Nybohm I, Waissi-Leinonen G, Mäenpää K, Leppänen MT, Kukkonen JWK, Werner D, Akkanen J. [Effects of activated carbon ageing in three PCB contaminated sediments: Sorption efficiency and secondary effects on *Lumbriculus variegatus*](#). *Water Research* 2015, 85, 413-421.

Copyright:

“©IWA Publishing 2015. The definitive peer-reviewed and edited version of this article is published in *Water Research*, 85, 413-421 2015 <http://dx.doi.org/10.1016/j.watres.2015.08.044> and is available at www.iwapublishing.com

DOI link to article:

<http://dx.doi.org/10.1016/j.watres.2015.08.044>

Date deposited:

21/09/2015

Embargo release date:

29 August 2016

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](#)

Effects of activated carbon ageing in three PCB contaminated sediments: sorption efficiency and secondary effects on *Lumbriculus variegatus*

Inna Nybom^{*1}, Greta Waissi-Leinonen¹, Kimmo Mäenpää¹, Matti T. Leppänen², Jussi V.K. Kukkonen³, David Werner⁴, Jarkko Akkanen¹

¹ Department of Biology, University of Eastern Finland (UEF), P.O. Box 111, FI-80101 Joensuu, Finland

² Finnish Environment Institute (SYKE), Ecotoxicology and risk assessment group, P.O.Box 35, FI-40014 University of Jyväskylä, Finland.

³ University of Jyväskylä, Department of Biological and Environmental Science, P.O.Box 35, FI-40014 University of Jyväskylä, Finland.

⁴ School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, England, United Kingdom.

* inna.nybom@uef.fi

ABSTRACT

The sorption efficiency and possible secondary effects of activated carbon (AC) (ø 63-200 µm) was studied with *Lumbriculus variegatus* in three PCB contaminated sediments applying long AC-sediment contact time (3 years). AC amendment efficiently reduced PCB bioavailability as determined with both, *L. variegatus* bioaccumulation test and passive samplers. However, dose related secondary effects of AC on egestion rate and biomass were observed (applied doses 0.25% and 2.5% sediment dry weight). The sorption capacity and secondary effects remained similar when the experiments were repeated after three years of AC-sediment contact time. Further, transmission electron microscopy (TEM) samples revealed morphological changes in the *L. variegatus* gut wall

microvilli layer. Sediment properties affected both sorption efficiency and secondary effects, but 2.5% AC addition had significant effects regardless of the sediment. In conclusion, AC is an efficient and stable sorbent to decrease the bioavailability of PCBs. However, sediment dwelling organisms, such as Oligochaete worms in this study, may be sensitive to the carbon amendments. The secondary effects and possible morphological changes in benthic organisms should not be overlooked as in many cases they form the basis of the aquatic food webs.

Keywords:

Activated carbon; sediment remediation; transmission electron microscopy (TEM); PCBs; PDMS method

1. Introduction

Light density black carbonaceous particles in the sediment (e.g. soot carbon, coal, coke and charcoal) are efficient sorbents for hydrophobic organic compounds (HOCs) and capable of reducing contaminant aqueous concentrations and bioavailability (Jonker and Koelmans 2002; Ghosh et al. 2003). Consequently synthetic black carbon amendments, such as activated carbon (AC), have been studied as an in situ remediation technique for stabilizing contaminated sediments. AC amendments have been shown to reduce biological and aqueous concentrations of a wide variety of HOCs e.g. polychlorinated biphenyls (PCBs) (Zimmerman et al. 2005; Sun and Ghosh 2008; Cho et al. 2009; Janssen et al. 2010; Kupryianchyk et al. 2013b), polycyclic aromatic hydrocarbons (PAHs) (Zimmerman et al. 2005; Cornelissen et al. 2006; Rakowska et al. 2013), dichlorodiphenyltrichloroethanes (DDTs) (Tomaszewski et al. 2008; Hale et al. 2009; Lin et al. 2014), and organotins such as tributyltin (TBT) (Brändli et al. 2009).

Along with the remediation benefits, secondary effects of AC have also been investigated. Reduced weight or inhibition in growth (Millward et al. 2005; Kupryianchyk et al.

2011; Janssen et al. 2012; Nybom et al. 2012; Kupryianchyk et al. 2013b) and decreased lipid content (Jonker et al. 2009; Janssen et al. 2011; Nybom et al. 2012) have been reported for several organisms with AC doses of 4% sediment dry weight (dw) or lower, and with higher doses (15-20% sediment dw) avoidance behavior has been observed (Jonker et al. 2009). Adverse effects have been seen especially on sediment-dwelling organisms exposed to AC amendments both externally through sediment and internally through ingested AC particles (Jonker et al. 2009; Nybom et al. 2012). Contrarily other studies have reported no effects on growth (Millward et al. 2005; Janssen et al. 2011; Kupryianchyk et al. 2011; Janssen et al. 2012; Josefsson et al. 2012), lipid content (Millward et al. 2005; Janssen et al. 2011; Janssen et al. 2012; Josefsson et al. 2012; Kupryianchyk et al. 2013b) or avoidance behavior (Kupryianchyk et al. 2011; Janssen et al. 2012) and through reduced sediment toxicity even some positive effects on survival in short term experiments has been observed (Kupryianchyk et al. 2011). The ecological responses were recently reviewed by Janssen and Beckingham (2013) and Kupryianchyk et al. (2015).

Despite of interest toward ecological responses the majority of the research has focused on assessing contaminant bioaccumulation. The mechanisms behind the AC induced ecological effects remain unclear. AC has been shown to bind nitrogen from the water (Janssen et al. 2012), but the observed avoidance behavior and reduction in feeding (Jonker et al. 2009; Nybom et al. 2012) indicates that AC may also affect sediment dwelling organisms through some other mechanisms. Sediment-dwelling organisms are relevant test species since they form the base of many aquatic food webs acting as an important food source for predatory invertebrates and fishes. Changes at the bottom of the food webs may alter not only the food web structure but also food chain transfer of HOCs (Figueiredo et al. 2014).

The present study investigates the effects of AC amendments on bioavailability of PCBs in three different sediments by applying *Lumbriculus variegatus* bioaccumulation tests, and passive sampling with silicone coated glass method. The secondary effects of the amendments on *L.*

76 *variegatus* were studied by determining egestion rate, reproduction, biomass and lipid content of the
77 worms when exposed to AC, and by preparing transmission electron microscopic (TEM) samples to
78 study internal structure changes.

79

80 **2. Materials and Methods**

81 **2.1 Organisms**

82 *L. variegatus* used as a test organism were reared at the University of Eastern Finland in the
83 Department of Biology. The rearing method has been described earlier (Leppänen and Kukkonen
84 1998a). Prior to the experiments the worms were acclimated in artificial fresh water for 24h. Small
85 and actively swimming worms were chosen by visual observation (Leppänen and Kukkonen
86 1998b). The weight of the worms at the beginning was evaluated by weighting a set of individuals
87 (minimum 23 worms) with the representative size 5-9 mg ww (Leppänen and Kukkonen 1998b)
88 prior to experiment.

89

90 **2.2 Sediments**

91 Three natural sediments from PCB contaminated areas in Southern Finland were chosen in the
92 experiment. The sediments were collected from the River Tervajoki (N 60° 82' 34" E 24° 63' 52",
93 TJ) and from Lake Kernaalanjärvi (N 60° 85' 44" E 24° 64' 21", KJ) located downstream of the
94 same water body. Some studies of the KJ water district have been previously published (Koponen et
95 al. 2003; Mäenpää et al. 2011; Figueiredo et al. 2014). A third sediment for the experiments was
96 collected from Viinikanlahti bay (N 61° 48' 97" E 23° 76' 70", VL) which is unrelated to the
97 previously mentioned water body. The PCB contamination in KJ and TJ relates to discharges of
98 PCB oils from a paper mill from 1956 to 1984 (Figueiredo et al. 2014). In VL a single source of
99 contamination cannot be named, but the contamination is a result of an extensive use and
100 production of PCB compounds prior to the 1970s. The sediments were sieved to a particle size

101 smaller than 1mm and stored at 5 °C prior to the experiments. Total organic carbon (TOC) and
102 black carbon (BC) content of the sediments was analysed with an Analytik Jena TOC analyser with
103 a solid sample module (Analytik Jena N/C 2100, Jena, Germany). Phosphoric acid (1M) was used
104 to remove the inorganic carbon from the sediment samples. BC content was determined according
105 to the chemical oxidation pre-treatment method (BC-chemox) described by Grossman and Ghosh
106 (2009).

107 Sediment samples were extracted for PCBs with acetone:hexane (1:1). The fresh
108 sediment was dried by applying Na₂SO₄, weighted in extraction thimbles and extracted with a
109 Soxtec system (1043 Extraction unit, Tecator AB, Höganäs Sweden) and analysed by using an
110 internal standard method. The procedure is described in detail in the Supplementary content. PCB
111 30 was used as internal standard and PCB 159 as an injection standard. Overall 20 PCB congeners
112 were analysed; 18, 28, 31, 44, 49, 52, 70, 101, 105, 110, 114, 118, 138, 149, 151, 153, 156, 157,
113 167 and 180. The extracted samples were analysed for PCB congeners with a gas chromatograph
114 (Hewlett- Packard series 6890) coupled with a mass selective detector (MSD) (Hewlett-Packard,
115 Avondale, PA, USA). The methods of analysis and quality assurance are the same as in Figueiredo
116 et al. (2014) and are described therein.

117

118 **2.3 Activated carbon**

119 Bituminous coal-based AC, Carbsorb® 40 (Chemviron carbon, England) was used in the
120 experiments. Prior to use the AC was ground and sieved to match the particle size range ø 63-200
121 µm. Based on the selected particle size fraction both internal and external exposure was expected,
122 since particles smaller than 100 µm are ingestible for *L. variegatus* (Nybom et al. 2012). The
123 particle size fraction of AC was verified by Nybom et al. (2012), stating that based on organic
124 petrography analysis most of the particles were in the nominal size range, although some smaller
125 particles were also detected. The mixing procedure may also cause some abrasion of AC particles,

126 but the effect is expected to be for a minor fraction of the total mass of AC applied (Nybom et al.
127 2012). The AC was amended to the sediment by presoaking it into the water over night followed by
128 mixing into the sediments with a stainless steel paddle driven by an overhead motor for 2h. AC was
129 added to the test sediments on a dw basis on dosages of 0.25% and 2.5%. Dosages were selected to
130 represent low and average level of AC addition generally recommended for remedial purposes (1-
131 5%) (Ghosh et al. 2011). Amended sediments were allowed to settle in closed glass vials for two
132 weeks (12-18 days), in darkness at 5 °C prior to the first experiments. Experiments were repeated
133 three year later, when the AC-sediment contact time (CT) was 1093- 1171 days. Correspondingly
134 the sediments were kept in dark, at 5 °C prior to the second experiment set. The control sediments
135 without AC amendment were processed accordingly.

136

137 **2.4 Experimental design and ecological responses**

138 **2.4.1 Experimental setup**

139 Both experimental sets, with the settling time of two weeks and three years were performed
140 according to the same methods. Experiments were conducted in standard conditions 20 ±1°C, 16:8
141 light:dark, (OECD, 2007). Sediment was weighted into a glass experimental vessel and artificial
142 fresh water, 1mM, pH 6.5-7.5 (OECD, 1992) was placed on top of the sediment, after which the
143 experimental units were allowed to settle for two days under aeration prior to the experiments.
144 Experimental units were aerated during the experiments. Oxygen content, water and sediment pH
145 and temperature was monitored weekly (MultiLine P4, Weilheim Germany). Part of the overlying
146 water in experimental units was changed when required with an objective to keep the water pH in
147 an acceptable range [6.0-9.0, (OECD, 2007)]. Ammonium level was measured for each water
148 change and at the end of the experiments.

149

150 **2.4.2 Ecological responses**

151 Studied ecological responses of *L. variegatus* were egestion rate, growth, reproduction and total
152 lipid content. Egestion rate (14 days, n=3) was studied according to the method described in Nybom
153 et al. (2012), and calculated as produced fecal pellets (dw, mg) per worm weight (dw, mg) and hour.
154 In the study of growth, reproduction and total lipid content (n=4), 50g of sediment (fw) was
155 weighted into a 200ml glass jar and filled with artificial fresh water. The duration of the experiment
156 was 28 days, after which the worms were sieved from the sediment (ϕ 200 μ m) and placed in clean
157 water for 6h to clean the gut content (Mount et al. 1999). The growth was studied as an alteration in
158 biomass (fw) and reproduction was measured by counting the worms. The reproduction factor was
159 calculated by dividing the number of the worms at the end by the number of worms applied at the
160 beginning. Total lipids were analysed gravimetrically (Parrish 1999), by using 2-propanol-hexane-
161 water (12:8:5; v:v:v) in lipid extraction. Ten worms were applied per experimental unit at the
162 beginning of the experiment.

163

164 **2.4.3 Transmission electron microscopic samples**

165 Transmission electron microscopic (TEM) samples were prepared to analyze possible internal
166 structure changes in *L. variegatus*. The samples were prepared from the worms exposed to the
167 sediment after three years CT (n=6). Duration of the exposure was 28 days and the samples were
168 handled as described earlier. TEM samples were prepared additionally from worms reared in
169 artificial sediment (OECD, 2007) with added *Urtica dioica* powder (0.5% sediment dw, (OECD,
170 2007) and limited food level (no *U. dioica*). Additional samples were prepared to study the effects
171 of feeding, since the adverse effects of AC amendments has been suggested to relate to the sorption
172 of nutritious compounds from the sediment (Janssen et al. 2012; Rakowska et al. 2012;
173 Kupryianchyk et al. 2013b). TEM samples were handled according to the method described by
174 Waissi-Leinonen et al. (2012). Briefly the samples were fixed with 4% glutaraldehyde and 0.2%
175 Na-cacodylate buffer (pH 7.5) 1:1 overnight in 5 °C temperature. Dehydration was made with

176 ascending acetone concentration followed by embedment into Epon resin (LX-112 Ladd Research
177 Industries, Williston US). The samples were cast and eighty-nanometer-thick slices were cut
178 vertically in the area of the gut, from the middle part of the worms. The samples were analysed with
179 a Zeiss 900 high-resolution TEM (West Germany). From TEM figures length and densities of gut
180 wall microvilli was calculated. Calculations were made from 6 replicate samples from which 400
181 microvilli were measured, and for density measurements a minimum 57 μm distance was examined
182 (Figure S1).

183

184 **2.5 PCB concentrations and bioaccumulation**

185 **2.5.1 Bioaccumulation of PCBs**

186 For the PCB bioaccumulation study 250 g of sediment were weighted into a 1l beaker and filled
187 with water. After two days settling time approximately 60 worms were applied per experimental
188 unit (approximately 0.5g fw, n=3). The duration of the bioaccumulation experiment was 28 days,
189 after which the worms were sieved from the sediment, allowed to clear the gut content for 6 h in
190 artificial fresh water, weighted and preserved by freezing. The worm samples were extracted for
191 PCBs with ultrasound method by acetone:hexane (1:1) using an internal standard method. The same
192 method has been applied for Chironomidae larvae and is described on detail by Mäenpää et al.
193 (2011).

194

195 **2.5.2 Determination of $C_{\text{Pol,Sed}}$, C_{free} and $C_{\text{Lip}\rightleftharpoons\text{Sed}}$**

196 The equilibrium concentrations of PCBs in the passive sampling polymer equilibrated with
197 sediment ($C_{\text{Pol,Sed}}$) were measured by using a passive sampling method with silicone coated glass
198 described in Mäenpää et al.(2011). Glass vials (20 ml) were internally coated with nominal 4, 8, and
199 16 μm layers of silicone polydimethylsiloxane (PDMS) (SilasticA, Dow Corning Corporation,
200 Midland, MI). The method was originally developed for PAHs (Reichenberg et al. 2008), and later

201 optimized for PCBs (Mäenpää et al. 2011). Coated jars were filled with sediment samples, with and
 202 without AC addition, and sealed tightly with a lid. The jars were rotated slowly (5rpm) in a roller-
 203 mixer in horizontal position at the room temperature without illumination for two weeks in order to
 204 achieve equilibrium of the analytes between the polymer and sediment. Then the analytes were
 205 extracted from the silicone by n-hexane. The extraction procedure was modified from the method
 206 described by Mäenpää et al.(2011) and is described in detail in the supplementary content. Analysed
 207 PCB congeners were ($\Sigma 16$); 18, 28, 31, 52, 44, 49, 101, 105, 110, 118, 138, 149, 151, 153, 156,
 208 180. Two parallel samples for each silicone thickness were prepared for all of the sediment samples
 209 (i.e. n=6 for each treatment).

210 PCB concentrations in the passive sampling polymer at equilibrium with sediment
 211 ($C_{Pol,Sed}$) were converted to freely dissolved concentration (C_{free}) using analyte-specific
 212 polymer/water partition ratios ($K_{Pol/W}$):

$$214 \quad C_{free} = \frac{C_{Pol \rightleftharpoons Sed}}{K_{Pol/W}} \quad (1)$$

215
 216 $K_{SilasticA/W}$ were taken from Smedes et al. (2009).

217 The concentrations in the passive sampler polymer at equilibrium with sediment
 218 ($C_{Pol,Sed}$) were converted to equilibrium partitioning concentrations in lipids ($C_{Lip \rightleftharpoons Sed}$) by
 219 multiplying with the analyte specific polymer/lipid partition ratios ($K_{Lip/Pol}$) as described in
 220 Mäenpää et al.(2011):

$$222 \quad C_{Lip \rightleftharpoons Sed} = C_{Pol \rightleftharpoons Sed} \times K_{Lip/Pol} \quad (2)$$

223
 224 $K_{Lip/Pol}$ were taken from Jahnke et al. (2008).

225

226 **2.6 Statistical analyses**

227 The results are expressed as mean $\pm$ standard deviation (sd). Two way analysis of variance
228 (ANOVA) was used to examine the effects of time and AC exposure, followed by Bonferroni
229 posthoc test to ascertain the difference between AC amended and unamended sediment.
230 Homogeneity of variances was tested by Levene's test and the normality was studied with Shapiro-
231 Wilks test. If homogeneity of variances or normal distribution of the data was not met or when n
232 was smaller than 4, nonparametric Kruskal-Wallis test was used, followed by Dunns posthoc test to
233 study the effects of AC amendment.

234 Statistical analyses were performed with GraphPad Prism 5.0 for Windows (GraphPad
235 Software, San Diego, CA, USA) and SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

236

237 **3. Results and Discussions**

238 **3.1 Sediment characteristics and experimental design**

239 Sediment dw, TOC and BC values are presented in Table S1. The water pH showed tendency to
240 decrease during the experiments, and it was adjusted by changing the water approximately once a
241 week. In VL the pH decrease was most intense and despite the water change pH below 6 was
242 occasionally measured. However obvious pH related ecological responses were not observed (the
243 worms were able to grow and no mortality was observed) and the decrease in pH was unrelated to
244 the AC addition. The oxygen level remained at the adequate level throughout the experiment due to
245 the aeration. Temperature remained stable and the variation among experimental units was
246 negligible. Slight ammonium formation was observed, but due to the low pH levels the assumption
247 can safely be made that ammonium had no effects on the organisms (Schubaur-Berigan et al. 1995).
248 During preservation sediment pH dropped slightly in all sediments regardless of the AC
249 amendments. Average water and sediment quality parameters are represented in supplementary
250 content (Table S2).

251 Comparison between the experiments executed at different times revealed that
252 datasets differed significantly between the years (with both ecological parameters and
253 bioaccumulation). AC addition, however, did not cause any consistent effect on sediments, meaning
254 that the sediments were not univocally improved or deteriorated during preservation, but the effects
255 of storage time differed among sediments. Some variation between experiments executed at
256 different times can be expected due to the natural variation caused by organisms, experimental
257 conditions and measurement accuracy. Despite the observed variation the effects of AC addition
258 was indisputable at both sampling times and the effect of AC addition in each sediment studied was
259 mostly the same.

260

261 **3.2 Ecological responses**

262 AC dose related responses in growth, reproduction and egestion rate were observed in all of the
263 studied sediments (Figures 1, S2-S3). Compared to the unamended sediment the biomass and
264 egestion rate decreased with increasing AC dose, but the responses were dependent on the sediment
265 type. The characteristics of the studied sediments differed (TOC, BC, dw) (Table S1) and the TOC
266 content of KJ sediment was considerably lower than in the other two sediments. This may explain
267 the magnitude of the ecological responses observed in KJ sediment.

268 In KJ 0.25% AC addition reduced egestion for 36% (two weeks CT) and 44% (three
269 years CT) compared to the unamended sediment, whereas in TJ and VL sediments the effects of the
270 low AC dose were less apparent. The higher AC dose of 2.5% had clear effects on egestion in all of
271 the studied sediments. The reduction in egestion rates with higher AC dose were KJ 90% (two
272 weeks CT) and 87% (three years CT), TJ 45% (both two weeks, and three years CT), and VL 86%
273 (two weeks CT) and 69% (three years CT) (Figure S2). Reduced egestion rates of *L. variegatus*,
274 induced by AC amendments, have been reported before, especially with ingestible AC particle sizes
275 (Jonker et al. 2009; Nybom et al. 2012). Egestion rates after three years were generally lower

276 compared to the egestion rates after two weeks CT, this phenomenon is discussed in more details in
277 supplementary content.

278 Growth and reproduction was most intense in TJ sediment. In unamended sediment
279 the biomass of the worms more than doubled during the experiment (increases of 2.96 ± 0.13 after
280 two weeks CT and 2.65 ± 0.09 after three years CT). Increase in biomass was observed also in
281 unamended KJ (1.49 ± 0.09 two weeks CT, 1.55 ± 0.09 three years CT) and VL (1.85 ± 0.08 two
282 weeks CT, 1.72 ± 0.10 three years CT) sediments. The low AC dose caused a significant reduction
283 in growth in KJ, whereas in TJ and VL the effect was less pronounced (Figure 1). The biomasses of
284 the worms exposed to 2.5% AC amended sediments were significantly lower compared to the
285 unamended sediments. In KJ (two weeks CT) and VL (two weeks and three years CT) the worms
286 lost weight during the experiments with the higher AC dose, whereas in TJ (two weeks and three
287 years CT) the worms were still gaining weight during the experiment despite the AC addition
288 although significantly less than in unamended sediment. Reduced sediment consumption correlates
289 with the observed effects on biomass (Figure S4). The biomass may also be affected by reduced
290 bioavailability of nutritious compounds sorbed by AC from the sediment (Jonker et al. 2004;
291 Schreiber et al. 2005; Janssen et al. 2012), altering the food availability or quality for *L. variegatus*.

292 Survival of organisms was high throughout the experiment and the number of worms
293 remained stable or increased in all experimental units (reproduction factor 1 or higher). Addition of
294 2.5% AC induced significant reduction in reproduction in all sediments after two weeks CT, but
295 significant effects were only observed in TJ after three years CT, where 0.25% AC addition reduced
296 reproduction by 55% and 2.5% AC addition by 81% compared to the unamended sediment (Figure
297 S3). Responses on *L. variegatus* reproduction may not be directly linked to the worm biomass.
298 Instead increased reproduction may also relate to hormesis-like response to environmental stress, a
299 phenomenon which has been observed for *L. variegatus* exposed to small doses of fluoranthene
300 (Landrum et al. 2002) and AC amendments (Nybom et al. 2012). Slight increase in reproduction

301 with increasing AC dose can be observed in KJ and VL after three years of CT although significant
302 effects were not observed. Due to the inconsistency of the responses, reproduction as an ecological
303 endpoint should be interpreted together with other ecological endpoints.

304 Lipid content did not correlate directly with AC dose in the sediment or other
305 ecological endpoints studied. The different sampling times, two weeks and three years CT, proved
306 to be pair wisely comparable, meaning that proportionally similar lipid contents were measured at
307 different sampling times. Only in TJ sediment a slight AC dose related trend in lipid decrease can
308 be seen, although no statistical difference was observed between the groups (Figure S5). Nybom et
309 al. (2012) observed significant AC induced reduction in lipid content in an experiment performed
310 with *L. variegatus* in artificial sediment, but no effects were observed in AC amended natural
311 sediment. They concluded that the method used for lipid extraction is sensitive to detect the increase
312 in lipids when the worms gain weight. However if the increase in weight is not sufficient or the
313 worms lose weight, the response in lipids may be undetectable because the proportion of storage
314 lipids becomes negligible in worm samples and diminishes the effects of AC amendments. In TJ the
315 growth of the worms was most intense, and although the worms gained weight also in KJ and VL
316 sediments the weight increase may have not been adequate for the response to be seen in lipids.
317 Sediment type has been shown to strongly affect *L. variegatus* lipids (Jonker et al. 2009; Nybom et
318 al. 2012), and with longer experiment duration or higher AC doses the effect could have been more
319 consistent also with the studied sediments.

320

321 **3.3 Transmission electron micrographs (TEM)**

322 TEM samples revealed clear differences between worms exposed to AC amended and unamended
323 sediment. The microvilli layer inside the gut wall of digestive tract was denser and longer in
324 unamended sediment compared to the AC exposed worms (Figures 2-3). Study performed with
325 clean artificial sediment with limited food supply revealed that available nutrients may affect the

microvilli length. The average microvilli length was 660 ± 126 nm with normal feeding and 611 ± 122 nm with limited food supply, statistically significant differences were observed between the two feeding levels. The microvilli measured in artificial sediment were shorter than observed in field collected sediments. Artificial sediment was prepared from finely ground peat, kaolinite and quartz sand (OECD, 2007). The shorter microvilli length compared to the natural sediments might be due to the sediment texture, containing sharp grains of quartz sand, possible causing damages to the worm gut microvilli layer. Density of microvilli was slightly lower in limited feeding conditions, but a significant difference between treatments was not found (number/ μm 5.056 ± 0.966 with normal feeding and 4.578 ± 0.725 with limited feeding level). Over all the effects caused by limited food availability were milder than observed in AC amended sediments.

As far as is known, previous studies describing secondary effects of AC amendments have not studied the morphological changes within the organisms. Waissi-Leinonen et al. (2012) reported changes in microvilli length and layer density in *Chironomus riparius* exposed to carbon nanoparticles (C_{60}), and concluded that the structural changes in the gut wall may hinder the absorption of food and nutrients, and thus affect the growth and development of the larvae. The results in artificial sediment in this study indicate that nutritional level may affect *L. variegatus* microvilli length and layer density. More pronounced effects observed with AC amended sediment, together with the fact that high AC doses inhibits sediment consumption, may indicate a direct mechanical damage caused by ingestion of AC particles. Reduced sediment consumption observed within this and previous studies (Jonker et al. 2004; Nybom et al. 2012) for sediments dwelling organisms could thus be a mechanism to avoid AC induced morphological damages.

347

3.4 PCB concentrations and bioaccumulation

The sum PCB concentrations varied from < 3 to >6 $\mu\text{g/g}$ sediment dw depending on the sediment and sampling time (Figure S6, Table S1). In KJ sediment penta -chlorinated PCB homologues

351 dominated and corresponded to more than a third of the total PCB sum. In TJ sediment tetra-, penta-
352 and hexa-chlorinated PCB homologues were most abundant corresponding each approximately to
353 one fourth of the total PCBs. In VL sediment the dominant PCB homologues were tri- and tetra-
354 chlorinated, and higher chlorinated PCBs were nearly absent. On the basis of the PCB analysis both
355 the homologue composition and concentrations remained relatively similar during the aging and
356 only small differences were observed between the sampling times (Figure S6).

357 Passive sampling approaches, such as polyoxymethylene (POM) (Beckingham and
358 Ghosh 2013; Oen et al. 2011; Janssen et al. 2011; Kupryianchyk et al. 2013a), low-density
359 polyethylene (PE) (Oen et al. 2011), semi permeable membrane devices (SPMDs) and polyethylene
360 devices (PEDs) (Cho et al. 2009) has been successfully applied in laboratory and field studies to
361 investigate the sediment pore water concentrations with AC amended sediments. Compared to the
362 previously mentioned approaches the great benefit of PDMS method applying multiple thicknesses
363 is that the thermodynamic equilibrium can be easily ensured. The method of internally coated jars
364 with multiple thicknesses was recently applied and validated for PCB contaminated sediments
365 (Mäenpää et al. 2011). After two weeks silicone-sediment contact time the mass of analytes was
366 proportional to the PDMS mass, which confirms the thermodynamic equilibrium between the
367 PDMS and sediment. Additionally the silicone coated glass method using multiple coating
368 thicknesses enables identification of different sources of error in sampling: sample depletion,
369 polymer abrasion and surface adsorption (Reichenberg et al. 2008). In this study, significant
370 polymer abrasion and surface adsorption were met. Corrections were applied for the data and those
371 are described in details in supplementary content (Figures S7-S8).

372 The results clearly demonstrate that AC addition reduced the freely dissolved
373 concentration (C_{free}) of PCBs in each sediment (Figure 4). The decrease of C_{free} was apparent
374 already after two weeks contact time of AC. The C_{free} of the lighter congeners appeared to decrease
375 more compared to more chlorinated congeners after two weeks contact time, which can be

376 attributed to the molecular size of different PCB congeners. After three years contact time, the C_{free}
377 was further decreased and also heavier congeners were sorbed by AC. The AC addition of 0.25%
378 reduced the $\sum C_{\text{free}}$ by approximately 20% or more. With higher AC concentration the reduction was
379 approximately 70% or more in all sediments, most reaching more than 90% reduction compared to
380 the unamended sediment (Figure 4). The extracted $\sum C_{\text{free}}$ concentrations and AC dose related
381 reductions in each sediment are shown in Table S4. Silicone coated glass method, measuring C_{free} at
382 equilibrium, was proven to be suitable to measure the effects of AC amendments in sediments. Thus
383 the method could be used for monitoring of PCB bioavailability in the field sites under remedial
384 actions with AC.

385 The PCB bioaccumulation reduced clearly with increasing AC dose in all sediments.
386 As observed in several studies before the AC amendments has high efficiency towards low
387 chlorinated PCB homologues (Cho et al. 2009; Beckingham and Ghosh 2011). Also in this study
388 the most significant reduction was observed with tri –chlorinated homologues followed by
389 decreased efficiency with increasing level of chlorination (Figure 5). Measurements done after two
390 weeks, and three years CT were comparable. The reduction of bioaccumulation was most
391 pronounced in KJ sediment and also in VL sediment bioaccumulation was greatly reduced by AC
392 addition. In TJ sediment AC addition of 0.25% did not decrease the PCB bioaccumulation after two
393 weeks CT, but after three years CT the reduction was 20%. The higher AC dose 2.5% decreased the
394 bioavailability 63% after two weeks CT and 17% after three years CT. The reason why the
395 bioavailability reduction was less efficient after longer CT in 2.5% amended TJ is unknown, similar
396 response were not observed in freely dissolved concentrations measured with coated vials.
397 Termination or reduction of sediment consumption may affect to the PCB bioaccumulation to some
398 extent, but if the biota measurements are compared with the measurements done with PDMS the
399 effects of feeding appears to be negligible on bioaccumulation. The lipid normalized PCB
400 concentrations and AC dose related reductions in each sediment are shown in Table S4.

401 Equilibrium partitioning concentrations in lipids ($C_{Lip \rightleftharpoons Sed}$) are estimates of the tissue
402 concentrations in the situation when the biota lipid pool is in chemical equilibrium with its
403 surrounding environment. Thus, the estimates are not aimed to be the accurate tissue concentration,
404 but rather guiding reference values for assessing bioavailability at the given field site. The
405 determined $C_{Lip \rightleftharpoons Sed}$ showed that the lipid normalized tissue concentrations ($C_{Bio, Lip}$) are expected to
406 decrease by the increasing AC dose, except in 0.25% treatment in KJ sediment where slight
407 increase might occur (Figure 6). Closer comparison between the actual $C_{Bio, Lip}$ and $C_{Lip \rightleftharpoons Sed}$ (Figure
408 S9) revealed that $C_{Lip \rightleftharpoons Sed}$ slightly overestimates the actually measured tissue concentrations. This
409 result is in accordance with earlier observations (Mäenpää et al. 2011; Jahnke et al. 2014; Mäenpää
410 et al. 2015b). The reasons for conservative estimates are discussed in Mäenpää et al.(2011) and
411 Mäenpää et al. (2015a) and can be attributed to the type of lipid phase that the estimates are based
412 on versus the biota lipids where the bioaccumulation actually occurs (neutral lipid vs. neutral/polar
413 lipids), biotransformation, or temperature effects. The $C_{Lip \rightleftharpoons Sed}$ appears to produce reasonable
414 estimates of the bioaccumulation in unamended as well as AC amended sediments. The method
415 could therefore be used for producing the reference values for biota concentrations when
416 monitoring PCB bioavailability in the field sites remediated with AC. Further research is still
417 required to study if the PDMS method can be successfully applied in situ and whether it can be used
418 to distinguish e.g. vertical pore water concentration as previously done with POM and PE (Oen et
419 al. 2011). For application to the field collected AC amended sediment samples attention needs to be
420 paid to the rotating time during which the equilibrium of the analytes between the polymer and
421 sediment is achieved, since shaking or rotation of the sediment may mimic a longer post remedial
422 stage than is actually reached on site (Kupryianchyk et al. 2013a).

423

424 **4. Conclusions**

425 Bioaccumulation and freely dissolved concentrations of PCBs were reduced significantly as a
426 consequence of AC addition. However clear adverse effects were observed in egestion rate and
427 biomass of *L. variegatus* in KJ sediment already at very low dose of 0.25% compared to the doses
428 generally intended for remedial purposes (1-5%) (Ghosh et al. 2011), and with all of the studied
429 sediments at an AC dose of 2.5% sediment dw. The adverse effects have been suggested to be
430 related to the reduced availability of nutritious compounds (Jonker et al. 2004; Schreiber et al.
431 2005; Janssen et al. 2012). AC amendment induced reductions or terminations of sediment
432 consumption together with observed morphological changes indicates that sediment dwelling
433 organisms are not only trying to avoid AC exposure by changing their feeding behavior but ingested
434 AC particles may also cause direct mechanical damages to the gut microvilli layer of the organisms.

435 In some cases with good reasons the ecological responses can be considered negligible
436 and the remediation potential of AC amendments more significant. However the AC induced effects
437 and especially the mode of action in organisms deserves attention. Sediment dwelling organisms
438 may be sensitive for the effects of AC amendments, because they are exposed both internally and
439 externally, and therefore they may be useful organisms to study the potential adverse effects and
440 mechanisms. The effects of AC amendments on sediment dwelling organisms should not be
441 overlooked, since aquatic oligochaetes are important species in many aquatic food webs and present
442 in many contaminated areas (Cho et al. 2009; Cornelissen et al. 2011). The three sediments used in
443 this study differed from each other by sediment characteristics (TOC content, dw), PCB content and
444 -congener distribution, and PCB bioavailability. Natural sediments even in the same water district
445 may vary a lot, and as shown also in this study, the AC amendment related remediation potential
446 and secondary effects are dependent on the sediment properties. Since the mechanisms behind the
447 observed secondary effects of AC amendments are not yet thoroughly understood precaution and
448 site-specific ecological impacts evaluation is recommended before a remediation approach is
449 implemented. In order to improve the remedial technique attention should be directed towards new

450 sorbent recovery methods (Han et al. 2015) and mixing procedures causing potentially less
451 ecological effects e.g. adding sorbent together with natural materials such as clay (Cornelissen et al.
452 2011).

453

454 **Acknowledgement**

455 This research was funded by the Maj and Tor Nessling foundation, Finnish Doctoral Programme in
456 Environmental Science and Technology (EnSTe) and Academy of Finland (project no. 258120). We
457 acknowledge Chemviron Carbon (England) from donating the activated carbons for the
458 experimental use. We thank Marja Noponen and Julia Keronen for their practical assistance and
459 advices. Anna-Liisa Karttunen is acknowledged for preparing the transmission electron microscopy
460 (TEM) samples. The anonymous reviewers are acknowledged for suggestions to improve the
461 original manuscript.

462

463

464

REFERENCES

- 465 Beckingham, B. and Ghosh, U. 2013. Polyoxymethylene passive samplers to monitor changes in
466 bioavailability and flux of PCBs after activated carbon amendment to sediment in the field.
467 Chemosphere 91 (10), 1401-1407.
- 468 Beckingham, B. and Ghosh, U. 2011. Field-Scale Reduction of PCB Bioavailability with Activated
469 Carbon Amendment to River Sediments. Environmental science & technology 45 (24), 10567-
470 10574.
- 471 Brändli, R. C., Breedveld, G. D., Cornelissen, G. 2009. Tributyltin sorption to marine sedimentary
472 black carbon and to amended activated carbon. Environmental Toxicology and Chemistry 28 (3),
473 503-508.
- 474 Cho, Y., Ghosh, U., Kennedy, A. J., Grossman, A., Ray, G., Tomaszewski, J. E., Smithenry, D. W.,
475 Bridges, T. S., Luthy, R. G. 2009. Field Application of Activated Carbon Amendment for In-Situ
476 Stabilization of Polychlorinated Biphenyls in Marine Sediment. Environmental Science &
477 Technology 43 (10), 3815-3823.
- 478 Cornelissen, G., Elmquist Kruså, M., Breedveld, G. D., Eek, E., Oen, A. M. P., Arp, H. P. H.,
479 Raymond, C., Samuelsson, G., Hedman, J. E., Stokland, Ø, Gunnarsson, J. S. 2011. Remediation of

480 Contaminated Marine Sediment Using Thin-Layer Capping with Activated Carbon—A Field
481 Experiment in Trondheim Harbor, Norway. *Environmental Science & Technology* 45 (14), 6110-
482 6116.

483 Cornelissen, G., Breedveld, G. D., Kalaitzidis, S., Christanis, K., Kibsgaard, A., Oen, A. M. P.
484 2006. Strong Sorption of Native PAHs to Pyrogenic and Unburned Carbonaceous Geosorbents in
485 Sediments. *Environmental Science & Technology* 40 (4), 1197-1203.

486 Figueiredo, K., Mäenpää, K., Leppänen, M. T., Kiljunen, M., Lyytikäinen, M., Kukkonen, J. V. K.,
487 Koponen, H., Biasi, C., Martikainen, P. J. 2014. Trophic transfer of polychlorinated biphenyls
488 (PCB) in a boreal lake ecosystem: Testing of bioaccumulation models. *Science of the Total*
489 *Environment* 466, 690-698.

490 Ghosh, U., Luthy, R. G., Cornelissen, G., Werner, D., Menzie, C. A. 2011. In-situ Sorbent
491 Amendments: A New Direction in Contaminated Sediment Management. *Environmental Science &*
492 *Technology* 45 (4), 1163-1168.

493 Ghosh, U., Zimmerman, J. R., Luthy, R. G. 2003. PCB and PAH Speciation among Particle Types
494 in Contaminated Harbor Sediments and Effects on PAH Bioavailability. *Environmental Science &*
495 *Technology* 37 (10), 2209-2217.

496 Grossman, A. and Ghosh, U. 2009. Measurement of activated carbon and other black carbons in
497 sediments. *Chemosphere* 75 (4), 469-475.

498 Hale, S. E., Tomaszewski, J. E., Luthy, R. G., Werner, D. 2009. Sorption of
499 dichlorodiphenyltrichloroethane (DDT) and its metabolites by activated carbon in clean water and
500 sediment slurries. *Water research* 43 (17), 4336-4346.

501 Han, Z., Sani, B., Akkanen, J., Abel, S., Nybom, I., Karapanagioti, H. K., Werner, D. 2015. A
502 critical evaluation of magnetic activated carbon's potential for the remediation of sediment
503 impacted by polycyclic aromatic hydrocarbons. *Journal of hazardous materials* 286 (0), 41-47.

504 Jahnke, A., Mayer, P., McLachlan, M. S., Wickstrom, H., Gilbert, D., MacLeod, M. 2014. Silicone
505 passive equilibrium samplers as 'chemometers' in eels and sediments of a Swedish lake.
506 *Environmental Science-Processes & Impacts* 16 (3), 464-472.

507 Jahnke, A., McLachlan, M. S., Mayer, P. 2008. Equilibrium sampling: Partitioning of
508 organochlorine compounds from lipids into polydimethylsiloxane. *Chemosphere* 73 (10), 1575-
509 1581.

510 Janssen, E. M. L. and Beckingham, B. A. 2013. Biological Responses to Activated Carbon
511 Amendments in Sediment Remediation. *Environmental Science & Technology* 47 (14), 7595-7607.

512 Janssen, E. M. L., Oen, A. M. P., Luoma, S. N., Luthy, R. G. 2011. Assessment of Field-Related
513 Influences on Polychlorinated Biphenyl Exposures and Sorbent Amendment using Polychaete
514 Bioassays and Passive Sampler Measurements. *Environmental Toxicology and Chemistry* 30 (1),
515 173-180.

516 Janssen, E. M. L., Croteau, M., Luoma, S. N., Luthy, R. G. 2010. Measurement and Modeling of
517 Polychlorinated Biphenyl Bioaccumulation from Sediment for the Marine Polychaete *Neanthes*

518 arenaceodontata and Response to Sorbent Amendment. Environmental science & technology 44 (8),
519 2857-2863.

520 Janssen, E. M. L., Choi, Y., Luthy, R. G. 2012. Assessment of Nontoxic, Secondary Effects of
521 Sorbent Amendment to Sediments on the Deposit-Feeding Organism *Neanthes arenaceodentata*.
522 Environmental Science & Technology 46 (7), 4134-4141.

523 Jonker, M. T. O., Suijkerbuijk, M. P. W., Schmitt, H., Sinnige, T. L. 2009. Ecotoxicological Effects
524 of Activated Carbon Addition to Sediments. Environmental science & technology 43 (15), 5959-
525 5966.

526 Jonker, M. T. O., Hoenderboom, A. M., Koelmans, A. A. 2004. Effects of sedimentary sootlike
527 materials on bioaccumulation and sorption of polychlorinated biphenyls. Environmental Toxicology
528 and Chemistry 23 (11), 2563-2570.

529 Jonker, M. and Koelmans, A. 2002. Sorption of polycyclic aromatic hydrocarbons and
530 polychlorinated biphenyls to soot and soot-like materials in the aqueous environment mechanistic
531 considerations. Environmental science & technology 36 (17), 3725-3734.

532 Josefsson, S., Schaanning, M., Samuelsson, G. S., Gunnarsson, J. S., Olofsson, I., Eek, E., Wiberg,
533 K. 2012. Capping Efficiency of Various Carbonaceous and Mineral Materials for In Situ
534 Remediation of Polychlorinated Dibenzo-p-dioxin and Dibenzofuran Contaminated Marine
535 Sediments: Sediment-to-Water Fluxes and Bioaccumulation in Boxcosm Tests. Environmental
536 science & technology 46 (6), 3343-3351.

537 Koponen, K., Huuskonen, S., Ritola, O., Venalainen, R., Tarhanen, J., Lindstrom-Seppa, P. 2003.
538 Muscle chemical content and hepatic biotransformation in bream (*Abramis brama*) and asp (*Aspius*
539 *aspius*) in a PCB-contaminated lake. Boreal Environment Research 8 (3), 203-213.

540 Kupryianchyk, D., Noori, A., Rakowska, M. I., Grotenhuis, J. T. C., Koelmans, A. A. 2013a.
541 Bioturbation and Dissolved Organic Matter Enhance Contaminant Fluxes from Sediment Treated
542 with Powdered and Granular Activated Carbo. Environmental Science & Technology 47 (10),
543 5092-5100.

544 Kupryianchyk, D., Rakowska, M. I., Roessink, I., Reichman, E. P., Grotenhuis, J. T. C., Koelmans,
545 A. A. 2013b. In situ Treatment with Activated Carbon Reduces Bioaccumulation in Aquatic Food
546 Chains. Environmental Science & Technology 47 (9), 4563-4571.

547 Kupryianchyk, D., Reichman, E. P., Rakowska, M. I., Peeters, E. T. H. M., Grotenhuis, J. T. C.,
548 Koelmans, A. A. 2011. Ecotoxicological Effects of Activated Carbon Amendments on
549 Macroinvertebrates in Nonpolluted and Polluted Sediments. Environmental science & technology
550 45 (19), 8567-8574.

551 Kupryianchyk, D., Rakowska, M. I., Reible, D., Harmsen, J., Cornelissen, G., van Veggel, M.,
552 Hale, S. E., Grotenhuis, T., Koelmans, A. A. 2015. Positioning activated carbon amendment
553 technologies in a novel framework for sediment management. Integrated Environmental
554 Assessment and Management 11 (2), 221-234.

555 Landrum, P. F., Gedeon, M. L., Burton, G. A., Greenberg, M. S., Rowland, C. D. 2002. Biological
556 Responses of *Lumbriculus variegatus* Exposed to Fluoranthene-Spiked Sediment. Archives of
557 Environmental Contamination and Toxicology 42 (3), 292-302.

558 Leppänen, M. and Kukkonen, J. 1998a. Factors affecting feeding rate, reproduction and growth of
559 an oligochaete *Lumbriculus variegatus* (Müller). 377 (1), 183-194.

560 Leppänen, M. T. and Kukkonen, J. V. K. 1998b. Relationship between reproduction, sediment type,
561 and feeding activity of *Lumbriculus variegatus* (Müller): Implications for sediment toxicity testing.
562 Environmental Toxicology and Chemistry 17 (11), 2196-2202.

563 Lin, D., Cho, Y., Werner, D., Luthy, R. G. 2014. Bioturbation Delays Attenuation of DDT by Clean
564 Sediment Cap but Promotes Sequestration by Thin-Layered Activated Carbon. Environmental
565 science & technology 48 (2), 1175-1183.

566 Mäenpää, K., Leppänen, M. T., Figueiredo, K., Tigistu-Sahle, F., Kakela, R. 2015a. Sorptive
567 Capacity of Membrane Lipids, Storage Lipids, and Proteins: A Preliminary Study of Partitioning of
568 Organochlorines in Lean Fish from A PCB-Contaminated Freshwater Lake. Archives of
569 Environmental Contamination and Toxicology 68 (1), 193-203.

570 Mäenpää, K., Leppänen, M., Figueiredo, K., Mayer, P., Gilbert, D., Jahnke, A., Gil-Allué, C.,
571 Akkanen, J., Nybom, I., Herve, S. 2015b. Fate of polychlorinated biphenyls in a contaminated lake
572 ecosystem: Combining equilibrium passive sampling of sediment and water with total concentration
573 measurements of biota. Environmental Toxicology and Chemistry, n/a-n/a.

574 Mäenpää, K., Leppänen, M. T., Reichenberg, F., Figueiredo, K., Mayer, P. 2011. Equilibrium
575 Sampling of Persistent and Bioaccumulative Compounds in Soil and Sediment: Comparison of Two
576 Approaches To Determine Equilibrium Partitioning Concentrations in Lipids. Environmental
577 Science & Technology 45 (3), 1041-1047.

578 Millward, R. N., Bridges, T. S., Ghosh, U., Zimmerman, J. R., Luthy, R. G. 2005. Addition of
579 Activated Carbon to Sediments to Reduce PCB Bioaccumulation by a Polychaete (*Neanthes*
580 *arenaceodentata*) and an Amphipod (*Leptocheirus plumulosus*). Environmental Science &
581 Technology 39 (8), 2880-2887.

582 Mount, D. R., Dawson, T. D., Burkhard, L. P. 1999. Implications of gut purging for tissue residues
583 determined in bioaccumulation testing of sediment with *Lumbriculus variegatus*. Environmental
584 Toxicology and Chemistry 18 (6), 1244-1249.

585 Nybom, I., Werner, D., Leppänen, M. T., Siavalas, G., Christanis, K., Karapanagioti, H. K.,
586 Kukkonen, J. V. K., Akkanen, J. 2012. Responses of *Lumbriculus variegatus* to Activated Carbon
587 Amendments in Uncontaminated Sediments. Environmental Science & Technology 46 (23), 12895-
588 12903.

589 Oen, A. M. P., Janssen, E. M. L., Cornelissen, G., Breedveld, G. D., Eek, E., Luthy, R. G. 2011. In
590 Situ Measurement of PCB Pore Water Concentration Profiles in Activated Carbon-Amended
591 Sediment Using Passive Samplers. Environmental science & technology 45 (9), 4053-4059.

592 OECD, 2007. Guidelines for testing chemicals: Sediment-water *Lumbriculus* toxicity test using
593 spiked sediment. 225. Paris, France.

594 OECD, 1992. Guidelines for testing chemicals: Fish, acute toxicity test. 203. Paris, France.

595 Parrish, C. C. (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples.
 596 In: Arts, M.T., Wainmann, B.C. (Eds.), Lipids in freshwater ecosystems, Springer, New York, 4.

597 Rakowska, M. I., Kupryianchyk, D., Grotenhuis, T., Rijnaarts, H. H. M., Koelmans, A. A. 2013.
 598 Extraction of sediment-associated polycyclic aromatic hydrocarbons with granular activated carbon.
 599 Environmental Toxicology and Chemistry 32 (2), 304-311.

600 Rakowska, M. I., Kupryianchyk, D., Harmsen, J., Grotenhuis, T., Koelmans, A. A. 2012. In situ
 601 remediation of contaminated sediments using carbonaceous materials. Environmental Toxicology
 602 and Chemistry 4 (31), 693-704.

603 Reichenberg, F., Smedes, F., Joensson, J., Mayer, P. 2008. Determining the chemical activity of
 604 hydrophobic organic compounds in soil using polymer coated vials. Chemistry Central Journal 2, 8.

605 Schreiber, B., Brinkmann, T., Schmalz, V., Worch, E. 2005. Adsorption of dissolved organic matter
 606 onto activated carbon—the influence of temperature, absorption wavelength, and molecular size.
 607 Water Research 39 (15), 3449-3456.

608 Schubaur-Berigan, M. K., Monson, P. D., West, C. W., Ankley, G. T. 1995. Influence of pH on the
 609 toxicity of ammonia to *Chironomus tentans* and *Lumbriculus variegatus*. Environmental Toxicology
 610 and Chemistry 14 (4), 713-717.

611 Smedes, F., Geertsma, R. W., van der Zande, T., Booij, K. 2009. Polymer-Water Partition
 612 Coefficients of Hydrophobic Compounds for Passive Sampling: Application of Cosolvent Models
 613 for Validation. Environmental science & technology 43 (18), 7047-7054.

614 Sun, X. and Ghosh, U. 2008. The effect of activated carbon on partitioning, desorption, and
 615 biouptake of native polychlorinated biphenyls in four freshwater sediments. Environmental
 616 Toxicology and Chemistry 27 (11), 2287-2295.

617 Tomaszewski, J. E., McLeod, P. B., Luthy, R. G. 2008. Measuring and modeling reduction of DDT
 618 availability to the water column and mussels following activated carbon amendment of
 619 contaminated sediment. Water research 42 (16), 4348-4356.

620 Waissi-Leinonen, G. C., Petersen, E. J., Pakarinen, K., Akkanen, J., Leppanen, M. T., Kukkonen, J.
 621 V. K. 2012. Toxicity of fullerene (C60) to sediment-dwelling invertebrate *Chironomus*
 622 *riparius* larvae. Environmental Toxicology and Chemistry 31 (9), 2108-2116.

623 Zimmerman, J. R., Werner, D., Ghosh, U., Millward, R. N., Bridges, T. S., Luthy, R. G. 2005.
 624 Effects of dose and particle size on activated carbon treatment to sequester polychlorinated
 625 biphenyls and polycyclic aromatic hydrocarbons in marine sediments. Environmental Toxicology
 626 and Chemistry 24 (7), 1594-1601.

627

Figure 1. Relative change in biomass of *Lumbriculus variegatus* in the study sediments (KJ, TJ and VL) as a function of sedimentary AC percentage after two weeks and three years of AC-sediment contact time (28 day experiment). The level 0 dashed line represents the weight of the worms at the beginning of the experiment. Box plot for 25-75 percentiles and median, the whiskers indicate minimum and maximum values mean value is indicated with + (n=4). *Significantly different compared to the unamended sediment $p < 0.05$.

Figure 2. *Lumbriculus variagatus* microvilli length and densities measured from transmission electron micrographs exposed to unamended and AC amended sediments (KJ, TJ and VL), AC-sediment contact time three years (28 day experiment). Box plot for 25-75 percentiles and median, the whiskers indicate minimum and maximum values mean value is indicated with + (n=6). *Significantly different compared to the unamended sediment $p < 0.05$.

Figure 3. Transmission electron micrographs (TEM) of *L. variegatus* microvilli layer exposed to unamended and AC amended study sediments (KJ, TJ and VL), AC-sediment contact time three years (28 day experiment). Magnification $\times 12000$, scale bar 2000nm.

Figure 4. $\sum$ PCB (16 congeners) freely dissolved concentration (C_{free}) determined by PDMS passive samplers in three different sediments (KJ, TJ and VL) as a function of sedimentary AC percentage

after two weeks (A) and three years (B) AC-sediment contact time. Note the difference in the scales of the graphs.

Figure 5. Σ PCB (20 congeners) extracted from worms ($\mu\text{g/g}$ worm lipid) exposed to three different sediments (KJ, TJ and VL) as a function of sedimentary AC percentage after two weeks (A) and three years (B) AC-sediment contact time (28 day experiment). *Significantly different (Σ PCB) compared to the corresponding unamended sediment $p < 0.05$. Note the difference in the scales of the graphs.

Figure 6. Estimation of equilibrium partitioning concentrations of PCBs in lipids ($C_{\text{Lip}=\text{Sed}}$) in the three different sediments (KJ, TJ and VL) as a function of sedimentary AC percentage after two weeks (A) and three years (B) AC-sediment contact time. $C_{\text{Lip}=\text{Sed}}$ were determined as described in Mäenpää et al. (2011) for the equilibrium concentrations in the passive sampler polymer equilibrated with sediment. The Σ PCB is for congeners 28, 31, 44, 49, 52, 101, 105, 110, 118, 138,

149, 151, 153, 156, 180 divided by their chlorination stage. Note the difference in the scales of the graphs.