Potential of the Soil Microbial Biomass C to Tolerate and Degrade Persistent Organic Pollutants

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Abstract: A 12-day incubation experiment with the addition of glucose to soils contaminated with persistent organic pollutants (POPs) was carried out in order to estimate the potential microbial activities and the potential of the soil microbial biomass C to degrade 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT), polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAHs). The microbial activities were affected in different ways depending on the type of pollutant. The soil organic matter also played an important role. The microbial activities were affected particularly by high concentrations of PAHs in the soils. Soil microorganisms in the PAHs contaminated soil used the added glucose to a lesser extent than in the non-contaminated soil, which in the contaminated soil resulted in a higher microbial biomass content during the first day of incubation. DDT, DDD and DDE, and PCB affected the soil microbial activities differently and, in comparison with control soils, decreased the microbial biomass C during the incubation. The increased microbial activities led to a significant decrease of PAH up to 44.6% in the soil long-term contaminated with PAHs, and up to 14% in the control soil after 12 days of incubation. No decrease of PAHs concentrations was observed in the soil which was previously amended with sewage sludges containing PAHs and had more organic matter from the sewage sludges. DDT and its derivates DDD and DDE decreased by about 10%, whereas the PCB contents were not affected at all by microbial activities. Studies on the microbial degradation of POPs could be useful for the development of methods focused on the remediation of the contaminated sites. An increase of soil microbial activities caused by addition of organic substrates can contribute to the degradation of pollutants in some soils. However, in situ biodegradation may be limited because of a complex set of environmental conditions, particularly of the soil organic matter. The degradability and availability of POPs for the soil microorganisms has to be estimated individually for each contaminated site.

Keywords: soil; microbial biomass C; respiratory activity; metabolic quotient (qCO_2) ; glucose; persistent organic pollutants (POPs); degradation

Organic contaminating compounds enter soils by atmospheric emissions, agricultural activities, leaching from waste deposits, industrial production, and other sources of pollutants (SEMPLE *et al.* 2006). Persistent organic pollutants (POPs) such as PCB, PAH, or DDT represent a danger for the soil environment, because it is difficult to estimate the risks for the soil, which are linked with undesirable

effects of contaminants. Pollutants can also cause changes in the availability of organic matter in the soil, they affect also the soil rhisosphere and pH (EDWARDS 2002). Pollutants can disturb soil microbial populations by direct or indirect toxic effects which can cause functional disturbance of their metabolism and subsequent death (BROOKES 1995). On the other hand, the pollutants in soils

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can be dispersed within the soil micropores and in this way their toxicity for microorganisms could be diminished. They can also be used by soil microorganisms as an energy substrate. However, rarely were there made studies concerning the effects of pollutants on the microbial biomass, substrate induced respiration, enzymatic activity, or nutrient transformations, all of which being important processes related to the soil fertility (Thompson *et al.* 1999; Edwards 2002).

The microbial biomass represents the living part of a soil and therefore it is sensitive to the soil properties, particularly to the carbon supply. Microbial characteristics are more sensible and more predictive in short-term effects of contaminants than are physical and chemical soil properties (JENKINSON & Ladd 1981; Brookes 1995; Nannipieri et al. 1997). Not enough relevant data are available on the effects of the addition of glucose to soils on the microbial growth in the POPs contaminated soils. However, the data based on experiments with heavy metal contaminated soils showed that soil microbial populations used more carbon from glucose for their respiratory activities while less carbon was incorporated into the microbial biomass. These experiments demonstrated that in soil with a high metal content, more substrate was diverted by microorganisms into catabolic processes at the expense of the anabolic ones (Chander & Joergensen 2001).

Studies on the microbial degradation of POPs can be useful for the development of methods aimed at remediation of the contaminated sites. However, *in situ* biodegradation may be limited because of a complex set of environmental conditions (BIDLAN & MANONMANI 2002).

DDT and its derivates have been shown to persist in the environment predominantly in the form of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane], DDD [1,1-dichloro 2,2-bis(4-chlorophenyl) ethane] and DDE [1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene]. DDD and DDE are transformation products of DDT (BOUL 1995), formed by microbial action (Wedemeyer 1966) or by chemical or photochemical reactions (Baxter 1990). The mechanism of microbial degradation of DDT to DDD is the reductive dechlorination under reducing (Johnson 1976; Rockind-Dubinsky et al. 1987) or under aerobic conditions (Nadeau et al. 1994).

Polychlorinated biphenyls (PCB) are a group of compounds previously used in transformers, capacitors, paints, and other industrial appliances. Bioremediation is considered a viable PCB removal strategy because many microorganisms can degrade PCB in diverse environments, including soils and sediments (FOCHT & REINEKE 2002). Low PCB bioavailability is a major obstacle that must be overcome before biotransformation can be considered a viable PCB removal option. PCB bioavailability is influenced by its low water solubility and strong adsorption to soil organic matter (Chou & Griffin 1987). Little is known about soil microbial populations responsible for PCB degradation or how these pollutants are affected by bioremediation treatments (Luo *et al.* 2005).

Many soils contaminated with polycyclic aromatic hydrocarbons (PAHs) contain PAH-degrading microorganisms. For instance, it was found that the microbial activity in the rhizosphere may increase their degradation (Johnson *et al.* 2005). On the other hand, the microbes are often limited in their degradation capability because of some limiting environmental factors (i.e., low aqueous solubility of PAHs, low bioavailability of PAHs, nitrogen or other nutrient limitation, high cocontamination levels such as pentachlorophenol (PCP) that can inhibit PAH biodegradation, etc.) (STRAUBE *et al.* 2003).

The aim of this research was to evaluate the effects of the addition of an easily minerilisable substrate (glucose) on potential soil microbial activities and on the microbial degradation of POPs in soils contaminated with different types of persistent organic pollutants.

MATERIAL AND METHODS

Soil samples. The experimental soils contaminated with different kinds of persistent organic pollutants and control non-contaminated soils with similar characteristics were sampled from the pot experiment (Table 1). Briefly, the pot experiment started in the spring 2005. The contaminated soils were taken from sites long-term contaminated with POPs (Polluted-DDT, Polluted-PCB, Polluted-PAH). Noncontaminated soils were chosen so as to have similar characteristics as the contaminated soils (Control-DDT, Control-PCB, Control-PAH). Radishes and carrots were grown in pots containing 6 kg of soil in the years 2005 and 2006, respectively. The soil Polluted-sew.sl-PAH was amended with sewage sludge containing 66 mg PAHs/kg in ratio 1:1. The pot experiment was carried out under natural weather conditions and the pots were regularly wetted with

Table 1. Basic characteristics of soils used for the incubation experiment

Soil	*Soil type	*Contaminant		*Ratio HA:FA	*C _{water} (mg/kg)		Dehydroge- nase activity (µg C/g soil)	Respiratory activity (µg C/g soil/h)
Polluted-DDT	Fluvisol	DDT, DDD, DDE	0.95	1:1.36	71	238.0	102.8	0.063
Polluted-PCB	Modal Cambisol	PCB ₇	1.8	1:1	56	191.1	104.6	0.087
Polluted-PAH	Fluvisol	PAHs	1.6	1:1	34	124.2	35.6	0.156
Polluted-sew. sl-PAH	Modal Cambisol	sewage sludge-PAHs	5.23	1:0.6	204	533.4	105.2	0.973
Control-PCB	Modal Cambisol	${\rm control}\ {\rm PCB}_7$	2.04	1:1	111	331.9	75.4	0.191
Control-DDT	Fluvisol	control DDT, DDD, DDE	1.43	1:1.7	99	256.4	208.4	0.165
Control-PAH	Arenic Cambisol	control PAHs	1.66	1:0.6	89	256.3	218.8	0.100

^{*}The data of soil characteristics were used with a kind permission of Ing. Radim Vácha, Ph.D., from Research Institute for Soil and Water Conservation, Czech Republic.

500ml of distilled water with regard to the climatic conditions. The soil samples for the incubation experiment were sampled from pots after the carrot harvest in 2006 (VÁCHA *et al.* in press).

Incubation experiment. Seven days prior to the start of the incubation experiment, three replications of each treatment (0.5 kg of soil on an oven dry basis) in one litre plastic jars were placed into 3 litre plastic containers tightly covered with fitting lids and conditioned at 28°C. The soils were preincubated at 40% water holding capacity (WHC) with a jar of 25 ml 1M NaOH to take up the CO₂-evolved and distilled water at the bottom of the container. At the beginning of the experiment, each soil replication was mixed with glucose in the dose of 1000 μ g C/g, and NH₄(SO₄), giving ratio C:N 10:1 was added. Thereafter, 3 × 50 g of soil of each treatment were weighed and separately incubated in 1000 ml tightly closed plastic pots containing 5 ml 1N NaOH to determine the evolved CO₂. The soils were incubated for 12 days. The microbial biomass contents were determined on days 0, 1, and 12 of the incubation. The respiratory activity was determined on days 0, 1, 5 and 12 of the incubation.

Analyses. The measurements of the soil microbial biomass C (B_c) were performed using the fumigation-extraction method (F.E.) according to Vance *et al.* (1987) procedure. The microbial biomass C was calculated from the relationship: $B_c = 2.64\,E_c$, where E_c is the difference between organic C extracted from the fumigated and non-fumigated treatments, both expressed as μg C/g oven dry soil.

 ${
m K_2SO_4}$ -extractable carbon was determined as the non-fumigated C of the microbial biomass measurements described by VANCE *et al.* (1987).

The CO_2 -C evolved was determined as the amount of carbon released as CO_2 after absorption in NaOH and precipitation with BaCl_2 and was analysed by titration with 0.25M HCl using phenolphthalein as indicator. The metabolic quotient ($q\mathrm{CO}_2$) was calculated according to Anderson and Domsch (1990) equation: $q\mathrm{CO}_2 = \mu\mathrm{g} \ \mathrm{C}/\mu\mathrm{g} \ \mathrm{B}_\mathrm{c}/\mathrm{h}$.

100 g of soil samples were frozen to -24° C before the start of the experiment and on day 12 of the incubation. Thereafter, the frozen soils were

Table 2. Concentrations of DDT, DDD and DDE in soils Polluted-DDT and Control-DDT before (Polluted-DDT/0, Control-DDT/0) and after 12 days (Polluted-DDT/12, Control-DDT/12) of incubation with glucose (in μ g/kg dm; dm – dry matter)

	Polluted-DDT/0	Polluted-DDT/12	Control-DDT/0	Control-DDT/12
DDD	3.79	3.43	< 1	< 1
DDE	59.5	51.3	1.41	1.86
DDT (sum)	27.2	24.5	5.42	6

Table 3. Concentrations of PCB in soils Polluted-PCB and Control-PCB before (Polluted-PCB/0, Control-PCB/0) and after 12 days (Polluted-PCB/12, Control-PCB/12) of incubation with glucose. (in μ g/kg dm; dm – dry matter)

PCB congener	Polluted-PCB/0	Polluted-PCB/12	Control-PCB/0	Control-PCB/12	
No. 28	< 5	< 5	< 5	< 5	
No. 52	< 5	< 5	< 5	< 5	
No. 101	< 5	< 5	< 5	< 5	
No. 118	< 5	< 5	< 5	< 5	
No. 138	10.8	9.55	< 5	< 5	
No. 153	7.67	8.51	< 5	< 5	
No. 180	10.1	10.6	< 5	< 5	
Summ	35.5	36	< 5	< 5	

transported to the accredited laboratory Aquatest, s.r.o. (Czech Republic) which carried out the measurements of POPs contents. The soils Polluted-DDT and Control-DDT were analysed for DDT, DDD, and DDE contents, soils Polluted-PCB and Control-PCB were analysed for PCB₇ (congeners 28, 52, 101, 118, 138, 153 and 180), the soils Polluted-PAH, Polluted-sew.sl-PAH and Control-PAH were analysed for PAHs contents (Fluoranthene, Benzo/b/fluoranthene, Benzo/k/fluoranthene, Benzo/a/pyrene, Benzo/ghi/perylene, Indeno/c,d/

pyrene, Fenanthrene, Anthracene, Pyrene, Benzo/a/anthracene, Chrysene, Naphtalene, Dibenzo/a,h/anthracene, Acenaphtene, Fluorene). The POPs concentrations are given in Tables 2–4.

RESULTS AND DISCUSSION

Soil microbial activities

A sharp increase of the soil microbial biomass C was observed in all variants studied during the first

Table 4. Concentrations of PAH in soils Polluted-PAH, Polluted-sew.sl-PAH and Control-PAH before (Polluted-PAH/0, Polluted-sew.sl-PAH/0, Control-PAH/0) and after 12 days (Polluted-PAH/12, Polluted-sew.sl-PAH/12, Control-PAH/12) of incubation with glucose (in mg/kg dm; dm – dry matter)

	Polluted- PAH/0	Polluted- PAH/12	Polluted-sew. sl-PAH/0	Polluted-sew. sl-PAH/12	Control- PAH/0	Control- PAH/12
Fluoranthene	11.2	5.98	1.26	1.51	0.066	0.055
Benzo/b/fluoranthene	3.7	2.12	2.71	2.7	0.053	0.041
Benzo/k/fluoranthene	2.09	1.16	1.33	1.34	0.021	0.017
Benzo/a/pyrene	3.91	2.43	2.65	2.65	0.044	0.037
Benzo/ghi/perylene	3.49	2.06	2.33	2.34	0.048	0.041
Indeno/c,d/pyrene	3.2	1.81	2.29	2.36	0.045	0.038
Phenanthrene	5.61	3.02	0.4	0.45	0.029	0.021
Anthracene	2.79	1.02	0.09	0.11	0.009	0.007
Pyrene	9.52	5.23	0.94	1.03	0.071	0.063
Benzo/a/anthracene	5.25	2.75	1.71	1.79	0.043	0.037
Chrysene	4.63	2.47	1.44	1.46	0.042	0.035
Naphtalene	0.8	0.9	0.2	0.1	0.04	0.04
Dibenzo/a,h/anthracene	1.07	0.56	0.89	0.94	0.012	0.009
Acenaphtenes	0.6	0.5	0.1	0.1	0.01	0.01
Fluorene	0.79	0.45	< 0.01	< 0.01	0.007	0.007
PAH total	58.7	32.5	18.3	18.9	0.535	0.456

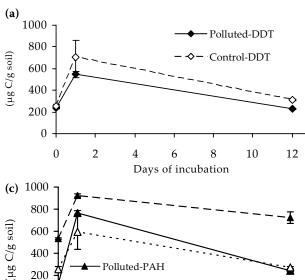
day of incubation with glucose (Figure 1). Microbial biomass C contents were lower in the DDT, DDD and DDE contaminated soil Polluted-DDT and in the PCB contaminated soil Polluted-PCB in comparison with the control soils Control-DDT and Control-PCB. The soil Polluted-PAH contaminated with PAH showed a larger microbial biomass C during the first day of incubation in comparison to non-contaminated soil Control-PAH. However, the largest microbial biomass C was found in the soil Polluted-sew.sl-PAH previously amended with sewage sludge.

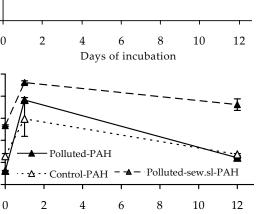
The concentrations of K_2SO_4 -extractable C (Figure 2) were higher during the first day of incubation with glucose in the soils Control-PCB and Control-DDT in comparison with carbon concentrations found in the contaminated soils Polluted-DDT and Polluted-PCB. On the contrary, compared to the soil Control-PAH, greater concentrations of K₂SO₄-extractable C were found during the first day of incubation in the polluted soil Polluted-PAH. Similarly as with the microbial biomass C, the highest K₂SO₄-extractable C concentrations were found in the soil Polluted-sew.sl-PAH.

Respiratory activity (Figure 3), despite the lower microbial biomass C contents, was higher during the first day of incubation in contaminated soils Polluted-DDT and Polluted-PCB in comparison with the soils Control-DDT and Control-PCB. On the contrary, respiratory activity in the soil Polluted-PAH was lower in comparison with the soil Control-PAH. The highest respiratory activity was found in the soil Polluted-sew.sl-PAH with the addition of sludges.

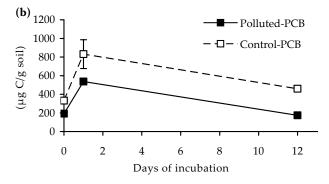
The values of the metabolic quotient (qCO_2) (Figure 4) were higher in the polluted soils Polluted-DDT and Polluted-PCB in comparison with the soils Control-DDT and Control-PCB. On the contrary, the qCO_2 was lower in the PAH contaminated soil Polluted-PAH than in the soil Control-PAH. The qCO_2 in the soil Polluted-sew.sl-PAH amended with sewage sludge was the lowest in comparison with other soil samples contaminated with organic pollutants and reached the maximum value 0.02 μg C/μg B_c/h during the first day of incubation (Figure 4c).

The pollutants may have direct or indirect effects on soil organisms through changing or contaminating their food supply. Through their direct or indirect toxicity to individual soil organisms, the pollutants can have drastic effects on the size of the soil populations and this is the effect that is most commonly measured in laboratory and field environmental studies (EDWARDS 2002). These effects can be accompanied by altered microbial activities as suggested by BROOKES (1995) and GILLER et al. (1998), who showed that microbial biomass in soils contaminated with heavy metals was usually lower in comparison with control soils, and that many times a higher respiratory activity and qCO_2 were observed as a measure of a greater energy demand for their maintenance and survival in the contaminated environment.





Days of incubation

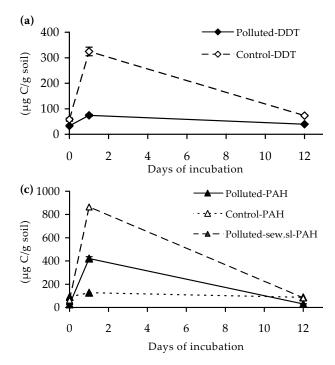


- (a) soils contaminated and non-contaminated with DDT
- (b) soils contaminated and non-contaminated with PCB
- (c) soils contaminated and non-contaminated with PAHs

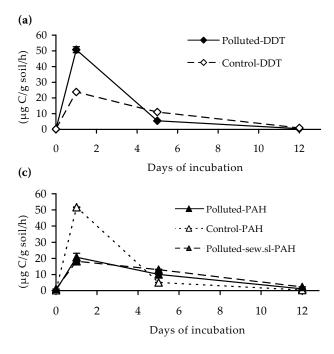
Figure 1. Microbial biomass C (µg C/g soil) dynamics in soils contaminated and non-contaminated with POPs during incubation with glucose; vertical bars represent the standard deviation

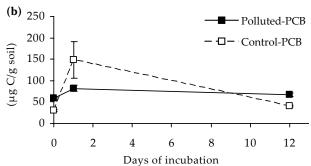
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Larger microbial biomass C and $\rm K_2SO_4$ -extractable C concentrations were found during the incubation with organic substrates in the heavy metal contaminated soil (Mühlbachová 2001), probably due to a less effective utilisation of the added substrate. This could be a possible explanation for the increased soil microbial biomass C and $\rm K_2SO_4$ -extractable C in the soil Polluted-PAH in comparison with the soil Control-PAH (Figures 1c, 2c). The toxicity of PAHs in the soil Polluted-PAH could cause a lower utilisation of the added glucose during the first 24 hours of the experiment. Therefore, the total microbial



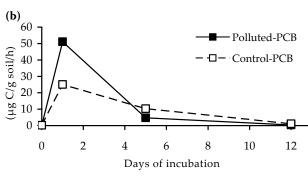


- (a) soils contaminated and non-contaminated with DDT
- (b) soils contaminated and non-contaminated with PCB
- (c) soils contaminated and non-contaminated with PAHs

Figure 2. ${\rm K_2SO_4}$ -extractable carbon (µg C/g soil) dynamics in soils contaminated and non-contaminated with POPs during incubation with glucose; vertical bars represent the standard deviation

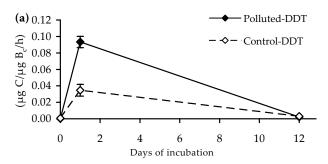
biomass C in the soil Polluted-PAH could, during the first day of incubation, exceed its contents in the soil Control-PAH which probably utilised glucose more quickly. As could be expected, the largest microbial biomass C and $\rm K_2SO_4$ -extractable C concentrations were found in the soil Polluted-sew. sl-PAH previously amended with sewage sludges and for this reason containing the greatest organic matter content of all soils.

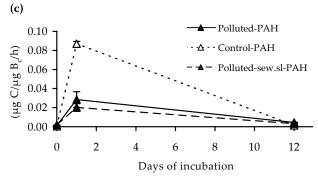
A different course was observed with the microbial biomass *C* in the soils Polluted-DDT or Polluted-PCB, which was lower during the first day

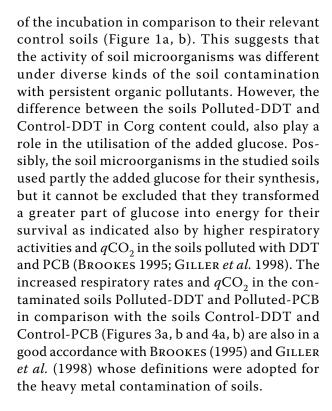


- (a) soils contaminated and non-contaminated with DDT
- (b) soils contaminated and non-contaminated with PCB
- (c) soils contaminated and non-contaminated with PAHs

Figure 3. Respiratory activity (μ g C/g soil/h) in soils contaminated and non-contaminated with POPs during incubation with glucose; vertical bars represent the standard deviation

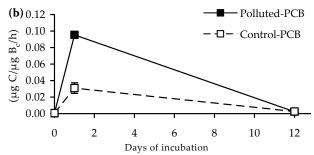






Persistent organic pollutants

The concentrations of persistent organic pollutants were determined before the start of the experiment and after 12 days (Tables 2–4). Lower concentrations of DDT, DDD, and DDE of 9.9%, 13.8%, and 9.5%, respectively, were found in the soil Polluted-DDT after 12 days of incubation in comparison with the measurements before the start of incubation. The



- (a) soils contaminated and non-contaminated with DDT
- (b) soils contaminated and non-contaminated with PCB
- (c) soils contaminated and non-contaminated with PAHs

Figure 4. Metabolic quotient (qCO_2) in soils contaminated and non-contaminated with POPs during incubation with glucose; vertical bars represent the standard deviation

soil Control-DDT did not show any decrease in the concentrations of these contaminants.

The concentrations of the measured congeners of PCB in the soil Polluted-PCB did not decrease during the incubation with glucose with the exception of the PCB congener No. 138, and the PCB concentrations in the soil Control-PCB remained lower than 5 μg PCB/kg soil over all the time of incubation.

The PAHs concentrations in the soil Polluted-PAH were lower in average by 44.6% after 12 days of incubation in comparison with the measurements before the start of incubation. The measurements of 2-3-ring PAHs and 4-6-ring PAHs showed that the decrease of PAHs ranged between 16% (acenaphtenes) and 63% (anthracene). The already low concentrations of PAHs in the soil Control-PAH decreased in average by 14.7% during the incubation. However, it was not possible to estimate precisely whether more simple PAHs were degraded under increased microbial activities to a larger extent then more complex PAHs. Despite the highest microbial activities of all soils during incubation, PAHs concentrations in the soil Polluted-sew.sl-PAH did not decrease.

As DDD and DDE are the transformation products of DDT (BOUL 1995) formed as a result of microbial activities, the results obtained indicate that the microbial pool enhanced by glucose addition contained microbial species able to degrade DDT to DDD and DDE. Carbon sources may influence the degradation rates in soils. It was found that the presence of glycerol, peptone, yeast extract

and tryptone soya broth completely eliminated DDT (BIDLAN & MANONMANI 2002). The degradation of DDT by about 10% in the soil Polluted-DDT suggests that the soil microorganisms were involved in the degradation process of DDT and contained microbial populations responsible for the degradation of DDT. Therefore, the addition of glucose contributed to the DDT, DDD, and DDE degradation in the soil studied.

There are many microorganisms which can degrade PCB in diverse environments, including soils and sediments (FOCHT & REINEKE 2002). However, little is known about the soil microbial populations responsible for PCB degradation or how they are affected by the bioremediation treatments (Luo et al. 2005). The soil Control-PCB did not exceed the detectable level of PCB and therefore was not evaluated. The total concentration of PCB in the soil Polluted-PCB did not decrease. It is not excluded that PCB in the soil Polluted-PCB could be adsorbed onto the soil organic matter and PCB bioavailability could be affected by its low water solubility (Chou & GRIFFIN 1987). Possibly, the microbial populations responsible for PCB degradation were not present in the soil Polluted-PCB, and therefore higher microbial activities after the glucose addition did not lead to the PCB degradation and to the decrease of its concentration in the soil Polluted-PCB.

The decrease of PAHs concentrations during the incubation with glucose was different in the soils Polluted-PAH and Control-PAH. The soil Polluted-PAH was a soil with a lower organic carbon concentration in comparison with the soil Polluted-sew.sl-PAH amended with sewage sludge and consequently containing a higher organic matter content. It is known that many soils contaminated with PAHs contain PAH-degrading microorganisms. These microbes are often limited in their degradation capacity because of some limiting environmental factors such as aqueous solubility of PAHs, low bioavailability of PAHs, nitrogen or other nutrient limitation, high co-contamination levels that can inhibit PAH biodegradation (STRAUBE et al. 2003). This fact could be a partial explanation why PAHs degraded well in the soil Polluted-PAH and partly also in the soil Control-PAH, whereas no decrease was found in the sewage sludges amended soil Polluted-sew.sl-PAH. It is not excluded that the different organic matter contents in these soils played an important role in the different solubility of PAHs in these soils. PAHs in the soil Polluted-sew.sl-PAH could also interact with non-aqueous phases and soil organic matter due to high hydrophobicity and solid-water distribution ratios and, as a consequence, became unavailable for microbial degradation since bacteria are known to degrade chemicals only when these are dissolved in water (Johnsen *et al.* 2005).

CONCLUSIONS

The contamination with persistent organic pollutants affected microbial activities in soils. Microbial activities were affected particularly by high concentrations of PAHs in soils as the soil microorganisms used the added substrates to a lesser extent than in the non-contaminated soil, which resulted in a higher microbial biomass content during the first day of incubation. DDT, DDD, and DDE in the contaminated soils affected the soil microbial activities differently while the microbial biomasses C of these soils, were lower during the incubation in comparison with the relevant control soils. Increased microbial activities led to a significant decrease of PAHs up to 44.6% in the soil Polluted-PAH, and up to 14% in the soil Control-PAH after 12 days of incubation. No decrease of PAHs concentrations was observed in the soil Polluted-sew.sl-PAH which contained more organic matter from the sewage sludges. DDT and its derivates DDD and DDE contents decreased by about 10%, whereas PCB were not affected by microbial activities.

Studies on the microbial degradation of POPs could be useful for the development of methods for the remediation of the contaminated sites. *In situ* biodegradation may be limited because of a complex set of environmental conditions, particularly the soil organic matter. The degradability and availability of POPs for soil microorganisms has to be estimated individually at each contaminated site.

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