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RESEARCH ARTICLE

Bioremediation trial on aged PCB-polluted soils—a bench study in Iceland

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Abstract Polychlorinated biphenyls (PCBs) pose a threat to the environment due to their high adsorption capacity to soil organic matter, stability and low reactivity, low water solubility, toxicity and ability to bioaccumulate. With Icelandic soils, research on contamination issues has been very limited and no data has been reported either on PCB degradation potential or rate. The goals of this research were to assess the bioavailability of aged PCBs in the soils of the old North Atlantic Treaty Organization facility in Keflavík, Iceland and to find the best biostimulation method to decrease the pollution. The effectiveness of different biostimulation additives (N fertiliser, white clover and pine needles) at different temperatures (10 and 30 °C) and oxygen levels (aerobic and anaerobic) were tested. PCB bioavailability to soil fauna was assessed with earthworms (*Eisenia foetida*). PCBs were bioavailable to earthworms (bioaccumulation factor 0.89 and 0.82 for earthworms in 12.5 ppm PCB soil and in 25 ppm PCB soil, respectively), with less chlorinated congeners showing higher bioaccumulation factors than highly chlorinated congeners. Biostimulation with pine needles at 10 °C under aerobic conditions resulted in nearly 38 % degradation of total PCBs after 2 months of incubation. Detection of the aerobic PCB degrading *bphA* gene supports the indigenous capability of the soils to aerobically degrade PCBs. Further research on field scale biostimulation trials with pine needles in cold environments is recommended in order to optimise the method for onsite remediation.

Keywords PCB · Aerobic bioremediation · Anaerobic bioremediation · Cold regions · Bench study · Volcanic soils · Bioavailability

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Introduction

One of the key functions of soils is to filter, absorb and transform various substances (Jones et al. 2010), e.g. soil pollutants such as polychlorinated biphenyls (PCBs). PCBs are organic hydrocarbons that have 1–10 chlorine atoms attached to biphenyl and 209 different congeners exist theoretically, of which 20–60 are most widely used (Safe 1994; Erickson 1997; BEST 2001; Abraham et al. 2002; Ohtsubo et al. 2004; Vasilyeva and Strijakova 2007). PCBs were produced between 1929 and late 1970s and used in industrial applications such as transformers, capacitors, hydraulic liquids, lubricants, flame retardants and plastics due to their thermal stability and low reactivity, low water solubility and high vaporisation temperature. They pose a serious risk to the environment with their bioaccumulation and biomagnification

potential (Erickson 1997; Fagervold et al. 2007; Jörundsdóttir 2009) and toxicity (Ross 2004; Ulbrich and Stahlmann 2004) and high adsorption capacity to the soil organic matter and clay particles.

Up to 85 % of the global biosphere is permanently exposed to temperatures below 5 °C (Margesin 2007). Soils of cold regions and environments are key players in pollution research due to currently experiencing faster and larger-scale environmental changes than any other areas on earth (UNEP/AMAP 2011). They accumulate pollutants from lower latitudes, which has a great impact on their people's traditional use of natural resources and food systems (Jones et al. 2010; UNEP/AMAP 2011). PCB biodegradation can take place through mineralization and cometabolism (Gomes et al. 2013): under anaerobic conditions, biodegradation occurs through dehalorespiration when bacteria capable of anaerobic respiration use PCBs as electron acceptors (Vasilyeva and Strijakova 2007), while aerobic degradation relies on oxidative destruction of PCBs with the help of various genes, e.g. on *bphA* and dehydrogenase (Wiegel and Wu 2000; Ohtsubo et al. 2004). In the biphenyl pathway, PCBs are first transformed to chlorobenzoic acid (CBA) by bacteria that uses biphenyl as a carbon and energy source and followed by CBA-degrading bacteria that transform the pollutant to less toxic forms (Ohtsubo et al. 2004). Research of cold soils has found that temperature is not the only governing factor of pollutant degradation and functioning of the microbial communities (Mohn et al. 1997; Master and Mohn 1998; Kuipers et al. 2003; Welander 2005; Aislabie et al. 2006; Lambo and Patel 2007; Zharikov et al. 2007), but knowledge of the dynamic soil environment, contaminants and contaminant degraders are of pivotal importance. Soil pH, close to neutral being optimal for degradation, has an effect on the adsorption of PCBs into organic matter and therefore bioavailability and biodegradation as well (Jota and Hassett 1991; Borja et al. 2005; Aislabie et al. 2006). Sufficient amount of carbon in easily consumable form, such as terpenes (Hernandez et al. 1997), is one of the necessities for improving the living conditions for dechlorinating microorganisms (Wiegel and Wu 2000; Ohtsubo et al. 2004; Aislabie et al. 2006). Furthermore, adequate amount of electron donors, such as nitrate (NO_3^-), is crucial to the rate, extent and route of any anaerobic reductive dehalogenation process (Tiedje et al. 1993; Wiegel and Wu 2000; Abraham et al. 2002; Borja et al. 2005).

Studies on PCB remediation of northern circumpolar soils are scarce (Lambo and Patel 2007; Kalinovich et al. 2012) and in Iceland, in particular, very limited research exists on soil pollution and bioremediation (Meyles and Schmidt 2005). The goal of the study was to test biostimulation methods for reduction of soil PCB concentrations. The specific aims were to investigate whether (1) PCBs adsorbed to these soils are bioavailable, whether (2) biological degradation activity in Icelandic sub-arctic soils could be increased at ambient temperature with both conventional (fertiliser N) and unconventional (white clover and pine needle) amendments and whether (3) increased temperature would augment bioremediation potential of the soils in question. Considering that climate is already getting wetter and warmer in Iceland (Björnsson et al. 2008) with increasing soil temperatures, it is imperative to study how such changes affect the soils of northern latitudes and hence the fate of pollutants within soils.

Materials and methods

Scene setting

The former North Atlantic Treaty Organization facility and United States Naval Air Station Keflavík (NASKEF) was situated at Keflavík International Airport in Iceland from the WWII until the autumn of 2006 (Almenna Consulting Engineers 2008 and 2010, personal communication). PCBs, mainly Aroclor 1260, were used at NASKEF in transformer oils in great quantities and were stored in old machinery at the army sales facilities but were mainly phased out of use during early 1990s (Almenna Consulting Engineers 2010, personal communication). The soil from the research area (63°57′29″N, 22°34′59″W) was divided into three pollution categories: more than 50 ppm (in microgram per gram dry weight) PCB, 1–50 ppm PCB and less than 1 ppm PCB; bioremediation of this study was applied to soils containing 1–50 ppm PCBs. The groundwater around the study site has been confirmed to contain no detectable PCBs (ÍSOR 2008). The climate in the study area is relatively mild, cold temperate oceanic. The mean annual precipitation is 1,100 mm and temperature 4.4 °C (Icelandic Meteorological Office database 2010, personal communication).

Soil samples

Soils from the area have been previously described by Arnalds (2004) and Arnalds et al. (2009) and classified as Brown Andosols. Soils were sampled according to NORDTEST Technical Report No. 329 (Karstensen et al. 1997). At the laboratory, soils were sieved (2 mm), stored in glass jars at 4 °C in the dark and allowed to equilibrate for 2 weeks prior to the experiment. A control soil was sampled from the same area and confirmed not to contain any detectable PCBs. Soils used in this research are characterised in Table 1. Soil moisture content was calculated from oven-dried samples (105 °C) and water-holding capacity (WHC) was determined according to Smith and Mullins (2001). Soil pH (H_2O) was measured in 1:5 soil/water suspension with a glass calomel electrode (Oakton pH/mV/°C Meter pH 1000 Series, Chicago, IL, USA). C_{tot} and N_{tot} were measured by thermal combustion (Elemental

Analyzer Vario MAX CN, Analysensysteme GmbH Germany) and since the studied soils contained no carbonates, C_{tot} was taken as C_{org}. Cation exchange capacity (CEC) was determined according to Blakemore et al. (1987) using Sampletek Vacuum Extractor (Macro Industries, INC). The exchangeable bases (Na^+, Mg^{+2}, K^+ and Ca^{+2}) were determined by gas diffusion with FIALAB 3500B (Fialab Instruments, USA) and NH_4-H in a flow injection FIAstar 5010 analyzer (Tecator, Hoganas, Sweden). The content (in percent) of ammonium oxalate extractable Al, Si, Fe and Mn were analysed by inductively coupled plasma optical emission spectrometry (Spectro, Germany). Allophane content was estimated by multiplying Si % by 6 (Parfitt 1990) and ferrihydrite by multiplying Fe % by 1.7 (Parfitt and Childs 1988).

Soil PCB analyses

Analysis of PCB in soil samples was undertaken according to NORDTEST Technical Report No. 329 guidelines (Karstensen et al. 1997, 1998). Following extraction and clean-up, the determination of total PCBs and individual PCB congeners were carried out with Agilent 6890 N GC with DB1701 column (60 m, 0.25 mm i.d., 0.25 μm film) equipped with an electron capture detector. A mixture of Aroclor 1260, 50, and 500 ppm in transformer oil (Accustandard, USA) were used to determine the total amount of PCBs by comparing and quantifying 20 different peaks in the mixture with a five-point standard curve (2, 10, 50, 100 and 500 ppm Aroclor 1260). The individual congeners were determined using nine individual PCB standards (#28, 52, 101, 118, 138, 153, 170, 180 and 187) from Accustandard, USA, using a six-point standard curve of 0.5, 2, 8, 25, 100 and 200 pg/μl of each congener. The method was originally developed by this laboratory, which also coordinated a ring test involving 21 laboratories (Karstensen et al. 1998). The method was tested using a PCB-contaminated certified reference soil (CRM481) from IRMM (BCR). The laboratory participates in two different quality assurance schemes every year, Quasimeme BT2 (PCBs and pesticides in marine biota) and AMAP (PCBs, pesticides, PBDEs in human serum), thus testing standard solutions.

Bioavailability of PCBs in soil to earthworms

The uptake of PCBs by earthworms (*Eisenia foetida*) was determined according to Hallgren et al. (2006). *E. foetida* is a widely used earthworm in bioavailability studies and results obtained may be considered as worst case scenarios (Hallgren et al. 2006). Earthworms and compost soil were confirmed to contain no PCBs. The earthworms were 3.0–9.5 cm long and weighed 0.11–0.67 g.

Microcosms were prepared by carefully mixing 20 g field moist polluted soil and 20 g of fresh compost soil in a glass jar. Deionised water (5 g) was added to achieve a suitably moist environment. Nine identical microcosms were prepared; three with unpolluted control soil, three with soil containing 25 ppm PCBs (final soil mixture, 12.5 ppm) and three with soil containing 50 ppm PCBs (final soil mixture, 25 ppm). Ten earthworms were weighed and added to each jar. The jars were covered with parafilm and placed in desiccators where the drying stones had been replaced by water to create stable humidity and no evaporation. The desiccators were stored in darkness at room temperature for a period of 10 days. After incubation, the earthworms were removed, rinsed and stored at −20 °C until analysis (Hallgren et al. 2006).

Extraction of PCBs from the earthworms was carried out by the Jensen extraction method (Jensen et al. 1983) as described in Ólafsdóttir et al. (1995), Jensen et al. (2003) and Ólafsdóttir et al. (2005). The total PCBs and individual PCB congeners were determined by gas chromatography as described above.

Presence of PCB-degrading bacterial genes and groups

Soil DNA was extracted in four replicates from 0.25 g of unpolluted control soil, soil containing 25 ppm of PCBs and soil containing 50 ppm of PCBs with PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA).

Table 1 Selected soil physicochemical properties presented as mean of three replicates (if standard deviation is not given, result is based on one replicate)

Soil PCB Concentration	MC %	WHC g/100 g	pH H2O	C_{org} %	N_{tot} %	C/N	CEC meq/100 g	*A* %	*F* %	*A*+*F* %
0 ppm	24.60	95.6 (6.93)	6.0 (0.01)	2.10 (0.00)	0.16 (0.00)	13 (0.57)	13 (0.57)	8.8 (0.07)	5.0 (0.06)	13.8 (0.12)
25 ppm	11.60	32.7	6.3 (0.02)	1.7 (0.10)	0.03 (0.00)	58 (6.33)	4.8 (0.18)	8.2 (0.42)	4.1 (0.20)	12.4 (0.61)
50 ppm	11.80	33.1	6.6 (0.01)	1.0 (0.00)	0.02 (0.00)	45 (2.28)	5.4 (0.20)	9.2 (0.16)	4.5 (0.05)	13.7 (0.21)
27 ppm mixture	19.90	56.8 (0.62)	6.9 (0.01)	2.0 (0.10)	0.14 (0.00)	15 (0.35)	7.9 (0.70)	12.5 (0.39)	6.7 (0.20)	19.2 (0.59)

MC moisture content, *WHC* water-holding capacity, *CEC* cation exchange capacity, *A* allophane, *F* ferrihydrite

Manufacturer's instructions were followed except for disrupting the cells by beadbeating with FastPrep instrument for 30 s at speed 5.5 m s^{-1}. Extracted DNA was quantified with Qubit Quant-iT dsDNA HS Assay kit by Qubit 1.0 fluorometer (Invitrogen, USA) and the quality was checked by agarose gel electrophoresis with ethidium bromide staining and NanoDrop ND1000 UV–vis spectrophotometer (NanoDrop Technologies, USA).

The presence of bacterial genes and taxa related to PCB degradation was tested by PCR with seven different previously published primer sets. Gene *bphA* has been associated with aerobic PCB degradation (Witzig et al. 2006), genes *fcbA*, *fcbB* and *ohb* with degradation of PCB dechlorination products (Rodrigues et al. 2001, 2006) and gene *cbrA* with anaerobic dechlorination by *Dehalococcoides* (Watts et al. 2005). Taxon-specific 16S ribosomal RNA gene primers were used to target the dechlorinating members of *Chloroflexi* (Wagner et al. 2009). The primer sequences and annealing temperatures are presented in Table 2. One microliter of the DNA extract (0.02–0.86 ng of DNA) was used as template with duplicate PCR reactions for each DNA extract. The reaction mixtures with final volume of 50 μl contained 0.2 mM of each dNTP (Finnzymes, Finland), 0.5 mM of both primers (Oligomer, Finland), 0.05 % of bovine serum albumin (BSA acetylated, Promega), 1×Biotools reaction buffer with 2 mM $MgCl_2$ and 1 U of DNA polymerase (Biotools, Spain). Peltier Thermal Cycler DNA Engine (MJ Research) was used for the amplification with the following programme: initial denaturation at 95 °C for 5 min, followed by 35 cycles (40 cycles for *cbrA*) of denaturation at 94 °C for 45 s, annealing at variable temperature for 1 min and elongation at 72 °C for 2 min. The presence and size of PCR product was checked on a 1.5 % agarose gel.

Bioremediation amendments

Soils used for bioremediation treatments were carefully mixed from polluted soil and control soil with a final concentration of 27 ppm PCBs (analysed in three technical replicates). Soils were incubated (aerobically and anaerobically) at 10 and 30 °C in heat-controlled incubators for 2 months in order to see if higher temperature increases the degradation of PCBs. Treatments included autoclaved sterile control, active control without amendments, 50 kg N ha^{-1}, 100 kg N ha^{-1}, pulverised white clover (*Trifolium repens*; 0.5 g dry mass in 10 g field moist soil) and pulverised pine needles (*Pinus contorta*; 0.5 g dry mass in 10 g field moist soil). After the additions, 10 g of field moist soil mixture was transferred to five replicate 20-ml amber vials (Agilent Technologies, Germany) that served as microcosms. Microcosms were covered with ultraclean screw cap with septa (Agilent technologies, Germany). The aerobic samples were adjusted to 60 % WHC for optimal microbial activity (Alexander 1999) and only closed loosely in order to let air in continuously to the samples. Anaerobic conditions were obtained by addition of 10 ml deionized water into the microcosms.

Soil dehydrogenase activity

Dehydrogenase enzymes are involved in the aerobic degradation of PCBs (Ohtsubo et al. 2004) through the biphenyl pathway. Dehydrogenase activity was determined according to the modified method of Trevors (1984). One gram of soil was placed into sterilised and foil-covered plastic 50-ml centrifuge bottles (Sarstedt, Germany). Ten milliliter of sterile substrate solution (0.1 % *p*-iodonitrotetrazolium chloride) and 0.5 M TES buffer (adjusted to pH 7.8 with 0.5 M NaOH) was added and samples were placed on an end-over shaker for

Table 2 Summary of primers used for PCR amplification of PCB degrading genes

Gene	Primer	Sequence	Primer annealing °C	Reference
bphA	bphAf668-3 bphAr1153-2	5′ GTTCCGTGTAACTGGAARTWYGC 3′ 5′ CCAGTTCTCGCCRTCRTCYTGHTC 3′	58 °C	Witzig et al. (2006)
fcbA	Forward Reverse	5′ AACTGATCCGCCGAGACAACATCC 3′ 5′ AGGCATTTTTCGAGACGCTTCA 3′	60 °C and 58 °C	Rodriques et al. (2001)
fcbB	Forward Reverse	5′ GGTCCAGCGCGAAATCCAGTC 3′ 5′ CCCCCGCACACCGCATCAAG 3′	55 °C	
ohb	F580*ohb* R580*ohb*	5′ GCGGACAAGCGTTTCGATACAGGA 3′ 5′ GCTTGCAGTTGCGCTTGATGAT 3′	58 °C	Rodriques et al. (2006)
cbrA	cbdbA84_f cbdbA84_r	5′ CTTATATCCTCAAAGCCTGA 3′ 5′ TGTTGTTGGCAACTGCTTC 3′	55 °C	Wagner et al. (2009)
cbrA	cluster 2a_f cluster 2a-1_r	5′ GTYTTCMAKGAYHTKGACGA 3′ 5′ TCRATTTMTYAGGYAKCAC 3′	48 °C	
Chloreflexi 16 s rRNA	fD1	5′ AGAGTTTGATCCTGGCTCAG 3′	62 and 57 °C	Weisburg et al. (1991)
	Dehal1265R	5′ GCTATTCCTACCTGCTGTACC 3′		Watts et al. (2005)

18 h at room temperature. After shaking, 10 ml of ethanol was added and samples were centrifuged (Universal 320R, Hettich, Germany) for 20 min at 4 °C and 2,700×*g*. Absorbance of the supernatant at 490 nm was measured with a linear spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences, Sweden). Standard curves were determined using 1, 2, 3 and 5 ppm iodonitetrazolium formazan.

Data analyses

Basic statistical analyses of the results were performed with SAS 9.1 for Windows and Excel Analysis ToolPak. In addition, PCB data was analysed with a non-parametric multivariate test based on Gower dissimilarity that gives equal weights to the different variables (congeners). The significance of the difference in congener abundance was evaluated by permutation test in the CAP programme (Canonical Analysis of Principal Coordinates, mode: Generalised Discriminant Analysis) available at http://www.stat.auckland.ac.nz/~mja/Programs.htm (Anderson and Robinson 2003). Statistical testing was based on 9,999 permutations.

Results

Bioavailability of PCBs in soil to earthworms

The earthworms in the control soil appeared to be in good condition throughout the study whereas the earthworms in the polluted soils faced higher mortality. Of the 30 earthworms added in the beginning of the experiment, only five remained alive in the 12.5 ppm PCB soil and nine in the 25 ppm PCB soil, compared to all remaining alive in the control soil. The PCB accumulation in earthworms, in 12.5 ppm PCB soil and 25 ppm PCB soil, respectively, is presented on the *x*-axis of Fig. 1a. Bioaccumulation factor (BAF, PCB concentration in earthworms; in milligram per gram of fresh weight) divided by PCB concentration in the soil (in milligram per gram of dry weight) for earthworms was 0.89 and 0.82 for earthworms in 12.5 ppm PCB soil and in 25 ppm PCB soil, respectively. The earthworms accumulated both higher chlorinated and less chlorinated congeners (Table 3). Congener abundance and proportions, as evaluated by generalised multivariate discriminant analysis without and with data standardisation, were significantly different in the two PCB levels ($P=0.027$ and $P=0.016$, respectively; Fig. 1). Earthworms accumulated PCB 153 in greatest quantity, as it was the congener of the highest concentration in both soil mixtures. The highly chlorinated PCB 153, 180 and 187 were accumulated in great quantity, as well as PCB 138 and 101. However, BAFs were highest for the least chlorinated congeners (Table 3).

Presence of PCB degrading bacterial genes and groups

Soil DNA concentration (average±standard deviation) varied from 2.14±1.57 μg g^{-1} soil fresh weight in unpolluted control soil to 0.13±0.04 in soil containing 25 ppm of PCBs to 0.36±0.06 in soil containing 50 ppm of PCBs. A single strong PCR product of the expected size (approximately 500 bp) was systematically produced with bphA primers from all the PCB-contaminated soil extracts with no or faint products from the unpolluted control soil. No PCR products were amplified from either contaminated or control soil DNA extracts with any of the primers related to dechlorination or degradation of dechlorination products.

PCB degradation and biological activity

PCB bioremediation trial

Large variation in total PCB concentration was observed after the bioremediation trial, possibly due to treatment effects on the extractability of the aged PCBs. Best reduction in total PCBs was obtained in the aerobic pine needle treatment at

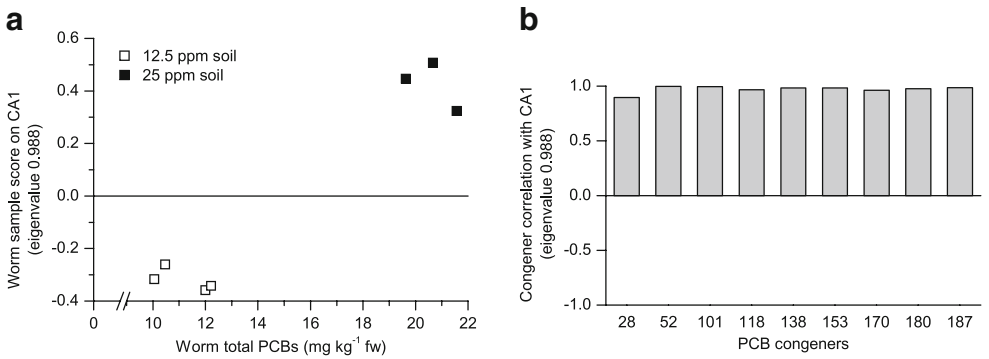

Fig. 1 **a** Distribution of the earthworm samples on the single canonical axis formed with discriminant analysis, testing the hypotheses that PCB congener uptake differs in *E. foetida* incubated in soil with different PCB contamination level (permutated *P* value 0.027). *x*-Axis is the total PCB uptake, which was below detection limit in worms incubated in clean control soil. **b** Correlations of the individual PCB congeners with the same canonical axis

Table 3 Earthworm PCB concentrations and bioaccumulation factors (BAFs) for individual congeners in 12.5 ppm PCB soil and 25 ppm PCB soil. Mean and standard deviation for three replicate microcosms (each with 10 worms) is presented

	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 170	PCB 180	PCB 187	Total PCBs
PCB										
12.5 ppm soil	0.22 (0.04)	0.15 (0.01)	0.68 (0.02)	0.26 (0.03)	0.93 (0.04)	1.49 (0.07)	0.59 (0.10)	1.16 (0.05)	1.22 (0.04)	11.19 (1.08)
25 ppm soil	0.34 (0.06)	0.25 (0.02)	1.34 (0.05)	0.43 (0.05)	1.75 (0.05)	2.75 (0.08)	0.94 (0.06)	1.93 (0.04)	1.92 (0.05)	20.62 (0.97)
BAF										
12.5 ppm soil	7.32 (1.20)	2.98 (0.16)	2.07 (0.05)	4.27 (0.51)	3.46 (0.13)	1.17 (0.05)	0.84 (0.14)	0.69 (0.03)	1.40 (0.04)	0.89 (0.09)
25 ppm soil	11.35 (2.05)	5.06 (0.37)	4.07 (0.16)	7.09 (0.92)	6.49 (0.19)	2.16 (0.06)	1.34 (0.08)	1.15 (0.02)	2.20 (0.06)	0.82 (0.04)

10 °C, which yielded a 38 % reduction in the total PCBs after 2 months, final concentration being 16.9 ppm (95 % confidence interval, 16.1–17.6 ppm) compared to 27.0 ppm at the beginning of the experiment. For the pine needle treatment, a reduction compared to sterile and non-sterile controls was observed also at aerobic 30 °C, whereas the other additives did not seem to improve PCB degradation in any incubation condition (Table 4).

The abundance of the different PCB congeners was significantly different in the untreated, anaerobically treated and aerobically treated samples (permutated $P=0.001$). In the constrained ordination, the aerobically treated samples received generally higher scores than the anaerobically treated ones on the canonical axis 1, with which congeners 28, 52 and 118 showed negative correlation (Fig. 2). Best reduction in total PCBs was obtained in the aerobic pine needle treatment at 10 °C, which yielded a 38 % reduction in the total PCBs after 2 months, final concentration being 16.9 ppm compared to 27.0 ppm at the beginning of the experiment (Table 4).

Soil dehydrogenase activity

The highest values for dehydrogenase activity were measured for white clover treatments and the second highest for the pine needles treatment (Fig. 3). All treatments with fertiliser addition (50 and 100 kg N ha^{-1}) resulted in decreased dehydrogenase activity. Activity was typically higher at 10 °C than at 30 °C. In general, PCB concentration had a significant negative correlation with dehydrogenase activity (Pearson $r=-0.67$, $p<0.001$).

Discussion and conclusions

Bioavailability of PCBs in soil to earthworms

PCBs in the studied soils were bioavailable to earthworms (*E. foetida*) increasing the risk of the mobility of pollutants to the surrounding environment. Earthworms process a large quantity of soil daily and in addition they may absorb pollutants through their thin external barrier, which may increase the bioaccumulation of PCBs in the food web when higher organisms, such as birds, consume earthworms (Ville et al. 1995). Explanations for the observed high bioavailability include the low organic matter content of the soils (e.g. Wågman et al. 2001), the low CEC of the studied soils compared to Icelandic soils in general (Sigurgeirsson et al. 2005) as well as lower allophane and ferrihydrite contents than reported by Guicharnaud and Paton (2006). Ideally, earthworms will be able to release some of the most recalcitrant parts of the pollutants and at the same time enhance the soil properties including porosity and aeration of the soil. In this study, the earthworms were able to absorb and accumulate both highly and less chlorinated PCB congeners (Fig. 1, Table 3). This is in accordance with other studies done on Aroclor mixtures (e.g. Tharakan et al. 2006). Interestingly, we found bioaccumulation factors to vary for the different congeners. BAF correlated negatively ($r=-0.766$, $P=0.01$) with the number of chlorines, suggesting that the test in fact indicated not higher accumulation but higher bioavailability of the less chlorinated congeners. To our knowledge, this is the first report of systematic patterns observed in BAFs for different PCB congeners.

PCB degradation and biological activity

Aroclor 1260 has previously been shown not to be susceptible to aerobic biodegradation (Crawford and Crawford 2005). In contrast, our study found aerobic biostimulation at 10 °C with pine needles result in 38 % reduction of total PCBs in 2 months. The success with pine needles could be explained by the increase in dehydrogenase activity and the terpenes in the pine needles acting like a natural substrate for biphenyl-degrading bacteria (Herdandez et al. 1997; Park et al. 1999). Lack of degradation due to white clover amendment may be related to flavonoids not being able to function as growth substrates for PCB-degrading bacteria as described in previous studies (Donnelly et al. 1994; Pieper 2005). Preliminary experiment treatments with plant detritus and roots did not result in degradation of the total PCBs in the samples, which

Table 4 Soil total PCBs and individual congeners after 2-month treatments, mean and standard deviations for 3 replicate microcosms presented

Temperature		Treatment	Total PCB mg/kg	PCB 28 mg/kg	PCB 52 mg/kg	PCB 101 mg/kg	PCB 118 mg/kg	PCB 138 mg/kg	PCB 153 mg/kg	PCB 170 mg/kg	PCB 180 mg/kg	PCB 187 mg/kg
		Before experiment	27.00 (3.54)	0.45 (0.00)	0.23 (0.01)	1.32 (0.10)	0.57 (0.02)	2.21 (0.19)	3.24 (0.28)	1.87 (0.17)	3.48 (0.29)	2.05 (0.21)
10 °C	Anaerobic	Sterile	54.05 (16.51)	0.30 (0.01)	0.24 (0.01)	1.44 (0.36)	0.40 (0.03)	3.22 (1.08)	4.71 (1.58)	2.85 (1.01)	6.17 (2.40)	3.26 (1.23)
		Control	55.36 (10.72)	0.28 (0.01)	0.23 (0.02)	1.48 (0.24)	0.39 (0.04)	3.56 (0.75)	5.17 (1.00)	3.08 (0.65)	6.78 (1.34)	3.40 (0.68)
		50 kg N/ha	46.36 (7.82)	0.31 (0.02)	0.22 (0.00)	1.33 (0.17)	0.39 (0.00)	2.96 (0.48)	4.30 (0.75)	2.60 (0.43)	5.54 (1.07)	2.87 (0.49)
		100 kg N/ha	56.93 (6.07)	0.32 (0.01)	0.34 (0.09)	1.54 (0.07)	0.42 (0.00)	3.55 (0.31)	5.31 (0.43)	3.10 (0.34)	6.92 (0.88)	3.56 (0.45)
		White clover	45.14 (5.27)	0.32 (0.01)	0.28 (0.01)	1.25 (0.09)	0.42 (0.02)	2.66 (0.18)	3.77 (0.30)	2.28 (0.14)	4.59 (0.33)	2.32 (0.16)
		Pine needles	49.67 (5.21)	0.33 (0.01)	0.31 (0.01)	1.49 (0.18)	0.45 (0.02)	3.21 (0.35)	4.40 (0.44)	2.65 (0.30)	5.29 (0.59)	2.68 (0.29)
10 °C	Aerobic	Sterile	42.64 (1.10)	0.22 (0.00)	0.22 (0.00)	1.28 (0.13)	0.35 (0.02)	3.00 (0.24)	4.00 (0.45)	2.49 (0.27)	5.42 (0.61)	2.94 (0.38)
		Control	41.81 (3.50)	0.24 (0.01)	0.24 (0.00)	1.22 (0.09)	0.34 (0.01)	2.65 (0.28)	3.88 (0.35)	2.17 (0.18)	4.80 (0.42)	2.63 (0.23)
		50 kg N/ha	61.77 (14.53)	0.22 (0.02)	0.26 (0.04)	1.76 (0.44)	0.39 (0.06)	4.30 (1.22)	5.59 (1.45)	3.34 (0.87)	7.52 (1.98)	3.93 (0.93)
		100 kg N/ha	48.59 (5.66)	0.24 (0.01)	0.25 (0.01)	1.37 (0.11)	0.36 (0.02)	3.25 (0.39)	4.33 (0.52)	2.56 (0.35)	5.60 (0.81)	3.03 (0.38)
		White clover	82.80 (29.37)	0.20 (0.02)	0.20 (0.11)	1.50 (1.17)	0.35 (0.17)	3.09 (2.41)	4.49 (3.18)	2.70 (1.77)	5.64 (3.67)	3.01 (2.14)
		Pine needles	16.85 (0.23)	0.20 (0.01)	0.14 (0.00)	0.62 (0.01)	0.25 (0.01)	1.06 (0.01)	1.55 (0.02)	0.93(0.01)	1.75 (0.02)	1.04 (0.01)
30 °C	Anaerobic	Sterile	55.23 (5.83)	0.28 (0.02)	0.27 (0.02)	1.53 (0.15)	0.41 (0.03)	3.47 (0.33)	4.95 (0.54)	3.06 (0.37)	6.21 (0.72)	3.03 (0.32)
		Control	43.01 (3.26)	0.29 (0.01)	0.26 (0.01)	1.23 (0.09)	0.39 (0.01)	2.72 (0.23)	3.83 (0.34)	2.29 (0.17)	4.60 (0.36)	2.37 (0.15)
		50 kg N/ha	51.94 (3.84)	0.30 (0.01)	0.28 (0.01)	1.35 (0.07)	0.42 (0.02)	3.12 (0.32)	4.16 (0.24)	2.61 (0.19)	5.18 (0.34)	2.64 (0.18)
		100 kg N/ha	40.14 (3.30)	0.30 (0.01)	0.26 (0.01)	1.17 (0.07)	0.39 (0.01)	2.58 (0.21)	3.42 (0.23)	2.23 (0.18)	4.29 (0.34)	2.24 (0.17)
		White clover	42.79 (2.04)	0.32 (0.02)	0.29 (0.01)	1.29 (0.06)	0.42 (0.02)	2.70 (0.18)	3.78 (0.18)	2.30 (0.11)	4.53 (0.18)	2.36 (0.11)
		Pine needles	51.15 (5.04)	0.32 (0.01)	0.28 (0.01)	1.36 (0.14)	0.37 (0.06)	2.95 (0.37)	4.21 (0.53)	2.65 (0.32)	5.35 (0.71)	2.73 (0.38)
30 °C	Aerobic	Sterile	37.14 (4.04)	0.20 (0.01)	0.18 (0.00)	1.13 (0.11)	0.31 (0.01)	2.23 (0.25)	3.29 (0.35)	1.99 (0.21)	4.13 (0.53)	2.22 (0.26)
		Control	46.13 (3.45)	0.19 (0.00)	0.18 (0.00)	1.25 (0.05)	0.32 (0.01)	2.79 (0.22)	4.16 (0.34)	2.63 (0.29)	5.33 (0.50)	2.86 (0.26)
		50 kg N/ha	49.35 (1.10)	0.19 (0.00)	0.18 (0.01)	1.37 (0.08)	0.32 (0.01)	3.09 (0.16)	4.51 (0.23)	2.78 (0.10)	5.76 (0.18)	3.10 (0.13)
		100 kg N/ha	46.12 (2.71)	0.18 (0.00)	0.17 (0.01)	1.27 (0.05)	0.31 (0.01)	2.89 (0.16)	4.14 (0.24)	2.51 (0.14)	5.37 (0.29)	2.89 (0.14)
		White clover	72.33 (5.08)	0.19 (0.00)	0.19 (0.02)	1.56 (0.35)	0.34 (0.05)	3.51 (1.30)	5.13 (1.54)	3.04 (1.32)	6.35 (2.70)	3.50 (1.25)
		Pine needles	27.06 (1.66)	0.17 (0.01)	0.14 (0.00)	0.80 (0.03)	0.24 (0.01)	1.63 (0.07)	2.37 (0.08)	1.44 (0.05)	2.94 (0.12)	1.67 (0.06)

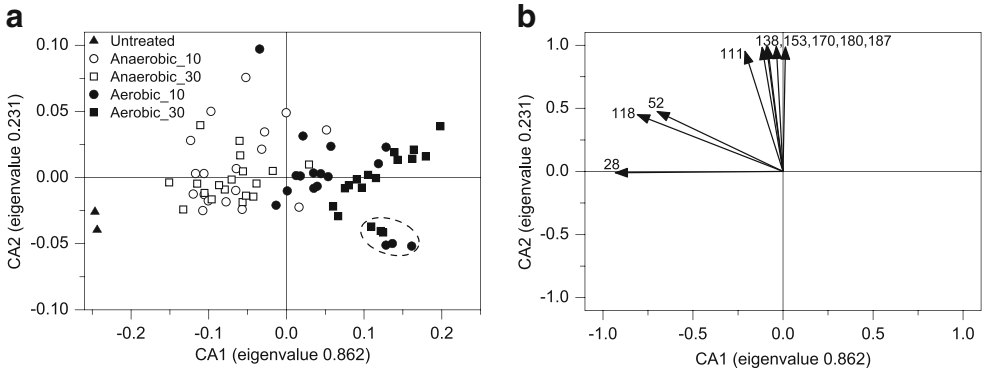

Fig. 2 Canonical plots showing the results of generalised discriminant analysis for PCB congener abundance in bioremediation experiment. **a** Visualises the distribution (scores) of the original and bioremediated-contaminated soil samples in constrained ordination, testing the hypothesis that PCB composition differs in untreated, anaerobically treated and aerobically treated samples (permutated *P* value 0.001). *Dash line* circles the aerobic pine needle treatments. **b** The correlation of the individual PCB congeners with the same canonical axes, illustrating their association to the separation of the differently treated samples

could indicate lack of easily available nitrogen for the microorganisms (Michel et al. 2001). Fertiliser treatments did not decrease total PCBs, possibly due to fertiliser adsorption to the allophane and ferrihydrite surfaces, which in turn would make the nutrients less available for active soil microorganisms (Shoji et al. 1993).

PCB degradation by cold-adapted bacteria has been reported previously (e.g. Welander 2005; Lambo and Patel 2007) and biodegradation in cold environments, including frozen soils (Aislabie et al. 2006), is far from impossible. Factors enhancing biodegradation are 60 % WHC (Aislabie et al. 2006), close to neutral soil pH (Wiegel and Wu 2000; Fava et al. 2003) and temperature (Wu et al. 1997; Guicharnaud et al. 2010). In this study, increase in temperature benefitted neither PCB degradation nor microbial dehydrogenase activity. The increase in dehydrogenase activity obtained after addition of white clover and pine needles is in agreement with Wilke and Bräutingam (1992). The observed strong amplification of the aerobic *bphA* gene furthermore indicates the indigenous capability of the soils to aerobically degrade PCBs. In aerobic oxidative PCB degradation, *bphA* encodes the first fundamental step of the biphenyl upper pathway, in which biphenyl is converted to dihydrodiol and further to CBA. Our inability to detect any genes related to anaerobic PCB degradation potential corroborates the negative results received with the anaerobic treatments. Together, these observations suggest that reductive dehalogenation might require bioaugmentation of effective anaerobic degrader bacteria into the studied soils.

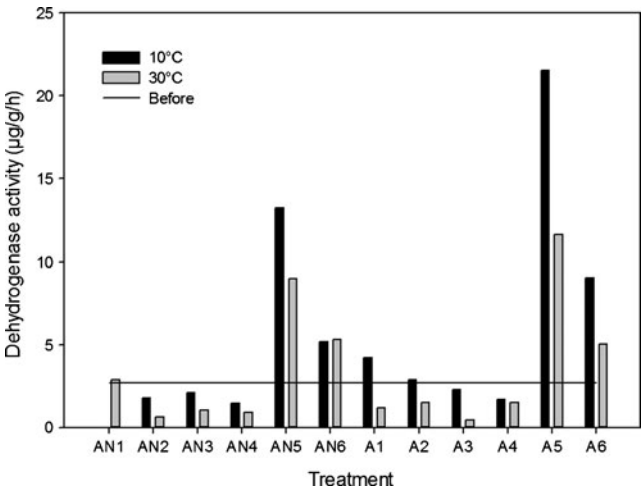

Fig. 3 Dehydrogenase activity before and after different bioremediation treatments at 10 and 30 °C. *Line* mean value of three replicate and columns represent a bulked sample from three samples. *A* aerobic, *AN* anaerobic, *1* sterile control, *2* active control, *3* 50 kg N ha^{-1}, *4* 100 kg N ha^{-1}, *5* white clover and *6* pine needles

Conclusions

This study demonstrated a 38 % aerobic biodegradation of PCBs with pine needle biostimulation at 10 °C in 2 months. These results give an indication that bioremediation at average Icelandic summer field temperatures, with appropriate biostimulation, could be feasible. This is supported by reported findings of the study by Guicharnaud et al. (2010), which showed that the biological properties of the Icelandic soils are adapted to work at low temperatures and governed by substrate availability to microorganisms rather than temperature. Earthworms accumulated both less and higher chlorinated PCBs effectively, which indicates their bioavailability in the studied Icelandic soils as well as risk of biomagnification of PCBs in the food chain.

The success with pine needle treatment is of great significance for bioremediation in cold environments: (1) since Aroclor 1260 has been considered as recalcitrant for aerobic degradation and for any microbial degradation previously, (2) the treatment working best at 10 °C gives good indications for remediation being successful at higher latitudes and (3)

terpenes, which are present in pine needles, may be seen as a feasible and sustainable stimulation method since they are natural materials, easily available in the northern circumpolar region and they can promote bioavailability of PCBs. Therefore, further research should focus on field-scale biostimulation trials with pine needles in order to optimise the method for onsite remediation.

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