



## Searching for novel modes of toxic actions of oil spill using *E. coli* live cell array reporter system – A Hebei Spirit oil spill study



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### HIGHLIGHTS

- LCA is a promising tool for assessment of complex environmental mixtures.
- Oil pollution can modulate several toxic response pathways, such as DNA damage and antibiotic responses in LCA.
- Hebei Spirit oil spill incident (Yellow Sea, 2007) still influences some locations along the western coastline of Korea.

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### ABSTRACT

Oil is a complex mixture of numerous compounds. Therefore, oil spills near shore can cause various adverse effects on coastal ecosystems. However, most toxicological assessments conducted on oil spill sites have focused on limited modes of toxic actions. In the present study, we utilized the *Escherichia coli* (*E. coli*) live cell array system (LCA) to identify novel modes of toxicities of the oil spill-affected sediments. For this purpose, sediment samples were collected from an area heavily polluted by Hebei Spirit oil spill (HSOS) incident of 2007. A total of 93 *E. coli* reporter genes were used to study responses to the chemicals in the mixture. *E. coli* K12 strains were exposed to extracts of oil or the sediment, and changes in gene expression were measured. Exposure to extracts of crude and weathered oil resulted in decreased expression in ~30% of tested genes. However, changes in expression observed after exposure to sediment extracts varied. Sediment extracts containing large concentrations of polycyclic aromatic hydrocarbons (PAH) caused down-regulation of >70% of the genes, while extracts containing lesser total concentrations of PAHs exhibited different trends: genes involved in drug resistance were generally up-regulated, while genes responsive to DNA damage were up-regulated in only two extracts. Results suggest that oil pollution can modulate several toxic response pathways related to DNA repair and antibiotic responses. Results from LCA obtained from the sediment and oil samples were different from those observed in the H4IIE-*luc* assay. Toxicological implications of such observations deserve further examination. Overall, LCA is a promising tool for screening samples and identifying potential modes of toxicities of environmental samples associated with oil spills.

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### 1. Introduction

Contamination of coastal ecosystems by petroleum products has been a long-standing problem worldwide (Burns and Smith, 1982; Volkman et al., 1992; Commendatore and Esteves, 2007; Boitsov

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et al., 2013). Oil spill accidents are significant contributors to contamination of the coastal environment (Walker et al., 2005). Because petroleum products are inherently complex mixtures of numerous hydrocarbons (Wang et al., 2003) modes of toxicity associated with petroleum derived components are also diverse and complicated.

The *Hebei Spirit* oil spill (HSOS) incident occurred in the Yellow Sea, about 10 km off the coast of Taean, South Korea, on 7 December 2007. The HSOS was the most serious oil spill episode in Korean history. Approximately 10,900 tons of crude oil was spilled into the sea over two days, affecting >375 km of coastal shoreline. After rigorous cleanup efforts, roughly 20% of the spilled oil was estimated to be removed within one year (Yim et al., 2012). Although the impacted area seems to have visually recovered from the accident, ongoing monitoring of the area showed that, depending on location, certain parts of this area are still being affected by residual petroleum hydrocarbons (Ji et al., 2011; Hong et al., 2012; Jung et al., 2012; Yim et al., 2012; Kim et al., 2013; Hong et al., 2014; KIOST, 2015). Indeed, oil has been detected sporadically along the most heavily impacted coastline (Hong et al., 2014; KIOST, 2015).

Oil spills can cause profound effects on ecosystems that can persist for a long time, often more than 25 years (Kingston, 2002). Thus, a long-term follow-up investigation to understand the status of the contamination and potential toxicological consequences of the spill is warranted. For HSOS, most assessments of toxicity have been focused on several specific modes of toxicities such as the pathway mediated by the aryl hydrocarbon receptor (AhR) and to a lesser extent, endocrine disruption and DNA damage (Ji et al., 2011; Liu et al., 2012; Hong et al., 2015; Jeong et al., 2015; Kim et al., 2016). Considering the complex composition of spilled petroleum hydrocarbons and structural changes during weathering, possible toxic modes of action associated with oil spills are expected to be diverse.

The *E. coli* live cell reporter array (LCA) system is a promising tool for identifying toxic modes of action of chemicals through use of a multi-targeted approach. The assay utilizes *E. coli* K12 MG1655 strains that are genetically engineered to respond to specific signals, for example, temperature changes or DNA damage (Zaslaver et al., 2006). Promoters that respond to external signals, or stressors, are fused to green fluorescence proteins (GFP) that act as reporters. The expression of GFP is a surrogate for the potential

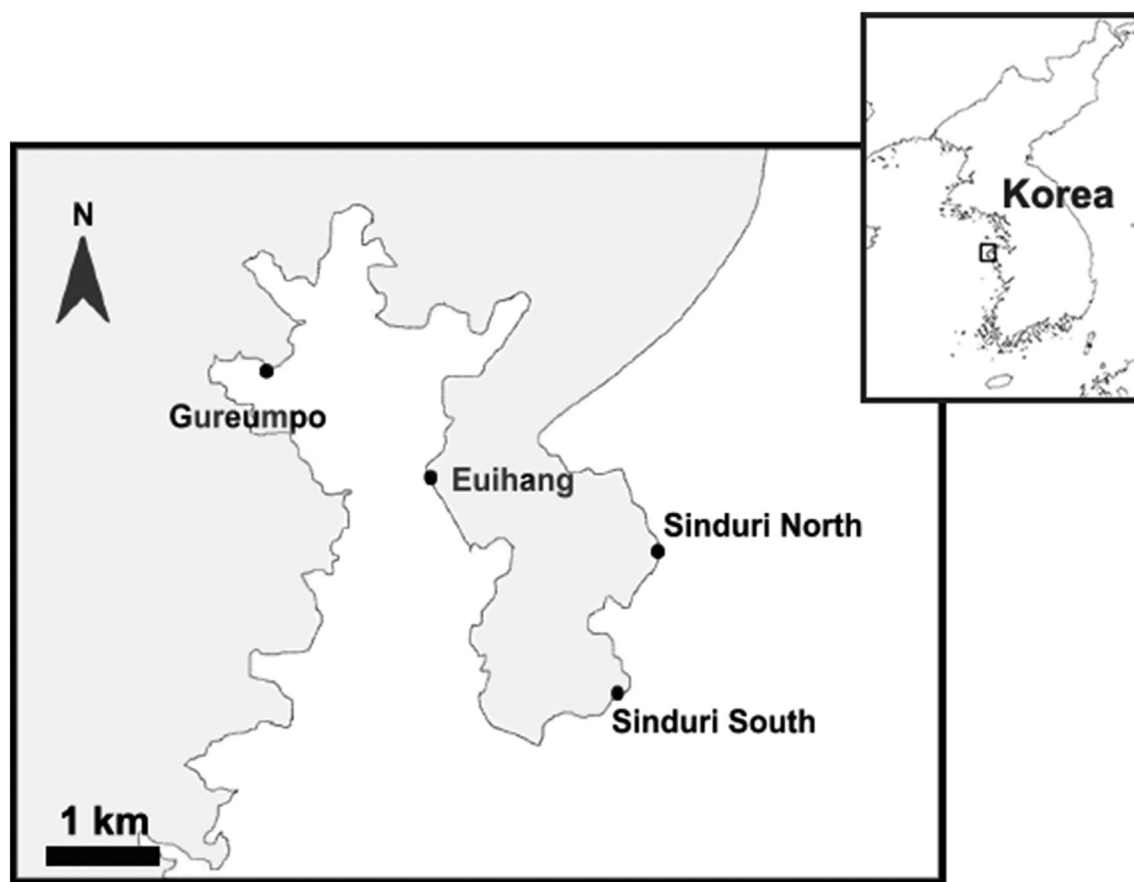


Fig. 1. Sampling locations along the Taean coastline of Korea.

Table 1  
Sampling location and characterization.

Location	Latitude	Longitude	Sediment type	Land usage	Sample ID
Gureumpo (GRP)	36°50'16.4"N	126°09'05.4"E	Sand	Rocky beach	GRP
Sinduri North (SN)	36°49'29.8"N	126°11'19.5"E	Mud	Adjacent to agricultural land	SN1, SN2
Sinduri South (SS)	36°48'49.1"N	126°11'04.2"E	Mud	Adjacent to agricultural land	SS1, SS2
Euihang (EH)	36°49'48.3"N	126°10'00.4"E	Mud	Adjacent to agricultural land and harbor	EH1, EH2

transcriptional changes associated with the prompters. This assay has been successfully applied to identifying potential toxicity pathways for chemicals such as triphenyltin chloride, brominated diphenyl ethers, novel flame retardants and naphthenic acid mixtures (Zhang et al., 2011; Su et al., 2012, 2013, 2014; Guan et al., 2016), and more recently for assessing contamination of surface waters (Hug et al., 2015). As a screening tool, LCA is less labor intensive, less time consuming, less costly, and can be effectively used to obtain information regarding possible modes of toxicity of a given stressor in real time compared to other methods such as microarrays and real time quantitative PCR (Zhang et al., 2011; Hug et al., 2015). Once identified, more quantitative measures, e.g., gene transcriptional changes and protein synthesis, can be applied to confirm the proposed mechanisms. In addition, LCA can be useful for clustering of chemicals or samples with similar mechanism of toxic action (Guan et al., 2016).

In the present study, LCA was used to evaluate extracts of sediments, collected from various locations within the area affected by the HSOS, as well as crude and artificially weathered oil to investigate possible effects of the oil spill at the genome level of organization. In addition, AhR and estrogen receptor (ER) transactivation assays, which aim at detecting specific modes of action, i.e., AhR-dependent and endocrine disruption pathways, respectively, were used to characterize the same samples for comparison with the results obtained by *E. coli* multi-targeted LCA. Through this process, not only the utility of the LCA in screening toxicity of oil spilled sediment was evaluated, but also possible modes of toxic action that warrant more quantitative investigation in the future could be identified. The results of this study will provide information on the applicability of LCA on environmental samples such as sediments from oil spilled areas, and on potential unidentified toxic pathways of oil spill remnants.

## 2. Materials and method

### 2.1. Sample collection and extraction

In September 2013, six years after the incident, sediments were collected from four locations along the Taean coastline that was affected by the HSOS, i.e., Gureumpo (GRP), Sinduri North (SN), Sinduri South (SS), and Euihang Harbor (EH) (Fig. 1, Table 1). Collection of sediments was biased toward those with visible signs of oil. At SN, SS, and EH, two separate samples were collected from each site, ~10 m apart. Collected sediments were transported to the laboratory and stored at  $-20^{\circ}\text{C}$  until preparation and analysis. Aliquots of 30 g of each sediment sample were extracted with 350 mL dichloromethane (Burdick and Jackson, Muskegon, MI, USA) on a Soxhlet extractor for 24 h, as described previously (Hong et al., 2015). After extraction, sediment extracts were concentrated to 5 mL, cleaned up by running through silica gel columns, and were used directly for chemical analysis. For biological analyses, the concentrated sample was evaporated and resuspended in equal volume of dimethyl sulfoxide (DMSO). Chemical and biological analyses were conducted for both crude and weathered oil (WO). Iranian Heavy crude oil (IHC), which was the major oil that was spilled, was artificially weathered by evaporation simulation technique (Fieldhouse et al., 2010).

### 2.2. Chemical analysis

Concentrations of individual PAH were measured in sediments as well as weathered and un-weathered samples of crude oil, by use of gas chromatography-mass spectrometry (GC/MS, Agilent 7890 GC, 5975C MSD, Agilent Technologies) according to previously reported methods (Hong et al., 2012).

### 2.3. Live cell array assay

The *E. coli* LCA assay was performed following previously published protocols with some modifications (Zhang et al., 2011; Su et al., 2014). Among the previously described *E. coli* K12 reporter strains, 93 strains that measure expression of genes responsive to environmental stressors (Onnis-Hayden et al., 2009) were selected for the present study (For the list of the strains, see Table S3). Cells were maintained in a 96-well stock plate then inoculated into a fresh 96-well plate (Corning, NY, USA) containing 250  $\mu\text{L}$  LB-Lennox plus 25 mg/L kanamycin (Inalco, Milano, Italy). Additionally two wells of promoterless strains for deducting background value and a well of blank were also included. Cells were incubated for 3 h at  $37^{\circ}\text{C}$ , then transferred to a 384-well black optical bottom plate (NUNC, Rochester, NY, USA) containing 72  $\mu\text{L}$  medium. Cells were then dosed with 3.75  $\mu\text{L}$  of the following; (1) DMSO as solvent control, (2) undiluted sediment extracts, or (3) crude or weathered oil diluted to 1/64 in DMSO. Preliminary test was conducted to check for cytotoxicity of the samples using the alamar blue assay as described previously (Guan et al., 2016). No cytotoxicity was observed in any of the sample concentrations tested, i.e., undiluted environmental samples of 5%  $v_{\text{sample}}/v_{\text{total}}$  or 1/64 of the oil samples. GFP intensity of each well was monitored every 10 min for 4 h by a Synergy H4 hybrid microplate reader (excitation/emission: 485 nm/528 nm) (BioTek Instruments Inc., Winooski, VT, USA). Three replicates were measured for the experiment. Classification and visualization of the gene expression were derived by the use of ToxClust, an R-based analytical method developed for clustering

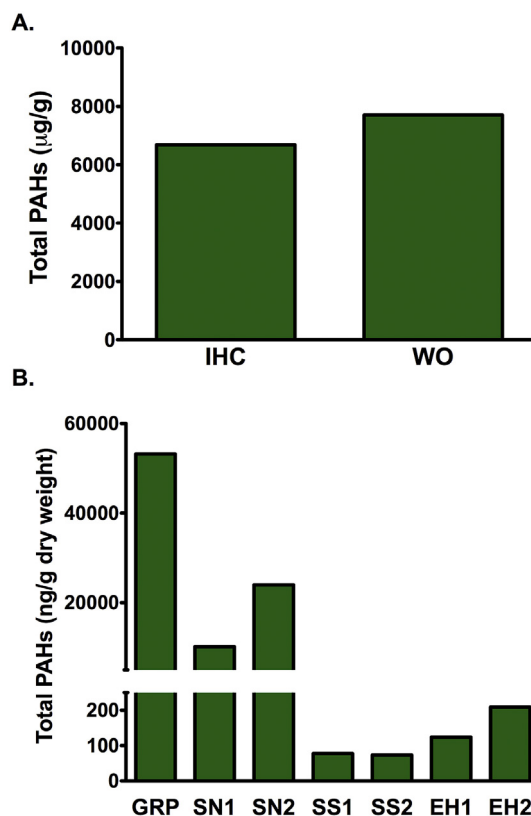
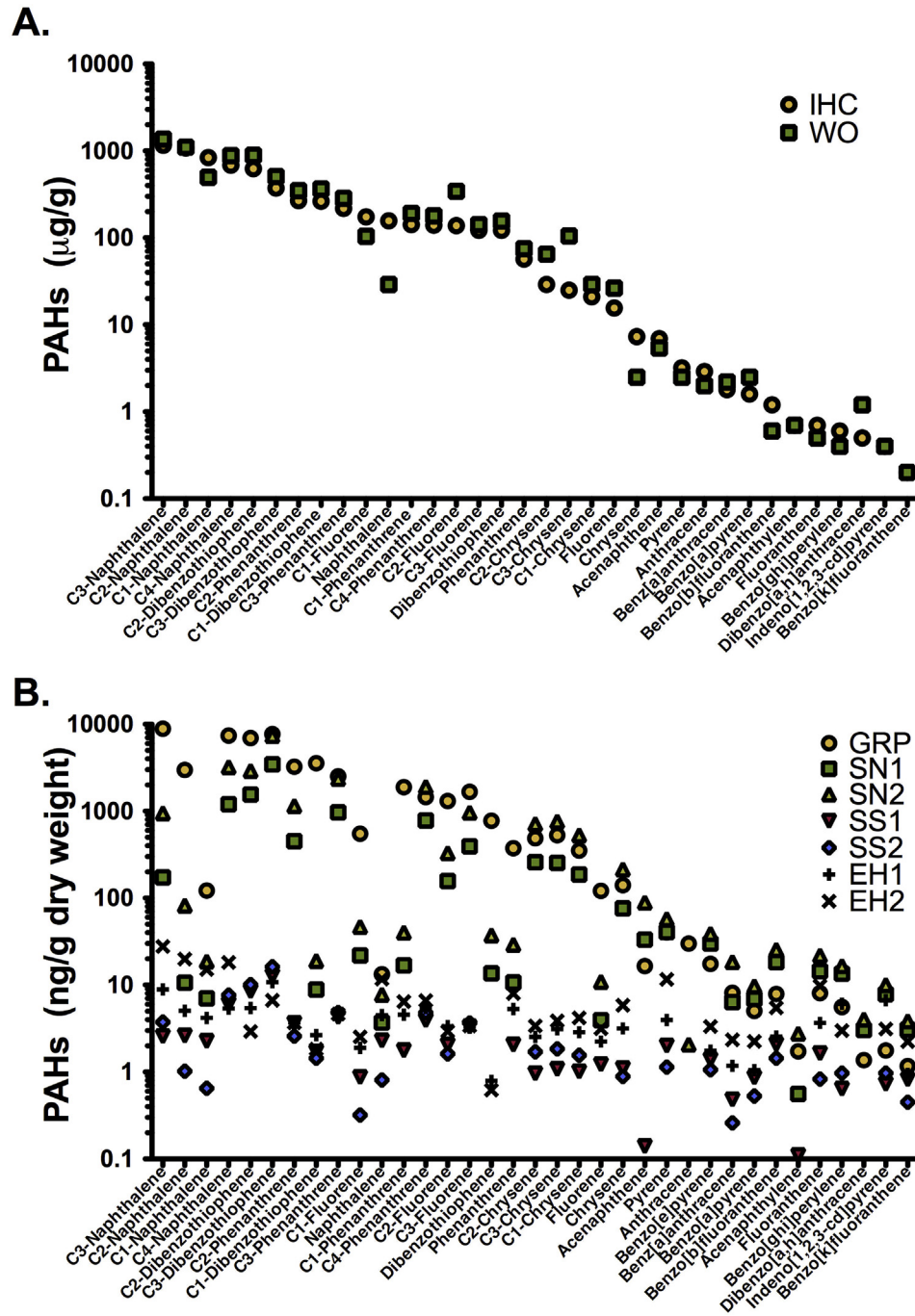


Fig. 2. Total PAHs measured in oil and the sediment extracts. (A) Artificial weathering of crude oil (IHC) resulted in slight increases in concentrations of PAHs measured due to about 28.8% loss of mass in weathered oil (WO). (B) Sediments from GRP and SN had greater concentrations of PAHs, whereas sediments collected from SS and EH sites contained lesser total concentrations of PAHs.



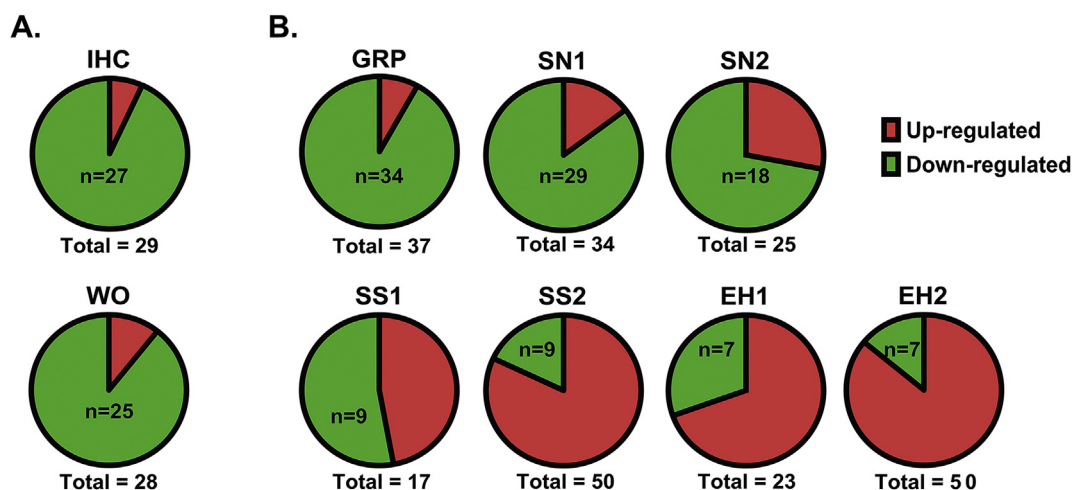
**Fig. 3.** Patterns of relative concentrations of PAHs in oil and extracts of sediments. (A) Crude (IHC) and weathered (WO) oil exhibited similar patterns of PAHs. Chemicals are listed in the order of concentrations of PAHs from greatest to least in IHC (left to right). (B) Profile of relative concentrations of PAH in extracts of sediments from sites with greater total concentrations of PAHs (GRP and SN) resembles the PAH composition of samples of crude oil. In contrast, samples with lesser total concentrations of PAH (SS and EH) exhibited little variation in concentrations of individual PAHs.

chemicals or environmental samples based on toxic responses (Zhang et al., 2009).

#### 2.4. *In vitro* bioassays for AhR and ER mediated toxicity

*In vitro* bioassays were conducted with H4IIE-*luc* cells and MVLN cells following previously published protocols (Hong et al., 2012; Liu et al., 2012). H4IIE-*luc* cells are a rat hepatoma cell line that has been transfected with drug response elements (DRE) and firefly luciferase reporter (Sanderson et al., 1996). MVLN cells are a human

breast cancer cell line that has been transfected with estrogen response elements (ERE) and firefly luciferase (Pons et al., 1990). Briefly, cells were seeded either at  $8 \times 10^4$  cells/mL (H4IIE-*luc*) or at  $1.25 \times 10^5$  cells/mL (MVLN) in 96 well plates, and incubated at 37.5 °C for 24 h. Following incubation, cells were dosed with 1 µL of either DMSO (solvent control) or undiluted sediment extract, or 1 µL of standards (TCDD at 30, 10, 3.3, 1.1, 0.33, and 0.11 pM; 17β-estradiol (E2) at 370, 123, 41, 13.7, 4.6, and 1.5 pM). A preliminary test with WST–1 cell proliferation assay confirmed that no cytotoxicity occurred at the doses tested. Cells were incubated at 37.5 °C



**Fig. 4. Identification of differentially expressed genes among 93 different strains of *E. coli* in the live cell array.** (A) Exposure to 1/64 dilution of IHC or WO resulted in a general decrease in expression of genes responsive to environmental stressors, compared to controls. (B) *E. coli* strains exposed to extracts of sediments exhibited variable responses. Sediments with greater concentrations of PAHs caused lesser expression of genes, whereas more genes were up-regulated in *E. coli* exposed to extracts of sediments with lesser concentrations of PAHs. Numbers below each pie chart represent total number of strains with more than two-fold changes in expression compared to controls. Numbers within each pie chart represents the number of genes down-regulated by exposure to oils or sediments.

for 72 h after dosing. At the end of the designated time, luminescence was measured with a microplate reader (Tecan, Infinite 200, Mannedorf, Switzerland). TCDD equivalents (TCDD-EQ) or E2-EQ were calculated from the dose-response relationships of samples tested (Villeneuve et al., 2000).

### 2.5. Statistical analyses

For the LCA experiment, linear regression analysis was used to identify genes with significant expression changes. Each promoter reporter strain's GFP response was fitted to a function of time with  $p < 0.001$ . For analyses of H4IIIE-*luc* and MVLN *in vitro* bioassays, normality was confirmed by the use of Kolmogorov-Smirnov test. Homogeneity of variances was confirmed by Levene's test. Then, analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was used. Linear regression was performed using R software (R Development Core Team, 2008), and ANOVA was performed on Prism (version 5.0a, GraphPad Software Inc., La Jolla, CA).

## 3. Results

### 3.1. PAH concentrations in the oil and sediment extract samples

Concentrations of 34 parent and alkyl-PAHs (total PAHs) in IHC and WO were comparable (Fig. 2A, Table S1), with the total amount of PAHs per gram slightly greater in WO. This can be explained by ~28.8% mass loss of the oil following artificial weathering. Most of the loss of mass during artificial weathering was due to evaporation of the liquid portion as well as more volatile components of oil. Total concentrations of PAHs in sediments varied, ranging from very small (SS and EH) to relatively great (GRP and SN) (Fig. 2B, Table S2). Total concentrations of PAHs in sediments were greater than average total concentrations of PAHs measured in sediments along the western coast of Korea (mean  $48.0 \pm 43.0$  ng/g dry weight, range 4.18–169 ng/g dry weight) (KIOST, 2015).

Compositions of PAHs in IHC and WO were also similar, except for a greater percentage of alkylated and heavier compounds from WO (Fig. 3A, Table S1). Composition of PAHs in sediments from GRP, SN1, and SN2 were similar to those of IHC and WO, albeit at less concentrations (Fig. 3B, Table S2). In contrast, the samples from SS1,

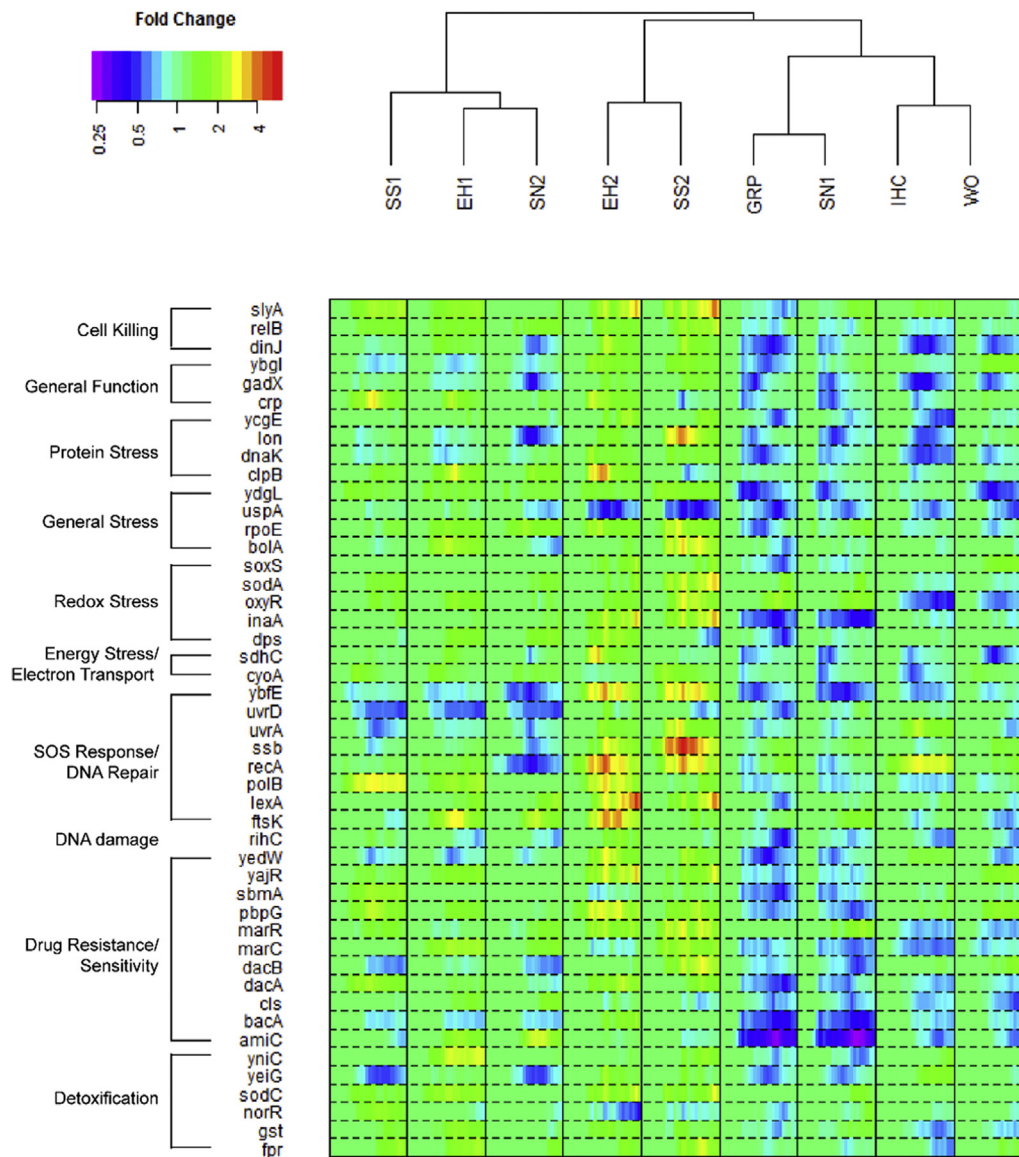
SS2, EH1 and EH2 contained small amounts of PAHs, with the majority less than 10 ng/g dry mass.

### 3.2. Profiles of expression of genes

With a few exceptions, exposure to extracts of IHC or WO resulted in overall lesser expression of target genes (Fig. 4A). Following exposure to IHC, only genes involved in DNA repair (*uvrA*, *recA*) were slightly up-regulated, whereas in cells exposed to WO, no apparent up-regulation was observed (Fig. 5). Exposure to extracts of sediments resulted in variable but sample-dependent responses (Fig. 4B). Samples with greater concentrations of PAHs (GRP, SN1, and SN2) generally exhibited down-regulation of the tested genes. Other sediments with lesser concentrations of PAHs generally caused up-regulation of the tested genes, with an exception of SS1. Exposure to SS1 and EH1 resulted in the fewest changes in expression of genes, whereas exposure to SS2 and EH2 resulted in the most changes. Genes that exhibited at least two-fold changes after exposure to extracts of sediments, including full names and functions, are listed in Table S4.

Multi-responsive genes exhibiting maximal changes of >2 fold in at least three of the seven sediments were further classified according to their biological pathways (Onnis-Hayden et al., 2009). Genes involved in xenobiotic responses (detoxification, and drug resistance/sensitivity) or repair of DNA damage were most affected (Fig. 6). Genes responding to redox and energy stress were also influenced, but directions of changes varied among samples (Fig. 5). Samples of oil, SN2, SS1, and EH1 caused few changes in these genes, whereas SS2 and EH2 samples caused changes in more genes. SN2, SS1, and EH1 showed similar changes in expression of each pathway. Alternatively, SS2 and EH2 induced expression changes in more redox and energy stress genes compared to other groups.

Time-course changes in expression of several selected genes (Fig. 5) show that GRP and SN1 exhibited similar trends, and were associated closely with those observed from both IHC and WO. For example, many of the down-regulated genes in GRP and SN1 samples were the same genes that were significantly down-regulated by exposure to IHC and WO (*dinJ*, *gadX*, and *bacA*). Exposure to extracts of both GRP and SN1 resulted in maximal effects on *inaA*, *bacA*, and *amiC*. In contrast, exposure to extracts of



**Fig. 5. Expression of 47 multi-responsive genes in *E. coli* during 4 h exposure to oil or extracts of sediments.** Fold changes in gene expression are expressed by the color gradient as shown on top left. Time course of the expression changes over 4 h period is indicated from left to right. The phylogeny at the top indicates relative similarity of the sediments and oil. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

EH2 and SS2 generally resulted in upregulation of the tested genes. Following exposure to these two sediment extracts, genes associated with SOS response and repair of DNA (eg. *ybfE*, *ssb*, *recA*, and *lexA*) were most up-regulated. Exposure to SN2, SS1 or EH1 resulted in down-regulation of several genes involved in SOS response and repair of DNA (eg. *ybfE*, *uvrD*, and *recA*). Similar patterns of changes were observed after exposure to extracts of GRP or SN1. Several genes, such as *polB*, *lexA*, *pgpG*, *marR*, *marC*, and *gst* were slightly up-regulated after exposure to extracts of SN2, SS1 or EH1.

### 3.3. In vitro bioassays for AhR- and ER-mediated toxicity

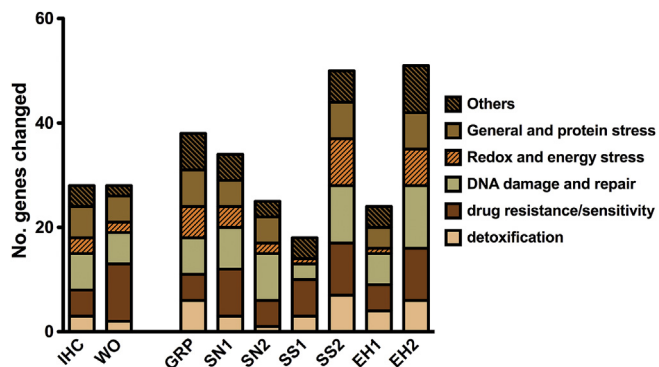
Potencies measured in the H4IIE-*luc* AhR transactivation assay were associated with the total PAH contents in the samples (Fig. 7). Extracts of sediments with greater total concentrations of PAHs exhibited greater responses, while those with lesser concentrations of PAHs caused lesser effects. Alternatively, results from the MVLN ER-binding affinity assay did not show such a clear association with

concentrations of PAHs in extracts. All samples induced similar extents of ER transactivation responses from the cells.

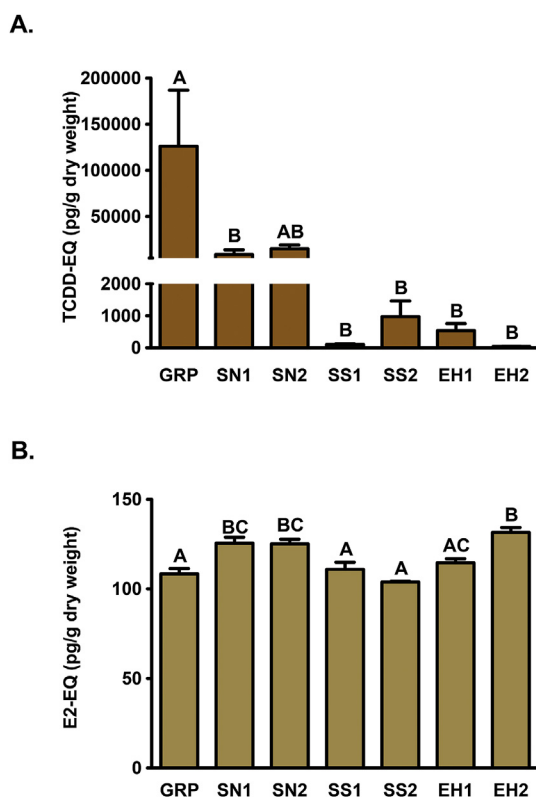
## 4. Discussion

Even six years after the HSOS accident, remnants of the oil spill are still present in some locations, such as GRP and SN, and can possibly cause adverse biological effects through several different modes of action. Contamination of the coastal environment by oil spill residues can persist (Burns et al., 1993; Su et al., 2013), and might have long-lasting effects on sediment ecosystems (Kingston, 2002). Therefore, monitoring long-term chemical (Figs. 2 and 3) and biological (Figs. 4–7) ramifications of oil spills is an important part of the management of coastal ecosystem post-environmental disasters such as the HSOS.

Reasons for differential profiles of relative concentrations of PAHs in extracts of sediments from locations SS and EH, compared to the oil samples and GRP and SN samples, could be due, in part, to



**Fig. 6. Classification of changed genes in the live cell array.** Only the genes that showed >2 fold up-regulation or down-regulation were classified. Pathway analysis of genes that changed in response to exposure to oil or sediment extracts demonstrated that genes mostly involved in xenobiotic responses (detoxification, drug resistance/sensitivity) or repair of damage to DNA were affected. In addition, genes responding to general and protein stress response were similarly affected. On the other hand, changes in expression of genes responding to redox and energy stress varied by sample.



**Fig. 7. In vitro bioassay of sediment samples.** (A) AhR activity, as shown by H4IIIE-*luc* assay closely resembles the total amount of PAHs present in the sediment samples. (B) Although activation of ER measured in MVLN cells show variation among samples, the levels are too low to result in any biological significance. Y-axis represents the standard equivalents (TCDD-EQ or E2-EQ) of the sediment samples.

anthropogenic activities along the coastline. The Taean coast of Korea is well populated and characterized by residential, farming, recreational, navigational, as well as industrial activities (Yim et al., 2007, 2012). Another possible reason would be changes in oil composition by the influence of weathering (Wolfe et al., 1994), as the weathering process may change the ratios of differentially alkylated PAHs (Hong et al., 2012). However, it is not clear whether the composition of PAHs can be the sole driver of expression

changes in the LCA. While EH1 and EH2 have similar ratios of differentially alkylated PAHs, patterns of expression of genes in the LCA were quite different. This observation suggests that factors other than weathering could also be responsible for the observed effects on expression of genes (Figs. 4–6).

LCA has been successfully employed to screen toxicities of chemicals (Onnis-Hayden et al., 2009; Gou et al., 2010; Zhang et al., 2011; Su et al., 2012, 2013; Guan et al., 2016) and surface waters (Hug et al., 2015). For the first time, we employed a subset of the LCA, i.e., “environmental stress genes,” to assess potential mechanisms of toxic action of extracts of sediments from oil contaminated coastline and complex mixtures of oil. Results of the LCA generally matched well with the chemistry results, i.e. total PAH (Fig. 2), and chemical composition of the sediment samples (Fig. S1), demonstrating the value of LCA as a suitable tool for screening oil contaminated sediments. Results of LCA varied among samples, but not among locations (Figs. 4–6), probably due to very heterogeneous composition of the sediment samples (Balba et al., 1998). For example, the profile of relative concentrations of PAHs in the extract of sediment from EH1 was similar to that of SS1, but not EH2. In addition, the profile of SN1 was more similar to GRP than SN2.

The pathway of response to DNA damage was one of the most affected pathways in this study. Damage to DNA is a well-recognized toxic endpoint for petroleum mixtures. Several studies examining damage to chromosomes in teleosts (Pilcher et al., 2014; Jeong et al., 2015) and genotoxicity in *in vitro* systems (Ji et al., 2011) have shown that repair of DNA is a crucial pathway that responds to oil-derived toxicities. In the present study, significant regulatory changes in the SOS response/DNA repair pathway were observed. Genes negatively regulated by *lexA* (*ybfE*, *uvrA*, *urvD*, *ssb*, and *recA*) were influenced, which indicated that the SOS response is the major pathway affected (Walker, 1984). Expressions of most responses were decreased, which implies shut-down of the cell's stress response system. EH2 and SS2 caused the opposite response of genes in the LCA compared to other sediment samples. For example, all samples except EH2 and SS2 caused up-regulation of *lexA* and down-regulation of genes negatively regulated by *lexA*. In contrast, EH2 and SS2 caused an initial increase in all *lexA*-regulated genes, with *lexA* induction lagging behind temporally. This is a classical profile of response to damage of DNA. Such responses were not correlated with total concentrations of PAHs in extracts of sediments. Specifically, sediments from locations SS1 and EH1, which had similar total concentrations of PAHs as did SS2 and EH2, did not elicit the same response. These results indicate that molecular level responses observed in LCA cannot be explained solely by total concentrations of PAHs in the sediment.

Genes that have been previously found to be antibiotic responsive and to contribute to antibiotic resistance, such as *pbpG*, *mrcR*, and *dacA* (Zhou and Rudd, 2013), were down-regulated by GRP and SN1, but up-regulated by other extracts of sediments. This observation shows that drug resistance genes are responsive to the samples with lesser concentrations of PAHs. However, at greater concentrations of PAHs, this pathway was shut down. In prokaryotes, these genes express proteins that respond to exogenous chemicals, such as antibiotics.

*UspA* was the only gene that was down-regulated by all treatments (Fig. 5). *uspA* is a universal stress protein that responds to DNA damage, oxidative stress, metal exposure, starvation, and other stresses (Siegele, 2005). Inactivation of *uspA* results in reduced growth during normal condition (Bochkareva et al., 2002). Similarly, *amiC*, which is involved in cell division (Heidrich et al., 2001), decreased the most in the GRP and SN1-treated groups, and showed slight decrease in SS1, SS2, and EH2-treated groups. Perhaps, chemical mixtures in extracts of sediments with greater

total concentrations of PAHs, as well as IHC and WO, forced *E. coli* to enter a dormant condition. This hypothesis would also explain the general decrease in most of the measured gene expressions in the samples with high total PAHs.

The results of the AhR binding affinity assay, a receptor-based, transactivation assay for dioxin like compounds correlated well with the presence of PAHs in the samples. This is not surprising since binding of ligands to the AhR is one of the major toxicity pathways of the aromatic PAHs (Wassenberg and Di Giulio, 2004). The ER-binding affinity assay, however, was not as effective in distinguishing estrogenic potencies of different samples. Recent studies reported that steroidogenesis or androgen receptor binding affinity were more sensitive endocrine pathways following exposure to PAHs (Monteiro et al., 2000; Frouin et al., 2007; Ji et al., 2011). Our observation with exposure of MVLN cells also showed that ER-mediated responses could not be explained by concentrations of PAHs in sediment or oil. It is likely that the weak ER-mediated responses observed here are from constituents other than PAHs.

The results reported here demonstrate the advantage of using *E. coli* LCA for environmental samples like sediment affected by oil contamination. Different samples could be sorted based on results of the LCA, and this clustering appears to be related to total concentrations of PAHs as well as the composition of different PAHs. There are a few caveats, however, that should be taken into account. First, LCA was not able to pinpoint the major stressor within each sample. Second, there is a possibility that responses observed in *E. coli* might be due to non-specific down-regulation simply due to high degree of toxicity of samples. Lastly, we only measured PAHs in our samples, and we do not know the contributions of non-PAH components of the samples on LCA, H4IIE-*luc*, or MVLN results. Nevertheless, LCA results reflect the actual biological responses that the cells may go through after exposure to different samples. LCA is useful for screening possible toxic mechanisms of action of given stressors; therefore this array can be used as an initial tier impact assessment tool, when understanding of toxic mechanisms is necessary.

Results of the H4IIE-*luc* assay were consistent with total concentrations of PAHs in samples (Figs. 2 and 7). However, in the LCA while very different expression patterns were observed in samples with greater total PAHs (GRP and SN1) compared to samples with lesser total concentrations of PAHs, the samples with similar total concentrations of PAHs (EH and SS), often showed different patterns of differential expression of genes in the LCA which suggest that the composition of PAHs cannot be the only factor that determined responses of the LCA to organic extracts of sediments. The H4IIE-*luc* assay is useful for general assessment of contamination that activates the AhR-mediated pathway, whereas LCA can provide more complex picture that can be applied to screen toxic samples with different modes of toxicity, and to identify possible modes of toxic actions in environmental samples.

In conclusion, the utility of the *E. coli* based LCA as a promising tool for assessing complex environmental mixtures, such as the sediments contaminated from the HSOS incident was demonstrated. Specifically, LCA shows a promise as an effective tool that screens toxic environmental samples, and describes their potential modes of toxicity. In addition, we found that some locations along the coastline are still under the influence of the HSOS, which suggests needs for long-term follow-up investigations along the Taean coastline.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.11.078>.

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## Searching for novel modes of toxic actions of oil spill using *E. coli* live cell array reporter system – A *Hebei Spirit* Oil Spill study

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Table S1. Chemical composition of crude and weathered oil ( $\mu\text{g/g}$  oil)

	Crude oil (IHC)	Weathered oil (WO)
Naphthalene	157.0	29.0
C1-Naphthalene	839.0	498.0
C2-Naphthalene	1083.0	1102.0
C3-Naphthalene	1160.0	1365.0
C4-Naphthalene	686.0	882.0
Acenaphthylene	0.7	0.7
Acenaphthene	6.9	5.4
Fluorene	15.6	26.4
C1-Fluorene	174.0	104.0
C2-Fluorene	138.0	343.0
C3-Fluorene	122.0	142.0
Dibenzothiophene	122.0	156.0
C1-Dibenzothiophene	264.0	365.0
C2-Dibenzothiophene	626.0	887.0
C3-Dibenzothiophene	374.0	508.0
Phenanthrene	57.0	75.0
C1-Phenanthrene	142.0	191.0
C2-Phenanthrene	268.0	348.0
C3-Phenanthrene	218.0	285.0
C4-Phenanthrene	140.0	179.0
Anthracene	2.9	2.0
Fluoranthene	0.7	0.5
Pyrene	3.2	2.5
Benz[a]anthracene	1.8	2.2
Chrysene	7.3	2.5
C1-Chrysene	21.0	29.0
C2-Chrysene	29.0	65.0
C3-Chrysene	25.0	105.0
Benzo[b]fluoranthene	1.2	0.6
Benzo[k]fluoranthene	0.2	0.2
Benzo[a]pyrene	1.6	2.5
Indeno[1,2,3-cd]pyrene	0.4	0.4
Dibenzo[a,h]anthracene	0.5	1.2
Benzo[g,h,i]perylene	0.6	0.4
Total PAHs	6688.6	7705.6

Table S2. Chemical composition of sediment extracts (ng/g dry weight)

	GRP <sup>1</sup>	SN1	SN2 <sup>1</sup>	SS1	SS2	EH1	EH2 <sup>1</sup>
Naphthalene	13.3	3.7	7.8	2.3	0.8	4.5	11.7
C1-Naphthalene	122.0	7.0	18.7	2.3	0.7	4.2	15.1
C2-Naphthalene	2980.0	10.6	82.3	2.6	1.0	5.1	19.9
C3-Naphthalene	8890.0	173.0	950.0	2.6	3.8	8.9	27.7
C4-Naphthalene	7410.0	1200.0	3230.0	6.0	7.6	5.4	18.2
Acenaphthylene	1.7	0.6	2.8	0.1	nd	nd	nd
Acenaphthene	16.5	33.3	89.1	0.1	nd	nd	nd
Fluorene	121.0	4.0	10.9	1.2	nd	2.2	3.2
C1-Fluorene	551.0	21.9	46.7	0.9	0.3	1.9	2.5
C2-Fluorene	1310.0	157.0	330.0	2.1	1.6	3.4	2.9
C3-Fluorene	1670.0	395.0	965.0	3.3	3.7	3.4	3.4
Dibenzothiophene	776.0	13.6	37.3	nd	nd	0.8	0.6
C1-Dibenzothiophene	3570.0	8.9	19.0	1.7	1.4	2.7	1.8
C2-Dibenzothiophene	6940.0	1560.0	2910.0	8.5	10.1	5.4	2.9
C3-Dibenzothiophene	7710.0	3450.0	7380.0	12.8	16.2	10.8	6.7
Phenanthrene	375.0	10.7	29.2	2.1	nd	5.3	8.0
C1-Phenanthrene	1890.0	16.8	40.2	1.8	nd	4.6	6.5
C2-Phenanthrene	3250.0	451.0	1150.0	3.7	2.6	3.7	3.7
C3-Phenanthrene	2520.0	968.0	2370.0	4.3	4.8	4.7	4.6
C4-Phenanthrene	1450.0	782.0	1910.0	3.9	4.8	5.4	6.6
Anthracene	29.9	nd	2.1	nd	nd	nd	nd
Fluoranthene	8.1	14.4	21.8	1.6	0.8	3.7	9.8
Pyrene	40.2	40.7	57.5	2.0	1.1	4.0	11.6
Benz[a]anthracene	8.1	6.37	18.5	0.5	0.3	1.2	2.4
Chrysene	141.0	76.2	215.0	1.1	0.9	3.2	5.9
C1-Chrysene	353.0	187	529.0	1.0	1.6	2.9	4.2
C2-Chrysene	488.0	259	714.0	1.0	1.7	2.5	3.4
C3-Chrysene	529.0	255	764.0	1.1	1.9	3.1	3.9
Benzo[b]fluoranthene	7.9	18.3	25.5	2.1	1.4	2.6	5.5
Benzo[k]fluoranthene	1.2	3.08	3.9	0.8	0.5	1.0	2.2
Benzo[e]pyrene	17.5	30	39.0	1.4	1.1	1.8	3.3
Benzo[a]pyrene	5.1	6.95	9.7	0.9	0.5	1.1	2.3
Indeno[1,2,3-cd]pyrene	1.8	7.82	10.0	0.7	1.0	6.7	3.1
Dibenzo[a,h]anthracene	1.4	3.04	4.0	nd	nd	nd	nd
Benzo[g,h,i]perylene	5.6	13.5	16.6	0.6	1.0	6.1	3.0
Total PAHs	53200.0	10200.0	24000.0	77.3	73.1	124.0	209.0

nd: not detected.

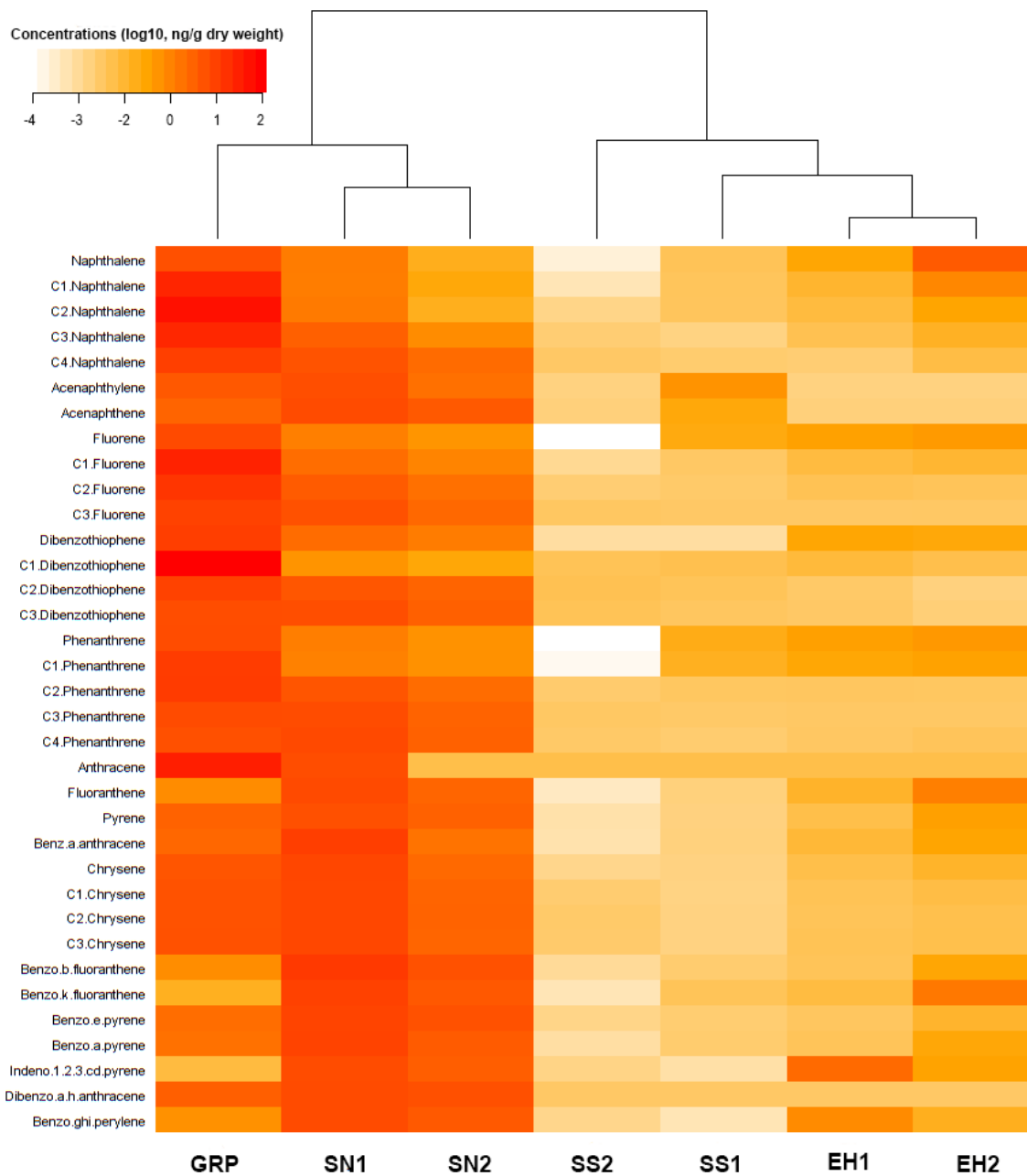


Figure S1. Quantitative expression of chemical composition of sediment extracts. Concentration of measured PAHs in sediment extract (Table S2) is expressed by the color gradient as shown on top left. Euclidean distance method was used for clustering.

Table S3. List of “environmental stress” genes used in LCA

position	gene name	Category	position	gene name	Category
A1	<i>amiC</i>	drug resistance/sensitivity	E1	<i>bacA</i>	drug resistance/sensitivity
A2	<i>rihC</i>	SOS response/DNA repair	E2	<i>dinJ</i>	Cell killing
A3	<i>uvrD</i>	SOS response/DNA repair	E3	<i>emrA</i>	drug resistance/sensitivity
A4	<i>dinD</i>	DNA damage	E4	<i>emrE</i>	drug resistance/sensitivity
A5	<i>crp</i>	General function Energy stress/electron transport	E5	<i>katE</i>	Redox stress
A6	<i>cyoA</i>	transport	E6	<i>marC</i>	drug resistance/sensitivity
A7	<i>dps</i>	Redox stress Energy stress/electron transport	E7	<i>marR</i>	drug resistance/sensitivity
A8	<i>sdhC</i>	transport	E8	<i>sanA</i>	drug resistance/sensitivity
A9	<i>uvrA</i>	SOS response/DNA repair	E9	<i>slyA</i>	Cell killing
A10	<i>yedW</i>	drug resistance/sensitivity	E10	<i>sodC</i>	detoxification
A11	<i>clpB</i>	Protein stress	E11	<i>soxR</i>	Redox stress
A12	blank		E12	<i>sulA</i>	SOS response/DNA repair
B1	<i>fpr</i>	detoxification	F1	<i>yebG</i>	SOS response/DNA repair
B2	<i>norR</i>	detoxification	F2	<i>yhjX</i>	drug resistance/sensitivity
B3	<i>uspA</i>	General stress	F3	promoterless	
B4	<i>recX</i>	SOS response/DNA repair	F4	<i>sbmA</i>	drug resistance/sensitivity
B5	<i>soxS</i>	Redox stress	F5	<i>entC</i>	Protein stress
B6	<i>tam</i>	detoxification	F6	<i>fepB</i>	Protein stress
B7	<i>cueR</i>	Protein stress	F7	<i>mdtK</i>	drug resistance/sensitivity
B8	<i>ycgE</i>	Protein stress	F8	<i>yajR</i>	drug resistance/sensitivity
B9	<i>yeiG</i>	detoxification	F9	<i>cmr</i>	drug resistance/sensitivity
B10	<i>dacB</i>	drug resistance/sensitivity	F10	<i>fsr</i>	drug resistance/sensitivity
B11	<i>cls</i>	drug resistance/sensitivity	F11	<i>zntA</i>	detoxification
B12	<i>yniC</i>	detoxification	F12	<i>grpE</i>	Protein stress
C1	<i>cdaR</i>	General function	G1	<i>yeaE</i>	detoxification
C2	<i>cspA</i>	cold shock	G2	<i>yeeV</i>	Cell killing
C3	<i>cspB</i>	cold shock	G3	<i>bolA</i>	General stress
C4	<i>dacA</i>	drug resistance/sensitivity	G4	<i>gst</i>	detoxification
C5	<i>inaA</i>	Redox stress	G5	<i>mrcB</i>	drug resistance/sensitivity
C6	<i>otsB</i>	General stress	G6	<i>pbpG</i>	General function
C7	<i>uspB</i>	detoxification	G7	<i>ssrA</i>	drug resistance/sensitivity
C8	<i>ada</i>	SOS response/DNA repair	G8	<i>yjfG</i>	Cell killing
C9	<i>dinB</i>	SOS response/DNA repair	G9	<i>dnaJ</i>	Protein stress
C10	promoterless		G10	<i>dnaK</i>	Protein stress
C11	<i>dinG</i>	SOS response/DNA repair	G11	<i>katG</i>	Redox stress
C12	<i>ftsK</i>	SOS response/DNA repair	G12	<i>lexA</i>	SOS response/DNA repair
D1	<i>gadX</i>	General function	H1	<i>lon</i>	Protein stress
D2	<i>mutT</i>	SOS response/DNA repair	H2	<i>ompC</i>	General function
D3	<i>nfo</i>	SOS response/DNA repair	H3	<i>oxyR</i>	Redox stress
D4	<i>recN</i>	SOS response/DNA repair	H4	<i>phoB</i>	drug resistance/sensitivity
D5	<i>sbmC</i>	SOS response/DNA repair	H5	<i>polB</i>	SOS response/DNA repair
D6	<i>sodB</i>	detoxification	H6	<i>recA</i>	SOS response/DNA repair
D7	<i>ybfE</i>	SOS response/DNA repair	H7	<i>relB</i>	Cell killing
D8	<i>ybgI</i>	General function	H8	<i>rpoD</i>	Protein stress
D9	<i>ydeO</i>	General function	H9	<i>rpoE</i>	General stress
D10	<i>ydgL</i>	General stress	H10	<i>sodA</i>	Redox stress
D11	<i>ahpC</i>	Redox stress	H11	<i>ssb</i>	SOS response/DNA repair
D12	<i>ahpF</i>	Redox stress	H12	<i>umuD</i>	SOS response/DNA repair

Table S4. Genes showing >2-fold changes after the exposure to sediment extracts<sup>2</sup>

Gene abbreviation	Gene description	Classification (Gene Ontology Terms)
<i>fpr</i>	<i>Ferredoxin-NADP+ reductase</i>	
<i>gst</i>	<i>Glutathione S-transferase</i>	
<i>norR</i>	<i>Transcription regulator for norVW, response to nitric oxide</i>	
<i>sodC</i>	<i>Superoxide dismutase, Cu, Zn, periplasmic</i>	detoxification
<i>yeiG</i>	<i>S-formylglutathione hydrolase, formaldehyde degradation</i>	
<i>yniC</i>	<i>2-deoxyglucose-6-P phosphatase</i>	
<i>amiC</i>	<i>N-acetylmuramoyl-L-alanine amidase, periplasmic</i>	
<i>bacA</i>	<i>Undecaprenyl pyrophosphate phosphatase</i>	
<i>cls</i>	<i>Cardiolipin synthetase</i>	
<i>dacA</i>	<i>D-alanine-D-alanine carboxypeptidase (penicillin-binding protein 5)</i>	
<i>dacB</i>	<i>D-alanine-D-alanine carboxypeptidase(penicillin-binding protein 4)</i>	
<i>marC</i>	<i>Multiple antibiotic resistance determinant</i>	drug resistance/sensitivity
<i>marR</i>	<i>Transcriptional repressor of multiple antibiotic resistance</i>	
<i>pbpG</i>	<i>Murein D-alanyl-D-alanine endopeptidase (Penicillin-binding protein7 and Penicillin-binding protein8)</i>	
<i>sbmA</i>	<i>Seinsitivity BI7 micorcin, peptide antibiotic transporter</i>	
<i>yajR</i>	<i>Putative transporter</i>	
<i>yedW</i>	<i>Putative response regulator</i>	
<i>rihC</i>	<i>Ribonucleoside hydrolase</i>	DNA damage
<i>ftsK</i>	<i>Filamentation, temperature sensitive, DNA translocase at septal ring sorting daughter chromosomes</i>	
<i>lexA</i>	<i>Global regulator ( repressor) for SOS regulon</i>	SOS response/DNA repair
<i>polB</i>	<i>DNA polymerase II</i>	
<i>recA</i>	<i>Multifunctional DNA recombination and repair protein</i>	

<i>ssb</i>	<i>Single-stranded DNA-binding protein</i>	
<i>uvrA</i>	<i>Excision nuclease subunit A</i>	
<i>uvrD</i>	<i>ATP-dependent 3'-5' DNA helicase II</i>	
<i>ybfE</i>	<i>CopB family protein, LexA-regulated,</i>	
<i>cyoA</i>	<i>Cytochrome <i>o</i> oxidase subunit II, lipoprotein</i>	Energy stress/electron transport
<i>sdhC</i>	<i>Succinate dehydrogenase (SQR) cytochrome b556</i>	
<i>dps</i>	<i>Stress-induced Fe-binding and storage protein</i>	
<i>inaA</i>	<i>Acid-inducible Kdo/WaaP family predicted kinase</i>	
<i>oxyR</i>	<i>Oxidative and nitrosative stress transcriptional regulator</i>	Redox stress
<i>sodA</i>	<i>Superoxide dismutase, Mn</i>	
<i>soxS</i>	<i>Global transcription regulator for superoxide response</i>	
<i>bolA</i>	<i>Stationary-phase morphogene, transcriptional repressor for mreB, dacA, dacC, and ampC</i>	
<i>rpoE</i>	<i>RNA polymerase sigma E factor</i>	General stress
<i>uspA</i>	<i>Global regulatory gene for stress response</i>	
<i>ydgL</i>	<i>SoxR iron-sulfur cluster reduction factor component</i>	
<i>clpB</i>	<i>Bichaperone with DnaK for protein disaggregation</i>	
<i>dnaK</i>	<i>Hsp70 molecular chaperone, heat-inducible</i>	Protein stress
<i>lon</i>	<i>DNA-binding ATP-dependent protease LA</i>	
<i>ycgE</i>	<i>Repressor of blue light-responsive genes</i>	
<i>crp</i>	<i>cAMP-activated global transcription factor</i>	
<i>gadX</i>	<i>Glutamic acid decarboxylase, transcriptional activator for gadA and gadBC, AraC family</i>	General function
<i>ybgI</i>	<i>GTP cyclohydrolase-like radiation resistance protein, metal-binding protein</i>	
<i>dinJ</i>	<i>Antitoxin for YafQ-DinJ toxin-antitoxin system</i>	
<i>relB</i>	<i>Antitoxin for RelE, Qin prophage</i>	Cell killing
<i>slyA</i>	<i>Activates cryptic hemolysin gene hlyE</i>	

## References

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