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# Best available technique for the recovery of marine benthic communities in a gravel shore after the oil spill: A mesocosm-based sediment triad assessment

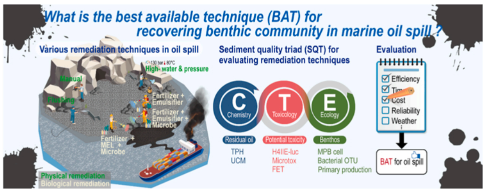
Taewoo Kim [a,1], Changkeun Lee [a,1], Junghyun Lee [a], Hanna Bae [b], Junsung Noh [c], Seongjin Hong [d], Bong-Oh Kwon [e], Jae-Jin Kim [f], Un Hyuk Yim [g], Gap Soo Chang [h], John P. Giesy [i,j], Jong Seong Khim [a,*]

[a] School of Earth and Environmental Sciences & Research Institute of Oceanography, Seoul National University, Seoul 08826, Republic of Korea
[b] GeoSystem Research Corporation, Gunpo 15807, Republic of Korea
[c] Department of Environment and Energy, Sejong University, Seoul 05006, Republic of Korea
[d] Department of Ocean Environmental Sciences, Chungnam National University, Daejeon 34134, Republic of Korea
[e] Department of Marine Biotechnology, Kunsan National University, Kunsan 54150, Republic of Korea
[f] Division of Environmental Science & Ecological Engineering, College of Life Science & Biotechnology, Korea University, Seoul 02841, Republic of Korea
[g] Oil and POPs Research Group, Korea Institute of Ocean Science and Technology, Geoje 53201, Republic of Korea
[h] Department of Physics and Engineering Physics, University of Saskatchewan, Saskatoon, SK S7N5E2, Canada
[i] Department of Veterinary Biomedical Sciences & Toxicology Centre, University of Saskatchewan,Saskatoon, SK S7N5B3, Canada
[j] Environmental Sciences Department, Baylor University, Waco, TX 76798-7266, United States

## HIGHLIGHTS

- Mesocosm study first adopting SQT revealed varied oil cleanup efficiencies.
- Physical cleanup effectively removed oil, but initially harmed benthic community.
- Mixed biological methods increased oil removal efficacy across SQT components.
- No treatment” recovered benthic community considerably, highlighting natural attenuation.
- Physical hand wiping combined with bioremediation showed the greatest oil recovery.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Ecotoxicological effects of spilled oils are well documented, but study of recovery of marine benthic communities is limited. Long-term recovery of hard bottom communities during physical and biological remediations after a spill was monitored. A 60-day experiment was conducted using a mesocosm with monitoring of eight endpoints by use of the sediment quality triad (SQT). First, physical treatment of hot water + high pressure flushing maximally removed residual oils (max=93%), showing the greatest recovery among SQT variables (mean=72%). Physical cleanup generally involved adverse effects such as depression of the microphytobenthic community

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[*] Corresponding author.
*E-mail address:* jskocean@snu.ac.kr (J.S. Khim).
[1] These authors contributed equally to this work.

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during the initial period. Next, biological treatments, such as fertilizer, emulsifier, enzyme and augmentation of the microbes, all facilitated removal of oil (max=66%) enhancing ecological recovery. Analysis of the microbiome confirmed that oil-degrading bacteria, such as *Dietzia* sp. and *Rosevarius* sp. were present. A mixed bioremediation, including fertilizer + multi-enzyme + microbes (FMeM) maximized efficacy of remediation as indicated by SQT parameters (mean=47%). Natural attenuation with "no treatment" showed comparable recovery to other remediations. Considering economic availability, environmental performance, and technical applicability, of currently available techniques, combined treatments of physical removal via hand wiping followed by FMeM could be most effective for recovery of the rocky shore benthic community.

## 1. Introduction

Oil spills can result in a wide range of adverse ecotoxicological effects on marine ecosystems, across diverse habitats of tidal flats, sandy beaches, and gravel shore (EPA, 1993; ITOPF, 2011). Spilled oils could reach and easily penetrate nearshore bottoms, varied though depending on the sedimentary environments such as mud content, sediment particle size, or organic and oxygen content. Permeation of oil into substrata could subsequently result in persistent, residual oil in surface and/or subsurface layers (Moore, 2016). Remaining, deeper subsurface oils can affect abundances and diversity of marine organisms (Hayes et al., 2010; Nixon et al., 2018; Yim et al., 2020). For example, after the *Hebei Spirit* spill in South Korea and the *Exxon Valdez* spill in Alaska, it took over 6 and 20 years, respectively, for benthic communities to recover and return to pre-spill status (Dave and Ghaly, 2011; Li et al., 2016).

Various techniques for remediation have been developed and employed to cleanup spilled oil (Azubuike et al., 2016; Dhaka et al., 2021). On hard bottoms, physical cleanups, such as hand wiping and hot water/high pressure flushing are essential for initial removal of stranded oils (ITOPF, 2011). Some biological treatments, which generally include applications of fertilizers (Nikolopoulou et al., 2007), emulsifier (Feng et al., 2006), multi-enzyme (Das et al., 2011) or augmenting microbial communities (Gao et al., 2011) are useful for longer-term cleanups. Typically, the primary focus on these methods of cleanup are rapid removals of spilled oils, but not considering receptor-oriented recovery of populations or communities (Siva et al., 1979). Historically, less attention has been paid to recovery of benthic organisms. For example, hot water/high pressure flushing, used in response to the *Exxon Valdez* spill, effectively removed stranded oils on hard surfaces, but severely damaged epibenthos (Mearns et al., 1993). Following the *Torrey Canyon* spill, use of dispersants caused unintended, greater toxicities to many organisms (Wardrop, 1991). Thus, a choice of timely method selection would be of great significance in effective cleanup implementation as oil spill response.

An intertidal zone with hard bottoms typically has great biodiversity, and its characteristics, including varying slopes, overhangs, and various textures of surfaces where various marine organisms can live. Spilled oil can penetrate into hard bottoms as a function of porosity related to gravel type and size. Finally, oil that penetrates gravel can form a relatively permanent surface or subsurface layer (Moore, 2016). Selection of the most appropriate techniques for remediation of hard bottom communities is challenging. For example, variations in oceanographic conditions, such as tide, current, geomorphology, sediment facies all influence spatiotemporal distributions and fates of sedimentary residual oils. Accessibility to the impacted shoreline, in particular, must be considered. On the shore, oil frequently coats hard surfaces such as rocks and gravel during the tidal range, accumulating in rock pools and cracks. Typically, the oil does not remain static, but is moved along the coast, eventually stranding in a safe spot. Access to the shorelines can be challenging at times, and special attention must be paid to worker safety in slippery areas, as well as to the hazards of waves and tides. In particular, the low load bearing qualities of such shorelines impede both vehicle and personnel transportation. That is the reason why relatively mobile techniques and equipment would be used to remove oil (ITOPF, 2011).

Since responses of benthic communities are important to overall recovery of marine ecosystems, remediation methods should be carefully chosen considering a number of factors. Thus, application of SQT assessment adopting lines of evidence approach, can be used to select the best available technique (BAT) for the remediation of sedimentary contamination by spilled oils. SQT is composed of three key components: chemical exposure (chemistry line of evidence (LOE)), toxicological effect (toxicology LOE), and benthic community health (ecology LOE) (Chapman, 1990, 1996). A single strategy or method cannot provide strong evidence for sediment toxicity, as causation cannot be established without integrating the three components. Nowadays, the ecological LOE contains a diverse array of benthic community, including microbiota and meiofauna (Khim et al., 2018, 2022). From a chemical perspective, total petroleum hydrocarbon (TPH) and unresolved complex mixture (UCM) could be targeted (Yim et al., 2012). Second, toxicity tests sensitive to polycyclic aromatic hydrocarbons (PAHs) and weathered PAHs should be conducted; The H4llE-*luc* bioassay (Martínez-Gómez et al., 2010), microtox® and fish embryo test (Lee et al., 2018a) have also been shown to be useful. Finally, ecological endpoints for multiple taxonomic groups relating to the benthic communities, such as diversity of the microbiome (Bourlat et al., 2013), microphytobenthos (MPB), and macrofauna can be monitored to determine potential ecological risks from oil spills (Lee et al., 2019a; Mohr et al., 2005). Overall, the integrated SQT approach is useful to accurately assess overall recovery of benthic communities in the vicinity of spills.

Enclosed experimental ecosystems, such as indoor mesocosms provide useful tools for examining effectiveness of cleanup and remediation. A recent mesocosm study successfully demonstrated the most appropriate biological methods for remediation of oil from soft bottoms and recoveries of marine benthic communities (Lee et al., 2019a). Here, as a continuing, but more in-depth effort, we evaluated physical and biological remediations, targeting recoveries of hard-bottom communities. The study adopted the advanced SQT approach, with multiple measures of chemical, toxicological, and ecological indicators across eight endpoints: (1) TPH, (2) UCM, (3) fish embryo mortality, (4) bacterial inhibition, (5) aryl hydrocarbon receptor (AhR) mediated potency, (6) MPB cell, (7) bacterial operational taxonomic unit (OTU), and (8) benthic primary production. Finally, the BAT for remediation of oil spills was carefully determined and discussed considering all eight indexes of the SQTs.

## 2. Materials and methods

### 2.1. Sample preparation

To simulate the gravel-covered coastal zone affected by oil spills, gravel with a diameter of 6.5 cm or less was collected from Gimhae on the southern coast of South Korea. We focused on the contamination of the gravels from oil exposure; therefore, the media used in the experiment were only composed of gravel. Collected gravel was introduced to seawater for 30 days in order to recruit marine organisms before initiating experiments (Fig. 1). Gravel was exposed to Iranian Heavy Crude oil (details in Table S1 of the Supplementary materials) at a volume ratio of 1:10 (oil: gravel) and was homogenized with a polytetrafluoroethylene stick. Gravel was shaken with oil until surfaces of gravel were evenly coated (physical remediation: set −1 d and biological remediation: set −30 d). In general, whilst, physical remediation is used to remove oil during the initial phases of remediation of oil spills, biological remediation can be applied to accelerate natural attenuation during the final phases of cleanup. Therefore, by setting the different weathering periods in each remediation, this study provided a more realistic simulation exposure of the gravel shore to oil.

### 2.2. *Mesocosm experiments*

The artificial tide system was designed to simulate tides on gravel-covered shores (Fig. S1). The tide control system was composed of three compartments: (1) one water storage tank with a dimension of 200 × 90 × 41 cm (W × L × H) and a capacity of ~700 L on the top (for thermal and dissolved oxygen control); (2) a set of 10 experimental aquariums with 45 × 35 × 40 cm per aquarium in the middle (to control water level, tidal cycle, and light condition); and (3) one wastewater tank with 200 × 70 × 80 cm in dimension and ~100 L of capacity at the bottom. Temperature was maintained constant (18–19 ℃) by use of the temperature control system. The volume of gravel in the aquarium portion of the mesocosm was identical to that of the gravel shore near where the mesocosm was installed. Natural seawater was supplied to experimental aquariums at 40 L for 12 h so that the gravel pile could be gradually submerged from the lower layer to the upper layer. This daily of horizontal supply and discharge of seawater at a 12 h interval, mimicking an in situ tidal condition, was repeated during the experimental period of 60 days without high-energy tidal action. The irradiance reaching the gravels was measured to be approximately 380–425 μmol $m^{-2}$ $s^{-1}$ from each aquarium's LED lamps (20 watts per lamp or aquarium).

Among the remediation techniques proposed by the International Tanker Owners Pollution Federation (ITOPF), 6 techniques were applied during the experiments (Fig. 1) (ITOPF, 2011). In particular, considering the applicable physical remediation techniques in a gravel and/or rocky shore from the manual of ITOPF (2011), we chose the techniques which

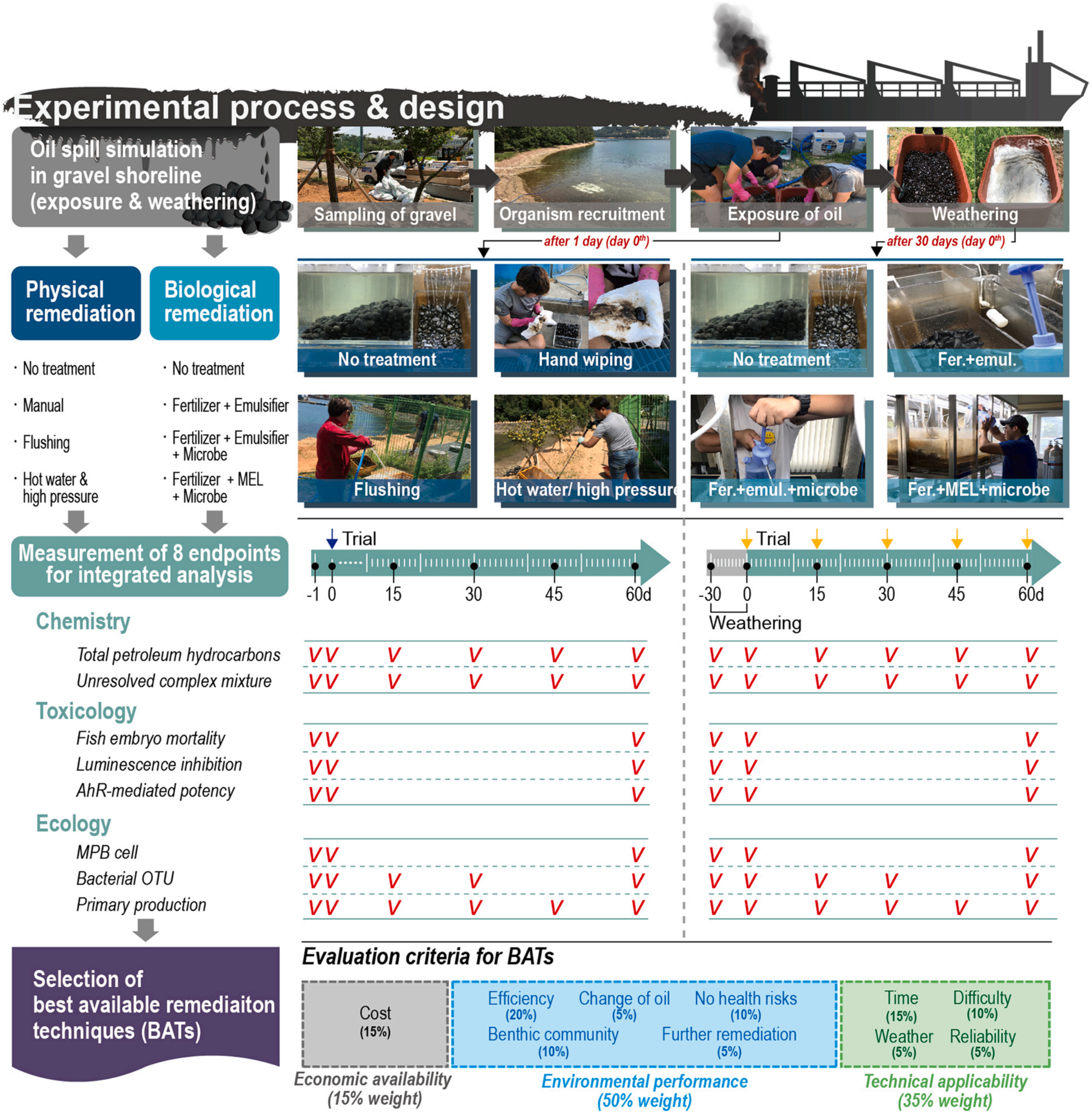


**Fig. 1.** Schematic of physical and biological remediation techniques, experimental conditions, and sampling design of this study. *Economic availability*: cost (relatively inexpensive); *environmental performance*: efficiency (95–99% removal rate of residual oil); health (reduction rate of potential toxicity and recovery of benthic community); further treatment (no further remediation required); *technical applicability*: time (removal of contaminant within days); level of difficulty (easy to maintain and operate); weather (favorable for application of method); reliability (the method works the majority of the time); change of oil (do not change physical/chemical characteristics of oil).

can be used in an indoor scale experiment (Table S2) possibly. They included 3 physical techniques; manual hand wiping (MA), flushing (FL), and hot water-high pressure (HW/HP). We analyzed the pressure in each remediation technique with (MA: 30 bars, FL: 20 bars, and HW/HP: 130 bars) by the in-line pressure gauge kit in each machine. Total 4 products were used in the biological remediation (fertilizer: oleophilic fertilizer, S200; emulsifier: Tween 80; multi-enzyme: Oil Spill Eater II; microbe: mixture (*Alcanivorax* sp., *Roseovarius* sp., *Corynebacterium variabilis*, *Dietzia* sp., *Sphingomonas yanoikuyae*, *Kyotococcus sedentarius*, *Bacillus aquimaris*, *Novosphingobium*, *Pentaromativorans*, and *Yarrowia lipolytica*)). To evaluate the mixed effects of biological remediation, 3 combined biological techniques; fertilizer + emulsifier (FE), fertilizer + emulsifier + microbe (FEM), and fertilizer + multi-enzyme + microbe (FMeM) were selected. In addition, natural attenuation during which no active treatments were applied (NT) was used to simulate responses to seawater circulation alone.

All treatments were applied at times equivalent to low tide, according to the tidal cycle in the area (34° 59.578'N, 128° 40.393'E) where the mesocosm was installed. Physical treatments were employed initially (0 d) and biological treatments were applied at the first low tide time on days 0, 15, 30, 45, and 60 (Table S3). Advantages and disadvantages of selected techniques are listed in Table S4.

## 2.3. Chemical assessments

### 2.3.1. Instrumental analysis of residual TPH and UCM in gravel

The analytical procedures for TPH and UCM in gravel followed methods used in previous studies (Yim et al., 2005; Yim et al., 2011). In brief, 120–140 g oil-contaminated gravel was mixed with anhydrous sodium sulfate (Sigma-Aldrich, St. Louis, MO) to remove water, and surrogate standard (*o*-terphenyl) was added. The samples were ultrasonically extracted for 15 min with 150 mL dichloromethane (Burdick & Jackson, Muskegon, MI), with three repeats. The extracts were concentrated to 1 mL under a gentle stream of nitrogen gas, and the internal standard (5α-androstane) was added. TPH and UCM were calculated by using an Agilent 7890 gas chromatograph equipped with a flame-ionization detector (Agilent Technologies, Santa Clara, CA) (Wang et al., 1994).

## 2.4. Toxicological assessment

### 2.4.1. Zebrafish (Danio rerio) embryo test

In order to clarify efficiencies of each remediation, bioassays were conducted at three times, including an initial oil exposure (−30 d), first application of each remediation (0 d), and final stages (60 d). Oil-contaminated gravel was extracted by DCM, and the extracts were substituted with dimethyl sulfoxide (~10 mg oil-contaminated gravel equivalent (GEq) $\mathrm{mL^{-1}}$). Before the solution was exposed to embryos, embryos with no abnormality in differentiation were selected. Using 12-well plates, the experiment was performed on 12 individuals with three repetitions. Individuals were exposed for 96 h in a culture system that was maintained at a constant temperature (26 °C). Every day, the mortality of each individual and developmental effect rates (spinal curvature and cardiac edema) were checked with a microscope (Lee et al., 2018a).

### 2.4.2. Vibrio fischeri (V. fischeri) bioassay

The *V. fischeri* bioassay was used to evaluate the potential toxicity of residual oil in gravel. Organic extracts from gravel were exposed to *V. fischeri*, confirming the effect of residual oil on the inhibition of luminescence using N-TOX (model 200; NeoEnBiz Inc., Bucheon, Korea), which is a commercial toxicity assessment kit (~10 mg GEq $\mathrm{mL^{-1}}$). The *V. fischeri* bioassay was conducted following the standard method specified by the Ministry of Maritime Affairs and Fisheries of South Korea (Lee et al., 2019b, 2019c).

### 2.4.3. H4llE-luciferase transactivation bioassay

The H4IIE-*luc* bioassay was performed to detect AhR-mediated potencies in oil-contaminated gravel according to previously published methods (Hong et al., 2012). In brief, trypsinized cells (~7.0 $\times 10^4$ cells $\mathrm{mL^{-1}}$) were seeded in 96-well plates and incubated for 72 h. The plates were then dosed with the appropriate standards (2,3,7,8-tetrachlorodibenzodioxin; 0.1% dose), samples (raw; 0.1% dose (~0.1 mg GEq $\mathrm{mL^{-1}}$)), and solvent controls (0.1% dimethylsulfoxide) for 72 h. After 72 h exposure, the results were expressed as relative luminescence units that were quantified using a Victor X3 multi-label plate reader (PerkinElmer, Waltham, MA).

## 2.5. Ecological assessment

Considering that organisms have different response rates to temporal variation of oil contamination, each data point is given as a different color, according to the endpoints (Yim et al., 2017). Because the lower the trophic levels respond more rapidly and responses to sedimentary contamination are complex, data points were assigned in this study in the following order: bacteria (n = 5) > MPB cells (n = 3), and meiofauna (n = 3). Because estimates of primary production were derived from the results of functions in various benthic organisms, we collected more data points than for other biological variables.

### 2.5.1. Identification of MPB and cell counting

Oil-contaminated gravel was collected in triplicate from each experimental aquarium. For diatom separation from gravel, 5% formalin solution was added to the collected gravels (5 of about 100 pieces of gravel in each aquarium) and thoroughly shaken. Only the supernatant was decanted into a beaker. After adding 10 mL distilled water to the remaining gravel, the water-gravel mixture was sonicated for 5 seconds. The supernatant was removed from the submerged samples, and 30 mL distilled water and 10 mL hydrochloric acid were added for the acid treatment. Samples, containing MPB cells, were heated and cooled to be neutralized for species identification and cell counting (until counting max of 100 cells) (Bae et al., 2020).

### 2.5.2. Bacterial meta-genomic analysis

The bacterial community was analyzed by extracting total genomic DNA from gravel, using a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). Sequencing was conducted using the Illumina MiSeq Platform with 16 S rRNA gene amplicons. The amplicons of V34 were prepared using the forward primer (16 S_341F: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and the reverse primer (16S_805R: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C). Quantitative Insights into Microbial Ecology was used to analyze the sequence data. Using the UCLUST algorithm, sequences were clustered by OTUs at a 97% identity threshold (Edgar et al., 2010). Taxonomic information was delegated by aligning sequences with the data from Ribosomal Database Project (RDP) (Cole et al., 2014).

### 2.5.3. Measurements of benthic primary production

Primary production of benthic algae was measured in each experimental aquarium (three repetitions) using a Diving-PAM fluorometer (Walz, Effeltrich, Germany) (Perkins et al., 2001). The maximum relative electron transport rate (rETRm) was determined as the product of Fq'/ Fm' and irradiance (Sakshaug et al., 1997). Fq' and Fm' denote the proportion of harvested photons driving photosynthesis and the light-adapted maximum fluorescence, respectively.

## 2.6. Multi-attribute utility theory (MAUT) analysis for selection of the best available technique for remediation of oil

MAUT analysis is a decision-making approach in multi-criteria decision analysis (MCDA). Various data were used as nine assessment factors (main 3 criteria: economic availability (Ea), environmental performance (Ep), and technical applicability (Ta)) within MAUT analysis. Each criterion was scaled from 0 (worst) to 1 (best) based on the average result. The best available remediation assessment was determined by use of MAUT, which was based on three previously proposed criteria (Dave and Ghaly, 2011; Zheng et al., 2019): *Ea* in terms of cost (relative inexpensive); *Ep* in terms of efficiency, effect on oil (physical and chemical change of properties), potential toxicity, recovery of benthic community, and further treatment (no further treatment required); *Ta* in terms of time (removal of oil within days), weather (favorable for application of method), reliability (the method works the majority of the time), and level of difficulty (easy to maintain and operate) (Fig. S2). Each criterion was weighted (15% for economic availability, 50% for environmental performance, and 35% for technical applicability).

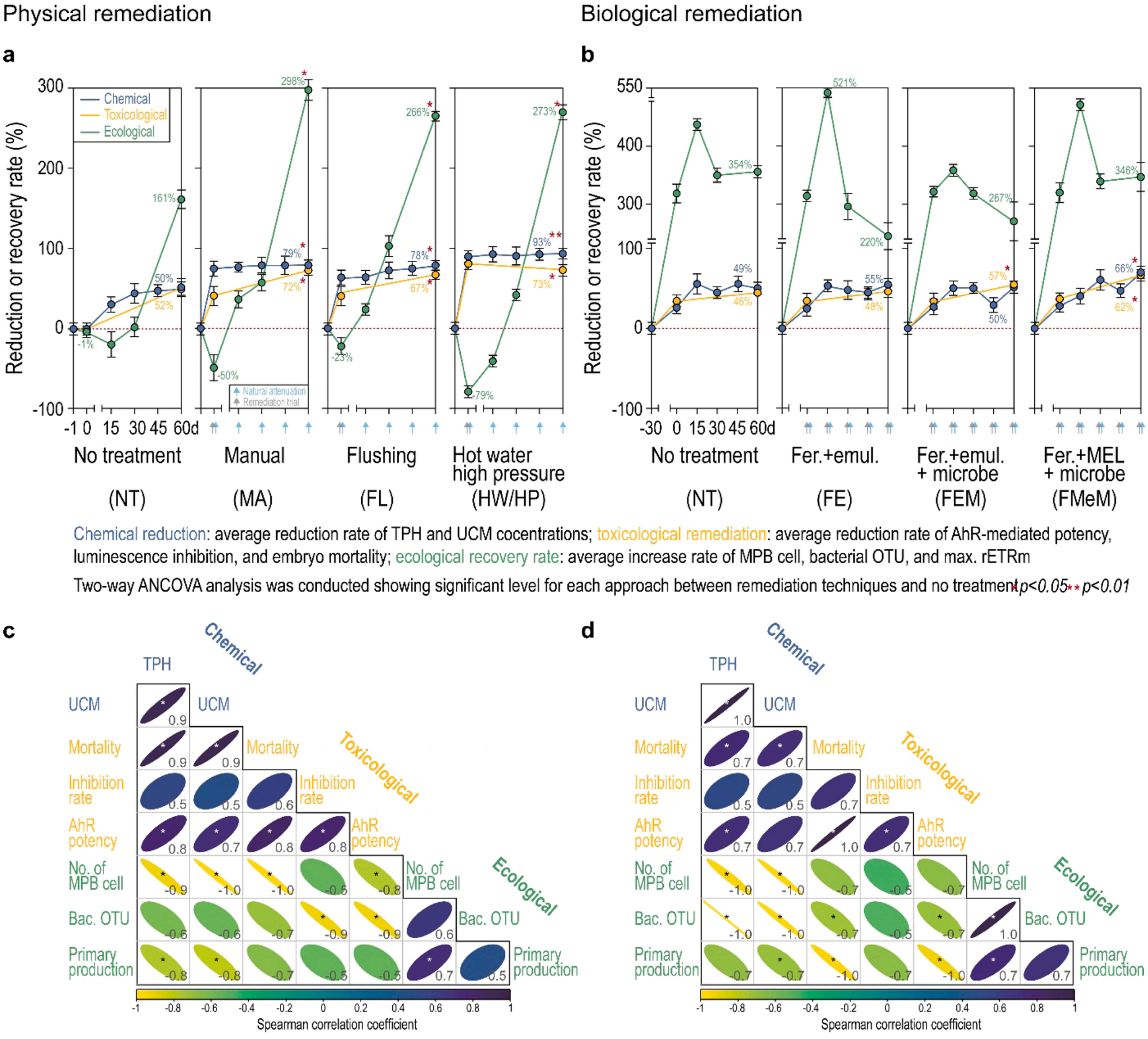
### 2.7. Statistical analysis

Data analyses were carried out using IBM SPSS software (version 23.0; SPSS Inc., Chicago, IL). The difference of recovery and reduction rate among each treatment was analyzed by analysis of covariance (ANCOVA). In all statistical analyses, *p* values less than 0.05 were considered to be statistically significant. Principal component analysis (PCA) to visualize the similarity between selected endpoint (concentrations of TPH and UCM, HI, and primary production) and bacterial abundance.

## 3. Results and discussion

### 3.1. Physical and biological remediations

Each efficiency was determined to increase (ecology) and/or



Chemical reduction: average reduction rate of TPH and UCM cocentrations; toxicological remediation: average reduction rate of AhR-mediated potency, luminescence inhibition, and embryo mortality; ecological recovery rate: average increase rate of MPB cell, bacterial OTU, and max. rETRm

Two-way ANCOVA analysis was conducted showing significant level for each approach between remediation techniques and no treatment * $p<0.05$ ** $p<0.01$

Spearman's correlation matrix was conducted showing the coefficient value and siginificant level * $p<0.05$

**Fig. 2. a–b:** Chemical and toxicological reduction rate and ecological recovery rate after implementation of each remediation technique (physical and biological remediation). **c–d:** Spearman's correlation matrix was conducted showing the coefficient value and significant level. A color-coded correlation scale is provided on the downward of each plot.

reduction rate (chemical and toxicity) as compared to the initial stage in order to evaluate the effect of using remediation techniques (Fig. 2). Physical remediation after exposure of oils to gravels resulted in time-dependent, recovery of all three components of the SQT, but efficiencies of recovery varied among the eight endpoints. Removal of hydrocarbons of 78–93% occurred within 60 days (Fig. 2a and S3a). After 60 d, rates of reduction of hydrocarbons of several treatments were significantly different from that of NT (MA: $p < 0.05$, FL: $p < 0.05$, and HW/HP: $p < 0.01$). Physical remediation seemed to mitigate toxicity of residual oil with an average reduction rate of 71% (MA: 72%; FL: 67%; and HW/HP: 73%) compared to the initial stage (−1d), which was significantly greater than that of NT (52% and $p < 0.05$) (Fig. 2a). Adverse effects on measures of ecosystem structure were observed soon after applications of physical remediation. In particular, the HW/HP treatment caused the greatest negative effects on ecological endpoints (mean = −79%) on the −1 d (Fig. S4a). However, the rate of recovery of structure of the benthic community increased as the seawater exchange promoted recruitment of micro-benthic organisms. Treatment MA resulted in the greatest (298%) recovery of the benthic community, followed by HW/HP (273%) and FL (266%) at the end of experiment (60 d), which almost doubled compared to that of NT (161%) (MA: $p < 0.05$, FL: $p < 0.05$, and HW/HP: $p < 0.05$).

Biological remediation, which was applied for 30 d, during which weathered oil was present on gravel (set treatment day as 0 d). Rate of removal of TPH was 49%, greater than NT when biological remediation techniques were applied. Removal of TPH of 50–66% was observed after 90 d (−30 d to 60 d) (Fig. 2b and S3b). Greatest reduction of toxicity of 62%, was observed for treatment FMeM, followed by FEM and FE, with reductions of 57% and 48%, respectively. Recovery of the benthic invertebrate community was greater with increases of all three ecological endpoints observed during the weathering period (Fig. S4b). On 60 d, the greatest ecological recovery of 346%, was observed for treatment FMeM followed by FEM and FE with improvements of 267% and 220%, respectively.

Reduction of residual oil was proportional to physical pressure given to each treatment. For example, reduction was greatest for treatment HW/HP (130 bars), followed by MA (30 bars), FL (20 bars), and NT (Table S5). Of the biological remediation treatments, TPH and UCM were reduced to the least concentrations by treatment FMeM, which indicated that combined use of fertilizers and augmenting the microbial community was effective at breaking down residual oil. However, compared to physical techniques, biological techniques resulted in more gradual reduction in TPH and UCM. This could be due to fertilizer, MEL, and microbe solutions stimulate growth of existing bacteria that were capable of degrading petroleum, which results in time-lagged promoting the natural degradation (Kim et al., 2005).

Reduction in toxicity, including mortality of embryos and AhR-mediated potency in gravel, was observed with the reduction of TPH after physical treatments (Fig. 2c, Tables S6, and S7) ($p < 0.05$). Embryo stages of development in fish have been found to be sensitive to exposure to oil, which especially includes various dioxin-like compounds, causing AhR activity (Johann et al., 2020). The *V. fischeri* bioassay is less sensitive to reduction of residual oil during the 90 days of experiment. For example, inhibition of luminescence relatively small responses (<50%), compared to fish embryo mortality (~100%), even when the concentration of TPH and UCM were greatest during the initial period. Results of previous studies have found that toxicity of oil to bacteria depends on constituents of the mixture other TPH or UCM (Brils et al., 2002; Adams, 2015). Toxic potency of residual oils decreased over 90 days (Fig. 2d, $p < 0.05$) when biological treatments were applied. For treatment FE, AhR-mediated potency did not decrease from 0 d to 60 d, even though TPH decreased (Tables S5 and S6). This is in accordance with the results reported by Johann et al. suggesting that emulsifier contributes to greater toxicity compared to native oils (Johann et al., 2020). Indeed the emulsifier does not appear to effectively remove the dioxin-like compounds affecting the AhR activity at the moment (Bach et al., 2005).

Ecological endpoints of SQT, initially included four targets, including MPB, bacteria, meiofauna, and primary production, generally encompassing key benthic taxonomic groups. Due to small numbers of individuals detected in control treatment (Fig. S5), meiofauna were not utilized to calculate rates or efficiencies of recovery. For physical treatments, MPB cells decreased within a day of removal of oil, although chemical concentrations of TPH and UCM decreased dramatically (Fig. S4a, S6a, and Table S8). This could be simply due to physical disturbance along with removal of oil from surfaces of gravel. MPB cells increased after 60 d during experiments, which confirms recolonization by MPB cells following proliferation after exposure to oil was less. This phenomenon was observed for all of physical treatments, but HW/HP showed relatively good recovery of MPB. Similar to physical treatment, the MPB cells after 0 d and/or 60 d in biological treatments showed significantly greater numbers of MPB cells compared to the initial exposure to oil (−30 d) (Fig. S4b). Application of emulsifiers was an effective at reducing residual oil, but adversely affected the MPB community (Fig. 2d, $p < 0.05$). For example, treatments FE and FEM, which contained emulsifiers, resulted in lesser numbers of MPB cells at the end of biological treatments even though concentrations of TPH and UCM decreased during that period. Toxic constituents of the emulsifiers affected the MPB community which offset the beneficial effects of dispersing residual oil (Hook and Osborn, 2012). In general, MPB species observed at the end of physical and biological treatments were dominated by a few taxa, including *Navicula* sp., *Amphora* sp. and *Nitzschia* sp. (Fig. S6 and S7), genera that have been reported to be resistant to effects of oil (Patrick and Palavage, 1994).

Changes in the bacterial community were observed during physical and biological remediations, that resulted in greater numbers of bacterial OTUs more quickly. In particular, rapid increase of bacterial OTUs during NT indicated faster recovery under natural conditions (Fig. S4a). During treatment with HW/HP, recovery of bacterial OTUs after 60 d was not clearly observed, and was eventually less than that initially (factor change: <1). Indices of bacterial diversity for HW/HP were less than those for other treatments. For instance, the Chao1 index decreased over 90 d (Table 1). The lesser *HI* (n17C/pristane and n18C/phytane) observed for treatment HW/HP resulted in a decrease in biodegradation of oil by bacteria (Fig. S8a). A combination of high temperature and pressure during the HW/HP treatment was the most effective in reducing concentrations of residual oil, but also resulted in adverse effects on natural bacteria (De Vogelaere and Foster, 1994). Some previous studies reported that the HW/HP method could damage directly to the habitat in which the marine organism lives, both in the short- and long-term periods (Paine et al., 1996; Pezeshki et al., 2000).

The number of bacterial OTUs in biological treatments also increased over 90 d of experiments, with final values being five-fold greater than initially stage (−30 d) (Fig. S4b). Oil-degrading bacteria were more abundant in biological treatments than physical treatments, (Table 1). In marine environments exposed to oil spills, abundances of hydrocarbon-degrading bacteria rapidly expands (Love et al., 2021). Relatively lesser and slow breakdown of residual oils in biological treatments than physical treatments minimize this response (Fig. S8b).

Primary production in the benthic environment, monitored in the remediation experiments to assessed functioning of microorganisms under the oil exposed environments tended to increase during both physical and biological remediations. Benthic, primary production increased 2- to 6-fold in all treatments over 60 d and was significantly ($p < 0.05$) and negatively correlated with concentrations of TPH and UCM (Figs. 2c and 2d), increasing as the amount of oil decreased. However, 30 days of pre-seawater exchange before the actual biological treatments resulted in the greatest benthic primary production across all biological treatments. Greater primary production was attributed to recruitment of MPB and bacteria during continuous seawater inputs and consumption of hydrocarbon by oil-degrading bacteria (Valentine et al., 2015).

Table 1
Shannon and Simpson diversity index, and Chao1 index for each of the sampling in physical and biological remediation techniques.

| Remediation techniques | Diversity and richness index of bacteria | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shannon | | | | | Simpson | | | | | Chao1 | | | | |
| **Physical** | **-1 d** | **0 d** | **15 d** | **30 d** | **60 d** | **-1 d** | **0 d** | **15 d** | **30 d** | **60 d** | **-1 d** | **0 d** | **15 d** | **30 d** | **60 d** |
| NT | 5.5 | 5.5 | 5.5 | 5.7 | 6.2 | 0.9 | 0.9 | 0.9 | 1.0 | 1.0 | 132 | 132 | 469 | 524 | 592 |
| MA | 5.5 | 6.6 | 5.4 | 4.9 | 5.5 | 0.9 | 1.0 | 0.9 | 0.9 | 0.9 | 132 | 233 | 360 | 455 | 612 |
| FL | 5.5 | 6.7 | 4.7 | 5.9 | 5.3 | 0.9 | 1.0 | 0.9 | 0.9 | 0.9 | 132 | 203 | 352 | 418 | 620 |
| HW/HP | 5.5 | 4.2 | 4.4 | 5.7 | 5.2 | 0.9 | 0.9 | 1.0 | 0.9 | 0.9 | 132 | 255 | 200 | 198 | 94 |
| Control | 3.7 | 3.7 | 4.5 | 4.9 | 6.4 | 0.8 | 0.8 | 0.8 | 0.9 | 1.0 | 387 | 387 | 721 | 538 | 229 |
| **Biological** | **-30 d** | **0 d** | **15 d** | **30 d** | **60 d** | **-30 d** | **0 d** | **15 d** | **30 d** | **60 d** | **-30 d** | **0 d** | **15 d** | **30 d** | **60 d** |
| NT | 1.8 | 5.2 | 6.1 | 4.7 | 5.8 | 0.4 | 0.9 | 1.0 | 0.9 | 0.9 | 142 | 306 | 673 | 523 | 839 |
| FE | 1.8 | 5.2 | 6.1 | 5.1 | 5.8 | 0.4 | 0.9 | 1.0 | 0.9 | 1.0 | 142 | 306 | 519 | 600 | 699 |
| FEM | 1.8 | 5.2 | 4.6 | 5.3 | 5.8 | 0.4 | 0.9 | 0.9 | 0.9 | 1.0 | 142 | 306 | 471 | 601 | 791 |
| FMeM | 1.8 | 5.2 | 5.6 | 5.0 | 5.9 | 0.4 | 0.9 | 0.9 | 0.9 | 1.0 | 142 | 306 | 516 | 575 | 731 |
| Control | 8.0 | 8.0 | 7.5 | 4.6 | 7.7 | 1.0 | 1.0 | 1.0 | 0.7 | 1.0 | 1443 | 1443 | 1412 | 1173 | 1181 |

NT: no treatment; MA: manual; FL: flushing; HP: hot water & high pressure; FE: fertilizer + emulsifier; FEM: fertilizer + emulsifier + microbe; FMeM: fertilizer + MEL + microbe.

### 3.2. *Dynamics in bacterial communities*

Relative abundances of bacterial 16S rRNA gene sequences discriminatively revealed variation in relative compositions of bacterial communities. This information was used to address dynamics in structures of bacterial communities during exposure to and weathering of oil (Fig. S9). On average, three phyla (Proteobacteria, Bacteroidetes, and Firmicutes) accounted for more than 85% of bacterial communities during both physical and biological treatments (Fig. S10). For physical treatments, the principal component analysis (PCA) showed that abundances of bacterial phyla were interrelated with residual oil (TPH and UCM), *HI* (nC17/pristine and nC18/phytane), and primary production in the correlation matrix (Fig. S11). Results of PCA suggested that the two principal components collectively accounted for 58.8% of the total variance. Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria were grouped with the concentrations of the residual oil (represented by Groups I–III). Three classes of Proteobacteria were specifically grouped with respect to different endpoints. First, beta-proteobacteria was positively loaded with residual oil, while alpha-proteobacteria were grouped with *HI* and primary production, which indicated biodegradation and functional recovery. In comparison, gamma-proteobacteria did not load to any endpoints.

Temporal variation between the groups was observed and was a of concentrations of residual oil and hydrocarbons (aliphatic) for each physical remediation technique (Fig. 3a). Bacterial Group I included Bacteroidia, Bacilli, Clostridia, beta-proteobacteria, and others, including unknown classes from Actinobacteria and Cyanobacteria, and generally consisted of early oil-degrading bacteria which changed in response to aliphatic hydrocarbons in TPH (Fig. 3b). Group II included gamma-proteobacteria exclusively which have multiple oil-derived hydrocarbons, both aliphatic and aromatic, degrading metabolic activities; genera included *Pseudoaltermonas*, *Alcanivorax*, *Thalassolituus*, and *Cycloclasticus*. For HW/HP treatment, relatively small abundance of bacteria associated with degradation of oil was detected because a large amount of oil was initially removed. In particular, the FL treatment seemed to promote the rapid removal of residual oil as well as natural degradation by bacteria when *HI* increased.

Samples dominated by Group III had greater *HI* and primary production later in the experiment period. The results showed that a greater availability of residual aromatics supported greater degradation of hydrocarbons and increased primary production by *Sulfitobacter* and *Erythrobacter* as phototrophic bacteria despite the effect of overall reduction in the amount of hydrocarbons. Results of previous studies have shown changes in bacterial communities during degradation of oil might be attributed to a preferences by various bacteria for hydrocarbon degradation intermediates as sources of carbon (Lea-Smith et al., 2015; Uribe-Flores et al., 2019). Changes in structures of bacterial communities would be dependent on compositions of hydrocarbons present in oil during process of degradation of oil, in the order of alkanes, cycloalkanes, aromatics, resins, and finally asphalt (Dubinsky et al., 2013).

Changes in bacterial communities also occurred in response to lessening concentrations of hydrocarbons with increasing *HI* and primary production during biological treatments. Results of PCA ordination showed that the two principal components collectively accounted for 81.5% of total variance (Fig. S11b). Changes in structures of bacterial communities were strongly associated with experimental periods. Bacteria in Group I, which mainly degrade aliphatic compounds decreased in response to a gradual decrease in TPH (Fig. 3c). After 30 d of pre-exposure (−30 d to 0 d), the relative abundance of Group II noticeably increased. *Alcanivorax*, *Salegentibacter*, *Muricauda*, and *Marinicella* in gamma-proteobacteria were the prominent degraders of hydrocarbons (aliphatic and aromatic hydrocarbons) and more prevalent than any other taxa detected (Fig. 3d). Bacteria in Group III began to dominate the community after 30 d. In particular, these bacteria included *Roseovarius* and *Erythrobacter*, which are psychrophilic photosynthetic organisms in addition to *Dietzia*. Appearances of these bacteria is indicative of a community optimized for degradation of complex organic matter and they dominated the community in the treatments in which microbial communities were augmented and enhanced, either directly or with fertilization.

In contrast to physical treatments, biological treatments had relatively great amounts of residual oil, which resulted in the dominance of aliphatic compound-degrading bacteria in Group II (30 d to 60 d). Biological remediations influenced compositions and enriched bacterial populations with the ability to use dispersed compounds as the growth substrates (Kleindienst et al., 2015). Furthermore, these treatments enhanced abundances and expressions of oil-degrading bacteria, such as *Alcanivorax* and *Roseovarius* (Tremblay et al., 2017; Procópio et al., 2020). Together, biological treatments and proliferation of oil-degrading bacteria from seawater input collectively contributed to the overall reduction of residual oils in a combined manner.

### 3.3. *Effectiveness of remediation techniques*

To evaluate the effectiveness of various methods of physical and biological remediation, ratios of eight target endpoints to negative control values (clean gravel) were determined (Fig. 4 and S12). The results (−30, 0, 1, 30, and 60 d) were used to determine the recovery from the implementation of remediation treatments and oil weathering, and to confirm the long-term effects. The HW/HP treatment showed the greatest recovery effectiveness (72%), since the initial implementation for 60 d, followed by MA (68%), NT (54%), and FL (53%) (Fig. 4a).

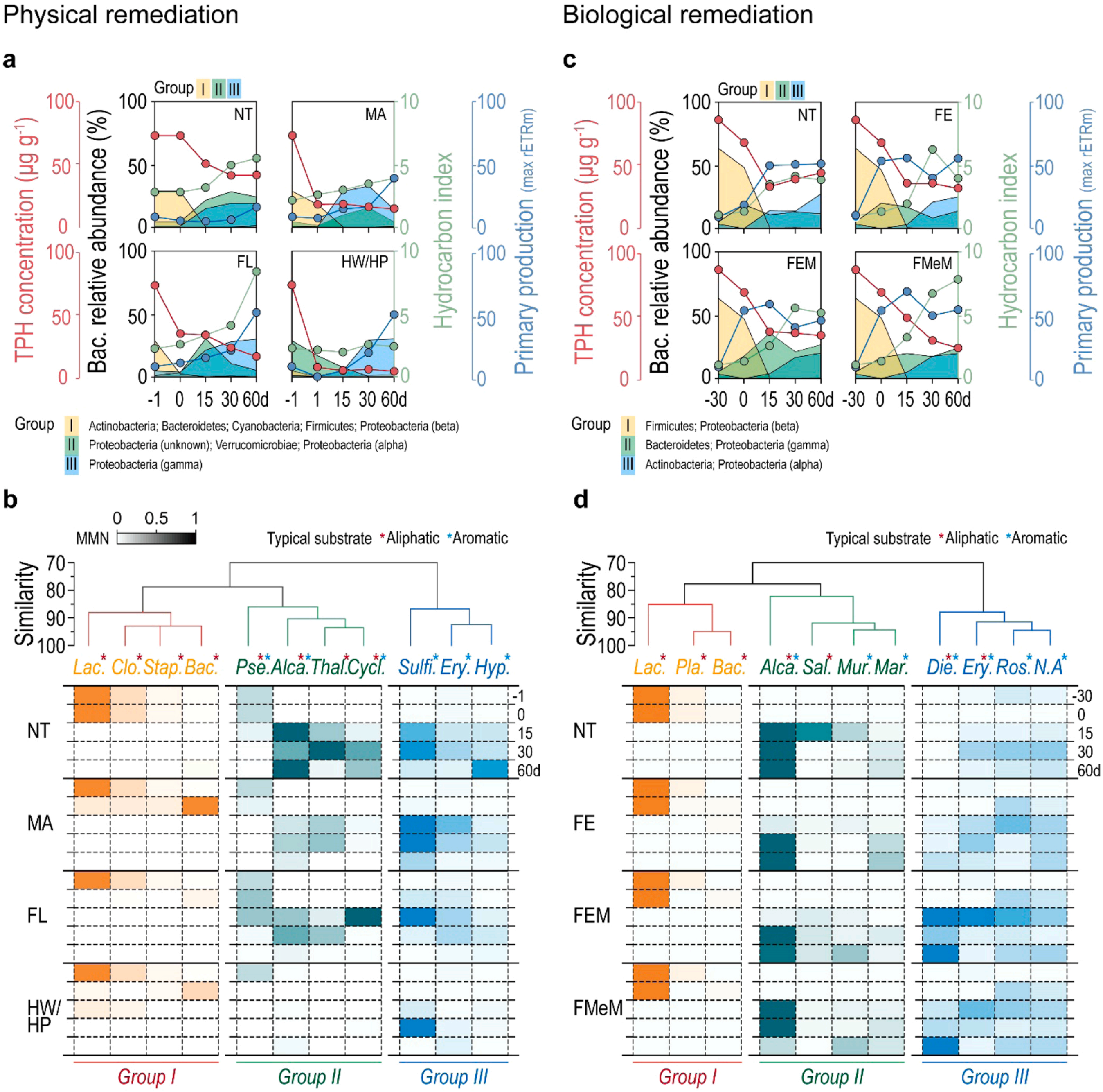


**Fig. 3.** Temporal variation of the most abundant bacteria in each treatment (**a**: physical remediation and **b**: biological remediation) and heat map showing changes to bacterial genera over the experiment periods (**c**: physical remediation and **d**: biological remediation). Genus name is provided for genera with over 3% of the relative abundance to the total for each remediation (*Lac.*: *Lactobacillus*; *Clo.*: *Clostridium*; *Stap.*: *Staphylococcus*; *Bac.*: *Bacillus*; *Pse.*: *Pseudoalteromonas*; *Alca.*: *Alcanivorax*; *Thal.*: *Thalassolituus*; *Cycl.*: *Cycloclasticus*; *Sulfi.*: *Sulfitobacter*; *Ery.*: *Erythrobacter*; *Hyp.*: *Hyphomonas*; *Pla.*: *Planococcus*; *Sal.*: *Salegentibacter*; *Mur.*: *Muricauda*; *Mar.*: *Marinicella*; *Die.*: *Dietzia*; *Ros.*: *Roseovarius*; and *N.A.*: unknown).

Except for MPB biomass and primary production, bacterial richness was adversely affected by HW/HP at 60 d (Fig. S12). After 60 d of all treatments, except for HW/HP, ecological endpoints first recovered rapidly, followed by toxicological endpoints. Although physical oil removal negatively influenced the marine life at the beginning of treatment, physical treatments accelerated the long-term recovery. In particular, ecological endpoints recovered by the MA treatment (100%) reached similar level to the control values. While the recovery of chemical endpoints were relatively great in the HW/HP treatment compared to other treatments, ecological endpoints showed little recovery. Furthermore, the HW/HP treatment had a lesser effectiveness for toxicological endpoints on 60 d (55.3%) compared to 0 d (79.2%), indicating certain unknown effect. When HW/HP treatment was implemented to wash oil-contaminated gravel, the washed oil appeared to penetrate into the deeper layers of sediment and caused potential toxicity (Street, 2011; Walther III, 2014). Overall, although physical treatments were initially effective at removing oil, the oil could remain beneath the surface and cause further adverse effects on surrounding environments.

Weathering oil-contaminated gravel for 30 d before biological treatments given generally increased the recovery of chemical, toxicological, and ecological endpoints, with mean efficiency of > 30%

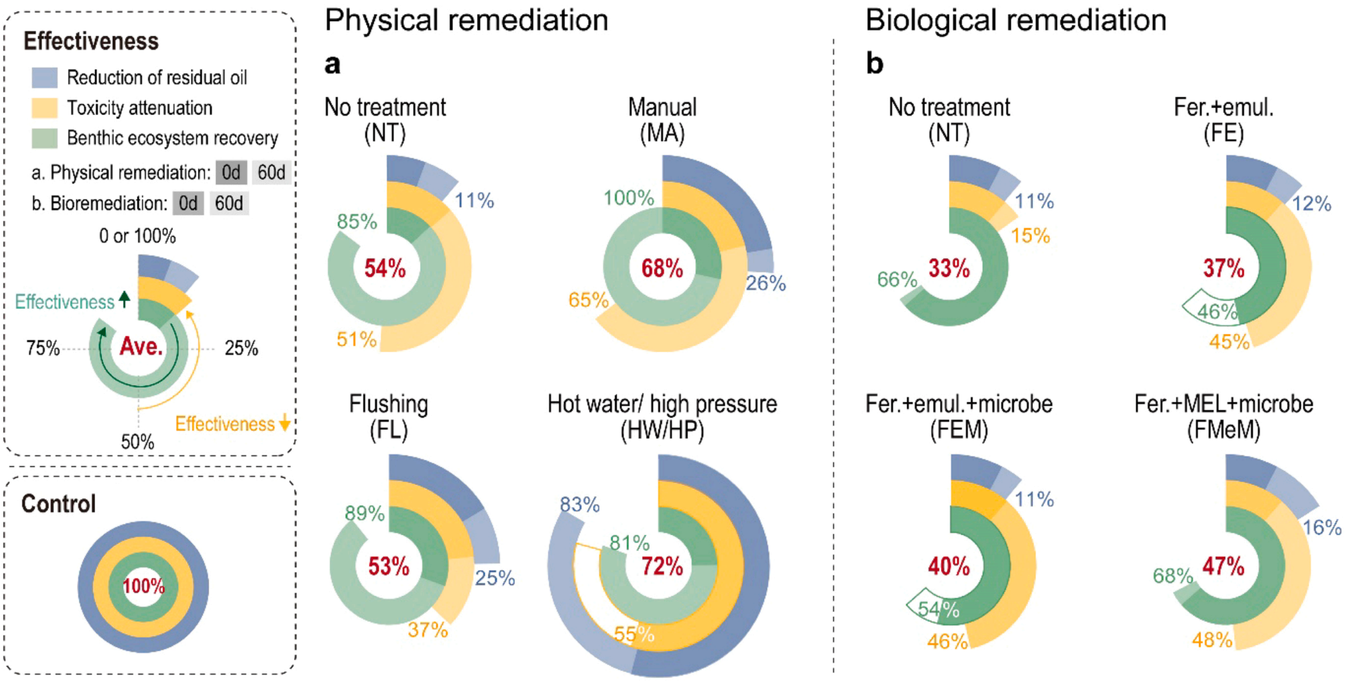


**Fig. 4.** Comparison of effectiveness based on integrated approaches with eight target elements. The integrated approaches included: 1) reduction of residual oil (TPH and UCM concentration), 2) toxicology attenuation (mortality, luminescence inhibition, and %TCDD$_{max}$), and 3) benthic ecosystem recovery (MPB cell, bacterial community richness, and primary production). Each element was calculated using the ratio to negative control.

(Fig. 4b). After 90 d, total recovery efficiency reached 47% in the FMeM treatment, which was greatest among the biological treatments. In the previous study, as in one of the series of this study, a combination of microbes was effective for remediation of the sedimentary contamination by oil. Interestingly, "no treatment" showed comparable recovery to others, indicating natural attenuation can promote soft bottom benthic community health (Lee et al., 2019a). Alternatively, the combined use of emulsifiers reduced both potential toxicity and residual oil, but adversely affected marine benthic organisms (Lönning et al., 1976). Primary production was less in the mixed solution with emulsifier at 60 d compared to 0 d (Fig. S12). Results of a previous study demonstrated that primary producers are sensitive to biological treatments (Hsiao et al., 1978). In particular, potential toxic effects by dioxin-like substances would increase after implementing the techniques, resulting in negative effects of emulsifiers on benthic organisms, including primary producers (Couillard et al., 2005; Rahsepar et al., 2016). Unlike physical techniques, the poor efficiency for reducing the total amount of residual oil could be attributed to incomplete removal of the residual oil by the dilution of the mixed solution due to seawater inflow. Overall, the present results suggested that residual oil cannot be removed completely by a sole application of biological techniques, but it is desirable to use physical techniques prior to implementation of biological treatments (Fox, 1996).

### *3.4. Best available remediation techniques*

Decision-making in response to spilled oil entails balancing economic constraints with the need to remove as much oil as possible from the spilled site while minimizing the environmental effects of the remediations used, thus optimizing environmental protection in the short and long term (Etkin, 2005). Natural attenuation is often as efficient as most active restoration alternatives and is cost-effective while minimizing the impacts of active remediation (Jahn and Robilliard, 1997). Results of the study presented here also indicated that no treatment was effective for long-term recovery of the area in which oil was spilled. While natural recovery has less effect on the environment compared to active remediations, it would be difficult to implement in areas, which are more heavily affected by oil spills. Accordingly, natural recovery might be suitable for the areas exposed to small quantities of oil spills and high energy tides (Etkin, 2002). An appropriate remediation technique should be carefully selected in a way to promote both short- and long-term recoveries while securing the immediate remediation at golden time.

In order to evaluate the best available techniques, we considered three main criteria, including economic availability, environmental performance, and technical applicability. The three criteria were subdivided into a total of 9 factors (Table S9). For physical remediation, the criteria with greater scores were different from treatment by treatment (Fig. 5a and Table 2). NT scored great in the economic availability category (0.15), which focuses on the lesser cost of removing residual oil. Various physical techniques used for remediation of oil are effective in restoring benthic ecosystems, but seem to be relatively more expensive for operating them compared to NT. Based on performance, treatment MA showed the greatest score (0.30). While MA had relatively lesser scores for economic availability, it scored well for environmental performance and technical applicability. Therefore, in comprehensive consideration of all three categories, MA was evaluated as the BAT among physical techniques tested during the present investigation (Fig. 5a). The HW/HP treatment was ranked next to MA because of a lesser score for technical applicability.

Based on the MAUT analysis, FMeM treatment scored greater in all categories compared to other biological techniques (Fig. 5a and Table 1). Other techniques scored similarly in environmental performance and technical applicability (0.18–0.27), but FMeM scored the greatest in environmental performance (0.45) because of a quick reduction of oil. The NT scored great in economic availability (0.15) but little in environmental performance and technical applicability, because of its relatively poor ability to remove residual oil and may incur additional remediation cost. However, on exposed shorelines with high-energy tides, the majority of oil is expected to be cleared within a seasonal cycle. Natural cleaning is similar to flushing in principle, but relies on the natural energy of the waves to give far larger quantities of water

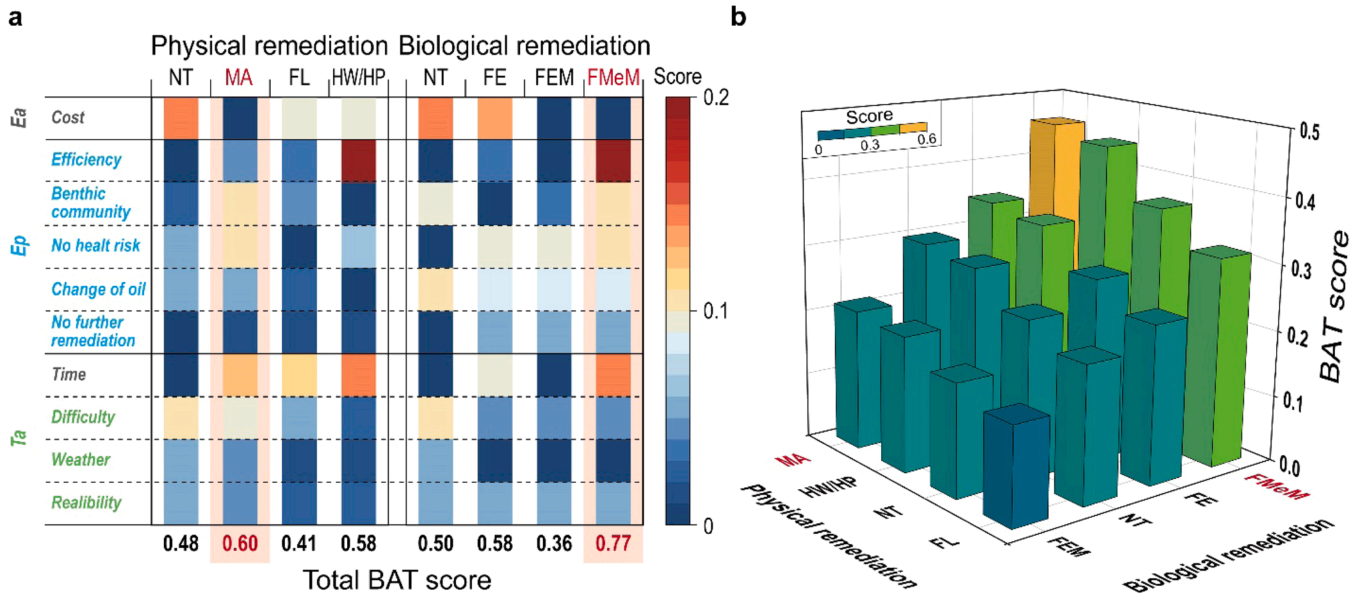


**Fig. 5.** Score diagram of the best available techniques (BATs). (**a**) Total score calculated from ten criteria using multi-attribute utility theory (MAUT) analysis for each technique. *Ea*: economic availability; *Ep*: environmental performance; *Ta*: technical applicability. (**b**) Evaluation of combined physical and biological remediation techniques based on multiplying each score in the oil spill simulation.

**Table 2**
Multi-attribute utility theory (MAUT) analysis for selecting the best available techniques for oil spill response.

| Remediation techniques | Method | [1]Ea | Rank | [2]Ep | Rank | [3]Ta | Rank | Ea+Ep | Rank | Ea+Ta | Rank | Ep+Ta | Rank | Ea+Ep+Ta | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Physical | NT | **0.15** | **1** | 0.13 | 3 | 0.20 | 4 | 0.28 | 3 | **0.35** | **1** | 0.33 | 3 | 0.48 | 3 |
| | MA | 0.00 | 4 | **0.30** | **1** | **0.30** | **1** | 0.30 | 2 | 0.30 | 4 | **0.60** | **1** | **0.60** | **1** |
| | FL | 0.09 | 2 | 0.11 | 4 | 0.21 | 2 | 0.21 | 4 | 0.30 | 2 | 0.32 | 4 | 0.41 | 4 |
| | HW/HP | 0.09 | 2 | 0.28 | 2 | 0.21 | 2 | **0.37** | **1** | 0.30 | 2 | 0.48 | 2 | 0.57 | 2 |
| Biological | NT | **0.15** | **1** | 0.15 | 4 | 0.20 | 3 | 0.30 | 3 | 0.35 | 2 | 0.35 | 4 | 0.50 | 3 |
| | FE | 0.14 | 2 | 0.18 | 3 | 0.27 | 2 | 0.31 | 2 | **0.40** | **1** | 0.45 | 2 | 0.58 | 2 |
| | FEM | 0.00 | 3 | 0.18 | 2 | 0.18 | 4 | 0.18 | 4 | 0.18 | 4 | 0.36 | 3 | 0.36 | 4 |
| | FMeM | 0.00 | 3 | **0.45** | **1** | **0.32** | **1** | **0.45** | **1** | 0.32 | 3 | **0.77** | **1** | **0.77** | **1** |

[a] Ea (economic availability): cost (relatively inexpensive)

[b] Ep (environmental performance): efficiency (95–99% removal rate of residual oil); health (reduction rate of potential toxicity and recovery of benthic community); change of oil (do not change physical/chemical characteristics of oil); necessity for further treatment (no further remediation required)

[c] Ta (technical applicability): time (removal of contaminant within days); level of difficulty (easy to maintain and operate); weather (favorable for application of method); reliability (the method works the majority of the time)

than could be delivered by pumps. Future research is needed to settle the high-energy tide system in the mesocosm system, and determine its potential for removing oil. Overall, based on biological techniques tested, FMeM was deemed to be the BAT. However, these biological technologies do not rapidly remove a large amount of residual oil in a short-time period, so they need to be employed in conjunction with physical techniques (Zengel et al., 2015).

Assuming an oil spill occurred on a gravel shoreline, the BAT could be selected by multiplying each previously obtained score from the MAUT analysis (Fig. 5b). When evaluating various remediation techniques in this study, the most effective remediation for oil was calculated as "MA + FMeM", which scored 0.46. The "HW/HP + FMeM" received a comparable score of 0.44, but a penetration of washed oil into the substrate layer and thus long-term adverse effects on the benthic community would be required. While employing various remediation techniques in this study, although various other factors, including labor requirement, workplace safety, and the post management should be included in evaluating each technique, quantitative studies were insufficient to include all of them. Therefore, the result of this study may not be suitable to all oil spill situations.

Physical remediation is conducted to recover sedimentary contamination by oil at initial stage. Biological remediation is applied to promote health of benthic communities (Tuler et al., 2007). Physical and biological remediation treatments should be applied differently depending on the habitat characteristics of the spilled site and it is recommended that the remediation technique suggested in this study would be applied to a gravel covered coast with an environmental sensitive index (ESI) of "6A" (Table S10) (Michel, 2013). When the oil spilled from sea is introduced to the coast, MA treatment proposed in this study can limit the spread of pollution by reducing the risk of oil landfilled in the coast by the wave or the wind (POSOW, 2013). In general, a combination of physical and biological techniques is well known to effectively remedy the oil spills (Byroade et al., 1981; Reed et al., 1995; Chen et al., 2019). According to the results of this study, the use of the initial implementation of MA and the use of FMeM treatment can promote the recovery of benthic community health avoiding further adverse effects. Further study to conduct the experiment as successive combining between physical and biological remediation is necessary for applying to in situ oil spill response. Final cleanup should be recommended to begin only as successive after the initial cleanup of the large-scale accumulated oil has been completed and all threats of new significant deposits have been removed (Venosa et al., 2004).

## 4. Conclusions

In conclusion, the present study successfully analyzed and demonstrated the integrated aspects of the efficiency of various remediation techniques in relation to early and long-term recovery of a gravel shore. Much environmental and technical science research has been under taken recently to better understand the evaluation of efficiency in the physical and biological remediation techniques. For removing the residual oil more effectively and eco-friendly, application of appropriate remediation techniques to be suitable the targeted shoreline is required. The selection of the techniques cannot be determined unless considering economic availability, environmental performance, and technical applicability. With experimental results, further study of encompassing economic, social, technical, and environmental aspects is needed to oil contaminated shoreline. The results provide guidelines on the techniques best suited for recovering an oil-contaminated gravel shore while facilitating the recovery of adversely affected benthic ecosystems, elsewhere.

## CRediT authorship contribution statement

**Taewoo Kim**: Conceptualization, Formal analysis, Statistical analyses, Visualization, Writing – original draft. **Changkeun Lee**: Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Junghyun Lee**: Writing – review & editing, Data review, Formal analysis. **Hanna Bae**: Investigation, Formal analysis. **Junsung Noh**: Review & editing, Project administration. **Seongjin Hong**: Investigation, Project administration, Writing – review & editing. **Bong-Oh Kwon**: Investigation, Project administration, Funding acquisition. **Jae-Jin Kim:** Investigation, Data curation. **Un Hyuk Yim**: Investigation, Project administration. **John P. Giesy**: Investigation, Writing – review & editing. **Gap Soo Chang**: Investigation, Writing – review & editing. **Jong Seong Khim**: Conceptualization, Investigation, Statistical analyses, Visualization, Writing – review & editing, Project administration, Funding acquisition, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.128945.

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<Journal of Hazardous Materials>

*Supplementary materials for*

# Best Available Technique for the Recovery of Marine Benthic Communities in a Gravel Shore after the Oil Spill: A Mesocosm-based Sediment Triad Assessment

Taewoo Kim[1], Changkeun Lee[1], Junghyun Lee, Hanna Bae, Junsung Noh, Seongjin Hong,
Bong-Oh Kwon, Jae-Jin Kim, Un Hyuk Yim, Gap Soo Chang, John P. Giesy, Jong Seong Khim[*]

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References

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[*] **Corresponding author.** *E-mail address*: jskocean@snu.ac.kr (J.S. Khim).

## Supplementary Tables

**Table S1**. General properties of Iranian Heavy Crude oil used in this study.

| **Properties** | **Unit** | **Value** |
|---|---|---|
| Density (15°C) | g $mL^{-1}$ | 0.9 |
| API Gravity | API | 30 |
| Dynamic Viscosity | mPa.s | 20 |
| Surface Tension (15°C) | mN/M | 26 |
| Interfacial Tension (15°C, 33psu) | mN/M | 22 |
| Sulphur Content | % w/w | 1 |
| Water Content | % w/w | < 0.025 |
| Total Petroleum Hydrocarbons (TPH) | µg $g^{-1}$ | 300,000 |
| Unresolved Complex Mixture | µg $g^{-1}$ | 190,000 |
| *n*-Alkane | µg $g^{-1}$ | 40,000 |
| Total 16 PAHs | µg $g^{-1}$ | 396 |
| Total alkylated PAHs | µg $g^{-1}$ | 12,216 |

**Table S2.** Applicable techniques for cleaning oil in gravel and/or rocky shore (ITOPF, 2011)

| Approach | Rocks and boulders | | Gravels and pebbles | |
|---|---|---|---|---|
| | **Accessible** | **Inaccessible** | **Accessible** | **Inaccessible** |
| **Stage 1** | Skimmers/pumps, vacuum trucks, flushing | Manual, Manual & sorbents | Skimmers/pumps, vacuum trucks, flushing | Manual, manual & sorbents |
| **Stage 2** | Pressure washing, sorbent materials, natural cleaning | Natural cleaning, hand wiping | Flushing, surf/cobble washing, mechanical, natural cleaning | Natural cleaning, hand wiping |
| **Stage 3** | Natural cleaning, pressure washing, sand blasting | Natural cleaning | Natural cleaning, surf/cobble washing, sand blasting | Natural cleaning |

**Table S3**. Summary of the experimental conditions and description of remediation techniques in this study.

| **Experimental conditions** | | | |
|---|---|---|---|
| Salinity | 28 – 30 psu | | |
| Dissolved oxygen | > 80% | | |
| pH range | 7.9 – 8.2 | | |
| Experimental duration (d) | 90 | | |
| Gravel size | *Φ* <60 mm | | |
| Crude oil | Iranian Heavy Crude oil | | |
| Volume ratio (gravel:oil) | 10:1 | | |
| **Remediation** | **Techniques** | **Description** | **Reference** |
| Physical- and biological- | Manual | Oil absorbent, handling, <30 bar | ITOPF (2011) |
| | Flushing | Spraying for 10 min, <20 bar | ITOPF (2011) |
| | Hot water & high pressure | M1002 DS, spraying for 3 min, 130 bar | ITOPF (2011) |
| | Fertilizer | Oleophilic fertilizer; S200, 15 mL per treatment | Nikolopoulou et al. (2007) |
| | Multi-enzyme liquid | Oil Spill Eater II, 15 mL per treatment | Das and Chamdram (2011) |
| | Emulsifier | Tween 80, 15 mL per treatment | Feng et al. (2011) |
| | Microbe solution | Microbial solution (bacteria mixture; *Alcanivorax* sp., *Roseovarius* sp., *Corynebacterium variabilis, Dietzia* sp*.*, *Sphingomonas yanoikuyae*, *Kyotococcus sedentarius*, *Bacillus aquimaris*, *Novosphingobium*, *Pentaromativorans*, and *Yarrowia lipolytica*), 50 mL per treatment | Gao and Zaki (2011) |

**Table S4**. Advantages and disadvantages of various remediation techniques for oil removal.

| Remediation techniques | Advantage | Disadvantage |
|---|---|---|
| Physical | | |
| [a] Manual | Application of all kind of oil | Moderate expensive |
| | No maintenance required | Labour intensive |
| | Effective as final clean up | Weather condition dependent |
| [b] Flushing | Application of all kind of oil | Moderate expensive |
| | Cost effective | Threaten for marine organism |
| | Less labour intensive | Weather condition dependent |
| [b] Hot water & high pressure | Application of all kind of oil | Moderate expensive |
| | Quick | Threaten for marine organism |
| | Less labour intensive | Possibility of second oil contamination |
| | | Weather condition dependent |
| Biological | | |
| [a] Fertilizer | Application of all kind of oil | Moderate Expensive |
| | No maintenance required | Disrupting balance of nutrient and pH |
| | Less labour intensive | Oil dependent |
| | | Indigenous bacteria dependent |
| [a] Emulsifier | Effective on wide range of oil | Potential toxicity |
| | Accelerating the degradation | Expensive |
| | Less labour intensive | Second pollution |
| [a] MEL | Non-aggressive | Slow recovery |
| | No maintenance required | Expensive |
| | Environmentally friendly | Oil dependent |
| [c] Microbe solution | Non-aggressive | Slow recovery |
| | Accelerating the degradation | Expensive |
| | Environmentally friendly | Required maintenance |

Data from [a] Dave and Ghaly (2011), [b] Baker (1995), and [c] Gudi et al. (2016).

**Table S5**. Temporal variation of sedimentary TPH and UCM concentration in the 60–90 days; a total of 10 treatments including positive controls.

| **Remediation techniques** | **Compounds** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TPH (ug g$^{-1}$) | | | | | | UCM (ug g$^{-1}$) | | | | | |
| **Physical** | **-1d** | **0d** | **15d** | **30d** | **45d** | **60d** | **-1d** | **0d** | **15d** | **30d** | **45d** | **60d** |
| NT | 70 | 70 | 51 | 41 | 39 | 42 | 56 | 56 | 39 | 31 | 29 | 24 |
| MA | 70 | 18 | 19 | 16 | 15 | 15 | 56 | 11 | 14 | 11 | 11 | 10 |
| FL | 70 | 30 | 33 | 24 | 23 | 17 | 56 | 23 | 26 | 19 | 15 | 15 |
| HW/HP | 70 | 8 | 5 | 6 | 5 | 9 | 56 | 3 | 2 | 2 | 1 | 1 |
| Control | 1 | 1 | 0.6 | 0.3 | 1 | 1 | 0.4 | 0.4 | 0.2 | 0.7 | 0.5 | 0.6 |
| **Biological** | **-30d** | **0d** | **15d** | **30d** | **45d** | **60d** | **-30d** | **0d** | **15d** | **30d** | **45d** | **60d** |
| NT | 86 | 68 | 73 | 33 | 39 | 32 | 52 | 35 | 58 | 27 | 35 | 28 |
| FE | 86 | 68 | 46 | 36 | 36 | 38 | 52 | 35 | 68 | 28 | 32 | 34 |
| FEM | 86 | 68 | 36 | 37 | 36 | 50 | 52 | 35 | 27 | 31 | 32 | 45 |
| FMeM | 86 | 68 | 44 | 48 | 30 | 39 | 52 | 35 | 41 | 36 | 26 | 35 |
| Control | 1 | 1 | 1 | 0 | 0 | 0 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 |

NT: no treatment; MA: manual; FL: flushing; HP: hot water & high pressure; FE: fertilizer + emulsifier; FEM: fertilizer + emulsifier + microbe; FMeM: fertilizer + MEL + microbe.

**Table S6**. Evaluating developmental effect (spinal curvature and cardiac edema) and mortality of the residual oil exposure on zebrafish embryo (in vivo bioassay).

| **Remediation techniques** | **Spinal curvature (%)** | | | **Cardiac edema (%)** | | | **Mortality (%)** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Physical** | **-1d** | **0d** | **60d** | **-1d** | **0d** | **60d** | **-1d** | **0d** | **60d** |
| NT | 42 | 42 | 36 | 58 | 58 | 44 | 94 | 94 | 14 |
| MA | | 28 | 14 | | 42 | 14 | | 72 | 10 |
| FL | | 8 | 6 | | 8 | 6 | | 53 | 13 |
| HW/HP | | 8 | 3 | | 3 | 3 | | 31 | 33 |
| **Biological** | **-30d** | **0d** | **60d** | **-30d** | **0d** | **60d** | **-30d** | **0d** | **60d** |
| NT | 3 | 19 | 36 | 3 | 17 | 36 | 69 | 72 | 55 |
| FE | | | 3 | | | 3 | | | 50 |
| FEM | | | 19 | | | 17 | | | 44 |
| FMeM | | | 31 | | | 36 | | | 39 |

NT: no treatment; MA: manual; FL: flushing; HP: hot water & high pressure; FE: fertilizer + emulsifier; FEM: fertilizer + emulsifier + microbe; FMeM: fertilizer + MEL + microbe.

**Table S7**. Evaluating AhR mediated potency of the residual oil by H4lIE-*luc* (in vitro bioassay).

| **Remediation techniques** | **%TCDD$_{max}$** | | |
|---|---|---|---|
| **Physical** | **-1d** | **0d** | **60d** |
| NT | 48.3±8.1 | 48.3±8.1 | 39.3±6.6 |
| MA | | 37.1±6.1 | 20.3±2.7 |
| FL | | 38.6±8.2 | 24.8±5.5 |
| HW/HP | | 12.1±2.1 | 19.8±4.3 |
| **Biological** | **-30d** | **0d** | **60d** |
| NT | 47.9±5.8 | 38.6±6.8 | 43.8±12.2 |
| FE | | | 48.7±2.1 |
| FEM | | | 38.5±1.1 |
| FMeM | | | 34.0±1.5 |

NT: no treatment; MA: manual; FL: flushing; HP: hot water & high pressure; FE: fertilizer + emulsifier; FEM: fertilizer + emulsifier + microbe; FMeM: fertilizer + MEL + microbe.

**Table S8**. Temporal changes of the number of diatoms in each treatment.

| Remediation techniques | Number of diatom | | |
|---|---|---|---|
| **Physical** | **-1d** | **0d** | **60d** |
| Control | 100 | 100 | 77 |
| NT | 18 | 18 | 75 |
| MA | 18 | 1 | 100 |
| FL | 18 | 7 | 52 |
| HW/HP | 18 | 1 | 90 |
| **Biological** | **-30d** | **0d** | **60d** |
| Control | 16 | 100 | 100 |
| NT | 16 | 72 | 100 |
| FE | 16 | 72 | 54 |
| FEM | 16 | 72 | 68 |
| FMeM | 16 | 72 | 100 |

**Table S9**. Modified evaluation criteria from Dave and Ghaly (2011) for marine oil spill remediation techniques.

| Criteria | | Definition | Score |
|---|---|---|---|
| Economic availability | Cost | Relatively inexpensive | 15 |
| Environmental performance | Efficiency | [a] 95-99% removal | 20 |
| | Health | [a] Reduction rate of potential toxicity | 10 |
| | | [a] Recovery of benthic community | 10 |
| | Change of oil | Do not change physical/chemical characteristics of oil | 5 |
| | Further treatment | No further treatment required | 5 |
| Technical applicability | Time | [a] Removes contaminant within days | 15 |
| | Level of difficulty | Easy to maintain and operate | 10 |
| | Weather | Favourable for application of method | 5 |
| | Reliability | The method works the majority of the time | 5 |

[a] Scoring from data in this study.

**Table S10**. Environmental sensitive index (ESI) types for estuarine settings modified from NOAA (2013).

| ESI no. | Estuarine |
|---|---|
| 1A | Exposed rocky shores |
| 1B | Exposed, solid man-made structures |
| 1C | Exposed rocky cliffs with boulder talus base |
| 2A | Exposed wave-cut platforms in bedrock, mud, or clay |
| 2B | Exposed scarps and steep slopes in clay |
| 3A | Fine- to medium-grained sand beaches |
| 3B | Scarps and steep slopes in sand |
| 3C | Tundra cliffs |
| 4 | Coarse-grained sand beaches |
| 5 | Mixed sand and gravel beaches |
| **6A** | **Gravel beaches (granules and pebbles)** |
| 6B | Riprap |
| 7 | Exposed tidal flats |
| 8A | Sheltered scarps in bedrock, mud, or clay |
| 8B | Sheltered, solid man-made structures |
| 8C | Sheltered riprap |
| 8D | Sheltered rocky rubble shores |
| 8E | Peat shorelines |
| 8F | None |
| 9A | Sheltered tidal flats |
| 9B | Vegetated low banks |
| 9C | Hypersaline tidal flats |
| 10A | Salt- and brackish-water marshes |
| 10B | Freshwater marshes |
| 10C | Swamps |
| 10D | Scrub-shrub wetlands; mangroves |
| 10E | Inundated low-lying tundra |

# Supplementary Figures

## Graphical design of indoor experiment

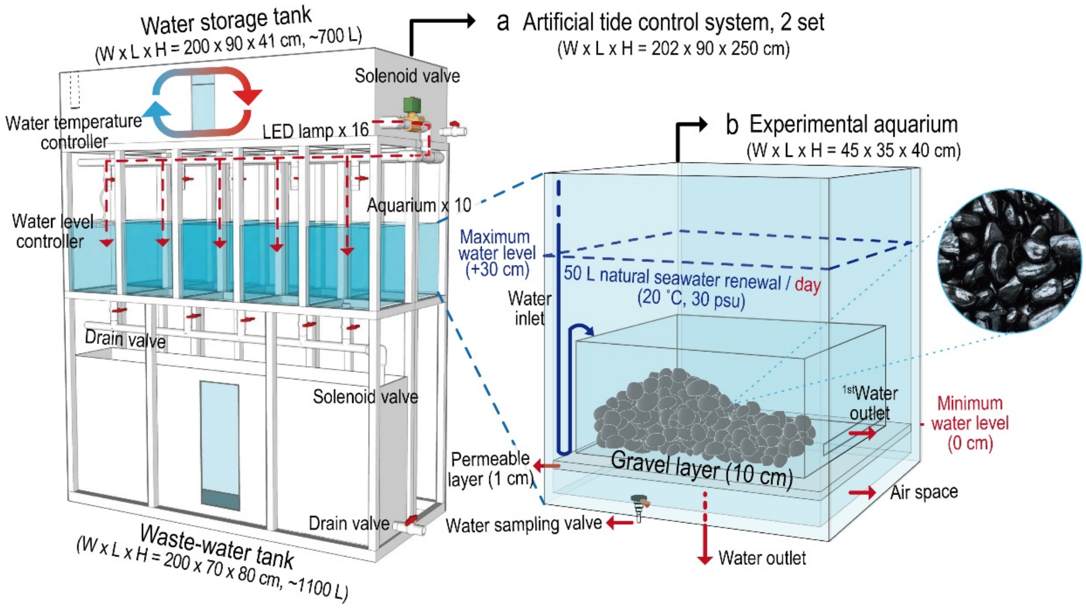


**Fig**. **S1**. Schematic of the artificial tide control system. **(a)** Artificial tide control system, and **(b)** experiment aquarium. Experimental conditions are detailed in Table S1.

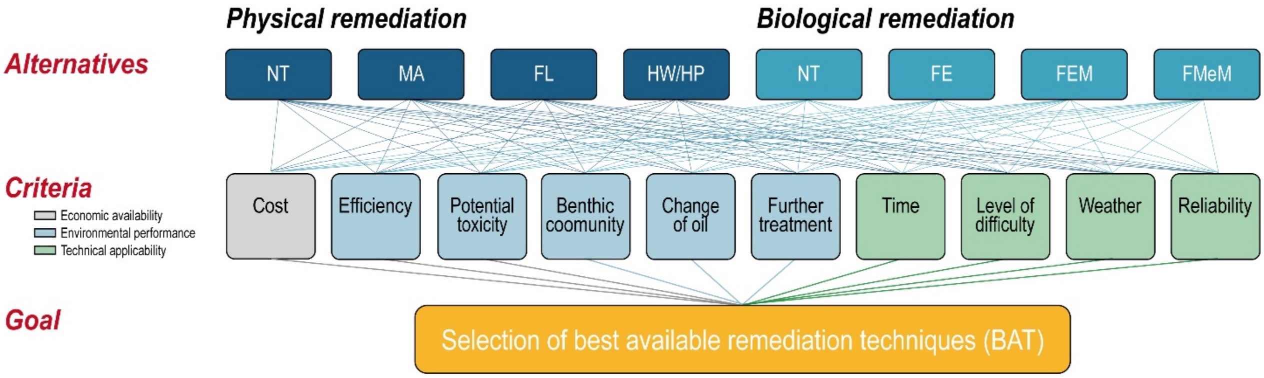
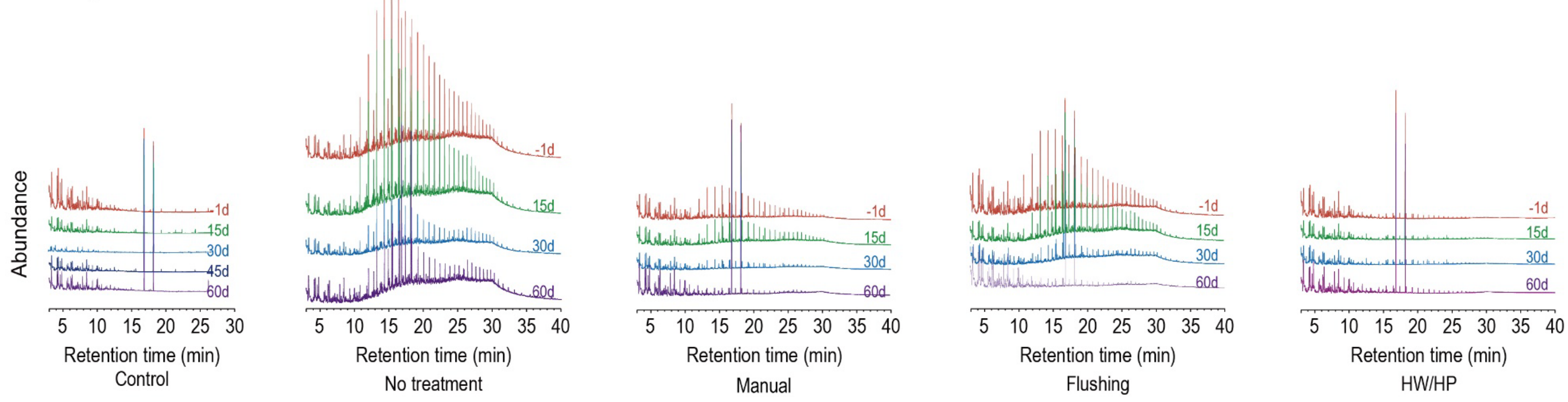


**Fig**. **S2**. Proposed criteria for best available techniques (BATs) in physical and biological remediation against the oil spills.

**a** ***Physical remediation***



**b** ***Biological remediation***

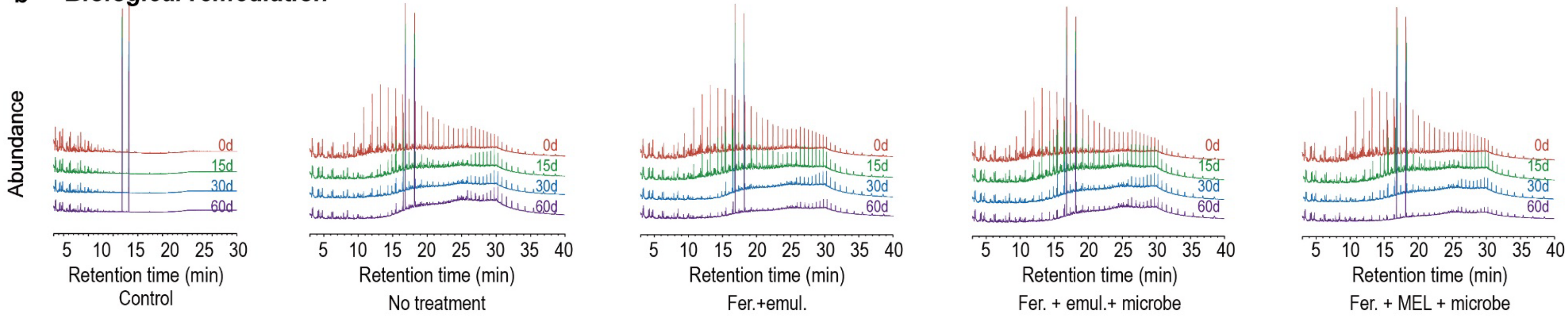


**Fig**. **S3**. Overall chromatograms obtained from gravel samples in each physical and biological remediation technique for oil removal from day 0 to 60 d.

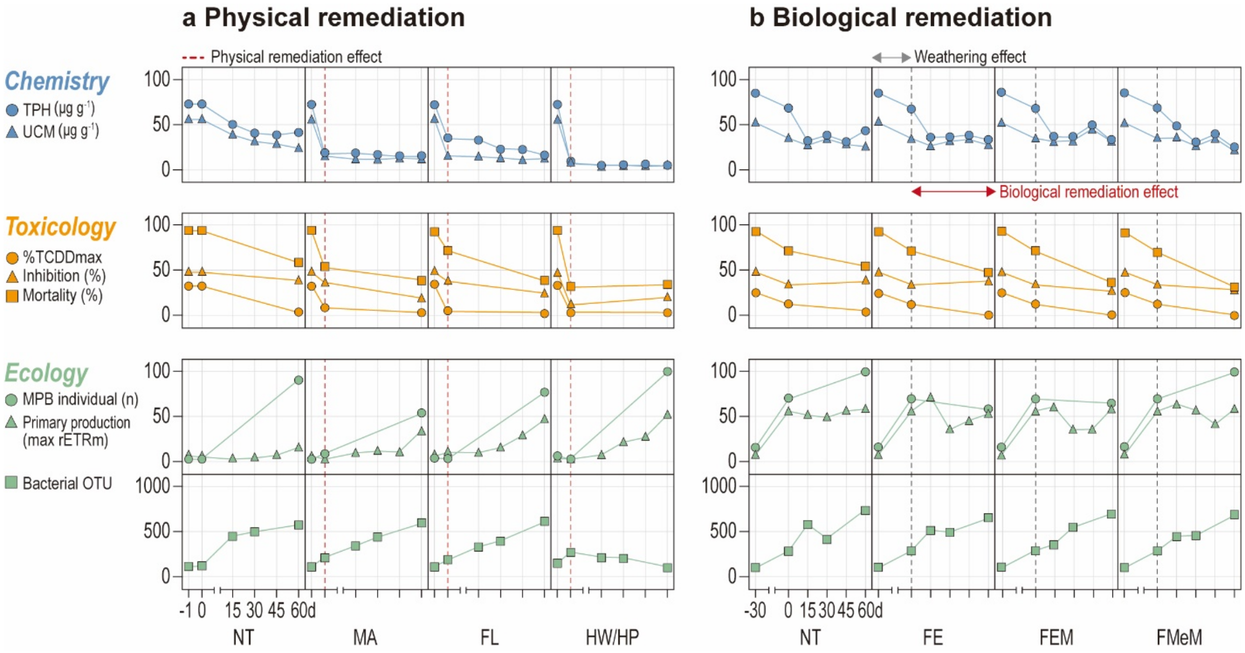


**Fig**. **S4.** Temporal variation in TPH and UCM concentrations (μg $g^{-1}$), zebrafish embryo mortality (%), *Vibrio fischeri* luminescence inhibition rate (%), AhR-mediated potency (%$TCDD_{max}$), MPB cell (n), primary production (P.P) (max rETRm), and bacterial OTUs measured in the experiments periods. Eight remediation techniques were implemented including control.

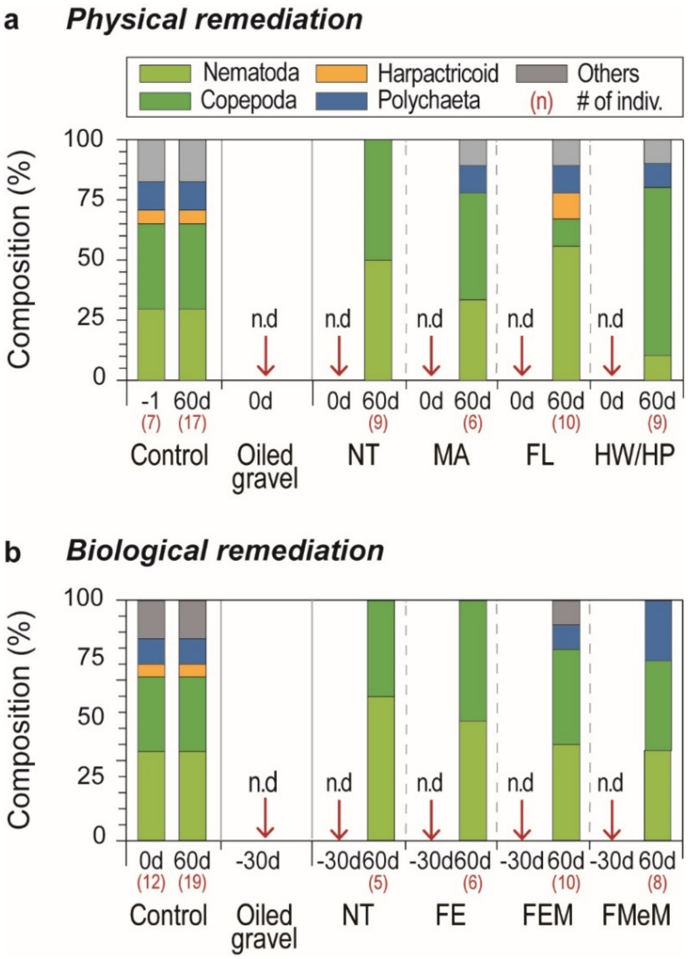


**Fig**. **S5**. Observed meiofauna and its composition in each treatment during the experiment.

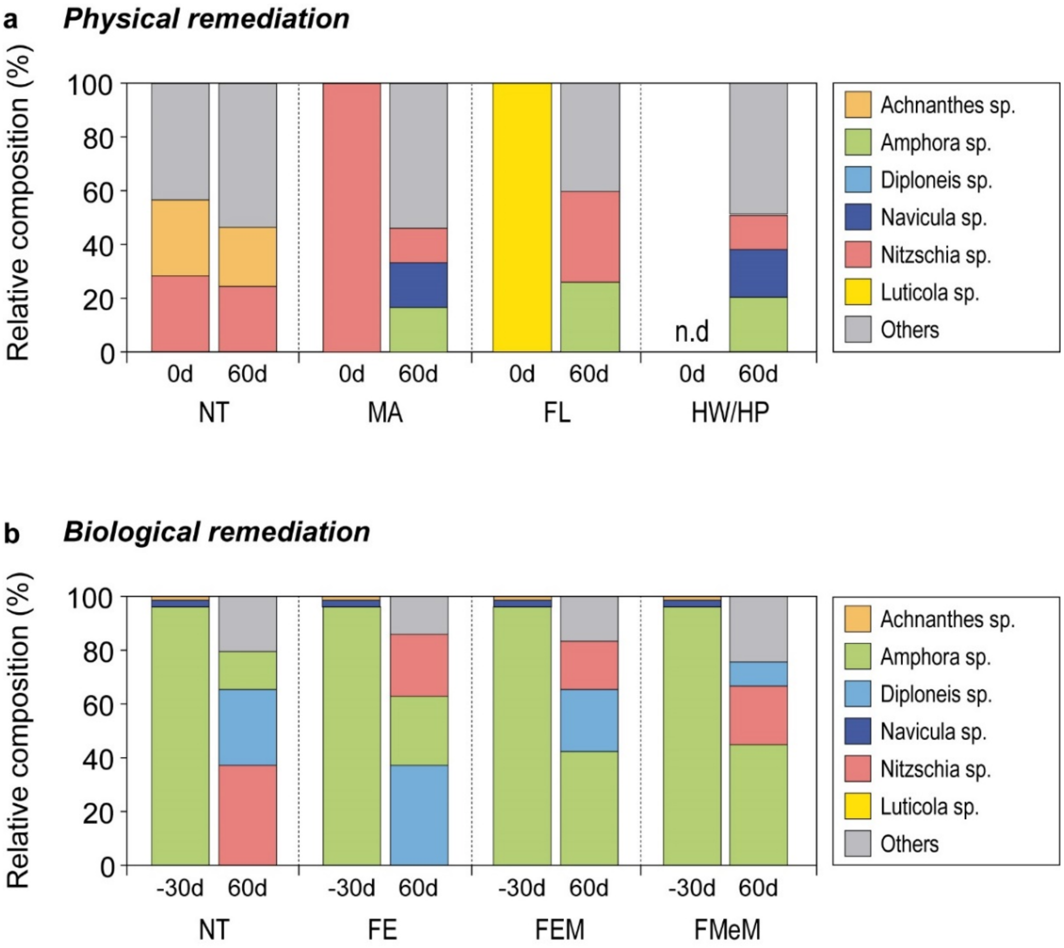


**Fig**. **S6**. Relative composition and abundance of the dominant diatom species in each treatment during the experiment.

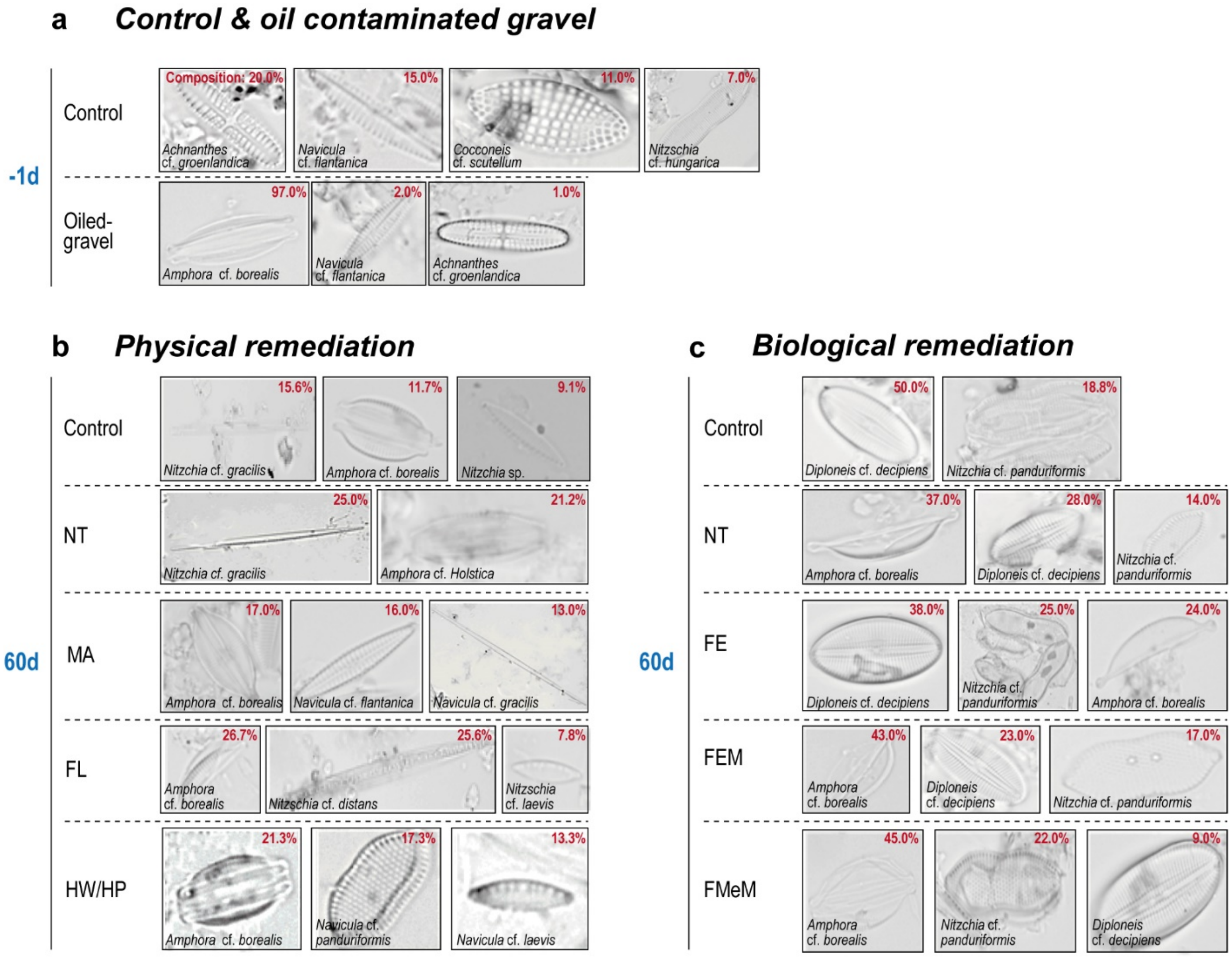


**Fig**. **S7**. Images of dominant species of diatom in each treatment.

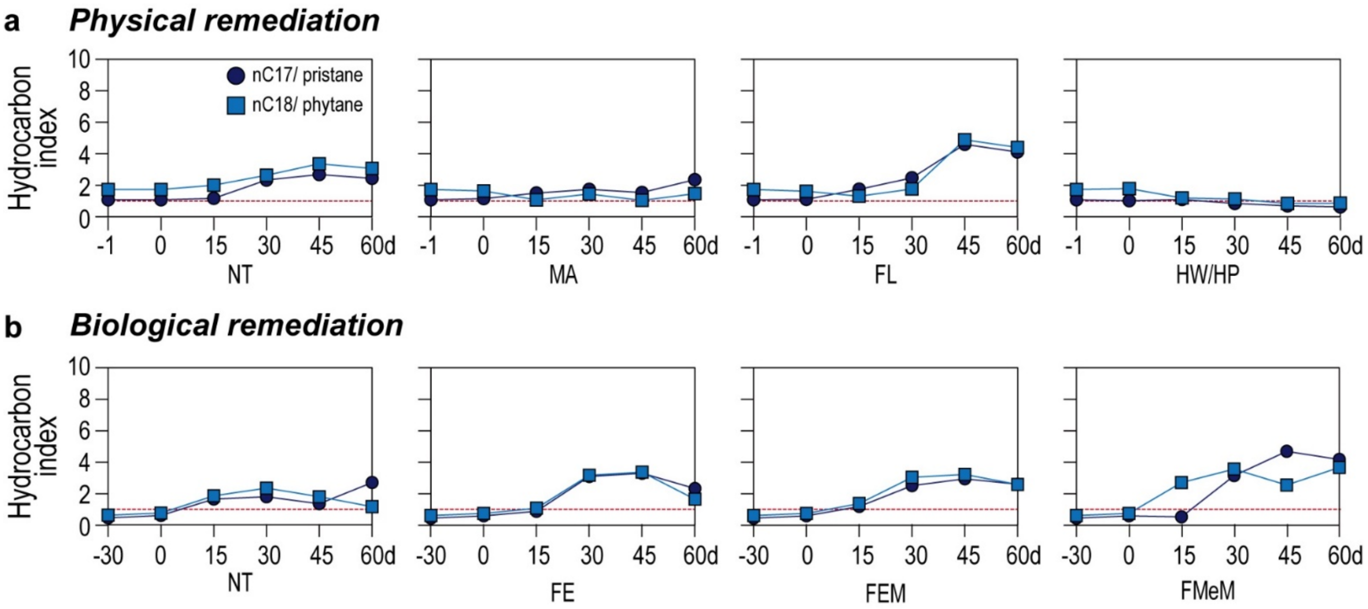
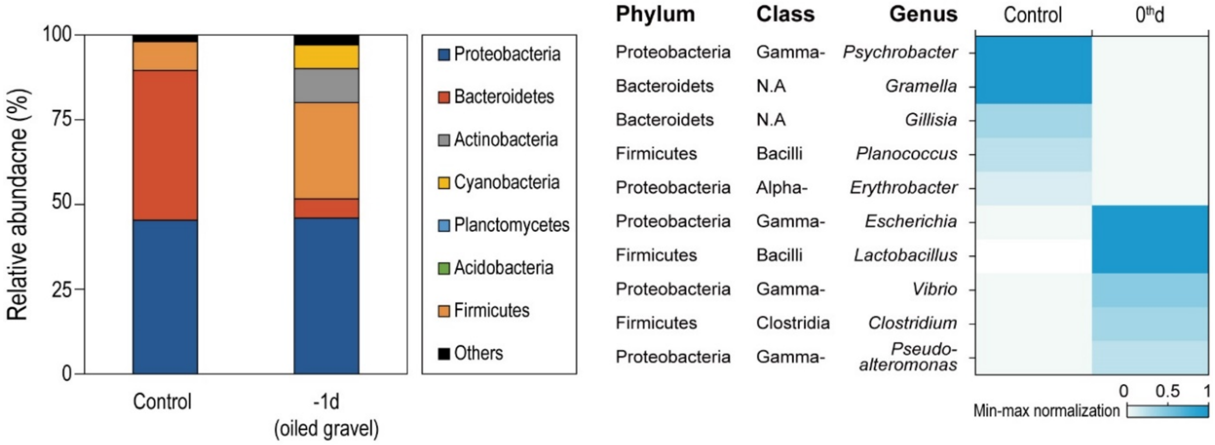


**Fig**. **S8**. Temporal variations of nC17/ pristine and nC18/ phytane in the oil-contaminated gravel in each treatment.

**a** ***Physical remediation***



**b** ***Biological remediation***

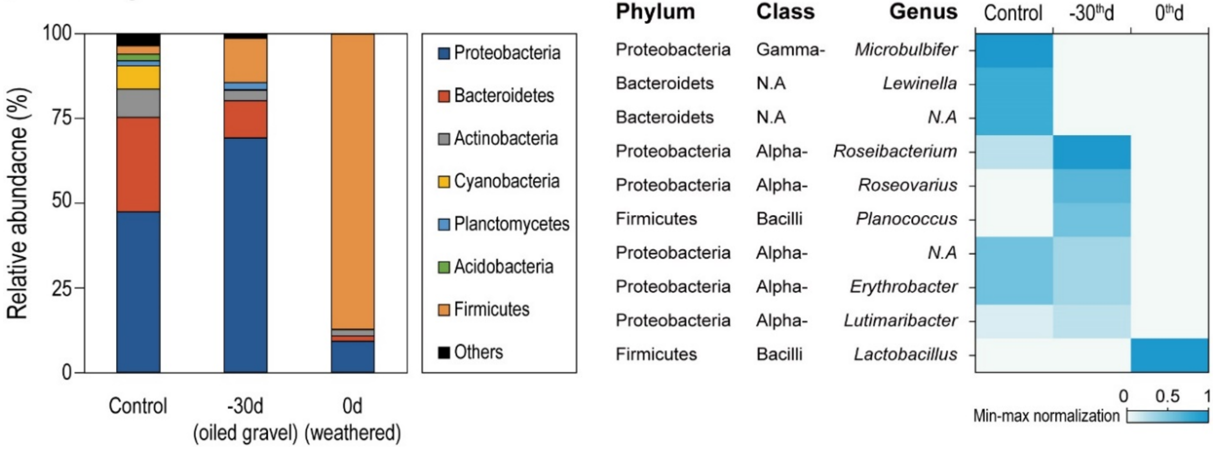


**Fig**. **S9**. Relative composition of bacterial community and dominant genus in the control and oil-contaminated gravel. **(a)** Negative control and oil-contaminated gravel, **(b)** negative control, oil-contaminated gravel, and oil-weathered gravel.

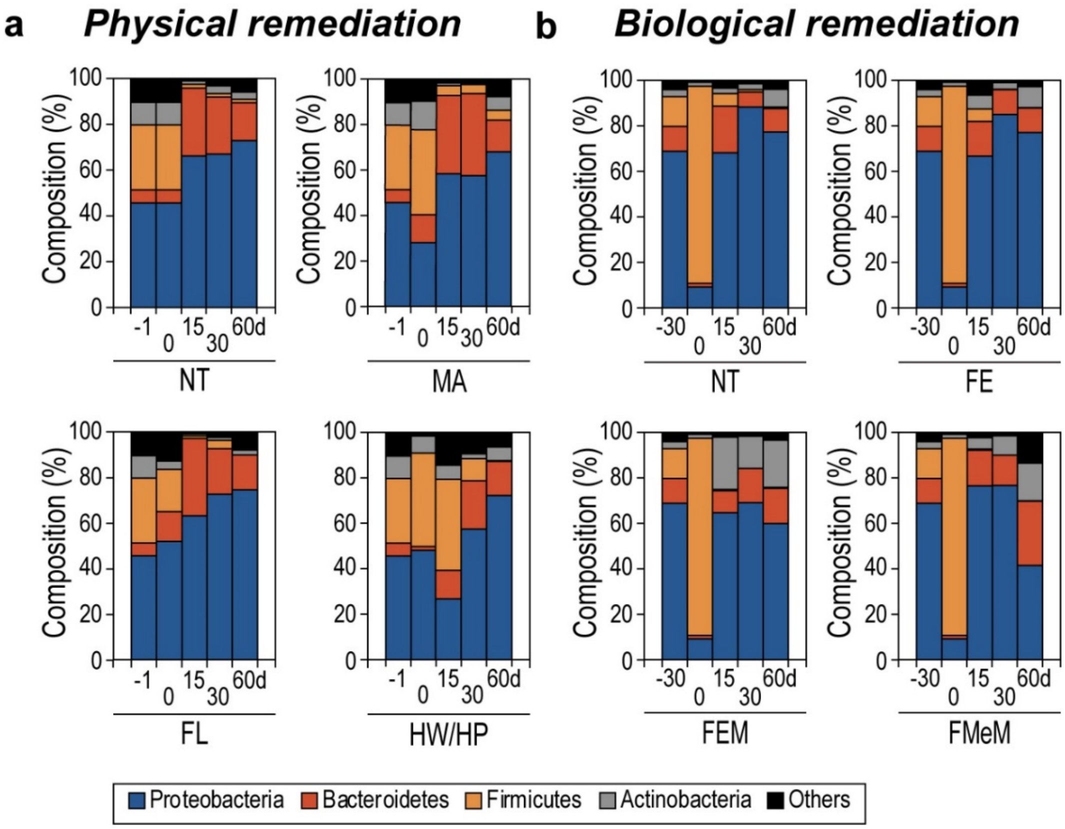


**Fig**. **S10**. Relative composition of bacterial community and dominant genus in each treatment from day 0 d to 60 d and -30 d to 60 d.

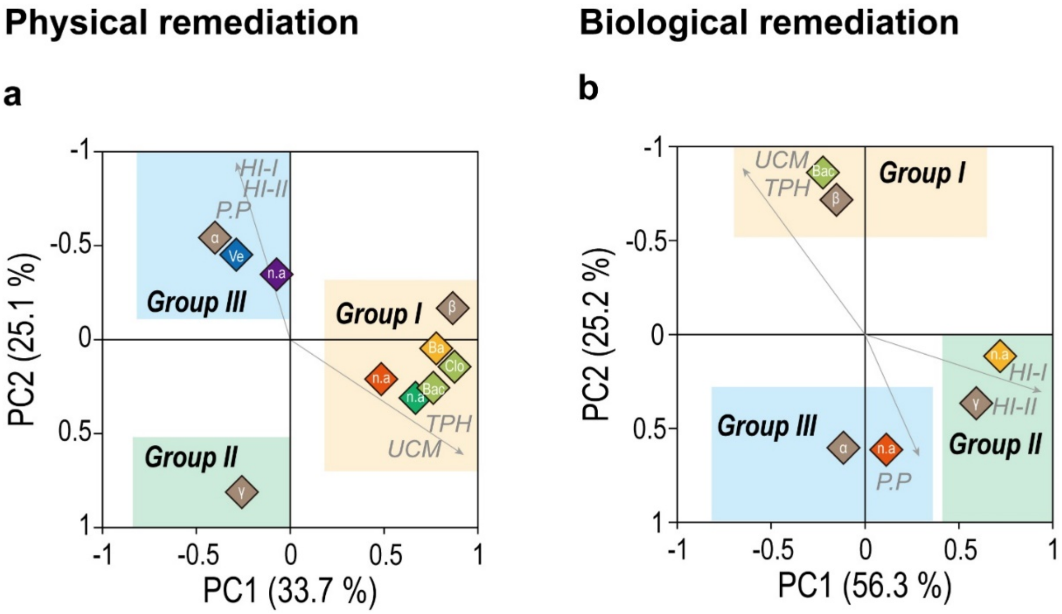
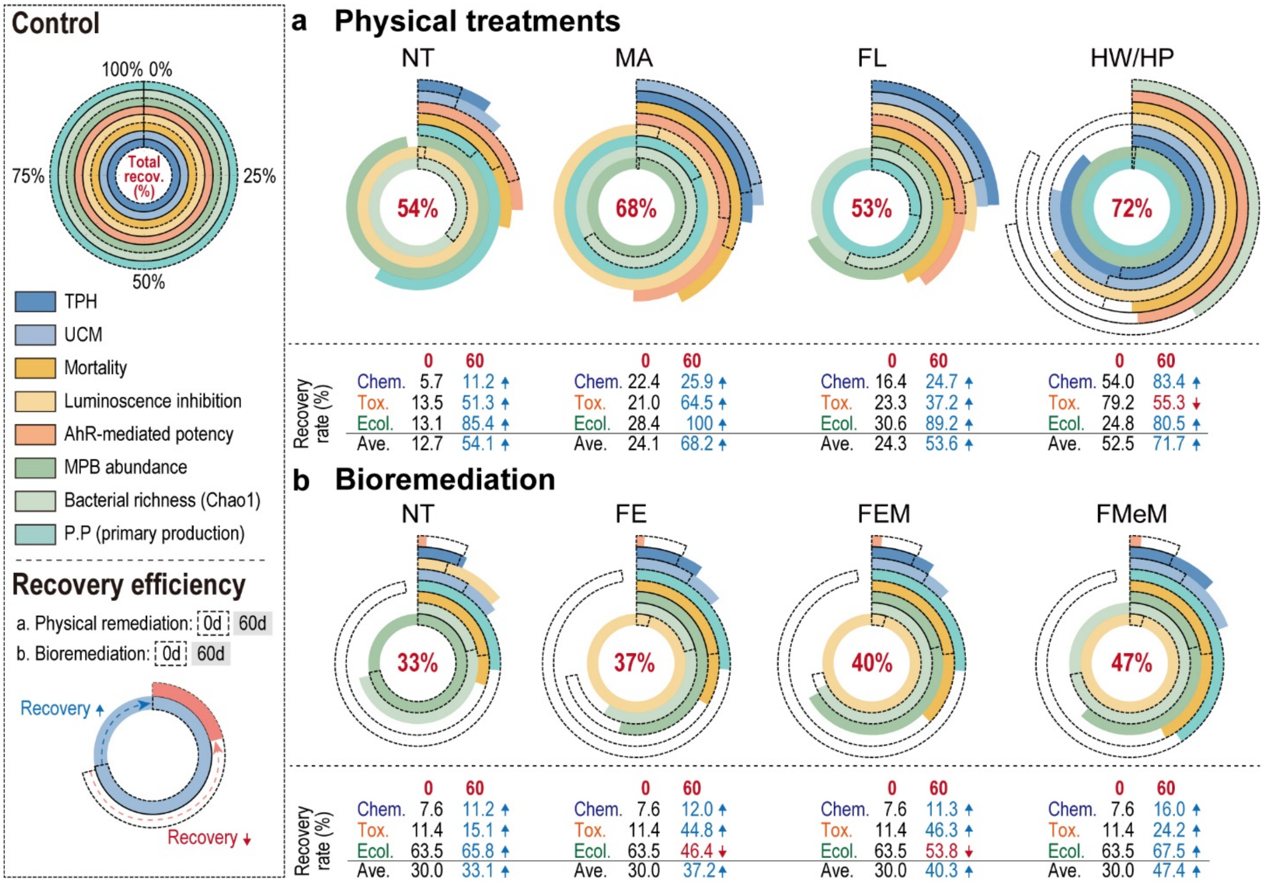


**Fig**. **S11**. Principal component analysis (PCA) ordination between the selected endpoint (TPH and UCM concentration, hydrocarbon index, and primary production) representatives of Group I–III (Diamond colored with red: *Actinobacteria*; yellow: *Bacteroidetes*; green: *Cyanobacteria*; light green: *Firmicutes*; brown: *Proteobacteria*; purple: N.A) (n.a: unknown; Ba.: *Bacteroidia*; Bac.: *Bacilli*.; Clo.: *Clostridia*; α: *Alphaproteobacteria*; β: *Betaproteobacteria*; γ: *Gammaproteobacteria*; Ve.: *Verrucomicrobiae*).



| | 0 | 60 |
|---|---|---|
| Chem. | 5.7 | 11.2 ↑ |
| Tox. | 13.5 | 51.3 ↑ |
| Ecol. | 13.1 | 85.4 ↑ |
| Ave. | 12.7 | 54.1 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 22.4 | 25.9 ↑ |
| Tox. | 21.0 | 64.5 ↑ |
| Ecol. | 28.4 | 100 ↑ |
| Ave. | 24.1 | 68.2 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 16.4 | 24.7 ↑ |
| Tox. | 23.3 | 37.2 ↑ |
| Ecol. | 30.6 | 89.2 ↑ |
| Ave. | 24.3 | 53.6 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 54.0 | 83.4 ↑ |
| Tox. | 79.2 | 55.3 ↓ |
| Ecol. | 24.8 | 80.5 ↑ |
| Ave. | 52.5 | 71.7 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 7.6 | 11.2 ↑ |
| Tox. | 11.4 | 15.1 ↑ |
| Ecol. | 63.5 | 65.8 ↑ |
| Ave. | 30.0 | 33.1 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 7.6 | 12.0 ↑ |
| Tox. | 11.4 | 44.8 ↑ |
| Ecol. | 63.5 | 46.4 ↓ |
| Ave. | 30.0 | 37.2 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 7.6 | 11.3 ↑ |
| Tox. | 11.4 | 46.3 ↑ |
| Ecol. | 63.5 | 53.8 ↓ |
| Ave. | 30.0 | 40.3 ↑ |

| | 0 | 60 |
|---|---|---|
| Chem. | 7.6 | 16.0 ↑ |
| Tox. | 11.4 | 24.2 ↑ |
| Ecol. | 63.5 | 67.5 ↑ |
| Ave. | 30.0 | 47.4 ↑ |

**Fig. S12**. Comparison of efficiency by the integrated approaches with eight target elements in each remediation.

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