GRASS LITTER DECOMPOSITION AND SOIL ANIMAL COLONIZATION: IMPACT OF BENZO(A)PYRENE AND PCB 52 IN FORMER SEWAGE FIELDS

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SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are organic chemicals ubiquitous in the environment. Because of their toxicity to man, their recalcitrance to degradation and their persistency in the environment, PAHs and PCBs are listed in the inventory of priority pollutants compiled by several Environmental Protection Agencies. The far greatest amount of PAHs and PCBs are stored in the soil. Despite of this fact, the amount of data available on the toxicity of these organic compounds to soil animals and soil processes is very scarce.

Aim of this thesis was to assess the response of soil fauna to a contamination of soil and litter with the chosen reference substances benzo(a)pyrene (BaP; PAH) and 2,2'-5,5' tetrachloro-biphenyl (PCB 52; PCB). The overall experimental approach was designed so to investigate the impact of BaP and PCB52 on terrestrial ecosystems at different level of biological organization: Surveyed were the reactions of single species but also the time course of selected soil processes. One further issue addressed to was the characterization of the behavior of the reference compounds exposed in the field by means of litter as carrier matrix.

Two litter decomposition experiments were the core experiments of this study. The first was carried out on a former sewage field (RefB field) near Berlin, Germany, employing the litter of the typical grass species of the study area (*Agropyron repens* L.). The second decomposition experiment was performed under semi-field condition in so-called Mitscherlich vessels. In this second trial, radio-labeled compounds were utilized to contaminate the litter, in contrast to the "cold" BaP and PCB 52 used in the field experiment. The concentrations of extractable parent compounds were monitored in both experiments.

After a brief exposure in the field, extractable concentration of BaP as well as of PCB 52 decreased sharply: After one year, only 15 % and 10% of the initial applied amounts, respectively, could be recovered by solvent extraction from the litter of the field experiment. The complete combustion of the litter from the Mitscherlich vessel experiment, however, revealed that 80% of the initially applied ^{14}C activity was still present in the BaP treatment, but only 50% in the PCB 52 variant. Moreover, the ^{14}C activity in the vessels treated with PCB 52 was almost evenly distributed between litter and the topmost soil layers, whereas 95 % of the recovered BaP activity was detected in the litter layer. Thin layer chromatographies of the organic phase of the litter solvent extraction were performed to assess the percentage amount of the non metabolized parent compound vs. the transformed metabolites. The overall picture points to a 'real' loss of PCB 52 and its metabolites during the exposure in the field, with the remaining ^{14}C activity in the litter still belonging to the parent compound. In contrast, BaP was metabolized to a higher extent, but the transformed metabolites were strongly bound to the organic matrix and did not move into the soil.

In the Mitscherlich vessels, the grass litter decomposed more slowly than in the field. After 9 months, 65% of the litter remained in these containers in contrast to 45 % on RefB area.

Nevertheless, the two decomposition experiments were comparable regarding the time course of the extractable amounts of BaP and PCB 52: The concentrations were related to the decomposition extent of the litter and not to the absolute time it had been exposed in the field.

The contamination of the litter with PCB 52 had no detectable effect on the litter decay rates up to the tested concentration of 11 mg PCB 52 x kg^{-1} litter. Benzo(a)pyrene, on the contrary, enhanced markedly the decomposition process, with maximum differences in the highly contaminated variants (100 mg BaP x kg^{-1} litter) of 18 % compared to the uncontaminated controls. The time course of the litter decay as affected by BaP displayed an effect curve typical for an environmental contamination with compounds that become less available or transformed with time: The decay rates were similar in the beginning, diverged with time, but approached the control values towards the end of the experiment.

The colonization of the litter by microarthropods revealed a differentiated response of single Collembola and oribatid mite species to the grass litter contamination with BaP and PCB 52. No species was found that reacted with decreased colonization densities to a contamination with BaP. In the PCB 52 treated litter, some species with low dominance displayed reduced individual densities. Euryoecious Collembola and Oribatida did not avoid the highly contaminated litter of both variants. Laboratory tests on the reproductive performance of Collembola were carried out according to ISO standard procedures: *Folsomia candida* displayed higher reproduction rates in soils contaminated with PCB 52 and BaP. Benzo(a) pyrene influences directly the reproduction rate of soil animals, probably via mimicry of steroid hormone structure. Summarizing the gained insight achieved within this thesis on the reaction of the decomposer community to a contamination with BaP and PCB 52, we may point to the reported higher colonization densities of soil organisms in the field BaP treatments as responsible for the higher litter decay rates.

In order to compare the results achieved by means of experimental spiking of litter and soil materials with the possible effects resulting from long-term contamination histories in the field, I investigated the fauna composition and activity in differently contaminated former sewage areas. Reforestation of the fields allowed for a diverse microarthropod fauna to colonize the soils, but the population structure of nematodes reflected the superficiality of the changes occurred, for all species present were typical for strongly disturbed sites.

Finally, in a first risk assessment, I applied simple extrapolation methods to the achieved data on the response of soil animals and soil processes to a contamination with BaP and PCB 52. In the investigated sites the maximum soil concentrations at which the soil decomposer community may still be protected from adverse effects are reached. Concerning their loads with organic contaminants, the sewage field soils have no security margin in respect to the compliance of protection targets as defined in the German Soil Protection Act.

ZUSAMMENFASSUNG

Polyzyklische Aromatische Kohlenwasserstoffe (PAKs) und Polychlorierte Biphenyle (PCBs) sind organische Stoffverbindungen mit einer ubiquitären Umweltverbreitung. Aufgrund ihrer Toxizität für den Menschen, ihrer geringen Abbaubarkeit und ihrer hohen Persistenz in der Umwelt werden PAKs und PCBs in den Listen prioritärer Schadstoffe vieler Länder geführt. Obwohl der größte Anteil der PAKs und PCBs in terrestrischen Ökosystemen gespeichert vorliegt, sind die Kenntnisse über die Toxizität dieser organischen Verbindungen für Bodenorganismen und Bodenprozesse sehr gering.

Ziel dieser Arbeit war es, die Auswirkungen einer Belastung von Streu und Boden mit den Referenzsubstanzen Benzo(a)pyren (BaP; PAK) und 2,2'-5,5'-Tetrachlorbiphenyl (PCB 52; PCB) auf die Bodenfauna zu charakterisieren und zu bewerten. Der experimentelle Ansatz wurde so gewählt, dass der Einfluss von BaP und PCB 52 auf Bodenorganismen auf verschiedenen Stufen biologischer Organisation untersucht werden konnte. Hierfür wurden sowohl die Reaktionen einzelner Arten erfasst, als auch der zeitliche Ablauf wichtiger Bodenprozesse aufgezeichnet. Ein weiterer Schwerpunkt der Arbeit bestand in der Charakterisierung des Verhaltens der gewählten PAK- und PCB-Referenzsubstanzen im Laufe der Freilandversuche.

Zwei Streuabbauversuche bildeten die Hauptuntersuchungen dieser Arbeit. Der erste Streuabbauversuch wurde auf einer ehemaligen Rieselfeldfläche (RefB) in der Nähe von Berlin mit standorttypischer Streu (*Agropyron repens* L.) durchgeführt. Der zweite Versuch fand dagegen unter semi-Freiland-Bedingungen in sogenannten Mitscherlich-Gefäßen statt: hier konnte radioaktiv markiertes BaP und PCB 52 eingesetzt werden, im Gegensatz zu den "kalten" Substanzen in den Freilandversuchen.

Bereits nach einer kurzen Exposition im Freiland sanken in der Streu die Konzentrationen an extrahierbarem BaP und PCB 52 stark ab. Nach einem Jahr konnten nur noch 15 % bzw. 10 % der applizierten Mengen durch Lösungsmittelextraktion nachgewiesen werden. Eine vollständige Verbrennung der Streu aus den Versuchen mit radioaktiven Referenzsubstanzen zeigte jedoch, dass 80 % der anfänglich applizierten ^{14}C-Aktivität weiterhin in den BaP-Varianten vorhanden war. In den PCB-Varianten konnten dagegen noch 50 % der ^{14}C-Aktivität nachgewiesen werden. Interessanterweise war die ^{14}C-Aktivität in den Varianten, die mit radioaktivem PCB 52 kontaminiert wurden, gleichmäßig zwischen Streu- und oberster Bodenschicht verteilt, wohingegen 95% der wiedergefundenen ^{14}C-Aktivität der BaP-Varianten in der Streu lokalisiert war. Um die ursprünglich eingesetzten Referenzsubstanzen von den im Versuchverlauf gebildeten Metaboliten trennen zu können, wurden Dünnschichtchromatographien der extrahierten organischen Phasen durchgeführt. Die Ergebnisse belegen einen hohen 'echten' Verlust von PCB 52 und seinen Metaboliten aus den untersuchten Substraten während der Exposition im Freiland. Benzo(a)pyren dagegen lag zwar zu einem höheren Anteil transformiert vor, aber sowohl die Ursprungssubstanz als auch die Metabolite

blieben stark an die organische Matrix gebunden.

Die Grasstreu wurde in den Mitscherlich-Gefäßen langsamer abgebaut als im Freiland. Nach 9 Monaten wurden 65 % der Streu in diesen Gefäßen wiedergefunden, im Gegensatz zu den 45 % im Abbauversuch auf der Fläche RefB. Dennoch sind die zwei Experimente in Bezug auf das Verhalten von BaP und PCB 52 vergleichbar: Die Konzentrationen der extrahierbaren Referenzsubstanzen korrelierten mit dem Abbaugrad der Streu und nicht mit der Länge der Streuexposition im Freiland.

Die Kontamination der Streu mit bis zu 11 mg PCB 52 x kg^{-1} hatte keine nachweisbaren Effekte auf die Dekomposition von *A. repens*. Benzo(a)pyren bewirkte dagegen eine deutliche Steigerung der Streuabbauraten, mit maximalen Abweichungen zwischen der hochkontaminierten Variante (100 mg BaP x kg^{-1} Streu) und der unbelasteten Kontrolle von 18 %. Die Dekomposition der BaP-belasteten Streu zeigte einen Prozessverlauf, der typisch für eine Umweltkontamination mit Substanzen ist, die mit der Zeit schlechter verfügbar oder transformiert werden. Die Abbauraten der hochkontaminierten Streu unterschieden sich zuerst nicht von den Kontrollvarianten, divergierten im Laufe des Experimentes, näherten sich aber zum Ende hin wieder den Werten der unbelasteten Streu.

Die Besiedlungsdynamik der Streu durch Mikroarthropoden belegte eine differenzierte Antwort der einzelnen Collembolen- und Oribatidenarten auf die Kontamination mit BaP und PCB 52. Es wurde keine Art gefunden, die mit verminderten Besiedlungsdichten auf Benzo(a)pyren reagierte. In den Streuabbaucontainern, die mit PCB 52 belastet wurden, wiesen dagegen einzelne Arten geringere Individuendichten auf. Euryöke Collembolen und Oribatiden vermieden nicht die Abbaucontainer mit hochkontaminierter Streu.

Der Einfluss von BaP und PCB 52 auf die Reproduktionsraten von Collembolen wurde in Labortests nach DIN-ISO-Vorschriften untersucht. *Folsomia candida* zeigte höhere Vermehrungsraten in den kontaminierten Böden. Besonders Benzo(a)pyren kann, aufgrund seiner strukturchemischen Ähnlichkeit mit steroiden Hormonen, direkt in das Reproduktionssystem von Organismen eingreifen.

Die höheren Besiedlungsdichten von Mikroarthopoden in der mit BaP kontaminierten Streu, die in den Freilandcontainern nachgewiesen wurden, könnten direkt für die beobachteten höheren Streuabbauraten verantwortlich sein.

Um die Ergebnisse der Studien, die mit experimentell belasteten Substraten durchgeführt wurden, mit möglichen Effekten einer oft langjährigen Kontaminationsgeschichte im Freiland vergleichen zu können, untersuchte ich die Zusammensetzung der Bodenfauna von verschiedenen ehemaligen Rieselfeldflächen. Besonders die Umgestaltungsmaßnahmen nach der Stilllegung der Flächen (z.B. Aufforstung) hatten einen großen Einfluss auf die Diversität der Oribatidenzönosen. Die Zusammensetzung der Nematodenfauna wies jedoch deutlich auf die Oberflächlichkeit der Veränderungen hin, die in den Böden stattgefunden hatten, da alle Arten typisch für stark gestörte Lebensräume waren.

Die Untersuchungen, die in dieser Arbeit durchgeführt wurden, waren in einem Verbundprojekt des BMBFs eingebettet. Die Ergebnisse der hier vorgestellten Studien und weiterer Arbeiten aus dem Verbundprojekt erweiterten deutlich die Datenlage zu den ökotoxikologischen Auswirkungen von Benzo(a)pyren und PCB 52, so dass ich eine erste Risikoabschätzung durchführen konnte. Die Ergebnisse einfacher Extrapolationsrechungen zeigen, dass auf den untersuchten Standorten bereits die maximalen Schadstoffkonzentrationen erreicht sind, unter denen die Bodenorganismen vor schädlichen Einflüssen noch geschützt werden können. Um natürliche Bodenfunktionen gemäß Bundes-Bodeschutzgesetz erhalten zu können, sind auf den Rieselfelder alle weiteren Einträge zu vermeiden, da die Böden keine Sicherheitsspanne bezüglich ihrer organischen Schadstoffbelastung aufweisen.

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LIST OF ABBREVIATIONS AND SYMBOLS

AET	actual evapotranspiration
AFS	ash free substance in a sample after oxidizer combustion
ANOVA	analysis of variance
BaP	benzo(a)pyrene, chosen reference substance from the polycyclic aromatic hydrocarbons
BaP 1; Bap2	BaP litter contamination variants (10; 100 mg BaP x kg^{-1} litter)
1BaP; 2BaP; 3BaP	BaP soil contamination variants (1.0;10;100 mg BaP x kg^{-1} soil)
BMBF	Bundesministerium für Bildung und Forschung (German Federal Ministry of Education and Research)
BTX	benzene toluene xylene
C/N	carbon to nitrogen ratio
CEC	Commission of the European Communities
Corg	organic carbon content of a sample
c-p1…c-p5	colonizers-persisters type classification for nematodes families
CSTE/EEC	Conference of State and Territorial Epidemiologists / European Economic Community. Here: Section of CSTE/EEC, the Scientific Advisory Committee on Toxicity and Ecotoxicity of Chemicals
CV	coefficient of variation
DDT	dichloro-diphenyl-trichloroethane
DIN	Deutsches Institut für Normung (German Institute for Standardisation)
DM	dry matter
D_N	individual dominance, relative frequency of species in a sample
DW	dry weight
EC	European Commission
EPA	Environmental Protection Agency
F (ANOVA)	value of the F-Distribution (after R.A. Fisher). Here: ratio of two variance estimates
FAME	Factorial Application Method, extrapolation method used in the calculation of threshold values in risk assessment procedures
DF (ANOVA)	degrees of freedom
FW	fresh weight
GG; MG	Grobgaze, coarse gauze litter decomposition containers (GG, 10 mm mesh size) ; Mittelgaze, medium gauze containers (MG, 1 mm mesh size)
HPLC	high-pressure liquid chromatography
ISO	International Organisation for Standardisation
K	Kontrolle, uncontaminated control experimental variant

KL	Kontrolle Lösungsmittel, solvent control experimental variant
Koc	partition coefficient between watery solutions and the organic carbon of the solid matrix
Kow	octanol water partition coefficient
LOEC	lowest observed effect concentration
MFO	mixed function oxigenase
MG	Mittelgaze, see GG; MG
MI	Maturity Index, classification index for nematode communities
MQ (ANOVA)	mean square sum
NEL	No Effect Level
nPAK	former sewage field, highly PAH contaminated
nPCB	former sewage field, highly PCB contaminated
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
PCB 52	2,2'-5,5' tetrachlorobiphenyl, chosen reference substance from the polychlorinated biphenyls
PCB 1 ; PCB 2	PCB 52 litter contamination variants (4; 40 mg PCB 52 x kg^{-1} litter) soil)
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
PVC	polyvinyl chloride
QS	Sörensen's quotient for simple comparison of species communities considers the range of species identical in both habitats
QSAR	quantitative structure activity relationship
R^2	coefficient of determination
Re	Renkonen's Index is a measure of the similarity in the dominance structures of two communities of species
RefB	former sewage field, reference area
SQ (ANOVA)	sum squared
TLC	thin layer chromatography
TVO	Trinkwasserverordnung, Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe, TrinkwV (German Ordinance for the Protection of Drinking Water Quality)
UBA	Umweltbundesamt (German Federal Environmental Protection Agency)
VDLUFA	Verband der Deutschen Landwirtschaftlichen Untersuchungs- und Forschungsanstalten (Association of German Agronomy Research Institutions)

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"*Conserving the biodiversity of soil organisms may be based on their current in-situ usefulness for soil processes, such as soil structure formation, decomposition…Another argument could be based on the 'intrinsic' value of soil organisms, apart from any utility, but soil organisms are not cuddly enough to be a prime focus of that type of attention*"

Meine van Noordwijk
"Decomposition: driven by nature or nurture?"
Applied Soil Ecology 4 (1996)

"Die meisten Bodentiere sind so klein, dass wir sie einfach übersehen. Das ist vielleicht gut so für die Ruhe unseres Gemüts"

Friedrich Schaller
"Die Unterwelt des Tierreiches" (1962)

1 GENERAL INTRODUCTION

The contamination of air, water, and soil with organic contaminants belonging to the polycyclic aromatic hydrocarbons (PAHs) and to the polychlorinated biphenyls (PCBs) is a topic of great environmental concern. These substances are listed in the inventory of priority pollutants compiled by Environmental Protection Agencies of several countries, being toxic to man, recalcitrant to degradation, very persistent in the environment and in part biomagnified in food chains. Some of the compounds can be transformed in the mammalian organism into potent carcinogens.

The distribution of PAHs and PCBs between the different environmental compartments points to the soil as the main repository sink for these organic compounds. In spite of the fact that the far greatest amount of all PAHs and PCBs determined in the environment is allocated in the topmost layers of the soil, knowledge about their toxicity in terrestrial ecosystems is very scarce. As VAN BRUMMELEN (1995) pointed out, "this absence of data is shocking", since soil organisms live in close contact to their surroundings and an impairment of their activity leads to the disruption of important processes of matter and energy cycling in terrestrial ecosystems.

The assessment of the impact of pollutants on ecosystems is often performed by means of simple toxicity testing, and the uncertainties arising from the extrapolation to the desired higher level of biological organization counteracted with the use of security factors. On the one side, every new added study result to the set of available data is an improvement in the accuracy of the prediction of pollutant impact on ecosystem level, since the uncertainty decreases the more ecotoxicological knowledge on the effect of a specific chemical exists.

On the other side, the protection of functional properties of ecosystems may not be accomplished by the most accurate analysis and protection of structural characteristics, as it is

the case when relating organisms' responses from single species test to ecosystem responses by means of extrapolation. At this point, the direct evaluation of toxicant effects at higher level of biological organization is required.

In this thesis, the effects of reference substances from the polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs) on the structure and activity of the soil fauna is investigated. The experimental plan was drawn so to cover the response of single organisms but also of selected processes to an environmental pollution with benzo(a)pyrene (BaP) and 2,2'-5,5' tetrachlorobiphenyl (PCB 52). The embedding of this work in a joint research project allows for the interdisciplinary evaluation of the obtained results; moreover, it facilitates the attempt of an estimation of the risks that may originate from a contamination with PAHs and PCBs for the investigated areas.

The research work was carried out on former sewage farm fields near Berlin, an area displaying extreme and patchy soil pollution with PAHs, PCBs and heavy metals, but being of particular interest for the urban planning needs of the expanding city.

1.1 Background

1.1.1 Ecotoxicology and Risk Assessment

The science of ecotoxicology has its ultimate interest in determining the effects of pollutants on ecosystems (FORBES & FORBES, 1994; STEINBERG *et al*., 1993). However, measuring directly the response of intact natural ecosystems to pollutants is difficult, and the complexity at this level of biological organization very high. Ecotoxicological research, therefore, focuses mainly on the structure of single ecosystem components, or on identifiable energy and matrix fluxes between them, that is on specific ecosystem processes (FORBES & FORBES, 1994).

Historically developed as an extension of the field of toxicology, ecotoxicology addressed the first concerns about potential undesiderable effects of chemicals in the environment with research approaches traditionally derived from single species tests. In contrast to toxicology, though, which aims at the protection of the human species, ecotoxicology concerns itself with the protection of "several millions of species scattered over a variety of habitats" (PERSOONE & GILLET, 1990). Furthermore, ecotoxicologists are rarely asked to assess threats posed by toxicants to individual species but to collective groups, to "multispecies systems interacting with each other and their physicochemical surrounds" (CALOW, 1998) "often referred to as ecosystems".

There is a clear inconsistency between the scarce availability of ecotoxicological data from others than single species tests (on account of both historical and practical reasons) and the need for prediction of effects at the ecosystem level. Currently, not enough experimental or theoretical evidence is available to justify the contention that physiological responses of

organisms or the loss of species can be related in a predictable way to ecosystem dynamics (FORBES & FORBES, 1994; MATHES, 1997).

This problem is particularly evident when results from ecotoxicological studies form the basis for ecotoxicological risk assessment and subsequently for risk management. With the exception of nature conservation objectives, which may aim at safeguarding endangered species, the loss of a few individuals from a population is usually not considered to be serious, as long as the proper functioning of the ecosystems remains unaffected. Protection targets in risk assessment procedures are often defined by means of valuable functions an ecosystem holds and exerts, which should not be impaired by toxicant action. But this, states CALOW (1998), "presumes that the ecological targets we want to protect are not only identified, but understood in terms of the properties associated with them that we should be interested in maintaining".

1.1.2 Soil Functions as Protection Targets

The soil is a highly complex compartment of terrestrial ecosystems. It has been expressly protected by law ever since the German Bundestag enacted the Federal Soil Protection Act (*Bundesbodenschutzgesetz, BBodSchG*) in 1998 with the purpose "to protect or restore the functions of the soil on a permanent sustainable basis" (DEUTSCHER BUNDESTAG, 1998). As defined by the Act, the soil performs valuable functions, harmful changes to which as posed by potentially toxic chemical substances should be avoided. Protection targets are, therefore, not the ecosystem integrity as such, but the ecosystem functions, defined in the Protection Act as "natural functions" and "functions useful to man".

Nevertheless, natural functions constitute the importance of the soil as a "habitat for animals and soil organism", a "part of natural systems, especially by means of its water and nutrient cycles," and as a "medium for decomposition, balance and restoration as a result of its filtering, buffering and substance-transformation properties" (Federal Soil Protection Act, English version, DEUTSCHER BUNDESTAG, 1998).

Increasing levels of concern regarding the potential occurrence of harmful changes to protected soil functions at growing pollutant loads are marked in the Ordinance accompanying the law (DEUTSCHER BUNDESTAG, 1999) by different threshold concentrations for single chemical compounds. Fundamental differentiation has been made between precautionary values, up to which safeguarded soil functions are not endangered, and distinctly higher trigger values. Trigger values are intended to mark the carrying capacity of soil ecosystems and the threshold concentrations to undesirable risk levels, at which the probability of harmful changes to soil functions is high.

Soil ecotoxicologists are asked to support these defined threshold levels with concrete chemical concentration data.

In this respect, challenging questions arise on which properties a soil ecosystem holds that are associated with the fulfilling of the safeguarded functions, and, moreover, which of these properties may be monitored in ecotoxicological studies. To consider this divergence and to identify characteristic structures and key processes in the soil ecosystem may ensure that what we measure in ecotoxicological tests ("measurement endpoints" *sensu* SUTER, 1993) is relevant for what we want to protect ("assessment endpoints" *sensu* SUTER, 1993).

In practice, threshold values are derived by extrapolation from the available, limited data sets of concentration-effect relationships for single substances on selected organisms. The effect assessment at the required ecosystem level may involve many extrapolation steps: From acute to chronic effects, from one species to many species, from direct to indirect effects, from one ecosystem to other ecosystems, and, finally, in time and space (VAN LEEUWEN *et al.*, 1996).

To overcome the uncertainties, safety or assessment factors are always applied when deriving "safe" concentrations for the ecological target to be protected (US-EPA, 1984; CSTE, 1994; CEC, 1996; KOOIJMAN, 1987; VAN STRAALEN & DENNEMAN, 1989; ALDENBERG & SLOB, 1991; WAGNER & LØKKE, 1991).

The more ecotoxicological data are available, the smaller the applicable safety factor becomes by which the apparent [No] Effect Level ([N]EL), derived from all species tested, is divided to account for the species that have not been tested. Refer to the works of CAIRNS (1992), CAMPBELL & HOY (1996), CHAPMAN *et al.* (1996), CRANE & NEWMAN (2000), LASKOWSKI (1995), and VAN STRAALEN *et al.* (1994) regarding the controversial question, which measurement endpoints and statistical quantities may be considered a pertinent basis for extrapolation and which may not.

The extrapolation from results of single species tests to the ecosystem level are appropriate with respect to any risk assessment for the protection target "habitat function" (*Lebensraum-funktion*, Soil Protection Act): The property of a sufficiently clean environment that offers soil organisms the opportunity to live and reproduce may be mirrored in an extensive data set from survival and reproduction tests.

However, given that this type of risk assessment procedure ultimately will follow a bottom-up resolution approach, it may not be appropriate to extrapolate from species (ecosystem structures) to processes (ecosystem functions in the narrower sense, STEINBERG *et al.*, 1993).

These methods generally assume that safeguarding the species within an ecosystem will safely ensure the maintenance of soil properties as required, e.g., in processes like "decomposition, buffering and transformation of substances" (*Transformatorfunktion*, Soil Protection Act) or in the "water and nutrient cycles".

Extrapolation methodologies based on species sensitivity distribution allow for the loss of few species in the ecosystem (e.g., 5%) at given concentrations (ALDENBERG & JAWORSKA, 2000;

WAGNER & LØKKE, 1991; VAN STRAALEN & DENNEMAN, 1989). This comes to meet remarks of being overprotective in respect to soil functioning, since species inventory may be redundant. But again, it can never be known for certain that is not just those few species lost which are "ecosystem engineers", or species with unexpected outstanding relevance for soil processes (e.g., GITAY *et al*., 1996; BRUSSAARD, 1998; LYONS & SCHWARTZ, 2001).

Altogether, it may be argued that “at each succeeding level of biological organization, new properties appear that would not have been evident by the most intense and careful examination of lower level of organization” (CAIRNS, 1983).

Predicting the consequences of pollutant-induced effects on ecosystems requires that the effects be examined at different levels of biological organization (e.g., VAN STRAALEN & LØKKE, 1997; MATHES *et al*., 1991): Understanding toxic mechanisms is achieved by progressing downward in complexity, whereas the outcome of a toxicological event can only be fully understood by progressing upward to the more complex system. Here, the ecotoxicological effects of pollutants can be integrated with the fate (transport, transformation, and breakdown) of the compound in the environment (FORBES & FORBES, 1994; VAN STRAALEN &VAN RIJN, 1998).

The more realistic ecological results of field studies are achieved by involving the responses to toxicant exposure of all the interacting biological variables, making the outcome of the study unique in respect to the actual environmental conditions. But although the results of field studies are not transferable to other ecosystems and, therefore, have a low predictive potential (PERSOONE & GILLET, 1990), it is of great importance to integrate so-called higher tier approaches in risk assessment procedures. Higher tier tests conducted in the field are not used as first screening tools, but are required to address specific questions (BOUTIN *et al*., 1995) raised by a particular toxicant in a given environment: They are carried out as "definitive assessment" (VAN DIJK *et al*., 2000).

1.1.3 Soil Organisms and Ecosystem Processes

The Decay of Organic Matter

Currently, the monitoring of the decomposition process of dead organic matter is being discussed as a tool for evaluating the potential long-term effects of persistent substances on terrestrial ecosystems at higher tiers (EC, 2000; EC, 1991, RÖMBKE & KULA, 1998; DE JONG, 1998).

The discussion focuses on the harmonization of pertinent test protocols, not on the outstanding importance of this process, which is unanimously acknowledged.

The decomposition of plant litter and animal debris is the counterpart to photosynthesis (HEAL *et al*., 1997): It makes nutrients and trace elements available again to plants. It closes the cycles

of energy and matter transfer throughout ecosystems, as elements supplied from primary producers in organic form enter the decomposer subsystem, either directly, or, when stored in perennial plant tissues or allocated in the herbivore subsystem, over time. Material is recycled again and again in the decomposer subsystem, strictly speaking until carbon mineralization is complete (ANDERSON, 1983). The breakdown of organic compounds results in smaller, partly inorganic molecules, but since these can in turn be polymerized to stable humic compounds, "decomposition yields both complex molecules and simple inorganic compounds" (VAN WEMSEN, 1992).

Decomposition is defined as the process of organic matter breakdown (structural decomposition) and element mineralization (chemical decomposition) (WEIGMANN, 1998). Next to abiotic influences, like oxidation or leaching, it is mainly living organisms that perform and regulate the decay of organic compounds. In the terrestrial environment, soil animals and soil microorganisms form the "decomposer community", and its activity, composition, and species interactions strongly influence development, productivity, and stability of terrestrial ecosystems (e.g., SWIFT *et al*., 1979; LUSSENHOP, 1992; BEARE *et al*., 1997; BRUSSAARD, 1998; MARAUN *et al*., 1998; SETÄLÄ *et al*., 1998; WOLTERS, 2000).

On one side, the decomposition of plant residues may be seen as fully determined by "their nature", that is by the "physical, chemical and biological qualities of the organic residues" (VAN NOORDWIJK, 1996). In this respect, several studies with varying litter qualities have shown the consequences of different tissue structures, nutrient and secondary plant metabolite contents (e.g., phenols, lignin) on the decay process. Specific ratios of, e.g., carbon to nitrogen, carbon to phosphate, or nitrogen to lignin in the starting material have been used to characterize litter quality, since they are fairly good predictors of the decay rates in different phases of the decomposition process. (COTRUFO *et al.*, 1998; FIORETTO *et al*., 1998; GALLARDO & MERINO, 1999; GILLON *et al*., 1999; GUNADI *et al*., 1998; KEENAN *et al*., 1996; SZUMIGALSKI & BAYLEY, 1996; WEDDERBURN & CARTER, 1999; WISE & SCHAEFER, 1994). A narrow C/N ratio of the litter entering the decomposer subsystem has been correlated with a high susceptibility to decomposer attack, in contrast to high lignin and low nitrogen contents. As decomposition proceeds, though, high nitrogen concentrations inhibit lignin degradation, the decay rate of which exerts the dominant control in the latter decomposition stages (BERG, 1986; BERG *et al*., 1993).

On the other side, the actual "biotic and abiotic environment has a considerable modifying effect" on the decay, so that it is additionally driven by the specific ecosystem "nurturing" in which decomposition takes place (VAN NOORDWIJK, 1996). Climate is surely the one abiotic parameter that most significantly shapes the course of decomposition, as shown in experiments with unified litter material exposed to soils of climate transects across Europe (BERG *et al*., 1995; BOTTNER *et al*., 1998; BOTTNER *et al*., 2000; MARTIN *et al*., 1997). BERG *et al*., (1993) could explain 70% of the site litter mass loss variability in including in combined data analyzes

the actual evapotranspiration (AET) and the temperature (summer or average annual) as independent factors.

The influence of "nature" or "nurture" on the decay cannot be this sharply separated, though. Firstly, even if at broader regional scales the spatial patterns of decay appear to be dominated by climatic variables, the climate of the site influences litter quality. High values of AET correlate, e.g., with maximum values for N, P, S, and K concentrations in litter of the same type (BERG *et al*., 1995).

Secondly, and even more significantly, litter of either different qualities or exposed in ecosystems with dissimilar climatic conditions is decayed by specific decomposer communities, the activities and species compositions of which are influenced by both.

In detailed studies on the impact of varying microclimatic conditions on decomposer activity, KÖCHY & WILSON (1997) found lower decomposition rates in litter that had been shaded during the exposure on prairie soil in contrast to unshaded litter of the same species. In forest environments, though, the reverse seemed to be the case, even if the differences were minimal. The effect of periodical climate variations on the decay process has been often surveyed, lately by BALLINI (1997) or FIORETTO *et al*. (2001), who found clear correlations between lower decomposer activity and strong summer drought in Mediterranean ecosystems. CORTEZ stated in 1998 that moisture regimes are important, but rather during the first stages of decomposition. Subsequently, sites with different decomposer colonization densities may diverge in their decay rates, because in his studies the impact of soil organisms increased distinctly and site-specifically with time.

In summary, applying exclusively abiotic parameters as decay rate predictors may help to estimate the gross course of the organic matter breakdown. It is of great interest, however, to look inside the "black box" of the decaying litter matrix: Species composition and organism interactions are influenced by their abiotic environment, but as a consequence of organism activity the impact of abiotic parameters on soil processes may be buffered as well as increased.

Role of Soil Fauna in the Processes of Decomposition and Element Cycling

Soil animals and soil microorganisms form the decomposer community in the decaying litter "black box".Bacteria, fungi, and the soil microflora contribute largely to the breakdown of dead organic matter. The soil microorganisms are capable of mineralizing organic compounds and are, therefore, finally responsible for chemical decomposition. Recent studies have shown that the amount of secreted microbial extracellular enzymes in soils correlated with organic matter decomposition rates, and that they represented "instantaneous measures of biochemical processes" responsible, e.g., for the hydrolysis of particular chemical litter compounds (MOORHEAD & SINSABAUGH, 2000).

The soil fauna comprises a variety of species belonging to diverse taxonomic units, with different body sizes, feeding preferences, and habitat preferences; correspondingly, soil animals may be classified according to each of the above-mentioned criteria. Here, I will concentrate on the more functional properties of soil animals, like feeding mode, and on microbial-faunal interactions. If not expressly noted otherwise, the following is based on the reviews and standard works of WEIGMANN, 1998; WEIGMANN, 1993; PETERSEN & LUXTON, 1982; DUNGER, 1983; DUNGER, 1998; HOPKIN, 1997; LUSSENHOP, 1992 and VERHOEF & BRUSSAARD, 1990.

The usual classification identifies phytophagous (feeding on plants), zoophagous (predators and animal parasites), and detritophagous animals (feeding on dead organic matter). To characterize the decomposer community, soil animals of the detritophagous group may be subdivided further, namely into macrophytophagous (feeding on relatively intact plant litter), saprophagous (feeding on decayed plant debris, humus, and microorganisms) and microphytophagous (specialized on bacteria and fungi) animals.

According to their feeding mode, soil animals of the macrophytophagous group may attack plant litter immediately after it enters the decomposer subsystem. Since at least 80% of the plant material is not assimilated, it re-enters the decomposer food web and is then processed by saprophagous animals and microorganisms. Here, one important function of the soil fauna for the cycling of energy and nutrients through soil ecosystems becomes evident: Soil animals that comminute plant litter, crack cell walls, and break up recalcitrant material supply saprophagous animals and microorganisms with substrates that have been preliminary processed, and, therefore, are more accessible and offer greater surface areas (SCHEU & WOLTERS, 1991; CORTEZ, 1998; CURRY & BYRNE, 1997).

When microorganisms colonize dead organic matter, decomposer animals like saprophagous and microphytophagous organisms may feed on bacteria, algae, and fungi. Soil animal grazing has been shown to stimulate microorganisms, especially by triggering compensatory growth and increasing the specific activity of the remaining microbes (up to 30%, HANLON & ANDERSON, 1980)

Soil animal casts are substrates displaying high microbiological activity; they have higher organic carbon contents, and nitrogen contents twice that of the surrounding soil (GUGGENBERGER *et al*., 1996; MARINISSEN & DIDDEN, 1997; MARINISSEN & HILLENAAR, 1997).

Moreover, the ratio of bacteria to fungi is increased in animal feces as compared to the non-ingested soil (SCHEU & PARKINSON, 1994; MCLEAN & PARKINSON, 1997).

Finally, the burrowing and mixing activity of soil animals leads to the transfer of organic matter to deeper soil layers, to increased soil pore volume, to the amelioration of soil aggregate properties, and to the transport of microbial propagules within the soil (SCHULZ & SCHEU,

1994; HASSALL *et al*., 1987; CORTEZ & BOUCHÉ, 1998; HAYNES & FRASER, 1998; SCHRADER *et al*., 1997).

The regulatory activity of soil fauna has been shown to act specifically on C and N mineralization rates of different soil horizons as well as of soils of different ecosystem types (MCGONIGLE, 1995; VEDDER *et al*., 1996; BRUSSAARD, 1998). The study results give evidence for fauna dependent nitrogen immobilization in the litter layer as well as nutrient supply retardation due to inclusion of organic matter in clay-humus complexes. In the upper soil layers, however, the fauna contributes to nitrogen mobilization processes and, due to its digging activity, to a higher carbon supply (WOLTERS *et al*., 1989; HASEGAWA & TAKEDA, 1996).

The overall picture based on investigational results presents the soil fauna as a dynamic control factor, which buffers abiotic parameter fluctuations in soil ecosystems.

Particularly interesting in this respect are studies on the maintenance of turnover rates through the activity of soil fauna. Net nitrogen mineralization, e.g., is kept constant at a given site by soil animal activity, even in periods of great drought (VERHOEF & DE GOEDE, 1985), when organic material with extremely low nitrogen contents enters the system (SCHEU & SCHULZ, 1994), or when nitrogen is supplied in surplus (SCHOLLE *et al*., 1995). Soil animal communities react with colonization density adjustments as soon as the litter amount supplied to the system changes. Therefore, the soil cenosis at a given site regulates exactly those processes, which shape the characteristic properties of the ecosystem (WEIGMANN, 1998).

The impact of specific soil animals on the decay and turnover rates of organic matter has been studied extensively. Corresponding to the importance in terms of species representation in terrestrial habitats as well as their modulation extent on decay process, research focused on the contribution of two major microarthropod groups: Collembola, or springtails (Insecta), and Oribatida or oribatid mites (Cryptostigmata: Acarina).

Collembola are wingless insects which species are adapted to the life in different soil depths. While surface forms may display colorful body drawings, deeper dwelling species are mostly white and have reduced eyes. Collembola are mostly saprophagous, feeding on small plant debris colonized by microorganisms; some surface species, however, may also feed on fresh fallen litter. Species switching to a microphytophagous-feeding mode are known as well. Generally speaking, the Collembola are not very specialized feeders, and they are flexible regarding feeding substrate quality. In Central Europe, around 1.000 species have been identified. Colonization densities of Collembola may reach 100.000 individuals per square meter in forest organic layers. In meadows, the numbers of individuals may be between 10.000 and 100.000 and in agricultural soils around 20.000 individuals per square meter can be found.

Oribatid mites are the most numerous mite group in soils. The number of species identified in Central Europe is approximately 1.000, with larger surface species living in decaying litter and

smaller species (down to 200 µm) dwelling in the soil. Adult oribatid mites are mailed with a chitin shield that protects them from predators. Many Oribatida species have a distinctly specialized feeding mode: Some feed, e.g., on specific fungal mycelia, some only on lichens, some on litter in advanced stages of decay. In meadows, oribatid mites are present in densities between 5.000 and 50.000 individuals per square meter; in forest soils with a raw humus layer, they may reach colonization densities up to 500.000 animals per square meter. Oribatid mites have high demands with respect to soil profile structure and integrity: In agricultural fields their densities rarely reach 1.000 individuals per square meter.

During the decomposition process, Collembola and Oribatida species show characteristic succession patterns in the decaying litter according to their feeding mode, which can be studied vertically through the different litter horizons, or chronologically over sampling periods (e.g., ANDERSON, 1971; BERG *et al*., 1998; BECK, 1983; HUBERT *et al*., 2000; IRMLER, 2000; NANNELLI, 1990; PEREIRA *et al*., 1998; PONGE, 2000).

In the past years, a great amount of work has been devoted to quantifying the impact of soil microarthropods, and especially of Collembola and Oribatida, on the decomposition of organic matter. I will not attempt at presenting a complete literature review. However, two of the main different research approaches should be looked at.

On one side, the influence of soil animals on the decay of litter has been studied extensively in so called litterbag approaches. Litter confined in bags or boxes with different size mesh barriers facilitates the examination of the amount decayed in the presence of differently sized soil animals. Mesh sizes < 0,2 mm allow microbial litter colonization only. Microarthropods mostly enhance litter decay, with increases in mass loss up to 40%. As often stated by the authors, the results of microbial contribution to mass loss may sometimes be overestimated because of the improved moisture conditions in the fine mesh bags (e.g., TIAN *et al*., 1998; BEARE *et al*., 1997; HENEGAN *et al*., 1998; SIMONOV & DOBROVOLSKAYA, 1994; REDDY *et al*., 1994; YAMASHITA & TAKEDA, 1998). Some authors reported reduced decomposition rates in litter bags with medium gauze size in contrast to fine mesh or coarse mesh size: Most likely, the grazing pressure of microarthropods on microorganisms becomes too high when not regulated by macroarthropod predation (HEISLER, 1994; TIAN *et al*., 1998; VREEKEN-BUIJS & BRUSSAARD, 1996).

On the other side, the influence that fauna composition, i.e., of different microarthropods species or of different diverse communities, exerts on ecosystem processes has lately been investigated in micro- or mesocosm approaches. Here, the modulating effect of the soil fauna on the litter decay rates and on nutrient cycling - described previously in this chapter - could be assessed and their significance quantified (KANDELER *et al*., 1999; EDSBERG, 2000, HUTHA *et al*., 1998; SETÄLÄ *et al*., 1998; SCHULZ & SCHEU, 1994; SULKAVA *et al*., 1996; MEBES & FILSER, 1998).

A completely different assessment of the role of soil animals in the decomposition process is obtained by bait-lamina tests (VONTÖRNE, 1990; LARINK & KRATZ, 1994). The experimental approach differs from litter decomposition studies in the field or in microcosms trials inasmuch as only the feeding activity of the soil fauna is accounted for by exposing a non-specific, highly standardized and readily available food source in the soil. The test is limited to a few weeks of exposure, so to prevent microorganisms' decay of the baits inserted in the upper soil centimeters by means of plastic sticks. The advantages of the test are the attainable standardization that allows the comparison of results from different environments, the low labor costs, and the supply of a large amount of feeding data, which can be biometrically evaluated. The bait-lamina test enjoys great popularity as a fast and standardized test of the overall activity of soil fauna (e.g., BAYER & SCHRADER, 1997; BODE & BLUME, 1995, 1997; FEDERSCHMIDT & RÖMBKE, 1994; GEISSEN & BRÜMMER, 1999; GEISSEN *et al*., 1997; HELLING & LARINK, 1995; KRATZ, 1994, 1998; LARINK, 1994).

Ecotoxicological Investigation of the Decomposer Community

Soil animals and soils processes linked to carbon and nutrient cycles have been the targets of ecotoxicological research since the early 1950s. In a milestone article on "Soil Pollutants and Soil Animals" EDWARDS stated in 1969 that "…into the shallow but ubiquitous environment (of soil invertebrates) modern agriculture now injects huge quantities of potent new substances: chemical pesticides." He asked: "How do these substances and other pollutants affect the complex, interrelated world of soil animals?"

This question, without rephrasing, is still valid today. It hasn't lost its relevance to the present, because in spite of the intensive research activities of the last decades, we cannot answer it accurately: The amount of different chemicals released into the environment is so great, that "even if the potentially dangerous compounds should be tested, a sufficiently large testing capacity is not available, and it would require enormous economic and scientific resources" (VAN STRAALEN & LØKKE, 1997). As presented in section 1.1.1 of this chapter, this dilemma was tackled at first by assessing the toxicity of substances that were intentionally released in the environment (e.g., pesticides) with fast and practicable single species tests.

The growing concern about further groups of substances - aside from those employed in plant protection – is mirrored by the increasing amount of existing data for the ecotoxicity of heavy metals, especially from the 1970s onwards, and for substances related to threats posed by "acid rain" to terrestrial ecosystems.

In a review of available data on ecotoxicological tests conducted with soil organisms for a list of priority substances compiled by the German Federal Environmental Protection Agency (UBA), PIEPER & KRATZ (2000) found 900 data sets that fulfilled the validity requirements

within the aim of that specific study[1]. The validity requirements concerned the pertinence, e.g., of the organisms tested in respect to a risk assessment for terrestrial environments, of the selected experimental set up or of the applied statistical result evaluation. The priority list of chemical included 10 metals (e.g., Lead, Cadmium, Chromium, Copper, Nickel, Zinc), 8 single organic compounds (e.g., aldrine, DDT, lindane, pentachlorophenol) and 5 groups of organic substances (volatile halogenated hydrocarbons, BTX [benzene, toluene, xylene], mineral oil hydrocarbons, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls). The aim of the UBA project was to derive threshold values for the soil function "habitat for soil organisms" (refer to section 1.1.2). Taking into account the minor ecological significance of results from single species tests, a ranking for preferential results to be included in the intended extrapolation to the ecosystem level was set up: If the results met the statistical requirements, records from field experiments were to be preferred over single species tests.

Half of the collected data were related to the impact of the selected priority chemicals on soil microorganisms, measured in endpoints like the activity of specific enzymes (e.g., dehydrogenase, proteolytic activity) or the respiration response to toxicant stress (e.g., CO_2 release from soil).

Soil animals were represented in the collected data set with 50 single species, but the overwhelming majority of the available ecotoxicity results concerned the sensitivity to heavy metals of the insect *Folsomia candida* (Collembola) and of the earthworm *Eisenia fetida* (Lumbricidae). For these two soil animal species, DIN ISO test protocols have been available for some years (DIN ISO 11267, 1999, and DIN ISO 11268-1-2-3, 1997, 2000, 1999, respectively) but their practicability had been proven even earlier, namely through the drafts of these protocols.

For higher tier assessment endpoints such as the survey of the organic matter decay under toxicant stress, only very few records for the selected chemicals were available. In the final database, less then 10 study results of this quality could be included. On one side, the exclusion of several important plant protection products from the list of priority pollutants, and, additionally, the limited availability of data from chemical registration procedures may have biased the literature search results. On the other side, because no standardized test protocols are yet available for higher-tier assessment endpoints, the data collection had to rely exclusively upon field or microcosm research investigations, which rarely were done at several concentration levels of the investigated compound that would allow for an estimation of a concentration-response curve.

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[1] The database "SoilValue" containing the researched effect concentrations on soil organisms for the selected priority pollutants is available at the German Federal Environmental Protection Agency; information under http://www.umweltbundesamt.de

Summarizing the results of the literature research carried out for the UBA project and within the scope of this dissertation, data on the impact of pollutants on the decomposition process are available for three rough categories of substances: for metals, for acid rain components, and for pesticides.

Reduced decomposition rates due to metal pollution have been reported, e.g., by NÜß (1993) for lead, by KRATZ *et al*. (1983) and WEIGMANN *et al*. (1985) for cadmium and by BOGOMOLOV *et al*. (1996) for copper. Experiments with material, i.e., either litter or upper soil horizons, from sites in the vicinity of pollutant sources have been done, e.g., by STROJAN (1978), BENGTSSON *et al*. (1988), or RÜHLING & TYLER, (1973) near zinc smelters or brass mills; and, e.g., by KRATZ & WEIGMANN (1987) or CARREIRO *et al*. (1999) on roadside shoulders near highways. In all the latter studies, the reported depressed rates of organic matter turnover refer to the impact of the metal mixture typical for the industrial or urban source, since the study materials were not spiked with single substances. The dominant pollutants in relatively high but realistic environmental concentrations in the vicinity of smelters were mostly copper and zinc; near highways, they were lead, cadmium, and zinc. The higher amount of only partly decayed leafs or pine needles observed at many of the contaminated sites in comparison to unpolluted areas resulted in a clear accumulation horizon in the organic soil layers. Quite impressive in this respect are the calculations given by BENGTSSON *et al*. (1988) for organic horizons in the vicinity of a brass mill: At their study site, "it would take 100 years to remove 50% of the organic matter of the litter in the polluted soil compared with 6 or 7 years in the unpolluted soil".

Concerning the threats posed by acid rain components especially to forest ecosystems, several projects studied the consequences of different acid salt loads on decomposition and nutrient cycling. These experimental set ups were sometimes accompanied by studies of possible countermeasures to the acid impact, such as lime treatments. The effect of liming on the organic matter turnover *per se* has been examined as well (BÅÅTH *et al*., 1980; GEISSEN & BRÜMMER, 1999; KUPERMAN, 1999; NÜß, 1993; SCHÄFER, 1986, WEIGMANN *et al*., 1989). In general, acid deposition decreased the litter decomposition rate, but increased the leaching of nutrients from the organic layers (e.g., calcium and potassium). Liming in turn enhanced the decay process of litter, at least up to a certain load, from which on faster organic matter turnover could not be observed. Higher losses of nitrogen in nitrate form from organic horizons, however, which accompanied the observed higher litter decay rates, have been identified as questionable in respect to the ecological benefits of liming measures (e.g., MARSCHNER, 1995).

The influence of plant protection products on organic matter decay has been studied, e.g., by EDWARDS (1969), HENDRIX & PARMELEE (1984), WEIGMANN *et al*. (1985), WERNER & CONRADY (1991), DE JONG (1998), RÖMBKE & KULA (1998), PAULUS *et al*. (1999), HEINZE *et al*. (2000) or CHEN *et al*. (2001). These studies are given by way of example, because, as stated

above, the research carried out within the framework of chemical registration or during the compilation of the guidance paper to the litter bag test protocol is very extensive. Some ecotoxicological dose-response curves for pesticide impact are consistently reproducible, so that these specific compounds are discussed as potential internal control substances for standardized litterbag test procedures (e.g., benomyl or carbendazim).

Plant protection products may influence the decay process of plant residues by directly affecting the densities of the decomposer organisms, but also by radically changing the organisms' microenvironments. While this holds true for all toxicant groups reviewed, the indirect effects of pesticide application on the decomposition of organic matter have been addressed more often in field research. This may be the case because the higher organic matter turnover rates sometimes observed under the influence of pesticides have been related more often to indirect than direct effects. This point needs to be considered further.

An often described indirect effect of pesticides may occur when herbicides, sprayed directly to the study fields, alter the nutrient contents of prematurely senescent leaves: high nitrogen concentrations in plant residues will accelerate their decay process. In contrast, treating "real" litter with herbicides in the laboratory prior to exposure in the field resulted in slower decomposition rates compared to uncontaminated litter (e.g., HENDRIX & PARMELEE, 1984).

Herbicide application in the field may additionally affect soil temperature as a consequence of canopy defoliation (SUFFLING & SMITH, 1979).

A different mode of indirect impact on litter turnover rates occurs when toxicants selectively affect soil organisms. Especially if the applied chemical has a specific mode of action, as is often the case for plant protection products, some species might react directly with decreased individual densities to the chemical exposure, but some might not. It is at this juncture that the decay rate of plant litter as an integrated measure of soil organism activity has to be linked to the structural and functional composition of the decomposer community. For closer theoretical reflections on this topic see BENGTSSON (1998), BRUSSAARD (1998), LAMONT (1995), OTHONEN *et al*. (1997), WARDLE & GILLER (1996).

Organisms without specific receptors and/or lower sensitivity may not be influenced by the applied toxicant, and, as a result of changed trophic relationships and altered food web balances in the decomposer community, might even increase their niche exploitation. Following the application of any chemical, the decay rates of organic matter might therefore be enhanced by the activity of some organisms such as saprophagous and microphytophagous Collembola, whose densities are no longer predator controlled by the gamasid mites directly affected by toxic impact (EDWARDS, 1969). Differentiated responses of specific soil microarthropods species in natural communities to pesticides, heavy metals, and acid rain components have been comprehensively studied, e.g., by BECK (1983), FRITSCH (1993), HÅGVAR (1984), HÅGVAR & AMUNDSEN (1981), HÅGVAR & ABRAHAMSEN (1980), HEUGENS

& VAN DAELE (1984), HENEGAN & BOLGER (1996), KRATZMANN *et al*. (1993), KOPESZKI (1992), OSLAR *et al*. (2001), PERRY *et al*. (1997), SALMINEN & SULKAVA (1996, 1997), SALMINEN & HAIMI (1997), WEIGMANN & KRATZ (1987), and WOLTERS *et al*. (1989).

Finally, as DAS & MUKHERJEE (2000) have so pithily formulated, "as organic substance of any kind cannot escape the onslaught of microbial degradation, pesticides are no exception". Microorganisms may readily use organic chemical compounds as carbon source, increase their numbers and activity in soil and litter layers and, therefore, trigger higher turnover rates of plant residues directly or indirectly by acting as an enhanced food source for soil animals. Exploitation by microorganisms of added "substrate" provided in form of organic chemical has been ascertained in many laboratory studies with liquid cultures and single chemical compounds, but also in field studies with more likely pesticide exposure and availability scenarios (e.g., TRABUE *et al*., 2001, RÜTTIMAN-JOHNSON & LAMAR, 1997; CHAUDRI *et al*., 2000).

No data are available on the impact of organic chemicals other than plant protection products on the litter decomposition process.

1.1.4 Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs)

The contamination of water, air, and soil with organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) is an issue of great environmental concern. Because of their recalcitrance to environmental degradation and low water solubility, these organic chemicals are very persistent in the environment. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls as groups as well as several individual compounds among them are contained in the list of environmental priority pollutants by the US-EPA and the German UBA (e.g., KEITH & TELLIARD, 1979).

Polycyclic aromatic hydrocarbons (PAHs) are made up of two or more fused benzene rings in linear, angular, or cluster arrangements and contain only carbon and hydrogen (see Figure 1). Several hundred mono- and heterocyclic PAHs occur naturally, but usually only 16 compounds are routinely analyzed as designated by the US-EPA or 6 by the German TVO (AURAND & HASSELBARTH, 1987). Molecular weights of PAHs range from 178 to 300. With increasing molecular weight, PAHs become more lipophilic, less soluble in water, and less volatile (MACAY *et al*., 1992a).

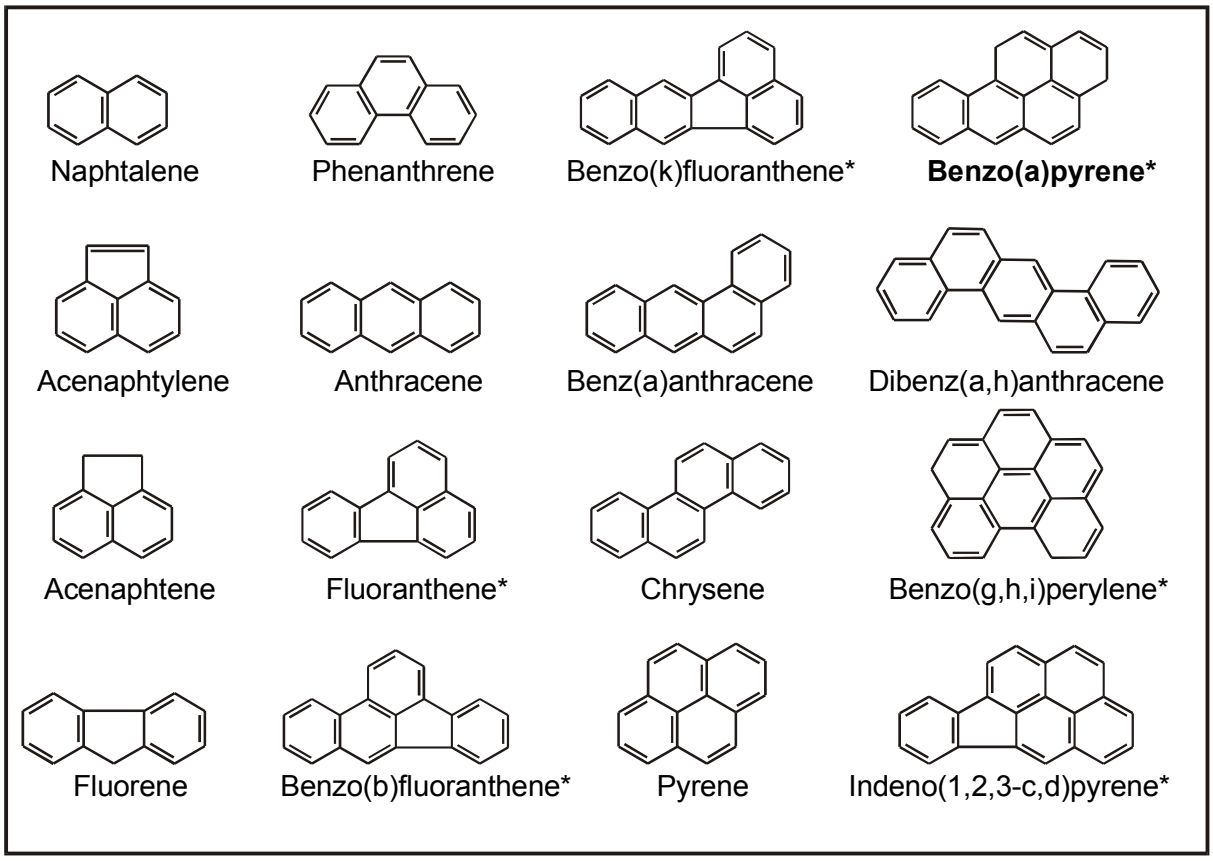

Figure 1: Structure of some polycyclic aromatic hydrocarbons (PAHs). Shown are the 16 PAHs measured according to the US-EPA list and the 6 PAHs (*) measured according to the German TVO. Figure from MARSCHNER (1997), modified.

Benzo(a)pyrene (BaP) has been very often measured as a reference substance in pollution profiles from contaminated and uncontaminated sites: It is a well-known potent carcinogenic compound and has been identified as such since the end of the 18th century. Benzo(a)pyrene has a molecular weight of 252 g, a high octanol/water partitioning coefficient K_{ow} (log K_{ow} range 6.0 to 6.5), a high partitioning coefficient between watery solutions and the organic carbon of the solid matrix K_{oc} (log K_{oc} range 5.3 to 6.7) and a low solubility in water (range 2.0 to 4.5 µg x l^{-1}). In the project described here, Benzo(a)pyrene (BaP) has been chosen as reference substance for the polycyclic aromatic hydrocarbons as well.

Polychlorinated biphenyls (PCBs) consist of a biphenyl ring with 10 positions (labeled 2-6 and 2'-6') where chlorine substitution may occur (see Figure 2). A total of 209 PCB congeners are possible and around 100 congeners have been reported in various commercial preparations and in environmental samples (MACAY *et al.*, 1992b). Beck *et al.* (1996) state that "the physicochemical properties, degradability and toxicity of PCBs are all related to their molecular structure".

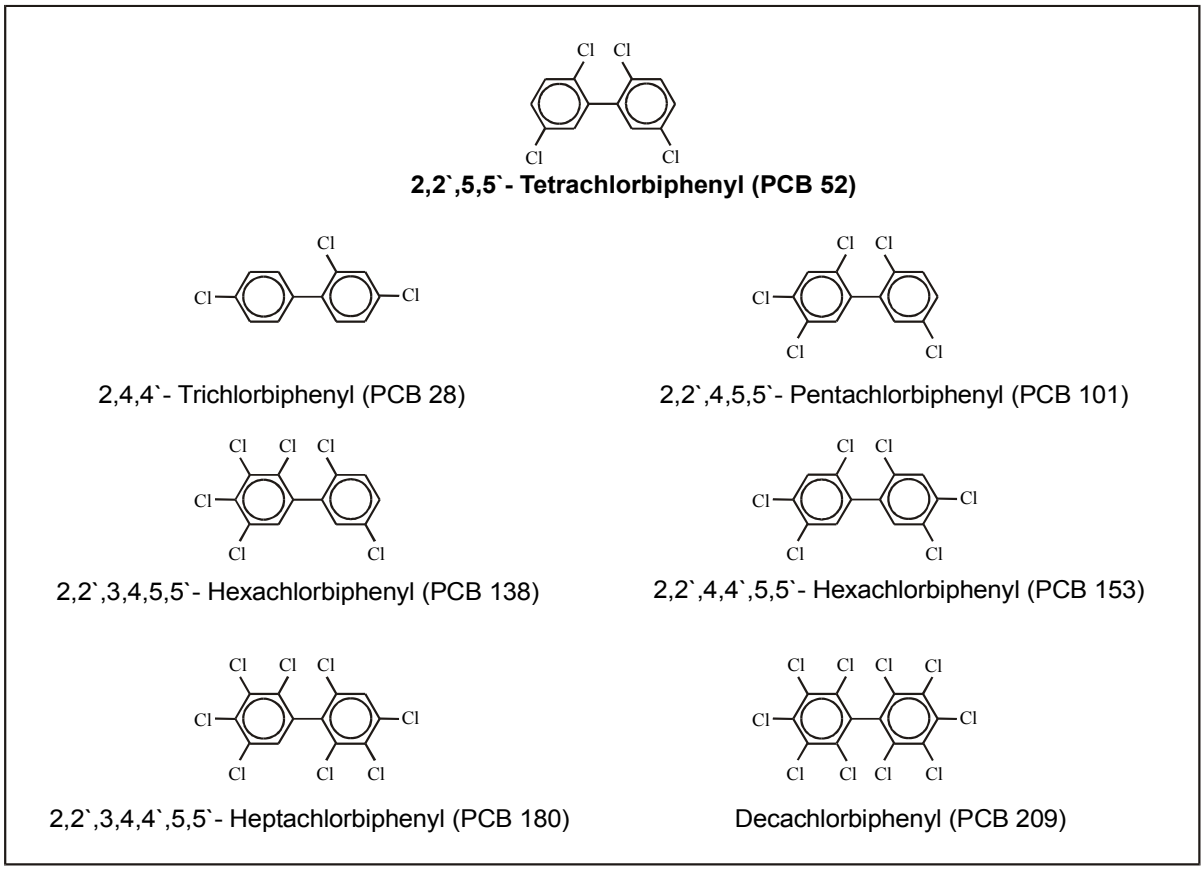

Figure 2: Structure of some polychlorinated biphenyls (PCBs). Shown are the 6 PCBs measured according to the German TVO and additionally PCB 209.

Higher chlorinated PCBs have a lower water solubility and vapor pressure and are more lipophilic than PCBs with lower chlorine substitution, which are less persistent. In this study, 2,2'-5,5' tetrachlorobiphenyl (PCB 52) was chosen within the PCB congeners as reference substance. PCB 52 has a molecular weight of 292 g, relatively low water solubility (range 6 to 120 µg x l^{-1}), a high octanol/water partitioning coefficient K_{ow} (log K_{ow} 5.8), and an intermediate K_{oc} partitioning coefficient (log K_{oc} range 4.7 to 5.4) among the PCB congeners.

PAHs originate in the high temperature pyrolysis of various naturally occurring organic materials: They are formed whenever organic substances exposed to high temperatures are incompletely combusted, e.g., during natural forest and prairie fires, volcanic activity, anthropogenic forest and agricultural fires, but especially during fossil fuel combustion. Even if PAHs may also be synthesized naturally by some plants and bacteria (e.g., WILCKE 2000), by far the greatest amounts released into the environment result from coal burning (e.g., EDWARDS, 1983; WILD & JONES, 1993; WILD & JONES, 1995), because fossil fuels contain considerable amounts of aromatic hydrocarbons formed during the incubation of organic matter under specific anoxic conditions.

Unlike PAHs, PCBs are not naturally occurring substances. They have been industrially synthesized since the beginning of the 19th century and have been used on account of their chemical properties as coolants, insulating materials, lubricants, softening and impregnating agents, but also as carriers for pesticides. The environmental contamination with PCBs occurs therefore solely through various anthropogenic activities, namely industrial discharge, poor waste disposal processes of closed cooling systems or used oil spills. On account of the concern stirred by alarming incidents, especially from the so called Yusho disease occurring in Japan 1969 where more than 1000 people were massively poisoned by PCB contaminated cooking oil, the open application of PCBs is nowadays almost generally prohibited. The use of PCBs in closed systems was allowed somewhat longer, in Germany until 1999.

Both groups of organic compound are ubiquitous in the terrestrial environment, as can be seen by the ranges of determined soil concentrations in Table 1. In spite of the research interest developed in the 1960s, only recently sufficiently sensitive analytical procedures have become available to reliably detect PAH and PCB concentrations in environmental matrices. In this respect, the retrospective analysis of archived samples has provided valuable insight into the influence of human activity on the inputs, environmental cycling, and time trends of these contaminants (JOHNSTON, 1997). Quite remarkable are the analyses of soil samples from 1840 to the 1990s from the Rothamsted Experimental Station in the United Kingdom: The increase in organic compound concentrations in the terrestrial environment can be clearly related to anthropogenic activities, which, by raising the atmospheric burden of PAHs and PCBs, increased their deposition during this century.

These processes are closely connected, given that the atmospheric storage of organic compounds is very small compared to their atmospheric input, which in the case of PAHs is the major flux into the environment. The assumption that the overall flux is directed "probably in the direction of the soil" (VAN BRUMMELEN, 1995) is largely supported by data from ALCOCK *et al*. (1994), HARRAD *et al*. (1994) and WILD & JONES (1995).

In their reviews, the authors emphasize that more than 90% of all PCBs and PAHs found in the (UK) environment are stored in the top 15 cm of the soil, even excluding, as in the case of PAHs, contaminated areas like gasworks sites or petroleum refineries. Soil is, therefore, assumed to be the most important repository sink for organic contaminants.

Table 1: Concentration range of PAHs and PCBs in soil matrices according to DÖRING (1998) and MARSCHNER (1997), complemented with data from BECK *et al.* (1996), EDWARDS (1983), JOHNSTON (1997), KRAUSS *et al.* (2000), TERYTZE *et al.* (1998), VAN BRUMMELEN *et al.*(1996) WILCKE (2000) and WILCKE & ZECH (1998).

	Polycyclic Aromatic Hydrocarbons		Polychlorinated Biphenyls
Soil matrices	Total of PAHs* mg x kg $^{-1}$ DW	Benzo(a)pyrene mg x kg $^{-1}$ DW	Total of PCBs* µg x kg $^{-1}$ DW
Uncontaminated reference sites	< detection limit to 0.1	< detection limit to 0.1	<detection limit to 160
Anthropogenic managed sites			
Meadows	0.3 to 7.2	< detection limit to 0.2	5 to 13
Organic layers of forests	0.8 to 20.0#	< detection limit to 0.5	6 to 150#
Agricultural soils	0.1 to 1.0	0.02 to 0.04	1 to 280
Agricultural soil with sewage sludge amendments	1.8 to 4.0	0.02 to 0.07	6 to 1270
Sewage sludge	1.0 to 80.0	0.1 to 0.4	20 to 9000
Sewage farm fields	0.2 to 7.5		200 to 2500
Urban soils	1.6 to 25.0	0.03 to 3.7	400 to 9700
Contaminated sites	up to 650	up to 650	

* Total of 6 PAHs, 6 PCBs respectively, according to TVO if not stated differently
# Total of 20 PAHs, 12 PCBs respectively (KRAUSS *et al.*, 2000)

Soil concentrations of PCBs and PAHs continuously increased in the last century and peaked at maximum loads in the 1960s and 70s. If the contemporary concentrations of PCBs and those of more volatile PAHs are similar to those in the 1940s, loads of higher condensed PAHs like benzo(a)pyrene don't appear to decrease in soil samples (JOHNSTON, 1997). Emission trends and production history of these compounds are in agreement with the time trend findings: The prohibition of PCBs use in open systems and the coal use at higher combustion temperatures in power houses have lowered their immission into the soil. Interestingly, in the light of the observed rapid losses of specific organic compounds from the soil samples during the last 30 years, soil appears as source of PCBs and low molecular weight PAHs emitted back to the atmosphere.

The fact that losses from soil ecosystem compartments may not only be attributed to volatilization to the atmosphere will be shown in the next section.

1.1.5 Behavior and Toxicity of PAHs and PCBs in Soils

Sorption and biodegradation

The soil specific load of single polycyclic aromatic hydrocarbons or polychlorinated biphenyls is, of course, dependent on the kind of immission exposure of the ecosystem. The different so-called contamination profiles of a site might directly point to a possible source of pollution, even though during transport a selection in the organic compound compilation may occur due to different half-lives or preferential binding of single substances to differently sized particles. Even so, some characteristic substances may point to a given source, because, e.g., pyrene, fluoranthene, and benzo(a)anthracene dominate profiles in flood areas (TERYTZE *et al*., 1998; TEEBAY, 1994), benzo(ghi)perylene are dominant in traffic exhausts (WILCKE, 2000), tetra- and heptachlorinated biphenyls are characteristic for the emission profiles of incineration plants and hexachlorobiphenyls for technical PCBs mixtures (e.g., WILCKE & ZECH, 1998).

On the other hand, the different structural properties of single PAHs and PCBs lead to a specific behavior of these compounds when involved in soil ecosystem processes, and, therefore, to different residence times in the different soil layers.

As suggested by their lipophilic properties, PAHs and PCBs entering terrestrial ecosystems are mostly either already bound to organic particles (e.g., sorbed to aerosols, wastewater, or sewage sludge particles), or are readily absorbed to organic substrates (e.g., needle and leaf waxes, plant litter). Even PAHs and PCBs bound to dust particles filtered by plants from the atmosphere enter the soil system when washed out by rain.

Some insight into the further fate and behavior of organic contaminants in the soil compartment has been gained from the survey of stratified organic layers in forest ecosystems (VAN BRUMMELEN *et al*., 1996; KRAUSS *et al*., 2000). Enrichment of soil organic matter with PAHs occurs during ageing, so that litter layers with undecayed leaves and needles (Ol-horizon) exhibit lower loads than the underlying partly decomposed litter (Of) and this again less than the lowermost organic layer (Oh), which may be strongly humified. This is not the case for PCBs: If, as for PAHs, the Ol-horizon shows the lowest concentrations found in the profile, Of- and Oh- horizon display similar PCBs loads, and no further accumulation occurs during humification of the organic matter.

In order to accumulate in the organic profile during decomposition and mineralization of plant litter, the half-lives of PAHs and PCBs compounds have to be greater than the half-lives of the organic matter itself, to which they are bound. Low molecular weight PAHs and lower chlorinated PCBs accumulate to a lesser extent in the organic layers of a soil profile than higher condensed/chlorinated compounds, since the latter display lower water solubility, lower volatility, and lower susceptibility to biological degradation.

Before addressing these issues, it should be noted that PAHs show a higher enrichment factor in organic soil profiles than PCBs, even when comparing compounds with similar chemical properties, e.g., the same range of measured log K_{ow} (Krauss *et al*., 2000). This has been attributed to stronger adsorption of PAHs to organic matter than of PCBs. Factors affecting the sorption of organic pollutants to the organic matter of soils have been reviewed lately by MARSCHNER (1999) and PIGNATELLO (2000). These authors refer in particular to underlying theoretical models of kinetic processes and to the influence of the physicochemical properties of the soil surroundings on sorption and desorption rates. SCOW & JOHNSON (1997), on the other side, refer in their review to the biological implications weaker or stronger bindings of organic pollutants, and subsequently higher or lower availability, have in soil ecosystems.

The sorption (and desorption) process of organic pollutants to and from the organic soil fractions generally exhibits two stages: A fast initial absorption of up to 50% of the compound, and a second phase in which the remaining chemical sorbs slowly over weeks and months to the organic matter without changing its chemical properties, i.e. without being metabolized. The formation of strongly adsorbed "bound residues" over time, which cannot be recovered from the solid phase by exhausting solvent extraction, has been related occasionally to the establishment of strong covalent bonds between PAHs and PCBs and the organic substance. On the other hand, since hydrophobic compounds are thought to form rather weak bonds with the soil substance, physical sequestration processes have lately been discussed as the rate-limiting factors. PAHs and PCBs may move slowly into soil intraaggregate pores or diffuse through the organic matrix itself, with the first fast sorption phase relating to interactions with the surface of the organic matter molecules.

According to this concept, however, sorbed chemicals should be extractable slowly but finally from the organic matrix, and this is not always the case. MARSCHNER (1999) suggests possibly coupled mechanisms, in which the slow diffusion of the chemical into organic molecules may become irreversible when structural changes of the organic matrices, due to drying out or microbial activity, trap the organic chemical inside them.

Chemicals like PAHs and to a slightly lesser extent PCBs that with time become either irreversibly sorbed to soil organic matter or are released with extremely slow kinetics, display decreasing fractions readily available for uptake and/or transformation by living organisms (SCOW & JOHNSON, 1997).

Bioavailability, which is the state of this available fraction, is a key issue in modulating the ecotoxicological effects of chemicals on soil organisms and it may alter massively the expected magnitude of toxicant impact (e.g., KAAG, 1998; SMIT & VAN GESTEL, 1996; SMIT *et al*., 1997). The critical question following from this may then be posed like ALEXANDER did 1995: "How toxic are toxic chemicals in soil?"

To complicate matters further, organic chemicals, unlike heavy metals, may basically be transformed and degraded by organisms living in the soil. I addressed this subject briefly when presenting the ecotoxicological results of research done on the impact of pesticides on soil organisms of the decomposer community. While the coupled process of biodegradation definitely involves a biological component, i.e., the actual existence and the activity rates of a microorganism population capable of degrading the chemical, it is important not to ignore the physicochemical component, i.e., the distribution and availability of the compound to the degraders. Sorption affects biodegradation kinetics by lowering the amount of potentially metabolized substrate at each time interval. Moreover, the second, slower diffusion into soil organic matter particles and the possible formation of "bound residues" manifest themselves in the lower amount of degraded compounds in the initial phase when the contact time between soil and organic chemical had been raised prior to degrading microorganism inoculation (GUERIN & BOYD, 1992). Biodegradation and biotransformation, therefore, may be limited by slow mass transfer from the soil organic matrix to the degrading microorganisms (e.g., ACHTNICH, 1999; BOSMA *et al*., 1997; GROSSNER *et al*., 2000; FRIEDRICH *et al*., 2000; HATZINGER & ALEXANDER, 1995; THIELE & BRÜMMER 1998).

In summary, the sorption behavior of an organic contaminant in soil influences its ecotoxicological impact on soil organisms by ultimately changing its bioavailability: The amount of potentially toxic substance may decrease over time due to sequestration by the organic matter fraction of the soil, but it may decrease as well due to biodegradation of the parent compound, a process in turn affected itself by the bioavailability of the chemical (e.g., CORNELISSEN *et al*., 2001; MANILAL & ALEXANDER, 1991; TANG & ALEXANDER, 1999).

But is there a "biological component" that may be involved in the degradation of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in soils? In the past years, a wide range of microorganisms have in fact been identified that are able to co-metabolize or mineralize several organic compounds from the PAH and PCB groups.

Experiments under laboratory conditions with activated sediments or soil slurries from contaminated sites showed the ability of some microorganisms, especially bacteria, to anaerobically dechlorinate PCBs and transform them into lower chlorinated congeners (e.g., YE *et al*., 1992; KIM & RHEE, 1997; WU *et al*., 1996). The dechlorination activity may be primed by adding PCBs to long-term contaminated substrates, but also by supplying certain plant compounds or root exudates (e.g., BEDARD *et al*., 1996; GILBERT & CROWLEY, 1997; VAN DORT *et al*., 1997). Many of the lower chlorinated congeners are then more susceptible to complete biodegradation by a variety of aerobic PCB-co-metabolizing, biphenyl-utilizing microorganisms, the activity of which has been examined individually (e.g.; BAXTER *et al*., 1975; BAXTER & SUTHERLAND, 1984; BEAUDETTE *et al*., 1998; GILBERT & CROWLEY, 1997; LAJOIE *et al*., 1994), in mixed indigenous bacteria and fungi populations, or in complex

microcosm studies (WAGNER-DÖBLER *et al*., 1998; DONNELLY & FLETCHER, 1995; HICKEY *et al*., 1993; KOHLER *et al*., 1988; ROJAS-AVELIZAPA *et al*., 1999).

In soils, PAHs are poorly degraded under anoxic conditions, and the enzymes predominantly involved in their transformation, partial degradation, or mineralization by microorganisms are the ones associated with oxidation pathways: oxigenases, dehyrogenases, and peroxidases (PARLAR & ANGERHÖFER, 1991). Bacteria may completely mineralize PAHs by initially cleaving the benzene rings with dioxigenases, and thus utilize high molecular compounds up to four benzene rings as sole carbon and energy source (MOSER & STAHL, 2001; KÄSTNER *et al*., 1994; SAMANTA *et al*., 2001; TANG & ALEXANDER, 1999). Many of the isolated strains are also able to partially degrade five-benzene-ring PAHs like benzo(a)pyrene when supplied with additional growing substrate, e.g., lower condensed compounds (KANALY *et al*., 2001; YE *et al*, 1996; MCNALLY *et al*., 1999; MOLINA *et al*., 1999; TONGPIM & PICKARD, 1999).

Fungi can transform a variety of PAHs, including benzo(a)pyrene, to polar metabolites through an enzyme system acting as oxigenase in the hydroxylation of organic compounds (reviewed by FABER *et al*., 2001). The most extensive studies have focused on so-called white rot fungi, though, which produce highly reactive extracellular enzymes (reviewed by, e.g., BARR & AUST, 1994; BLANCHETTE, 1995; KÄSTNER *et al*., 1993; POINTING, 2001). The lignin peroxidases, mangan peroxidases, and laccases involved in the fungal degradation of lignin are not substrate specific, transform PAHs through enzymatic combustion, and may lead to complete co-metabolic mineralization of highly condensed PAHs like benzo(a)pyrene (e.g., BALDRIAN *et al*., 2000; COLLINS *et al*., 1996; POTHULURI *et al*., 1995; IRIE *et al*., 2001).

The effect of microorganism activity on the degradation of organic contaminants and the formation of non-extractable residues in soils has been investigated in microcosms studies, frequently with ^{14}C-labeled parent compounds (e.g., GROSSER *et al*., 1995; KANALY *et al*., 1997). The formation of oxidation products has been surveyed focusing on the effects of soil amendments that might enhance the degradation and detoxification of the organic contaminants in the field. The planting of soil and the establishment of an active rhizosphere (BINET *et al*., 2000; GÜNTHER *et al*., 1996; FANG *et al*., 2001), the amendment of soil with humic acids (HADERLEIN *et al*., 2001), with straw (BENOIT & PRESTON, 2000; JOERGENSEN *et al*., 1997; MARTENS *et al*., 1999) or with compost mixtures (KÄSTNER *et al*., 1999; WISCHMANN & STEINHART, 1997) enhanced the mineralization of monitored compounds, but also the formation of the so-called "bound residues". Only in some studies the adsorption of the parent compound to specific organic matrices was so high that it markedly reduced the bioavailability to the degrading microorganisms (e.g., BOYLE *et al*., 1998).

Addressing the enhanced formation of non-extractable chemical fractions, KÄSTNER *et al*. (1999) formulated the theory of a biogenic residue formation responsible for the incorporation of especially PAH metabolites into humic substances and for the origin of "humic substance-

like macromolecules". SMITH *et al*. (1999) also described the preferential strong binding of PAH metabolites to soil.

Supplementing contaminated soil with uncontaminated organic matter activates co-metabolic degradation pathways especially of high-molecular mass PAHs: Essential for this stimulated degradation is the mineralization of the carbon from the added organic substrates (KÄSTNER & MAHRO, 1996).

There are no studies in which the behavior of PAHs and PCBs was studied by exposing the chemical directly to the degradation activity of microorganisms with a natural organic matrix as carrier. Both kinds of organic substrates, the litter and the organic pollutants, may undergo simultaneous degradation by the autochthonous microorganisms present in the field.

Toxicity of PAHs and PCBs to soil animals

Unlike the long-standing research activities on the response of microorganisms to soil pollution with compounds from the polycyclic aromatic hydrocarbon and polychlorinated biphenyl groups, at the beginning of this project in 1994, virtually no data were available on the impact of PAHs and PCBs on soil animals.

Research on the toxicity of polychlorinated biphenyls focused on the observed accumulation of these compounds in organisms of aquatic food chains (reviewed by VAN WEZEL, 2000; JARMAN *et al.*, 1996), starting with polluted sediment feeders and stretching to fish and birds, otters and seals. PCBs are lipophilic substances and, unlike PAHs, do not undergo substantial metabolization in vertebrate organisms: they are therefore accumulated, mainly in fatty tissues. In humans, PCBs are neurotoxic and in addition to developmental neurotoxicity and impairments of the immunoresponse cause chronic diseases like chloracne, thymus atrophy, and liver damage. The higher chlorinated compounds are suspected to be genotoxic and carcinogenic, as well as teratogenic because they are able to cross the placenta. PCBs have received much attention recently because of their endocrine-disrupting effects, which is thought to play an important role in the impaired sexual differentiation of fish, birds, reptiles, and mammals (e.g., KESTER *et al*., 2000; BROUWER *et al*., 1999; LEISEWITZ & KAMRADT, 1996).

Direct effects of PCBs bound to soil have been tested with commercial technical mixtures, mostly Aroclor 1254, or with soil from contaminated sites. The exposure of rats, earthworms, and isopods to PCB contaminated substrates has been shown to induce immunological responses as measured by the activity of selected biomarkers (BILLERET *et al*, 2000; BUNN *et al*., 1996; KÖHLER *et al*., 1999; SUZUKI *et al*., 1995).

For soil animals, standard ecotoxicity endpoints like the determination of the effect concentration, at which 50% of the observed parameter is impaired (EC_{50}; LC_{50} for survival)

aside from the results from the joint research project and presented in this thesis, have up to now been published, to my knowledge, only in one paper.

MEIER *et al*. (1997) reported about the reproduction success of the earthworms *Lumbricus rubellus* and *Eisenia fetida andrei* in PCB contaminated soil prior and after remediation. The earthworms were clearly impaired by the PCB concentration of the polluted soil (144 mg x kg^{-1}; EC_{50} about 90 mg x kg^{-1}), but the reproductive success was not improved by the soil clean up with solvent extraction. Because the soil after remediation had a concentration of 2 mg PCB x kg^{-1} soil, it is unlikely that PCB was solely responsible for the toxicant effect. In test systems with complex fauna communities, NIEMANN & DEBUS (1996) and PARMELEE *et al*. (1997) found responses to PCB contamination in a concentration range between 50 and 2500 mg x kg^{-1}. After 56 days the number of nematodes decreased by 25 % in soil contaminated with 50 mg PCB 52 x kg^{-1} soil compared to the unpolluted control. Increased bioavailability of PCB was probably given in this test setup, because the soil was mixed 2:1 with agar (NIEMANN & DEBUS, 1996). PARMELEE *et al*. (1997) could observe a drastic decline in the abundance of soil microarthropods like oribatid mites and Collembola in a soil microcosm treated with Aroclor 1254. At a concentration of 2500 mg x kg^{-1}, the Oribatida individual densities decreased by 85 % compared to the controls.

In contrast to the PCBs, biomagnification in the food chain has not been observed for PAHs, probably because higher developed predator organisms are able to transform them. Aromatic compounds are commonly present in the environment and the food of animals and this could have been "a selective force for efficient detoxification enzymes" (VAN BRUMMELEN, 1995).

The observed mode of action of PAHs in the animal organism has been often been described as resembling the so-called baseline-toxicity of non-polar narcotics: The substance affects cellular structure by accumulating on membranes and disrupting processes of osmoregulation and neurotransmission. Substances with this mode of action require no specific receptors and their action is thought to depend exclusively on their lipophilic properties. Their toxicity should, therefore, be predictable by parameters like the octanol/water partitioning coefficient. Recently published results on the toxicity of PAHs on the collembolan *Folsomia fimetaria* L. (SVERDRUP *et al*., 2001) seem to confirm this hypothesis: When effect concentrations were recalculated as soil pore-water concentrations, they showed a significant correlation with the log K_{ow}-values of the substances tested. The EC_{50} for reproduction ranged between 14 mg fluorene x kg^{-1} soil and 51 mg fluoranthene x kg^{-1} soil. Values for pyrene and phenathrene were within this range (16 and 30 mg x kg^{-1}, respectively). Fluoranthene and phenanthrene toxicity was even below the estimated values derived by a quantitative-structure-activity-relationship (QSAR) for non-polar narcosis as mode of action. The same workgroup performed equivalent tests with the enchytraeid *Enchytraeus crypticus*, and found this species less sensitive than the collembolan (EC_{50} range 50 to 91 mg x kg^{-1}; SVERDRUP *et al*., in press).

CROUAU *et al*. (1999) determined for the Collembola species *Folsomia candida* L. in a standard toxicity test according to ISO 11267 an EC_{50} for reproduction of 175 mg phenanthrene x kg^{-1} soil.

All results described above were obtained in freshly spiked soils and represent a worst-case assessment of a compounds' toxicity. On the other side, CROUAU *et al*. (1999) observed a decreased LOEC (lowest observable effect concentration) when employing a natural sandy soil instead of the artificial soil recommended in the standard test procedure, which is supplemented with organic carbon and kaolinite.

Some observed effects in ecotoxicological experiments with PAHs, however, point to another mode of action of the compounds. VAN STRAALEN & VERWEIJ (1991) fed the isopod *Porcellio scaber* with food contaminated with benzo(a)pyrene. In males, the growth efficiency decreased with increasing BaP concentrations from 11% in the controls to 1.7% in the variants contaminated with 125 mg BaP x kg^{-1} food. In females, however, an increase in growth efficiency was observed at intermediate concentrations (1 to 5 mg BaP x kg^{-1} food), which was significant and amounted to a doubled assimilation efficiency from the food. This response was discussed as resembling an "hormesis" effect, which has been often observed in organisms when exposed to low levels of growth inhibitors and has been related to an overcompensative reaction to stress.

Experiments of VAN BRUMMELEN & STUIJFZAND (1993) and VAN BRUMMELEN *et al*. (1996) had similar results: While a distinct reduction in growth was observed at the highest tested BaP concentration (58% inhibition at 316 mg BaP x kg^{-1} food), experiments with females only showed a clear concentration-related response to BaP concentrations: the proportion of females that became gravid increased markedly. The overall stimulatory effect on brooding success was not accompanied by reduced weights of the mothers nor by decreased numbers of juveniles per successful brood nor by lower survival of the juveniles upon starvation.

This clear enhancement of reproduction could not be related to hormetic effects, which do not normally show a dose-response relationship, but disappear at higher concentrations. Since PAHs, like PCBs, are compounds known to interfere with the endocrine systems of animals, the structural resemblance between higher condensed PAHs like BaP and steroid hormones like estradiols or ecdysteroids could have led to a direct interaction of the tested compounds with the hormonal balance of the animals. PAHs need to undergo metabolization prior to interfering with the hormone regulation in organisms.

Only phenolic compounds, which result from an initial PAH attack by the mixed function oxigenase (MFO) enzymatic system, can bind to hormonal receptors. The MFO system, which contains cytochrome P-450 and cytochrome P-450 reductase, is responsible for the transformation of PAHs to polar metabolites.

The highly reactive intermediate products may bind to macromolecules like DNA, forming adducts that eventually are responsible for the carcinogenic potential of the parent compounds. The existence of the MFO detoxifying enzymatic system has been demonstrated in many soil animals (e.g., BERGOUT *et al*., 1991; HODGSON, 1983).

The results reported here are the only ones available about the toxicity of PAHs and PCBs on soil animals, aside from the further insight into the ecotoxicological impact of benzo(a)pyrene and PCB 52 gained in the joint research project in which this study was embedded. The results of this interdisciplinary research work will be comprehensively presented in the discussion.

1.2 Aim of the Study and Outline of the Thesis

The overall experimental approach chosen for this work was the assessment of the effects of reference substances from the polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs) on the structure and activity of soil animals by means of examination at different levels of biological organization. To realize this investigational goal, a study plan was drawn up that combined field experiments and surveys, as well as so-called semi-field experiments in microcosms and laboratory tests.

The influence of benzo(a)pyrene (BaP) and 2,2'-5,5'- tetraclorobyphenyl (PCB 52) on the litter decomposition process was studied in the field as well as under semi-field conditions.

As representatives of the decomposer community, the response of Collembola and Oribatida to explicitly spiked substrate was analyzed, both in connection with the litter decay process in the field as well as in laboratory experiments.

The evaluation of the soil animal cenosis of different PAH and PCB polluted areas, finally, permit the analysis of long-term anthropogenic impact on the soil decomposer community.

The specific aims of the study were to:

- Assess the behavior of the selected reference substances BaP and PCB 52 in grass litter serving as organic carrier matrix
- Survey the decomposition process of grass litter as influenced by increasing loads of BaP and PCB 52
- Investigate the response of Collembola and Oribatida by means of their colonization densities in BaP and PCB 52 contaminated litter
- Carry out standard laboratory toxicity tests with BaP and PCB 52 spiked substrates, and to
- Compare different long-term PAH and PCB contaminated areas by means of their soil animal species composition, cenosis structure and activity levels of simple functional parameters

The work was embedded in a joint research project of the Federal Ministry of Education and Research (BMBF). In the joint project "Soil Biological Investigations of the Effects and the Distribution of Organic Compounds (PAHs, PCBs) in Typical Conurban Ecosystems"[2] , 8 workgroups from 5 institutions cooperated: The research focus of the individual projects covered the topics of mobilization and transport of chemicals in soils, chemical transfer from soil to plants and ecotoxicological investigations with higher plants, microorganisms and soil animals. A central coordination guaranteed the common utilization of defined soil types, spiking variants, experimental field plots, and chemical analytic services (see common final report KRATZ & BROSE, 1998).

All research activities of the joint project were carried out either locally on former sewage field farms near Berlin, Germany, or with material collected there and placed at the disposal of all working groups.

The project outlined in this thesis, the "Soil Zoology" project, was conducted at the Soil Science Department of the Technical University Berlin and tightly coupled with the research work of the Microbiology Group (BROSE *et al*., 1997): In contrast to the other joint research projects, our attention was focused more on organisms and processes in the litter layer than in the underlying soil layers.

The central experiments were 2 litter decomposition experiments: One was carried out in the field on the central investigational area RefB of the joint research project; the second in so-called Mitscherlich vessels under semi-field conditions. The experiment in the RefB area was set up to run slightly over one year with six sampling dates. Replicates were sampled at the same dates for this study and for the microbiological survey. The semi-field experiment lasted 10 months and comprised one destructive sampling at the end of the study period.

Litter from the field experiment in the RefB area was contaminated with non-labeled benzo(a)pyrene and PCB 52, in contrast to the employment of ^{14}C-labeled BaP and PCB 52 in the Mitscherlich vessels. The concentrations of extractable parent compounds were monitored over the duration of the decay experiments; the radio labeled litter was additionally extracted to determine the amount and the nature of the fractions recovered in the different phases and organic matrices.

The results of the **survey of the BaP and PCB 52 concentrations in the litter of both decomposition experiments are presented in section 3.1.**

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[2] "Bodenbiologische Untersuchungen zur Wirkung und Verteilung von organischen Stoffgruppen (PAK, PCB) in ballungsraumtypischen Ökosystemen" was supported by the Federal BMBF with Reference No. 07 OTX 08

The course of the grass litter decay in the RefB area is presented in section 3.2 for uncontaminated litter and litter spiked with BaP and PCB 52. The specific decomposition rates of the control litter and the litter of the contaminated variants were calculated according to decay models. Analyses of variance and multiple regression calculations served to analyze the response of the decay rate parameters to the chemical impact. The concentrations of C and N in the decomposing litter were determined over the course of the experiment.

Section 3.3 documents the response of soil animals to litter and soil contamination with BaP and PCB 52. The colonization densities of Collembola and Oribatida in the litter of the different contaminated variants in the decomposition experiment in the RefB area give insight into the particular response of different species to the applied chemical compounds.

Single species standard laboratory toxicity tests with Collembola according to ISO protocols demonstrate the reaction of the reproductive rate of *Folsomia candida* to soils spiked with BaP and PCB 52.

The comparison of former sewage fields, which display varying pollution profiles and loads due to long term contamination with different levels of untreated wastewater, was achieved by means of the survey of the oribatid and nematode fauna and of their feeding activities. **The faunistic characterization of differently managed sewage field areas is formulated in Section 3.4**

In section 4, the results of the investigation are discussed in the light of the formulated aims, and future research needs are pointed out.

2 MATERIALS AND METHODS

2.1 Characterization of Investigated Sewage Field Areas

Because the long-term contamination process of the sewage fields occurred via the depositing of untreated waste water with varying intensity, the clearly differing degrees of accumulation of the compound groups to be investigated is mirrored by the heterogeneous concentrations of other environmentally relevant chemicals in the soils (see Table 2). Aside from the heterogeneous expression of important pedobiological parameters (C_{org}, pH), different areas also exhibit different concentrations of heavy metals (e.g., cadmium, lead, zinc). These in part clearly exceed levels that can be considered biologically harmless. In addition, the sewage field areas are characterized by different vegetation stands (quack grass meadows, plantations of willow, poplar, and pines) with characteristic degrees of soil shading, microstructure and nutrient supply.

With this degree of heterogeneity, an activity related analysis of the PAH and PCB impact on vegetation, soil fauna, and soil processes of the former sewage fields is almost impossible. In order to derive concentration-effect relationships for selected reference substances, studies with spiked materials were performed on a central investigational area (RefB). Benzo(a)pyrene (BaP) and PCB 52 were selected as reference substances. Research activities in the central investigation area RefB with the reference substances BaP and PCB 52 were coordinated within the joint research project (see section 1.2)

Another focus of the studies were areas that exhibited decidedly different PAH and PCB soil pollution patterns due to different management practices. In order to designate areas with significant differences in contamination, sewage field sites were preselected based on existing physicochemical and pedobiological data (MARSCHNER & SCHLENTHER, 1994; KRATZ & MARSCHNER, 1994; KRATZ, 1992).

The highest contamination levels for PAH and PCB were measured at the sample sites nPAK and nPCB, respectively (see Table 2). Thus, these sites served as comparison areas to assess differences between the effects of long-term contamination and the “fresh” spiking of the RefB soil and litter.

Table 2: Ranges of important pedological parameters and pollutant loads of the sewage field soils in Berlin-Buch and Hobrechtsfelde (from MARSCHNER & SCHLENTHER, 1994; MARSCHNER *et al.,* 1997) and characterization of the focus areas of the joint research project RefB, nPAK, and nPCB (from KRATZ & MARSCHNER, 1995)

	Vegetation	pH	Corg	C/N	Pb	Cd	PAH*	PCB**
		$CaCl_2$	%		mg x kg^{-1}	mg x kg^{-1}	µg x kg^{-1}	µg x kg^{-1}
Range of sewage field soils		4.3-5.8	1.3-8.2	9.5-12.6	52-390	2.2-44	88-7480	0-2560
Investigated areas								
RefB	Quack grass	5.5	1.7	9.8	70	4.9	370	200
nPAK	Poplar	4.9	3.7	11.9	334	6.5	3180	223
nPCB	Partly bare soil	4.3	5.5	9.9	116	22.5	2140	804

* Σ of 6 PAHs, according to TVO

** Σ of 6 PCBs, according to BALLSCHMITER & ZELL (1980)

2.1.1 Vegetation of the Main Field Study Area RefB

The selected main study area RefB is part of a sewage field classified as contaminated at low to intermediate levels (see Table 2). It is located northwest of Hobrechtsfelde and wastewater was continuously deposited here under extremely intensive farming conditions until 1984. After the cessation of farming, levies and several seepage basins were leveled and afforestation attempts were conducted with different tree species across the entire area.

An overview of the species growing in the RefB area was obtained by vegetation surveys which were conducted in parallel to the project, starting in October of 1993 (Table 3). The dominant plant species was found to be quack grass, *Agropyron repens* L.[3], which reaches a degree of coverage of close to 100% in the entire fenced study area (65 x 45 m).

Inside this area, experimental plots of a size of 20 m² each were created (see section 2.2.1). They were examined separately prior to the onset of the experiments to establish their baseline contamination with PAH and PCB (see section 2.2.2) and to survey their vegetation.

[3] *Elymus repens* (L.) Gould according to ZENTRALST. FLOR. KARTIERUNG DER BRD (NORD) (1993).

Table 3: Consolidated species list and degree of coverage for the plants at the central study site RefB in Hobrechtsfelde (County of Barnim). The vegetation analysis was done approximating the technique by BRAUN-BLANQUET (1964).

Species	Common name (selected)	Presence	Degree of coverage
Experimental plots			
Agropyron repens L.	Quack grass	very numerous	95-100%
Agrostis stolonifera L.	(Creeping) bentgrass	up to 50 Individuals	< 5%
Poa angustifolia L.	Narrow-leaf meadow grass	up to 50 Individuals	< 5%
Additionally on whole RefB site			
Calamagrostis epigejos (L.)	Wood small-reed	sporadic	-
Convolvulus arvensis L.	(Field) bindweed	"	-
Melandrium album (Mill.)	White campion	"	-
Sisymbrium loeselii L.	Tall hedge mustard	"	-
Urtica dioica L.	Big string nettle	"	-
Acer negundo L.	Box elder	single	-
Acer saccharinum L.	Silver maple	"	-
Ceratodon purpureus c.f.	Fire moss	"	-
Areas with soil disturbance			
Apera spica-venti (L.)	Loose silky-bent	sporadic	-
Capsella bursa-pastoris (L.)	Shepherd's purse	"	-
Chenopodium album L.	Lamb's quarters	"	-
Euphorbia cyparissias L.	Cypress spurge	"	-
Rumex acetosella L.	Red sorrel	"	-

2.1.2 Biomass Assessment for *Agropyron repens*

According to AUHAGEN *et al.* (1994), the Agropyretea repentis species associations constitute almost 48% of the plant communities of former sewage field areas. The so-called typical form of these associations, characterized by species poorness and a monotonous habitus, was present in the RefB area.

Being the dominant plant species in the main study area RefB and a species characteristic for former sewage field areas, *Agropyron repens* L. was selected for the litter decomposition studies.

The standing crop of *A. repens* was assessed in the fall of 1993. At this time of the year, the aboveground plant parts no longer contribute to the live plant biomass (HAHN *et al.* 1979). Therefore, it was assumed that most of the annually produced *A. repens* litter in the area would consist of the still standing dry stalks and leaves.

A. repens exhibits two different reproductive aspects. With the exclusively vegetative aspect, distribution and reproduction are achieved solely through rhizomes and the fields achieve a higher standing crop (see Table 4). Areas in which *A. repens* forms stalks bearing inflorescences are somewhat less densely covered. Only about one third of the values for the standing crop that can be observed for the vegetative aspect are achieved with predominantly generative reproduction. Based on a survey of the *Agropyron repens* plant cover of the experimental plots, a mean value of about 600 g dry matter (DM) per square meter was determined, as these plots did not contain any large areas providing only the generative aspect.

In late fall of 1993 the litter for the decomposition experiments was harvested, freed from sand particles, and dried at 40 °C.

Table 4: Standing crop of *Agropyron repens* L. on the RefB area on Oct. 16, 1993. For each area three sections of 50 x 50 cm each were sampled.

Areas	Reproductive aspect	Fresh Weight	Dry Weight (40 °C)		Dry weight (105 °C)	
		g x m^{-2}	g x m^{-2}	% FW	g x m^{-2}	% FW
A	generative	814	306	37.6	300	36.8
B	vegetative	2470	734	29.7	721	29.2
C	generative+ vegetative	1716	537	31.3	522	30.4
Mean standing crop		1667	526		514	
Mean standing crop without Area A		2093	635		621	

2.2 Litter Decomposition Experiments on Sewage Field Area RefB

2.2.1 Experimental Variants

For the *Agropyron repens* litter decomposition studies and the accompanying faunistic and floristic studies, 16 experimental plots were set up on RefB central study area (see Figure 3, following page).
Two experimental plots were set up for each of the 8 variants listed below. Litter decomposition containers were then deployed on them as follows:

- Untreated litter in litter decomposition containers was placed on experimental plots of the RefB area (sewage fields background contamination):

  Untreated Control **K**

- Litter moistened with acetone in decomposition containers was placed on RefB plots with sewage fields background contamination but treated as well with solvent:

  Control with solvent **KL**

- Litter contaminated with benzo(a)pyrene (BaP) and PCB 52 in litter decomposition containers was placed onto additionally spiked plots:

BaP litter concentrations	**BaP 1**:	10 mg x kg^{-1} DM
	BaP 2:	100 mg x kg^{-1} DM
PCB 52 litter concentrations	**PCB 1**:	4 mg x kg^{-1} DM
	PCB 2:	40 mg x kg^{-1} DM

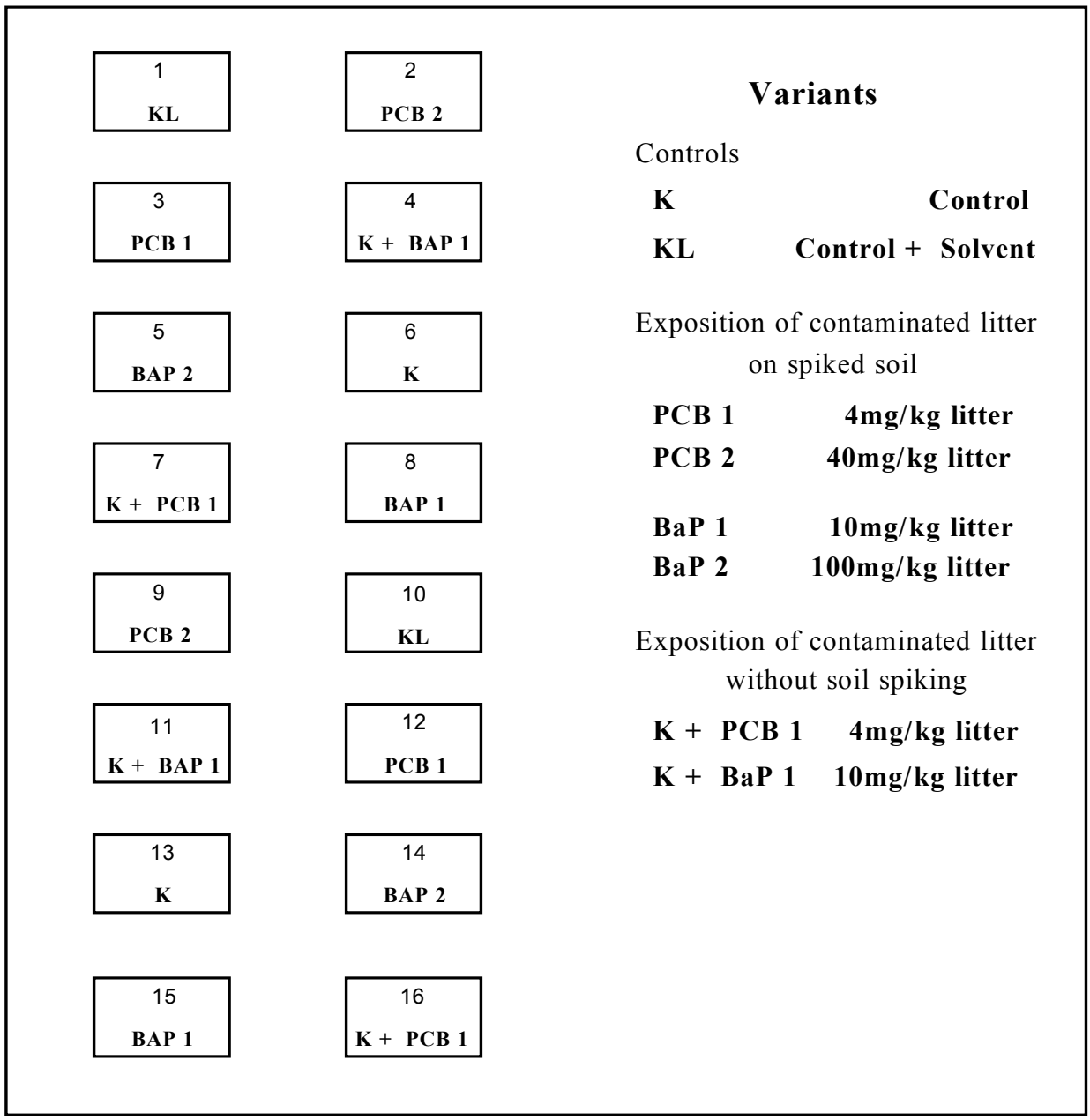
- Litter containers with BaP and PCB 52 contaminated litter were placed on plots exhibiting the background contamination of sewage fields:

BaP litter concentrations	**K + BaP 1**:	10 mg x kg^{-1} DM
PCB 52 litter concentrations	**K + PCB 1**:	4 mg x kg^{-1} DM

Thus, the experimental design provided for the investigation of two possible forms of contamination:

- ➔ A worst case study in which soil and litter matrix are contaminated (variants BaP 1 and BaP 2 and PCB 1 and PCB 2);
- ➔ A study of the response to a hot spot contamination of the *Agropyron repens* litter on "clean" areas (variants K+BaP1 and K+PCB 1).

Figure 3: Map of plots and experimental variants. The total fenced area was 65 x 45 m, experimental plots were 4 x 5 m and spaced 4 m apart.



2.2.2 Background Contamination of Areas and Application of Chemicals

For the reference substances BaP and PCB 52 the background contamination of the topsoil (0 to 30 cm) of the plots was measured prior to the onset of the experiments. For BaP it was found to be between 50 and 150 µg x kg^{-1} DM, with one outlier of 230 µg x kg^{-1} DM. This area was designated as one of the controls. The average concentrations of PCB 52 fell between 20 and 60 µg x kg^{-1} DM.

In order to achieve a homogenous load of the litter layer and to reduce the contamination heterogeneity in the experimental plots, it was necessary to apply the investigated chemicals in the field. This application was intended not to cause a higher load than that which had previously been found in the neighboring sewage fields (KRATZ & MARSCHNER, 1994).

The amount to be applied was calculated based on the mean litter biomass of *Agropyron repens* (600 g DM / m², see Table 4). As approved by the Department of Environmental Protection, Environmental and Regional Policy of the Land Brandenburg, a total of 1.76 g BaP and 0.704 g PCB 52 was applied to 160 m² of the main study area RefB. The top soil in the plots was spiked according to the central soil spiking procedure of the joint research project using sea sand, evenly applied to the soil surface, as a carrier (REESE-STÄHLER *et al.*, 1995). Spiking was done at the beginning of the vegetation period in March 1994, shortly after the dry and still standing stalks of *A. repens* had fallen down and the new stalks were sprouting.

2.2.3 Litter Decomposition Containers

The *Agropyron repens* litter was exposed in decomposition containers according to KRATZ (1991). These decomposition containers have a diameter of 13 cm and a height of 5 cm. The 5 cm high sides of the containers have 16 holes with a diameter of 2 cm each. The litter decomposition containers were filled with amounts of dry litter that were equivalent to the litter biomass previously found in areas of the same size (8 g per container; dried at 40 °C).

Several different gauze mesh sizes can be used to line the litter decomposition containers. For this study, decomposition containers with two types of gauze were employed. These types were referred to as medium gauze (MG, 1 mm mesh size) and coarse gauze (GG, 10 mm mesh size). Using these different mesh sizes, the contribution of microorganisms and mesofauna and that of macrofauna to the decomposition process can be distinguished.

About 2 weeks after the exposure was initiated, mice damaged some of the containers, especially the medium gauze ones. In case of substantial damage, the containers were removed; if the damage was minor, it was documented and the containers were continued to be used.

Following this incident the litter decomposition containers were protected from recurrent mice interference by means of a cage made of plasticised zinc meshed wire (height 30 cm, mesh size 10 mm). Because not only the lining of the side holes but also part of the bottom gauze of the

containers had been damaged, the soil surface was covered with meshed wire (15 x 15 cm) as well. Care was taken to ensure continued close contact between the container gauze and the soil.

Litter mass loss was assessed at 6 sampling dates in the course of the experiment.

For each experimental variant 6 replicates were sampled for each type of gauze (3 per plot), resulting in 96 litter containers for each sampling date. For the mycological studies conducted by the Microbiology Workgroup of the Technical University Berlin (BROSE *et al.*, 1997) 3 additional containers were harvested for each variant at each sampling date.

The field fresh remaining litter thus obtained was weighed and dried stepwise up to 40 °C in an modified extraction apparatus according to KEMPSON *et al.*, (1963), in order to survey the microarthropods present. Prior to the dry weight determination the litter was freed from sand particles.

2.2.4 Spiking and Chemical Analysis of the Litter

Of the 864 containers of the litter decomposition experiment on RefB area, 720 litter containers were treated. Except for the untreated controls, the entire amount of litter was to be evenly moistened with solvents or an adequate mixture of acetone and chemicals. To achieve this, each litter charge was spiked separately with 1 ml of acetone in which the different amounts of BaP or PCB 52 had been solved. Eight grams of litter at a time were spread on aluminum foil and sprayed with an airbrush gun (Hansa AeroPro 302). After the acetone had evaporated (24 h) the litter charges were placed in the decomposition containers.

For the determination of the initial concentrations, 3 samples per variant were set aside and stored at –20 °C until the BaP and PCB 52 was analyzed.

Residue Analysis of BaP and PCB 52

Prior to the chemical analysis, the litter samples were ground in a plant grinder (Retsch ZM 1, 0.2 mm mesh size sieve). Samples of the remaining litter were treated separately rather than pooled, in order to obtain information regarding the variability among litter containers and between the two plots in the field used for the same variant. Further sample preparation and the analysis of BaP and PCB 52 were done at the laboratories of the Institute of Ecological Chemistry of the Federal Biological Research Centre (BBA) at Berlin-Dahlem.

The extraction protocol for the plant matrix was developed to resemble the VDLUFA (1993) protocols for soil samples. Approximately 2 g litter were mixed with 50 ml bidestilled water, 20 g sodium chloride, 50 ml acetone, and 25 ml petroleum ether and subsequently shaken for 16 hours.

PCB 52 was measured gaschromatographically with electron capture detection (detection limit in the litter extracts 100 µg x kg^{-1}DM). BaP was determined via HPLC (detection limit for the diode array detector 300 µg x kg^{-1} and 10 µg x kg^{-1} litter DM for the fluorescence detector, respectively). Extract purification, sample preparation for analysis and the device settings are described in detail in VOLK (1995), REESE-STÄHLER *et al*. (1995) and FROST *et al.* (1997).

At least 2 litter samples were analyzed per sampling time and experimental variant. These samples were split into two samples for extraction and were processed in parallel from then on.

C/N Determination

The ground (0.2 mm) and dried (at 105 °C) litter was weighed in tin cartridges (3 parallel samples per litter container, weighed portion 1.3 to 2.5 mg) and were catalytically combusted in a C/N analyzer (Carlo Erba 1500) under an atmosphere of pure oxygen.

With this method, CO_2 and N_2 molecules are separated gaschromatographically. The percentages of C and N are assessed with a thermal conductivity detector. Atropine was used as reference substance.

2.3 Litter Decomposition Experiments with ^{14}C-BaP and ^{14}C-PCB 52

2.3.1 Experimental Variants in the Mitscherlich Vessels

To further investigate the behavior of BaP and PCB 52 during the decomposition of contaminated litter, container experiments with ^{14}C labeled substances were conducted on site at the BBA in Berlin-Dahlem. The BBA maintains a separate area for experiments with radioactively labeled substances under semi-field conditions.

Into so-called Mitscherlich vessels, which include a basin mounted below the actual container for collecting leachate, 30 kg of the RefB soil were filled (see section 2.1, p. 46 for a description of the soil). The soil was moistened immediately after the weighing into the vessels and then regularly during the following weeks. Thus, the containers exhibited plant growth at the beginning of the experiments 10 weeks later. In addition to *Agropyron repens* L. (quack grass) there were occurrences of *Urtica dioica* L. (stinging nettle), *Capsella bursa-pastoris* L. (shepherd's purse) and *Anthemis arvensis* L. (corn chamomile).

Table 5: Controls and litter spiking variants for the vessel experiments

Variant	Treatment
K	Control
KL	Control + solvent (acetone)
PCB	40 mg PCB 52 x kg^{-1} litter DM
BaP	100 mg BaP x kg^{-1} litter DM

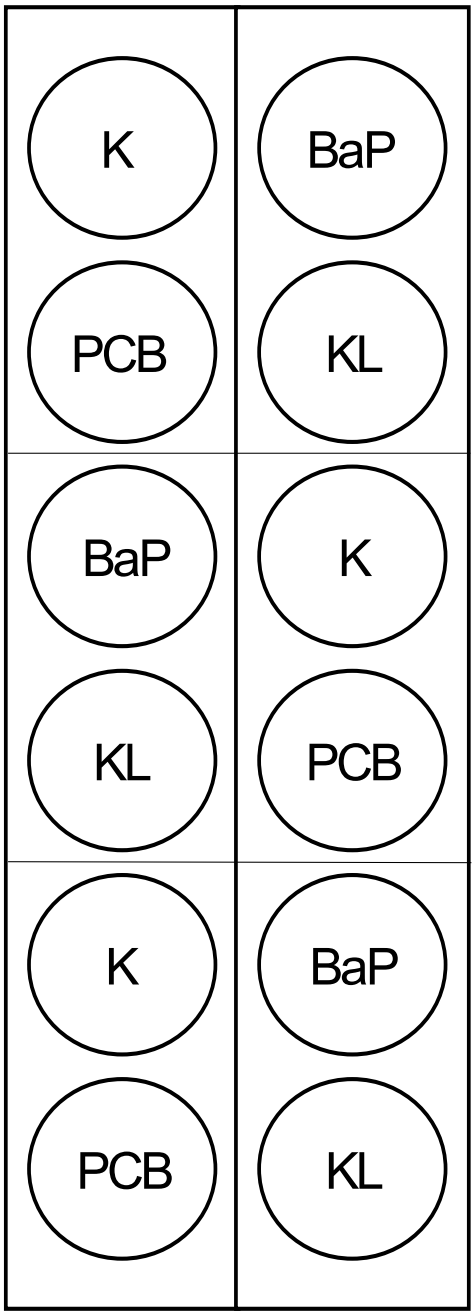


Figure 4: Variants in the Mitscherlich Vessel Experiment with ^{14}C-BaP and ^{14}C-PCB 52 contaminated litter

At the BBA site in Dahlem, 12 Mitscherlich vessels (3 repetitions each per variant, see Figure 2 and Table 5) were placed inside an open cage for field studies with radioactive substances where they were subject to natural meteorological conditions. Onto the soil surface of each of the vessels, 3 litter decomposition containers were placed. The decomposition containers were filled with 8 g of *Agropyron repens* litter each, as in the field litter decomposition experiment carried out on the central study area RefB (see section 2.2.3).

Spiking of the litter was done according to the procedure described in section 2.2.4. However, for each batch 9 g of litter were spiked instead of 8 g, with 1 g being used to determine the initial concentration. For each variant an acetone solution containing the study chemicals corresponding to the target concentration was prepared.

^{14}C-BaP (Sigma; purity: 98%; specific activity: 11.25 kBq/mg; ^{14}C-labeled at C7) was added to the unlabeled BaP, resulting in a nominal concentration of 100 mg BaP x kg^{-1} litter and 10 kBq per container. Each of the Mitscherlich vessels of the "BaP" variant was exposed to 30 kBq via the 3 decomposition containers placed onto its soil surface.

The target concentration for the decomposition containers of variant “PCB” was 40 mg PCB 52 x kg^{-1} litter and 20 kBq, resulting in 60 kBq per Mitscherlich vessel (PCB 52 by KfA Jülich, purity: 98%, specific activity: 58.19 kBq/mg; evenly labeled rings).

The litter decomposition containers were exposed on August 29, 1996, and harvested on June 16, 1997. The litter was weighed fresh and then dried to constant weight at 40°C after being cleaned from sand particles.

The soil underneath the litter containers was removed down to 0.5 cm across the container area. The soil layers down to 15 cm below the surface was sampled underneath each litter container with a core sampler (diameter 5.5 cm). Separate samples were taken at 0.5 to 5 cm, 5 to 10 cm and 10 to 15 cm.

After drying (40 °C), roots and stones were removed and the soil dry weight was determined. The samples were broken up separately with a mortar and pistil. From the top layer (0 to 0.5 cm) only the stones were removed, since the particulate loss from the litter layer was to be included in the assessment.

Litter and soil samples were weighed into ceramic vessels (approximately 100 mg and 2 g, respectively) and combusted in a Biological Material Oxidizer (OX 500, Harvey Instrument Corporation). The oxidizer combusts the organic materials in a stream of oxygen gas at a temperature of 900 °C. It then passes the combustion products through a series of catalysts at 680 °C and then traps the $^{14}CO_2$ directly in vials containing scintillation liquid (Oxisolve, Zinsser Analytik). Subsequently the amount of radio labeled carbon was measured in a liquid scintillation counter (LS 6500, Beckmann). Based on the specific activity of the substances added, the content of those substances in the samples was calculated.

The ash-free substance of the litter and soil samples was determined based on the weight of the cooled off combusted sample matrix

2.3.2 Thin Layer Chromatography

Samples of the litter that had been exposed for 9 months in the Mitscherlich vessel were extracted using the same method that had been applied to the non-labeled BaP and PCB 52 samples from the field experiment on sewage field area RefB (see section 2.2.4). Here, the aim of the "cold" extraction was to compare the extractable amounts of the applied reference substances with the total amount of radioactivity present in the litter, which had been determined by means of sample combustion and scintillation counts (see above).

The organic phase of the extraction was subsequently analyzed by Thin Layer Chromatography (TLC). The ^{14}C labeled compounds were characterized using 60 Å, 200 x 200 mm silica gel plates with fluorescent indicator (F254, Merck).

Organic extracts (5 ml) containing ^{14}C-PCB 52 were reduced to 1000 µl under a stream of N_2 and then applied to the plates in 20 mm bands 20 mm apart from each other. For the ^{14}C-BaP variant, 2000 µl aliquots of the organic phase were applied directly to the plates. Reducing these extracts to 1000 µl resulted in subsequent poor phase separations.

Unlabeled standards were dot-applied onto plates and were run together with the organic extracts in toluene until the solvent had reached 16 and 18 cm for the ^{14}C-BaP and ^{14}C-PCB extracts, respectively. The Rf-value for ^{14}C-PCB 52 and ^{14}C-BaP was 0.78 while other ^{14}C-compounds remained at the origin. The distribution of the ^{14}C activity was analyzed using an Automatic TLC Linear Analyzer (LB 284-2, Berthold). Radiochromatographs are evaluated with a spot sensitive counter, which allows for simultaneous detection of the total ^{14}C activity over the chomatogram. The inherent resolution of the scan analysis is 0.25 mm.

2.4 Laboratory Reproduction Test with *Folsomia candida*

For the assessment of the subacute effects of BaP and PCB 52 on Collembola, reproduction laboratory tests with *Folsomia candida* (Willem 1902) were performed according to DIN ISO guideline 11267 (DIN ISO, 1999; RIEPERT, 1996).

Toxicological endpoint of the test is the number of juveniles (F1 generation) at the end of a 4-week experiment. Subadult Collembola are exposed to a sublethal concentration of the test chemical which has been mixed into soil or artificial soil, resulting in an exposure to the chemical that covers egg laying, emergence, and the first larval state.

Non-contaminated granulated baker's yeast is sprinkled on the bottom of the experimental containers to feed the animals. The mean number of juveniles per test container (5 parallels) serves as a measure of the reproductive rate. The mortality of the parental generation should not exceed 20%, the number of juveniles should reach at least 100 per container, and the coefficient of variation of the reproductive rate in the control containers should not exceed 30%. Soil pH-value is measured before and at the end of the experiment in replicated test containers set up for this purpose.

Deviating from DIN ISO guideline, contaminated soil from the sewage fields was used as test substrate instead of the artificial soil. The artificial soil prescribed by the DIN ISO guideline contains not only fine quartz sand (68 to 69%) but also sphagnum peat (10%) and kaolinite (20%). Under these conditions, the adsorption of the test chemical to the test matrix might have been increased and their bioavailability decreased compared to the sewage field soils, which have especially low clay content.

The soils used were investigational soils from the joint research project: "RefB" from the central investigational sewage field area was used as the control (see Table 2), RefB soil spiked with BaP or PCB 52 spiked provided the contamination variants (see Table 6). Spiking soil procedure with sea sand as carrier is described in REESE-STÄHLER *et al.* (1995) and FROST *et al.* (1997).

Two runs of the reproduction test with *F. candida* were carried out: One was performed 2 years, a second run 6 years after spiking of the soil. The aim was the detection of possible reductions in the toxicity of the substance after long-term storage of the spiked soils.

Table 6: Spiked soil variants, target concentrations and concentrations in the soil 2 and 6 years after spiking.

Variants and concentrations	RefB	1 P52	2P52	3 P52	1 BaP	2 BaP	3 BaP
BaP mg x kg^{-1}							
target concentrations	**0.1**	0.1	0.1	0.1	**1.0**	**10**	**100**
after 2 years	**0.1**	0.1	0.1	0.1	0.6	9.6	-
after 6 years	**0.1**	0.1	0.1	0.1	**0.5**	**8.0**	**86.4**
PCB52 mg x kg^{-1}							
target concentrations	**0.02**	**0.20**	**2.0**	**20.0**	0.02	0.02	0.02
after 2 years	**0.02**	**0.18**	**1.9**	**20.0**	0.02	0.02	0.02
after 6 years	**0.02**	**0.18**	**1.3**	**18.7**	0.02	0.02	0.02

2.5 Faunistic Studies

The primary objective of the joint research project was the investigation of the effects of organic chemicals on soil organisms and soil processes. Thus, a general survey of the soil fauna of the former sewage fields in Berlin-Buch could be done only at selected points and has to be considered provisional.

In the central study area RefB, the Collembola (springtails) and Oribatida (oribatid mites) of all litter decomposition containers were surveyed at each sampling date, resulting in a data set for the colonization densities in spiked and control litter for one year of exposure.

To achieve a comparative state assessment of long-term contaminated sewage fields based on the characterization of the biocenosis (see section 1.2), different areas were so selected as to represent specific soil pollutant loads based on their diverse previous use (see Table 2).

The colonization densities of litter and soil dwelling Oribatida were investigated at two times (fall of 1995, spring of 1996) in the areas RefB, nPAK and nPCB. Samples were taken with a core-sampling device (5.5 cm diameter) down to a depth of 5 cm. In the fall of 1995 additional

samples were taken for a survey of the nematode fauna. Dr. G. Korthals of the Wageningen Agricultural University, Netherlands, studied these samples.

Microarthropods were extracted from the litter of the decomposition containers using an extraction apparatus according to KEMPSON *et al.* (1963). Soil samples, on the other hand, were processed in a modified extraction device according to MACFADYEN (1961). These samples were smaller, making them suitable for this method that allows for higher numbers of replicates to be extracted at the same time. In both extractors the animals were trapped in saturated picric acid (1:1 dilution) and subsequently transferred to 75% ethanol.

First, the animals from the samples were sorted into Collembola, Oribatida, and other groups, using a binocular stereo-zoom microscope (GSZ Zeiss Jena, up to 40x magnification). All of the oribatids and the members of the Entomobryidae and Isotomidae families from the Collembola group were identified down to the species level. For the species determination of the oribatid mites the identification keys and writings by WILLMANN (1931), SELLNIK (1929, 1960), STRENZKE (1950,1951), WUNDERLE *et al.* (1990), and MIKO & WEIGMANN (1996) were used.

In cases of uncertain identification, Dr. Ch. Kehl and Prof. Dr. G. Weigmann offered to inspect the subjects in question. Their help is gratefully acknowledged. Data regarding the ecology of oribatid mites were taken from STRENZKE (1952), KNÜLLE (1957), RAJSKI (1967, 1968), MORITZ (1962), and WEIGMANN & KRATZ (1982).

Identification of Collembola species was done according to FJELLBERG (1980) and GISIN (1960).

2.6 Bait Lamina Test

In accompanying studies to the faunistic work done on the areas RefB, nPAK and nPCB, the soil fauna feeding activity was determined. As opposed to litter decomposition studies, which can be performed using matrices typical for the investigated site but which are very labor intensive, the bait lamina test (VONTÖRNE 1990, LARINK & KRATZ, 1994) delivers a large amount of feeding data, which can be evaluated biometrically and are associated with estimable error rates. This test basically consists of bait substrates that are deposited in holes drilled into special PVC sticks and then exposed in the soil or inside the soil cover. Feeding traces then are visible when light shines through the PVC sticks.

Here, 16 cm long, thin PVC sticks (Terra Protecta GmbH) with 16 holes 0.5 cm apart were used. They were inserted into the soil so that the uppermost hole was positioned 0.5 cm beneath the soil surface. This approach allowed for the differential evaluation of feeding activities on the bait substance down to a depth of 8 cm.

The bait substance used was a mixture of cellulose, bran, and activated charcoal (70:27:3), which represents a non-specific, highly standardized and readily available food source. In each of the investigational areas, the bait lamina test was carried out with 3 to 4 so-called base groups. Each base group consists of 16 bait lamina strips exposed in a square of 4 by 4 strips, 10 cm apart.

Originally, the bait lamina test was used "only to determine zootic feeding activity…" (VONTÖRNE in DUNGER & FIEDLER, 1989, P. 259) and the duration of the experiments was limited to 2 weeks in order to prevent microbial bait decomposition. In cooperation with other workgroups of the joint research project, tests of different exposure times were done, and it could be shown that feeding activities determined in longer experiments also may correlate rather well with data from faunistic studies (e.g., number of enchytraeids present, ACHAZI *et al.*, 1997).

2.7 Data Analysis

2.7.1 Evaluation of the Litter Decomposition Experiments

Analysis of Variance

Using the analysis of variance (ANOVA) approach, the effect of the applied chemicals on the litter decomposition process was investigated. The variable "litter decomposition" was described by the logarithm of the dry weight of the remaining litter. Separate analyses were performed for the following groups: PCB variants with coarse gauze, PCB variants with medium gauze, BaP variants with coarse gauze, and BaP variants with medium gauze.

As previously described in section 2.2.3, 6 decomposition containers were harvested per sampling time and experimental variant. In order to be able to assess the influence of the spatial variability of the experimental plots, 3 containers each were harvested from the two plots for a single variant (factor "block", see also Figure 3). The different chemical concentrations (treatment levels) were included in the calculations as steady factors ("BaP" and "PCB") that were assigned to the two blocks in the RefB area (nested ANOVA).

The overriding importance of time for the decomposition process was taken into account by conducting an analysis of covariance using the sampling date as a variable prior to the analysis of variance. Because the analysis of covariance executes a regression calculation with the litter decomposition data of the entire experimental period, the logarithm of the litter weight was used to describe the exponential decomposition curve (see also Decomposition Models below).

All calculations were done with the SPSS© software suite, version 9.0.

In cases where no significant influence of the experimental plot as such on the litter decomposition rate was discernible and where the analysis of variance resulted in rejection of the null hypothesis (all mean values of the factorial levels are equal), the data for each

sampling date were compared separately using single factorial one way ANOVA and the SHEFFÉ test or the DUNNETT-C test as *a posteriori* test procedures for equal or different variances, respectively.

If the data to be compared belonged to only two variants, the T test was used with *n* as the maximum of *a posteriori* conductible tests, defined by the number of mean values k (n = 0.5 x k)

Decomposition Models

Exponential decomposition models are often used to describe the litter decomposition process. Using decomposition constants *k*, a process is described that occurs evenly over time and that has a steep initial phase representing the decomposition of the readily decomposable litter components. Additionally, a second pool of slow decaying organic matter may be included in the calculation, allowing for a long decomposition process of recalcitrant litter remains (equation 1). In contrast to this double exponential model, very slow decomposing components are generally integrated in a single exponential model with a constant term, which describes the apparent convergence of the weight loss towards a constant value.

$$w = w_1 \times e^{-k_1 t} + w_2 \times e^{-k_2 t} \qquad \text{(Equation 1)}$$

where

w	= remaining litter (% of initially exposed)
$w_1 + w_2$	= initially exposed litter (initial weight= 100%)
$k_{1,2}$	= decomposition constants
t	= time (days)

In addition to the model described above, a linear model was used in which two decomposition phases were fitted using the necessary decomposition constants. This was done because the stagnation of the decomposition process, which was apparent from the data of the early summer months immediately after the start of the experiment, could not be described by fitting the exponential model. This stagnation was likely to be a result of the extreme dryness and high soil temperatures.

$$w = w_0 + k_L \, t \qquad \text{(Equation 2)}$$

where

w	= remaining litter (% of initially exposed)
w_0	= initially exposed litter (initial weight= 100%)
k_L	= linear decomposition constant
t	= time (days)

The decomposition constants used in the exponential and the linear model of equations 1 and 2 are different. In the exponential model k_1 and k_2 represent the percentage weight loss per existing litter per unit of time. In the linear model, k_L represents the percentage weight loss per initial litter per unit of time.

Multiple Regression

If the qualitative and quantitative relationships between one dependent variable and one or more independent variables are to be analyzed, multiple regression calculations can be applied (overview in SACHS, 1992; BACKHAUS *et al.*, 1990). Based on the data set obtained in the litter decomposition experiments, the influence of experimental factors (acetone treatment, different substance concentrations) and of environmental factors (moisture) on the decomposition of *A. repens* litter and on the densities of selected microarthropod species was to be investigated and quantified.

The following is a general regression equation for the analysis of a (statistical) sample:

$$Y = b_0 + b_1X_1 + b_2X_2 + ...+ bjXj \qquad \text{(Equation 3)}$$

where

Y	=	regressand or dependent variable
b_0	=	constant term, intersection of the line and the y axis
bj	=	regression coefficient of regressor j, or independent variable
Xj	=	regressor j, or independent variable

Not all aspects of the variation of Y (dependent variable) can be explained by the selected independent variables. The influence of non-recorded factors in the context of the analysis is termed residuals (e) and is included in the regression equation. Simplified:

$$Y = b_0 + b_1X + e \qquad \text{(Equation 4)}$$

A measure for the assessment of the goodness of fit of the estimation is the coefficient of determination, which represents the ratio of the explained variation to the total variation. The method employed was stepwise regression, where single variables are added to the calculation one after another. The first regression calculation is done with the variable most strongly correlated to the dependent variable. The influence of the factors included is defined qualitatively as well as quantitatively (based on the change in the coefficient of determination).

The results of linear regression calculations need to be interpreted carefully, since they represent a linear relationship between the expression of a biological parameter (here: the decomposition rate or the species densities) and its biochemical and physical environment.

The responses of living organisms to the expression of an environmental factor across the entire possible variation range may result in an increase, the attainment of an optimum value and eventually in a decrease of the measured quantity, even within the boundaries of the tolerable range of that environmental factor. Thus, even an existing strong linear relationship may disappear once certain values are reached, and then may reappear with an inverted sign.

2.7.2 Evaluation of the Faunistic Studies

In cases where the faunistic data from the field and laboratory studies could not be considered as normally distributed after transformation (e.g., log x + 1), the H test by KRUSKAL-WALLIS was used instead of an analysis of variance (ANOVA). The H test is a non-parametric single factor analysis of variance applied to determine whether there are significant differences in the medians of the different factorial levels (overview in SACHS, 1992). In cases where the H test resulted in rejection of the null hypothesis, i.e., where at least one factorial level belonged to a different population, the individual groups were compared *a posteriori* by means of the NEMENYI test.

If the data were normally distributed, statistical differences between factor levels (here, contamination variants) were assessed by a one-way ANOVA followed by a SHEFFÉ test or a DUNNETT-C test *a posteriori* for equal or different variances, respectively.

The MACFADYEN extraction provided the absolute numbers of Collembola and Oribatida contained in the litter and soil samples. The **densities** with which the animals were found in the litter of the decomposition containers were graphically represented as number of individuals x 100 g^{-1} litter.

The constancy, which represents the percentage of samples in which a given species was found, may serve as a measure for the equitability of the colonization of one species in the samples. If the constancy is calculated based on sample numbers obtained over a period of time, it is equivalent to the site constancy of the species (MORITZ, 1963). According to STRENZKE (1952) the occurrence of a given species in a sample expressed as a percentage of the total number of animals present can be classified as in Table 7.

Table 7: Classification of constancy, according to STRENZKE (1952), modified according to MORITZ (1963)

Constancy class	% of samples	Constancy
5	81 to 100	always
4	61 to 80	most of the time
3	41 to 60	often
2	21 to 40	rare
1	1 to 20	very rare

The oribatid **cenoses** of the sample sewage field areas RefB, nPAK and nPCB were, unlike the litter decomposition container studies, compared qualitatively: The numbers of species present and the ecological preferences of the species found served to characterize the different habitats. In the literature, species are often classified by their ecological habitat requirements, especially requirements regarding habitat parameters that are considered as distribution controlling factors.

STRENZKE (1952), KNÜLLE (1957), MORITZ (1963), RAJSKI (1967, 1968), and others used a species' tolerance to the fluctuation of a factor and the position of the species' optimum rage on the scale to classify species as ecological types. In the following compilation, special emphasis was placed on the requirements of oribatid mites for moisture and pH value of the substrate.

A species with a relatively narrow ecological amplitude regarding the fluctuation of a given environmental parameter is characterized by the prefix "steno" added to the adjective describing the respective factor, and this species is found only in habitats that provide optimum conditions for it. If a species on the other hand is described as "eury…" regarding a given parameter it has a relatively high plasticity for deviations from the optimum value, if an optimum value can be determined at all.

The position of the ecological optimum of a species on the respective scale has been described (for moisture and pH of the substrate) by STRENZKE (1952) and RAJSKI (1967, 1968) as listed below. The respective scale consists of the range of values found for a given parameter in the investigational area.

For example, if a species is considered oligoeuryionic it will be found with high constancy in samples with a low pH value but also, leas frequently, in substrates of a different pH.

Table 8: Classes and associated values for the parameters moisture and pH value according to STRENZKE (1952) and RAJSKI (1967, 1968)

		Associated Values	
Factor	Definition	STRENZKE (1952)	RAJSKI (1967, 68)
Water content	oligohygric	dry substrate	1.0 to16.6% (w/w)
	mesohygric	moist substrate	5.7 to54.0% (w/w)
	polyhygric	wet substrate	16.8 to 84.6% (w/w)
pH value	oligoionic	<6.5	<6.5
	mesoionic	6.5-7.5	6.5-7.5
	polyionic	>7.5	-

Indices for the description of species communities can serve as a measure for the similarity of two sites in their species composition or dominance structure (e.g., see MÜHLENBERG, 1993).

The **individual dominance (D_N)** was calculated as a measure of the relative frequency of species in a sample:

$$D_N = \frac{a}{c} \times 100 \qquad \text{(Equation 5)}$$

where

a = number of individuals of species a in the community

s = total of all individuals of all species in the community.

Sörensen's quotient (QS) considers the numbers of the species occurring in two habitats as well as the total number of species in those habitats and, therefore, serves as a simple comparison of species communities:

$$QS(\%) = \frac{2G}{S_A + S_B} \times 100 \qquad \text{(Equation 6)}$$

where

G = number of species occurring in both areas

S_A, S_B = number of species in area A and B, respectively.

This quotient assumes values between 0% (no species in common) and 100% (range of species identical in both habitats).

Renkonen's Index (Re), on the other hand, constitutes a measure of the similarity of the dominance structures of two species communities.

$$\text{Re}(\%) = \sum_{i=1}^{G} \min D_{A,B} \qquad \text{(Equation 7)}$$

where

G = number of species occurring in both areas

i = species i

min D_A, D_B = the smaller dominance value (D_N) for each of the species common to both areas A and B

Thus, this index is based on similarities in the occurring species as well as on similarities in their dominance structures.

3 RESULTS

3.1 Behavior of PCB 52 and BaP in the Litter Matrix

3.1.1 BaP and PCB 52 Levels in the Litter of the Field Experiment

The spiking procedure, described in chapter 2.2.4 and used to contaminate the litter for the field decomposition experiments on RefB area, led to different results depending on the substance sprayed.

For benzo(a)pyrene (BaP), 100% of the desired target concentrations in the litter could be achieved in the lower contamination variant (BaP 1, target concentration of 10 mg x kg^{-1} litter) and 90% in the BaP 2 litter (target concentration 100 mg x kg^{-1} litter).

In contrast, the concentrations for PCB 52 in the litter at the beginning of the exposure were only half (low contamination level PCB 1) and only one third (high contamination level PCB 2) that of the desired concentrations.

The decreases in the concentrations of BaP and PCB 52 in the litter over the course of decomposition are shown in Figure 5 and Figure 6, respectively. The values given are that for the extractable amounts of BaP and PCB 52 from the litter matrix. The proportions of the different processes that may have contributed to the losses of extractable compounds (e.g., mineralization, leaching, fixation/immobilization) cannot be differentiated and are integrated into the decreasing concentration curves.

In the beginning, the curves were steeper than those for the litter decomposition as such (see chapter 3.2). After approximately 100 and 140 days, respectively, the concentrations of the extractable portions of PCB 52 and BaP reached a plateau level at which they remained until the end of the experiment. The final concentrations in the litter after 400 days of exposure for both PCB 52 and BaP were between 10% and 15% of the initial values. The PCB variants often showed a higher variability among parallel samples than the BaP variants.

An exponential curve fit gave the best results in estimating the concentrations of extractable BaP and PCB 52 over time (see equations in Figure 5 and Figure 6, coefficients of determination $R^2 \geq 0.98$). In the high contamination litter variants, detectable concentrations of BaP and PCB 52 decreased with exponential rate constants of 0.018 and 0.022, respectively.

The absolute amounts of PCB 52 and BaP measured in the litter decomposition containers are listed in Table 9. The litter, which serves as carrier matrix for the applied chemicals, is decomposed itself over the course of the experiment. Note that the variants BaP 1 and BaP 2 exhibited faster litter decomposition (see Figure 15, p. 67) than the PCB variants.

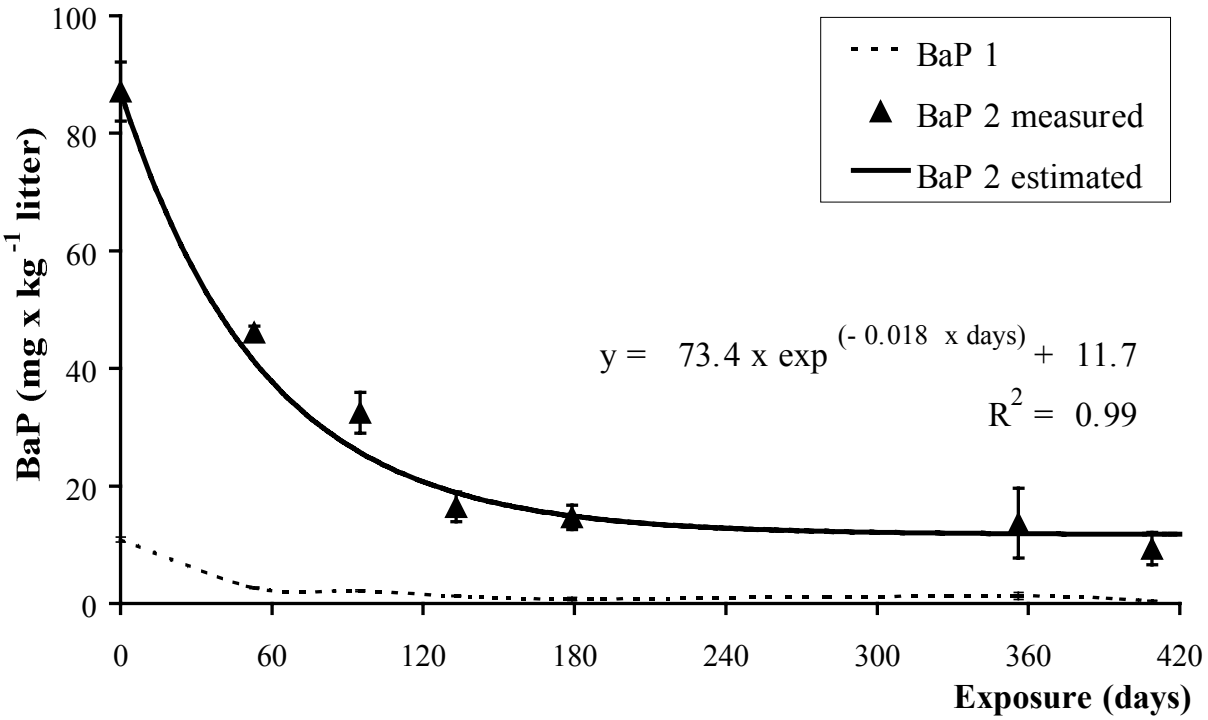


Figure 5: BaP levels in litter during exposure. Mean values and their mean deviations for the variants BaP1 (target concentration 10 mg x kg^{-1} litter) and BaP 2 (target concentration 100 mg x kg^{-1} litter)

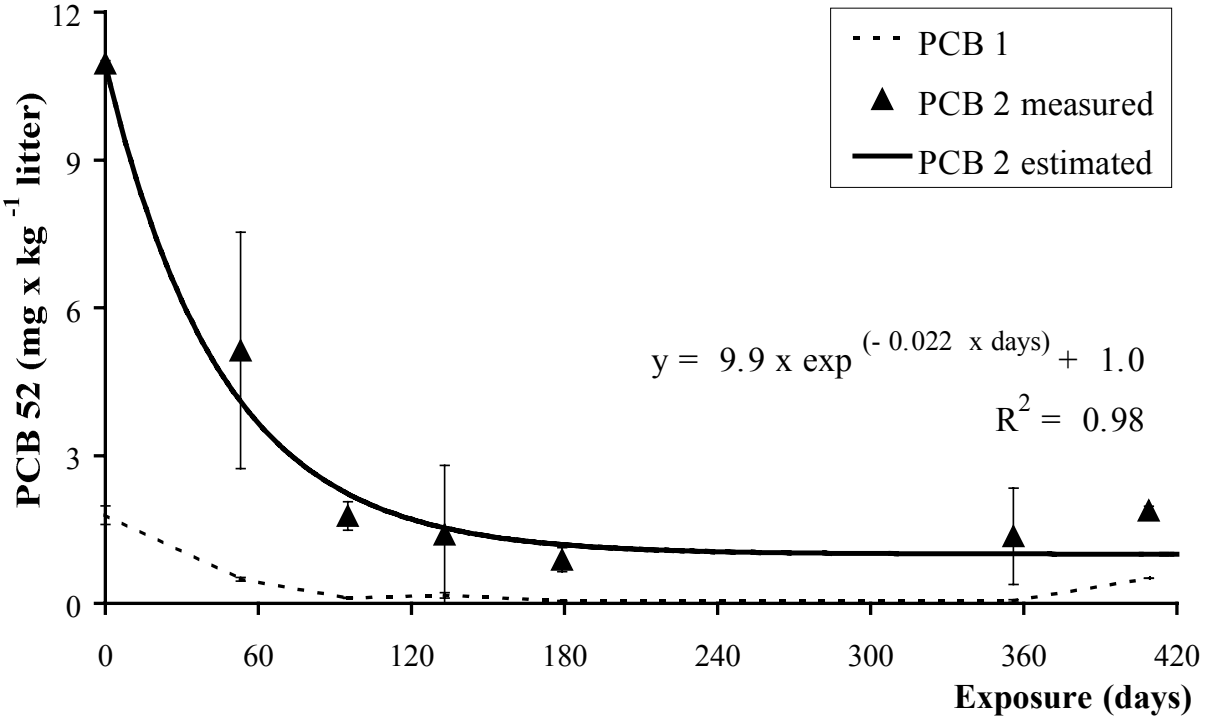


Figure 6: PCB 52 levels in litter during exposure. Mean values and their mean deviations for the variants PCB 1 (target concentration 4 mg x kg^{-1} litter) and PCB 2 (target concentration 40 mg x kg^{-1} litter)

Both PCB 52 and BaP were more stable or the amounts present more readily extractable in the variants with the higher spiking levels of the chemicals. The BaP 1 as well as the PCB 1 litter exhibited a significant reduction of the amount of extractable chemical after brief exposure in the field. After 50 days only 19% and 22% of the initial amounts of BaP and PCB 52, respectively, could be detected in the litter. At this time, approximately 80% of the initial amount of litter remained in the decomposition containers.

With the highly contaminated litter, however, at the first sampling date, 36% of the PCB 52 and 42% of the BaP could be extracted. The reason for the difference in behavior of both chemicals at the two contamination levels cannot be found in the litter decomposition process, because the differences in litter weight between the contamination levels 1 and 2 for both variants was negligible at the first sampling date.

Table 9: Existing amounts of PCB 52 and BaP in the remaining *A. repens* litter in the coarse gauze containers. Listed are mean values from two containers and their mean deviations. Weight of litter at the beginning of the experiments: 8 g

		PCB 52 (µg/container)		BaP (µg/container)	
Sampling date		PCB 1	PCB 2	BaP 1	BaP 2
--	Start	14.34 ± 1.51	87.67 ± 0.46	87.40 ± 3.40	697.0 ± 40.2
1.	53 days	3.17 ± 0.12	31.45 ±13.24	17.18 ± 0.16	290.2 ± 11.5
2.	95 days	0.72 ± 0.06	11.21 ± 1.66	13.84 ± 0.11	205.9 ± 25.6
3.	133 days	0.90 ± 0.29	7.42 --	6.99 ± 0.69	89.5 ± 11.2
4.	179 days	0.21 ± 0.01	3.99 ± 1.01	3.38 ± 1.20	55.9 ± 11.8
5.	356 days	0.19 ± 0.09	5.28 ± 3.65	4.98 ± 2.42	48.1 ± 15.4
6.	409 days	0.01 ± 0.00	7.22 ± 0.52	1.89 ± 0.35	32.9 ± 10.1

3.1.2 ^{14}C-PCB 52 and ^{14}C-BaP Behavior in the Mitscherlich Vessels

Distribution of the Radio labeled Substances

The exposure of BaP and PCB 52 in the field (RefB area) using *A. repens* litter as carrier matrix resulted in a rapid reduction of the extractable amount of chemical within a few months (see Figure 5 and Figure 6). However, this process was not observed in soils that had been spiked at the same time and were regularly tested for BaP and PCB 52 to assess their storage stability (see FROST *et al.*, 1997). Over a course of 3 years, the PCB 52 levels (spiked at 0.2, 2, and 20 mg x kg^{-1}) decreased only by 10% and 30%, respectively. After the same period of time, more than 80% of the initial amounts of BaP (spiked at 1, 10, and 100 mg x kg^{-1}) could be extracted from the soils.

A semi-field experiment was conducted to investigate whether the higher losses of BaP and PCB 52 in the field decomposition experiment on RefB area were due to mineralization and/or leaching from the litter layer, or a result of immobilization/fixation processes resulting in non-extractable "bound residues" (see chapter 1.1.5, p. 36).

The amount of activity applied to the *A. repens* litter and then exposed in so-called Mitscherlich vessels, is shown in Table 10 for the BaP and PCB variants. Along with the spiking efficiency, the recovered amounts from the litter and the soil underneath the litter containers after approximately 9 months of exposure are listed.

Unlike with the chemical application for the litter decomposition experiment onto RefB (see chapter 3.1, p. 66) here the target levels for both BaP and PCB 52 were not sufficiently achieved. If in the previous experiment almost 100% of the desired BaP levels had been reached, only approximately 50% of the amount applied of ^{14}C-BaP were detected in the litter at the beginning of the experiment in the Mitscherlich vessels.

For ^{14}C-PCB 52, an adsorption to the walls of the litter storage vessels was observed when the latter were rinsed and tested for contamination with the spiking substances. If corrected by this amount, the initial amounts of activity would be approximately on third higher than those reported in Table 10. It was impossible to tell whether the PCB 52 recovered from the vessel walls had been deposited there after volatilizing along with the acetone from the litter, or whether the deposition on the walls was a result of the direct contact between litter and walls. The former assumption is supported by the fact that no wall depositions were found in the storage vessels of the BaP spiked litter, BaP being considerably less volatile than PCB 52.

Table 10: Activity (^{14}C-BaP and ^{14}C-PCB 52) applied and recovered after exposure from litter and soil of the Mitscherlich vessels

Variant	Matrix		Depth (cm)	Activity Σ (KBq)	% of the initial activity (as measured)	% of the initial activity (nominal)	% of the total recovered amount
BaP	Initial Litter		-	48.0	100	53.3	-
	After exposure:	Litter	-	35.6	74.1	39.6	94.2
		Soil	0 - 0.5	1.46	3.04	1.62	3.86
			0.5 - 5	0.39	0.81	0.43	1.02
			5 - 10	0.20	0.41	0.22	0.53
			10 - 15	0.14	0.28	0.15	0.36
PCB 52	Initial Litter		-	50.2	100	27.9	-
	After exposure:	Litter	-	15.7	31.3	8.74	63.5
		Soil	0 - 0.5	3.55	7.06	1.97	14.3
			0.5 - 5	5.17	10.3	2.87	20.9
			5 - 10	0.31	0.62	0.17	1.25
			10 - 15	n.d.	-	-	-

n.d.: Activity of the sample below detection limit

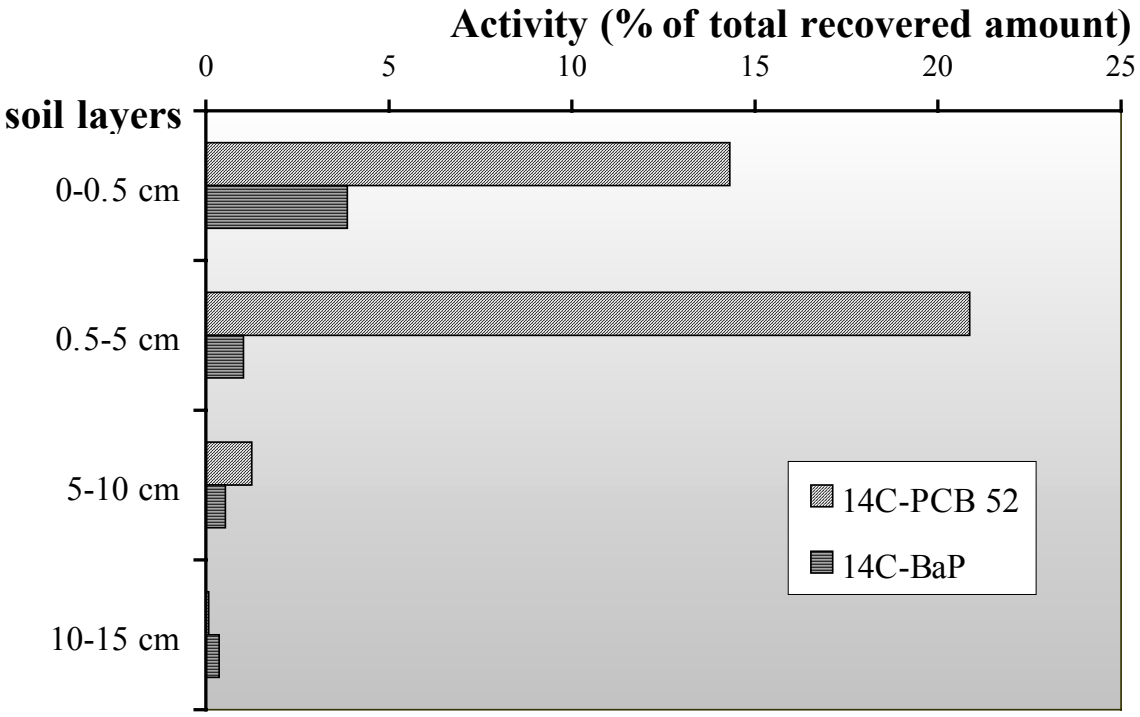


Figure 7: ^{14}C-Activity in the soil layers after exposure of contaminated litter on the soil surface. Shown are the percentages of the recovered activity; differences to 100% are the parts associated with the litter layer.

After the litter had been exposed on the surface of the Mitscherlich vessels filled with RefB soil for 9 months (see chapter 2.3.1, p. 54), a total of approximately 79% of the applied ^{14}C-BaP and 50% of the ^{14}C-PCB 52 were detected in litter and soil (see Table 10).

Based on the total recovered activity, 94% of the ^{14}C-BaP, but only 63% of the ^{14}C-PCB 52 remained in the litter layer. For the PCB variant, 18% of the amount of chemical applied at the beginning of the experiment, equaling 35% of the amount of total recovered activity after 9 months, was found in the soil beneath the litter decomposition containers (see Table 10 and Figure 7).

To account for the different weights of the sampled layers, the activity concentrations of ^{14}C-BaP and ^{14}C-PCB 52 from the litter and soil samples were plotted in Figure 8. In order to be able to compare the different matrices, concentrations are given as activity (Bq) per gram of ash-free substance (AFS), i.e., corrected for the weight of the ash after combustion in the oxidizer.

For all of the litter samples, the content of ash-free substance was approximately 96% at the beginning of the experiment and approximately 94.5% after the exposure (percentage by weight). The uppermost soil layer (down to 0.5 cm depth) contained 8.5% ash-free substance, the deeper layers down to 15 cm contained 4.5% (percentage by weight). No differences were observed between the samples from different variants.

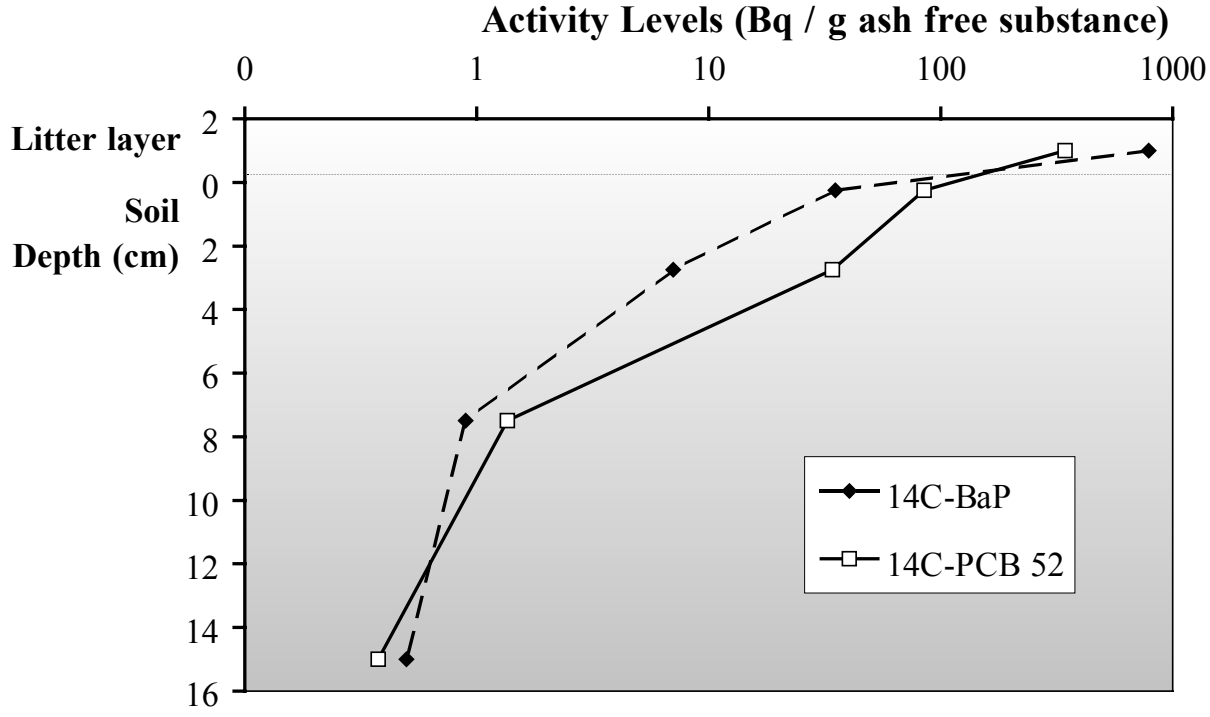


Figure 8: Activity levels (as Bq per gram of ash-free substance, see text) in litter and soil beneath the decomposition containers after 9 month of exposure of contaminated litter

After 9 months of exposure the activity level of ^{14}C-BaP in the litter layer was approximately 800 Bq x g^{-1} AFS (see Figure 8, note the logarithmic scale). In the soil layer down to 0.5 cm the activity had decreased to approximately 35 Bq x g^{-1} AFS and in the layer beneath it (down to 5 cm depth) to approximately 7 Bq x g^{-1} AFS.

The activity levels of ^{14}C-PCB 52 in litter were around 340 Bq x g^{-1} AFS, in the topmost soil layer approximately 85 Bq x g^{-1} AFS. Thus, the activity levels of ^{14}C-PCB 52 in the upper most half centimeter of soil was equivalent to approximately 25% of the litter concentration, and those of ^{14}C-BaP were equivalent to only 4%.

The differences in the behavior of the two radio labeled substances were even more pronounced in the soil layer down to 5 cm depth: The activity level of PCB 52 at approximately 35 Bq x g^{-1} AFS was 10% that of the litter, for BaP the soil activity was equivalent to 1% of that of the litter.

Assuming that the recovered ^{14}C still represents the original substances rather than metabolites formed in the meantime, the activity levels can be transformed into substance concentrations using the specific activity of the substances applied, yielding the values shown in Figure 9 and Figure 10 for BaP and PCB 52, respectively.

The initial levels for BaP were slightly above half that of the target concentrations (approximately 57 mg x kg^{-1} AFS litter or 55 mg x kg^{-1} total weight, instead of 100 mg x kg^{-11}). The extremely high standard deviations of the data were not only seen between samples of different litter batches, but also between individual stalks from a single sample. This was no longer the case after 9 months, and the deviations for the exposed litter shown in Figure 9 represent the differences between the ^{14}C-BaP levels of the three containers per Mitscherlich vessel. The litter samples from a given decomposition container were at this time relatively homogenous.

The BaP levels were higher after the litter had been exposed than at the beginning of the experiment. However, this means that unlike in the litter decomposition studies conducted in the RefB area with "cold" substances" (see chapter 3.1.1, Figure 5 and Figure 6), the BaP in the Mitscherlich vessel remained and accumulated in the litter during the decomposition process.

Underneath the litter containers, a significant increase in BaP levels was only observed in the top soil layer (approximately 3.1 BaP x kg^{-1} AFS soil, or 0.26 mg x kg^{-1} total soil DW). In the soil layer underneath, down to 5 cm, the levels were at 0.62 mg x kg^{-1} AFS or 0.04 mg x kg^{-1} total soil DW, representing a negligible increase compared to the original levels of the RefB soil (see, chapter 2.2.2, p. 52).

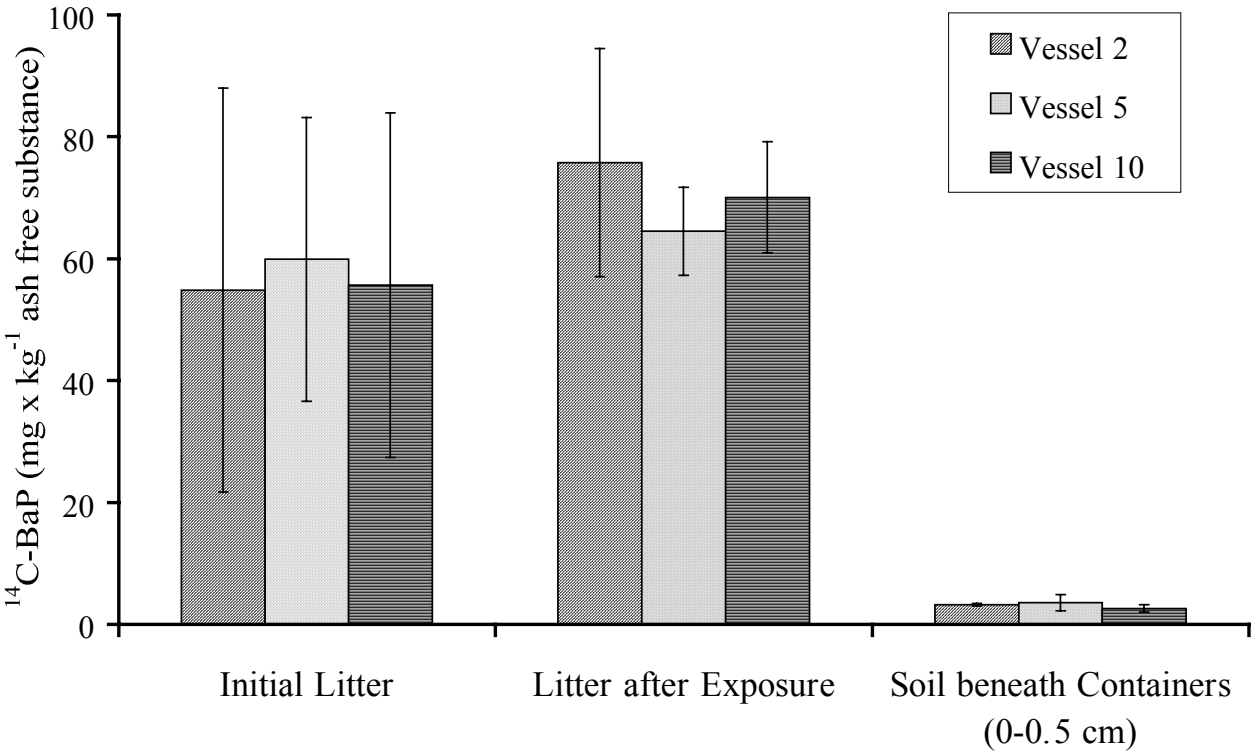


Figure 9: ^{14}C-BaP levels in the initial litter and in litter and soil after 9 months of exposure. Shown are the mean values and standard deviations for the 3 containers set out on the surface of each Mitscherlich vessel.

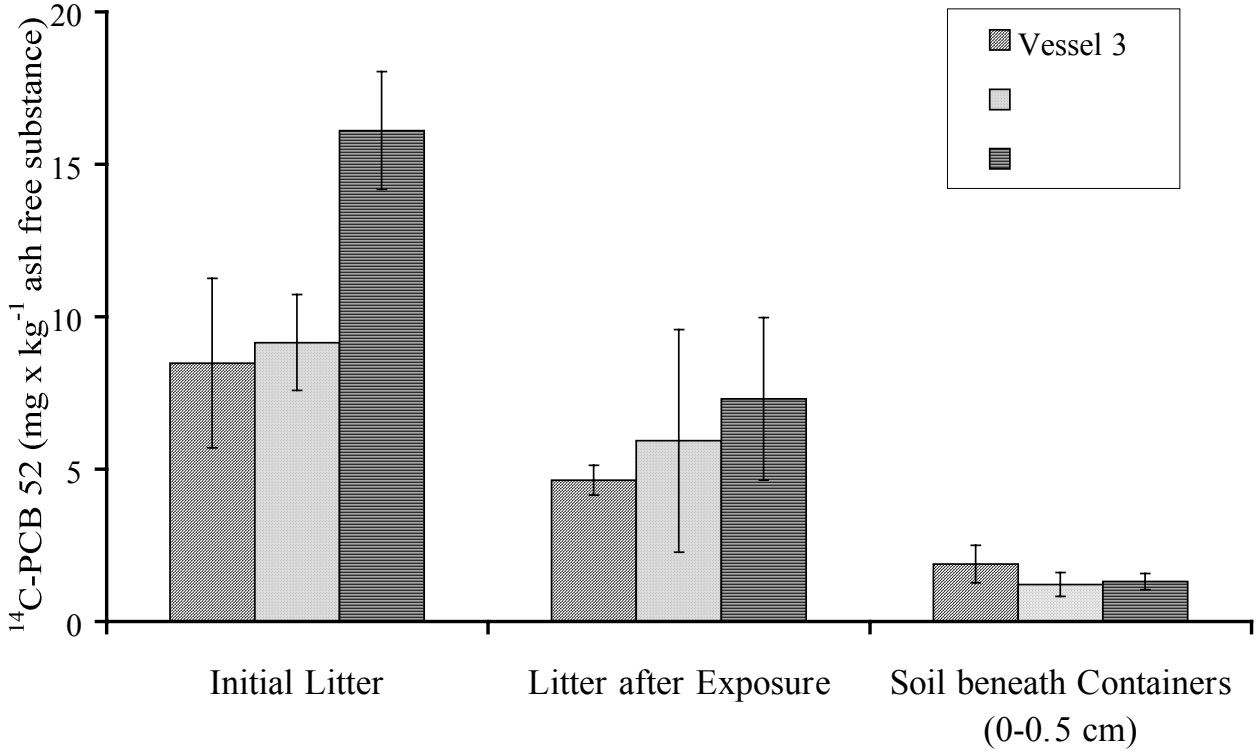


Figure 10: ^{14}C-PCB 52 levels in the initial litter and in litter and soil after 9 months of exposure. Shown are the mean values and standard deviations for the 3 containers set out on the surface of each Mitscherlich vessel.

The 3 Mitscherlich vessels of the PCB variant differed strongly in their initial spiked load (see Figure 10): For 2 of these vessels the levels were around 9 mg x kg^{-1} AFS, but for the third one the initial concentration was 17 mg x kg^{-1} AFS. After the litter had been exposed, these differences were no longer quite as pronounced, but a trend for differences in concentration corresponding to the initial levels prevailed. The mean level of PCB 52 in the litter after exposure was 6 mg x kg^{-1} AFS, in the soil immediately beneath the decomposition containers it was 1.5 mg x kg^{-1} AFS soil or 0.12 mg x kg^{-1} of total soil DW. The substance levels in the layer underneath (5 cm depth) were of the same order of magnitude as those of ^{14}C-BaP in the soil of the BaP variant, in the case of PCB 52 this being twice as high as the original levels in the RefB soil (0.59 mg x kg^{-1} AFS or 0.03 mg x kg-1 of total soil DW).

Thin Layer Chromatography

Some of the litter that had been exposed for 9 months in the Mitscherlich vessels was extracted using the same method that had been applied for the non-labeled BaP and PCB 52 samples from the field experiments on sewage field area RefB.

The distribution of the activity across the different extraction phases of ^{14}C-BaP and ^{14}C-PCB 52 contaminated litter is shown in Table 11.

The coefficients of variation shown in Table 11 overall are rather large, they refer, however, to differences between the individual batches of litter from different containers, not to the parallel extractions for a given litter batch. The total recovery, i.e., the sum of all activity recovered from the different phases, was very close to the amount of activity recovered when non-extracted litter was combusted in the oxidizer.

Table 11: Distribution (%) of activity between the different extraction phases after extraction according to the VDLUFA protocol (1996). The activity remaining in the extraction flask is included in the rinse. Shown are the mean values from 9 litter samples from different containers and their standard deviation.

	Distribution of Activity (%)			
Litter spiked with	Solid Phase	Organic Phase	Aqueous Phase	Rinse
^{14}C-BaP	47.6 ± 13.3	43.1 ± 17.5	2.7 ± 19.6	6.6 ± 38.1
^{14}C-PCB 52	32.7 ± 16.5	58.7 ± 9.9	1.4 ± 44.9	7.2 ± 53.1

Based on the total recovery, 15% more activity was extracted into the organic phase for ^{14}C-PCB 52 than for ^{14}C-BaP. In contrast, there are hardly any differences between the amounts of activity recovered in the aqueous phases for the different contamination variants.

Almost 50% of the activity in the BaP and 33% of the activity in the PCB variant could not be extracted from the litter (solid phase) into the organic phase. According to general definitions, these parts constitute therefore the amounts of "bound residues" as detected by this extraction method.

The results of the thin layer chromatography analyses of the organic extracts of both variants are presented in Table 12. Listed are the percentages of the total activity recovered in the organic phase that could be assigned to metabolites formed during the exposure of the litter in the Mitscherlich vessels or, respectively, to extractable parent compounds.

In the BaP treatment, 60% of the ^{14}C activity detected were non-metabolized BaP and 28% were metabolites that did not migrate with the solvent and remained at the plate origin (see Figure 11). Comparing the organic phase of the litter extracts from the different variants, 12% more ^{14}C activity was recovered as unchanged PCB 52 than it was the case for BaP.

If the outcome of the "cold" extraction adjusted according to the chromatography results, the amount of ^{14}C BaP extracted as parent compound is 25.8% of the total ^{14}C activity detected in the litter (43.1% x 60.0%, see Table 11 and Table 12).

In contrast, ^{14}C PCB 52 accounts for 42.4% of the total radioactivity measured in the litter of the PCB variants (58.% x 72.3%).

Table 12: Characterization of the ^{14}C activity recovered in the organic phase of the extractions. Shown are means of 6 samples and their standard deviation.

	Distribution of Activity (% of ^{14}C extracted in the organic phase)	
Litter spiked with	^{14}C as Metabolite Compounds (Origin)	^{14}C as Parent Compounds (Rf-value ≅ 0.78)
^{14}C-BaP	27.7 ± 3.5	60.0 ± 5.9
^{14}C-PCB 52	12.1± 3.0	72.3 ± 1.9

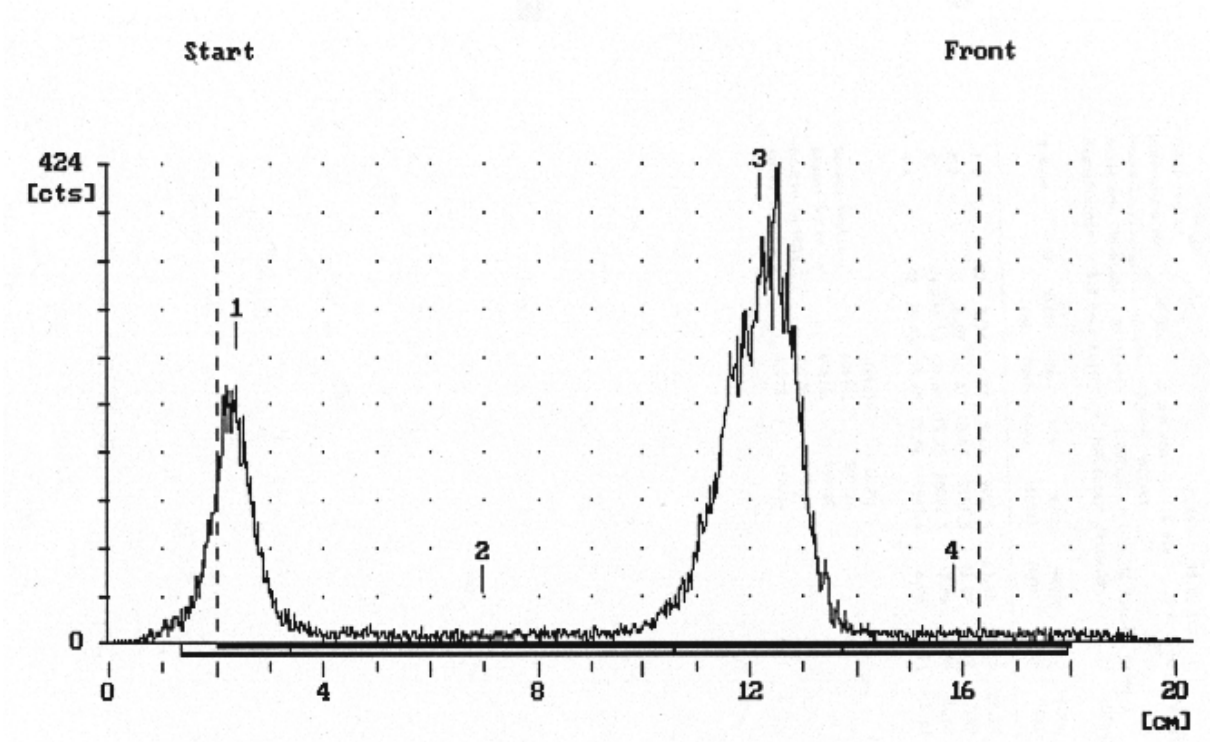


Figure 11: Radiodensitometric scan of TLC separations of the organic phase of the litter extraction. Shown is by way of example one single extract of the litter spiked with ^{14}C-BaP.

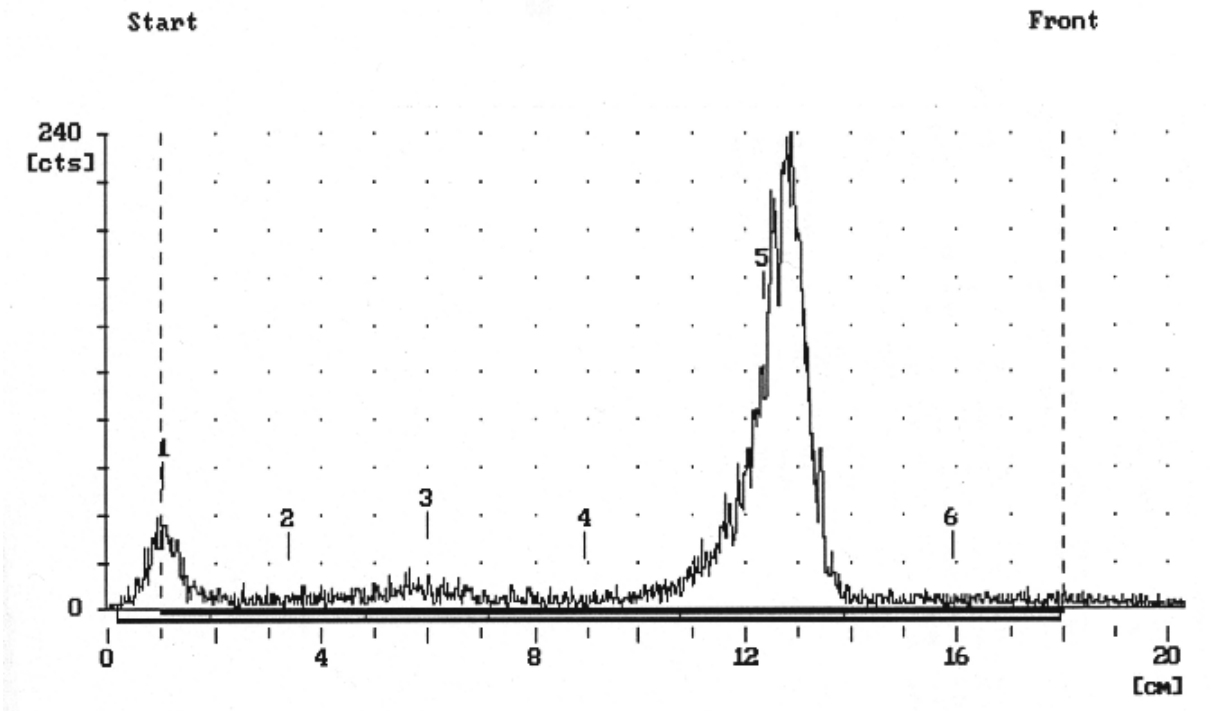


Figure 12: Radiodensitometric scan of TLC separations of the organic phase of the litter extraction. Shown is by way of example a single extract of the litter spiked with ^{14}C-PCB 52.

3.2 Decomposition of *Agropyron repens* Litter in the Field Experiment

3.2.1 Uncontaminated Litter

After 1 year, approximately 50% (by weight) of the non-contaminated litter was decomposed in the containers lined with the coarse gauze (10 mm, GG). Litter treatment with acetone did not have any statistical significant effects on litter decay (see Table 13), although the litter weight in the acetone controls (KL, see Figure 13) was slightly lower than in the uncontaminated controls.

During the dry summer period (2nd sampling date in July and 3rd sampling date in August), the decomposition process in the containers with the coarse gauze slowed down. This could not be seen so clearly in the containers lined with medium gauze (MG).

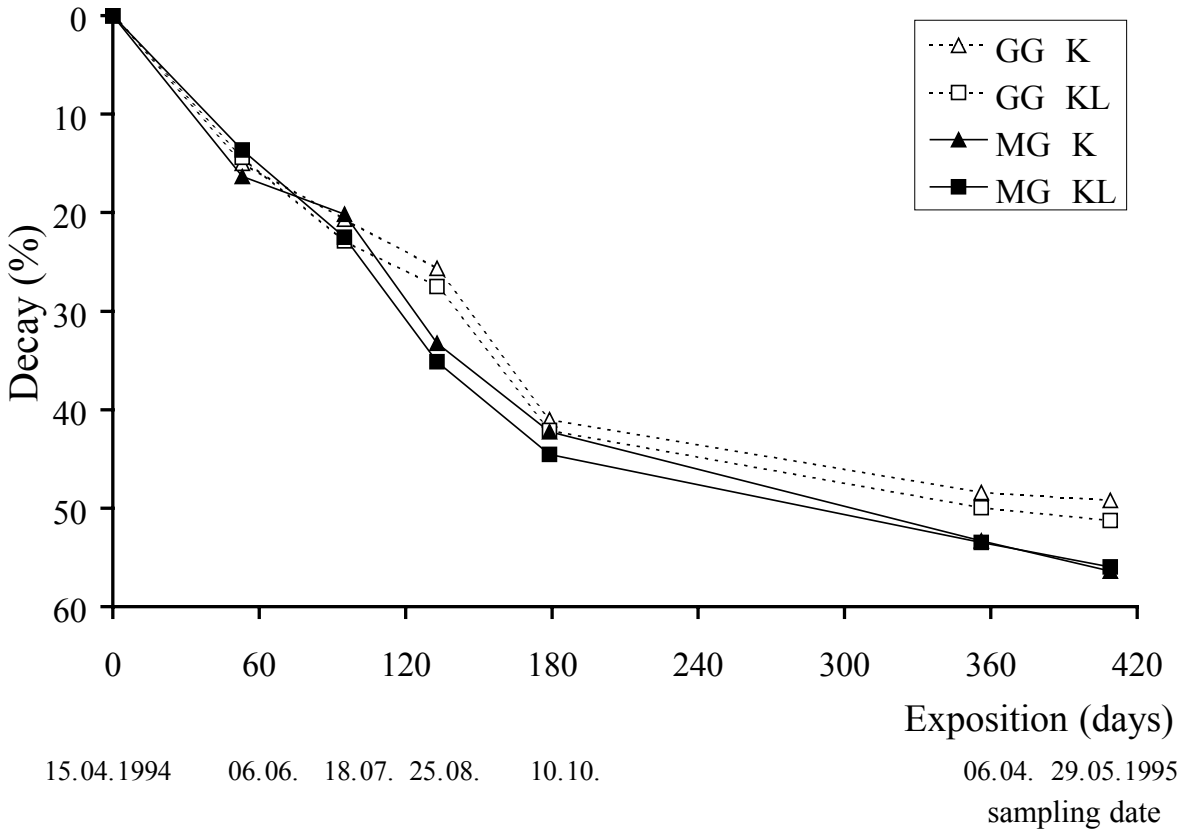


Figure 13: Decomposition of *A. repens* litter in the litter composition containers of variants K (control) and KL (control with solvent) in percent during the exposure from April 15, 1994, until May 29, 1995. MG medium gauze (1 mm)
GG coarse gauze (10 mm)

The litter decomposition processes in the medium and coarse gauze containers, while similar, were influenced by the mesh size. As shown in Table 13, the results of the analysis of variance calculated with the entire data set from the decomposition containers with untreated litter, point out the gauze mesh size as the only factor with a statistically significant effect on litter decomposition ("gauze", $p<0.001$), next to time (covariate "sampling date"). Neither the acetone treatment of the litter nor the experimental field block from which the containers were removed accounted significantly for a part of the data variability.

At the sampling dates 3, 5, and 6 the control containers with medium gauze exhibited a significantly higher decomposition rate than the respective containers with coarse gauze (one way ANOVA, $p \leq 0.05$). This result was not true to expectations, considering that the coarse gauze allows for a higher loss rate of litter fragments from the containers as well as a free access to the litter container by macro-arthropods, which could enhance litter decomposition.

At these sampling dates, the water content of the litter in the medium gauze containers was significantly higher than that in the coarse gauze containers (data not shown).

Table 13: Result of the analysis of variance for the decomposition of *A. repens* litter in the litter composition containers of variants K (control) and KL (control with solvent)
Factors included: Duration of exposure (days, sampling date), experimental plot (block), gauze mesh size (gauze) and solvent treatment (acetone).

	SQ	DF	MQ	F	sign. of F
Covariate	1.189	1	1.189	851.191	<0.001
Sampling date	1.189	1	1.189	851.191	<0.001
Model	0.020	3	0.007	4.864	0.003
Block	0.000	1	0.000	0.051	0.822
Gauze	0.018	1	0.018	12.994	<0.001
Acetone	0.002	1	0.002	1.547	0.216
Explained	1.210	4	0.302	216.446	<0.001
Rest	0.170	122	0.001		
Total	1.380	126	0.011		

SQ sum squared; DF degrees of freedom; MQ mean square sum

3.2.2 Effect of Contaminants on Litter Decomposition

The decomposition rates of the litter spiked with PCB 52 did not differ from the controls at any of the sampling dates. This was true for the coarse gauze containers (see Figure 14) as well as for the medium gauze containers (data not shown).

The decay curves of litter contaminated with PCB 52 progressed remarkably close to the control litter ones, including the stagnation of the decomposition in the dry summer months. With PCB 52 at the applied concentrations having no detectable effects on litter decomposition, these results show a good correspondence for the data from different field experimental plots on RefB area.

In contrast to the contamination of litter with PCB 52, the effect of the BaP litter contamination on the decomposition was pronounced (see Figure 15 following page, Table 14 and Table 15). In the higher concentration variant (BaP 2), faster decomposition was seen as early as 53 days after the litter was exposed in the coarse gauze containers.

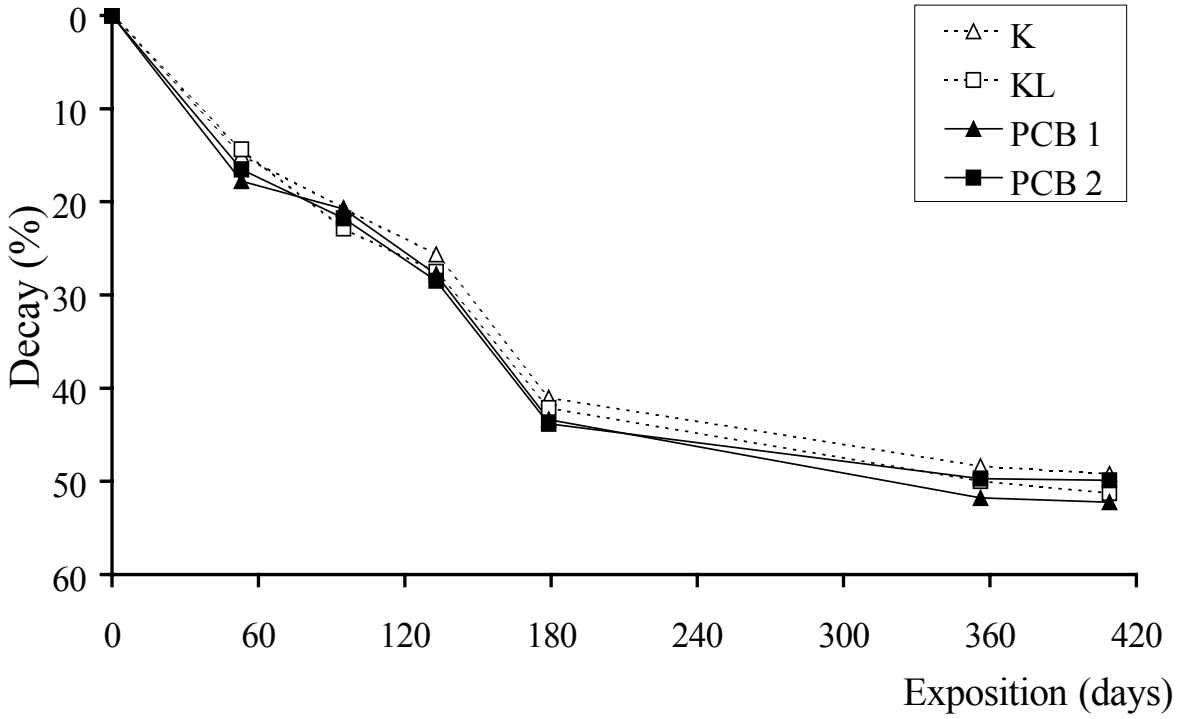


Figure 14: Decomposition in percent of the *A. repens* litter in the control containers (K, KL) and in the PCB containers: PCB 1= 4 mg PCB 52 x kg^{-1} litter
PCB 2= 40 mg PCB 52 x kg-1 litter

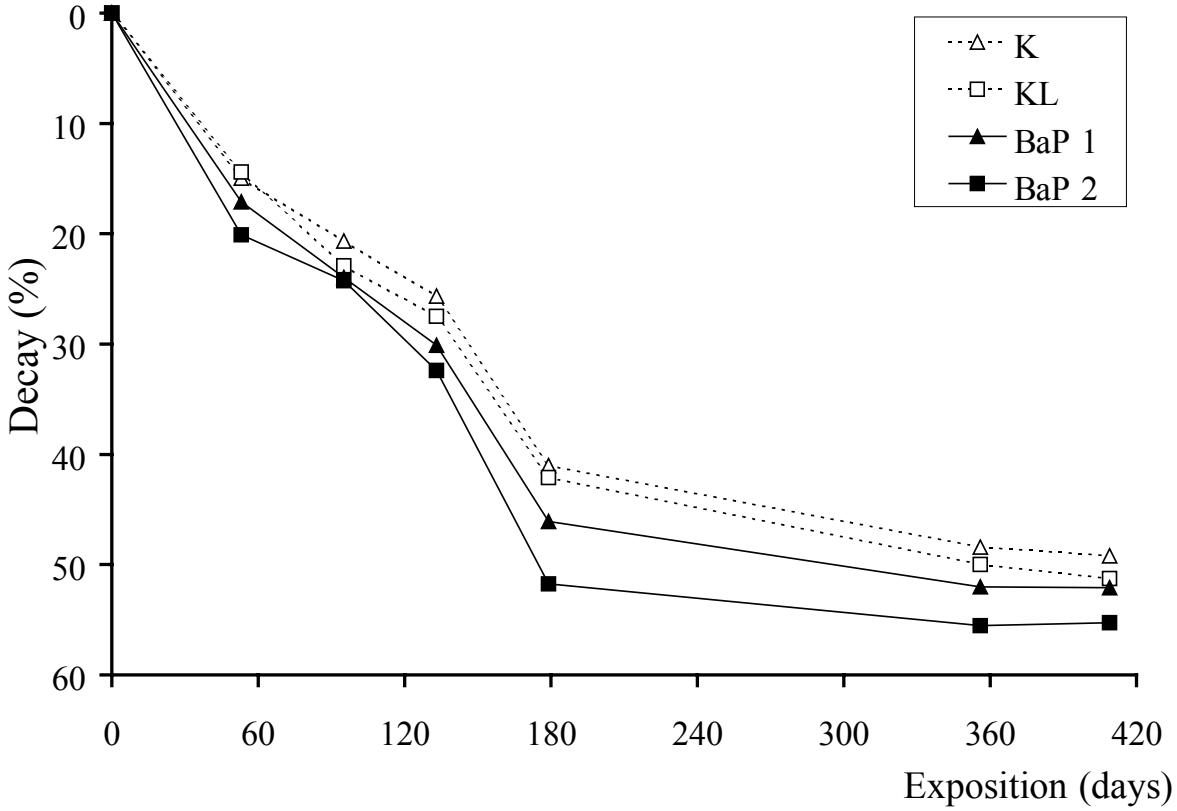


Figure 15: Decomposition in percent (coarse gauze) of the *A. repens* litter in the control containers (K, KL) and in the BaP containers: BaP 1= 10 mg BaP x kg^{-1} litter
BaP 2 =100 mg BaP x kg-1 litter

The analysis of variance emphasizes the impact of the BaP contamination on the decomposition process (see Table 14 and Table 15). After quantification of the effect of the sampling date via covariant analysis (regression calculation) the effect of BaP on litter decomposition was highly significant, independently of the plot position in the field. Interactions between the factors “BaP contamination” and “block” were not significant and were not included in the final analysis.

Because the variability of the data from the medium gauze containers was more pronounced than that of the coarse gauze containers, and because the former containers had been more severely damaged by mice (potential source of error) only the litter decomposition data from the coarse gauze containers were analyzed in more detail.

For the decomposition data from the coarse gauze containers significant differences (one way ANOVA, $p \leq 0.05$) were found between the variants BaP 2 and KL for the 1^{st}, 4^{th}, 5^{th}, and 6^{th} sampling date. Compared to the control, significant differences were observed from the 3^{rd} sampling date on until the end of the experiment. Starting at the 4^{th} sampling date, the variant BaP 2 also exhibited higher decomposition rates than the variant BaP 1 (see Figure 15).

Table 14: Result of the analysis of variance for the decomposition of *A. repens* litter in coarse gauze containers.
Variants included: Control (K), Control with solvent (KL), BaP 1, and BaP 2. Factors included: Duration of exposure (days, sampling date), experimental plot (block) and chemical treatment (BaP)

	SQ	DF	MQ	F	Sign. of F
Covariate	0.982	1	0.982	566.611	<0.001
Sampling date	0.982	1	0.982	566.611	<0.001
Model	0.050	3	0.017	9.627	<0.001
Block	0.000	1	0.000	0.098	0.754
BaP	0.050	2	0.025	14.392	<0.001
Explained	1.032	4	0.258	148.873	<0.001
Rest	0.206	119	0.002		
Total	1.238	123	0.010		

SQ sum squared; DF degrees of freedom; MQ mean square sum

Table 15: Result of the analysis of variance for the decomposition of *A. repens* litter in medium gauze containers. Variants included: Control (K), Control with solvent (KL), BaP 1, and BaP 2. Factors included: Duration of exposure (days, sampling date), experimental plot (block) and chemical treatment (BaP)

	SQ	DF	MQ	F	sign.of F
Covariate	1.293	1	1.293	488.893	<0.001
Sampling date	1.293	1	1.293	488.893	<0.001
Model	0.030	3	0.010	3.732	0.013
Block	0.001	1	0.001	0.511	0.476
BaP	0.028	2	0.014	5.343	0.006
Explained	1.323	4	0.331	125.022	<0.001
Rest	0.310	117	0.003		
Total	1.633	121	0.013		

SQ sum squared; DF degrees of freedom; MQ mean square sum

The difference in the amount of remaining litter between the highly contaminated litter and the control litter after 400 days was approximately 12%.

The decomposition rates of the lower concentration BaP variant (BaP1) were always higher than those of the control, but only at the 4th sampling date statistically significantly so.

The difference between point contamination (spiked litter, K + BaP 1) and area contamination (litter as well as soil cover contaminated, BaP 1) could not be quantified (see section 2.2.1, p. 50). In a direct comparison of the variants, only the effect of the high BaP concentration (BaP 2) on litter decomposition was statistically significant. Regardless whether soil and litter (BaP 1) or only the litter (K + BaP 1) had been spiked, statistically speaking the litter was decomposed identically in both variants.

Variability Between the Field Plots

If the decomposition data from the coarse gauze containers are divided into 2 sets (blocks) according to the experimental plot setup in area RefB, at the beginning of the experiments the differences between K/KL and BaP 2 were only significant at the $\alpha = 10\%$ level. This is attributable to a high degree of variability among the areas, but also to the lower sample size. The longer the experiment lasted, the closer the values obtained from the two areas used for the same variant became. At the first sampling date the probability p for the data from both areas used for the same variant being from the same statistical population was $p = 0.12$. A year later it had increased to $0.56 \leq p \leq 1.00$. Therefore, the litter mass loss data for the first sampling date were not included in the analyses of variance, since data sets with inhomogeneous variances do not meet the ANOVA statistical requirements.

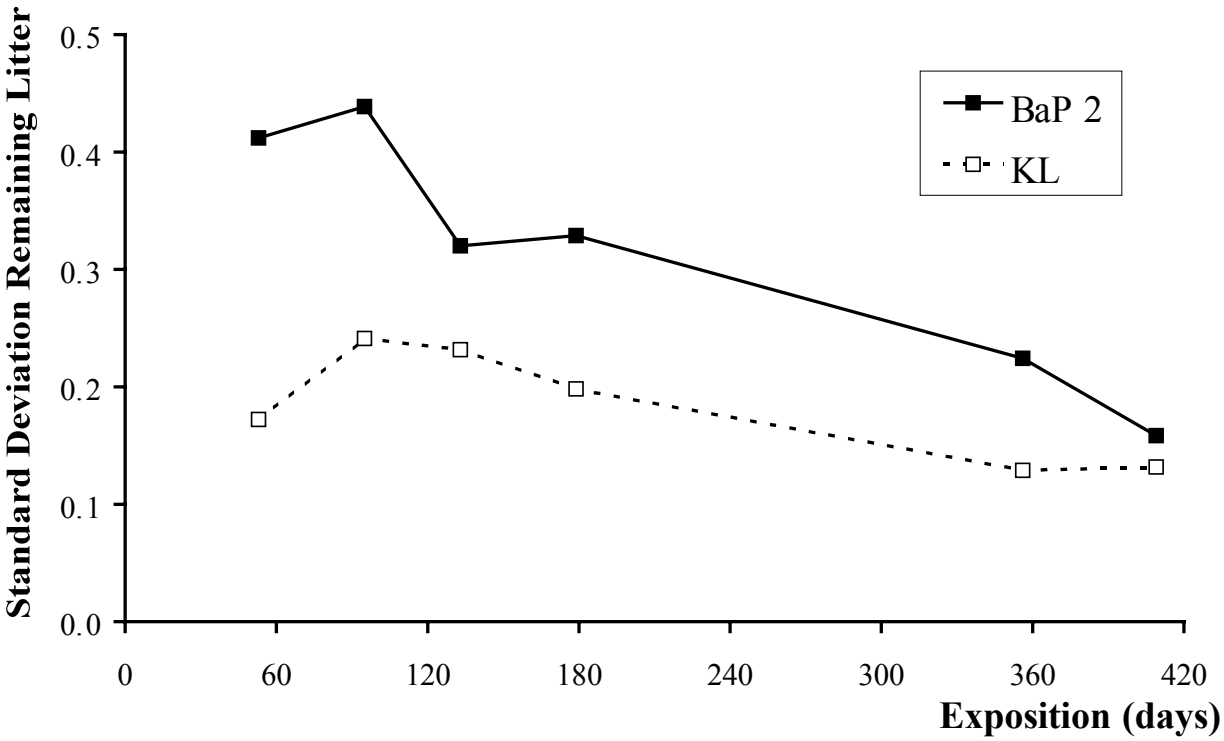


Figure 16: Changes in the standard deviation of the remaining litter data (g) in the course of the experiment for the variants control with solvent (KL) and BaP 2.

Although the variability between the two areas associated with a given variant decreased with time (especially in case of the spiked variant, see Figure 16), and thus the statistical certainty for the difference between the variants increased, the absolute difference between the mean values for the variants BaP 1 and BaP 2 as compared to the controls seemed to become smaller towards the end of the experiment (see Figure 15).

Decomposition Models

If a two-phase linear model is fitted to the data for the variants K and BaP 2 from the litter decomposition containers with coarse gauze (see chapter 2.7.1, Equation 2), the faster decomposition of the spiked litter is apparent from the higher decomposition constant for the first phase (see Table 16). Decomposition of 25% of the initially exposed litter (remaining litter y = 75%) was seen in the control containers after 113 days, and in the BaP 2 containers after 87 days.

During the second, "slower" decomposition phase on the other hand, 75% of the uncontaminated litter were decomposed after 595 days (y = 25%), for the litter spiked with BaP this level was reached after 925 days. This would be the case assuming in both variants 50% of litter remain at the beginning of the second phase. Thus, the mean values of the two variants exhibited increasing differences during the first phase of the process, but as decomposition continued, the absolute values tended to converge again. The smaller decomposition constant that was calculated for the BaP variant during the second phase of the process may continue to lead to an amount of non-decomposable organic matter residues comparable to that in the control litter containers, provided the mineralization of the plant matrix is less than 100%.

Table 16: Linear decomposition models according to Equation 2 (page 61) for the data of the significantly different variants K and BaP2 (100 mg BaP x kg^{-1} litter). Phase 2 began after approximately 200 days.

Variants	First phase of decay ("fast")		Second phase ("slow")	
	Linear model	R^2	Linear model	R^2
K	y = -0.199 x days + 97.4	0.87	y = -0.042 x days + 67.6	0.64
BaP 2	y = -0.262 x days + 97.8	0.87	y = -0.027 x days + 52.9	0.71

y = remaining litter in % of initial weight

In order to be able to compare these results with the data found in the literature, in addition to the linear model a double exponential model was fitted to the decomposition data (Table 17, see also chapter 2.7.1, Equation 1).

With this model, decomposition constants were calculated as weight loss per day per remaining litter, and the initial steep slope in the curve represents the fast decay of readily decomposable litter components. Additionally, a second pool of slow decaying organic matter is included in the calculation from the beginning, allowing for a long decomposition process of recalcitrant litter remains. With a single exponential model, very slow decomposing components are generally integrated in the models with a constant term.

As shown in Figure 17, fitting the model to the decomposition data of the non-contaminated litter (K), results in the estimate of two decomposition constants associated with two pools of litter components. The second decomposition constant in the model is very small, but determines the decay of a large part of the uncontaminated grass litter (50%).

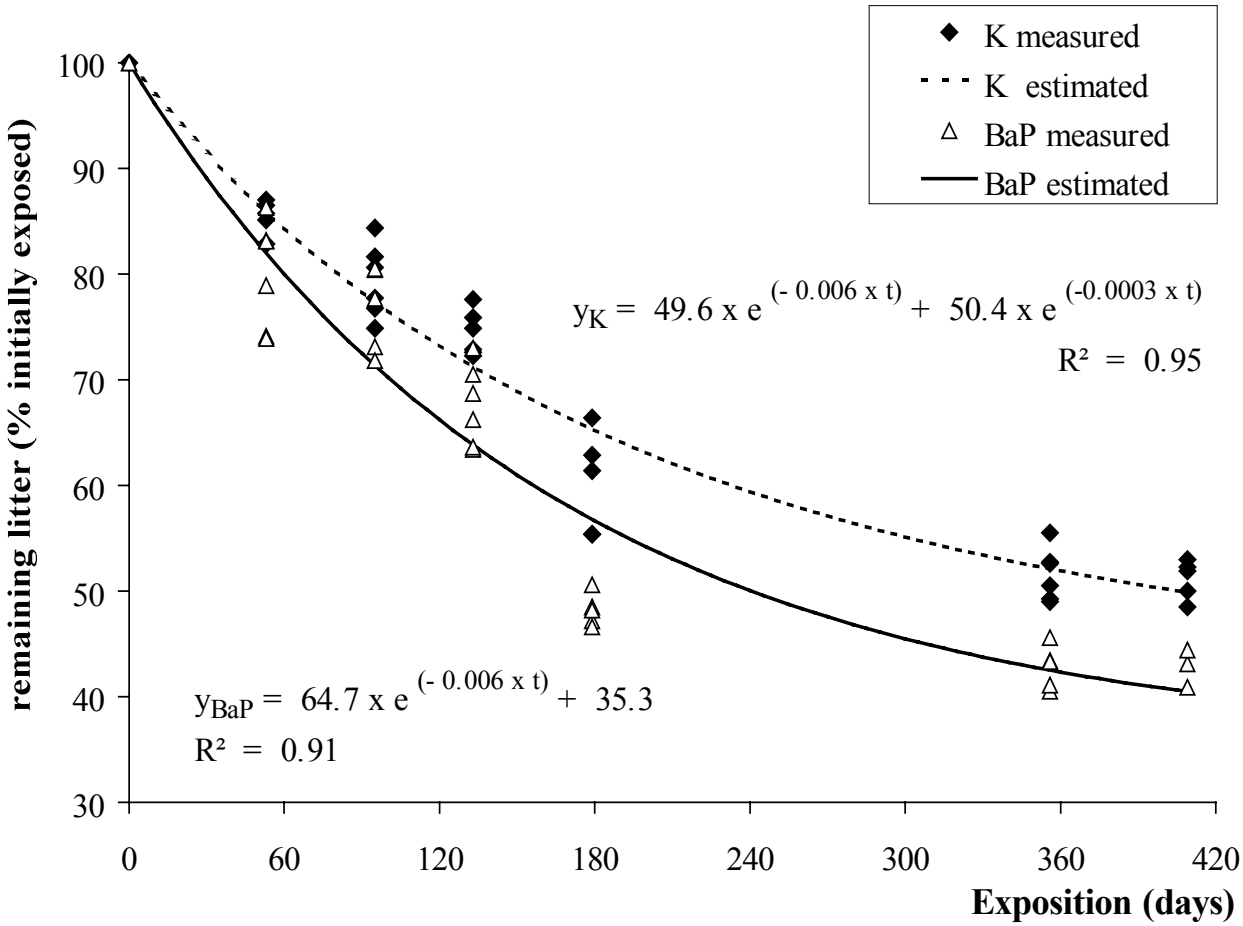


Figure 17: Exponential decomposition models fitted to the litter weight values obtained during exposure in the field (equation 1, page 61). The weight of the remaining litter is given as % of the initial weight. y_K = remaining litter of the control variant at time t; y_{BaP} = remaining litter of the spiked variant BaP 2 at time t

By contrast, the best goodness of fit for the decomposition data of the contaminated litter (BaP 2) resulted from calculations that did not include a second decomposition constant in the model (estimated as "0"). According to the equation, 65% of the contaminated litter decomposed exponentially with a decomposition rate similar to that of the non-contaminated litter (k = 0.006). In the control variants, however, only 50% of the litter was associated with this faster decay constant.

In spite of the high coefficient of determination, however, exponential fitting of the data could not reflect the slower decomposition in the dry summer months, which occurred during a phase of the process in general associated with high mass loss. Excluding the data from these extremely dry sampling dates, the convergence of the mean values of the variants towards the end of the experiment could be modeled fairly well (see Figure 18).

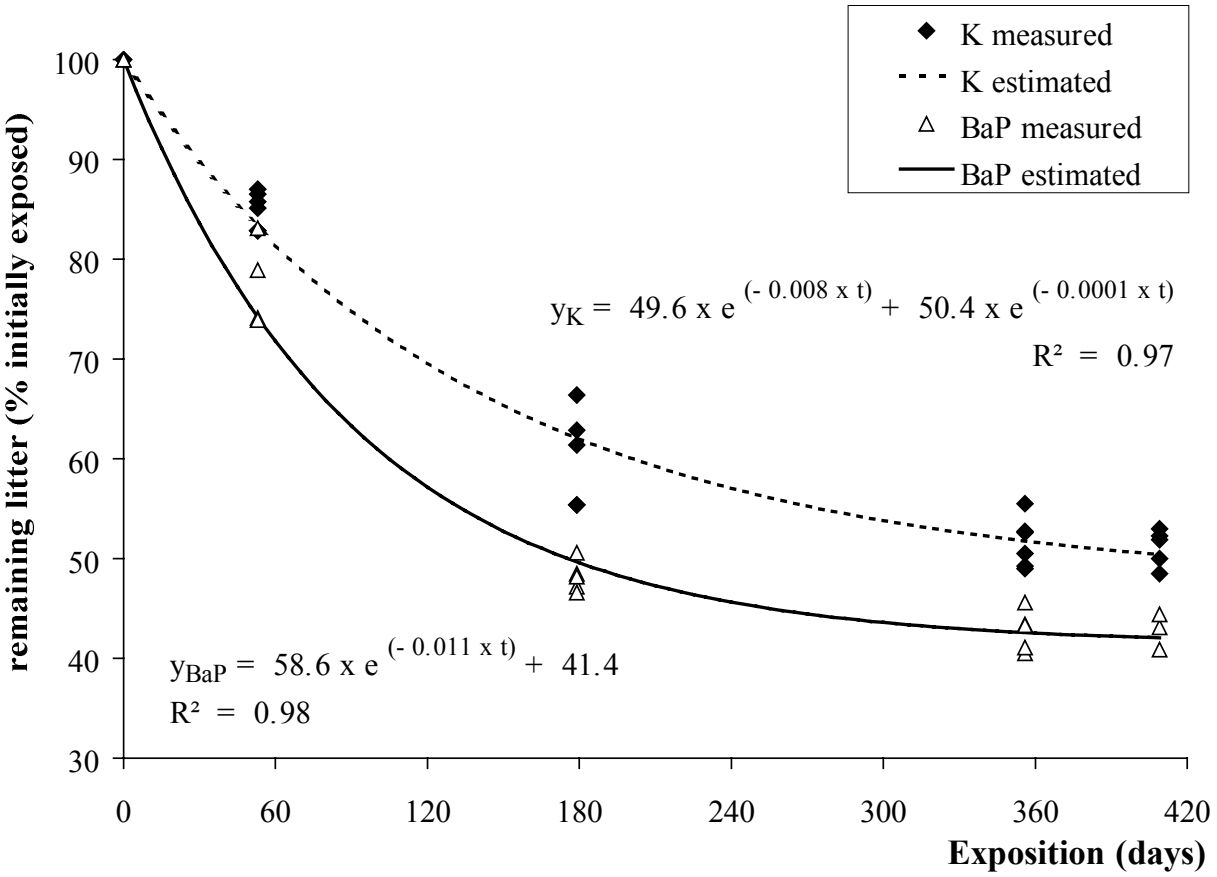


Figure 18: Exponential decomposition model fitted to the litter weight values obtained during exposure in the field, excluding the 2nd and 3rd sampling date (equation 1, page 61). The weight of the remaining litter is given as % of the initial weight. y_K = remaining litter of the control variant at time t; y_{BaP} = remaining litter of the spiked variant BaP 2 at time t

Table 17: Exponential decomposition models according to Equation 1, page 61, for the data of the significantly different variants K and BaP 2 (100 mg BaP x kg^{-1} litter)

Variants	Exponential Model	R^2
K	$y_K = 49.6 \times e^{(-0.008 \times days)} + 50.4 \times e^{(-0.0001 \times days)}$	0.97
BaP 2	$y_{BaP} = 58.6 \times e^{(-0.011 \times days)} + 41.4$	0.98

y = remaining litter as % of initial weight

The differences in weight remaining between control litter (K) and spiked litter (BaP 2) over the decomposition experiment are plotted in Figure 19. The estimated curve was calculated by means of the decomposition model parameter shown in Table 17 and Figure 18.

To demonstrate the climatic constraint on the decay process of the *A. repens* litter given in the early summer month, the decomposition constants for control litter K and spiked litter BaP 2 are related to the rainfall amount fallen from April to early September 1995. The picture shows the constantly higher decay rates of the litter contaminated with BaP in contrast to the control in the first phase of the decomposition process, the retardation of the overall grass decomposition rates in summer, and the increase of litter loss rates 5 months later at the onset of the rain period in fall (Figure 20, next page).

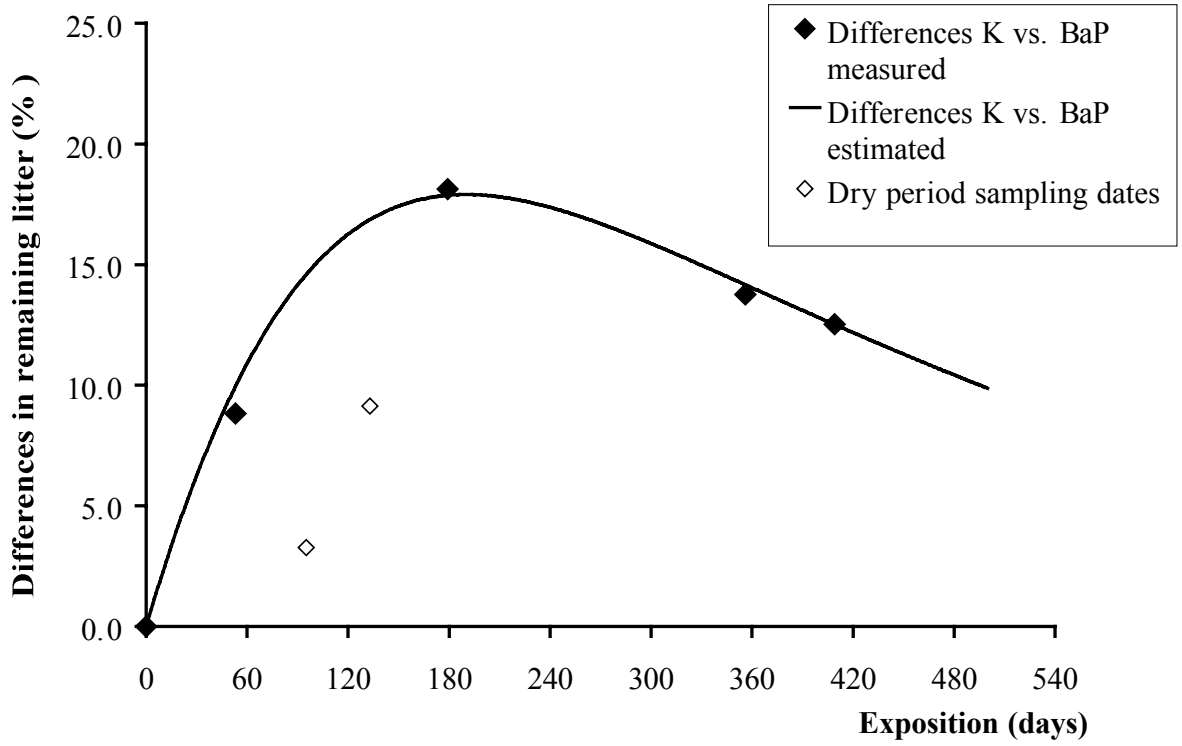


Figure 19: Mean differences in % litter remaining between highly contaminated variant (BaP 2) and control (K).

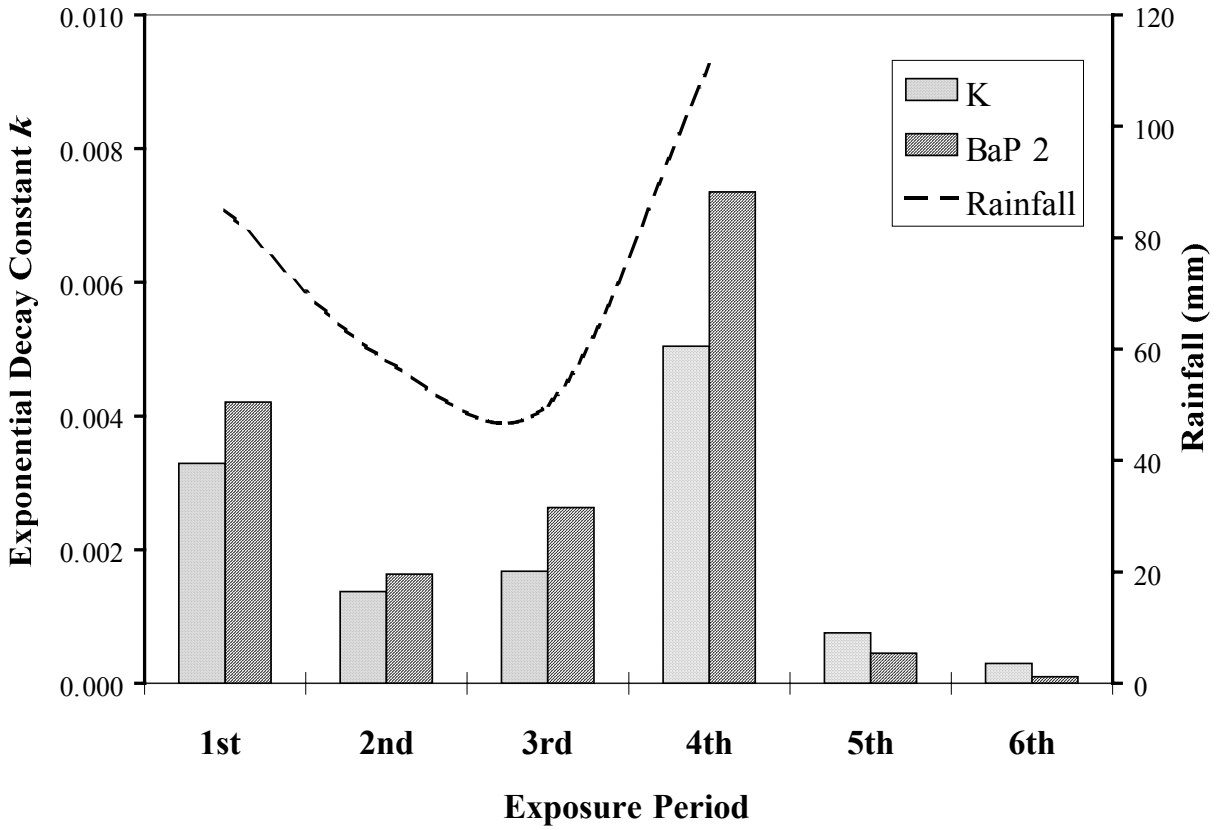


Figure 20: Changes in the single exponential decay constants for the control and the highly contaminated variant BaP 2 as related to the rainfall in the summer period (April till September) in Berlin Buch. Climatic data from BOWO (1997).

Multiple Regressions

The effect of BaP on the complex process of litter decomposition may be masked by other factors, especially when studies are conducted in the field. Therefore, multiple regression analysis was done for the medium gauze and coarse gauze containers, in order to uncover additional influence factors and to quantify their effect on the decomposition process of *A. repens* litter (see Table 18 and Table 19).

In the coarse gauze containers, the BaP contamination of the litter was found to be the parameter that could explain most of the variability in the decomposition data at all sampling dates. The coefficients of correlation and of determination increased over the course of the experiments and reached maximum values of 0.84 and 0.71, respectively, at the last sampling date. This fact certainly is attributable to the observed decrease in area variability that may have masked the effect of BaP. In some cases, the sample water content and the acetone treatment were figured into the calculations, but they never contributed to the increase of the coefficient of determination with more than 0.2.

Table 18: Multiple regression analyses for the coarse gauze containers (GG). Listed are parameters showing an effect on the variability of the decomposition data, multiple R, multiple R^2, and level of significance.
Independent variables: BaP concentration (BaP), water content at beginning of sampling (%W), acetone concentration (AC) and degree of damage to containers (M)

Sampling date		Parameter	Multiple R	Multiple R^2	Signif.
1.	53 days	BaP	0.55	0.31	0.004
		BaP + %W	0.71	0.50	0.009
2.	95 days	AC	0.47	0.23	0.032
3.	133 days	BaP	0.53	0.29	0.007
		BaP + AC	0.63	0.40	0.051
4.	179 days	BaP	0.76	0.58	0.000
		BaP + AC	0.82	0.68	0.032
5.	356 days	BaP	0.78	0.62	0.000
		BaP + %W	0.87	0.76	0.007
6.	409 days	BaP	0.84	0.71	0.000

In the medium gauze containers, on the other hand, the BaP variable rarely was the one included first in the stepwise regression. Here, the damage to the containers caused by mice at the beginning of the experiments exhibited a greater effect than in the calculations for the coarse gauze containers and resulted in obvious litter weight losses.

The water content of the litter at the harvesting date contributed to an increased coefficient of determination in a manner similar to that of the coarse gauze calculations. The great variability of the medium gauze containers was not sufficiently explained by the selected parameters: Especially the calculations for the data obtained at later sampling dates have very low R^2 values.

Table 19: Multiple regression analyses for the medium gauze containers (MG). Listed are parameters showing an effect on the variability of the decomposition data, multiple R, multiple R^2, and level of significance. Independent variables: BaP concentration (BaP), water content at beginning of sampling (%W), acetone concentration (AC) and degree of damage to containers (M)

Sampling date		Parameter	Multiple R	Multiple R^2	Signif.
1.	53 days	M	0.61	0.37	0.005
		M + %W	0.75	0.56	0.019
		M + %W + BaP	0.80	0.64	0.009
		M + %W + BaP + AC	0.86	0.75	0.025
2.	95 days	BaP	0.57	0.34	0.000
		BaP +%W	0.69	0.48	0.013
		BaP + %W + M	0.78	0.61	0.039
3.	133 days	%W	0.54	0.29	0.036
		%W + M	0.65	0.42	0.049
4.	179 days	BaP	0.61	0.38	0.002
		BaP + %W	0.72	0.52	0.026
5.	356 days	M	0.39	0.36	0.004
6.	409 days	%W	0.42	0.19	0.059

C/N Ratio of the Litter

At the beginning of the experiment the C/N ratio of the exposed litter was relatively wide (C/N ≅ 43, see Table 20). It is possible that in addition to an early nutrient shift inside the plants toward the subsurface parts (HAHN *et al.*, 1979) leaching may have occurred in the dead stalks above ground before the harvesting.

The C/N ratio decreased as N concentrations in the litter increased during the exposure (see Figure 21). The courses of development of N concentrations and C/N ratios for the variants control (K), litter treated with solvent (KL), and litter spiked with PCB 52 resembled each other. However, the litter from the higher concentration BaP variant (BaP 2) exhibited a higher N content during the first half of the experiment (see Table 20; at both the 2nd and 3rd sampling date, $p \leq 0.05$ vs. K), causing a narrower C/N ratio. At the final sampling date the N concentrations in the litter of the different variants had converged and differences in the C/N ratios were no longer statistically distinguishable.

Table 20: C/N ratio and N concentration in the litter during the first half and towards the end of the experiment. Mean values for 3 samples and the standard deviation are shown.

Sampling date		Untreated Control (K)		Spiked Litter (BaP2)	
		C/N	N%	C/N	N%
Start	-	42.7 ± 3.8	1.17 ± 0.03		
1.	53 days	33.0 ± 6.1	1.51 ± 0.26	26.4 ± 4.1	1.85 ± 0.33
2.	95 days	32.5 ± 4.3	1.51 ± 0.16	26.3 ± 3.9	1.84 ± 0.08
3.	133 days	32.3 ± 1.7	1.47 ± 0.09	27.9 ± 1.2	1.83 ± 0.08
6.	409 days	21.5 ± 2.5	2.23 ± 0.29	19.4 ± 0.3	2.24 ± 0.08

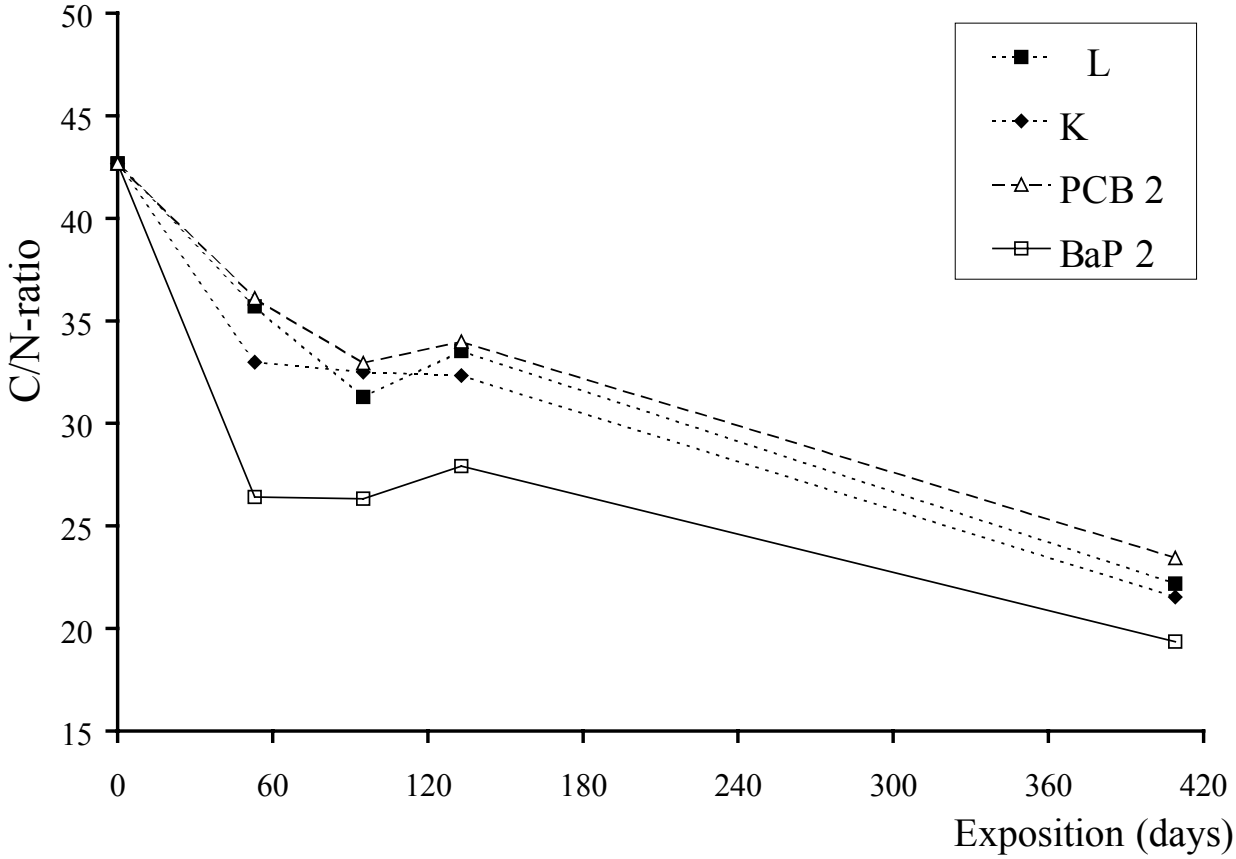


Figure 21: C/N ratio in *A. repens* litter in the course of the experiment

3.3 Impact of PCB 52 and BaP on soil animals

The basic study approach taken for identifying and quantifying the effects of chemical contamination on the edaphon has been described in section 1.2.

The litter decomposition studies yielded an insight into the course this very important soil process may take, and they pointed to discrepancies that may be attributed to the BaP contamination. This part of the study treats the litter layer as a black box, the functions of which are recorded summarily (BECK, 1983).

The analysis of the structure of the soil biocenosis as part of the decomposer community on the other hand is intended to gain some initial insight into the mechanisms of activity of the investigated chemicals. Results showing different microarthropod densities in controls and contaminated litter of the decomposition experiment, however, should be confirmed by laboratory experiments. In addition to the faunistic studies accompanying the litter decomposition experiment on area RefB, laboratory standard tests with Collembola were performed.

To assess the effects of BaP and PCB 52 in soils that have not been spiked recently, several point assessments of the soil fauna in differently contaminated sewage field areas (RefB, nPAK and nPCB) were carried out. These results, not obtained by experimental spiking of soil or litter, are presented in section 3.4.

3.3.1 Laboratory Reproduction Tests with *Folsomia candida*

The reproduction performance of Collembola under chemical stress was assessed in the laboratory by means of a standard ISO test with *Folsomia candida* (Willem, 1902) (see section 2.4, page 57). Toxicological endpoint is the number of juveniles (F1 generation) at the end of a 4-week experiment. Test substrates were uncontaminated and with BaP and PCB 52 spiked soils from the sewage field central study area RefB (see Table 6).

The first run of the laboratory reproduction test with *F. candida* was done 2 years after spiking the soil. Tested variants were the sewage field soil RefB as control, one spiked soil from the BaP variants (“2 BaP”, 10 mg BaP x kg^{-1} soil) and one from the PCB variants ("2 P52“, 2 mg PCB 52 x kg^{-1} soil).

The results of the first reproduction test are shown in Table 21. At the end of the experiment 97.5 ± 5% of the adult Collembola were recovered, satisfying the test requirements for exposure to sublethal levels of the chemicals to be tested. The validity criterion regarding the coefficient of variation of the juvenile number in the control was fulfilled (25%). Variants 2 PCB and 2 BaP had coefficients of variation of 20.1% and 37%, respectively.

Table 21: First reproduction test 2 years after soil spiking. Number of *Folsomia candida* juveniles in the reference control soil and contaminated soils. Shown are the mean values of 5 replicates and the standard deviation

	Soil				
	RefB	2 P52		2 BaP	
Test species	Juveniles	Juveniles	Sign.	Juveniles	Sign.
Folsomia candida	348.8 ± 85.2	642.0 ± 129.2	0.043	620.0 ± 227.9	0.021

Compared to the RefB control, the number of juveniles in the BaP contaminated soil was increased by approximately 78% (p=0.04; U-Test). In the PCB 52 variant, the enhanced reproduction was at 84% even more evident (p=0.02; U-Test).

The second run of the reproduction test with *F. candida* was performed 6 years after spiking of the soil. In this test block, all spiked variants were tested (see Table 6, page 58), including the RefB soil as control and an "internal" control with the artificial soil according to the DIN ISO 11267 guideline (1999).

As in the first test performed, the mortality of the adult Collembola was lower than 20%, even when the highest tested concentrations were 10 times higher. Collembola reproduced well in the RefB soil of the sewage fields and the juvenile numbers after 4 weeks were very similar to the ones in the artificial soil internal control. The coefficient of variation of the juvenile number in the control soils was lower than 15%, with an excellent replicate performance of the RefB soil containers (CV = 1.9%).

The results of the second reproduction test with *F. candida* are listed in Table 22 for the control and the PCB variants, in Table 23 for controls and BaP spiked soils.

Table 22: Second reproduction test 6 years after soil spiking. Number of *Folsomia candida* juveniles in the controls and PCB contaminated soils. Shown are the mean values of 5 replicates and the standard deviation

	Soil									
	artificial soil		RefB		1 P52		2 P52		3 P52	
Test species	Juveniles	Sign.*	Juveniles	Sign.	Juveniles	Sign.	Juveniles	Sign.	Juveniles	Sign.
F. candida	473,8 ± 31,9	**a**	468,6 ± 8,8	**a**	359,4 ± 69,2	**b**	143,4 ± 22,6	**c**	365,6 ± 70,4	**b**

* means followed by different letters are statistic significantly different (ANOVA, p < 0,05)

Table 23: Second reproduction test 6 years after soil spiking. Number of *Folsomia candida* juveniles in the controls and BaP contaminated soils. Shown are the mean values of 5 replicates and the standard deviation.

	Soil									
	Artificial Soil		RefB		1 BaP		2 BaP		3 BaP	
Test species	Juveniles	Sign.	Juveniles	Sign.	Juveniles	Sign.	Juveniles	Sign.	Juveniles	Sign.
F. candida	473.8 ± 31.9	**a**	468.6 ± 8.8	**a**	338.6 ± 30.4	**b**	393.5 ± 20.7	**b**	382.6 ± 14.0	**b**

* Means followed by different letters are statistic significantly different (ANOVA, $p < 0,05$)

There were no difference in the number of juveniles between RefB and artificial soil. The two controls, however, differed from all the spiked soils: Collembola displayed a higher reproduction rate in the uncontaminated soils (DUNNET-C test, $p< 0,05$).

In the PCB variants, the intermediate concentration ("2 P52", Table 22) showed significantly lower reproduction rates than the controls and the other contamination levels. The number of juveniles did not reach half the values of the other soils spiked with PCB 52. Note that the reproduction rate in the variants 1 P52 (0.2 mg x kg^{-1}) and 3 P52 (20 mg x kg^{-1}) are similar.

Increasing levels of BaP in the soil did not affect Collembola 6 years after spiking. The lowest number of juveniles was found in the lowest contamination variant (1 BaP, Table 23). No statistic significant differences could be detected between the BaP variants up to a concentration of 100 mg BaP x kg^{-1} soil (3 BaP). However, all BaP-spiked soil showed lower reproduction rates than the two controls, similar to the soil spiked with PCB 52.

In order to find an explanation for the lower reproduction rates in variants with intermediate chemical loads, the relationship between numbers of juveniles and mean measured pH-values of the different soils was analyzed (Figure 22).

Slightly lower pH-values than the recommended standard pH of 6.0± 0.5 seemed to affect the reproduction performance of *F. candida*. During the experiment, soil pH in all variants decreased in general by 0.2 units (data not shown). Because the sewage soils were not pH-adjusted, only the artificial soil was within the standard boundaries. The low pH of the intermediate contamination variants 2 P52 is surprising, given that the spiked soil originally was RefB soil.

In Figure 22, the lower reproduction rate in the PCB variant with low pH compared to the other PCB spiked soil is evident. Even in the 1 BaP variant, which exhibits slightly lower pH-values than the other BaP spiked soils, juvenile numbers were reduced compared to the 2 BaP or the 3 BaP variant.

The soils of the 1 BaP and 2 P52 contamination variants were the only two accidentally stored over the 6 years at different water contents: If all other variants showed a loss of max. 1% weight when dried at 105°C, the weight loss for 1 BaP and 2 P52 was 2.5 % and 4,2 %, respectively.

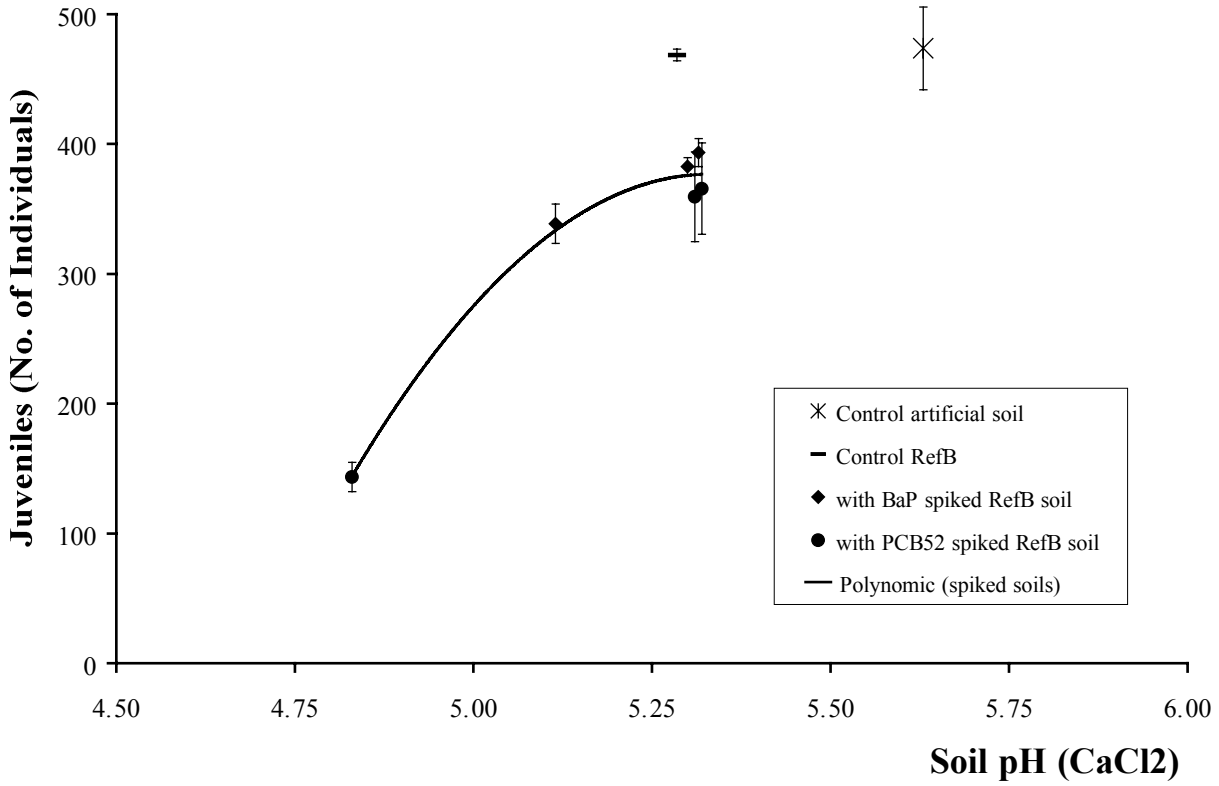


Figure 22: Juvenile numbers as related to the pH-values of the tested soils. Shown are the mean values of 5 replicates and the standard deviations as error bars. A trend curve was plotted through the values of the spiked soils.

3.3.2 Colonization of the Litter Decomposition Containers

As described in section 2.5 (page 58), the colonization densities of microarthropods in the decomposition containers were assessed on the same sampling dates on which the remaining litter weights were determined.

From the Collembola group, representatives of the Entomobryidae, Isotomidae and Sminthuridae families were determined to the species level. The oribatid mite cenosis on the other hand was comprehensively characterized, not only in the decomposition containers but also in the soil samples of areas RefB, nPAK and nPCB (see chapter 2.5).

Table 24 and Table 25 (see below) list the Collembola and oribatid mite species, respectively, which were identified in the litter of the decomposition containers.

Refer to Annex I for an ecological typefication of all Collembola and Oribatida determined based on literature research.

Collembola and Oribatid Mites Species

The Collembola species that were identified in the litter decomposition containers from the field experiment on RefB area are listed in Table 24, along with their constancy.
The sampling dates 1 to 4 were evaluated, representing an investigational period of 6 months. The data regarding species constancy in the samples refer either to the total number of samples (see Table 24, A) or to the samples other than those of the 2nd sampling date which fell into an extremely dry period and offered poor colonization conditions for the Collembola of the litter layer. The constancy was classified according to the percentage of samples in which a given species was present (see 2.7.2, page 63).

Table 24: List of the Collembola species identified and their constancy in the litter samples from the decomposition containers

	% of the samples and constancy*	
Species	A	B
Entomobrya multifasciata (Tullberg, 1871)	92.6% (5)	93.1%(5)
Entomobrya nivalis (Linné, 1758)	-	-
Sminthurus nigromaculatus Tullberg, 1872	62.6% (4)	61.0% (4)
Isotoma notabilis Schäffer, 1896	45.9% (3)	61.2% (4)
Isotoma anglicana Lubbock, 1873	41.0% (3)	50.8% (3)
Lepidocyrtus cyaneus Tullberg, 1871	13.5% (1)	17.5% (1)

*Constancy was calculated A) based on the samples of all sampling dates, and
B) from all samples except those obtained in 2nd sampling date

The oribatid mite species identified in the litter of the decomposition containers are listed in Table 25. The column "occurrence" in Table 25 summarizes the findings of the different oribatid mite species, not only in the litter of the RefB area but also in the soil and in litter of the studied sewage fields areas as follows:

A in all of the investigated sewage field areas

R only in the open, grass grown RefB area

P only in the with poplar covered nPAK area

This typefication recurs also in Table 29, were the oribatid cenoses from the different sewage fields are compared.

Table 25: Consolidated species list and occurrence of oribatids in the litter of the decomposition containers. Constancy classification: from 5 = always; to 1 = very rarely, see 2.7.2, page 63).

Species	Sampling site litter container	Occurrence*
Tectocepheus sarekensis Trägardh, 1910	5	A
Liebstadia similis (Michael, 1888)	3	
Punctoribates punctum (C.L. Koch, 1849)	2	R
Ramusella insculpta (Paoli, 1908)	1	A
*Carabodes labyrinthicu*s (Michael, 1879)	1	R
Ceratocetes mediocris Berlese, 1908	1	R
Oppia nitens (C.L. Koch, 1835)	1	R
Scheloribates laevigatus (C.L. Koch, 1836)	1	R
Metabelba pulverosa Strenzke, 1953	1	

*see text

Colonization Densities in the Control and Spiked Litter

Abundances of Collembola and oribatid mite species in the litter decomposition containers are shown in Table 26. They are sorted by sampling date and experimental variant.

The numbers of individuals found at the first sampling date show that Collembola and Oribatida rapidly colonized the containers (approximately 10000 and 1000 individuals per square meter after 50 days). After the very dry early summer season (2nd sampling date),

oribatid colonization densities reached a similar level as in the spring. Collembola numbers, however, remained mostly below the colonization densities of the first sampling date.

Figure 23 shows that the water content of the litter at sampling date may be responsible for the lower Collembola individual numbers later in the experiment. Such a relationship was not found for individual densities of the Oribatida group.

In comparison to data found in the literature, the abundances in the containers are to be considered rather low. Characteristic values for Collembola and Oribatida individual numbers in the upper horizon of coniferous forests have been reported to be between 50 to 100 x 10^3 x m^{-2} for each group (e.g., PETERSEN & LUXTON, 1982; NÜß, 1994). In cultivated fields, the average density has been reported to drop to 20000 Collembola x m^{-2} with oribatid mites hardly colonizing such disturbed soils (EHRNSBERGER, 1993).

However, it needs to be considered that in the experiments described here the colonization densities are referring only to the litter layer in the decomposition containers, and not, as commonly done, to the organic and upper soil layers of a given site.

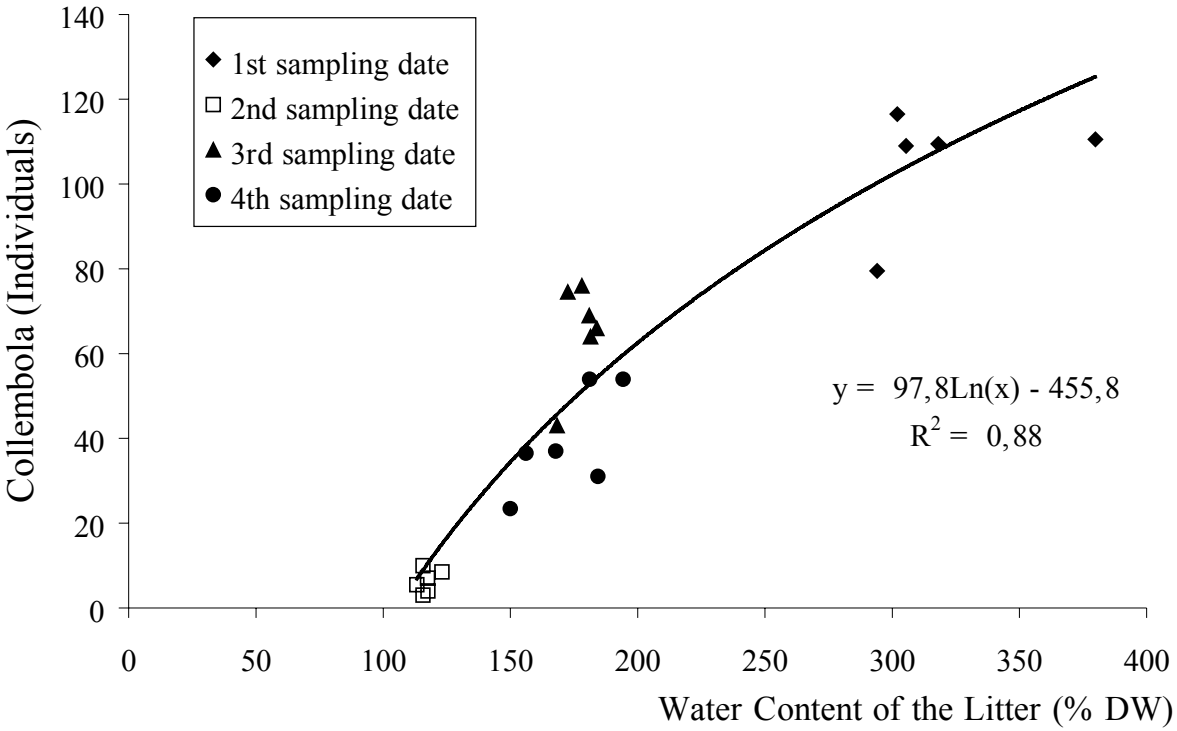


Figure 23: Collembola individual numbers in the litter of the decomposition containers as related to the water content of the litter at sampling date.

The total individual densities of Collembola and Oribatida did not suggest any possible treatment effects from the applied chemicals PCB 52 and BaP.

Considering the Collembola as a group, the high abundances found on the first sampling date in the acetone control KL are conspicuous, but they were not observed again during the remainder of the experiment. After the 2nd sampling date, the oribatid mites had higher abundances in the BaP 2 variant, but this also was true, to a lesser extent, for the PCB 2 variant.

Thus, more detailed studies at the species level are called for. Colonization patterns were more closely examined for species that were found in sufficient densities and were not considered chance occurrences.

In Figures 24 to 27, the individual numbers of different species found in the litter of the experimental variants over the course of the experiment are shown. Because of the different decomposition rates in the control and BaP variants (see Figure 15, page 80), mean numbers of individuals per 100 g litter are given. The mean densities over 4 sampling dates shown in the figures are to provide a first overview. Statements regarding any possible effects of the investigated chemicals are only given after taking all available information into account (e.g., area variability, species constancy over time).

Table 26: Individual densities (Ind. X m-2) of the determined Collembola and Oribatida in the litter of the investigated variants

	1st sampling date (53 days)						2nd sampling date (95 days)						3rd sampling date (133 days)						4th sampling date (179 days)					
	K	KL	PCB1	PCB2	BaP1	BaP2	K	KL	PCB1	PCB2	BaP1	BaP2	K	KL	PCB1	PCB2	BaP1	BaP2	K	KL	PCB1	PCB2	BaP1	BaP2
Collembola																								
Isotomidae																								
I. anglicana	888	763	388	600	900	1275		38		13	50	13	25						300	45	50	75		163
juv. *I.* cf. *anglicana*		13	50		30	63		13	13		25	13							175				138	113
I. notabilis	1263	2250	550	850	100	1175					13		300	688	415			288	1075	362	513	1780	113	1325
juv. *I.* cf. *notabilis*																			13	50				
Isotomidae				13									30		13								100	
Entomobryidae																								
E. multifasciata	288	175	450	488	263	600	163	188	175	450	175	650	213	125	491	427	256	625	800	845	688	850	1400	1263
juv. *E.* cf. *multifasciata*	50													138			30		188	138	313	388	30	113
E. nivalis													13											
L. cyaneus	188	50	13	25	150	188					13						15	13	88				13	38
juv. *L.* cf. *cyaneus*	237				13	13																		50
Sminthuridae																								
Sminthurinus spec.	3650	10475	4513	1600	6688	1875		13	13	50	13		788	3475	1226	1734	1222	1225	125	30	100	271	288	
S. nigromaculatus	63	25	38	75	38	38	75	150	138	75	113	100	75	150	151	126	256	238	138	30	63	150	138	150
Sminthuridae																	30		100	30				
Poduridae	1250	2763	1575	900	713	2050			38	63			1038	38	132	75	166	850	638	136	25	60	725	363
Onychiuridae	825	5763	925	3113	1213	250			13			13	3438	4725	9321	2839	498	2338	138	45			13	75
Σ	8702	22277	8502	7664	10108	7527	238	402	390	651	402	789	5920	9339	11749	5201	2473	5577	3778	1711	1752	3574	2983	3653
Oribatida																								
C. labyrinthicus			13																					
L. similis	438	13	13	1210	400	1038	50			75	75	238	679		321	302	377	1300	13	15		181	100	100
O. nitens																		13						
P. punctum	450	13	13		25	113							75			90	57	150	13			15	25	25
R. insculpta													15			30		25					13	13
S. laevigatus										13								13						
T. sarekensis	325	1138	138	325	138	100	63	175	13	38		138	588	347	321	1133	151	788	400	347	300	422	325	700
Belbidae																		13						
M. pulverosa		50																25						38
Galumnidae					13											13								
Σ	1213	1214	177	1535	576	1251	113	175	13	126	75	376	1357	347	642	1568	585	2327	426	362	300	618	463	876

For the Collembola species *E. multifasciata* and *S. nigromaculatus* (see Figure 24 A and B) higher abundances in the BaP contaminated litter was noted. For *E. multifasciata,* this was the case in both experimental plots of the BaP 2 variants at all sampling dates. In the BaP 2 treatment, individual numbers were on average three times higher than in the control.
S. nigromaculatus reached in both BaP contaminated litter variants densities one third higher than in the control or the litter contaminated with PCB.

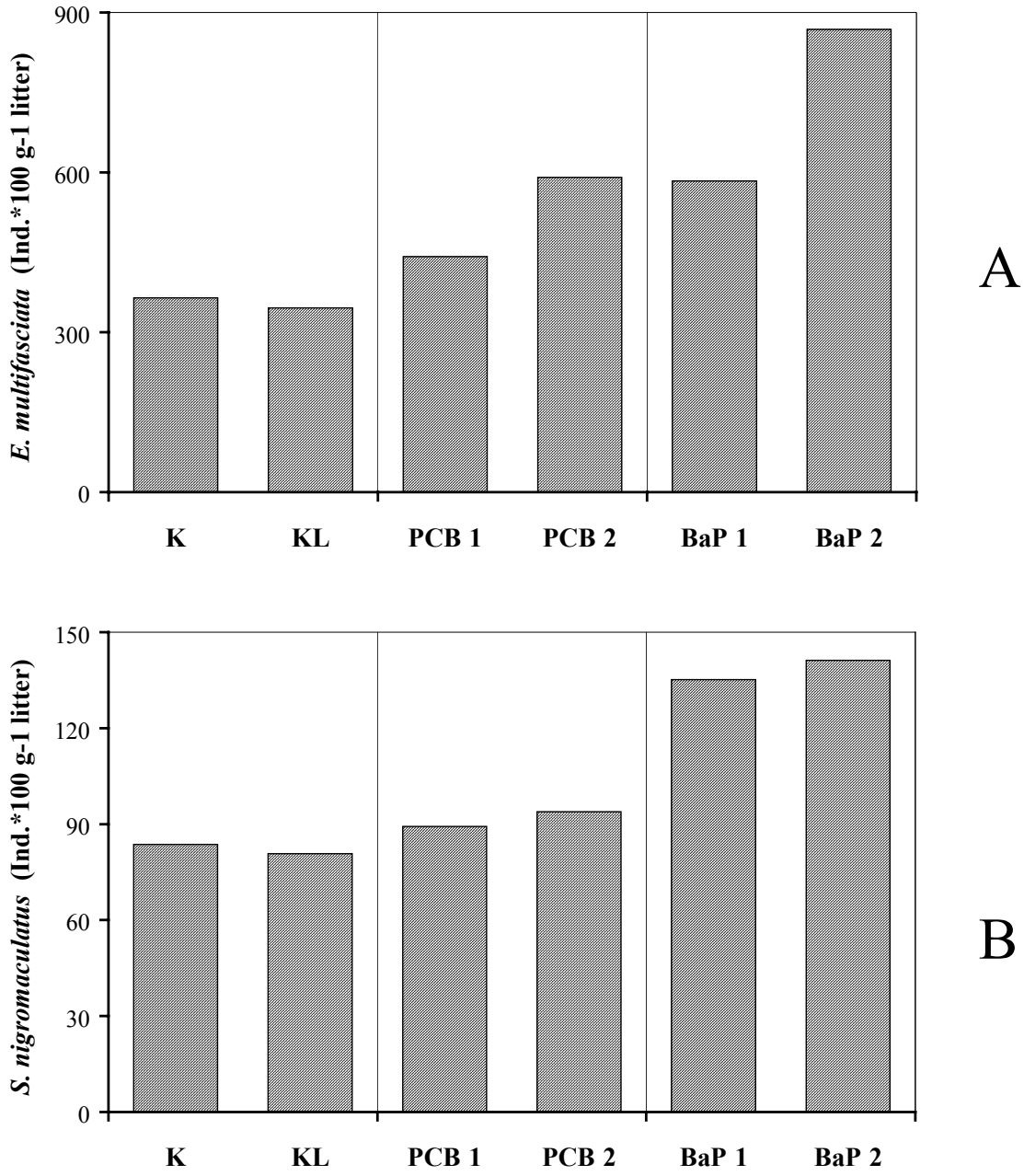


Figure 24: Abundances of A) *E. multifasciata* and B) *S. nigromaculatus* in the litter decomposition containers of the different variants. Shown are the totals of the mean densities from 4 sampling dates.

In contrast, *I. anglicana* and *L. cyaneus* (Figure 25) reached similar individual numbers in controls and BaP variants. However, considering the entire study period, this species exhibited lower densities in the PCB 52 variants. Similar to the distribution of the oribatid species *P. punctum* (following page), the acetone treated control KL was colonized by *I. anglicana* and *L. cyaneus* with lower abundances than the untreated control.

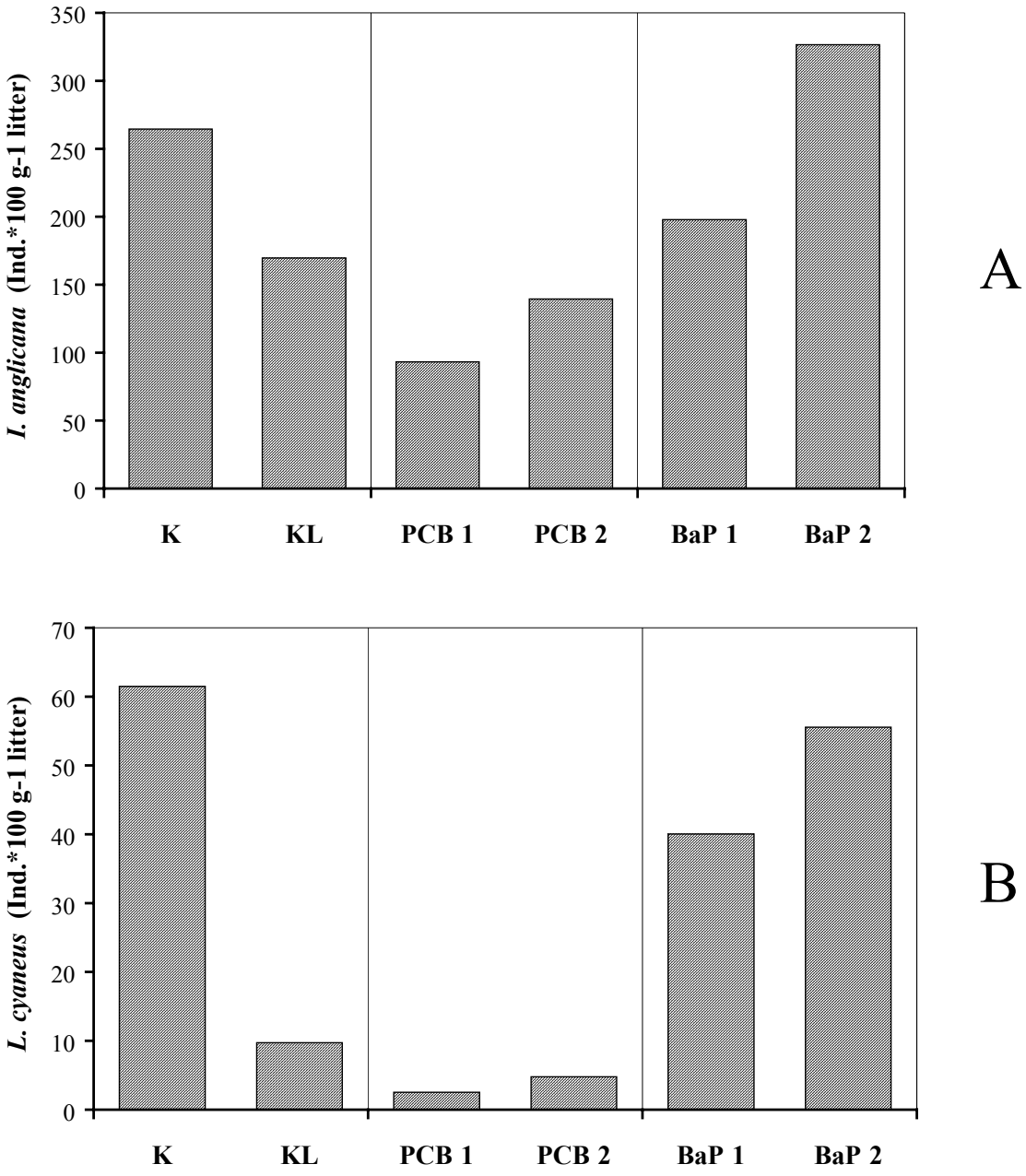


Figure 25: Abundances of A) *I. anglicana* and B) *L. cyaneus* in the litter decomposition containers of the different experimental variants. Shown are the totals of the mean densities of individuals from 4 sampling dates.

The oribatid mite *P. punctum* reached abundances comparable to those in the controls only in the highly contaminated BaP litter variants. The distribution of individuals of this species across the different variants was almost identical to that of *L. cyaneus.* The oribatid mite *L. similis* colonized the litter containers of the highly contaminated BaP and PCB variants to a greater extent than the control containers and the low contaminated litter. Its behavior is similar to that of the species shown in Figure 27 (following page), apart from the low densities in the acetone control KL.

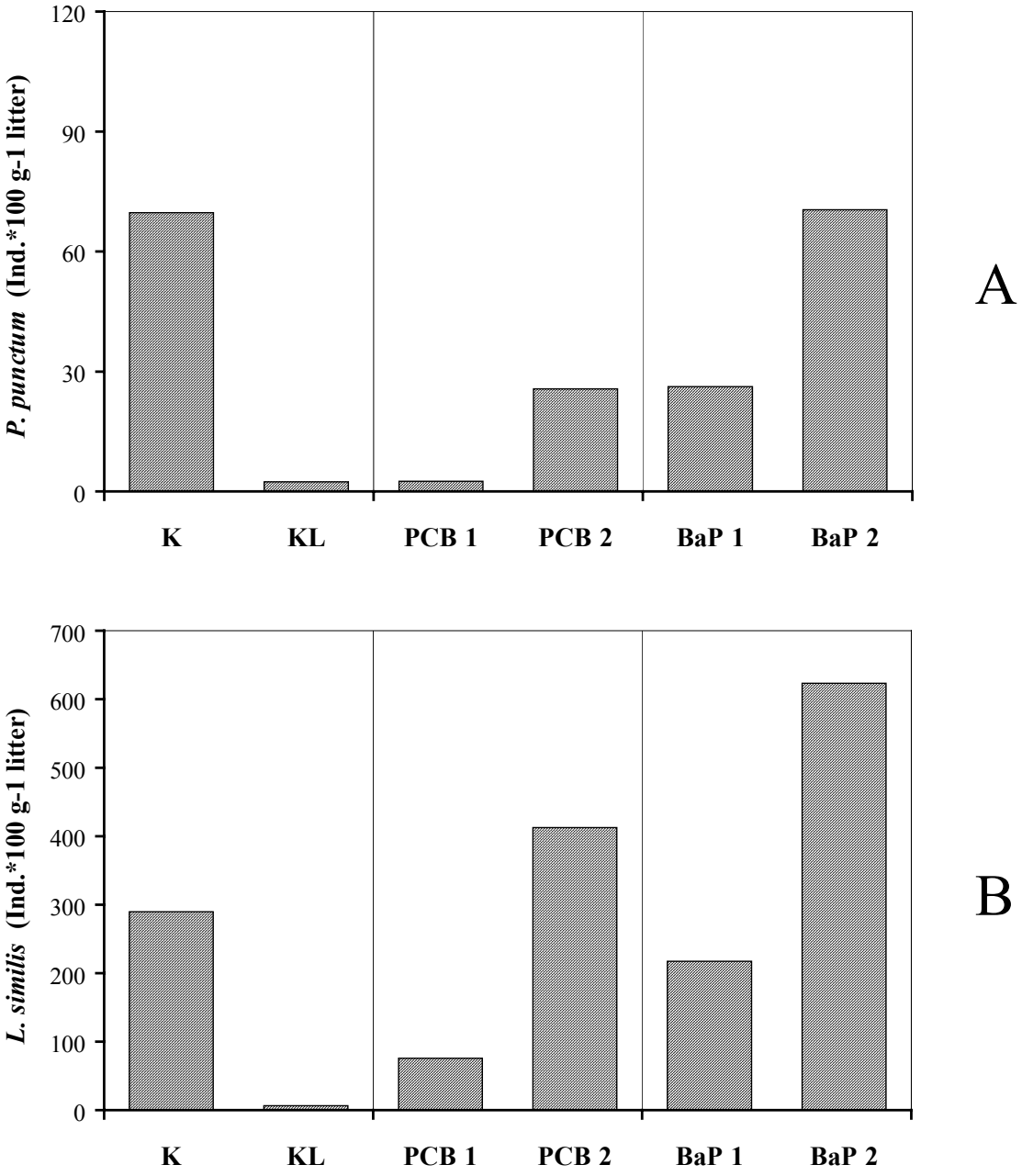


Figure 26: Abundances of A) *P. punctum* and B) *L. similis* in the litter decomposition containers of the different experimental variants. Shown are the totals of the mean densities of individuals from 4 sampling dates.

The oribatid mite most frequently and constantly found in the litter samples was *T. sarekensis.* It reached densities in the contamination variants PCB 2 and BaP 2 equal to or higher than those in the controls. In this respect it is similar to the Collembola *I. notabilis* (Figure 27, B). *L. similis* (previous page), *T. sarekensis,* and *I. notabilis* showed similar colonization patterns, with rather low densities in contamination variants BaP 1 and PCB 1. *T. sarekensis* and *I. notabilis,* however, colonized the plots of variant KL similarly to or more extensively than the untreated controls.

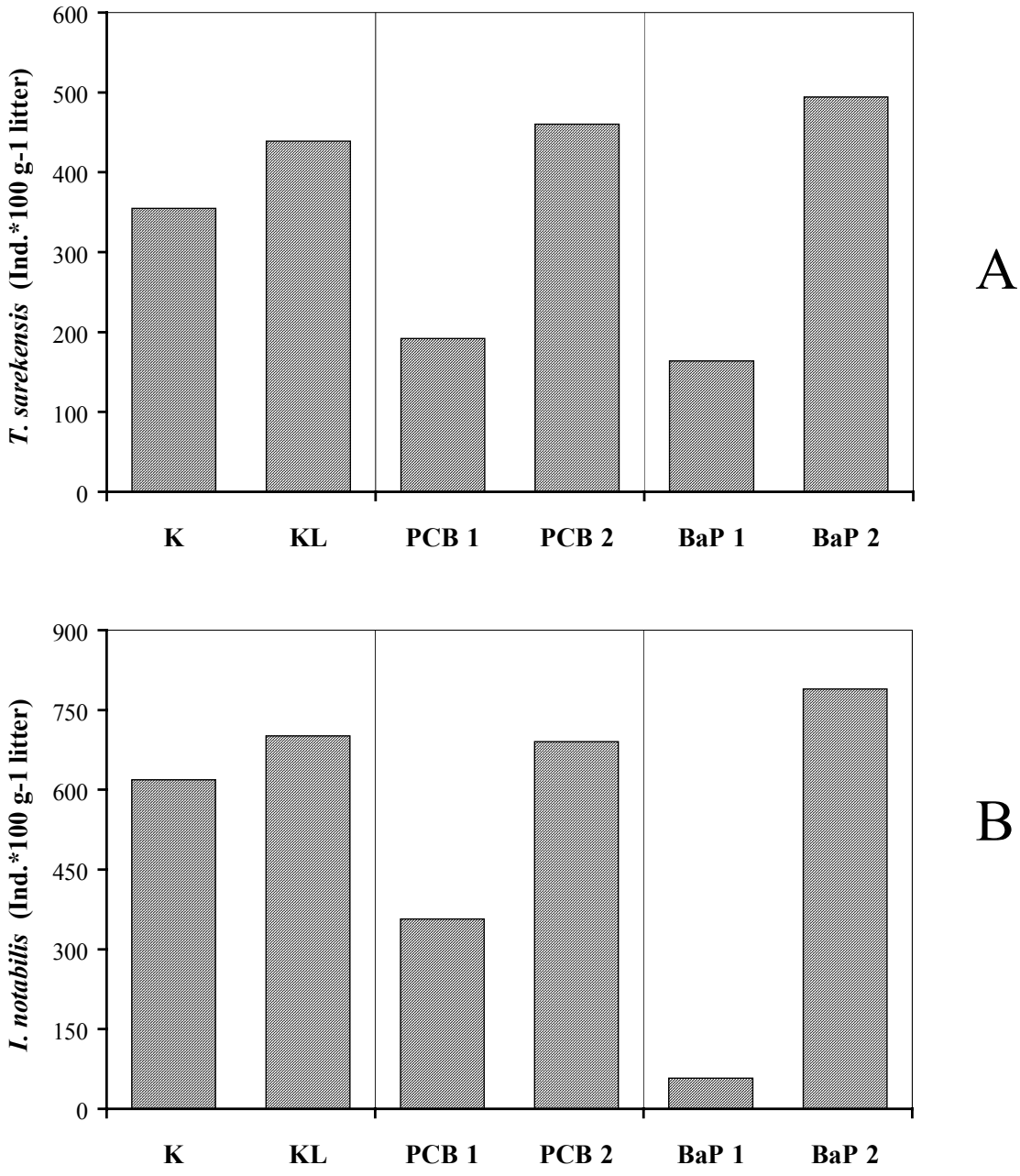


Figure 27: Abundances of A) *T. sarekensis* and B) *I. notabilis* in the litter decomposition containers of the different experimental variants. Shown are the totals of the mean densities of individuals from 4 sampling dates.

Based on the different distribution patters of total numbers of individuals shown in Figure 24 to Figure 27, species were classified in three groups as described below. It was evaluated whether the differences apparent in the total values over the entire period of litter exposure could be detected at each separate sampling date, and whether they could be statistically verified.

Species with Elevated Abundances in the BaP Decomposition Containers (Group I)

E. multifasciata (Figure 24, A) was the only Collembola species found in large numbers on all sampling dates in the containers and which exhibited the same tendencies in its distribution among the different experimental variants throughout the experiment. In addition, after transformation of the data, the densities were normally distributed (x = log10 (x+1)). The introduction of the term (x+1) into the equation allows the inclusion of the many samples in which no animals were found. For all other species the abundances as such were too low, or the number of blank samples (0 individuals) so high that the data could not be successfully transformed. The distribution was then similar to a normal distribution cut off at the 0 value that is often described in the literature for faunistic data (BERTHET & GERARD, 1965).

After the data had been transformed, the abundances of *E. multifasciata* were tested by means of analysis of variance to see whether the factors „sampling date“, „BaP contamination“ and „block“ (from which the field samples were taken – see area map on page 51) contributed significantly to the observed variability in the data. In a first calculation, substrate moisture was included as well, but interestingly enough proved not to be a significant factor (drought tolerance? see Species Ecology, Annex I).

The analysis of variance (see Table 27) suggested that in addition to the sampling date the BaP contamination had a significant effect on the abundances of *E. multifasciata.* Significant interactions were observed between the factors “sampling date” and “block”. This may have been due to abundance variations in the experimental plots of one variant that did not change simultaneously over the course of the experiment. The expression of the BaP effect was not dependent on the sampling date (no interactions). However, the high F values of the factor “block x BaP” suggest, that the chemical treatment effect may have been different in both plots of a given variant (not significant).

In addition, multiple regression calculations using the abundances of *E. multifasciata* as dependent variable and the factors „BaP contamination“, „substrate moisture“ and „block“ as the independent variables were done for each sampling date (not shown). Only for the data of the 3rd sampling date, no model could be found that could be considered significant beyond the sample. The calculations for the sampling dates 1 and 2 showed only BaP to be a significant variable, with a coefficient of variation of $0.54 \leq R \leq 0.64$ ($0.08 \leq p \leq 0.01$).

For the data of the 4th sampling date the variables “water content” and “block” were included, increasing the multiple R to 0.77 ($p = 0.04$ for variable "BaP").

Table 27: Results of the analysis of variance for the abundances of *E. multifasciata* in the litter decomposition containers.
Variants included: Control (K), Control & solvent (KL), BaP 1, and BaP 2. Parameters considered: days of exposure (date), experimental plot (block) and chemical treatment (BaP)

Variance	SQ	DF	MQ	F	Sign. of F
Model	5.792	6	0.965	10.847	<0.001
Sampling date	3.606	3	1.202	13.506	<0.001
Block	0.251	1	0.251	2.824	0.100
BaP	1.935	2	0.967	10.869	<0.001
2-way-interactions	1.588	11	0.144	1.622	0.111
Sampling date*Block	0.959	3	0.320	3.592	0.018
Sampling date*BaP	0.196	6	0.033	0.367	0.898
Block*BaP	0.432	2	0.216	2.429	0.095
Explained	7.380	17	0.434	4.878	<0.001
Rest	6.408	72	0.089		
Total	13.788	89	0.155		

SQ sum squared; DF degrees of freedom, MQ mean sum squared

The elevated abundances of the Collembola species *S. nigromaculatus* in the litter of the BaP variants (Figure 24, B) resulted from an increased colonization of these containers by the 3rd and 4th sampling date. At sampling dates 1 and 2, abundances in the variants BaP1/PCB1 and BaP2/PCB 2 were remarkably similar.

In contrast to *E. multifasciata*, for *S. nigromaculatus* there was no increase in numbers of individuals in the BaP contaminated litter on any sampling dates during the decomposition experiment. On the 3rd sampling date, however, the increase in abundances in the experimental plots of the BaP 1 and BaP 2 variants were similar and on average abundances were twice as high as in the controls (see Table 26, page 99).

Species with Reduced Abundances in the PCB Decomposition Containers (Group II)

Throughout the entire study period, the Collembola species *I. anglicana* and *L. cyaneus* as well as the oribatid *P. punctum* colonized the litter contaminated with PCB 52 with fewer numbers of individuals than the control litter or that of the BaP 2 variant.

The Collembola *I. anglicana* took a position between the classification groups I and II. In the BaP 2 variant this species showed higher abundances than in the control, but at the same time the densities in the PCB 52 variants were slightly lower (see Figure 25). *I. anglicana* was found in the containers of all variants only on the 1st sampling date, making a comparison of differences rather difficult. On the 1st sampling date the colonization densities were similar in all variants with the exception of the BaP containers, where on average 100 animals more per square meter were found. After the dry period in June, the *I. anglicana* population did not recover until the 4th sampling date, and then only in the untreated control and in the BaP variants. Here, many juveniles colonized the litter too, which was not the case in the PCB 52 containers (see Table 26). The differences between the variants K, PCB 1, and PCB 2 on the 4th sampling date could statistically be verified at the $\alpha = 10\%$ level (U test, $p = 0.03$ and 0.06).

The population development of *L. cyaneus* (totals for the year in Figure 25, B) was very similar to that of *I. anglicana*: The highest abundances in the containers were found at the first sampling date. In contrast to *I. anglicana*, the numbers of individuals in the control and the BaP variants did not differ at this time, but were clearly higher than those of the PCB variants. During the remaining study period, *L. cyaneus* was found in the litter of the BaP containers and in the control only. After the breakdown in the dry summer months, the PCB litter was not colonized again by this species. Due to the overall low colonization densities, though, no statistical evaluation of the data was possible.

The oribatid *P. punctum* (Figure 26, B) was also found only scattered in the PCB 52 containers later in the year. However, *P. punctum* was found more regularly in the litter from all decomposition containers during the study period, with no density maximum in spring. The high numbers of individuals in the untreated control (K, totals for the year in

Figure 26, A) are not to be considered representative for all of the sampling dates: They are based only on occurrences on the one sampling date, furthermore from only one of the 2 control plots. As with *L. cyaneus,* the low constancy of this species in the samples did not enable any statistic verification.

Species that Showed High Abundances in the Highly Contaminated Litter

The oribatid species *L. similis* (Figure 26, B) was found with high constancy in the litter and, over the entire study period, was present with higher numbers of individuals in the variants BaP 2 and, slightly less, PCB 2. The abundances of *L. similis* in the litter samples of the different variants were compared using non-parametric tests, because the data could not be normalized by transformation. When the sampling dates were studied separately, significant differences between the variants K and BaP 2 were found at the $\alpha = 10\%$ level. This probably was due to the frequent null values (see evaluation of the *E. multifasciata* occurrences, group I). When the entire study period is considered, however, there is a significant difference between the variants K and BaP 2 ($p = 0.03$). In contrast, the differentiation of abundances between the control and PCB 52 containers suggested in Figure 26 could not be statistically confirmed: Higher numbers of individuals in the PCB contaminated litter were found only on the 1st sampling date and were not significantly different from the control.

There were no clear trends in the *I. notabilis* (Figure 27, B) colonization of the different containers. The two plots for a given variant never showed the same abundances. Thus, the observed differences in the total number of individuals were caused by area variability rather than by the chemical treatment, with the latter possibly being masked by the former.

Also, the distribution pattern of *T. sarekensis* (Figure 27, A) individuals was very heterogeneous across the samples from the different variants: In some cases the species occurred evenly in parallel samples, but on other sampling dates null samples could be found alongside samples with extremely high numbers of individuals. This phenomenon was already described by USHER (1975) for the very similar species *T. velatus*. Also, the differences between the treatment variants showed different signs, making the interpretation of the distribution pattern rather speculative.

Finally, it should be noted that *I. notabilis* and. *T. sarekensis* were the only species studied here which were found in high numbers in the acetone treated control (KL) containers. It is unlikely that the acetone treatment at the employed concentrations (1 ml sprayed over 8 g litter and then evaporated) had a significant direct impact on the soil fauna. Thus, these observations probably were due to the heterogeneity of the areas (see above) and the population dynamics of certain species. The high *I. notabilis* and *T. sarekensis* abundances in the KL variant were based mostly on the extremely high values of the first sampling date, which in turn were based only on the colonization of one of the two experimental plots. If the decomposition containers were rapidly colonized by high numbers of individuals of these two euryoecious species, it is possible that species with a slower developmental process and/or a lower potential for spreading were not able to build higher population densities at first.

3.4 Faunistic Comparison of the Areas RefB, nPAK and nPCB

3.4.1 Oribatid Mites

In the fall of 1996 and spring of 1996, soil samples were taken from the sewage field areas RefB, nPAK, and nPCB and their oribatid mite colonization densities were studied.

Table 29 lists the oribatid mite species that were found in 2 soil samplings of the areas RefB, nPAK and nPCB. The percentage of samples from a given habitat in which the species occurred is characterized by constancy classes (see 2.7.2, page 63). The data for the 3 sewage field areas can be compared among each other. The constancy values from the litter containers (RefB-S), however, are listed for the sake of completeness only and represent an independent data set: They are based on different sample densities and investigational periods.

The sampling sites are listed once more in Table 28 (for soil parameters see Table 2, page 47).

Table 28: Sampling sites for the characterization of the oribatid mite fauna of the sewage fields

Sampling site name	Area	Vegetation	Sampling
RefB-S	RefB	*A. repens*	litter in decomposition containers
RefB-B	RefB	*A. repens*	soil covered with litter
nPAK	nPAK	poplar plantation	soil covered with litter
nPCB	nPCB	partially bare	soil

In the nPCB area both the larger areas without any vegetation as well as spot exhibiting scarce grass vegetation were sampled. The oribatid mite occurrences in Table 29 refer only to the spots with vegetation because no oribatid mites were found in any of the samples of the bare areas.

Table 29: Consolidated species list and occurrence of oribatids in all studied sewage field areas. Occurrence is characterized by the constancy of the species in the samples from the habitats studied (constancy classification: 5 = always, 1 = very rarely, see Table 7 page 63).

	sampling sites				
Species	nPCB	S/RefB	RefB	nPAK	Occurrence*
Oppiella nova (Oudemans, 1902)	2		2		
Microppia minus (Paoli, 1908)	3		5	3	A
Tectocepheus sarekensis Trägardh, 1910	5	5	5	5	A
Ramusella insculpta (Paoli, 1908)	3	1	2	2	A
*Carabodes labyrinthicu*s (Michael, 1879)		1			R
Punctoribates punctum (C.L. Koch, 1849)		2	2		R
Ceratocetes mediocris Berlese, 1908		1	1		R
Oppia nitens (C.L. Koch, 1835)		1	1		R
Scheloribates laevigatus (C.L. Koch, 1836)		1	1		R
Liebstadia similis (Michael, 1888)		3	3	3	
Metabelba pulverosa Strenzke, 1953		1	1	3	
Suctobelbella subcornigera Forsslund, 1941				3	P
Eupelops occultus (C.L. Koch, 1836)				2	P
Suctobelbella acutidens lobata Strenzke, 1951				2	P
Liacarus coracinus (C.L. Koch, 1841)				1	P
Galumna lanceata Oudemans, 1900				1	P
Oribatella quadricornuta (Michael, 1880?)				1	P
Pergalumna nervosa (Berlese, 1915)				1	P
Suctobelbella subtrigona (Oudemans, 1916)				1	P
Trichoribates novus Sellnick, 1928				1	P

*see text section 3.3.2, page 95

The composition of the oribatid mite communities from the different areas is shown in Table 30 and Table 31 and the distribution of the dominance percentages among the occurring species in Figure 28. Descriptions of the synusiae are based on literature review of the ecology of the species that can be found in Annex I.

Table 30: Oribatid mites of the areas 1) RefB and 2) nPCB, sorted by their respective abundances. Assignment of species to the synusiae of the habitats given and codes modified according to STRENZKE (1952), supplemented according to KNÜLLE (1957), MORITZ (1963), RAJSKI (1967, 1968), and WEIGMANN & KRATZ (1982). Characteristic species of the synusiae or frequently accompanying species are labeled **A! .**

1) RefB		**Eury-oecious**	**Meadows**		**Forests**			**Dry Habitats**
Species	**Abundance** (Ind. x m^{-2})	0	II	IIb with	IV	IVa moist	IVb dry	V
T. sarekensis	23158	✓	+	+	+	+	+	+
M. minus	13455			+			+	
L. similis	2378		**A!**	+				+
P. punctum	1527		+	+			+	+
O. nova	649	✓	+	+	+	+	+	+
R. insculpta	263		?	?				
M. pulverosa	90		+	+		+		
O. nitens	44							**A!**
S. laevigatus	35		**A!**	+		+		+
C.labyrinthicus	26			+			+	+
C. mediocris	26		**A!**	+				
Total	41651	**2**	**3**					**1**

2) nPCB		**Eury-oecious**	**Meadows**		**Forests**			**Dry Habitats**
Species	**Abundance** (Ind. x m^{-2})	0	II	IIb with litter	IV	IVa moist	IVb dry	V
T. sarekensis	17687	✓	+	+	+	+	+	+
M. minus	2527			+			+	
R. insculpta	1158		?	?				
O. nova	105	✓	+	+	+	+	+	+
Total	21477	**2**						

In the RefB habitat 11 species were found (Table 30-1), of which only *C. labyrinthicus* was confined to the litter from the decomposition containers. Of the species determined, two (18%) are described in the literature as truly euryoecious. They represented 57% of all individuals in this area.

T. sarekensis was the oribatid mite species with the highest abundance and constancy in the RefB area, not only in the soil samples but also – as discusses above – in the litter decomposition containers. In the nPCB area *T. sarekensis* was the most frequent species as well, only in the nPAK area the predominant species was *M. minus* (see Table 31).

Three species from the RefB area (27%), which only represent close to 6% of the individuals, are characteristic species of meadows and pastures. A yet to be confirmed classification of *R. insculpta* as belonging to this synusia would increase the percentage of the character species dominance to 36%. The other species, especially *M. minus* and *M. pulverosa*, often have been reported in woody habitats as well. However, in the RefB area no characteristic species for the synusiae of the forests could be found.

It is remarkable, that 7 of the 11 species found also may colonize dry epiphytic and epilithic habitats, especially *O. nitens* and according to STRENZKE (1952) also *C. labyrinthicus*. From the list of the 5 most common species in the RefB area, only *M. minus* does not occupy this niche, probably because of its preferences for deeper soil layers.

The nPCB area was extremely poor in species (Table 30). The partially bare soil was inhabited by only 4 species, which were only found in the sections on which grass was growing. The moss carpet found in this area did not provide a sufficient habitat, not even for a few individuals. Although *M. minus* prefers deeper soil layers, not even this species could be found in samples of soil without vegetation.

In the areas where scarce grass growth was present, *T. sarekensis* was able to establish abundances comparable to those found in the RefB sewage field soil. In these sections, *R. insculpta* even exhibited higher colonization densities than in RefB.

The clearly euryoecious species *O. nova* was found in the RefB and nPCB areas, but not in the nPAK area. Thus, 2 of the 4 species found in the nPCB area are euryoecious and represent almost 85% of all individuals. No characteristic species of any synusiae could be found in these samples (*R. insculpta*?).

In the nPAK area (Table 31), with its poplar stand and grass cover as undergrowth, the lowest colonization densities of all 3 sewage field areas studied were found. In contrast, the Oribatida community of this soil comprised 14 species (plus one species from the belboidea family) that were not a simple extension of the inventory found in the RefB area.

The species identity (Sörensen's quotient, Equation 6, page 53) for the areas RefB and nPAK was 38.5%. The dominance identity (75%, according to Renkonen, see Equation 7, page) on the other hand showed that there was a rather pronounced agreement of the dominant species of both habitats. Differences in the species community are caused in part by the occurrence of characteristic species of the synusiae of woody habitats (20%) that were not found in the RefB area. *P. nervosa*, *G. lanceata,* and *S. subtrigona* find their optimum conditions in soil with a

tree or shrub stand. *L. coracinus*, too, prefers woody habitats, but is also found, like the majority of the species of this synusia that were found in the nPAK area, in meadows with enhanced litter coverage.

Another factor is the increase in characteristic species of the synusia of meadows and pastures. These are the species, which, except for *L. similis*, were not found in the RefB area. *E. occultus*, *S. acutidens lobata,* and *T. novus* were found only in the nPAK area, *S. laevigatus* and *C. mediocris* were missing here. The latter two species, however, were found only with low constancies in the RefB area, their absence, therefore, should not be overrated. The percentage of characteristic species of meadows and pastures in the nPAK area was 26%, the same percentage as in the RefB area. These species represent approximately 10% of the individuals found.

Table 31: Oribatid mite species of the nPAK area, sorted by abundances. Assignment of species to the synusiae of the habitats given and codes modified according to STRENZKE (1952), supplemented according to KNÜLLE (1957), MORITZ (1963), RAJSKI (1967, 1968), and WEIGMANN & KRATZ (1982). Characteristic species of the synusiae or frequently accompanying species are labeled **A! .**

nPAK		**Eury-oecious**	**Meadows**		**Forests**			**Dry Habitats**
Species	**Abundance** (Ind. x m^{-2})	0	II	IIb with litter	IV	IVa moist	IVb dry	V
M. minus	7580			+			+	
T. sarekensis	6510	✓	+	+	+	+	+	+
L. similis	1193		**A!**	+				+
M. pulverosa	474		+	+		+		
S. subcornigera	474	✓	+	+	+	+	+	+
P. nervosa	456					**A!**		
E. occultus	228		**A!**	+				+
R. insculpta	175		?	?				
L. coracinus	140			+	+		+	+
S. a. lobata	105		**A!**	+				
T. novus	105		**A!**	+				
G. lanceata	70			+	**A!**	+	+	+
S. subtrigona	70			+	**A!**	+	+	
O. quadricornuta	35						+	**A!**
Belboidea	281							
Sum	17896	**2**	**4**		**2**	**1**		**1**

The percentage of characteristic species of forests, on the other hand, was only a little over 4%. Among the 5 most common species there were 2 species that are considered euryoecious (*S. subcornigera*, however, was found only in this habitat) and 2 accompanying species of both the neutrophilic and acidophilic synusiae (*M. minus* und *M. pulverosa*). *L. similis* was the only frequently occurring characteristic species and achieved similar dominances in the RefB and nPAK habitats (approximately 6%, see Figure 28).

Approximately 40% of the species occurring in the nPAK area have also been found in samples of dry habitats (see Table 31, column V). This holds especially true for *O. quadricornuta.* Even more conspicuous was the fact that close to 75% of all species, including the character species of different synusiae, also can be found in meadows with a generous litter cover, e.g., in forest meadows or in the soil cover of carrs (group IIb). This variant of the synusia II as differentiated by Strenzke (1952, page 48) exhibits „... a character combining that of the synusia of the moist meadows and the acidophilic synusiae of the peatlands and woods, corresponding to the transitional nature of its habitats. Only one species in this area, *Belba corynopus*, clearly prefers the habitats presented here...“, witch, though, was not found in the nPAK area.

Figure 28 shows the graduated dominance percentages of the different species per the total number of individuals in the three sewage field areas examined.

In the RefB and nPCB sewage fields *T. sarekensis* was most dominant at 55% and 82%, respectively. In the RefB area the difference to the second most frequent species was approximately 23%. In the nPCB area, on the other hand, the margin to the second most frequent species was more than 70%, *M. minus* achieving a dominance percentage of only 12%.

According to the second basic biocenotic rule by THIENEMANN (1956, in REMMERT, 1980), in a given habitat with extreme conditions only few species will be found, but these will achieve high colonization densities. In undisturbed habitats, however, the ratio of species and individuals will be more balanced (ENGELMANN, 1978). In undisturbed habitats, several species will exhibit moderately high percentages of dominance, and the community will not be dominated by a single species with very high abundances.

The nPCB area may well be viewed as an extreme example of dominance graduation of a disturbed habitat. The RefB area was a little more balanced, but *T. sarekensis* and *M. minus* together represented 88% of all individuals here as well. In the nPAK area these species combined achieved again a dominance percentage of 79%, but neither of them by itself contributed more than 50% to the individual number. Of all the habitats examined here, the nPAK area possibly offered the greatest variety of conditions, but still cannot, based on the dominance structure of the oribatid mite cenosis be classified as undisturbed.

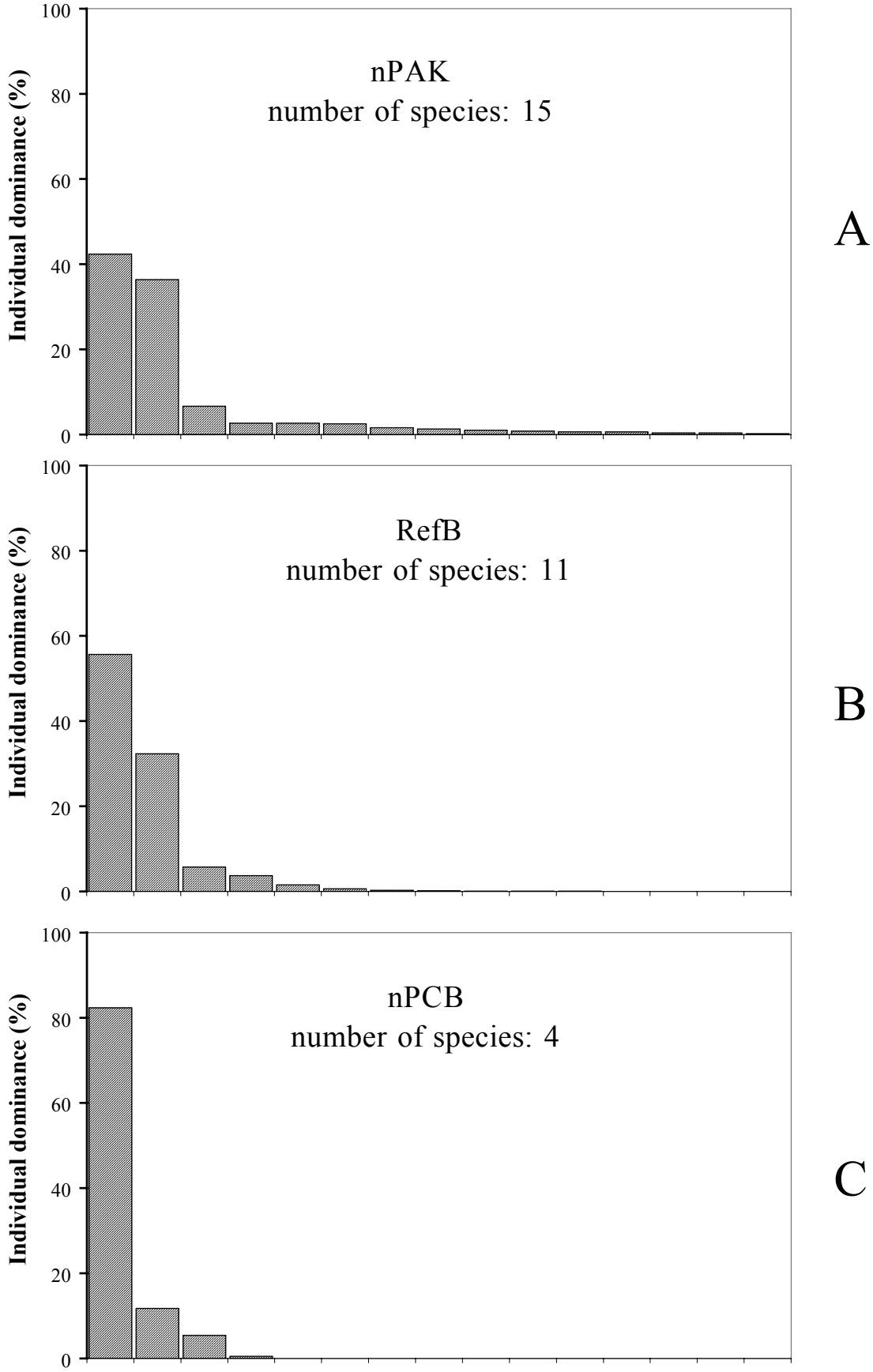


Figure 28: Dominance graduation of the species in the sewage field areas studied. A) nPAK, B) RefB and C) nPCB

3.4.2 Nematodes

The nematode fauna of the 3 sewage field areas was surveyed once (see chapter 2.5, page 58). As with the oribatid survey, in the nPCB area sections with as well as without vegetation were sampled. Table 32 lists the sum parameters for the characterization of the nematode fauna in the habitats. These data were supplied by Dr. G. Korthals of the Wageningen Agricultural University, The Netherlands.

According to KORTHALS (personal communication) the nematode abundances found in the RefB and nPAK areas are typical for the colonization of grassland ecosystems.

In the nPCB area, in contrast, the numbers of individuals are much lower. Here, the sections with grass vegetation were colonized more strongly by the nematodes than the bare sections. The spectrum of species was extremely poor, and the dominances of the different feeding types were considerably shifted. While the RefB and nPAK areas had a percentage of fungivore nematodes of approximately 8%, in the PCB area this percentage rose to 28% and 52% depending on whether these sampled soils had vegetation or was bare.

In the bare sections of the nPCB area, plant parasites and bacteriovores combined made up less than 50% of individuals – in contrast to the 90% dominance percentage achieved in the RefB and nPAK areas. The nPCB area was the only one in which the plant parasite *Paratylenchus* occurred. This species had been found in several studies occurring with increased numbers in areas contaminated with heavy metals (KORTHALS, personal communication). In none of the samples representatives of the Dorylamidae family were found. This fact suggests general environmental stress for the nematode population of the sewage fields.

The maturity index (MI) according to BONGERS (1990) is based on a classification of the nematode families according to their ability for colonizing new habitats. With this approach, values between 1 and 5 from a c-p scale (colonizers - persisters) are assigned, with low values characterizing pronounced R-strategists. The MI is the weighted average of all c-p values. Early serial states of a population are thought to have a low MI. The maturity index shifts to higher values when K-strategists (high c-p) increase in numbers later on in the succession.

A given nematode population can respond to a disturbance in two ways. On the one hand, nematode families with low c-p values may increase. If the stress factors are eutrophication, fertilization, or contamination with oil, especially the c-p 1 families (enrichment opportunists) may dominate (type I according to KORTHALS *et al.*, 1996). On the other hand, a different pattern has been observed in studies of very acidic or heavy metals contaminated soils. In this cases, the nutrient supply is not high enough for c-p 1 families to greatly proliferate. Instead, the taxa from the c-p 2 group (general opportunists) dominate. These can not only survive under unfavorable conditions but often are even able to increase their abundances (type II). In both cases the MI decreases, no matter which response pattern the community shows after the disturbance. MI values below 2 suggest that the former response type I was triggered and point

to eutrophication. Values around 2 in combination with a poorness of species diversity, on the other hand, suggest a contamination of the soils, e.g. by heavy metals, and a response reaction of type II.

Looking at the maturity index for the entire nematode population (Table 32) the higher values in the nPCB area compared to RefB and nPAK are conspicuous, especially those for the bare sections. However, if the additional information of the taxa distribution according to their c-p values is taken into consideration, different response patterns become apparent. Based on its MI of 2 and the dominance of the c-p 2 families, the nPCB area may be considered characteristic for a response after a disturbance of type II.

The areas RefB and nPAK, which do not differ from each other, also had high percentages of families from the c-p 2 group. Here, however, 30% of the species present belong to the c-p 1 group, which rapidly respond to good nutrient supplies. Their MI values under 2 also suggest a response of Type I, mirroring the eutrophication of the sewage field areas.

In the nPCB area, in contrast, the effect of the good nutrient supply due to the long-term deposition of untreated wastewater seems to have been masked by other stress factors.

Table 32: Sum parameters for the characterization of the nematode fauna in the sewage field areas RefB, nPAK and nPCB

	RefB	nPAK	nPCB	
Parameter			grown	bare
Mean Number of Taxa	17.3	17.0	8.0	6.5
Densities (Nematoda * 100 g^{-1}FW Soil)	2300	3100	2410	280
Maturity-Index MI	1.68	1.67	1.83	1.97
Percentage of colonizer/persister types				
c-p 1	32.6	33.4	17.0	2.5
c-p 2	67.1	66.6	83.0	97.5
c-p 4	0.3	0.0	0.0	0.0
Percentage of feeding types				
Plant parasites	23.3	21.0	12.3	2.5
Bacterial feeders	67.7	71.8	60.0	44.5
Fungivores	8.8	7.2	27.7	52.5
Carnivores	0.2	-	-	-

3.4.3 Feeding Activity of the Soil Fauna

The feeding activities of the soil dwelling animals were studied using the bait lamina test (see section 2.6, p. 59). This was done to supplement the investigation on the soil fauna colonization of the sewage field areas with the results of a summary functional parameter.

In the RefB and nPCB areas the feeding activities were assessed several times, since these areas were part of the study program of several workgroups in the joint research project (e.g. ACHAZI *et al*., 1997). The feeding activities in the sewage field areas overall can be considered as low (Table 33). Only in the nPAK area the overall feeding activity of 14% in the spring was comparable to literature results from investigated meadows. It should be noted that the higher biological activity - in comparison to the RefB or nPCB area - assessed with the bait lamina test does not correlate with higher colonization densities for the oribatid mites.

Figure 29 shows the distribution of the feeding activities across the soil profile. In fall and spring, the RefB area displayed low feeding activities compared to the nPAK area, especially in the uppermost soil layers. Here, the soil fauna of the nPAK area fed on 30% of the baits in one month, compared to less than 10% in the RefB soil.

To evaluate the depth distribution pattern of different high feeding activities, the overall percentage of bait fed was set to 100%. The relative depth distribution of the feeding activities is shown in Figure 30. Although in spring the total activity in the nPAK area was 4fold higher than that in the RefB area, the two investigated sites display similar activity profiles when the feeding rate is normalized to the absolute amount of fed baits. The profile is graduated down in soil depth, the highest values being at the soil surface. In area RefB, the assessment period in fall shows a bait feeding rate almost identical for all investigated depths, with slightly higher activities in the deeper range (see Figure 29). Normalizing the data to 100% baits fed leads to an activity percentage distribution with a reversed course as compared to the spring period (Figure 30).

Table 33: Percentage of soil fauna feeding activity as assessed with the bait lamina test normalized to 28 days of exposure

	Assessment Period	
Investigated Area	Fall	Spring
RefB	3.3 %	2.6 %
nPAK		13.7 %
nPCB	< 1%*	4 %*

* Data from ACHAZI *et al*. (1997).

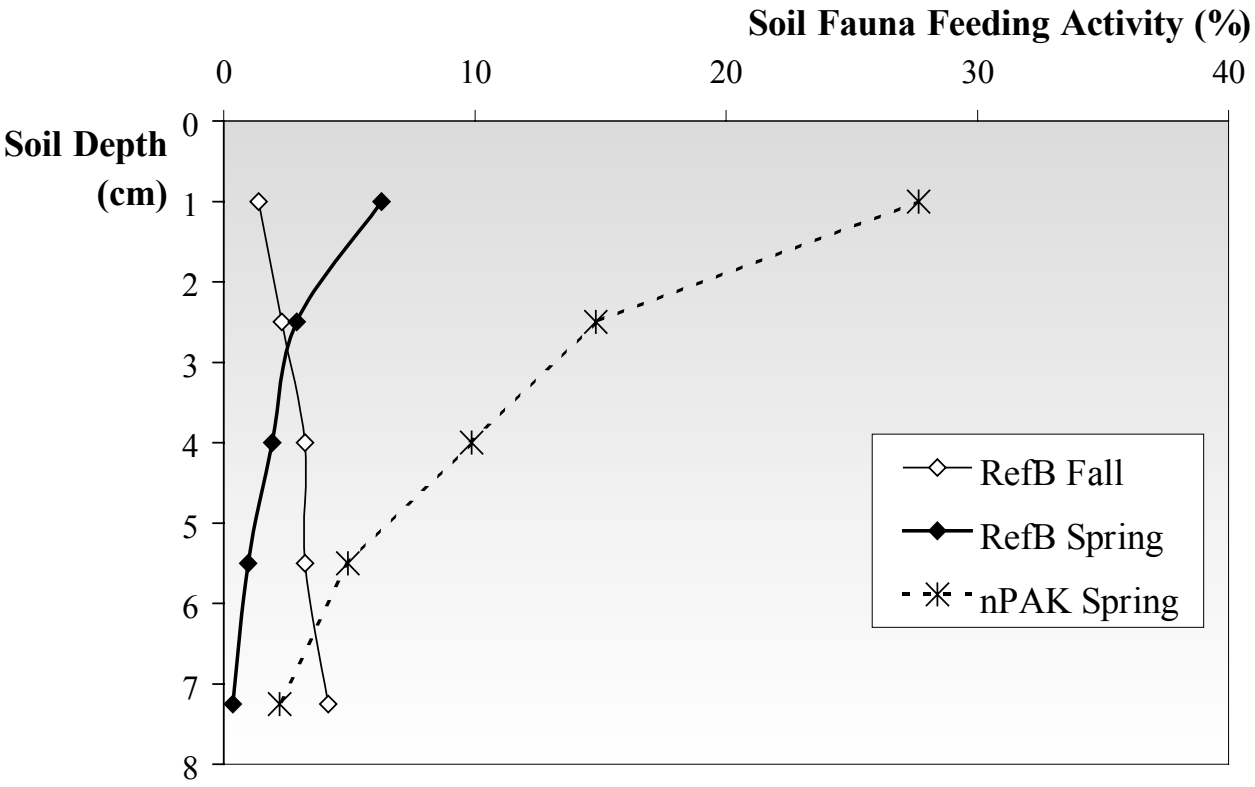


Figure 29: Distribution of the soil fauna feeding activity in the depth profile studied (fall of 1995 and spring of 1996). Shown are mean values of the activity measured in 3 subsequent baits.

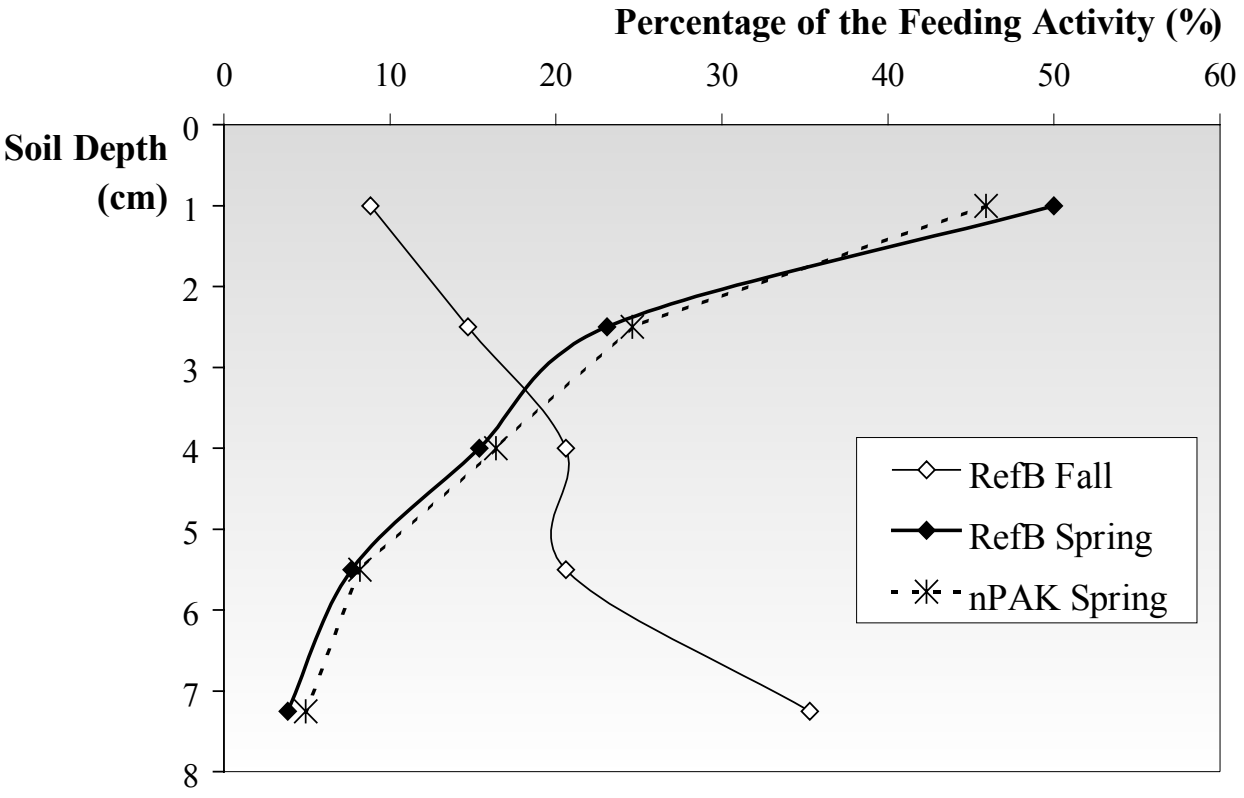


Figure 30: Relative distribution of the soil fauna feeding activity in the depth profile studied. Shown are mean values percentage activity measured in 3 subsequent baits.

4 DISCUSSION

The preceding chapters have demonstrated that organic chemicals have a differentiated impact on soil organisms and the decomposition of litter.

The selected reference substances benzo(a)pyrene (BaP) for the polycyclic aromatic hydrocarbons (PAHs) and PCB52 for the polychlorinated biphenyls (PCBs) behaved differently in the investigated litter layer than in soil. The decrease in the amounts extractable from spiked litter over the investigational period was marked.

BaP had an enhancing effect on the litter decomposition process, and PCB 52 did not show any detectable impact at the concentrations applied. The faster litter decay in the initial phase of the process was mirrored by increased individual densities of specific Collembola and Oribatid mite species in the litter spiked with BaP. In contrast, some soil animal species reduced their colonization of the litter contaminated with PCB 52, especially in later decay phases. None of the surveyed species showed decreased individual densities in response to a contamination with BaP.

The assessment of the effects a long-term contamination might have on the soil fauna of differently contaminated former sewage field demonstrated the prevailing influence of management practices on the type of soil animal cenosis developing in the disturbed soil and litter layers.

4.1 Benzo(a)pyrene and PCB 52 in the Litter Matrix

Every single charge of the grass litter to be exposed in the decomposition containers was spiked with an acetone solution containing the required different amounts of BaP and PCB 52. This procedure was chosen to achieve an even distribution of the compounds among litter charges belonging to one variant. For the first decomposition experiment with non-labeled substances, this approach was successful for BaP, but not for PCB 52. Following the application of the ^{14}C-labeled chemicals for the second set of decomposition experiments in the Mitscherlich vessels, the actual concentrations measured were half and one third, respectively, that of the target concentrations. These results question in general the suitability of spray application methods outside closed chambers as employed in this study, even for the application of substances of relatively low volatility. The litter was placed under exhaust hoods to allow for the acetone to volatilize prior to the transfer to the containers. When radio labeled substances were used, safety precautions required the exhausts to be closed and in operation. Volatilization of the compounds may have occurred alongside the evaporation of the solvent, explaining the higher loss of PCB 52 compared to BaP and the poor spiking efficiency in the contamination procedures with the ^{14}C-labeled substances. Determining actual initial concentrations is of unquestionable importance, and no contamination experiment should be

based on the assumption that the nominal target concentrations are the loads effectively present in the spiked substrates.

For purposes of further discussion, the contamination levels of BaP in the litter of the field decomposition experiment are referred to as 100 mg x kg^{-1} in the high and 10 mg x kg^{-1} in the low spiking variant. For PCB 52 they are 11.5 mg x kg^{-1} and 2.0 mg x kg^{-1}, respectively. The initial concentration levels in the Mitscherlich experiment under semi-field conditions were 55 mg BaP x kg^{-1} litter and 11.5 mg PCB 52 x kg^{-1} litter.

In the litter exposed in the field, the extractable amounts of BaP and PCB 52 decreased exponentially with time, and the apparent plateau concentrations were, compared to the initial loads, firstly higher for BaP than for PCB 52 (15% vs. 10%) and secondly achieved sooner in the PCB 52 litter. This can be seen in the higher loss rate of extractable PCB 52 and in the higher proportion of the substance that was affected by this "disappearance" rate. The percentage reduction in the measurable concentrations was more marked in the lower than in the higher contamination variants, even though in the high contamination variants the absolute amount lost was greater. This was observed by BEIGEL *et al*. (1999), too, who thought the loss to be limited by slow mass transfer of a higher amount of the compound strongly sorbed to the organic matrix. An influence of different application rates *per se* was observed by WOLTER *et al*. (1997) as well: The authors point to the spiking procedure as being responsible for the slower loss from higher contaminated substrates, because the same matrix surface may be coated by multiple layers of the applied compounds and the lowermost strata, therefore, may not be available for simultaneous desorption or transformation processes.

While after 50 days of exposure in the field on average only 20% and 40%, respectively, of the chemical litter concentrations in the low and high spiking variants were detectable, approximately 80% of the initially exposed grass litter remained in the decomposition containers. Because in soil there were clearly lower losses observed in measurements assessing the storage stability of these compounds (FROST *et al*., 1997), the reduction of the extractable percentages of BaP and PCB 52 in the litter has to be explained by the different structure and exposure conditions of the carrier matrix itself.

On the one hand, the contaminated litter was exposed in the topmost soil layer to strong varying abiotic factors (light, moisture), which may account for a quicker degradation of the chemicals in these experiments than in the soil stored under dry conditions. Especially PCB 52 has been shown to be rather volatile compared to BaP: MARSCHNER *et al.* (1997) and FROST *et al*. (1997) monitored considerable losses of PCB 52 in experiments of this joint research by means of trapping the fraction that evaporated from soil.

Moreover, PCB 52 may have been subjected to photochemical dechlorination when exposed to the soil surface, because it has been shown to be instable when exposed to light (e.g., BAXTER & SUTHERLAND, 1984).

On the other hand, the litter itself is a decomposable substrate and subject to considerable metabolic processes, which may also result in the formation of strong bound residues, which are not released by solvent extraction. It was impossible to determine the amounts of non-extractable percentages of the BaP and PCB 52 residues still present in the litter without radiochemical methods.

The experiment with ^{14}C-labeled compounds conducted to answer these questions suggests that both assumptions are correct. The litter as such was decomposed clearly slower in the isolated Mitscherlich vessels than in the open field. After 9 months, approximately 35% of the litter had been decomposed under semi-field conditions: The decay rate was therefore half that of the field experiment in the RefB area. Compared to the conditions in the open field, colonization by soil animals in the Mitscherlich vessels was limited.

By means of complete combustion of the organic matrix at the end of the experiment, 80% of the applied ^{14}C-activity could be recovered in the litter of the BaP variant, and 50% in the litter of the PCB 52 variant. Thus, reduced biological activity as mirrored by the lower litter decomposition rates may have been responsible for the significantly lower losses of the applied chemicals in the litter of the vessel experiments. As pointed out by KÄSTNER *et al*. (1999), higher biological activity results in higher degradation rates of the monitored compounds, but also in a higher amount of bound residues formed. Both processes result in a smaller fraction of the applied chemicals that can be recovered by solvent extraction.

When solvent extracted by the same methods applied in the experiment with non-labeled substances, only 43% of the BaP but 60% of PCB 52 present in the solid litter matrix could be recovered in the organic phase. The percentage of the substances remaining in the litter, therefore, has to be considered bound residues with respect to the extraction method used in the study. Again, analysis of the recovered organic phase by means of thin layer chromatography revealed that only 60 % of the ^{14}C-activity detected in the BaP variant was still the non-metabolized parent compound. In the PCB variant, non-metabolized PCB 52 accounted for more than 72% of the ^{14}C-activity of the organic phase from the litter extract.

BOYLE *et al*. (1998) and SMITH *et al*. (1999) postulated in experiments with ^{14}C-labeled BaP that the amount of radioactivity remaining in the bulk matrix is in form of metabolites, which during transformation have been bound to the organic matter of the soil. Following this assumption in recalculating the extraction results of the litter exposed in the Mitscherlich vessels, BaP as parent compound would account for 25% of the total activity found in the litter after 10 months. Surely, BaP as parent compounds accounts for 25% of the activity extractable from the litter. Based on the initial activity concentrations, and since 20% of ^{14}C-activity could not be detected neither in the litter nor in the uppermost soil layers underneath by complete combustion, 18.5 % of the applied BaP was found as extractable parent compound in the litter after 9 months.

Relating these recovery rates to the ongoing process of litter degradation and not to the absolute time elapsed in the studies, the decomposition experiment in the field and the one performed in the Mitscherlich vessels become more comparable in respect to the behavior of the studied chemicals. At the point at which 35% of the litter was decomposed in the field (approximately 140 days), the extractable BaP amounted to slightly less than 20% of the initially applied chemical, a value absolutely in the same range of the results of the experiments under semi-field conditions.

In the case of PCB 52, 50% of the applied radioactivity was distributed between the litter and the soil underneath it at the end of the Mitscherlich vessel experiment, but the litter contained only around 30% of this amount. Of the 30% of the activity recorded in the litter, 58% were extractable by solvent. In the organic phase of the extraction, however, more than 70% of the activity was found to belong to non-metabolized parent compounds, which is quite a different picture than the one seen in the BaP variants. There, the amount of activity present at the end of the experiment was clearly higher than in the PCB variant, and it was almost completely confined to the litter, but a smaller proportion of it could be extracted and identified as unmetabolized BaP.

In the end, 13% of the PCB 52 applied in the litter decomposition containers was still parent compound present in the litter layer after 9 months exposure in the Mitscherlich vessels. Again, this value is in the same range as the concentrations of extractable PCB 52 from the litter of the field experiment in the RefB area.

We don't know to which fraction the part of radioactivity that remained in the litter bulk matrix should be assigned, because no further extraction and destruction of the organic matrix by means of sonication or saponification procedures was performed. It has to be assumed, however, that these massive interventions would destroy organic compounds like PCBs and make them unrecoverable from the solid phase.

In conclusion, it can be said that the behavior patterns of PCB 52 and BaP differed from each other as follows:

- In the PCB 52 variants, more activity was mobilized from the litter layer to which it was applied. It was detected in the soil layer underneath the litter and reached soil depths of 10 cm after 9 months, and 35% of the total recovered activity was found in the soil layers down to 5 cm. In contrast to this distribution profile, 94% of the recovered activity in the BaP contaminated variants was detected in the litter layer at the end of the experiment, only 6 % had moved into the soil layers.
- The extractable fraction of parent compounds from the litter was higher for PCB 52 than for BaP. After 9 months, more than 72% remained as unmetabolized PCB 52, but unmetabolized BaP accounted for only 60% of the recovered activity.

- The losses of applied activity were greater in the PCB 52 variants than in the BaP variants. 80% of the initial activity was detected in soil and litter contaminated with BaP, but only 50% was recovered from the PCB 52 experiments.
- Monitoring the decreasing curves of the selected reference substances in the litter of decomposition experiment in the RefB area revealed merely slightly higher loss rates of extractable PCB 52 than of BaP. The survey of the radiolabeled substances demonstrated that BaP is more strongly bound to the litter matrix and therefore not extractable; PCB 52, by contrast, is actually lost from the system.

In the soil, the maximum losses for extractable PCB 52 were 30% after 6 years; In the litter of this study 90% was lost after 1 year of exposure. Because the soils, in which the storage stability was determined, were kept under conditions quite dissimilar from field exposure (outdoors, but shielded from rainfall and slowly drying out) it is appropriate to also consider the results from a laboratory study of MARSCHNER (1997), who performed column experiments with soils from the joint research project lasting 14 months. The maximum losses attributed to parent compounds leaching from the columns were found in the acidified variants and accounted for 0.2 % of the initial loads of PCB 52 and 0.0002 % of initial amounts of BaP. The total amounts recovered by solvent extraction after the experiments were higher for PCB 52 than for BaP (almost 100% and 63%, respectively). If this appears at first sight to stand in contrast to the litter extraction results presented in this work, it should be noted that MARSCHNER (1997) points out volatilization as the major source of PCB 52 losses from contaminated soils during storage in the field (i.e., CHIARENZELLI *et al*., 1997). During his experiments, however, the soil was filled in columns with small surface area and high water saturation. The potential for volatilization losses was, therefore, much smaller than from the litter loosely exposed on the soil surface in decomposition containers.

As seen in the litter extraction, when PCB 52 was not lost from the system, it was almost completely extractable as parent compound. BaP, by contrast, has a lower water solubility (see the minor leaching losses in MARSCHNER, 1997, and the negligible transport from litter into the soil in the present work) and lower vapor pressure. The substance is conservative in respect to mobilization, but it is readily adsorbed to the organic matter of soil and litter. Lower recovery percentages are in the case of BaP due to lower extraction efficiency, but parent compounds and metabolites are still present in the organic matrix, as demonstrated in the ^{14}C-experiments of this thesis.

4.2 Response of the Decomposer Community to BaP and PCB 52 contamination

4.2.1 Litter Decay

The second aim of this project was to assess the process of litter decomposition as influenced by the reference substances BaP and PCB 52. To start with, a closer examination of the decay process of the selected litter as such is provided.

In the central investigational area RefB, the decomposition of grass litter was a slow process. Decay rates similar to those found in the former sewage field with the typical grass species *Agropyron repens* would be those of straw in colder climates as described by KANAL (1995) and SMITH & JACKSON (1987). In comparison to the quack grass litter examined in the decomposition experiment in the RefB area, however, the barley, rye, and wheat litters examined in those studies (*Hordeum vulgare* L., *Secale cereale* L., *Triticum aestivum* L.) exhibited a far higher C/N ratio (95-140 vs. 45).

DICKINSON (1983) found daily decomposition rates between 0.41% and 1.09% for the grass species *Agrostis tenuis* Sibht., *Holcus lanatus* L., and *Antoxanthum odoratum* L. The lowest decay rates observed by DICKINSON (1983) occurred during a dry summer period, as it was the case in the present study, but they were twice as high as the initial decomposition rate of the *A. repens* litter in the RefB area.

CORNELISSEN (1996) reported an average decomposition of 40% after 4 months for Graminaceae. Considering that those results were found in studies utilizing gauze bags with fine mesh sizes (0.3 mm) and that the exposure took place during winter, a period with lower biological activity, the comparable decomposition (approximately 30% after 4 months) for quack grass observed in containers with a coarse mesh size of 10 mm starting in spring seems rather low.

On the one hand, no differences were observed in the decomposition of the quack grass litter in medium and coarse gauze containers in the RefB area. This can be attributed to the fact that the sewage field soils might rank among the “mesofauna type” of soils (SCHAEFER, 1990), which display only low abundances of macroarthropods, and, therefore, show no further litter losses from the coarse gauze containers that may be attributable to the presence of bigger soil animals. This assumption is supported by the extremely low Lumbricidae colonization densities observed in the investigated sewage fields by KRATZ & THIELEMANN (1994). The limited species diversity in the enchytraeid population (HECK & ACHAZI, 1995), however, suggested that the structure of the mesofauna in the sewage field soils was disturbed as well. The survey of the microarthropod fauna of different sewage field areas, the result of which will be discussed in section 3.4.2, reveal a poor species diversity for Oribatida and a disturbed Nematoda population structure, too.

At single sampling dates, the litter mass loss was even higher in the containers lined with medium gauze than in the coarse gauze containers. Several authors refer to the improvement of the moisture condition in the finer gauze containers that prevent the litter from drying out. This assumption is supported by the higher water content of the litter in the medium gauze containers in the RefB area at those sampling dates at which the decay rates were higher than in the coarse mesh containers.

The moisture conditions in the summer period shortly after exposure of the litter put a constraint on the decomposition process. This was elucidated in the correlations between the decay rates of the litter in the coarse mesh containers at the single sampling dates and the amount of precipitation during the corresponding periods. The highest decay rates were observed in the fall at the end of the dry summer period, an infrequently reported result considering that the decomposition process is assumed to follow an exponential mass loss curve with the fast initial phase accounting for the commonly observed higher decomposition activity of easily degradable compounds. The higher mass losses in the fall - compared to the slow litter degradation in the summer - could be also related to the onset of a late leaching phase triggered by the beginning rainfall. Losses due to abiotic mobilization of soluble compounds from the litter and washing out of small organic particles can account for 20 % of the weight loss during the first weeks of exposure as described by TIETEMA & WESSEL (1994). Microorganisms will have taken up especially the easily degradable litter compounds by the late sampling dates.

Regarding the experimental contamination of the litter, the non-detectable effect of PCB 52 is remarkable. As mirrored in the chosen lower initial spiking loads for the PCB 52 variants as compared to the BaP variants, it was expected that the chlorinated compound would display a higher toxic impact on the soil decomposer community than the condensed hydrocarbon with no functional group substitution.

On the other hand, initial loads of PCB 52 were evidently lower than aimed for, and extractable concentrations dropped sharply after short exposure of the litter in the field. Since there are no reported data on the impact of PCBs on the decay of organic matter, evaluation of the results from the experiments in the RefB area and in the Mitscherlich vessels (NOEC in both experiments ≥11.5 mg PCB 52 x kg^{-1} litter) is difficult.

In the joint research project, WILKE & KOCH (1996) reported a negative impact of PCB 52 on the dehydrogenase activity in the spiked RefB soil (LOEC = 39 mg PCB 52 x kg^{-1} soil). The contamination of soil without prior pollution and with lower organic matter content (no irrigation with sewage water) resulted in a lowest observable effect concentration of 4.9 mg PCB 52 x kg^{-1} soil, which is 1/10 of the LOEC for the RefB soil. BROSE *et al*. (1997), on the other hand, could measure only a stimulation of the activity of microorganisms related to the transformation of nitrogen in soil. The nitrification was enhanced by PCB 52 when measured

in spiked RefB soils that were exposed in the field for several months (LOEC 2.0 mg PCB 52 x kg^{-1} soil). In test systems with agar as growth medium, NEUMEISTER *et al*. (1996) found inhibition in the growth of the fungus *Arthrobothrys oligospora* at rather low PCB 52 concentrations (LOEC 1.0 mg x kg^{-1} agar), but the effect could not be detected when adding RefB soil to the test matrix.

Relating these outcomes to the number of experiments conducted in the joint research project and resulting in no detectable effects up to the maximum tested concentrations (40 mg PCB 52 x kg^{-1} soil), the activity of microorganisms in the soils from the sewage fields seems not to have been severely impacted by PCB 52.

Unlike PCB 52, BaP had an effect on the litter decomposition in the field: it enhanced it. The contaminated litter from the higher spiking BaP 2 variant (initial concentration 100 mg x kg^{-1} litter) exhibited a higher degradation rate than the control litter. After 6 months of exposure in the RefB area, 18% more of the remaining litter was found in the untreated controls than in the treated variants. During the second half of the experiment, however, the untreated litter was decomposed more quickly than the litter contaminated with BaP. At the end of the experiments, the differences in the quantities of litter present in the containers of control and BaP variants amounted to 12 %.

Fitting double exponential equations to the remaining litter weights resulted in decay rate constants that were only slightly higher for BaP litter than control litter. The fraction of the litter, however, that was decayed at these rates, was clearly larger in the BaP treatment than in the uncontaminated litter. While the fitting of double exponential models resulted in the determination of two decay rate constants in the control litter, accounting for a fast decaying and an extremely slow decaying fraction, respectively, exponential decay models for the BaP contaminated litter pointed to a smaller amount of residual litter at the end of the experiment than it was the case for the uncontaminated grass, but this residue seemed not to be decayed any further.

This may explain why the two variants at the end of the experiments approached each other. As VAN NOORDWIJK (1996) summarized the research results on litter decomposition, it is still subject to debate whether slower decaying material contributes more to soil organic matter pools than fast decomposing material, because slowly decaying litter "may contribute to the same pools, but more slowly".

In regard to the amount of readily degradable compounds in the *A. repens* grass litter these considerations can be interpreted as follows: If those compounds are decayed faster in the litter contaminated with BaP than in the controls, the stage in the decomposition process at which the remaining compounds are recalcitrant to degradation is faster reached. When assessed at the same time intervals, the control litter will, in contrast, display higher remaining amounts of

degradable litter constituents, which still are fraction of that part of the decomposition process measurable by mass loss.

Similar time courses in litter decay curves of matrices contaminated with organic compounds have been reported, e.g., by PAULUS *et al*. (1999) and HEINZE *et al*. (2001). The effects of the plant protection compound Dimilin (diflubenzuron) on the litter decay rates became smaller towards the end of the experiments and "a normalization in litter decomposition" was evident (PAULUS *et al*., 1999). In the investigation of HEINZE *et al*. (2001), the insecticide Dursban (chlorpyrifos) had an enhancing effect on wheat straw and mustard litter decomposition. After 6 weeks of exposure, the contaminated variants showed higher decay rates, but 8 weeks later the amounts of remaining mustard litter in the control containers were the same as in the contaminated variants. Straw was decomposed more slowly (30% in 100 days) and at the end of the experiment the enhanced litter decay was still evident in the different litter weights, even though here, too, the differences were becoming smaller. As HEINZE *et al*. (2001) state, "the effects were earlier detectable in the mustard litter because it decomposes faster".

The authors of the above-cited studies relate the recovery of the decomposition process to the decrease and, finally, to the disappearance of detectable fractions of the applied organic chemicals. In the investigations in the RefB area, a possible relationship between the decreasing extractable BaP concentrations in the litter over time and the shape of the decomposition curves can be assumed too.

After approximately 200 days of litter exposure in the field, the differences between the controls and the contaminated variants were most pronounced. At this point, however, the percentage of extractable BaP from the litter had already clearly decreased. However, it has to be assumed that the ecosystem is somewhat slow in expressing the effects caused by BaP contamination. If this is the case, at the beginning of the experiment the differences between controls and contaminated variants should be small, grow to be visible as time elapses, and later become smaller again. “Effect curves” like that are single peak curves skewed to the left, with the initial positive slope being controlled by the uptake rate of the substance into the responding system and its resistance, and the negative slope then being controlled by the decrease of the chemical’s concentration in the environment and the elasticity of the system.

Similar relationships have been described based on observations of the time course of body burdens in soil animals exposed to degradable chemicals, that is, to a "diluted pulse" of chemical loads (WIDIANARKO & VAN STRAALEN, 1996). The uptake of the substance in the organisms is reduced over time, but the internal concentration reaches a delayed maximum compared to the decreasing concentrations of the chemical in the environment. This means, that the probability for the occurrence of a response to the chemical is not at its highest at the beginning of the experiment. Instead for an individual organism, here I express these relationships for a complex system: Regardless of which members of the decomposer

community react to the BaP contamination, the strength of the effect that BaP has on the decomposer community over time is described by an “effect curve” which is characteristic for a diluted pulse type of contamination and mirrors the decreasing environmental concentration of the active chemical.

4.2.2 Response of Soil Organisms

But how do the organisms involved in litter decomposition react to a contamination with BaP and PCB 52 at the species level? One further aim of this project was to detect possible effects of the investigated chemicals by means of the survey of the faunal densities in the contaminated and control litter decomposition containers in the RefB area.

The determined Collembola and Oribatida species exhibited clearly distinct distribution patterns in the litter from the various contamination variants. I have classified the different patterns into 3 modes of response. Some species colonized the BaP spiked litter with higher abundances than the control litter (Group I). These included drought-tolerant collembolan species (e.g., *Entomobrya multifasciata*) and those oribatid mites that were present in the litter throughout the study period.

Considering all sampling dates, other species were not able to establish high population densities in the litter contaminated with PCB 52. At the same time, there was no difference between the control and the litter of the BaP variants (Group II). Except for the collembolan *Isotoma anglicana*, though, the species responding in this way generally displayed low colonization densities compared to the soil animals described as belonging to the first group. When looking at the time course of the grass litter colonization, it can be seen that the populations of the species reacting to PCB 52 collapsed during the summer (dry period sampling dates) and apparently did not re-colonize the PCB contaminated litter in fall.

Explicitly euryoecious species such as the Oribatida *Tectocepheus sarekensis* and the Collembola *Isotoma notabilis* did not avoid the highly contaminated litter of the BaP and PCB 52 variants (Group III).

The influence of the diversely structured Collembola and Oribatida populations in the different variant on the litter decomposition process is difficult to assess. The decreased abundances of some species in the PCB 52 contaminated litter is not reflected by lower decomposition rates – those variants did not differ in this regard from the controls. It has to be taken into account that the potential effects of the PCB 52 contamination on the microarthropod population manifested themselves only at the later sampling dates, and that the decomposition process by that time had already slowed down considerably.

In the BaP contaminated litter, for any and all of the species examined, the abundances were either similar to those found in the controls or decidedly higher. Not one species was found that responded with decreased colonization densities to a contamination of the litter with BaP.

This observation agrees with the results of monospecies test from laboratory studies in invertebrates performed by VAN STRAALEN & VERWEIJ (1991) and VAN BRUMMELEN *et al.* (1996). In toxicity assessments for 5 different PAHs with the isopods *Porcellio scaber* and *Oniscus asellus*, the authors demonstrated for higher condensed PAHs, and especially for BaP, an enhancing effect on reproduction as evidenced by an elevated number of healthy juveniles. The effect was dose-related, with a LOEC of 31.6 mg BaP x kg^{-1} food and maximum increase in brooding success of 43 % compared to the control at 360 mg BaP x kg^{-1} food.

In the laboratory tests performed within the scope of this study with the collembolan *Folsomia candida*, an enhancement of the reproductive performance could also be observed. In the test, carried out 2 years after the soil had been spiked with BaP and PCB 52, the increase in the number of juveniles compared to the control was in the BaP contaminated soil 78% and in the PCB 52 variant 84%. In this first test run, only one spiked variant per compound was tested: The LOEC is here given as statistic significant difference to the control and amounts to 2 mg PCB 52 x kg^{-1} soil and 10 mg BaP x kg^{-1} soil.

In the second run of the test, six years after the soil spiking, *F. candida* did not show a concentration related response neither to increasing loads of PCB 52 nor to those of BaP. The spiked soil differed from the controls inasmuch as the reproduction of the Collembola was reduced to 80% of the values in the RefB control and artificial soil.

The enhancement of the reproduction rates observed for Collembola in the contaminated soils of the RefB area 2 years after spiking might be related to an interference of the studied compounds with the hormonal balance of the animals. The clear enhancement of reproduction in isopods when exposed to food contaminated with BaP was not related by VAN BRUMMELEN *et al.* (1996) to so called hormetic effects, since this stress reaction often observed at subacute activity levels of chemicals does not normally show a dose-response relationship, but disappears at higher concentrations. It is possible that the structural similarity of higher condensed polycyclic aromatic compounds with steroid hormones is responsible for the induction of the observed effects. Phenolic metabolites of PAHs are known to interfere with hormone receptors, and soil animals have been shown to posses detoxifying enzymatic systems capable of metabolizing organic chemicals (ACHAZI *et al*., 1998). But, as VAN BRUMMELEN *et al.* (1996) pointed out in 1996, the direct impact of BaP on the endocrine system is not demonstrated: "So far most of the work is on vertebrates, and the evidence is only circumstantial". However, the metabolization of PAHs into compounds mimicking hormone structure may occur outside of the animal organisms, as PANTER *et al*. (1999) demonstrated for non-estrogenic steroid metabolites in sewage effluents: By minimal bacterial activity they were converted back into a more potent, active estrogen form.

Effects on the hormonal balance of several animal species are known for PCBs as well (see, e.g., BROUWER *et al*., 1999; LEISEWITZ & KAMRADT, 1996). However, within the framework of

this project they were observed directly only in the laboratory experiments. In the field, an increase in microarthropod colonization densities in the grass litter contaminated with PCB 52 that could be related to the higher reproduction rates of *F. candida* in the laboratory tests with contaminated soil could not be observed. It should be remembered, however, that the initial extractable concentration for PCB 52 amounted to half of those determined in the soil and they decreased very fast during exposure of the litter in the field.

The observed increased colonization density of microarthropod species in the BaP variants may have contributed to the higher losses of litter mass, since soil animals do exert a controlling function over the decomposition process through litter comminution, browsing of fungal mycelia and enhancement of microorganism activity. The differences in the litter decomposition rates between the control variants and the litter contaminated with BaP are consistent, but they are of an order of magnitude that may well be attributable to a higher mesofauna activity (e.g., BEARE *et al*., 1997; TIAN *et al*., 1998).

However, because microbial activity accounts ultimately for the mineralization of organic matter, the results of the accompanying microbiological studies to the decomposition experiment are of special interest. BROSE *et al*. (1997) studied the succession of the litter colonizing micro fungi in the different variants of the litter decomposition experiment in the RefB area. When comparing the litter contaminated with BaP to the control litter, a shift in the dominance percentages of the species present could be demonstrated. No differences were detected between the uncontaminated litter and the PCB 52 variants.

Clearly saprophytic species that become more important during the course of the degradation process are found earlier and with a higher colonization index in the BaP 2 contaminated litter compared to the controls. The total population percentage species following this response pattern represents more than 30% of all isolates studied. On the one hand, some fungal species were already decreased in numbers at the late sampling date in the BaP variants, while in the controls they only then reached the climax of their colonization intensity. On the other hand, species whose fructification frequency decreases anyway as the decomposition process progresses, were observed with lower frequency in the BaP contaminated litter than in the controls. These observations may be indicative of an accelerated succession due to the more advanced degree of litter decomposition in the BaP variants.

Interpreting these results as merely accelerated succession due to the faster decomposition, though, suggests that the fungal population only reacted to the accelerated substrate decay, as evidenced by the appearance of species characteristic for later colonization phases, and that the population itself was not a controlling factor in the decomposition process. However, since organic chemicals do present a potential growth substrate for microorganisms, it is well conceivable that species with special enzymatic makeup were selectively promoted. A shift in

microorganism population structure has been reported in soils following oil spills (e.g., MACNAUGHTON *et al*., 1999).

Reviewing potential biological degradation pathways for PAHs, KÄSTNER *et al*. (1993) differentiated between complete mineralization (bacteria, intracellular), co-metabolic degradation (bacteria and fungi, mostly intracellular) and degradation caused by unspecific radical induced oxidation processes (fungi, extracellular). A possible selective use of BaP from the litter matrix by bacteria and fungi able to intracellularly co-metabolize aromatic compounds could explain the increase in litter decomposition only if the litter itself provided the co-substrate needed for the initial enzymatic BaP attack. On the other hand, oxidation by extracellular, extremely reactive but rather unspecific, so-called lignolytic enzymes might result in an additional break down of the litter matrix to which the spiked substances were bound.

Altogether, the enzymes involved in the metabolism of PAHs are not dependent on a specific nutritional status of the microorganism environment, unlike, e.g., the process of lignin degradation itself that requires N-limiting conditions. They have been shown to be inducible in bacteria and fungi by exposing the substrate to single PAH compounds prior to degradation experiments or cross-induced by the simultaneous presence of additional PAHs (e.g., BOYLE *et al*., 1998; MOLINA *et al*., 1999). Moreover, the presence of extremely reactive enzymes has been detected not only in highly specific individual microorganisms strains but also in a variety of soil inhabiting fungi and bacteria (RODRIGUEZ *et al*., 1996; SALICIS *et al*., 1999). The ability to degrade PAHs in fungal-bacterial co-cultures from sites with a history of pollution may be even more pronounced than the efficiency of species that are known to achieve good degradation results in laboratory enrichment cultures but are not competitive in the soil environment in the field (BOONCHAN *et al*., 2000; COLOMBO *et al*., 1996; RAVELET, 2001).

The fungal species characteristic for later stages of the decomposition process may have been found in the BaP contaminated litter at earlier sampling dates because of their specific enzymatic makeup: They gain a competitive advantage over species utilizing only readily available substrates by means of their capability of breaking up recalcitrant compounds. The highly reactive extracellular enzymes synthesized in this process possess the same activity mechanisms as those catalyzing the oxidation of BaP.

It should be remembered that the litter contaminated with BaP displayed an increase in the percentage of nitrogen immobilized compared to the control and the litter contaminated with PCB 52. Control and PCB 52 variants were very similar in respect to the C/N ratios of the litter matrix.

Other results on microorganisms' activity from the joint research project are given by BROSE *et al*. (1997), who determined - as for PCB 52 - an enhancement of the nitrification activity in RefB soil spiked with BaP, but a reduction in respiration measured after cellulose addition.

Both measurement endpoints had a LOEC of 100 mg BaP x kg^{-1} soil. WILKE & KOCH (1996) determined a LOEC for a reduction in dehydrogenase activity of 10 mg BaP x kg^{-1} soil.

4.2.3 Conclusions

Summarizing the gained insight in the reaction of the decomposer community to a contamination of the grass litter with benzo(a)pyrene and PCB 52, a scheme may be drawn, in which the response of the measured endpoints are related to the process of litter decomposition.

The effects of benzo(a)pyrene on the decomposer community are shown in Figure 31, next page.

Micro fungi colonizing the litter react with a shift in their colonization pattern: Clearly saprophytic species (30% of all isolates) are found earlier and with a higher colonization indexes in the BaP contaminated matrix. Their direct effect on the decomposition could have been the utilization of the litter as co-substrate for the initial transformation of BaP, as well as the decay of the litter matrix to which the organic compound is bound by means of PAH oxidizing extracellular enzymes.

An enhancement of the microorganisms colonization in the litter contaminated with BaP can affect the individual densities of microarthropods, since especially fungi are preferred food of many microphytophagous soil animals. This could explain the colonization of the litter by microarthropod species displaying clearly enhanced individual numbers as observed in the grass litter contaminated with BaP.

Benzo(a)pyrene can also influence directly via mimicry of steroid hormone structure the reproduction rate of soil animals, as observed in the laboratory, and be therefore directly responsible for the increased litter colonization.

Higher animal densities and activity affect directly the decomposition process by comminuting higher amounts of litter. Indirectly, higher microarthropod grazing pressure on fungi mycelia and the enhanced transport of microorganisms' propagules may in addition enhance the activity of fungi and bacteria in the litter.

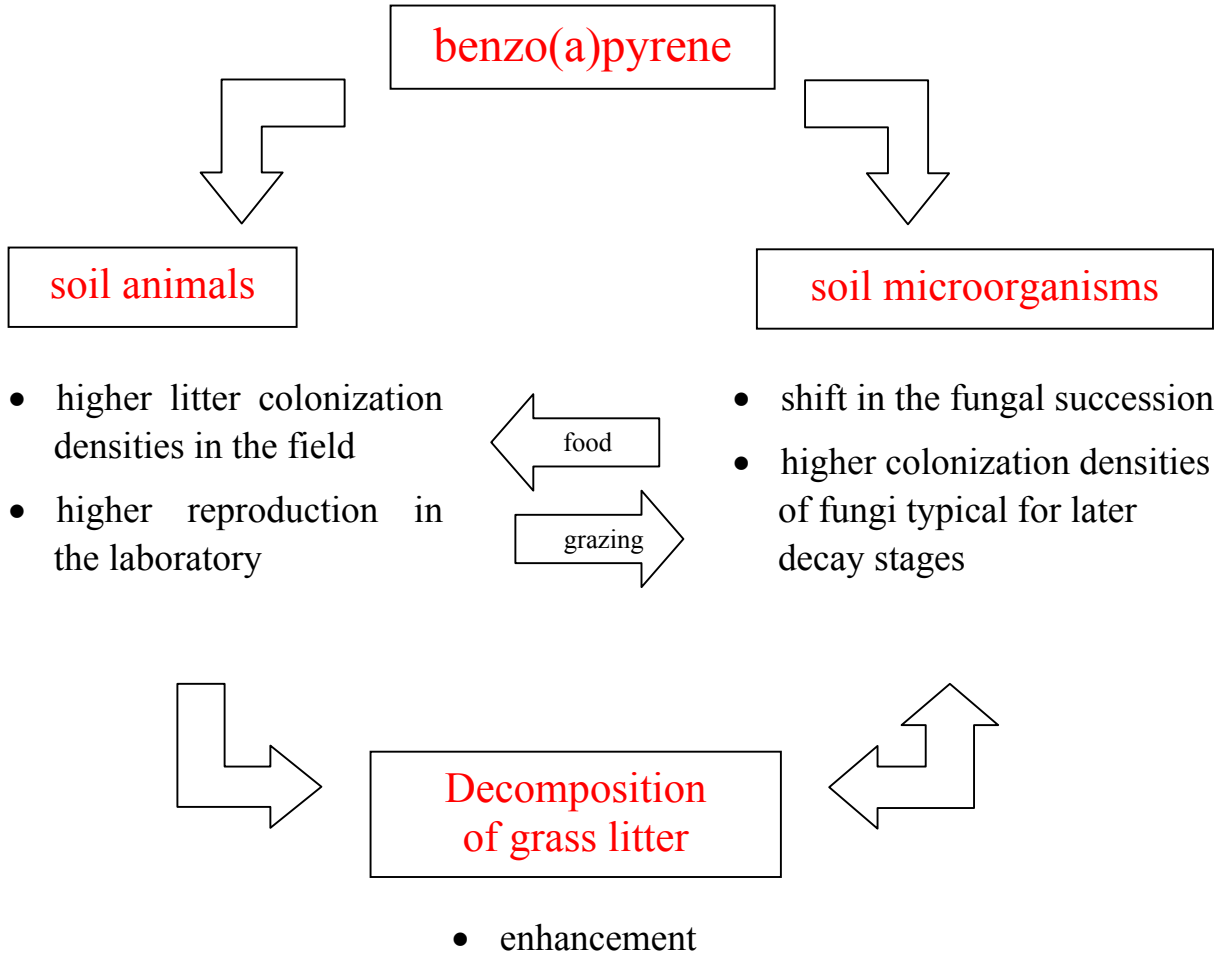


Figure 31: Scheme of the effects of a litter contamination with Benzo(a)pyrene on the decomposer community as related to the response of the litter decomposition process

In contrast to BaP, PCB 52 showed no effects on the litter decay of *A. repens* grass (s. Figure 32, next page). The scheme of the interactions between soil animals and soil microorganisms in the decomposer community is unchanged, i.e., the mutual influence mechanisms on the litter decomposition process. The direct effect of PCB 52, though, is undetectable in the field.

The micro fungi colonized the litter in a pattern similar to the control. An effect of specific species on the litter decay process is on account of the results in the joint research project improbable, but not impossible, since only a part of the litter microorganisms were surveyed.

In the field, Collembola and Oribatida showed no rapid reaction to a contamination with PCB 52, only at the sampling dates in fall, species with low dominance did not recolonize the litter of the PCB variant. The outcome of reduced microarthropod densities is not reflected in differences in litter remaining compared to the control. The decomposition process was, though, approaching the slow decay phase with low mass loss that persisted till the end of the experiment.

The enhanced reproduction of the Collembola *F. candida* measured in the laboratory trials did not find any correspondent effect in the field. The exposure to PCB 52 in the laboratory test occurred though the soil matrix, since clean food was provided. This could be an indication that the influence of organic compounds on soil animals are not based solely to an enhancement of food resources through microorganism colonization increases.

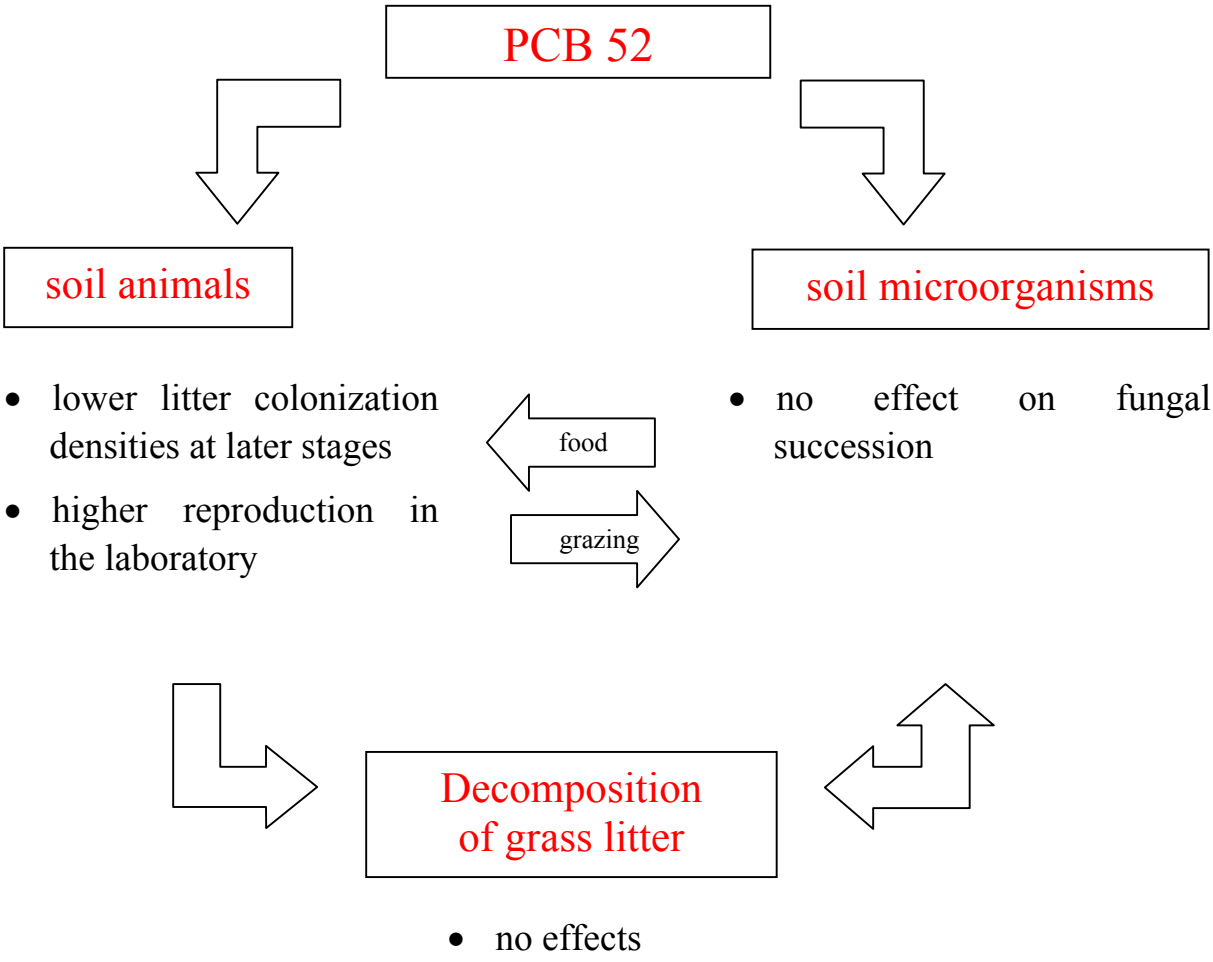


Figure 32: Scheme of the effects of a litter contamination with PCB 52 on the decomposer community as related to the response of the litter decomposition process

The apparent LOEC for PCB 52 in the field ranks between 2 and 20 mg x kg^{-1} soil. Applying a simple constant security factor of 100 as recommended by the FAME method of the US-EPA (1984) or the European Community (CEC, 1996), the protection of the decomposer community from adverse effect caused by a contamination with PCB 52 would be accomplished if soil concentrations do not exceed 0.02 mg x kg^{-1}. This is a very conservative estimate, since the performance of decomposition tests did not reveal any adverse effects up to a concentration of 11.5 mg PCB 52 x kg^{-1} litter.

Should these result be recalculated in base of the organic content of the matrix, though, the estimated LOEC for the soil would lay clearly lower compared to the LOEC for the litter: approximately around 0.2 mg PCB 52 x kg^{-1}soil.

The FAME method allows for the employment of a security factor of 10 in the case results from field trials are available. The calculation of a safe level for the soil decomposer community derived from the decay experiments delivers again a Predicted No Effect Concentration (PNEC) of 0.02.

For benzo(a)pyrene, the LOEC determined in the field was between 10 and 100 mg x kg^{-1} . The extrapolation based on the FAME method with a security factor of 100, by which the lowest obtainable effect concentration should be divided, results in a PNEC of 0.1 mg BaP x kg^{-1} soil. Again, by evaluating the impact of BaP on an integrated process as the decomposition of organic matter, the recalculated LOEC accounting for the lower organic matter content of soils would lay around 1.0 mg x kg^{-1} and the PNEC derived applying a security factor of 10 around 0,1 mg BaP x kg^{-1} soil.

Comparing these results to the actual concentrations of benzo(a)pyrene and PCB 52 found in the investigated sewage field areas near Berlin (Germany), and assessing the ratio of the Predicted No Effect Concentration (PNEC) to the actual Predicted Environmental Concentration (PEC), reveals that the ratio is nearly 1.

A ratio far below 1 is estimated as fairly sure, but values approaching 1 indicate an increased level of concern regarding the potential occurrence of harmful changes to protected targets. Concerning their loads with organic contaminants, the sewage field soils have no security margin in respect to the compliance of the protection targets "soil as habitat for soil organisms" ("*Lebensraumfunktion*", see p.19) and "soil as medium for decomposition and transformation" ("*Transformatorfunktion*").

4.3 Comparison of Sewage Field Areas with Different Contamination

The faunistic studies that were conducted in former sewage field areas displaying different soil pollution loads and profiles were intended to facilitate a comparative assessment of the current state of long-term contaminated areas with the results of the experimental contamination of soil and litter matrices with PAHs and PCBs.

The areas investigated were selected in base of their outstanding contamination profiles regarding the soil pollution with PAHs (sewage field "nPAK") or PCBs (sewage field "nPCB") in comparison with the central investigational area of the joint research project "RefB". Soil characteristic of the investigated sewage field are listed in Table 1, p. 35. Surveyed was the composition of the oribatid mite and of the soil nematode fauna.

Considering the structure of the oribatid cenosis first, definite differences between the three study areas are obvious.

The reference area of the joint research project, RefB, which at the time of this writing presents itself as a closed quack grass meadow in spite of several reforestation attempts, is characterized by index species of the synusiae of meadows and pastures. The species spectrum, however, is reduced, and the cenosis is dominated by a single euryoecious species. The vast majority of the oribatid mite species is also capable of colonizing dry habitats such as moss carpets on walls or roofs (see Annex I; STRENZKE, 1952; MORITZ, 1963; RAIJSKI, 1967, 11968; WEIGMANN & KRATZ, 1982).

The poplar covered area nPAK exhibited the highest species diversity of oribatid mites, and its cenosis was of a pronounced transitional character from the synusia of the forests to that of the meadows. This was certainly due to the management history and the heterogeneous structure of the vegetation of this habitat compared to those of the grass-covered areas. The reforestation had taken place not long before the study was started, and the soil characteristics, especially the structure of the organic layers were, e.g., in comparison to the RefB area, only slightly altered. An increase in typical forest species may be expected in the future, and the character of the synusia observed at this stage may very well represent merely a transitional period. On the other hand, it is possible that the oribatid mite community will preserve its transitional state over a longer period of time, due to the isolated position of the habitat in terms of its vegetational structure. The isolated position may result in a continuous invasion of species of the synusias of the meadows and pastures from the neighboring open meadow areas, which resemble in their species spectrum the RefB area.

The significantly higher contents of PAHs and heavy metals, especially of lead, in the soils of the nPAK area did not result in an observable species impoverishment of the oribatid mite cenosis compared to the fauna of the RefB area.

The topmost soil layer of the partially bare nPCB area, in contrast, was hardly colonized by oribatid mites at all. The few, mostly euryoecious species that could be found were only detected in samples from spots that had at least some grass cover. Although these findings might be attributed to the patchy vegetation structure, the same conclusion cannot be drawn regarding the drastically reduced nematode densities observed in these soils as well.

Nematodes that, unlike the oribatid mites, are euedaphic, were found in similar densities in the RefB and nPAK soils. Their population structure as well was largely similar for the significantly differently covered areas. The lack of a vegetation cover in the nPCB area is clearly a cause of the reduced number of plant parasitic nematodes found here, but the minimal nematode densities as such are more likely to be a result of the different contamination profile of this soil.

This assumption is supported by an analysis of the survival strategies of the nematode species found. Here, euryoecious pioneering species were observed that clearly differed from the "nutritional opportunists" of the RefB and nPAK areas. While the latter document an eutrophication of the studied areas, the dominance of the very resistant "generalists" found in the nPCB field suggest a long-term soil pollution (KORTHALS *et al*., 1996).

The results of the faunistic study are in agreement for all of the animal groups included. Based on its habitat, the nematode population characterized the former sewage fields as disturbed and eutrophied areas. Inclusion of the oribatid mite cenosis allowed for a more differentiated image, which documented the effects of recent measures as well, such as the reforestation of the nPAK area. However, in the case of extreme habitats as the vegetation free spots in the nPCB field, no oribatid mites were present. Here the survey of the nematode fauna enables the detection of a pollution response that deviates from the typical reaction to eutrophication found in the soils of the central investigational area RefB and on nPAK.

The accompanying assessment of the feeding activity of the soil fauna by means of the bait-lamina test confirms and complements the results of the faunistic survey.

All investigated former sewage field display a low feeding activity as assessed by the bait-lamina test. Only the reforested area nPAK reached values of an overall feeding activity above 10 % normalized to 28 days. BODE & BLUME (1997) found that if soil moisture is sufficient, fertilization measures would have a positive impact on the feeding activity. In the dry weather periods the lack of water masked the effect of the addition of liquid manure, and the feeding activity was similar to the values found in the nPAK area, but with an exposure lasting only 10 days. BAYER & SCHRADER (1997) found an average feeding activity of 10% within 10 days of exposure in a gray soil on loess. The feeding activity was reduced by heavy soil compaction caused by cultivation measures, resulting in minimum values of approximately 6% within 10 days when heavy equipment was used frequently.

As for the results of the soil fauna survey, no adverse effect could be detected in the nPAK areas; on the contrary the feeding rates were 4 times higher than in the soils of the RefB or the nPCB field. LARINK (1994) assessed too an enhancement in the feeding activity in soils treated with organic chemicals: In his experiments, the application of two different plant protection products resulted in an clear increase of the number of fed baits. FEDERSCHMIDT & RÖMBKE (1994) tested the impact of carbendazime in meadows and found no detectable influence when comparing the overall feeding rate of treated plots and controls. However, the depth distribution of the soil fauna activity was changed: The uppermost soil layer was avoided by the animals, and this resulted in a higher feeding rate in deeper soil layers.

This was tested for the results obtained in this study from the different contaminated sewage field areas. Again, the distribution profile in the field nPAK showed the typical shape for uncontaminated plots, with highest feeding rates in the topmost soil layer decreasing with soil

depth. Only in late fall the distribution of the feeding activity in the soil showed a reversed shape as compared to the spring period.

This agrees with distribution data for oribatid mites in soil samples from different microhabitats in the sewage field areas studied. The “valleys” and “ridges” in the topsoil that had been created by plowing of the sewage fields were populated with different abundances during different seasons. In fall, the majority of the oribatid mite species studied was only found in the “valley” samples, while the “ridges” remained unpopulated. In spring on the other hand the by approximately 20 cm elevated “ridges”, were preferred. A pronounced downward migration at the onset of colder weather is known for many soil animals (e.g., STRENZKE, 1952; RAJSKI, 1967, 1968).

Considering the results of the fauna composition and activity survey in sewage field differently contaminated and with a long-term pollution history, the contamination of the soils with PAHs up to a load of 3.2 mg x kg^{-1} did not result in a detectable impairment of soil fauna activity nor of its diversity. The management history had a far higher impact on the development of a soil structure that offers soil animals improved habitat conditions: Shading of the soil, higher quality litter, and a developed organic layer.
The investigation of soils contaminated with PCBs up to a concentration of 0,8 mg x kg^{-1} showed a response of the soil animals, but a definitive clarification of the factors causing the patchy, partially bare soil structure in the nPCB field could not be settled.
Interestingly, the assessment of the faunal feeding rates in the sewage field soils illustrated that the absolute amount of animal present did not determine the height of the activity: More diverse communities showed the higher feeding rates, even displaying lower individual densities.

4.4 Future research

The investigations performed in the joint research project and within the aims of this thesis resulted in a marked increase in the amount of available information on the impact of PCB 52 and benzo(a)pyrene on soil organisms and soil processes. Nevertheless, especially for PCB 52, the data set is still meager and doesn't allow for a sound application of risk assessment methods that are based, e.g., on the sensitivity distribution of the species living in the soil.

Therefore, further research on the effect of organic chemicals apart from plant protection products in terrestrial ecosystems is asked for. In the evaluation of the results of this thesis, special issues turned out to be recurring questions. Surely, as VAN STRAALEN & LØKKE (1997) pointed out, "some questions asked by ecotoxicologists seem to return over and over without the prospect of an answer. It could be argued that these questions may not be the right ones, and that we should ask other questions instead". Nevertheless, when addressing new experiments following issues might be taken into consideration:

- **It is still unclear how the loss of single species affects the proper functioning of soil processes**. The discussion focuses to-date on the relative importance of species as structural units as opposed to the different functions a specific species exerts when involved in soil processes.
- **The role of the soil fauna in the sorption, desorption and transport of toxic compounds in soil is completely unknown**. Through comminution of the organic matrix, sorptive organic molecule as, e.g., dissolved organic carbon, may be released from the bulk soil and increase solubility and transport of organic contaminants in soil.
- In evaluating the results of the experiments with BaP and PCB 52, increased responses of the observed endpoints were addressed to in the same manner as decreased responses. This procedure has been controversially discussed. But, as BENGTSSON (1998) points out, if high decomposition rates were in the past generally assessed as good, the latest discussions on conserving the stability of the soil carbon storage may reverse the situation. **The assessment of process rates enhancements for the stability of soil ecosystems should be cleared**.
- Increased sorption of hydrophobic organic contaminants over time has been occasionally taken in consideration in risk assessment procedures for contaminated sites (e.g., KELSEY & ALEXANDER, 1997). However, it would be of great usefulness if this issue could be related to the time course of toxicity of organic compounds as PAHs and PCBs for soil animals. As proposed by VAN STRAALEN & VAN RIJN (1998) for pesticides, **an ecotoxicological recovery time may be calculated for hydrophobic organic contaminants due to the degradation or transformation of the parent compounds**.

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6 ANNEX 1

6.1 Ecology of the Identified Collembola and Oribatid Mite Species

6.1.1 Collembola

***Entomobrya multifasciata* (Tullberg, 1871)**

E. multifasciata has been described as an extremely drought resistant species (HÜTHER, 1961) which has also been found in dunes and in areas without vegetation (ELLIS, 1974). However, FJELLBERG (1980) and PALISSA (1964) also describe incidences in forest litter and tree stumps. The preferred biotope according to WILLER, 1995 is a dry meadow habitat.
In the *A. repens* litter *E. multifasciata* was present throughout the entire period, including the dry summer months. There was a high constancy for this species in all the samples from the experiment.

***Entomobrya nivalis* (Linné, 1758)**

E. nivalis is distinguished from *E. multifasciata* only by their body markings, and the latter has been declared a possible variation of *E. nivalis* by some (CHRISTIANSEN, 1958 in GISIN, 1960; FJELLBERG, 1980). Therefore, special emphasis was placed on the suspected habitat vicariance (ELLIS, 1974) of these species. *E. nivalis* also is considered drought resistant, but is most often found in habitats with vegetation, because it feeds on the algae growing on tree bark (ALLMEN & ZETTEL 1982). Thus, preferred biotopes of this species are tree bark and crowns.
During the entire investigational period, only one sample contained *E. nivalis*.

***Isotoma anglicana* Lubbock, 1873**

For a long time, *I. anglicana* was deemed a variation of *I. viridis* (GISIN, 1960). FJELLBERG (1980), however, emphasized the morphological and ecological differences between both species. Especially the occurrence of *I. anglicana* juveniles early in the year and the reaching of their reproductive age in fall of the same year contrast the phenology of *I. viridis*. RUSEK (1984) characterized *I. anglicana* as a hygrophilous species with high numbers of individuals occurring in hydric meadow habitats. FJELLBERG (1980) on the other hand found *I. anglicana* in numerous different habitats, making the species a likely ubiquist (WILLER, 1995).
In the study described here, *I. anglicana* was found in the litter containers in spring and fall.

***Isotoma notabilis* Schäffer, 1896**

I. notabilis occurs in many different habitats, making the species a ubiquist one. The species seems to respond plastically to substrate moisture, because its occurrence has been reported for hydric as well as dry grassland (RUSEK, 1984) and for wet to hydric forest biotopes (WEIGMANN, 1984). *I. notabilis* is unanimously described as a species of slightly acidic to

neutral soils, which responds well to lime treatments (BÅÅTH *et al.*, 1980; HÅGVAR & ABRAHAMSEN, 1980; HUTHA *et al.* 1983; KRONSHAGE, 1991; NÜß, 1994). The species also prefers humous substrates (GISIN, 1960; PALISSA, 1964).
I. notabilis was found frequently at high numbers in the litter from the decomposition containers, with the exception of the sampling date in the very dry spring.

***Lepidocyrtus cyaneus* Tullberg, 1871**
L. cyaneus is mainly found in open areas or in woods with a rather low stand density (NÜß, 1994). This species has reportedly been found in grassland (GISIN, 1960; ELLIS, 1974; RUSEK, 1984), in vineyards (HÜTHER, 1961), in tree trunks and compost (FJELLBERG, 1980), and in near-natural woods of low density (KRONSHAGE, 1991).
Here, relatively few individuals of *L. cyaneus* were found in the litter in spring and fall.

***Sminthurus nigromaculatus* Tullberg, 1872**
Preferred biotopes of *S. nigromaculatus* are open habitats with different degrees of moisture (WILLER, 1995). *S. nigromaculatus* may be habitat vicariant with *S. viridis*, but in contrast to the latter prefers natural meadows and uncultivated fields.
Here, this species was found at all sampling dates with moderate numbers of individuals and with high constancy.

6.1.2 Oribatida

***Carabodes labyrinthicus* (Michael, 1879)**
STRENZKE (1952) characterized *C. labyrinthicus* as accompanying species in the synusiae of peat lands and forest soils, where it can be found rarely or with a low constancy. The species is more frequent on solid substrates and in dry sand soils. WILLMANN (1931) also described *C. labyrinthicus* as characteristic species of the xerophile habitats on lichens, bark and branches. KNÜLLE (1957) on the other hand classified *C. labyrinthicus* in the group (VII) having its optimum in fresh to moist forest litter, but could also be found in soil samples of sandy Ericaceous heath and swamp forests.
RAJSKI (1968) showed the species' moisture optimum to fall into mesohabitats. Thus, in summary *C. labyrinthicus* can be characterized as oligoeuryhygric (STRENZKE, 1952).
Regarding substrate pH, STRENZKE (1952), MORITZ (1963), and RAJSKI (1968) listed habitats of lower values as preferred, characterizing *C. labyrinthicus* as either oligostenoionic or oligoeuryionic. WEIGMANN & KRATZ (1982) described *C. labyrinthicus* as mainly occurring in broadleaf and coniferous forests with acidic soils and in mesophilic deciduous broadleaf forests. Ancillary occurrences have been reported for ruderal fields, areas of perennial herb vegetations, forest clearances, and in dry and semi-dry grassland meadows.

In the sewage field areas, solitary occurrences of *C. labyrinthicus* were found in the *A. repens* litter of the decomposition containers from the RefB field.

***Ceratocetes mediocris* Berlese, 1908**

STRENZKE (1952) described *C. mediocris* as a mesoeuryionic characteristic species of the synusia of reed fields and hygrophilic sweet grass meadows, where it could be found in singular or numerous occurrences. Scattered occurrences have been reported for forest meadows as well. The ecological behavior of *C. mediocris* closely resembles that of *L. similis*, the latter, however, being more frequent. The same ecological classification of *C. mediocris* as index species of meadow soils was agreed on by FRENZEL (1936 in STRENZKE, 1952), KNÜLLE (1957), RAJSKI (1968), and WEIGMANN & KRATZ (1982). RAJSKI (1968) named the grassland synusia after this species and described *C. mediocris* as the characteristic species occurring exclusively in this type of habitat. *C. mediocris* does tolerate variation in substrate moisture but is found less frequently in drier habitats (mesopolyhygric).

In addition to the main occurrences in fresh meadows and pastures as well as moist meadows, WEIGMANN & KRATZ (1982) reported ancillary occurrences including those in ruderal fields, areas of perennial herb vegetations, forest clearances, in dry and semi-dry grassland meadows and in dry epiphytic and epilithic habitats.

Solitary occurrences of *C. mediocris* were found in the open RefB area.

***Eupelops occultus* (C.L. Koch, 1836)**

E. occultus has been described unanimously in the literature as the index or characteristic species of the meadow soil fauna (STRENZKE, 1952; KNÜLLE, 1957; RAJSKI, 1968). *E. occultus* shows some plasticity regarding substrate moisture but generally avoids dry habitats (mesoeuryhygric: STRENZKE, 1952; mesopolyhygric: RAJSKI, 1968). RAJSKI (1968) emphasized that *E. occultus* is the only species of the genus inhabiting open areas.

E. occultus prefers slightly acidic to neutral soils (polyeuryionic) and according to WEIGMANN & KRATZ (1982) has been reported mainly for the plant formations „fresh meadows and pastures“ and „moist meadows“.

E. occultus was only found in the samples from the nPAK area.

***Galumna lanceata* Oudemans, 1900**

G. lanceata appears to be colonizing only woody and scrubby habitats and soils that are at least covered by litter (STRENZKE, 1952; KNÜLLE, 1957; RAJSKI, 1968; WEIGMANN & KRATZ, 1982). RAJSKI (1968) referred to *G. lanceata* as a typical forest species, rarely to be found outside its optimum range.

According to RAJSKI , *G. lanceata* tolerates only moderate variations from medium substrate moisture and definitely prefers habitats with acidic soils (mesostenohygric or mesopolyhygric and oligostenoionic). WEIGMANN & KRATZ (1982) confirmed the preference for litter-covered

soils in their list of main occurrences, but not the exclusive confinement to these habitats. Not only have ancillary occurrences of *G. lanceata* been reported for humid and wet forests as well as bog forests, but the species has also been found in dry epiphytic and epilithic habitats (e.g., lichen carpets on rocks) and in areas without vegetation.
Scattered occurrences of *G. lanceata* were observed in the nPAK area.

***Liacarus coracinus* (C.L. Koch, 1841)**
Just like *G. lanceata L. coracinus* has been reported almost exclusively in woody or shrubby habitats or habitats with a dense litter coverage. According to STRENZKE (1952) *L. coracinus* is a mesoeuryhygric accompanying species of the synusiae of forests and forest meadows, avoiding very wet habitats. According to KNÜLLE (1957), too, *L. coracinus* belongs to the group (3) having its optimum in dry to fresh, rarely moist covers of woody habitats. RAJSKI (1968) was not able to find the species in open habitats but pointed out that this was true merely for studies in northern Europe: In southern Europe occurrences of *L. coracinus* in meadows have indeed been reported.
Regarding pH this species is considered either oligostenoionic (RAJSKI, 1968) or oligoeuryionic (STRENZKE, 1952).
Here, *L. coracinus* was only found in the nPAK area.

***Liebstadia similis* (Michael, 1888)**
STRENZKE (1952) described *L. similis* as characterizing species of the synusia of reed fields, meadow soils, and hygrophilic sweet grass meadows, where it could be found in singular or numerous occurrences. The species apparently avoids acidic podsol soils. In the habitats investigated by RAJSKI (1968) and KNÜLLE (1957) the highest constancies were found in open habitats with moderately to slightly acidic soil reactions (5.7<pH<6.8).
Average moisture in substrates is the optimum for *L. Similis.* In studies by RAJSKI (1968) the species exhibited only moderate tolerance of wetness. In summary, *L. Similis* can be described as mesoeuryhygric and mesoeuryionic.
According to WEIGMANN & KRATZ (1982) the species mainly occurs in ruderal fields, areas of perennial herb vegetations, forest clearances, fresh meadows and pastures, as well as moist meadows. Ancillary occurrences have been reported for weed formations on cultivated land, oligotrophic peatlands and bog forests, moist and wet forests, as well as broadleaf and coniferous forests with acidic soils.
Here, *L. similis* was found with relatively high constancy in litter and soil samples from the sewage field areas studies, with the exception of the partially bare nPCB area.

***Metabelba pulverosa* Strenzke, 1953**
The rather late original description in 1953 led to certain difficulties in relating existing information regarding the ecological requirements of *M. pulverosa*. In the writings by RAJSKI

(1967) *M. pulverosa* Strenzke, 1953 was described as equivalent to *M. pulverulenta* C.L. Koch, 1840. STRENZKE (1952) mentioned only the latter and described it as accompanying species of neutrophilic and acidophilic synusiae in habitats with relatively dense litter coverage (forest meadows, broadleaf and coniferous forests).
According to STRENZKE (1952) *M. Pulverosa* shows some plasticity regarding moisture, RAJSKI (1967), however, pointed out that occurrences outside the „meso range" do no justify the classification as mesoeuryhygric and that mesostenohygric would be a better characterization. WEIGMANN & KRATZ (1982) listed main occurrences of *M. pulverosa* in forests and ancillary occurrences in perennial ruderal fields, fresh meadows and pastures, as well as moist meadows.
Here, *M. pulverosa* was found in litter and soil samples of all sewage field areas except for the nPCB area.

***Microppia minus* (Paoli, 1908)**
In the literature, *M. Minus* has been described as a species of not-too-moist and acidic forests (STRENZKE, 1952; KNÜLLE, 1957; RAJSKI, 1968; MORITZ, 1962). The species has, however, also been found in not-too-acidic meadows, steppes, and open habitats.
M. minus has been described as rather plastic regarding substrate moisture (mesoeuryhygric: STRENZKE, 1952; mesopolyhygric: RAJSKI, 1968) and as oligostenoionic to oligoplastic regarding pH (MORITZ, 1962).
M. minus lives in the deeper soil layers. WEIGMANN & KRATZ (1982) showed main occurrences of *M. Minus* in fresh meadows and pastures as well as moist meadows, in broadleaf and coniferous forests with acidic soils and in mesophilic deciduous broadleaf forests.
Here, *M. minus* was found in soil samples only. This was true for the open areas RefB and nPCB as well as for the nPAK area with its poplar stand.

***Oppia nitens* (C.L. Koch, 1835)**
According to STRENZKE (1952), *O. nitens* has been found in „moss and the like", but like its subspecies *myrmecophila* most often occurs in rotting wood, in not-too-moist decaying plant substrates containing lignin and cellulose, and has been found several times in aged hay. STRENZKE (1952) himself found the species in heaps of fallen leaf on overgrown rubble. In Germany, according to WEIGMANN & KRATZ (1982) *O. Nitens* only shows ancillary occurrences in mesophilic deciduous broadleaf forest and fir forests, as well as in small dry epiphytic and epilithic habitats.
In the study described here, scattered occurrences of *O. nitens* were detected in the *A. repens* litter in the decomposition containers from the RefB site.

Oppiella nova (Oudemans, 1902)

STRENZKE (1952) listed this species as *Oppia neerlandica* (Willmann) - *Oppia corrugata* (Berlese) and called it „the most common oribatid in the area!“. This species is found in scattered as well as mass occurrences as a euryoecious accompanying species in all synusiae (“with the exception of submerged water plants and saline soils”). KNÜLLE (1957) described the species as “without clearly apparent optimum conditions”, and RAJSKI (1968) and MORITZ (1963) were able to find it in all of the habitats they investigated, but described a preference for medium moisture.

Regarding pH *O. nova* is euryplastic. Most often *O. nova* is characterized as a species of the deeper layers of soil. However, the species has been reported to migrate somewhat across the profile (RAJSKI, 1968), making it unlikely that it is confined to a single soil horizon. WEIGMANN & KRATZ (1982) emphasized the eurytopic behavior of *O. nova* because the main occurrences of this species span all plant formations from weed formations on cultivated land and short-lived ruderal vegetation to fresh meadows and pastures to mesophilic deciduous broadleaf forests.

O. nova was found only rarely in the soil samples of the RefB and nPCB areas.

Oribatella quadricornuta **(Michael, 1880)**

STRENZKE (1952) pointed out, that *O. quadricornuta* had been considered identical to *O. calcarata* for a long time. In contrast to the solid substrate preferring *O. quadricornuta*, the latter has been found only in 0 horizons of biotopes with tree stands. MORITZ (1963), too, emphasized the raw humus habitat selection with a low pH of *O. calcarata*. According to WEIGMANN & KRATZ (1982), only ancillary occurrences of *O. quadricornuta* have been reported for Germany, these being in broadleaf and coniferous forests with acidic soils.

Here, scattered occurrences of *O. quadricornuta* were found in samples of the nPAK area.

Pergalumna nervosa **(Berlese, 1915)**

According to STRENZKE (1952), *P. nervosa* is a characteristic species of the synusia of moist and wet soils in peatland and acidophilic forests.

According to RAJSKI (1968), however, the definition of the ecological requirements of this species is not quite as obvious: *P. nervosa* may be a species that prefers a generous soil cover and is more likely to be found in still green plant litter. KNÜLLE (1957) described the species’ occurrence as limited to fresh habitats, making *P. nervosa* likely to be found in not-too-wet woody habitats. Acidic soils covered with a generous amount of litter seem to provide optimum conditions for this species.

Here, scattered occurrences of *P. nervosa* were found in the nPAK area.

***Punctoribates punctum* (C.L. Koch, 1849)**

FRENZEL (1936 in STRENZKE, 1952) considered *P. punctum* one of the index species of the meadow soil fauna, because it occurred frequently in these habitats. The species has been found in meadow and cultivated field soils, and less frequently in forest soils. STRENZKE (1952) himself characterized the species as a frequently occurring mesohygric and euryionic accompanying species of the synusia of reed fields and hygrophilic sweet grass meadows. RAJSKI (1968), too, considered an intermediate degree of moisture as the optimum for *P. Punctum* and pointed out that the density of individuals decreased where artificial irrigation was employed. MORITZ (1963) considered this species a member of the group (2) that tolerates a medium to high moisture content of the substrate and selects "quality" humus as its habitat (no occurrences in raw humus).

Although STRENZKE (1952) described *P. punctum* as euryionic, MORITZ (1963) seems to have found it more frequently in substrates with a high pH. The latter reports may have been due to the selection of the investigated habitats: MORITZ focused mainly on forest habitats in which the preferred types of humus are found only in areas of high pH. A different explanation would be provided by the tolerance regarding varying moisture conditions that STRENZKE (1952) observed for this species at higher pH values. If the pH is low, it occurs only in not-too-moist habitats.

According to WEIGMANN & KRATZ (1982) main occurrences of *P. punctum* are in fresh meadows and pastures as well as in mesophilic deciduous broadleaf forests. They reported ancillary occurrences in ruderal fields, areas of perennial herb vegetations, forest clearances and other habitats.

Here, *P. punctum* was found only in the RefB area, where it occurred as well in the *A. repens* litter of the decomposition containers as in the soil samples.

***Ramusella insculpta* (Paoli, 1908)**

It was only with the help of Prof. G. Weigmann that *R. insculpta* (described as *Dameosoma insculptum* by Paoli) could be correctly identified. This species is not mentioned in the works quoted above, thus there is hardly any information regarding its ecological requirements available. It is possible that this species often has been confused with *Oppia clavipectinata* - STRENZKE (1952) described a possible erroneous identification in detail. WEIGMANN (personal communication) also found *R. insculpta* relatively frequently in sewage field areas in Berlin-Gatow and in meadow soils. *R. insculpta* is not contained in the list of German oribatid mite species and their ecological characterizations by WEIGMANN & KRATZ (1982).

Here, *R. insculpta* was found with low to medium constancy in the litter and soil samples of all of the sewage field areas.

***Scheloribates laevigatus* (C.L. Koch, 1836)**

STRENZKE (1952) described *S. laevigatus* as euryionic and mostly euryoecious accompanying species of the synusiae of the grass fens and hygrophilic sweet grass meadows. FRENZEL (1936, in STRENZKE, 1952), too, considered it an index species of the meadow soil fauna.

With respect to its moisture preference, KNÜLLE (1957) classified *S. laevigatus* in the group (XIII) preferring fresh to wet substrates. This opinion was essentially shared by STRENZKE (1952), MORITZ (1963), and RAJSKI (1968). RAJSKI (1968) went as far as calling the species "mesopolyeuryhygric". FRANZ (1954, in RAJSKI, 1968) was able to find high numbers of individuals of *S. laevigatus* even in severely contaminated industrial habitats.

Here, only scattered occurrences of *S. laevigatus* were found in the litter of the decomposition containers and the soil samples from the RefB area.

***Suctobelbella acutidens lobata* Strenzke, 1951**

This species was found in single to moderately frequent occurrences in meadow soils by STRENZKE (1952). It is rarely found in soils lightly covered by litter and is thus described as characteristic species of the synusia of reed fields, grass fens, and hygrophilic sweet grass meadows. The occurrence of *S. acutidens lobata* is severely limited by a high water content of the substrate. In the samples of MORITZ (1963) the species was found alongside other species the occurrence of which is controlled by a combination of fresh water regimes and low pH. According to WEIGMANN & KRATZ (1982), only ancillary occurrences of *S. acutidens lobata* have been reported for ruderal fields, areas of perennial herb vegetations, forest clearances, and in fresh meadows and pastures as well as moist meadows.

Here, *S. acutidens lobata* was found in the nPAK area only.

***Suctobelbella subcornigera* Forsslund, 1941**

According to the literature (STRENZKE, 1952, KNÜLLE, 1957 MORITZ, 1963), *S. subcornigera* is one of the most commonly found species in the investigated areas. It is a very constant accompanying species of the neutrophilic and acidophilic synusiae and is rather plastic regarding pH and moisture (mesoeuryhygric, oligoeuryionic). According to MORITZ (1963), however, the species avoids pronounced raw humus and very wet 0 horizons. The main occurrences for this species range from perennial ruderal vegetation to dwarf scrub heaths and moist meadows to mesophilic deciduous broadleaf forests.

Here, *S. subcornigera* was found in the nPAK area only .

***Suctobelbella subtrigona* (Oudemans, 1916)**

Just like *S. subcornigera,* MORITZ (1963) considered *S. subtrigona* to belong to a group of species with optimum conditions including a fresh water regime, low pH and a dense litter cover (no raw humus). STRENZKE (1952) listed this species as *Suctobelba intermedia* and considered it an accompanying species mainly of the synusiae of acidophilic forests, less often

of reed fields and riparian meadows. According to him, the species does, however, tolerate a broader range of pH values. Main occurrences in moist and wet forests as well as in mesophilic coniferous and broadleaf forests with acidic soils. Ancillary occurrences include ruderal vegetation, fresh to moist meadows and pastures (WEIGMANN & KRATZ, 1982).
Just like *S. subcornigera* and *S. acutidens lobata*, *S. subtrigona* was found in samples of the nPAK area only.

***Tectocepheus sarekensis* Trägardh, 1910**
STRENZKE (1952) did not distinguish *T. sarekensis* from its sister species *T. velatus.* In recent years a heated discussion of the question, whether there really are two species, has been started once again. Some authors view *T. sarekensis* and *T. velatus* as variants of the same species, claiming that there morphological specification is caused by the respective habitats, and that intermediate forms exist. The sewage field samples, however, were evaluated differentiating between the two species. True to expectations, *T. sarekensis* was, in contrast to *T. Velatus,* present in large numbers in the sewage field samples. According to RAJSKI (1968) the former exhibits a high constancy in meadow habitats. While *T. velatus* prefers woody habitats and is very tolerant of variations in moisture, the distribution of *T. sarekensis* is limited to fresh habitats.
According to MORITZ (1963), this species prefers humous (organogenic) soils with a slightly acidic to neutral reaction, high water content and less strongly shaped plant litter („brushwood litter, wood and root remains"). The subgroup that *T. sarekensis* belongs to is euryplastic regarding pH and selects habitats with richer humus content of the soil. WEIGMANN & KRATZ (1982) listed as habitats for main occurrences ruderal fields, areas of perennial herb vegetations, forest clearances, fresh meadows and pastures, moist and wet forests, as well as mesophilic coniferous and broadleaf forests with acidic soils.
Here, *T. sarekensis* also was found with high constancy in the soil of those sections of the nPCB area that showed scarce vegetation, and in many samples from the sewage field areas was the most abundant oribatid mite species.

***Trichoribates novus* Sellnick, 1928**
In the literature, *T. novus* unanimously has been described as a grassland inhabitant (meadows, grass fens, saline meadows). The optimum for this species is formed by fresh to wet conditions (STRENZKE, 1952; KNÜLLE, 1957; "medium" moisture: RAJSKI, 1968) as well as by a soil reaction near the neutral range.
Here, *T. novus* was found in the nPAK area only.

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SELBSTÄNDIGKEITSERKLÄRUNG

Hiermit versichere ich, dass ich die vorliegende Dissertationsarbeit selbstständig erarbeitet, verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.