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Assessing ecotoxicological effects of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF in soil using *Allivibrio fischeri*

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Abstract

The toxic effect of dioxins and dioxin-like compounds have largely been studied using in vivo techniques; however, in vivo studies can be limited when rapid screening is required. Microtox® can be used as a rapid ecotoxicity assessment tool for dioxins in the environment, but studies on the assessment of dioxins and dioxin-like compounds using bioluminescent bacteria *Allivibrio fischeri* are limited. This study investigated the potential of using *A. fischeri* for assessing different dioxins and dioxin-like compounds, and the toxic effects of soils contaminated with dioxins and dioxin-like compounds were tested using different fractions of dioxins in soil to determine the appropriate way of assessing the toxic effects of contaminated soils. The results show that *A. fischeri* can potentially be used as a test species for rapidly evaluating toxic effects of dioxins and dioxin-like compounds in the environment. With the soil used in this study, the toxic effects of the water extracts (i.e., mobile fraction of dioxins) and the soil slurries (i.e., bioavailable fraction of dioxins) were similar to that of the controls. This suggests that the toxicity assessment of the organic extracts (i.e., total amount of dioxins) can be inappropriate in a managerial perspective, as the mobile or bioavailable fraction of contaminants in soils is often more of concern than the total amount of contaminants present in soils. Overall, when *A. fischeri* are to be used for a rapid toxicity assessment of dioxins-contaminated soils, different fractions of dioxins need to be assessed for better management of the contaminated soils.

Keywords: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, Dioxins, *Allivibrio fischeri*, Ecotoxicity

Introduction

Dioxins and dioxin-like compounds are toxic persistent organic pollutants (POPs) that are present in the global environment. They have been generated as byproducts of various industrial activities including incineration processes. Once they are released, they are transported from one environmental media to another environmental media and persist in the environment [1]. This will eventually impose adverse effects on organisms inhabiting in the environment, and biomagnification occurs through the food chain. The toxic effects of dioxins and dioxin-like compounds are determined relative to a reference compound, usually 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), and the World Health Organization (WHO) determined the human and mammalian toxic equivalency factors (TEFs) for dioxins and dioxin-like compounds [1]. For example, the TEF for 2,3,7,8-TCDD is 1, and the other dioxins and dioxin-like compounds have the TEFs 1 or lower than 1. For the mixtures of dioxins and dioxin-like compounds, the total toxic equivalent (TEQ) is calculated by summing the products of the concentration of each compound and TEF of that compound, thus, the resulting value represents the total 2,3,7,8-TCDD-like activity of the mixture [1].

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Environmental contaminants that have potential endocrine activity such as dioxins and dioxin-like compounds have been tested using in silico, in vitro, and in vivo techniques in order to detect potential endocrine activity [2]. When TEFs are set, in vivo studies using animal species are preferred as they assess both toxicokinetic and toxicodynamic aspects of the compound of interest [1, 2]. However, whether short-term in vivo or long-term in vivo is conducted, in vivo studies can be limited when rapid screening of the contaminants is required as they usually require relatively longer exposure times than in vitro studies and often the test animal species require longer growth period. Also, in vitro studies using various cell lines require facilities that can maintain appropriate conditions for the cell growth and toxicity tests [3]. Therefore, in vivo and in vitro cell line assays may not be appropriate when rapid in situ toxicity assessment is requested.

Among various ecotoxicity tests that can be used to determine toxic effects of environmental contaminants, Microtox® that uses the changes in the bioluminescence of *Allivibrio fischeri* (previously known as *Vibrio fischeri*) upon exposure to environmental contaminants has been widely used to assess ecotoxicity [4, 5]. With dioxins, recombinant bioluminescent strains of *Escherichia coli* were used to study the toxicity mechanisms of four dioxins and dioxin-like compounds [6]. However, studies on toxic effects of dioxin and dioxin-like compounds in the environment using *A. fischeri* are limited [7–9]. A few studies tried to determine the toxic effects of mixtures of dioxins and dioxin-like compounds extracted from biochar or sediment, and they used organic solvent [7, 8] or NaCl solution [9]. In other words, the assessment of the toxic effects of the total amount of dioxins extracted using organic solvents or the fraction of dioxins extracted using NaCl solution was tried. But the assessment of dioxins and dioxin-like compounds using *A. fischeri* needs more studies to be able to use Microtox® as a rapid ecotoxicity assessment tool for dioxins in the environment.

Therefore, this study was set to investigate the potential of using Microtox® for differentiating the toxic effects of one dioxin compound from another dioxin compound. Also, the toxic effects of soils contaminated with dioxins and dioxin-like compounds were tested using different phases of soil to determine the appropriate way of assessing the toxic effects. Based on the TEFs determined by the WHO, three compounds with higher TEFs were used as target compounds in this study.

Materials and methods

Chemicals

The target compounds used in this study are 2,3,7,8-TCDD (TEF of 1), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD; TEF of 1), and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF; TEF of 0.3), and these chemicals were chosen based on the TEFs provided by the WHO [1]. The standard solutions of 2,3,7,8-TCDD (50 μg mL^{-1}, purity 98.7%), 1,2,3,7,8-PeCDD (50 μg mL^{-1}, purity 100.0%), and 2,3,4,7,8-PeCDF (5.0 μg mL^{-1}, purity 100.0%) prepared in toluene were purchased from Sigma-Aldrich. These chemicals dissolved in toluene were extracted and then dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, ACS reagent grade) to prepare the stock solutions for toxicity tests. The initial concentrations of the prepared stock solutions were 467 μg L^{-1}, 429 μg L^{-1}, and 50 μg L^{-1} for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF, respectively.

Contaminated soil sample preparation

A soil sample without any known contamination history with dioxins and dioxin-like compounds were collected and sieved through a 2 mm sieve. The soil sample texture was loam with 41.7% sand, 45.7% silt, and 12.6% clay. The background concentrations of Pb, Cu, As, Cr, Zn, and Ni, measured by using inductively coupled plasma-optical emission spectroscopy (ICP-OES), were 17, 10, 5.9, 25, 57, and 15 mg kg^{-1}, respectively. The dioxin-contaminated soil samples were prepared by spiking 1 mL of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, or 2,3,4,7,8-PeCDF standard solution to 10 g soil. The initial concentrations of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, or 2,3,4,7,8-PeCDF in the contaminated soil samples were 5, 5, and 0.5 μg g^{-1}, respectively. The soil samples without spiking dioxin-like compounds were used as controls.

Toxicity tests using *Allivibrio fischeri*

Toxicity tests were carried out using bioluminescence bacteria *A. fischeri* according to the ISO 11348-3:2007 method. The changes in the bioluminescence before and after exposure to dioxins were measured using the Microtox M500 (Modern Water INC, Delaware, USA). The acute toxicity was determined by following the 81.9% basic test method for the liquid samples [10, 11], and the Basic Solid-Phase Test with nine serial dilutions of the test solution for the soil samples [12]. With contaminated soil samples, the toxicity tests were carried out in three phases of water extract, solid-phase, and organic extract to assess the bioavailability and potential mobility of contaminants in soil [13]. The water extracts were used to test the mobile fraction of the contaminants, while the soil slurry were used to test the bioavailable fraction of the contaminants [13]. The organic extracts are used to represent the total organic toxicity of the contaminated soil [13]; however, in this study, the toxicity obtained using the dioxin standards was used to represent the total organic toxicity of dioxins in contaminated soils.

The water extracts were prepared by mixing the contaminated soil and deionized water at 1:5 (w/v) solid-to-liquid ratio for 24 h followed by centrifugation to sample the supernatant. The solid-phase samples were prepared by making soil slurry at 1:5 (w/v) solid-to-liquid ratio. Each of the contaminated soil samples was mixed with deionized water to make the solid-phase samples. For toxicity tests, *A. fischeri* were exposed to the dioxin samples for 5, 15, and 30 min, and three replicates were used for each condition. The half maximal effective concentration (EC50) for each condition was determined using the Microtox Omni software.

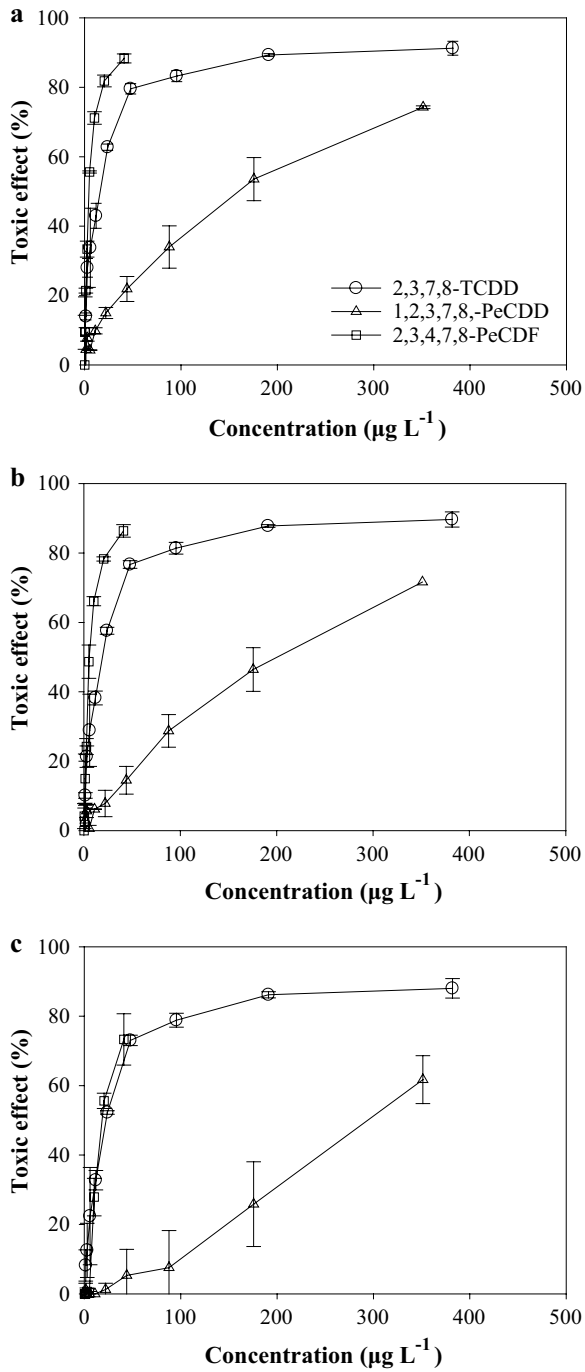

Fig. 1 Toxic effects of 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD), and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) on *Allivibrio fischeri* after **a** 5 min exposure, **b** 15 min exposure, and **c** 30 min exposure

Results and discussion

Toxic effects of dioxins on *A. fischeri*

Figure 1 shows the toxic effects of three dioxin-like compounds on *A. fischeri* with respect to the dioxin concentration. With increasing concentration, the toxic effects increased for all the three dioxin-like compounds studied (Fig. 1). After 5 min and 10 min exposures, the toxic effects of 2,3,4,7,8-PeCDF were greater than the other two compounds (Fig. 1a, b). But after 30 min exposure, the toxic effects of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF were similar (Fig. 1c). For all three exposure times, the toxic effects of 1,2,3,7,8-PeCDD were the lowest (Fig. 1). The EC50 values also increased with increasing exposure time from 5 min to 30 min, suggesting that the toxic effects are decreasing with increasing exposure time (Fig. 2). After 5 min exposure, the EC50 values for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF were 13, 140, and 4.4 μg L^{-1}. Among three dioxin-like compounds, 2,3,4,7,8-PeCDF had the lowest EC50 values, which suggests that it has the greatest toxic effects (Fig. 2). According to the human and mammalian TEFs recommended by the WHO, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD have the TEFs of 1, which means the toxic effects of 1,2,3,7,8-PeCDD are similar to that of

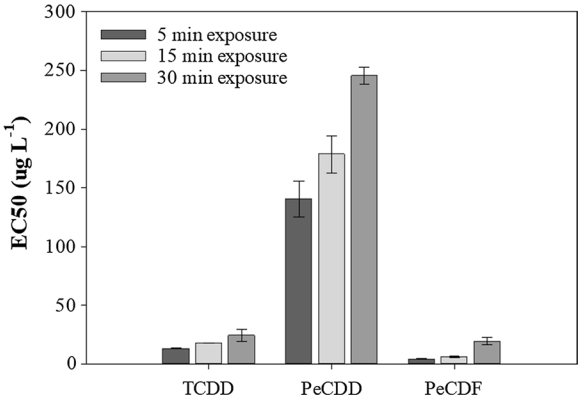

Fig. 2 Half maximal effective concentration (EC50) of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) determined using *Allivibrio fischeri* after different exposure times

2,3,7,8-TCDD when expressed in terms of 2,3,7,8-TCDD. On the other hand, 2,3,4,7,8-PeCDF has the TEF of 0.3, which suggests that 2,3,4,7,8-PeCDF has lower toxic effects than 2,3,7,8-TCDD. Overall, we observed greater toxic effects on *A. fischeri* with 2,3,4,7,8-PeCDF than with 2,3,7,8-TCDD or 1,2,3,7,8-PeCDD. Furthermore, the TEF for PCB 126 (i.e., 0.1) is even lower than the three dioxin-like compounds used in this study, and the TEFs of the other PCB congeners are lower by several orders of magnitudes. Previous study determined the EC50 values of 12 polychlorinated biphenyls (PCBs) congeners including PCB 126 using *V. fischeri* to be 1.4 ± 0.4 μg L^{-1} [8]. The 12 PCB congeners (i.e., EC50 of 1.4 ± 0.4 μg L^{-1}) were more toxic than the three dioxin-like compounds tested in this study (i.e., EC50 of 5.5–140 μg L^{-1}), although the TEFs of PCBs are lower than the three compounds tested in this study. Another study showed that the TEFs recommended by the WHO and the TEFs determined using the recombinant cell line (H4IIE-luc) were different [14]. For example, the TEF for 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin determined using the H4IIE-luc cell line system was higher than the TEF recommended by the WHO, and some other congeners had lower TEFs than the WHO recommended TEFs [14]. Such differences were attributed to the differences in in vivo and in vitro test conditions that might have different metabolic capacity, bioavailability, or exposure time [14]. Overall, the results show that *A. fischeri* can potentially be used as a test species for evaluating toxic effects of dioxin-like compounds in the environment.

Toxic effects of soil contaminated with dioxin-like compounds

The toxic effects of the soil samples contaminated with dioxin-like compounds were determined in three phases. The toxic effects of the organic extracts represent the total organic toxicity of the contaminant soil samples, so the toxic effects using the standards shown in Fig. 1 can

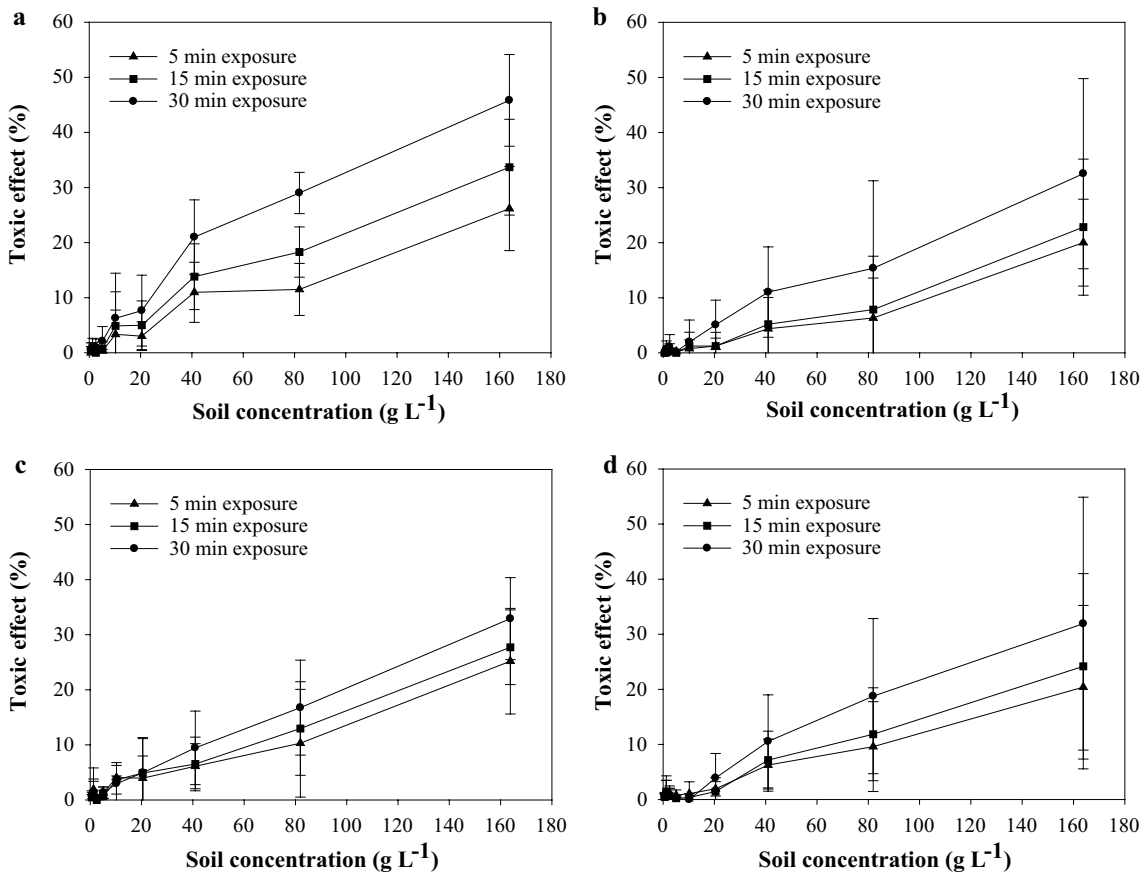

Fig. 3 Toxic effects of water extracts obtained from soil samples contaminated with dioxin-like compounds on *Allivibrio fischeri*. **a** Control soil (i.e., not contaminated with dioxin-like compounds), **b** 2,3,7,8-tetrachlorodibenzodioxin (TCDD)-contaminated soil, **c** 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD)-contaminated soil and **d** 2,3,4,7,8-pentachlorodibenzofuran (PeCDF)-contaminated soil. The data were obtained from four replicate samples

be used to represent the toxicity of the organic extracts. The toxic effects of the water extracts and the soil slurries were shown with respect to the soil concentration (i.e., soil concentration in water that was used to obtain the water extracts or soil slurries) rather than the dioxin concentration.

The toxic effects of the water extracts (i.e., mobile fraction of dioxins) from the contaminated soil samples were similar to that of the control soil samples (p value > 0.05) (Fig. 3). This suggests the low potential mobility of dioxins in the contaminated soil samples. The maximum toxic effects observed in this study ranged between 32–46% based on the average toxic effects regardless of the presence of dioxin-like compounds (Fig. 3). Thus, the estimated EC50 values were either > 100% or could not be determined in the studied conditions (Fig. 3). In particular, the soil 2,3,4,7,8-PeCDF concentration was 10 times lower than that of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, but the toxic effects were similar. This suggests that the toxic effects observed in the control and contaminated soils are due to other water-soluble chemicals such as heavy metals [15]. The toxic effects of soil water extracts can also be related to soil properties such as pH, organic carbon contents, and iron oxides that can affect the soil soluble fraction of potentially toxic elements in soils [15–17]. Dioxins are hydrophobic, and the solubility of dioxins in water is considered to be negligible; thus, they tend to adsorb on soil particles rather than staying dissolved in water. Therefore, it is unlikely that the water extracts obtained from the soil samples contaminated with dioxin-like compounds contain dioxins that may impose noticeable toxic effects. The average toxic effects seem to be increasing with increasing exposure time; however, they were statistically similar (Fig. 3). Overall, the results show that the toxic effects of the soil samples contaminated with dioxin-like compounds due to the mobile fraction of dioxins in soils may be negligible.

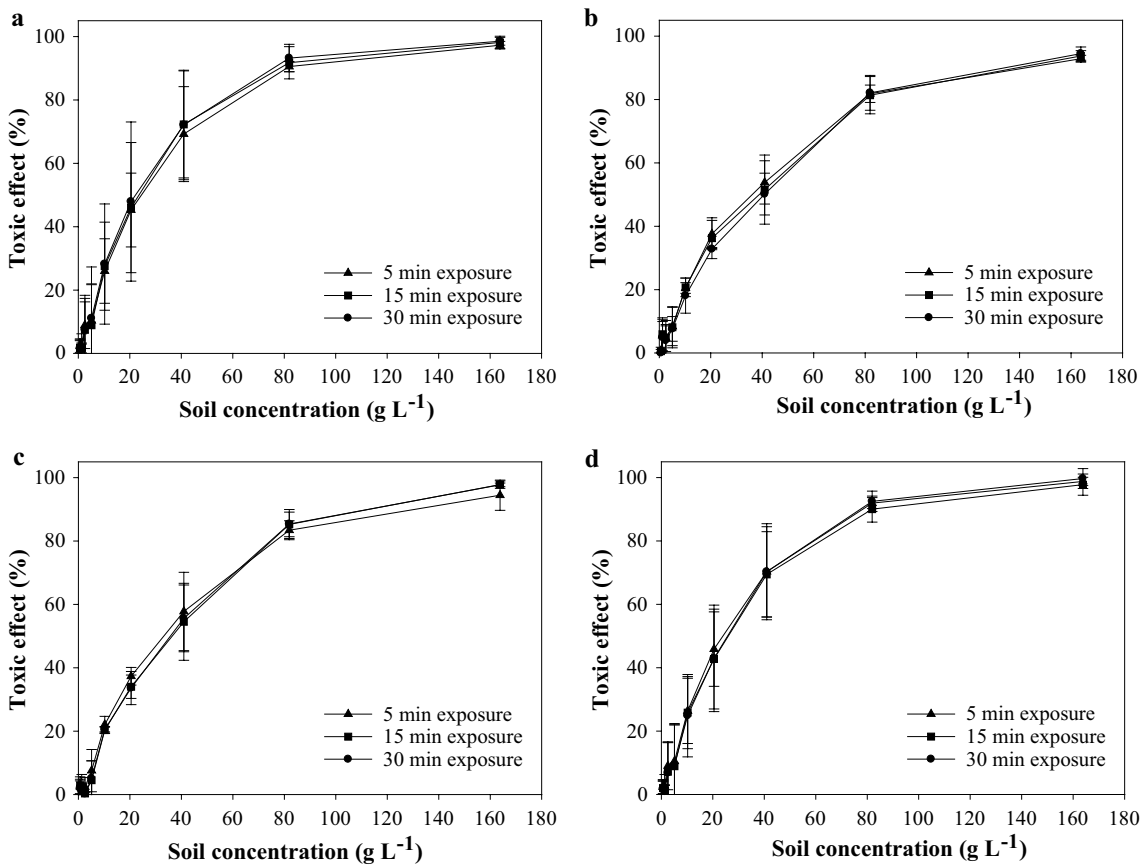

Fig. 4 Toxic effects of soil slurries obtained from soil samples contaminated with dioxin-like compounds on *Allivibrio fischeri*. **a** Control soil (i.e., not contaminated with dioxin-like compounds), **b** 2,3,7,8-tetrachlorodibenzodioxin (TCDD)-contaminated soil, **c** 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD)-contaminated soil, and **d** 2,3,4,7,8-pentachlorodibenzofuran (PeCDF)-contaminated soil. The data were obtained from four replicate samples

Figure 4 shows the toxic effects of the soil slurry made from the contaminated soil samples. The toxic effects increased with increasing soil concentration, and the toxic effects were similar regardless of the exposure times (p-value > 0.05) (Fig. 4). Also, the toxic effects of the contaminated soil samples were similar to that of the control soil samples. Considering the lower soil 2,3,4,7,8-PeCDF concentration than that of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, the toxic effects of the 2,3,4,7,8-PeCDF-contaminated soil can be considered to be greater than the other contaminated soils. Similar to the water extracts, there was no significant difference between the toxic effects of the contaminated soil samples and the control soil samples (Fig. 4). However, the toxic effects of the soil slurries were greater than the water extracts. This can be attributed to the increased bioavailable fractions of contaminants in the soil samples due to direct contact between the bacteria and the soil sample [12]. In other words, the toxic effects of the bioavailable fraction were greater than the toxic effects of the mobile fraction of the dioxins in the soil samples (i.e., the water extracts) [13]. Also, the luminescence of the bacteria can be affected by the color and turbidity of the samples as well [12].

Previous study showed that the toxic effects due to the bioavailable and mobile fractions of various organic contaminants in different soils ranged 7.20–100% and 0.0–10%, respectively, of the organic extract toxicity [13]. This shows that the soil slurry toxicity can be affected by the soil properties [13]. Thus, the toxic effects of dioxins and dioxin-like compounds in different soils need to be determined, and Microtox® can be a rapid way of assessing the toxicity.

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Authors' contributions
All authors designed the experiments together, and SJ and HJH conducted the experiments. SJ and EHJ analyzed and interpreted the results, and prepared the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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References

1. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M (2006) The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93:223–241
2. Coady KK, Biever RC, Denslow ND, Gross M, Guiney PD, Holbech H, Karouna-Renier NK, Katsiadaki I, Krueger H, Levine SL, Maack G (2017) Current limitations and recommendations to improve testing for the environmental assessment of endocrine active substances. Integr Environ Asses Manag 13:302–316
3. Borgert CJ, Mihaich EM, Quill TF, Marty MS, Levine SL, Becker RA (2011) Evaluation of EPA's tier 1 endocrine screening battery and recommendations for improving the interpretation of screening results. Regul Toxicol Pharmacol 59:397–411
4. Roig N, Sierra J, Rovira J, Schuhmacher M, Domingo JL, Nadal M (2013) In vitro tests to assess toxic effects of airborne PM10 samples. Correlation with metals and chlorinated dioxins and furans. Sci Total Environ 443:791–797
5. Abbas M, Adil M, Ehtisham-ul-Haque S, Munir B, Yameen M, Ghaffar A, Shar GA, Tahir MA, Iqbal M (2018) *Vibrio fischeri* bioluminescence inhibition assay for ecotoxicity assessment: a review. Sci Total Environ 626:1295–1309
6. Min J, Pham CH, Gu MB (2003) Specific responses of bacterial cells to dioxins. Environ Toxicol Chem 22:233–238
7. Lyu H, He Y, Tang J, Hecker M, Liu Q, Jones PD, Codling G, Giesy JP (2016) Effect of pyrolysis temperature on potential toxicity of biochar if applied to the environment. Environ Pollut 218:1–7
8. Salizzato M, Bertato V, Pavoni B, Ghirardini AV, Ghetti PF (1998) Sensitivity limits and EC50 values of the *Vibrio fischeri* test for organic micropollutants in natural and spiked extracts from sediments. Environ Toxicol Chem 17:655–661
9. Lewandowski K, Witt M, Kobusińska M, Niemirycz E (2014) Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and pentachlorophenol (PCP) in bottom sediments of the Port of Gdansk. Oceanol Hydrobiol St 43:312–323
10. Hwang SK, Jho EH (2018) Heavy metal and sulfate removal from sulfate-rich synthetic mine drainages using sulfate reducing bacteria. Sci Total Environ 635:1308–1316
11. Lee SY, Kang HJ, Kwon JH (2013) Toxicity cutoff of aromatic hydrocarbons for luminescence inhibition of *Vibrio fischeri*. Ecotox Environ Safe 94:116–122
12. Campisi T, Abbondanzi F, Casado-Martinez C, DelValls TA, Guerra R, Iacondini A (2005) Effect of sediment turbidity and color on light output measurement for Microtox® basic solid-phase test. Chemosphere 60:9–15
13. Prokop Z, Nečasová A, Klánová J, Čupr P (2016) Bio availability and mobility of organic contaminants in soil: new three-step ecotoxicological evaluation. Environ Sci Pollut R 23:4312–4319
14. Lee KT, Kim SK, Kim HJ, Kim YR, Jeong KH, Khim JS, Giesy JP, Lee JS (2010) Relative potency estimation of dioxin-like activity by dioxins, furans, and co-planar PCBs using chemically activated luciferase gene expression assay (H4IIE-luc Assay). Toxicol Environ Health Sci 2:12–17
15. Rocha L, Rodrigues SM, Lopes I, Soares AMVM, Duarte AC, Pereira E (2011) The water-soluble fraction of potentially toxic elements in contaminated soils: relationships between ecotoxicity, solubility and geochemical reactivity. Chemosphere 84:1495–1505
16. Yang K, Jeong S, Jho EH, Nam K (2016) Effect of biogeochemical interactions on bio accessibility of arsenic in soils of a former smelter site in Republic of Korea. Environ Geochem Hlth 38:1347–1354
17. Jho EH, Youn Y, Yun SH (2018) Effect of CO_2 exposure on the mobility of heavy metals in submerged soils. Appl Biol Chem 61:617–623

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