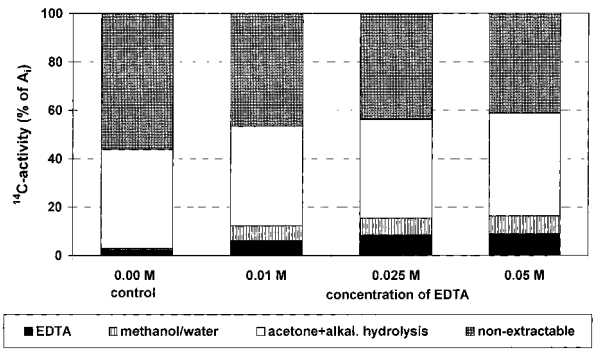

FIGURE 4. Effect of resolving metal−organic complexes by a treatement with EDTA at different concentrations on the fate of nonextractable residues of [^{14}C]pyrene (sample: BS−Pyr). Fractionation of the ^{14}C activity (extractable with EDTA, methanol/water, acetone and alkaline hydrolysis, and nonextractable) within the soil samples.

turnover rate in the soil (2−5% per year; data for undisturbed soils under moderate climatic conditions; *25*). Experiments with artificially produced pollutant/humic complexes revealed that covalently bound pollutants as aniline and chlorinated phenols can be mineralized to approximately the same amount as the humic substances itself (*26, 27*). The reduction of the nonextractable ^{14}C fraction observed in this study was also slightly higher than the given average natural humus turnover rate due to the optimized incubation conditions, the disturbed nature of the soil samples, the particular soil material, or the particular high activity of the autochthonous soil microorganisms. Another reason might be that the soil still contained a fraction of mineralizable but poorly available parent PAH molecules (i.e., trapped pollutants). However, the presumed release of some (formerly trapped) parent PAH never did lead to a net increase of the mobile and extractable ^{14}C fraction.

Impact of Physical Stress Factors. The experiments on the influence of frost and rapid temperature variations were conducted with soil containing nonextractable residues of [^{14}C]pyrene. The extreme alternations of temperature did not influence the amounts of nonextractable [^{14}C]pyrene residues (Figure 3). At the beginning of the incubation, 25% of the initial ^{14}C activity was extractable by solvents (thereof 3% in the methanol/water and 22% in the acetone extract) and 9% by the alkaline hydrolysis. At the end of the incubation period (i.e., after 4 months), about 92% of the initial ^{14}C activity was still detectable in the soil while the mineralized fraction amounted to 8 or 9% of the initial ^{14}C activity. About 63% of the initial activity was still present in a nonextractable form in both the control sample and in the treated soil samples (temp A and B). The solvent-extractable part decreased during the period of incubation slightly by about 5%.

Drying and rewetting of the soil also did not have a remarkable effect on the stability of the nonextractable residue fraction (data not shown). The collected data therefore allowed the conclusion that environmental factors such as repeatedly freezing and thawing—though they may influence the formation of nonextractable residues in soil (*8−10*)—did not destabilize nonextractable residues once they are formed.

Impact of Chemical Stress Factors. Another feasible worst case situation that was necessary to test was that the soil organic matter could become destabilized by chemical agents. A possible approach to evaluate this worst case situation was to treat the soil with EDTA, a compound that may extract the stabilizing divalent metal ions from metal−organic complexes (*17*). A soil sample with nonextractable residues of [^{14}C]pyrene was therefore treated with EDTA solutions and subsequently extracted as described above. It can be seen from Figure 4 that the application of increasing concentrations of EDTA solutions led to an increase of ^{14}C activity in the EDTA−soil extracts. In the control sample, treated by water rather than by EDTA solution, only 2% of the total initial ^{14}C activity was detectable and less than 1% in the methanol/water extract. However, about 9% of the initial ^{14}C activity became extractable by a 0.05 M EDTA solution. The subsequent soil extraction with methanol/water recovered another 8% of the initial ^{14}C activity. In contrast to all the other stress tests mentioned so far, EDTA was also able to mobilize perceptible amounts of ^{14}C activity from the nonextractable residue fraction. The amount of nonextractable ^{14}C activity was reduced with increasing concentrations of EDTA. The treatment with 0.05 M EDTA reduced the nonextractable ^{14}C residue fraction by 15 percentage points to an amount that was 41% of the inital ^{14}C activity (Figure 4), while 56% of the total ^{14}C activity remained nonextractable in the water-treated control sample.

The observed EDTA effect could be explained by two different hypotheses. The first explanation could be that the released ^{14}C activity consisted mainly of [^{14}C]PAH molecules that had been merely physically entrapped within the soil matrix. These molecules could have been set free due to the destruction of the spatial structure of the soil organic matter. It has been shown recently that humus does in fact contain such cage-like structures where especially hydrophobic substances such as PAH could have been sequestered or encapsulated (*24*). Another explanation of the remobilizing effect of EDTA could be that the released ^{14}C activity was part of unknown soil carbon molecules that were solubilized by the EDTA treatment. To check the latter hypothesis, the EDTA extracts were precipitated and analyzed in more detail. It was found that the ^{14}C activity in the supernatant was in fact reduced by more than 50% with either precipitation method. Though the soil organic matter fractions cannot be precipitated quantitatively, the experiment indicated that the released ^{14}C activity was probably mainly due to the elution of [^{14}C]carbon that was associated or bound to organic soil molecules. A liquid/liquid extraction of the EDTA solution with hexane confirmed the assumption. No ^{14}C activity was detectable in the solvent hexane after the extraction. This showed that neither original PAH molecules nor nonpolar PAH metabolites could be the carrier of ^{14}C activity in the EDTA extracts. The risk that may be involved with the presumed attachment of the ^{14}C residue activity to the dissolved organic matter (DOM) of the soil must be carefully addressed in future studies since it is well-known that water-soluble humic substances can act as carriers for

these organic xenobiotics over long distances (*28*, *29*). It is therefore necessary first to study in more detail the chemical identity and stability of the newly formed DOM−[^{14}C]PAH residue complexes and, second, to evaluate the (eco)-toxicological effects of these DOM−PAH residues.

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