

Impact of bio-palladium nanoparticles (bio-Pd NPs) on the activity and structure of a marine microbial community

Peer-reviewed author version

Nuzzo, Andrea; HOSSEINKHANI, Baharak; Boon, Nico; Zanaroli, Giulio & Fava, Fabio (2017) Impact of bio-palladium nanoparticles (bio-Pd NPs) on the activity and structure of a marine microbial community. In: ENVIRONMENTAL POLLUTION, 220, p. 1068-1078.

DOI: 10.1016/j.envpol.2016.11.036

Handle: <http://hdl.handle.net/1942/23255>

Manuscript Number:

Title: Impact of bio-palladium nanoparticles (bio-Pd NPs) on the activity and structure of a marine microbial community

Article Type: Research Paper

Keywords: Bio-Pd NPs; Nanoparticles; Ecotoxicity; Marine sediment; Bacterial community structure

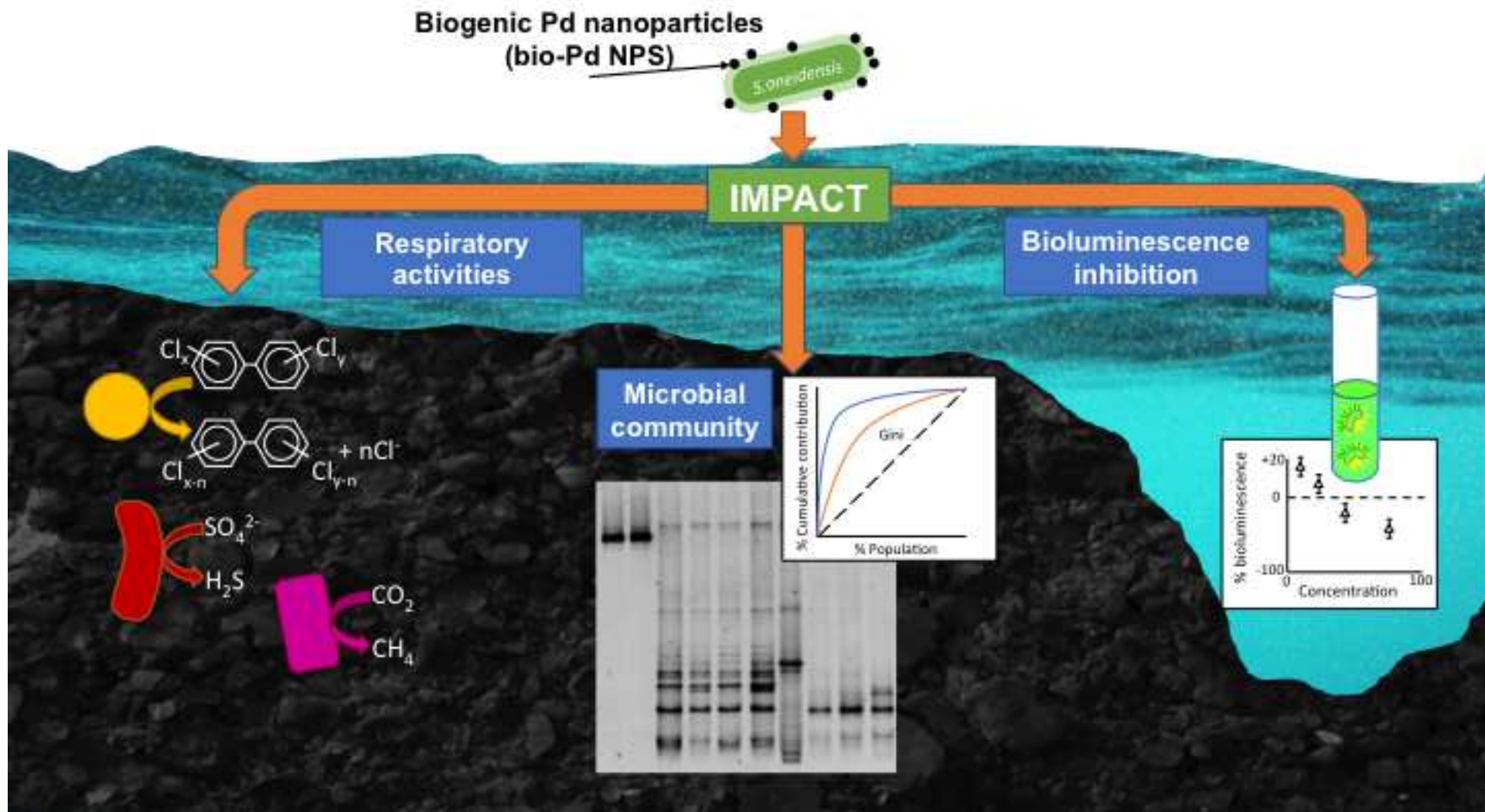
Corresponding Author: Dr. Giulio Zanaroli, Ph.D.

Corresponding Author's Institution: University of Bologna

First Author: Andrea Nuzzo

Order of Authors: Andrea Nuzzo; Baharak Hosseinkhani; Nico Boon; Giulio Zanaroli, Ph.D.; Fabio Fava

Abstract: Biogenic palladium nanoparticles (bio-Pd NPs) represent a promising catalyst for organohalide remediation in water and sediments. However, the available information regarding their possible impact in case of release into the environment, particularly on the environmental microbiota, is limited. In this study the toxicity of bio-Pd NPs on the model marine bacterium *V. fischeri* was assessed. The impacts of different concentrations of bio-Pd NPs on the respiratory metabolisms (i.e. organohalide respiration, sulfate reduction and methanogenesis) and the structure of a PCB-dechlorinating microbial community enriched from a marine sediment were also investigated in microcosms mimicking the actual sampling site conditions. Bio-Pd NPs had no toxic effect on *V. fischeri*. In addition, they had no significant effects on PCB-dehalogenating activity, while showing a partial, dose-dependent inhibitory effect on sulfate reduction as well as on methanogenesis. No toxic effects by bio-Pd NPs could be also observed on the total bacterial community structure, as its biodiversity showed a NPs dose-dependent increase compared to the not exposed community. In addition, resilience of the microbial community to bio-Pd NPs exposure was observed, being the final community organization (Gini coefficient) of samples exposed to bio-Pd NPs similar to that of the not exposed one. Considering all the factors evaluated, bio-Pd NPs could be deemed as non-toxic to the marine microbiota in the conditions tested. This is the first study in which the impact of bio-Pd NPs is extensively evaluated over a microbial community in relevant environmental conditions, providing important information for the assessment of their environmental safety.



Highlights:

- Bio-Pd NPs are deemed not toxic in standard *V. fischeri* ecotoxicity test
- Impact of Bio-Pd NPs tested on microbial community in its native sediment and water
- Bio-Pd NPs impact is limited to few respiratory activities
- Microbial community is resilient to bio-Pd NPs

28 **Abstract**

29 Biogenic palladium nanoparticles (bio-Pd NPs) represent a promising catalyst for organohalide
30 remediation in water and sediments. However, the available information regarding their possible
31 impact in case of release into the environment, particularly on the environmental microbiota, is limited.
32 In this study the toxicity of bio-Pd NPs on the model marine bacterium *V. fischeri* was assessed. The
33 impacts of different concentrations of bio-Pd NPs on the respiratory metabolisms (i.e. organohalide
34 respiration, sulfate reduction and methanogenesis) and the structure of a PCB-dechlorinating microbial
35 community enriched from a marine sediment were also investigated in microcosms mimicking the
36 actual sampling site conditions. Bio-Pd NPs had no toxic effect on *V. fischeri*. In addition, they had no
37 significant effects on PCB-dehalogenating activity, while showing a partial, dose-dependent inhibitory
38 effect on sulfate reduction as well as on methanogenesis. No toxic effects by bio-Pd NPs could be also
39 observed on the total bacterial community structure, as its biodiversity showed a NPs dose-dependent
40 increase compared to the not exposed community. In addition, resilience of the microbial community
41 to bio-Pd NPs exposure was observed, being the final community organization (Gini coefficient) of
42 samples exposed to bio-Pd NPs similar to that of the not exposed one. Considering all the factors
43 evaluated, bio-Pd NPs could be deemed as non-toxic to the marine microbiota in the conditions tested.
44 This is the first study in which the impact of bio-Pd NPs is extensively evaluated over a microbial
45 community in relevant environmental conditions, providing important information for the assessment
46 of their environmental safety.

47

48

49

50 **Keywords**

51 Bio-Pd NPs, Nanoparticles, Ecotoxicity, Marine sediment, Bacterial community structure

52

53

54

55 **Capsule**

56 Bio-Pd NPs are non-toxic towards *V.fischeri* and have a limited impact on a marine microbial
57 community in site-mimicking microcosms.

58 **Introduction**

59 Nanoremediation is the application of reactive nanoparticles (NPs) for the detoxification of
60 environmental matrices from pollutants (Karn et al., 2009); a long list of different nanomaterials have
61 been applied so far in nanoremediation of contaminated water and soils, such as nanoscale zero-valent
62 iron (nZVI) (Theron et al., 2008; Zhang, 2003), Pd/Fe bimetallic NPs, carbon nanostructures and many
63 more (Theron et al., 2008). These NPs show very high reactivity and different properties compared to
64 their bulk counterparts, due to their size and innovative surface coatings, allowing efficient and
65 controllable degradation activities, especially against recalcitrant pollutants (Macé et al., 2006;
66 Serrano, 2010). NPs of zerovalent iron represent a typical example of this approach, especially for the
67 decontamination of groundwater from organic solvents, pesticides, dyes and polychlorinated
68 hydrocarbons as well as inorganic ions (Zhang, 2003); more recently, other metal NPs have emerged as
69 novel catalyst in nanoremediation approaches, called zerovalent palladium nanoparticles (Pd-NPs).

70 Pd nano-catalysts were efficiently used for reductive dechlorination of several contaminants (Ukisu
71 and Miyadera, 2003). Pd-NPs catalyse the reductive dehalogenation of the contaminant through the
72 development of radical cathodic hydrogen which chemically dehalogenates the molecule (Choi et al.,
73 2009; De Windt et al., 2005); additionally, NPs of zerovalent Pd can be obtained through
74 bioprecipitation in bacterial cultures and they are referred to as *biopalladium nanoparticles* (bio-Pd
75 NPs) (Baxter-Plant et al., 2003; De Windt et al., 2005). Bio-Pd NPs have been effectively used for the
76 degradation/transformation of contaminants such as heavy metals, halogenated organic solvents and
77 pesticides in different environments, such as groundwater, wastewater, air, soil, sediments (De Corte et
78 al., 2012) constituting promising catalysts (Hennebel et al., 2009) in an effective cradle to cradle
79 approach which combine recovery of this precious metal from wastewater and a sustainable approach
80 in pollutant nanoremediation (Hennebel et al., 2012).

81 Among halogenated compounds, polychlorinated biphenyls (PCBs) are persistent organic pollutants
82 with toxic effects on all trophic levels, including humans (Safe, 1993). Due to their high
83 hydrophobicity, recalcitrance to biodegradation, biomagnification capabilities throughout the food
84 chain and toxic effects on both the environment and the human health, PCBs are still included in the
85 Priority Organic Pollutants list (Stockholm Convention, 2004). After more than 30 years from
86 worldwide production banishment, PCB pollution is still present in marine environments, especially in
87 sediments (Fernández and Grimalt, 2003). In these environmental compartments some microorganisms
88 of the phylum *Chloroflexi* are able to use them as final electron acceptor. Through this activity,

89 chlorine atoms are sequentially removed from the highly chlorinated PCB congeners leading to the
90 accumulation of lower chlorinated ones which have usually lower toxicity and can be more easily
91 degraded in aerobic conditions (Sowers and May, 2013; Zanaroli et al., 2015). This process known as
92 microbial reductive dechlorination, has been mainly studied in freshwater sediment cultures (Bedard,
93 2008; Field and Sierra-Alvarez, 2008; Wiegel and Wu, 2000), whereas less is known regarding these
94 activities in marine sediments (Fava et al., 2003a, 2003b; Zanaroli et al., 2015, 2006). In addition,
95 microbial reductive dechlorination processes are extremely slow (Wiegel and Wu, 2000) and
96 enhancement strategies must be developed for bioremediation approaches to compete with the
97 traditional remediation technologies, which are environmentally and economically unsustainable (Khan
98 et al., 2004; Perelo, 2010).

99 Bio-Pd NPs have been recently suggested as a promising approach to enhance PCB degradation also in
100 marine environments, where they can be synthesized by some indigenous marine microorganisms
101 (Hosseinkhani et al., 2014b). Using these biologically precipitated NPs, a complete dechlorination of
102 TCE was achieved in synthetic marine water and marine slurries of water and sediments (Hosseinkhani
103 et al., 2014a, 2014b) as well as an extensive dechlorination of Aroclor 1254 PCBs to mainly
104 monochlorobiphenyls (Hosseinkhani et al., 2015). However, concerns are rising around the
105 environmental remediation approaches using these NPs (Grieger et al., 2010; Karn et al., 2009; Otto et
106 al., 2008; Sánchez et al., 2011); metal NPs might have toxic effects on humans and on the
107 environment, due to their small dimensions, which allow them to penetrate easier and faster into the
108 cell space (Jiang et al., 2009; Schrand et al., 2010). Pd- NPs, in particular, have an inhibiting effect on
109 *E. coli* and *S. aureus* (Adams et al., 2014), kiwifruit pollen development (Speranza et al., 2010) and
110 peripheral blood mononuclear cells (Petrarca et al., 2014). It is therefore necessary to evaluate risk
111 factors of Pd-NPs as well as other metallic NPs via standardized tests before their application (Nowack
112 and Bucheli, 2007; Wiesner et al., 2006). Particularly, the information on their impact on the active
113 microbial community (Farré et al., 2008; Sánchez et al., 2011), which is the most important player in
114 bioremediation processes, is missing.

115 Therefore, the aim of this work was to assess the ecological toxicity of bio-Pd NPs in marine
116 environment using Microtox[®] standardized acute toxicity test (Ma et al., 2014) and to evaluate their
117 impact on a PCB-dehalogenating marine microbial community in its contaminated sediments of origin.
118 In particular, the effects on the main respiratory metabolisms of the examined microbial community
119 (i.e., dechlorination, sulfate reduction and methanogenesis), as well as on the total bacterial community
120 structure were assessed.

121

122 **Materials and Methods**

123 ***Preparation of bio-Pd NPs***

124 Preparation of bio-Pd NPs was performed through bioprecipitation with *Shewanella oneidensis* pure
125 cultures according to De Windt, et al. (2005).

126

127 ***Ecotoxicity tests on V. fischeri***

128 Acute toxicity of bio-Pd NPs was measured by assessing luminescence inhibition of the marine Gram-
129 negative bacterium *V. fischeri* (strain NRRL B-11177) with a Microtox® M500 (Modern Water
130 Monitoring, Cambridge, UK) after 5, 15 and 30' exposure to serial 2-fold dilutions of a bio-Pd NPs
131 solution at 9.5 ppm (equivalent to 50 mg/kg dry sediment condition). The luminescence signals were
132 normalized against an active control with no bio-Pd NPs using the MicroTox Omni Software,
133 according to the 81.9% Basic Test Protocol provided by the manufacturer. The concentration of the
134 sample (ppm) which produces a 50% decrease in bioluminescence after exposure is designated as the
135 effective concentration EC₅₀.

136

137 ***Microcosm preparation and sampling***

138 The impact of bio-Pd NPs on a PCB-dechlorinating marine microbial community previously enriched
139 from a sediment collected from the Venice lagoon (Italy) was tested by sub-culturing the community in
140 anaerobic 70 ml slurry microcosms prepared as follows. First, sediment from the Venice lagoon was
141 autoclave-sterilized at 121°C for 1 h on 3 consecutive days with incubation at 28 °C between each
142 autoclaving treatment. 1600 ml of site water were purged in a 1 l Erlenmeyer flask with filter-sterilized
143 N₂:CO₂ (70:30) with a Hungate-similar apparatus for 2 h under vigorous magnetic stirring. Then,
144 degassed water was filter-sterilized and added to the autoclaved sediment to obtain a 20 % (dry w/v)
145 suspension and the resulting slurry was mixed and purged as described above for 2 additional hours.
146 Seventy ml aliquots of sediment slurry were then withdrawn while mixing and purging, and transferred
147 into 120 ml serum bottles and sealed with Teflon-coated butyl stoppers under N₂:CO₂ flush. Three sets
148 of microcosms were prepared: i) a set inoculated (5% v/v) with the PCB dechlorinating culture and
149 spiked with the PCB mixture Aroclor 1254 (from a 20 g/l stock solution in acetone) at a final
150 concentration of 1 g/kgdw; ii) a set inoculated (5% v/v) with the same culture but not spiked with
151 PCBs (equal amount of PCB-free acetone added instead of the PCB stock solution); iii) a set non-

152 inoculated (i.e., sterile) and spiked with Aroclor 1254 at a final concentration of 1 g/kgdw. The two
153 sets spiked with PCBs comprised the following conditions: i) not amended microcosms (biologically
154 active and sterile controls), ii) microcosms amended with H₂:CO₂ (70:30) atmosphere, as control to
155 investigate the effect of hydrogen, required for bio-Pd catalytic activity, on respiratory activities and
156 the community structure (inoculated, i.e., biologically active set) and as additional negative control for
157 PCB chemical dechlorination (not inoculated, i.e., sterile set), (iii) microcosms amended with H₂:CO₂
158 (70:30) atmosphere and bio-Pd NPs at 5 mg/kgdw and iv) amended with H₂:CO₂ (70:30) atmosphere
159 and bio-Pd NPs at 50 mg/kgdw, to study the effect of different bio-Pd NPs concentrations on the
160 community activity and structure (biologically active set) and the PCB dechlorination activity of bio-
161 Pd NPs (sterile set). The inoculated set that was not spiked with PCBs included all conditions above
162 except for the not amended microcosms, and was set up to confirm the effect of bio-Pd NPs on the
163 activity and structure of the microbial community in the absence of organic contaminants. Triplicate
164 microcosms were set up for each condition. All microcosms were incubated statically in the dark at
165 28°C and periodically sampled according to the procedure described in Zanaroli et al. (2012a) to
166 analyse i) the volume and composition of the biogas produced in the microcosm headspace, ii) the type
167 and concentrations of PCBs in the sediment, iii) the concentration of sulfates in the water phase, iv) the
168 structure of the microbial community.

169

170 ***PCB extraction and analytical procedures***

171 PCB extraction was performed in duplicate from each replicate culture. PCBs were batch extracted
172 from 0.3 mL aliquots of sediment slurry with 3 volumes (0.9 ml) of hexane:acetone (9:1), 0.150 ml of
173 elemental mercury and octachloronaphtalene (OCN) at final concentration of 0.4 ppm were added
174 according to Fava et al. (2003). The qualitative and quantitative analyses of the extracted PCBs were
175 performed with a GC-ECD under the analytical conditions described elsewhere (Fava et al., 2003a).
176 Qualitative analysis of the freshly spiked PCBs and their possible dechlorination products was
177 performed by comparing the retention time (relative to OCN) of the peaks obtained from the analyses
178 of the sediment organic extracts with those of PCBs occurring in Aroclor 1242 and Aroclor 1254 PCB
179 standard mixtures analysed under identical conditions. Quantitative analyses of each PCB congener
180 were performed by using the GC-ECD response factor of each target PCB obtained through linear five-
181 points calibration curves of Aroclors 1254 and 1242 (in the range 1 to 50 ppm each mixture) as
182 described in Fava et al. (2003a). Response factors were verified monthly. PCB concentrations
183 (µmoles/kgdw), average number of Cl per biphenyl and dechlorination rates (µmoles of Cl released per

184 kgdw per week) were calculated assuming co-eluting congeners to be present in equal proportions as
185 described in previous works (Zanaroli et al., 2010).

186 Biogas production was measured at each sampling with an airtight glass syringe, while its composition
187 was determined via μ GC-TCD as described previously (Scoma et al., 2011). Sulfate concentration in
188 the water phase was determined with IC-CD as described in (Fava et al., 2003a); linear 5-point
189 calibration curves (1.0–25.0 ppm range) for SO_4^{2-} , Cl^- , NO_3^- and NO_2^- were obtained by using mixtures
190 of these compounds.

191

192 ***Community analysis by PCR-DGGE of the 16S rRNA gene***

193 Slurry samples (2 ml) were centrifuged at 10,000g for 10 minutes and the water phase was discarded;
194 metagenomic DNA was extracted from the wet sediment (approximately 250 mg) recovered DNA was
195 extracted with the PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) according
196 to the protocol provided by the manufacturer. Sediment samples suspended in the bead solution
197 supplied with the kit were treated with 4.5 μl of a 100 mg ml^{-1} Proteinase K solution from *Streptomyces*
198 *griseus* (Sigma-Aldrich, Milano, Italy) and 8.2 μl of a 100 mg ml^{-1} of chicken egg Lysozyme (Sigma-
199 Aldrich, Milano, Italy) solution at 37°C under shaking at 150 rpm for 30 min, prior to cell lysis step
200 described in the provided protocol.

201 For DGGE analysis, 16S rRNA genes of the bacterial community were PCR amplified from the
202 metagenomic DNA with the GC-clamped forward primer GC-357f (5'-
203 CGCCCGCCGCGCCCCGCGCCCGGCCCGCCCGCCCCCGCCCCCTACGGGAGGCAGCAG-3')
204 and the reverse primer 907r (5'-CCGTCAATTCCTTTGAGTTT-3') (Sass et al., 2001) with PCR
205 conditions described elsewhere (Zanaroli et al., 2012b). DGGE of bacterial amplicons (approximately
206 400 ng DNA per lane) were performed with a D-Code apparatus with a denaturing gradient from 40%
207 to 60% denaturant as described in Zanaroli et al. (2010). Gels were stained with SYBR Green I
208 (Sigma-Aldrich, Milano, Italy) and their image captured in UV transilluminator with a digital camera
209 supported by a Gel Doc apparatus (Bio-Rad, Milan, Italy).

210 Community richness (Rr) and community organization (Co) indexes were calculated from DGGE
211 image analysis as described in literature (Marzorati et al., 2008; Read et al., 2011; Wittebolle et al.,
212 2009). In particular, the range-weighted richness was calculated from the total number of bands in the
213 pattern and the denaturing gradient comprised between the first and the last band of the pattern,
214 whereas the community organization was derived from Pareto-Lorenz (PL) evenness curves and the
215 respective Gini coefficient times 100.

216

217 **Results**

218 The impact of bio-Pd NPs was assessed firstly on a model marine bacterium, *V.fischeri*, by assessing
219 eventual inhibition of its bioluminescence. Then, tests focused on the eventual impact of bio-Pd NPs on
220 the main respiratory metabolisms and the structure of a PCB dechlorinating marine microbial
221 community. For this purpose, anaerobic sediment sub-cultures were set-up, in the presence and in the
222 absence of spiked PCBs either: i) in absence of any amendments (unamended controls); ii) in presence
223 of 70% hydrogen atmosphere, which is necessary for the catalytic activity of bio-Pd NPs; iii) in
224 presence of 70% hydrogen atmosphere and two different concentrations of bio-Pd NPs.

225

226 ***Acute toxicity standard tests with V. fischeri***

227 To assess the acute toxicity of bio-Pd NPs on the evolution of a marine microbial community, a
228 standard test was performed by assessing of the decrease in luminance of *V. fischeri* in presence of
229 different concentrations of nanoparticles. A slight inhibition of luminescence (12%) was detected only
230 after 5 minutes of incubation in the presence of the highest bio-Pd NPs concentration (Fig 1). In all
231 other cases (both in terms of bio-Pd concentration and exposure time) stimulating effects were
232 detected, as luminescence was higher than non-amended control (percentages of inhibition below 0%).
233 Therefore, no EC₅₀ could be determined. Such a biostimulation effect might be due to the presence of
234 some cell debris associated to the bio-Pd NPs. Therefore, bio-Pd NPs do not result to be toxic at the
235 concentrations used in this experiment, that were previously shown to be effective in the
236 dehalogenation of different organohalides in marine sediments (Hosseinkhani et al., 2015).

237

238 ***Respiratory metabolisms***

239 The unamended control (hydrogen free and bio-Pd NPs free) showed the highest dechlorination
240 activity among the whole PCB-spiked, biologically active microcosms: the average number of chlorine
241 atoms per biphenyl molecule decreased from 5.1 to 4.3 in 18 weeks of incubation, leading to the
242 depletion of 54±3% of penta- to octachlorinated congeners at the maximum rate of 243±16 µmol Cl
243 removed week⁻¹ kgdw⁻¹ (Fig 2). The presence of hydrogen in the headspace, either alone or in
244 combination with bio-Pd NPs, decreased remarkably the dechlorination rates, all of which were less
245 than half of unamended microcosms (107±37, 118±75 and 97±27 µmoles of chlorine removed kgdw⁻¹
246 week⁻¹ for hydrogen-amended microcosms with no bio-Pd NPs, bio-Pd-NPs at 5 mg/kgdw and bio-Pd

247 NPs at 50 mg/kgdw, respectively). The final average number of chlorine substitution after 18 weeks of
248 incubation resulted to be 4.7, 4.7 and 4.9, corresponding to depletion percentages of highly chlorinated
249 congeners as low as 25%, 24% and 21%, for the hydrogen-amended microcosms with no bio-Pd NPs,
250 bio-Pd-NPs at 5 mg/kgdw and bio-Pd NPs at 50 mg/kgdw, respectively (Fig 2). The dechlorination
251 specificity led to a predominant accumulation of 24-25-CB and 24-34/236-25-CB (14 mol %) (Fig 3a).
252 In addition, the high residual concentration of the main penta-chlorinated biphenyls of spiked Aroclor
253 1254 mixture indicates that the dechlorination process was still incomplete when the incubation
254 stopped. Coherently with the lower activities in all hydrogen-amended microcosms, and regardless of
255 bio-Pd NPs, lower percentages of tetra- and tri-chlorinated congeners were detected at the end of
256 incubation with a specificity apparently not influenced by bio-Pd NPs (Fig. 3). From these
257 observations, bio-Pd NPs do not seem to impact the microbial dechlorination activities. No
258 dechlorination was detected in any of the sterile microcosms, regardless of hydrogen atmosphere and
259 bio-Pd NPs amendments (Fig 2).

260 Regarding other respiring metabolisms, sulfate-reduction activities were marked in all biologically
261 active microcosms: the initially occurring sulfates (2.5 g/l average) were completely consumed in the
262 not-amended active controls after 6 weeks of incubation. A similar sulfate consumption was detected in
263 microcosms amended with hydrogen only, while sulfate was completely consumed only after 9 weeks
264 of incubation in microcosms supplemented with hydrogen and bio-Pd NPs at 5 mg/kgdw, and by the
265 end of incubation in microcosms supplemented with hydrogen and 50 mg/kgdw of bio-Pd NPs (Fig
266 4a). Thus, a dose-dependent inhibition of bio-Pd on sulfate reduction was observed. Similar dose-
267 dependent effect of bio-Pd on sulfate reduction was also detected in the PCB-free sediment
268 microcosms (Fig 4a).

269 No sulfate reduction, nor methane accumulation, were observed in the sterile microcosms throughout
270 incubation (data not shown). In PCB-dechlorinating microcosms, hydrogen atmosphere stimulated
271 methanogenesis compared to the not amended control, leading to a production of 27 ml of methane in
272 the bio-Pd NPs-free microcosms versus 6 ml in the unamended control after 18 weeks on incubation
273 (Fig 4b). In addition to the positive effect of hydrogen, however, a negative effect of bio-Pd NPs on
274 methanogenesis was detected, since 17 ± 1 and 13 ± 1 ml of methane were produced respectively in the
275 microcosms amended with 5 mg/kgdw and 50 mg/kgdw of bio-Pd NPs, corresponding to 62% and
276 48%, respectively, of the methane produced in the bio-Pd NPs-free control (Fig 4b). Similar dose-
277 dependent inhibition by bio-Pd NPs on methanogenesis was observed in the microcosms without
278 PCBs, as the methane production decreased of 44% and 59% compared to hydrogen-amended bio-Pd

279 NPs-free microcosms, in the presence of 5 mg/kgdw and 50 mg/kgdw bio-Pd NPs, respectively (Fig
280 4b).

281 282 ***Influence of bio-Pd NPs on the microbial community composition***

283 To further verify the impact of bio-Pd NPs on the total microbial community, PCR-DGGE approach
284 was used to determine variations in terms of Community organization (Co) and Richness (Rr)
285 throughout incubation. PCR-DGGE analyses were carried out on the bacterial communities of
286 biologically active microcosms at the beginning, at the half and at the end of incubation (0, 9 and 18
287 weeks, respectively) (Figs 5 and 6).

288 A marked increase of community richness was detected in all hydrogen-amended dechlorinating
289 microcosms, and especially in presence of bio-Pd NPs, which exhibited a dose-dependent increase
290 effect. The final richness (Rr) values were 25, 30, and 29 for microcosms with hydrogen only,
291 hydrogen and bio-Pd NPs at 5 mg/kgdw and hydrogen and bio-Pd NPs at 50 mg/kgdw, respectively,
292 while only 21 in unamended active controls (Fig 5). The community organization gradually decreased
293 over time in the unamended controls, as the Co decreased from 40 to 32; on the contrary, all
294 dechlorinating microcosms amended with hydrogen showed a transient decrease in evenness, followed
295 by a successive re-organization, to reach final Co similar to values measured in the unamended
296 dechlorinating control (Fig 5). Thus, bio-Pd NPs did not seem to exhibit any effect on the community
297 organization of the microbial dechlorinating community, while apparently stimulating a richer
298 microbiota.

299 The same analysis was also performed on microcosms without PCBs (Fig 6) revealing again a dose-
300 dependent increase effect by bio-Pd NPs on the richness of the bacterial community at the end of the
301 incubation. In the absence of PCBs a decrease in community organization was observed over time.
302 This suggests that the community re-organizes, possibly because of the loss of some selective pressure
303 exerted by PCBs and thus of the competitive advantage of the organohalides respiring members, which
304 possibly favoured other species as the dechlorinating species decay (Fig 6). However, no differences
305 were observed in the community organization of bio-Pd amended and bio-Pd free microcosms,
306 indicating the bio-Pd NPs do not affect significantly the community structure.

307
308
309 **Discussion**

310 The use of bioPd-NPs as remediation catalyst is currently gaining a momentum, providing an effective
311 strategy for the treatment of recalcitrant compounds such as azo dyes (Quan et al., 2015) or
312 organohalides (Hosseinkhani et al., 2014b; Mertens et al., 2007) in several contaminated matrices, such
313 as wastewater (Hennebel et al., 2012), groundwater (De Corte et al., 2012) and sediments
314 (Hosseinkhani et al., 2015). NPs in general may pose risk for aquatic environments (Moore, 2006) and
315 their delivery, either by *in situ* application or by accidental release, might result in unwanted effects
316 which would eventually invalidate their benefits (Grieger et al., 2010; Karn et al., 2009; Schrand et al.,
317 2010). The evaluation of the risk/benefit ratio is not trivial, due to the lack of standard experimental
318 conditions and analytical protocols for nanoparticles in general (Crane et al., 2008; Grieger et al., 2010)
319 which persists in spite of a huge research need, still unaddressed (US-EPA 2014, 2007). In addition, the
320 literature regarding Pd-NPs toxic effect is quite scarce. It is known that they can exert antimicrobial
321 effects (Adams et al., 2014) and that they might be toxic for some superior organisms (Lüderwald et
322 al., 2016; Petrarca et al., 2014) but not for others (Kovrižnych et al., 2013; Shah and Belozerova,
323 2009). In this study, a standard *V. fischeri*-based ecotoxicity assay has been implemented, being
324 currently referred as a standard test also for NPs toxicity (Sánchez et al., 2011). The test is more
325 sensitive than other standard tests reported, such as *D. magna* toxicity (van Beelen, 2003), highly
326 specific and recommended to investigate the toxicity of various metals. To the best of our knowledge,
327 this is the first study in which such assay was used on Pd-NPs and the obtained results are dismissing
328 hypotheses of toxic effect of these NPs on marine bacteria.

329 Some limitations characterize standard ecotoxicity assays since they usually miss the interplay between
330 different factors, affecting both the environmental fate of the delivered/dispersed nanoparticles and
331 their interaction with the pollutant and the organisms (Nowack and Bucheli, 2007). To the best of our
332 knowledge, no information on the toxicity of Pd-NPs over environmental microbial communities is
333 available. Moreover, the effect of the natural organic matter, which occurs in complex environments
334 such as aquatic sediments, should not be underestimated: it is well known that organic matter not only
335 can change the catalytic properties of metal NPs, but can also affect exposure and toxicity to
336 (micro)organisms (Wang et al., 2016). In addition, the bio-Pd NPs used for this study are embedded in
337 the cell wall of killed *S. oneidensis* and this might influence their toxicity to bacteria, by limiting their
338 entry into the cells or altering their interaction with the cell surface and the sediment organic matter; on
339 the other hand, the cell lysis might generate free bio-Pd NPs over time, thus changing their potential
340 impact. Therefore, prior actual delivery or accidental leaks into the environment, and regardless of their
341 catalytic efficiency, bio-Pd NPs environmental impacts need to be assessed, i.e. on microbial activity

342 and on microbial community structure. For all of these reasons, thorough tests were required in
343 matrices close to the environmental conditions and for longer incubation time than current standard
344 tests. Microcosms studies are a good compromise between the complexity of environmental matrices
345 and the need to get preliminary data on the impact of NPs over microbial communities (Echavarri-
346 Bravo et al., 2015), which then need be thoroughly investigated further in mesocosms (Holden et al.,
347 2016). Therefore, tests were performed in microcosms of actual marine sediments and water,
348 inoculated with a known dehalogenating microbial community in biogeochemical conditions close to
349 those occurring in the real site: this allowed to evaluate both the catalytic activity and the impact of
350 bio-Pd NPs on the microbial community under environmental conditions and in a time frame suitable
351 for slow-growing anaerobic microorganisms. A culture enriched in organohalide respiring members
352 was selected because bio-Pd NPs could be used to complement biological reductive dechlorination
353 activities with catalytic dehalogenation in nanoremediation approaches.

354 In this study, a lack of catalytic activity by bioPd-NPs over PCBs was evidenced in the sterile sediment
355 microcosms. A similar lack of activity was already shown to occur for bioPd-NPs in another anoxic
356 sediment of the Venice Lagoon area, where it was due to some inhibiting effects of the environmental
357 matrix, i.e., sulfide-poisoning phenomena (Hosseinkhani et al., 2015). This further supports the
358 assumption that bio-Pd NPs are effective catalysts in low-sulfidogenic sediments, i.e., in presence of
359 low sulfate concentration or under more aerobic conditions (Hosseinkhani et al., 2015). The complex
360 matrix of sediments might as well have played an inhibiting role, since the environmental distribution,
361 fate and activity of many NPs could be influenced by phenomena of sequestration, adsorption and
362 chemical inactivation by humic acids (Shah and Belozerova, 2009; Wang et al., 2016). Further studies
363 could be performed on different sediments not affecting the catalytic activity of bioPd-NPs to exclude
364 their toxic effect on microbiota in effectively catalytic conditions; however, inactivation of bio-Pd NPs
365 is a possible occurrence in the actual sediment used in this study, making relevant the evaluation of
366 bio-Pd NPs impact on microbial communities independently from their activity.

367 A negligible effect of bio-Pd NPs was observed on organohalide respiration. It has been shown that
368 other zero-valent metal NPs, known to be effective enhancers of PCB reductive dechlorination
369 activities in marine sediments, such as zero-valent iron NPs (nZVI), (Zanaroli et al., 2012b) do exhibit
370 also a series of ecotoxic effects in the environment, recently reviewed extensively by Lefevre, et al.
371 (2015), which include severe impacts on organohalides respiring communities in groundwater (Barnes
372 et al., 2010) and soil (Tilston et al., 2013), either through shifts in the microbial composition (Tilston et
373 al., 2013) or direct inhibition (Z. M. Xiu et al., 2010). A dose-dependent inhibitory effect exerted by

374 bioPd-NPs was evident on sulfate reduction and methanogenic activities. Literature data on the effect
375 exerted on these respiratory metabolisms in anaerobic microbial communities by Pd-NPs or similar
376 nanoparticles, such as nZVI, are limited and often contradictory: either inhibition of sulfate reduction
377 activities (Barnes et al., 2010) or increase in sulfate reducing bacteria population (Kirschling et al.,
378 2010), either increase in methane production (Hu et al., 2015) or its inhibition (Huang et al., 2016)
379 were reported. Studies investigating the mechanisms of interaction between bio-Pd NPs and cells of
380 different microbial species might be useful to understand why bio-Pd NPs exert an inhibitory effect on
381 some respiratory metabolisms rather than others.

382 Microbial community analysis is a fundamental tool to investigate toxicity hypotheses on
383 environmental microbial communities (Sánchez et al., 2011). Particularly, diversity highly influences
384 microbial community efficiency (Tilman et al., 2014), but also evenness plays an important role under
385 selective stress (Wittebolle et al., 2009). It would be expected that, in presence of a toxic compound,
386 both indexes would significantly decrease in a marine community (Johnston and Roberts, 2009), due to
387 a selective growth of the resistant community members. BioPd-NPs, instead, only slightly altered the
388 structure of the microbial community. In addition, they had a clear positive effect on community
389 richness, while causing only a temporary shift in its community organization, which was reversed at the
390 end of incubation. These observations clearly indicate that bioPd-NPs have no significant effect of on
391 the overall microbial community. In other studies, more remarkable changes in the microbial
392 community composition with nZVI NPs were reported, which were interpreted as a toxic effect
393 (Kirschling et al., 2010; Tilston et al., 2013; Z. ming Xiu et al., 2010); however, in these cases no
394 analysis of the community richness and community organization was performed, not allowing a clear
395 comparison of the overall effects of bioPd-NPs and nZVI-NPs on microbial communities. Also, the
396 different catalytic mechanisms of these two NPs (nZVI are reagents, while Pd-NPs are catalysts) may
397 explain the differences in the toxic behaviours, as the peculiar microbial community structure and the
398 interplay among environmental factors and NPs can do (Lefevre et al., 2015). Finally, microbial
399 communities in marine sediments often demonstrate the capacity to resist to perturbations; even when
400 ecological shifts are observed, particular metabolic functions of the community might be uncoupled,
401 making the community eventually resilient to external forces (Bowen et al., 2011). The marine
402 sediment community used in this study showed resiliency to bio-Pd NPs. Although this property might
403 mask possible toxic effects on some specific members of the community, combined with the lack of
404 toxicity to *V. fischeri* and the limited influence on few respiration activities observed, this information
405 further supports the lack of significant impacts of bio-Pd NPs on marine bacteria. Overall, these data

406 also point out the importance of combining multiple tests addressing different types of effects (standard
407 ecotoxicity assays, monitoring of metabolic activities and community structure) to evaluate the impact
408 of NPs on the environmental microbiota.

409

410 **Conclusions**

411 Different approaches have been used to evaluate the effect of bio-Pd NPs on marine microbes and
412 communities, taking into consideration their main metabolic activities, their biodiversity and
413 community structure. Bio-Pd NPs do not exert toxicity towards the bioluminescent marine bacterium
414 *V. fischeri*. They may have limited inhibitory effects selectively towards specific respiratory
415 metabolisms, such as sulfate reduction and methanogenesis, but not organohalide respiration. On the
416 other hand, an increase of the community biodiversity along with no permanent effects on its
417 community organization have been observed, indicating the lack of a significant impact on the
418 microbial community. Overall, these data combined dismiss hypotheses of ecological impact of bio-Pd
419 NPs on marine microbiota. The use of a combination of different approaches tailoring as many factors
420 as possible should be implemented when investigating the possible impact of NPs in the environment.

421

422 **Acknowledgements**

423 This study was supported by the European Commission [grant number 266473] (ULIXES Project).

424

425 **References**

426 Adams, C.P., Walker, K. a., Obare, S.O., Docherty, K.M., 2014. Size-Dependent Antimicrobial Effects
427 of Novel Palladium Nanoparticles. PLoS One 9, e85981. doi:10.1371/journal.pone.0085981

428 Barnes, R.J., Riba, O., Gardner, M.N., Singer, A.C., Jackman, S.A., Thompson, I.P., 2010. Inhibition
429 of biological TCE and sulphate reduction in the presence of iron nanoparticles. Chemosphere 80,
430 554–562. doi:10.1016/j.chemosphere.2010.04.033

431 Baxter-Plant, V.S., Mikheenko, I.P., Macaskie, L.E., 2003. Sulphate-reducing bacteria, palladium and
432 the reductive dehalogenation of chlorinated aromatic compounds. Biodegradation 14, 83–90.
433 doi:10.1023/A:1024084611555

434 Bedard, D.L., 2008. A case study for microbial biodegradation: anaerobic bacterial reductive

435 dechlorination of polychlorinated biphenyls-from sediment to defined medium. *Annu. Rev.*
436 *Microbiol.* 62, 253–70. doi:10.1146/annurev.micro.62.081307.162733

437 Bowen, J.L., Ward, B.B., Morrison, H.G., Hobbie, J.E., Valiela, I., Deegan, L.A., Sogin, M.L., 2011.
438 Microbial community composition in sediments resists perturbation by nutrient enrichment. *ISME*
439 *J.* 5, 1540–1548. doi:10.1038/ismej.2011.22

440 Choi, H., Al-Abed, S.R., Agarwal, S., 2009. Catalytic Role of Palladium and Relative Reactivity of
441 Substituted Chlorines during Adsorption and Treatment of PCBs on Reactive Activated Carbon.
442 *Environ. Sci. Technol.* 43, 7510–7515. doi:10.1021/es901298b

443 Crane, M., Handy, R.D., Garrod, J., Owen, R., 2008. Ecotoxicity test methods and environmental
444 hazard assessment for engineered nanoparticles. *Ecotoxicology* 17, 421–437. doi:10.1007/s10646-
445 008-0215-z

446 De Corte, S., Hennebel, T., De Gussemé, B., Verstraete, W., Boon, N., 2012. Bio-palladium: From
447 metal recovery to catalytic applications. *Microb. Biotechnol.* 5, 5–17. doi:10.1111/j.1751-
448 7915.2011.00265.x

449 De Windt, W., Aelterman, P., Verstraete, W., 2005. Bioreductive deposition of palladium (0)
450 nanoparticles on *Shewanella oneidensis* with catalytic activity towards reductive dechlorination of
451 polychlorinated biphenyls. *Environ. Microbiol.* 7, 314–325. doi:10.1111/j.1462-
452 2920.2005.00696.x

453 Echavarri-Bravo, V., Paterson, L., Aspray, T.J., Porter, J.S., Winson, M.K., Thornton, B., Hartl,
454 M.G.J., 2015. Shifts in the metabolic function of a benthic estuarine microbial community
455 following a single pulse exposure to silver nanoparticles. *Environ. Pollut.* 201, 91–99.
456 doi:10.1016/j.envpol.2015.02.033

457 Farré, M. La, Pérez, S., Kantiani, L., Barceló, D., 2008. Fate and toxicity of emerging pollutants, their
458 metabolites and transformation products in the aquatic environment. *TrAC - Trends Anal. Chem.*
459 27, 991–1007. doi:10.1016/j.trac.2008.09.010

460 Fava, F., Gentilucci, S., Zanaroli, G., 2003a. Anaerobic biodegradation of weathered polychlorinated
461 biphenyls (PCBs) in contaminated sediments of Porto Marghera (Venice Lagoon, Italy).
462 *Chemosphere* 53, 101–9. doi:10.1016/S0045-6535(03)00438-7

463 Fava, F., Zanaroli, G., Young, L.Y., 2003b. Microbial reductive dechlorination of pre-existing PCBs
464 and spiked 2,3,4,5,6-pentachlorobiphenyl in anaerobic slurries of a contaminated sediment of

465 Venice Lagoon (Italy). *FEMS Microbiol. Ecol.* 44, 309–18. doi:10.1016/S0168-6496(03)00069-2

466 Fernández, P., Grimalt, J.O., 2003. On the Global Distribution of Persistent Organic Pollutants. *Chim.*
467 *Int. J. Chem.* 57, 514–521. doi:10.2533/000942903777679000

468 Field, J. a, Sierra-Alvarez, R., 2008. Microbial transformation and degradation of polychlorinated
469 biphenyls. *Environ. Pollut.* 155, 1–12. doi:10.1016/j.envpol.2007.10.016

470 Grieger, K.D., Fjordbøge, A., Hartmann, N.B., Eriksson, E., Bjerg, P.L., Baun, A., 2010.
471 Environmental benefits and risks of zero-valent iron nanoparticles (nZVI) for in situ remediation:
472 risk mitigation or trade-off? *J. Contam. Hydrol.* 118, 165–83. doi:10.1016/j.jconhyd.2010.07.011

473 Hennebel, T., De Corte, S., Verstraete, W., Boon, N., 2012. Microbial production and environmental
474 applications of Pd nanoparticles for treatment of halogenated compounds. *Curr. Opin. Biotechnol.*
475 23, 555–561. doi:10.1016/j.copbio.2012.01.007

476 Hennebel, T., Simoen, H., Windt, W. De, 2009. Biocatalytic dechlorination of trichloroethylene with
477 bio-palladium in a pilot-scale membrane reactor. *Biotechnol. Bioeng.* 102, 995–1002.
478 doi:10.1002/bit.22138

479 Holden, P.A., Gardea-Torresdey, J.L., Klaessig, F., Turco, R.F., Mortimer, M., Hund-Rinke, K., Cohen
480 Hubal, E.A., Avery, D., Barceló, D., Behra, R., Cohen, Y., Deydier-Stephan, L., Ferguson, P.L.,
481 Fernandes, T.F., Herr Harthorn, B., Henderson, W.M., Hoke, R.A., Hristozov, D., Johnston, J.M.,
482 Kane, A.B., Kapustka, L., Keller, A.A., Lenihan, H.S., Lovell, W., Murphy, C.J., Nisbet, R.M.,
483 Petersen, E.J., Salinas, E.R., Scheringer, M., Sharma, M., Speed, D.E., Sultan, Y., Westerhoff, P.,
484 White, J.C., Wiesner, M.R., Wong, E.M., Xing, B., Steele Horan, M., Godwin, H.A., Nel, A.E.,
485 2016. Considerations of Environmentally Relevant Test Conditions for Improved Evaluation of
486 Ecological Hazards of Engineered Nanomaterials. *Environ. Sci. Technol.* 50, 6124–6145.
487 doi:10.1021/acs.est.6b00608

488 Hosseinkhani, B., Hennebel, T., Boon, N., 2014a. Potential of biogenic hydrogen production for
489 hydrogen driven remediation strategies in marine environments. *N. Biotechnol.* 31, 445–50.
490 doi:10.1016/j.nbt.2014.04.005

491 Hosseinkhani, B., Hennebel, T., Van Nevel, S., Verschuere, S., Yakimov, M.M., Cappello, S., Blaghen,
492 M., Boon, N., 2014b. Biogenic nanopalladium based remediation of chlorinated hydrocarbons in
493 marine environments. *Environ. Sci. Technol.* 48, 550–557. doi:10.1021/es403047u

494 Hosseinkhani, B., Nuzzo, A., Zanaroli, G., Fava, F., Boon, N., 2015. Assessment of catalytic

495 dechlorination activity of suspended and immobilized bio-Pd NPs in different marine conditions.
496 *Appl. Catal. B Environ.* 168-169, 62–67. doi:10.1016/j.apcatb.2014.12.014

497 Hu, Y., Hao, X., Zhao, D., Fu, K., 2015. Enhancing the CH₄ yield of anaerobic digestion via
498 endogenous CO₂ fixation by exogenous H₂. *Chemosphere* 140, 34–9.
499 doi:10.1016/j.chemosphere.2014.10.022

500 Huang, Y.-X., Guo, J., Zhang, C., Hu, Z., 2016. Hydrogen production from the dissolution of nano zero
501 valent iron and its effect on anaerobic digestion. *Water Res.* 88, 475–480.
502 doi:10.1016/j.watres.2015.10.028

503 Jiang, W., Mashayekhi, H., Xing, B., 2009. Bacterial toxicity comparison between nano- and micro-
504 scaled oxide particles. *Environ. Pollut.* 157, 1619–1625. doi:10.1016/j.envpol.2008.12.025

505 Johnston, E.L., Roberts, D.A., 2009. Contaminants reduce the richness and evenness of marine
506 communities: A review and meta-analysis. *Environ. Pollut.* 157, 1745–1752.
507 doi:10.1016/j.envpol.2009.02.017

508 Karn, B., Kuiken, T., Otto, M., 2009. Nanotechnology and in situ remediation: A review of the benefits
509 and potential risks. *Environ. Health Perspect.* 117, 1823–1831. doi:10.1289/ehp.0900793

510 Khan, F.I., Husain, T., Hejazi, R., 2004. An overview and analysis of site remediation technologies. *J.*
511 *Environ. Manage.* 71, 95–122. doi:10.1016/j.jenvman.2004.02.003

512 Kirschling, T.L., Gregory, K.B., Minkley, E.G., Lowry, G. V., Tilton, R.D., 2010. Impact of nanoscale
513 zero valent iron on geochemistry and microbial populations in trichloroethylene contaminated
514 aquifer materials. *Environ. Sci. Technol.* 44, 3474–3480. doi:10.1021/es903744f

515 Kovrižnych, J. a, Sotníková, R., Zeljenková, D., Rollerová, E., Szabová, E., Wimmerová, S., 2013.
516 Acute toxicity of 31 different nanoparticles to zebrafish (*Danio rerio*) tested in adulthood and in
517 early life stages - comparative study. *Interdiscip. Toxicol.* 6, 67–73. doi:10.2478/intox-2013-0012

518 Lefevre, E., Bossa, N., Wiesner, M.R., Gunsch, C.K., 2015. A review of the environmental
519 implications of in situ remediation by nanoscale zero valent iron (nZVI): Behavior, transport and
520 impacts on microbial communities. *Sci. Total Environ.* 565, 889–901.
521 doi:10.1016/j.scitotenv.2016.02.003

522 Lüderwald, S., Seitz, F., Seisenbaeva, G.A., Kessler, V.G., Schulz, R., Bundschuh, M., 2016.
523 Palladium Nanoparticles: Is There a Risk for Aquatic Ecosystems? *Bull. Environ. Contam.*
524 *Toxicol.* doi:10.1007/s00128-016-1803-x

525 Ma, X.Y., Wang, X.C., Ngo, H.H., Guo, W., Wu, M.N., Wang, N., 2014. Bioassay based luminescent
526 bacteria: interferences, improvements, and applications. *Sci. Total Environ.* 468-469, 1–11.
527 doi:10.1016/j.scitotenv.2013.08.028

528 Macé, C., Desrocher, S., Gheorghiu, F., Kane, A., Pupeza, M., Cernik, M., Kvapil, P.,
529 Venkatakrishnan, R., Zhang, W., 2006. Nanotechnology and groundwater remediation: A step
530 forward in technology understanding. *Remediat. J.* 16, 23–33. doi:10.1002/rem.20079

531 Marzorati, M., Wittebolle, L., Boon, N., Daffonchio, D., Verstraete, W., 2008. How to get more out of
532 molecular fingerprints: practical tools for microbial ecology. *Environ. Microbiol.* 10, 1571–81.
533 doi:10.1111/j.1462-2920.2008.01572.x

534 Mertens, B., Blothe, C., Windey, K., De Windt, W., Verstraete, W., 2007. Biocatalytic dechlorination
535 of lindane by nano-scale particles of Pd(0) deposited on *Shewanella oneidensis*. *Chemosphere* 66,
536 99–105. doi:10.1016/j.chemosphere.2006.05.018

537 Moore, M.N., 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic
538 environment? *Environ. Int.* 32, 967–976. doi:10.1016/j.envint.2006.06.014

539 Nowack, B., Bucheli, T.D., 2007. Occurrence, behavior and effects of nanoparticles in the
540 environment. *Environ. Pollut.* 150, 5–22. doi:10.1016/j.envpol.2007.06.006

541 Otto, M., Floyd, M., Bajpai, S., 2008. Nanotechnology for site remediation (including fact sheet).
542 *Remediat. J.* 19, 99–108. doi:10.1002/rem.20194

543 Perelo, L.W., 2010. Review: In situ and bioremediation of organic pollutants in aquatic sediments. *J.*
544 *Hazard. Mater.* 177, 81–9. doi:10.1016/j.jhazmat.2009.12.090

545 Petrarca, C., Clemente, E., Di Giampaolo, L., Mariani-Costantini, R., Leopold, K., Schindl, R., Lotti, L.
546 V, Mangifesta, R., Sabbioni, E., Niu, Q., Bernardini, G., Di Gioacchino, M., 2014. Palladium
547 Nanoparticles Induce Disturbances in Cell Cycle Entry and Progression of Peripheral Blood
548 Mononuclear Cells: Paramount Role of Ions. *J. Immunol. Res.* 2014, 1–8.
549 doi:10.1155/2014/295092

550 Quan, X., Zhang, X., Xu, H., 2015. In-situ formation and immobilization of biogenic nanopalladium
551 into anaerobic granular sludge enhances azo dyes degradation. *Water Res.* 78, 74–83.
552 doi:10.1016/j.watres.2015.03.024

553 Read, S., Marzorati, M., Guimarães, B.C.M., Boon, N., 2011. Microbial Resource Management
554 revisited: successful parameters and new concepts. *Appl. Microbiol. Biotechnol.* 90, 861–871.

555 doi:10.1007/s00253-011-3223-5

556 Safe, S., 1993. Toxicology, structure-function relationship, and human and environmental health
557 impacts of polychlorinated biphenyls: progress and problems. *Environ. Health Perspect.* 87, 1219–
558 20.

559 Sánchez, A., Recillas, S., Font, X., Casals, E., González, E., Puentes, V., 2011. Ecotoxicity of, and
560 remediation with, engineered inorganic nanoparticles in the environment. *TrAC Trends Anal.*
561 *Chem.* 30, 507–516. doi:10.1016/j.trac.2010.11.011

562 Sass, A.M., Sass, H., Coolen, M.J., Cypionka, H., Overmann, J., 2001. Microbial communities in the
563 chemocline of a hypersaline deep-sea basin (Urania basin, Mediterranean Sea). *Appl. Environ.*
564 *Microbiol.* 67, 5392–402. doi:10.1128/AEM.67.12.5392-5402.2001

565 Schrand, A.M., Rahman, M.F., Hussain, S.M., Schlager, J.J., Smith, D.A., Syed, A.F., 2010. Metal-
566 based nanoparticles and their toxicity assessment. *Wiley Interdiscip. Rev. Nanomedicine*
567 *Nanobiotechnology.* doi:10.1002/wnan.103

568 Scoma, A., Bertin, L., Zanaroli, G., Fraraccio, S., Fava, F., 2011. A physicochemical-biotechnological
569 approach for an integrated valorization of olive mill wastewater. *Bioresour. Technol.* 102, 10273–
570 9. doi:10.1016/j.biortech.2011.08.080

571 Serrano, E., 2010. Nanotechnology and the Environment. *Mater. Today* 13, 55. doi:10.1016/S1369-
572 7021(10)70089-4

573 Shah, V., Belozerova, I., 2009. Influence of metal nanoparticles on the soil microbial community and
574 germination of lettuce seeds. *Water. Air. Soil Pollut.* 197, 143–148. doi:10.1007/s11270-008-
575 9797-6

576 Sowers, K.R., May, H.D., 2013. In situ treatment of PCBs by anaerobic microbial dechlorination in
577 aquatic sediment: are we there yet? *Curr. Opin. Biotechnol.* 24, 482–8.
578 doi:10.1016/j.copbio.2012.10.004

579 Speranza, A., Leopold, K., Maier, M., Taddei, A.R., Scoccianti, V., 2010. Pd-nanoparticles cause
580 increased toxicity to kiwifruit pollen compared to soluble Pd(II). *Environ. Pollut.* 158, 873–882.
581 doi:10.1016/j.envpol.2009.09.022

582 Stockholm Convention, 2004. Listing of POPs in the Stockholm Convention (updated May 2013)
583 [WWW Document]. URL
584 <http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx>

585 Theron, J., Walker, J. a, Cloete, T.E., 2008. Nanotechnology and water treatment: applications and
586 emerging opportunities. *Crit. Rev. Microbiol.* 34, 43–69. doi:10.1080/10408410701710442

587 Tilman, D., Isbell, F., Cowles, J.M., 2014. Biodiversity and Ecosystem Functioning. *Annu. Rev. Ecol.*
588 *Evol. Syst.* 45, 471–493. doi:10.1146/annurev-ecolsys-120213-091917

589 Tilston, E.L., Collins, C.D., Mitchell, G.R., Princivalle, J., Shaw, L.J., 2013. Nanoscale zerovalent iron
590 alters soil bacterial community structure and inhibits chloroaromatic biodegradation potential in
591 Aroclor 1242-contaminated soil. *Environ. Pollut.* 173, 38–46. doi:10.1016/j.envpol.2012.09.018

592 Ukisu, Y., Miyadera, T., 2003. Hydrogen-transfer hydrodechlorination of polychlorinated dibenzo-p-
593 dioxins and dibenzofurans catalyzed by supported palladium catalysts. *Appl. Catal. B Environ.* 40,
594 141–149. doi:10.1016/S0926-3373(02)00148-0

595 US Environmental Protection Agency, 2014. Technical Fact sheet – Nanomaterials At a Glance
596 [WWW Document]. Off. Solid Waste Emerg. Response, EPA 505-F-14-004. URL
597 [http://nepis.epa.gov/Exe/ZyNET.exe/P100MWS9.TXT?ZyActionD=ZyDocument&Client=EPA&](http://nepis.epa.gov/Exe/ZyNET.exe/P100MWS9.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&)
598 [Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=](http://nepis.epa.gov/Exe/ZyNET.exe/P100MWS9.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&)
599 [n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&](http://nepis.epa.gov/Exe/ZyNET.exe/P100MWS9.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&)
600 [ExtQFieldOp=0&XmlQuery=&](http://nepis.epa.gov/Exe/ZyNET.exe/P100MWS9.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&)

601 US Environmental Protection Agency, 2007. Nanotechnology white paper [WWW Document]. Sr.
602 Policy Counc. EPA 100/B-07/001. URL [http://www.epa.gov/osainter/pdfs/nanotech/epa-](http://www.epa.gov/osainter/pdfs/nanotech/epa-nanotechnology-whitepaper-0207.pdf)
603 [nanotechnology-whitepaper-0207.pdf](http://www.epa.gov/osainter/pdfs/nanotech/epa-nanotechnology-whitepaper-0207.pdf)

604 van Beelen, P., 2003. A review on the application of microbial toxicity tests for deriving sediment
605 quality guidelines. *Chemosphere* 53, 795–808. doi:10.1016/S0045-6535(03)00716-1

606 Wang, Z., Zhang, L., Zhao, J., Xing, B., 2016. Environmental processes and toxicity of metallic
607 nanoparticles in aquatic systems as affected by natural organic matter. *Environ. Sci. Nano* 3, 240–
608 255. doi:10.1039/C5EN00230C

609 Wiegel, J., Wu, Q., 2000. Microbial reductive dehalogenation of polychlorinated biphenyls. *FEMS*
610 *Microbiol. Ecol.* doi:10.1016/S0168-6496(00)00014-3

611 Wiesner, M.R., Lowry, G. V., Alvarez, P., Dionysiou, D., Biswas, P., 2006. Assessing the risks of
612 manufactured nanomaterials. *Environ. Sci. Technol.* 40, 4336–4345. doi:10.1021/es062726m

613 Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P.,
614 Verstraete, W., Boon, N., 2009. Initial community evenness favours functionality under selective

615 stress. *Nature* 458, 623–6. doi:10.1038/nature07840

616 Xiu, Z. ming, Jin, Z. hui, Li, T. long, Mahendra, S., Lowry, G. V., Alvarez, P.J.J., 2010. Effects of
617 nano-scale zero-valent iron particles on a mixed culture dechlorinating trichloroethylene.
618 *Bioresour. Technol.* 101, 1141–1146. doi:10.1016/j.biortech.2009.09.057

619 Xiu, Z.M., Gregory, K.B., Lowry, G. V., Alvarez, P.J.J., 2010. Effect of bare and coated nanoscale
620 zerovalent iron on tceA and vcrA gene expression in *Dehalococcoides* spp. *Environ. Sci. Technol.*
621 44, 7647–7651. doi:10.1021/es101786y

622 Zanaroli, G., Balloi, A., Negroni, A., Borruso, L., Daffonchio, D., Fava, F., 2012a. A Chloroflexi
623 bacterium dechlorinates polychlorinated biphenyls in marine sediments under in situ-like
624 biogeochemical conditions. *J. Hazard. Mater.* 209-210, 449–57.
625 doi:10.1016/j.jhazmat.2012.01.042

626 Zanaroli, G., Balloi, A., Negroni, A., Daffonchio, D., Young, L.Y., Fava, F., 2010. Characterization of
627 the microbial community from the marine sediment of the Venice lagoon capable of reductive
628 dechlorination of coplanar polychlorinated biphenyls (PCBs). *J. Hazard. Mater.* 178, 417–26.
629 doi:10.1016/j.jhazmat.2010.01.097

630 Zanaroli, G., Negroni, A., Häggblom, M.M., Fava, F., 2015. Microbial dehalogenation of
631 organohalides in marine and estuarine environments. *Curr. Opin. Biotechnol.* 33, 287–295.
632 doi:10.1016/j.copbio.2015.03.013

633 Zanaroli, G., Negroni, A., Vignola, M., Nuzzo, A., Shu, H.-Y., Fava, F., 2012b. Enhancement of
634 microbial reductive dechlorination of polychlorinated biphenyls (PCBs) in a marine sediment by
635 nanoscale zerovalent iron (NZVI) particles. *J. Chem. Technol. Biotechnol.* 87, 1246–1253.
636 doi:10.1002/jctb.3835

637 Zanaroli, G., Pérez-Jiménez, J.R., Young, L.Y., Marchetti, L., Fava, F., 2006. Microbial reductive
638 dechlorination of weathered and exogenous co-planar polychlorinated biphenyls (PCBs) in an
639 anaerobic sediment of Venice Lagoon. *Biodegradation* 17, 121–9. doi:10.1007/s10532-005-3752-
640 7

641 Zhang, W.X., 2003. Nanoscale iron particles for environmental remediation: An overview. *J.*
642 *Nanoparticle Res.* 5, 323–332. doi:10.1023/A:1025520116015

643 **Figure Captions**

644 **Figure 1.** Bioluminescence inhibition of *V. fischeri* in acute toxicity tests after 5, 15 and 30 minutes
645 exposure to different dilutions of bio-Pd NPs in marine filter-sterilized water. The highest
646 concentration of bio-Pd NPs corresponds to 81.9% of the 50 mg/kgdw concentration at which bio-Pd
647 NPs were applied in the microcosms experiment. Inhibition is calculated in percentage against control
648 with no bio-Pd NPs. Values are means of duplicate analysis.

649 **Figure 2.** Dechlorination of Aroclor 1254 PCBs over time in the biologically active (inoculated) and
650 sterile (non inoculated) sets of spiked microcosms. Values are means of triplicate microcosms, with
651 error bars representing standard deviation.

652 **Figure 3.** Concentrations of spiked PCB congeners and their dechlorination products constituting more
653 than 1% w/w of total PCBs at the end of incubation in the biologically active (white bars) and sterile
654 (black bars) sets of spiked microcosms. (a) unamended microcosms; (b) hydrogen-amended
655 microcosms; (c) microcosms amended with hydrogen + bioPd 5mg/kgdw; (d) microcosms amended
656 with hydrogen + bio-Pd 50 mg/kgdw. Values are means of triplicate microcosms with error bars
657 representing standard deviation.

658 **Figure 4.** Sulfate concentration (a) and methane production (b) in the biologically active sets of
659 microcosms spiked and not spiked (No PCB) with Aroclor 1254. Values are means of triplicate
660 microcosms with error bars representing standard deviation.

661 **Figure 5.** Upper panel: DGGE profiles of the total bacterial community in the biologically active,
662 PCB-spiked set of microcosms at weeks 0, 9 and 18. In each lane, PCR products obtained from the
663 three replicate microcosms were pooled. Lower panel: analysis of the Community organization (Co
664 expressed as Gini percentage times 100, bars) and richness (diamonds) for each DGGE lane.

665 **Figure 6.** Upper panel: DGGE profiles of the total bacterial community in the biologically active,
666 PCB-free set of microcosms at weeks 0, 9 and 18. In each lane, PCR products obtained from the three
667 replicate microcosms were pooled. Lower panel: analysis of the Community organization (Co
668 expressed as Gini percentage times 100, bars) and richness (diamonds) for each DGGE lane.

Figure 1
[Click here to download high resolution image](#)

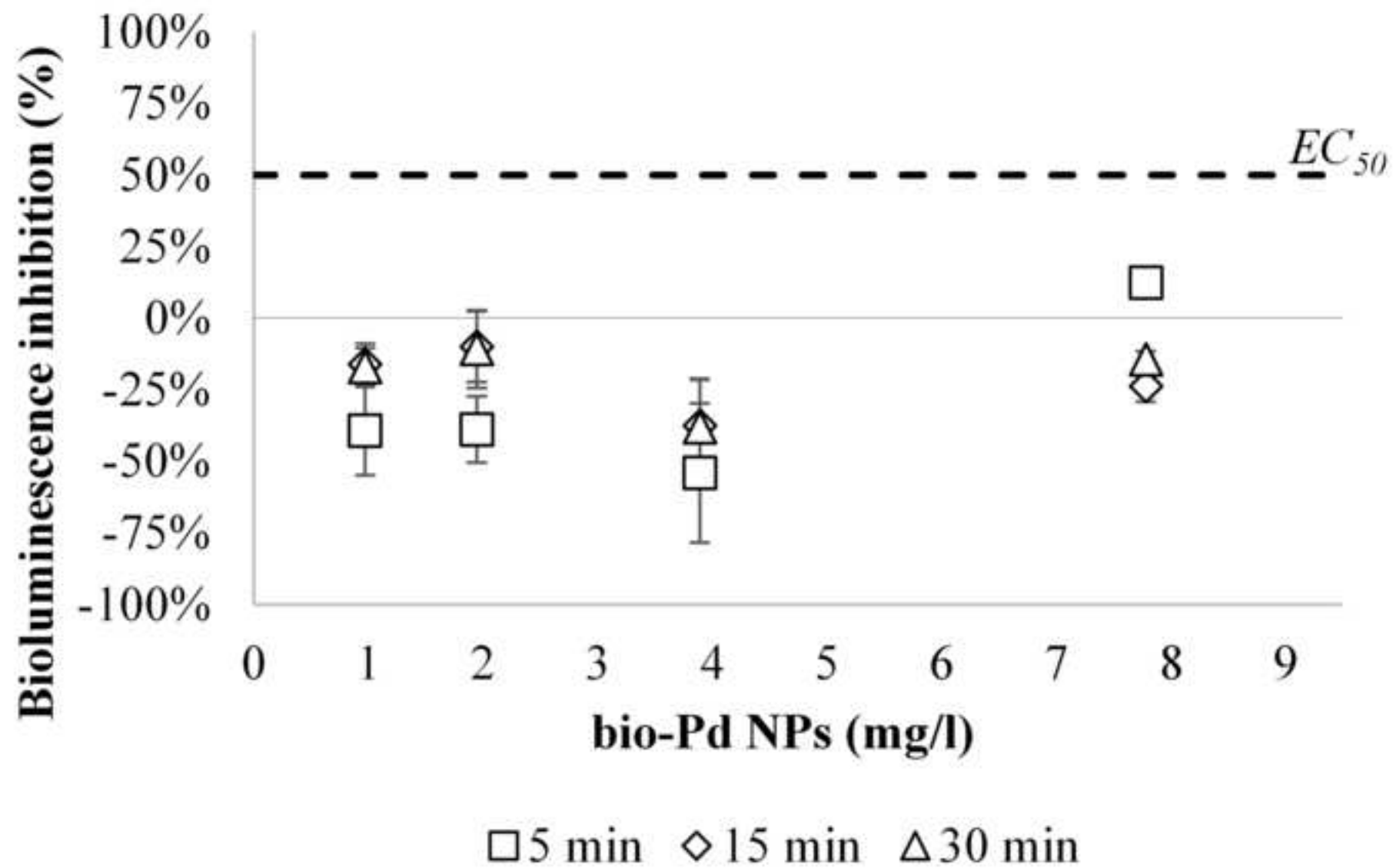


Figure 2
[Click here to download high resolution image](#)

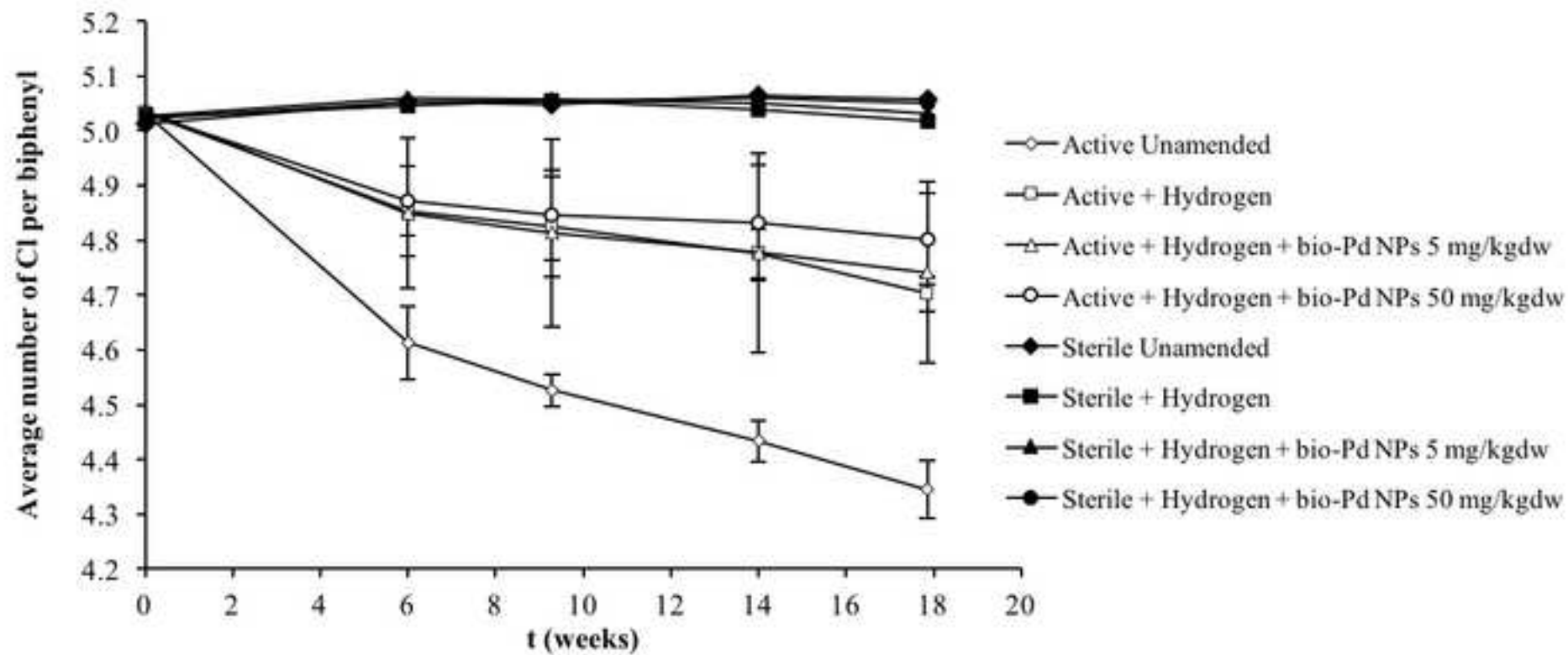


Figure 3
[Click here to download high resolution image](#)

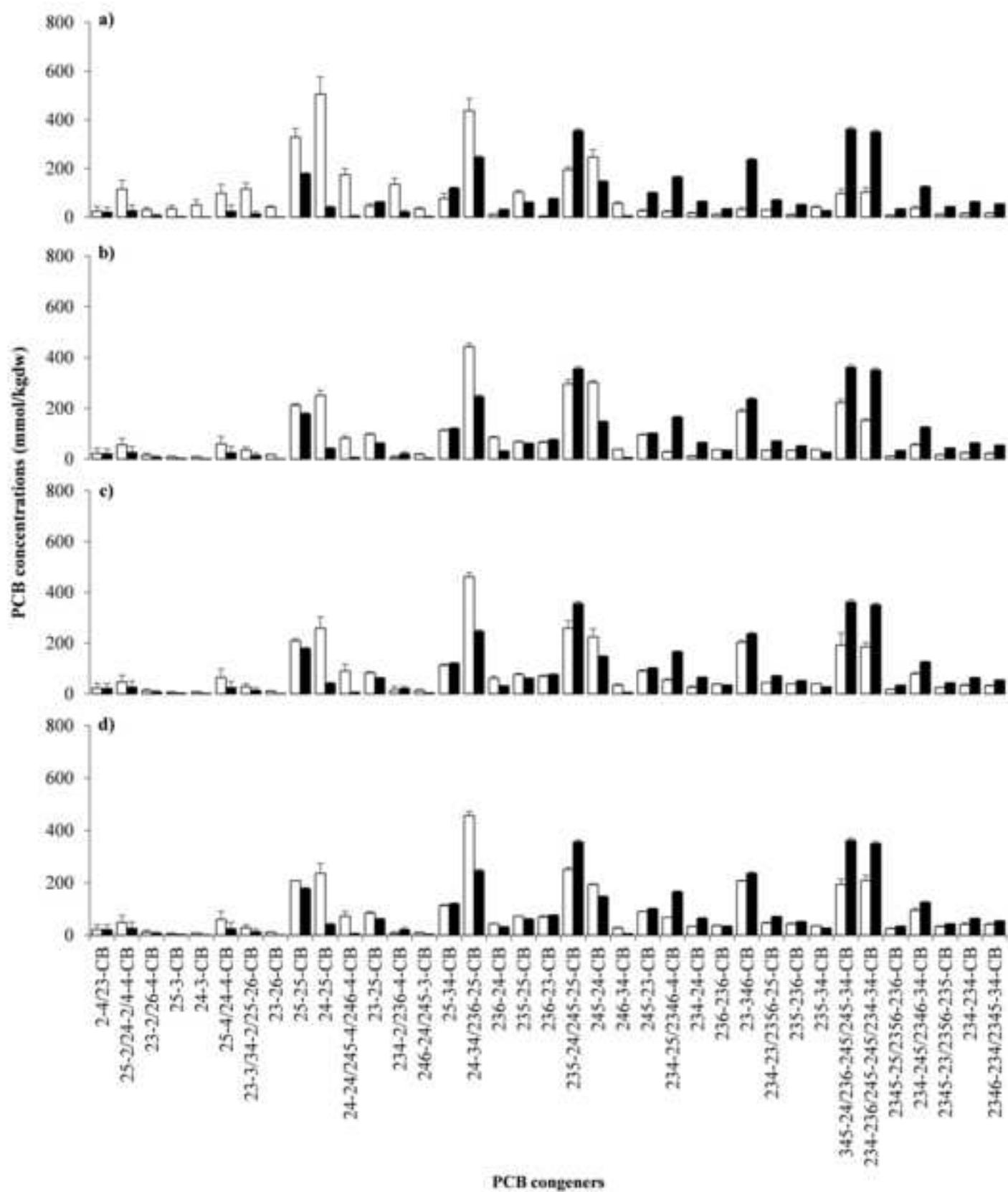


Figure 4
[Click here to download high resolution image](#)

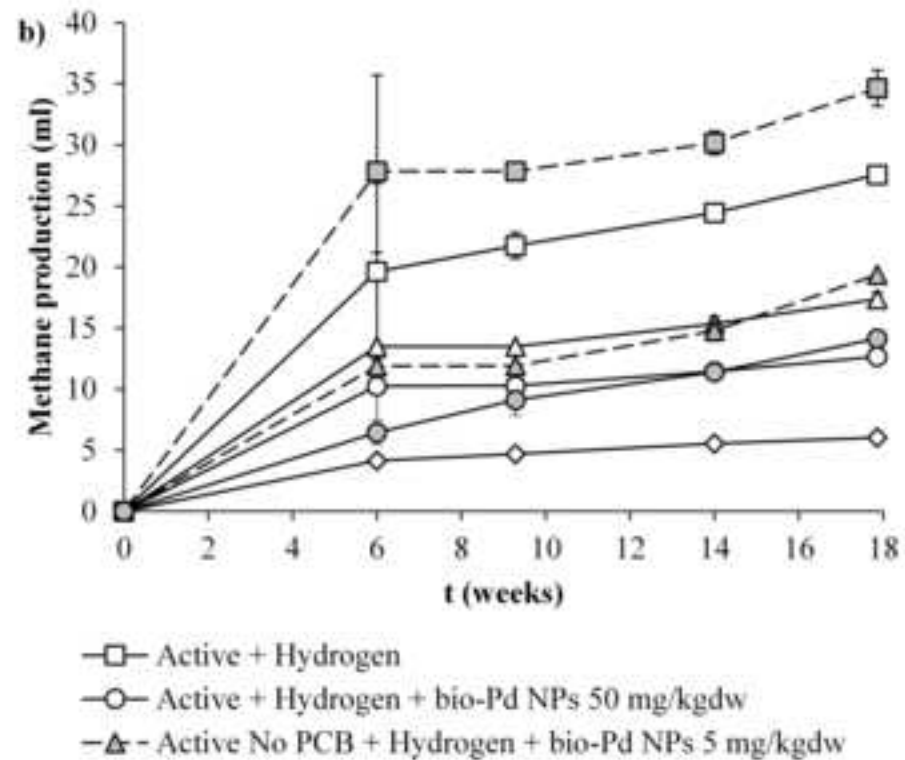
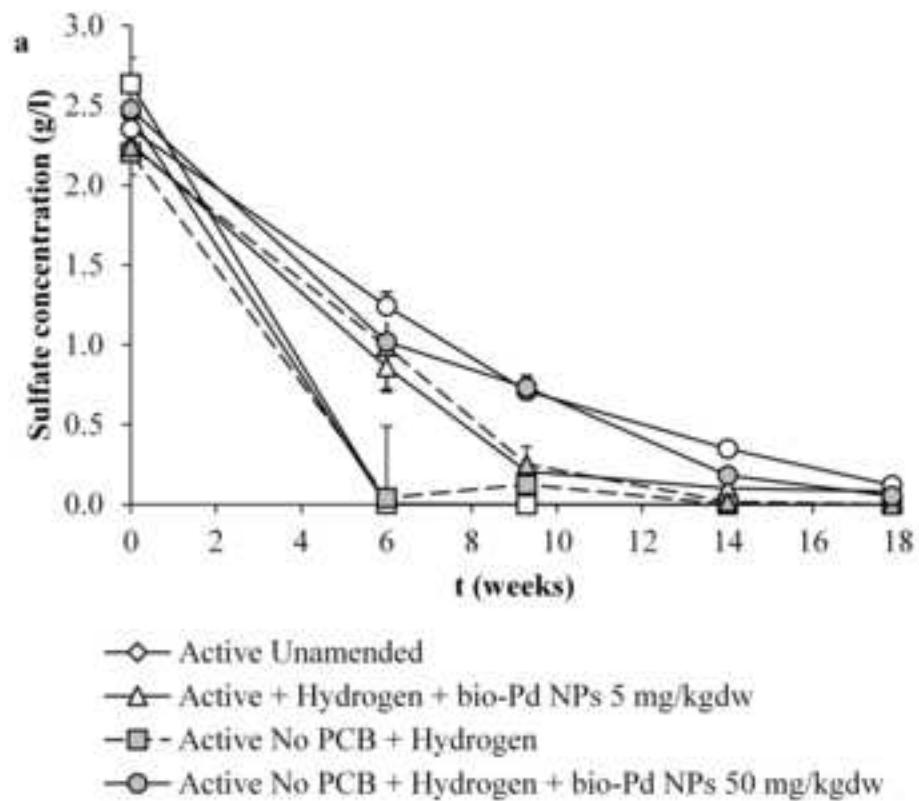


Figure 6
[Click here to download high resolution image](#)

