이학박사 학위논문

Integrated ecological risk assessment for the persistent toxic substances in contaminated sediments from the Korean coastal waters: A multiple lines of evidence approach

한국 연안해역 오염퇴적물 내 잔류성독성물질의 생태위해성 통합평가: 다중증거접근법

2020년 8월

서울대학교 대학원

지구환경과학부

이 정 현

Integrated ecological risk assessment for the persistent toxic substances in contaminated sediments from the Korean coastal waters: A multiple lines of evidence approach

지도 교수 김 종 성

이 논문을 이학박사 학위논문으로 제출함

2020년 8월

서울대학교 대학원

지구환경과학부

이 정 현

이정현의 이학박사 학위논문을 인준함

2020년 8월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

위 원 _____ (인)

위 원 _____ (인)

ABSTRACT

The coastal ecosystems of Korea are subject to a broad range of adverse impacts from anthropogenic activities. Coastal sediments are particularly vulnerable to pollution by chemicals because they act as terminal sinks for persistent and hydrophobic toxicants. Furthermore, contaminated sediments potentially cause harmful effects on not only benthic animals but also pelagic organisms through sedimentary resuspension and bioturbation processes. Thus, it is crucial to ensure high sediment quality to maintain the environmental health of marine organisms, which can be evaluated using ecological risk assessments (ERA). Complexity of anthropogenic influences on coastal sediments necessitates use of an integrated assessment strategy for effective interpretation and subsequent management of ecological risk associated with chemical contaminants. In this study, an enhanced, the multiple lines of evidence (LOEs) approach for sediment assessment, that combined use of chemical contamination, biological effect, and benthic community structure in the sediment was used to assess spatiotemporal changes and ecological risks of persistent toxic substances (PTSs) including polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and styrene oligomers. Various bioassays, environmental DNA based assessments for benthic microbial communities, and several ecological quality (EcoQ) indices of macrobenthic community structure were also implemented.

The concentrations of targeted PTSs in the study areas were generally half that of previous years in recent years. Compared to the inner region, the concentrations of PTSs in sediments from the outer regions were significantly lower. These decreasing trends seemed to be associated with the implementation of pollution control measures and the management of toxic substances in South Korea. Strong aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)-mediated potencies were documented in the mid-polar and polar fractions of sediment extracts. AhR-mediated potencies in sediments also declined with PAH concentration over the sampling time interval, whereas ER-mediated potencies increased. Thus, over the sampling period, the input of ER agonists appears to have been substantial and continuous, especially in Masan Bay.

Potency balance analysis demonstrated that only small portions of AhR- and ER-mediated potencies could be explained by identified known chemicals, including PAHs and APs. Thus, target chemicals were not a major AhR- or ER- agonist in sediments of the study area. Thus, to identify potential toxic chemicals in samples,

non-targeted full-scan screening analyses (FSA; GC- and LC-QTOFMS) were performed. As a result, enoxolone was first identified as a novel AhR agonist in the sediment of Masan Bay. Enoxolone had a relative potency of 0.13 compared to benzo[*a*]pyrene (1.0) in the H4IIE-*luc* bioassay. Nonylphenols were also detected, with this group being associated with membrane damage influencing the viability of microalgae. Non-polar compounds were strongly associated with inhibiting the bioluminescence of *Vibrio fischeri* and having lethal effects on the embryos of *Danio rerio*. Overall, the selected endpoints and FSA demonstrated the toxicological properties of complex environmental mixtures comprehensively.

To evaluate the ecological effects of pollutants on benthic microbial communities, the relative abundance of Planctomycetes could be used as an indicator of sedimentary contamination by PAHs and metals. Based on correlation analyses, cadmium and ER-mediated potencies were more associated with bacterial abundance at the taxonomic level of class compared to other PTSs and metals. EcoQ indices tended to reflect PTS contamination of macrobenthic communities in the region. Ratio-to-mean (RTM) values obtained from the three LOEs indicated that the quality of sediments from the offshore area of the bay had recovered more over the 16-year period compared to inland areas. The RTM values of the benthic indices was similar to those obtained in the chemical analysis; however, changes across the study period were less pronounced. Thus, while chemical concentrations have the potential to decline rapidly, benthic communities require much longer to recover. Other conditions, such as metal contamination and/or hypoxia in bottom water, represent additional anthropogenic pressures on the benthic community. Overall, the present work demonstrated assay- and endpoint-specific variation and sensitivity for the potential toxicity of chemical mixtures in sediments, reaffirming the utility of "the multiple LOEs approach" in ERA. In conclusion, the characterization of *in situ* bacterial communities could provide a useful baseline for monitoring and assessing sediment quality in an integrated manner.

Keywords: Coastal pollution, Persistent toxic substances, *In vitro* and *in vivo* bioassays, Benthic community, Sediment triad assessment, Advanced effect-directed analysis

Student Number: 2015-30986

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ace	Acenaphthene
Acl	Acenaphthylene
AhR	Aryl hydrocarbon receptor
AMBI	Azti marine biotic index
Ant	Anthracene
APEOs	Alkylphenol ethoxylates
APs	Alkylphenols
BaA	Benzo[*a*]anthracene
BaP	Benzo[*a*]pyrene
BbF	Benzo[*b*]fluoranthene
BghiP	Benzo[*g,h,i*]perylene
BkF	Benzo[*k*]flouranthene
BQI	Benthic quality index
CAP	Canonical analysis of principal coordinates
CCME	Canadian council of ministers of the environment
Chr	Chrysene
Co-PCBs	Coplanar-polychlorinated biphenyls
DBahA	Dibenz[*a,h*]anthracene
DCM	Dichloromethane
DM	Dry mass
DMSO	Dimethyl sulfoxide
E2	17β-estradiol
EBMs	Effects-based methods
EcoQ	Ecological quality
EDA	Effect-directed analysis
eDNA	environmental DNA
EEQs	E2 equivalent concentrations
EQR	Ecological quality ratio
ER	Estrogen receptor

FCM	Flow cytometry
FDA	Fluorescein diacetate
FET	Fish embryo toxicity test
Fl	Fluoranthene
Flu	Fluorene
FS	Forward scatter
FSA	Full-scan screening analysis
GC-MSD	Gas chromatography-mass selective detector
H′	Shannon-Wiener diversity index
HMW	Higher-molecular weight
HPLC	High performance liquid chromatography
IC	Industrial complex
IcdP	Indeno[*1,2,3*-cd]pyrene
ISQG	Interim sediment quality guidelines
LMW	Lower-molecular weight
LODs	Limits of detection
LOEs	Lines of evidence
M-AMBI	Multivariate-azti marine biotic index
MOE	Ministry of environment
MOMAF	Ministry of maritime affairs and fisheries
Na	Naphthalene
NP	Nonylphenol
NP1EO	Nonylphenol monoethoxylate
NP2EO	Nonylphenol diethoxylate
4-*t*-OP	4-*tert*-octylphenol
OP1EO	4-*tert*-octylphenol monoethoxylate
OP2EO	4-*tert*-octylphenol diethoxylate
PAHs	Polycyclic aromatic hydrocarbons
PCA	Principal component analysis
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
Phe	Phenanthrene

PI	Propidium iodide
PTSs	Persistent toxic substances
Py	Pyrene
QSAR	Quantitative structure–activity relationship
RePs	Relative potency values
REs	Raw extracts
RP-HPLC	Reverse-phase high performance liquid chromatography
RTM	Ratio-to-mean
SCR	Sihwa coastal reservoir
SD1	1,3-diphenylpropane
SD2	cis-1,2-diphenylcyclobutane
SD3	2,4-diphenyl-1-butene
SD4	trans-1,2-diphenylcyclobutane
SDs	Styrene dimers
SOs	Styrene oligomers
SS	Surrogate standards
SS	Side scatter
ST1	2,4,6-triphenyl-1-hexene
ST2	1e-phenyl-4e-(1-phenylethyl)-tetralin
ST3	1a-phenyl-4e-(1-phenylethyl)-tetralin
ST4	1a-phenyl-4a-(1-phenylethyl)-tetralin
ST5	1e-phenyl-4a-(1-phenylethyl)-tetralin
ST6	1,3,5-triphenylcyclohexane
STP	Sewage treatment plant
STs	Styrene trimers
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TIE	Toxicity identification and evaluation
TEQs	TCDD equivalent concentrations
TOF MS	Time-of-flight mass spectrometry
TPLMS	Total pollution load management system
TPS	Tidal power station
WQ	Water quality

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1.

INTRODUCTION

1.1. Backgrounds

In Korea, nearly all coastal marine environments adjacent to municipalities have been exposed to broad range of adverse impacts, especially during the period of rapid industrialization since the 1970s (Halpern et al., 2008; Xu et al., 2015; Hong et al., 2016). Coastal sediment is an important part of marine environments, providing habitat and food resources for the benthos. It can also act both as source and sinks of various persistent toxic substances (PTSs) that are introduced to coastal waters from a myriad of point and non-point sources (Hollert et al., 2003; Chapman et al., 2007; Luo et al., 2009; Dai et al., 2014) (Figure 1.1). Accordingly, contaminated sediments with PTSs might cause significant adverse effect on various marine organisms, especially to epi- and endo-benthic fauna as well as pelagic organisms through sedimentary resuspension and bioturbation processes (Holler et al., 2002; Schulze-Sylvester et al., 2016) (Figure 1.1). Additionally, as PTSs commonly have semi-volatile and relatively lipophilic characteristics, they can accumulate in marine organisms, becoming biomagnified through the food chain (Wania and Mackay, 1996; Oost et al., 2003). Thus, maintaining of sediment quality is crucial to maintain environmental health, and has become one of the focuses in environmental regulations (Davoren et al., 2005).

Because anthropogenic influences on coastal sediments are complex, ecological risk assessments (ERA) of integrated sediment assessment strategies must be accurate (Davoren et al., 2005). The ERA process that aims to identify potential ecological hazards, due to the exposure to chemical and non-chemical stressors, including contaminants (USEPA, 1992). ERAs include three fundamental steps: identification of stressors that are related to problem formulation; characterization of exposure and ecological effects; and risk characterization (Table 1.1). In terms of ERA, the “traditional” sediment quality triad (SQT) approach allows possible ecological impacts to be determined using multiple lines of evidence (LOEs). SQT includes three components: (1) exposure (chemical analysis; chemical LOE), (2) effects assessment (bioassays; toxicological LOE and benthic community structure analyses; ecological LOE), and risk characterization (weight of evidence determinations) (Long and Chapman, 1985; Chapman, 1990, 2006) (Table 1.1 and Figure 1.2).

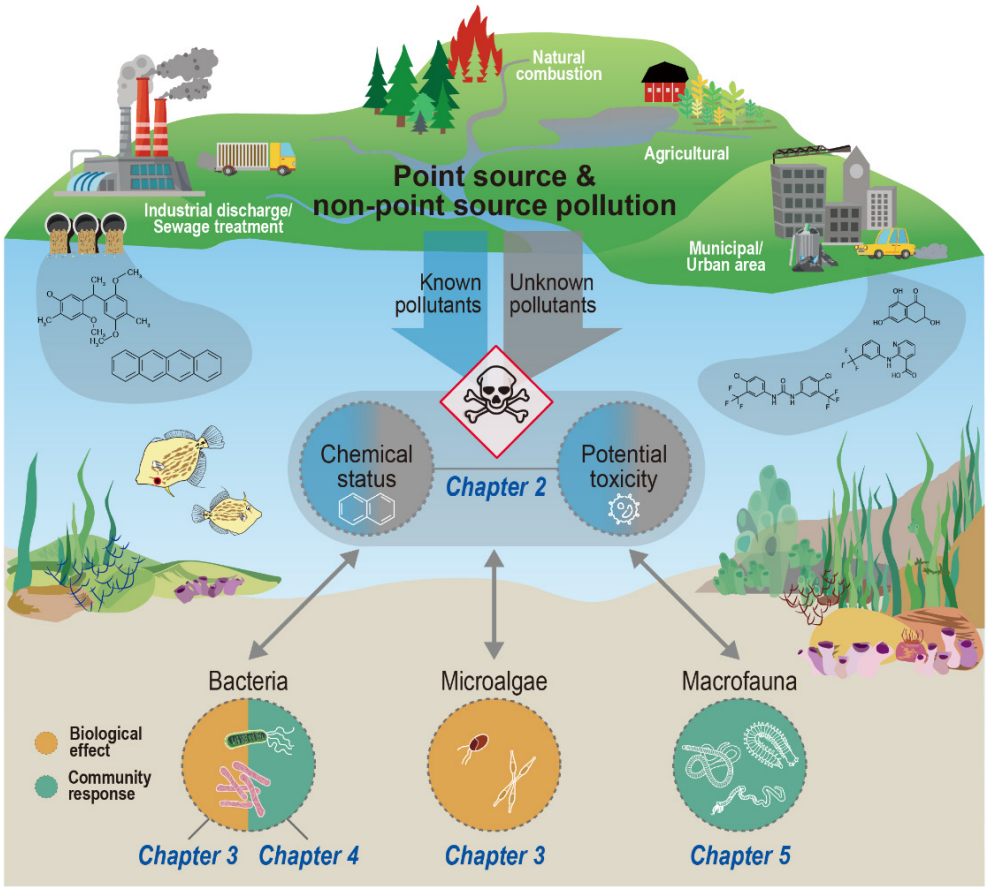


Figure 1.1.
Illustration of sources of various pollutants in the coastal environment and the concept and components of this study.

Table 1.1.

Interpretation of traditional triad approach for ecological risk assessment (related in Table 6.1).

No.	Lines of evidence			Interpretation	References
	Chem.	Tox.	Ecol.		
1	+	+	+	Contaminant-induced degradation in field evident	a, b
2	+	-	-	Contaminants unavailable to organisms in the field	c
3	-	+	-	Unmeasured factors contributing to toxicity	d, e, f, g
4	-	-	+	Effects on benthos not due to sediment contamination	h, i
5	+	+	-	Toxic contaminants probably stressing sediment-dwelling organisms	a
6	-	+	+	Unmeasured chemicals contributing to toxicity	-
7	+	-	+	Toxicity tests not sensitive enough	i

Chem.; Chemical contamination, Bio.; Biological effect, Ecol.; Benthic community structure, (+); employed approach. (-); Not considered approach, [a] Long and Chapman (1985), [b] Chapman (1990), [c] May et al. (1975), [d] Hollert et al. (2002), [e] Karuppiah and Gupta (1996), [f] Svenson et al. (1996), [g] Hollert et al. (2003), [h] Richard et al. (1985), [I] Lachmund et al. (2003). Orange box indicated that used approaches in this study.

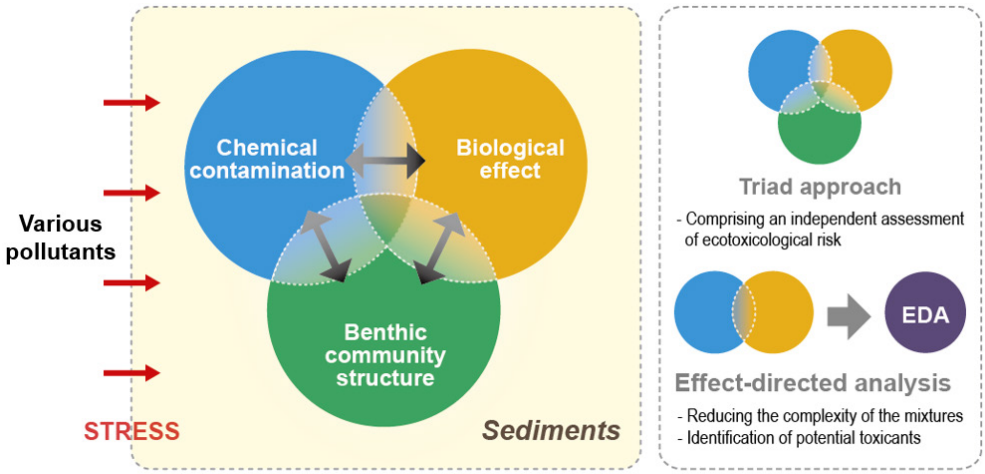


Figure 1.2.
Concept of the integrated sediment assessment used, which combines data from chemical contamination (concentrations and composition), biological effect (*in vitro* and *in vivo* bioassays), as well as benthic community structure (*in situ* studies). In contrast to the original triad approach by Chapman (1990), effect-directed analysis (EDA) were additionally performed.

While the traditional SQT LOE has evolved, two LOEs of the SQT continue to be universally used: chemical and toxicological LOEs. The third component, ecological LOE, was initially restricted to evaluating the structure of the benthic community (Long and Chapman, 1985; Chapman et al., 1987a, Chapman, 1990). However, it was subsequently clarified that this LOE was not restricted to benthos, but rather dealt with the broad category of alterations to resident communities (Chapman, 1996). However, definitive conclusions cannot be obtained in all cases, as causation cannot be definitively determined without further research. Ultimately, the "traditional" SQT is based on correlation, not causation. The "traditional" and additional LOEs, such as effect-directed analysis (EDA), fit into risk assessment frameworks (Figure 1.2), that also include causation. Causation LOEs generally include additional studies once an SQT has been completed. Consequently, the "traditional" SQT serves as a screening-level risk assessment, with causation being examined at a higher tier, in which a more detailed risk assessment is provided (Hill et al., 2000).

With respect to diagnostic methods for identifying causative toxicants in complex environmental samples, such as sediments, EDA is regarded as a useful tool (Brack, 2003; Hong et al., 2016a). This analysis involves progressive fractionations, which reduces the complexity of the mixture, allowing focused chemical analysis on selected fractions that exhibit significant bioactivity (Brack, 2003; Weiss et al., 2009). However, since they do not provide information on all bioactive fractions and/or endpoints-specific variations or sensitivity of applied bioassays, such a targeted chemical analysis has limitations (Jeon et al., 2017; Lee et al., 2017). Alternatively, to fill the gaps, recently ERA has been challenged to adopt multiple lines of evidence approach, by employing effects-based methods (EBMs) along with full-scan screening analysis (FSA) (Babić et al., 2018; Brack et al., 2019). EBMs are regarded as holistic approaches to complement *in vitro* bioassays and can include a battery of *in vivo* bioassays targeting some important pelagic communities of fish, invertebrates, and algae (Brack et al., 2019). FSA is frequently practiced by use of high-resolution mass spectrometry, such as time-of-flight mass spectrometry (TOFMS) and Orbitrap ultrahigh-resolution mass spectrometry, which can detect untargeted compounds and potentially toxic substances in environmental mixture samples (Gallampois et al.,

2015; Schymanski et al., 2015; Xiao et al., 2016; Tousova et al., 2017).
Furthermore, these techniques can provide accurate masses from which the formula of chemicals can be determined, and putative structures can be derived by use of the MS/MS and libraries.

For chemical LOE, it is important to examine the occurrence, fate, and distribution of PTSs in sediments (Khim and Hong, 2014). Studies on concentrations of PTSs in sediments from the same region over long periods of time could be informative about changes in environments associated with anthropogenic activities. The PTSs in the sediments of Korean coastal waters have been extensively monitored only since the mid-1990s (Figure 1.3). Among various coastal areas, Masan Bay and Sihwa Bay have been identified as hot spots for coastal pollution, and were designated special management areas (SMAs) in 1982 and 2000, respectively (Figure 1.3) (Khim and Hong, 2014). However, most of the policies and regulations implemented in Korea are for management of water quality not for sediment. More importantly, chemical concentrations are not sufficient to demonstrate biological effects, because they do not provide information about the potential adverse effects on aquatic organisms.

For a comprehensive assessment, bioassays combined with chemical analyses are needed to infer probable adverse biological effects (Chapman, 2007). Bioassays utilized in the present study cover three mechanisms of actions including specific-, baseline-, and reactive-toxicity (Jia et al., 2019). *In vitro* bioassays comprise receptor gene assays for measuring "specific toxicity" of AhR agonists, such as polycyclic aromatic hydrocarbons (PAHs) (H4IIE-*luc* bioassay) and the bacterial test (*Vibrio fischeri* assay), which is related to the energy metabolism of a bacterium, was used for "baseline toxicity" measurements (Brack et al., 1999; ISO, 2007; Jia et al., 2019) (Table 1.1). *In vitro* bioassays are characterized by being rapid, cost-effective, sensitive, and reproducible assays (Kammann et al., 2005). However, despite the obvious advantages of *in vitro* assays for the determination of toxicological profiles of sediments, results of *in vitro* bioassays can explain only a small portion of overall toxic potencies in extracted samples and is limited with respect to predicting *in vivo* toxicity, which requires assessment of tissue-specific toxicity, adaptive response, and metabolic conversion to predict effects on fitness of individuals (Massei et al., 2019).

Study area	Chapter 2	3	4	5
Sihwa Bay	a		a	
Lake Asan/Sapgyo			b	
Taean Coast			c	
Geum River			d	
Yeongsan River			e	
Masan Bay		f		f

Survey year	'98	'16	'10-	'98
	'15		'14	'14
# of samples	*81*	*48*	*60*	*72*

Data from total 261 samples

Figure 1.3.
Mini review of traditional study efforts and historical trend in number of the sediment assessment by number of publications of each component of target approach (Scopus was used). studies on persistent toxic substances (PTSs) in sediments of Korean coastal waters over the past two decades, including 90 reports published since 1997, based on the literature survey (Scopus).

Thus, in addition to *in vitro* bioassays, several *in vivo* toxicity tests which cover broad range of modes of action, and represent different levels of biological organization including microalgae and fish embryo, for "reactive toxicity" could be conducted. Microalgae (including diatoms, dinoflagellates, and cyanobacteria) are known not only to be highly sensitive to the contamination of marine ecosystems in response to various PTSs, but also food source for small, free-swimming crustaceans or fish larvae, referred to as zooplankton (Jeong, 1999). Hence, it is very important to identify the toxic effects of microalgae. Each bioassay exhibits different sensitivities to chemical contaminations in sediments; thus, the combination of a battery of bioassays provides a better assessment of the sediment contaminations (Maltby et al., 2005; Brack et al., 2019). However, chemical and toxicological analyses cannot capture community-level effects observed in natural systems. The utility of such results is enhanced when it can be integrated more closely with ecological data. Hence, to obtain a fuller understanding, it is preferable to bring together different LOEs.

Ecological effects are an alternative line of evidence, enabling the responses of taxa to be examined under relevant environmental conditions (e.g. spatio-temporal varied exposure to multiple stressors). To predict ecological effects, studies have tended to focus on structures of communities of benthic organisms (Wernersson et al., 2015). Benthic organisms represent ecological receptors ideal for assessment of sediment quality because they are exposed both directly and indirectly by contaminated sediments (Chapman et al., 2013). Generally, responses of benthic organisms to environmental factors are assessed by use of biotic indexes of numbers of taxa and individuals, diversity, and tolerance, which provide an integrated evaluation of alterations caused by exposure to multiple stressors. And also, studies over gradients of stressors (both spatially and temporally) can provide thresholds for adverse effects for single or multiple stressors (Brock et al., 2008; Coffey et al., 2014; Schipper et al., 2014). However, it is disadvantageous that values of biotic indices are not always sensitive to effects of some stressors and there are concerns regarding subjectivity of interpretations of indices (Lim et al., 2007).

Also, traditionally community-level data have only examined a small proportion of a system's true diversity by focusing on large, easily quantifiable

organisms (e.g. diatoms, fish and macrobenthic invertebrates). In doing so, the diversity and functionality of a community held within the microbiota have often been ignored.

Within benthic communities, microbial communities play an important role in biogeochemical cycling (Fischer and Pusch, 2001), decomposition of contaminants, and in providing other functions necessary for sustaining aquatic ecosystems (Reed and Martiny, 2013; Xie et al., 2017). Microbial communities, including bacteria, are extremely sensitive to changes in physicochemical conditions, such as temperature, pH, salinity, and concentrations of contaminants (Herlemann et al., 2011; Gibbons et al., 2014; Xie et al., 2016, 2017). Bacteria respond rapidly to changing environmental conditions and adapt their degree of activity, diversity, or community structure (Xie et al., 2016, 2017). This means that the composition of *in situ* bacterial communities can potentially be used to monitor key elements of sediment quality. By using environmental DNA (eDNA) metabarcoding, most microbial communities can be identified (Amann et al., 1995). Thus, approaches that use metagenomic level analysis to characterize the complexity of microbial ecosystems in sediments could provide a rapid and efficient way to identify and monitor benthic microbial communities and enumerate individual taxa (Sharmin et al., 2013; Gibbons et al., 2014). Improvements in methods for metabarcoding eDNA, in concert with advances in bioinformatics analysis, could provide a promising approach for improving ERA (Zhang, 2019).

1.2. Objectives

In this dissertation, an integrated ecological risk assessment using multiple lines of evidence in order to make a best-judgment weight of evidence decisions for PTSs in contaminated sediments from the Korean coastal waters through Chapter 2 to 6. Key questions that required resolving or clarification in current sediment assessment research, along with specific objectives and workflow, are stated as follows (Table 1.2 and Figure 1.4):

1. **Chapter 2**

   Determined long-term changes (> 15-year gaps) in distributions of PTSs and assessed their potential toxic (AhR- and ER-mediated potencies) effects in sediments from Sihwa Bay

2. **Chapter 3**

   Enhanced effect-directed analysis was conducted to identify the toxicity profile of contaminants in sediments by use of multiple bioassays and evaluate assay-specific relative potencies (RePs) of tentative AhR agonists in sediments from Masan Bay

3. **Chapter 4**

   Conducted comprehensive assessments with chemistry, *in vitro* bioassays, and eDNA-based benthic bacterial communities in relation to PTSs in sediment collected over space (5 regions) and time (5 years).

4. **Chapter 5**

   Integrated sediment assessment using triad approach (chemical, bioassays, and ecological investigations) and characterization of AhR- and ER-mediated potencies in coastal sediments.

Finally, conclusions including summary, environmental implications and limitations, and future research directions are provided in Chapter 6.

Table 1.2.

Summary of the key questions that have not been resolved or clarified in current sediment assessment research. Approaches and objectives for each Chapter are suggested.

Subject		Key question	Ch.	Approach
I	*In vitro* toxicity to sediment pollution	Any changes in distributions & potential toxic effects of PTSs **over time**? *(Sihwa Bay, 1998 vs. 2016)*	2	Chem. Tox. Mass balance analysis
II	*In vitro* and *in vivo* toxicities to sediment pollution	Any differences in variations and/or sensitivities cross **assays/endpoints**? *(Masan Bay, 2015)*	3	Chem. Tox. Effect-directed analysis
III	Benthic bacterial community response to sediment pollution	Any changes in ecotoxicological effects and benthic microbial community associated with PTSs for **5 years**? *(West coast of Korea, 2010-2014)*	4	Chem. Tox. Ecol. Sediment triad approach
IV	Macrobenthic community response to sediment pollution	Any changes in ecotoxicological effects and benthic community responses associated with PTSs over **15 years**? *(Masan Bay, 1998 vs. 2015)*	5	Chem. Tox. Ecol. Sediment triad approach

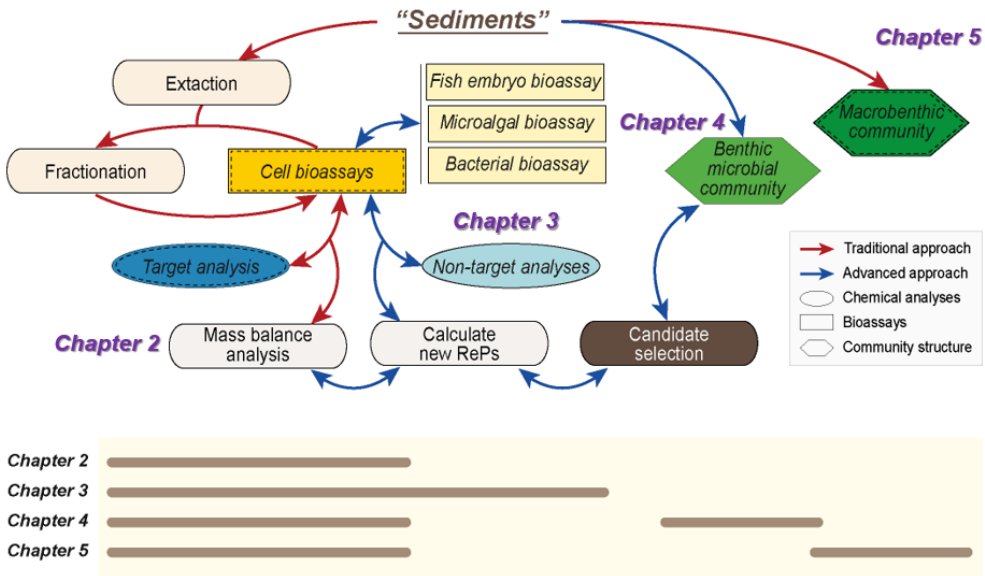
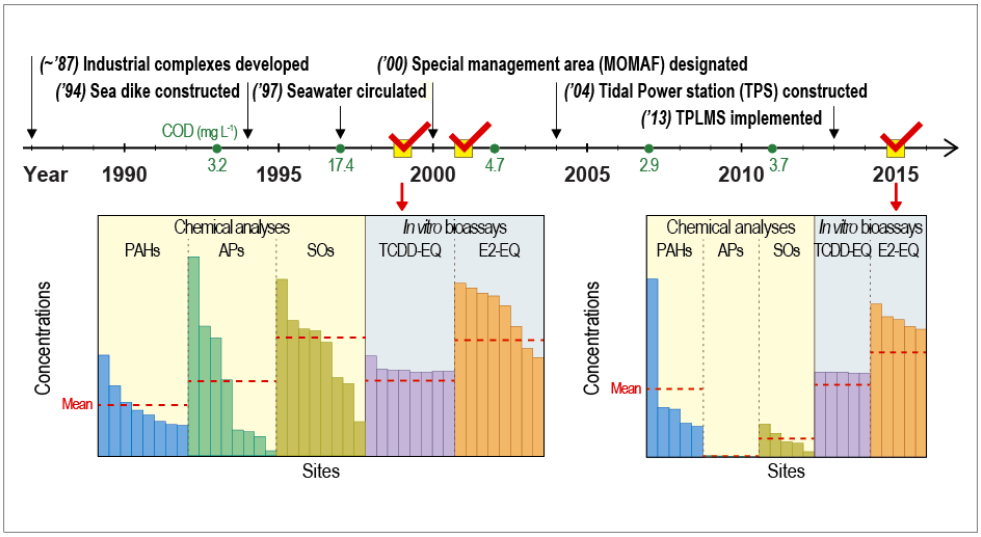


Figure 1.4.
Schematic diagram to investigate the chemical (target and non-target), toxicities (cell, bacterial, microalgae, and fish embryo), and ecological (benthic microbial and macrobenthic community) assessments in contaminated coastal sediment.

CHAPTER 2.

LONG-TERM CHANGES IN DISTRIBUTIONS OF DIOXIN-LIKE AND ESTROGENIC COMPOUNDS IN SEDIMENTS OF LAKE SIHWA, KOREA: REVISTED MASS BALANCE



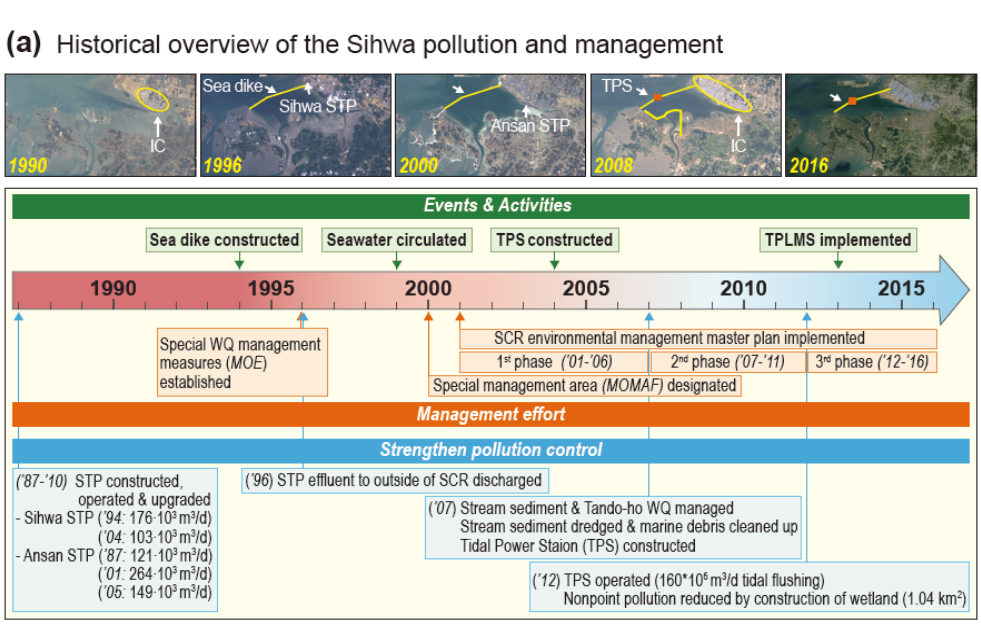
This chapter has been published in Chemosphere.

Lee, J., Hong, S., Yoon, S. J., Kwon, B.-O., Ryu, J., Giesy, J. P., Allam, A. A., Al-khedhairy, A. A., Khim, J. S. Long-term changes in distributions of dioxin-like and estrogenic compounds in sediments of Lake Sihwa, Korea: Revisited mass balance. *Chemosphere* 2017, **181**, 767–777. http://dx.doi.org/10.1016/j.chemosphere.2017.04.074

2.1. Introduction

Lake Sihwa is an artificial lake located on the west coast of Korea that was formed in 1994 following the construction of a dike, which separates it from the sea (Figure 2.1a). The original purpose of the lake was to supply water to industrial complexes and the surrounding cities, such as Siheung, Ansan, and Hwaseong (Khim and Hong, 2014; Lee et al., 2014). However, after embankment in 1994 the dike stopped flow of tidal currents, the water quality was subject to environmental deterioration because, in parallel to rapid growth of the population and industrial development (Yoo et al., 2006; Lee et al., 2014). In addition, various chemicals have been continuously discharged into the lake via several inland creeks. Thus, the Korean government enforced strategies to redress this problem, such as providing seawater circulation and since 1996 relocating the discharge from sewage treatment plants to outside the dike (Figure 2.1a). In 2000, the Korean government designated Lake Sihwa as a special coastal management zone and in 2011 constructed a tidal power station (TPS) to increase tidal mixing (Lee et al., 2014), and finally in 2013 implemented a total pollution load management system. Consequently, water quality of Lake Sihwa has somewhat improved, but contamination of sediments by persistent toxic substances (PTSs) still remained (Jeon et al., 2017; Meng et al., 2017).

Several previous studies have reported the presence of classic PTSs, especially polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs), in sediments of Lake Sihwa (Khim et al., 1999a; Li et al., 2004a, 2004b; Hong et al., 2010; Choi et al., 2011) (Figure 2.2a). PAHs are aryl hydrocarbon receptor (AhR) agonists, and are considered priority pollutants because of their mutagenicities and carcinogenicities (Lotufo and Fleeger, 1997). APs such as nonylphenol (NP) and octylphenol (OP) are degradation products of alkylphenol ethoxylates (APEOs), and mimic estrogen. Consequently, these compounds can disrupt hormonal balance (Koniecko et al., 2014) mediated by estrogen receptor (ER) dependent mechanisms (Behnisch et al., 2001; Giesy et al., 2002). Thus, use and production of APs have been banned, or their uses are strictly regulated (Soares et al., 2008).



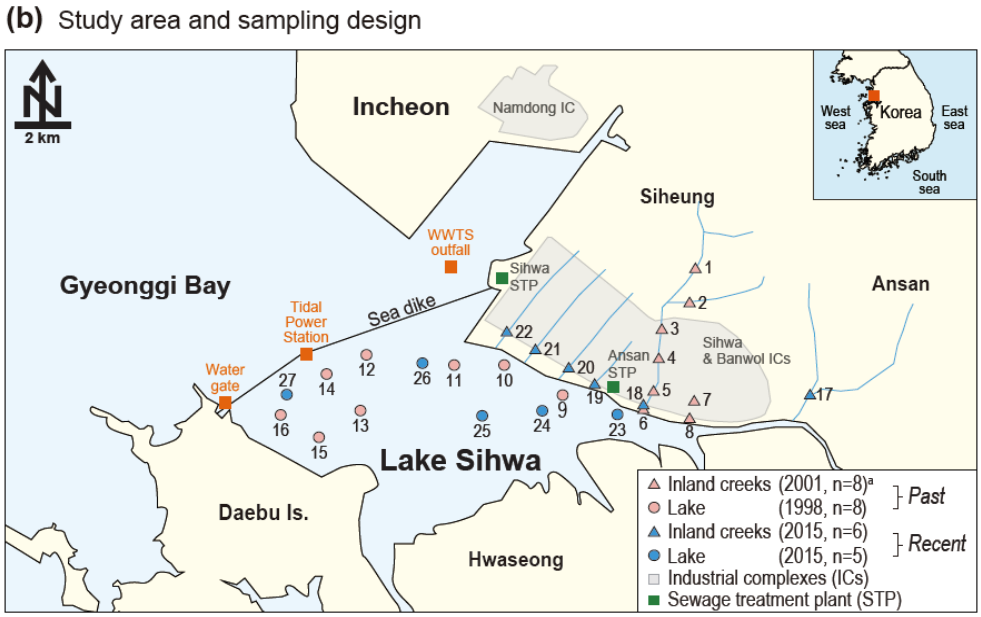


Figure 2.1.
(a) Satellite images and brief summary of the events/activity related to Lake Sihwa, including management effort and pollution control by the Korea Government (1987–recent). IC: Industrial complex; STP: Sewage Treatment Plant; TPS: Tidal Power Station; TPLMS: Total Pollution Load Management System; WQ: Water Quality; MOE: Ministry of Environment; SCR: Sihwa Coastal Reservoir; MOMAF: Ministry of Maritime Affairs and Fisheries. (b) Map showing the locations of the study areas in the inland creeks in 2001 (1–8) and 2015 (17–22), and sediment sampling sites from inside of dike in 1998 (9–16) and 2015 (23–27). [a] Data from Koh et al. (2005).

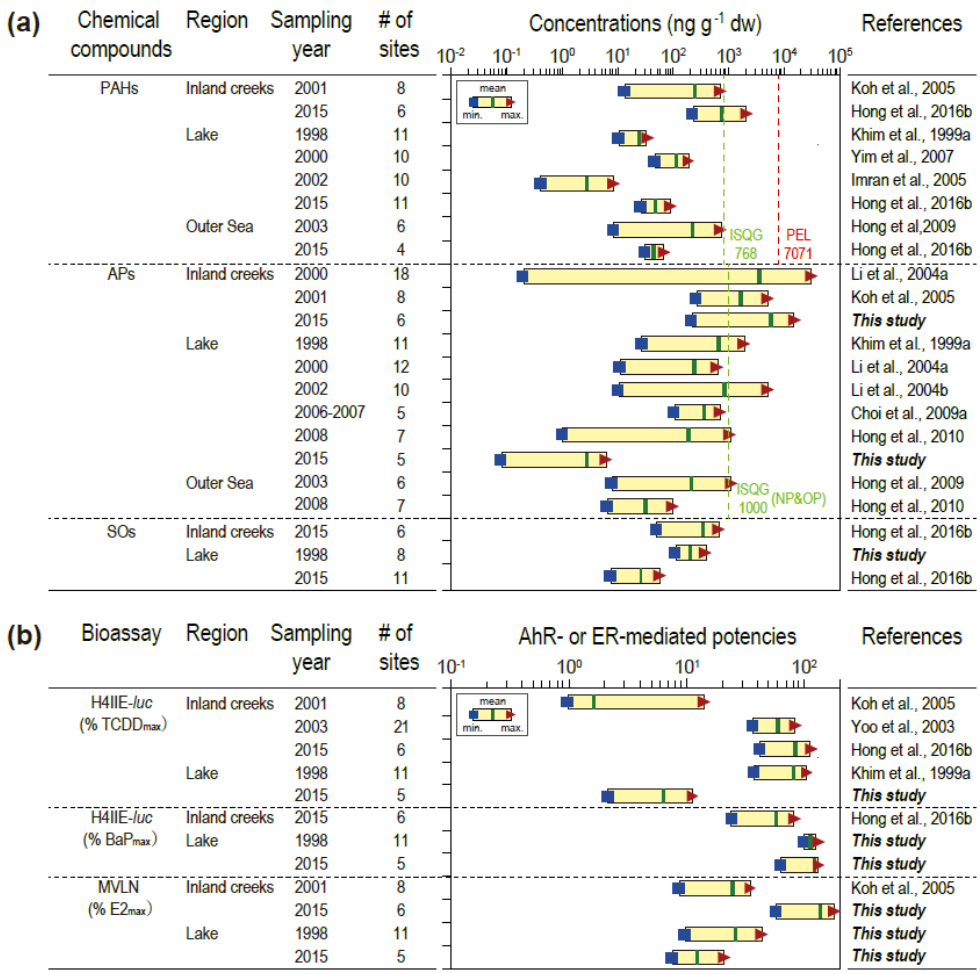


Figure 2.2.

Summary of concentrations of: (a) polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and styrene oligomers (SOs); (b) AhR- and ER-mediated potencies in sediments from inland creeks, inner bay, and outer bay around Lake Sihwa.

Recently, emerging PTSs, such as styrene oligomers (SOs), have been detected in sediments of Lake Sihwa (Hong et al., 2016b; Lee et al., 2016) (Figure 2.2a). SOs, including styrene dimers (SDs) and styrene trimers (STs) are produced by decomposition of polystyrene plastic (Ohyama et al., 2001; Yanagiba et al., 2008; Kwon et al., 2015), particularly during thermal decomposition (Kitamura et al., 2003; Kwon et al., 2014). Despite growing concern about contamination of marine environments by plastics, few studies have surveyed concentrations of SOs in coastal sediments (Kwon et al., 2015; Hong et al., 2016b). Furthermore, there have been no previous surveys of SOs in sediments of Lake Sihwa before 2010s. Thus, studies on distributions of SOs and their toxicities, such as endocrine disrupting effects are needed.

Objectives of the present study, results of which are presented here, were to: 1) investigate changes in classic PTSs (PAHs and APs) and emerging chemicals (SOs) in sediments from Lake Sihwa and nearby inland creeks, 2) identify the sources of selected target PTSs by compositional analyses, 3) evaluate AhR- and ER-mediated potencies associated with sediment extracts, and 4) assess the relative contributions of targeted compounds in sediments to total induced *in vitro* activities. Identification and quantification of individual compounds by use of gas chromatography (GC)-mass selective detector (MSD) and quantification of AhR- and ER-agonists by use of *in vitro* bioassays using recombinant cells with a luciferase reporter gene were applied simultaneously. The results of this study provide information on relatively long-term changes of several classes of environmental pollutants and associated biological responses in coastal sediments, highlighting adaptive management and control for some persistent and emerging PTSs.

2.2. Materials and Methods

2.2.1. Sampling and sample preparations

Sediments were collected from Lake Sihwa and nearby creeks in Korea (Figure 2.1b). A total of 27 batches of samples from three surveys conducted over a 15-year period were collected. These samples included archived samples collected from Lake Sihwa in January 1998 and newly collected samples from inland creeks and the lake in 2015 (Figure 2.1b). The sites from which samples were collected during 2015 were the same as those from which samples had been collected in 2001 (Hong et al., 2016b). To investigate long-term changes in occurrences and distributions of selected PTSs, the eight samples of sediments collected from inland creeks (S1–S8) in 2001 (Koh et al., 2005) were compared to samples collected from the same areas of creeks during 2015. Due to differences in sampling sites in creeks between 2001 and 2015 (Figure 2.1b), site-specific comparison was limited. Most of the sites inside Lake Sihwa receive PTSs from similar sources, thus trends in long-term, temporal changes between creeks and lake could be observed and comparisons made.

All samples were immediately transferred to the laboratory and stored at -20 °C until analyses. Samples collected in 1998 were freeze-dried and kept at -20 °C for 18 years. To obtain better insight on differences in concentrations of target chemicals in sediments and to avoid technical or methodological biases, samples that had been collected in 1998 were extracted and analyzed together with the 2015 samples.

Detailed descriptions of sample preparation for chemical analyses and bioassays have been published previously (Hong et al., 2012, 2015). In brief, 10 g of freeze-dried sediments were extracted for 16 h with 350 mL dichloromethane (Burdick & Jackson, Muskegon, MI) by use of Soxhlet extractor. To remove elemental sulfur, extracts were treated with activated copper powder (Sigma Aldrich, Saint Louis, MO) and concentrated to 1 mL. For *in vitro* assays, the aliquot of extract was exchanged into dimethyl sulfoxide (DMSO, Sigma-Aldrich) by use of differential volatilization.

2.2.2 Instrumental analysis

Concentrations of PAHs, SOs and APs in organic extracts of sediments were quantified using an Agilent 7890 GC equipped with a 5975C MSD (Agilent Technologies, Santa Clara, CA). Details of instrumental conditions for analyses of PAHs, SOs, and APs and their full chemical names (with abbreviations) and structures were provided in Table 2.1 and Figures 2.3–2.5. A total of 15 PAHs were quantified, including acenaphthylene (Acl), acenaphthene(Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Py), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]flouranthene (BkF), benzo[*a*]pyrene (BaP), indeno[*1,2,3*-cd]pyrene (IcdP), dibenz[*a,h*] anthracene (DBahA), and benzo[*g,h,i*]perylene (BghiP). APs were also quantified, including NPs (isomeric mixture), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), 4- *tert*-octylphenol (4-*t*-OP), 4-tert-octylphenol monoethoxylate (OP1EO), and 4-*tert*-octylphenol diethoxylate (OP2EO).

Limits of detection (LODs) for PAHs, SOs, and APs in sediments were calculated as 3.707 × standard deviation of the standard sample were analyzed. LODs for PAHs, SOs, and APs ranged from 0.2 to 1.3 ng g^{-1} dry mass (dm), from 0.2 to 0.9 ng g^{-1} dm, and from 0.1 to 0.9 ng g^{-1} dm, respectively (Table 2.2). Analytical method blank samples were quantified with each set of samples. Mean concentrations of PAHs, SOs, and APs in blank samples were all less than LODs. Five surrogate standards (SS; acenaphthene-d10, phenanthrene-d10, chrysened12, and perylene-d12 for PAHs and SOs; bisphenol A-d16 for APs) were used to assess the recoveries of target chemicals. Mean recoveries of five SS were generally within the acceptable range (83−102% for PAHs and SOs; 77% for APs; detailed in Table 2.2). Accuracy of determination of PAHs was assessed by use of certified reference material (CRM 1941b; marine sediment, Gaithersburg, MD) and recoveries ranged 74−112% (mean = 93%; n = 3).

Table 2.1.

GC-MSD instrumental conditions for the determination of polycyclic aromatic hydrocarbons, styrene oligomers, and alkylphenols.

Target Compounds	Polycyclic aromatic hydrocarbons	Styrene oligomers	Alkylphenols
	Naphthalene (Na) Acenaphthylene (Acl) Acenaphthene (Ace) Fluorene (Flu) Phenanthrene (Phe) Anthracene (Ant) Fluoranthene (Fl) Pyrene (Py) Benzo[*a*]anthracene (BaA) Chrysene (Chr) Benzo[*b*]fluoranthene (BbF) Benzo[*k*]fluoranthene (BkF) Benzo[*a*]pyrene (BaP) Indeno[*1,2,3*-cd]pyrene (IcdP) Dibenz[*a,h*]anthracene (DBahA) Benzo[*g,h,i*]perylene (BghiP)	1,3-diphenylpropane (SD1) *cis*-1,2-diphenylcyclobutane (SD2) 2,4-diphenyl-1-butene (SD3) *trans*-1,2-diphenylcyclobutane (SD4) 2,4,6-triphenyl-1-hexene (ST1) 1e-phenyl-4e-(1-phenylethyl)-tetralin (ST2) 1a-phenyl-4e-(1-phenylethyl)-tetralin (ST3) 1a-phenyl-4a-(1-phenylethyl)-tetralin (ST4) 1e-phenyl-4a-(1-phenylethyl)-tetralin (ST5) 1,3,5-triphenylcyclohexane (ST6)	4-*tert*-octylphenol (4-*t*-OP) 4 -*tert*-octylphenol monoethoxylate (OP1EO) 4-*tert*-octylphenol diethoxylate (OP2EO) Nonylphenol (NP) Nonylphenol monoethoxylate (NP1EO) Nonylphenol diethoxylate (NP2EO)
GC/MSD system	Agilent 7890A GC and 5975C MSD		
Column	DB-5MS (30 m long × 0.25 mm i.d. × 0.25 μm film thickness)		
Gas flow	1.0 mL min^{-1} (He)		
Injection mode	Splitless		
MS temperature	180 °C		
Detector temperature	230 °C		
Injection volume	2 μL		1 μL
Oven temperature	1. 60 °C hold 2 min 2. Increase 6 °C/min. to 300 °C 3. 300 °C hold 13 min		1. 60 °C hold 5 min. 2. Increase 10 °C/min. to 100 °C 3. Increase 20 °C/min. to 300 ° C 4. 300 °C hold 6 min.

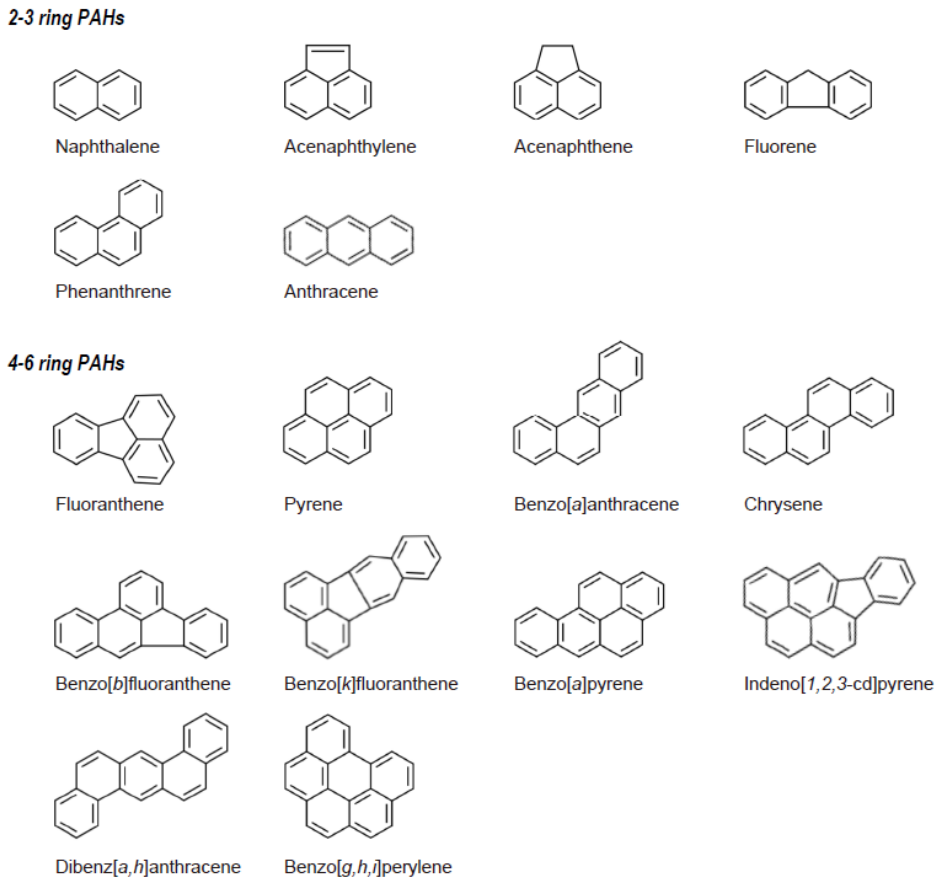


Figure 2.3.
Chemical structure of the targeted PAHs (polycyclic aromatic hydrocarbons) in the present study may be expected to have the potential to cause adverse effects through the aryl hydrocarbon receptor (AhR) mediated mechanism of action.

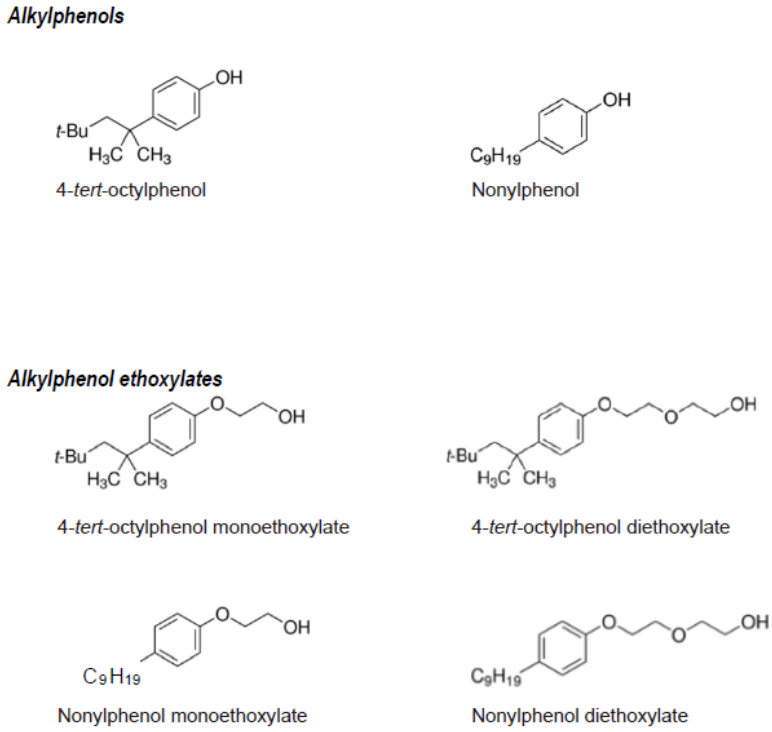


Figure 2.4.
Chemical structure of the targeted APs (alkylphenols and alkylphenol ethoxylates) in the present study may be expected to have the potential to cause adverse effects through the estrogen receptor (ER) mediated mechanism of action.

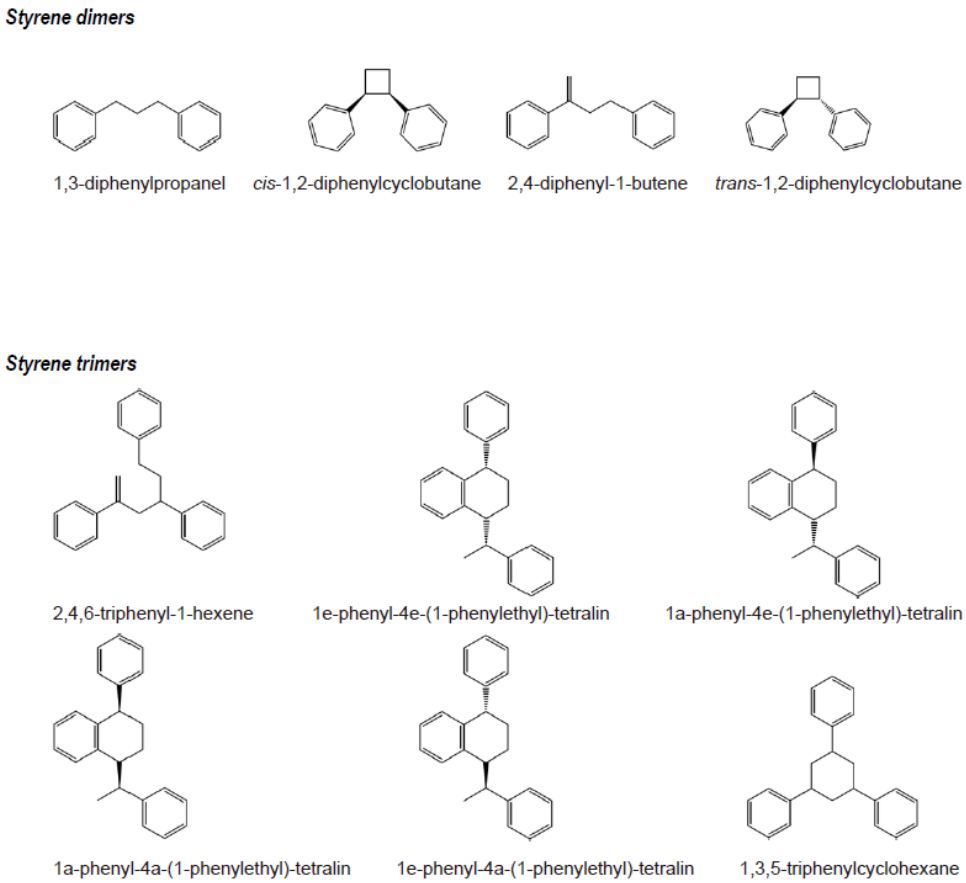


Figure 2.5.
Chemical structure of the targeted SOs (styrene dimers and styrene trimers) in the present study may be expected to have the potential to cause adverse effects through the aryl hydrocarbon receptor (AhR) mediated mechanism of action.

Table 2.2.
QA/QC data for sedimentary PAHs, SOs, and APs measured in the present study.

Compounds	Method detection limit (ng g^{-1} dm)	Surrogate recovery (%, n = 7)
PAHs and SOs		
Acl	0.78	
Ace	0.89	
Flu	1.32	
Phe	0.95	
Ant	0.40	
Fl	0.95	
Py	0.90	
BaA	0.62	
Chr	0.95	
BbF	0.66	
BkF	0.86	
BaP	0.69	
IcdP	0.72	
DbahA	0.17	
BghiP	0.61	
SD1	0.34	
SD2	0.65	
SD3	0.94	
SD4	0.23	
ST1	0.57	
ST2	0.53	
ST3	0.30	
ST4	0.49	
ST5	0.32	
ST6	0.34	
Ace-d10		[a] 82.9 ± 10.1
Phe-d10		102.3 ± 22.2
Chr-d12		97.6 ± 14.6
Pery-d12		92.9 ± 20.4
APs		
4-*t*-OP	0.07	
NPs	0.93	
t-OP1EO	0.09	
NP1EOs	0.45	
t-OP2EO	0.09	
NP2EOs	0.76	
BPA-d16		76.8 ± 18.7

[a] Mean ± SD.

2.2.3 *In vitro* bioassays

The H4IIE-*luc* bioassay was preformed according to previously published methods (Hong et al. 2012, 2015, 2016b). Dilution factors for samples were determined from the results of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Yoo et al., 2006), samples with the percentage of live cells > 80% were used for the bioassay. Trypsinized cells (~7 × 10^4 cells mL^{-1}) from a culture plate was seeded in 60 interior wells of a 96-well plate by adding 250 mL per well (Figure 2.6a). Metabolically labile compounds, such as PAHs or stably AhR binding compounds, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar-polychlorinated biphenyls (Co-PCBs) were identified with two exposure durations (4 and 72 h) in H4IIE-*luc* bioassay (Louiz et al., 2008; Lee et al., 2013; Larsson et al., 2014). After overnight incubation, the cells were exposed to 0.25 mL of extracts of sediments per well (0.1% dose). After 4 h and 72 h exposure, luminescence produced by luciferase was measured by use of a Victor X3 multi-label plate reader (PerkinElmer, Waltham, MA). The detailed molecular mechanisms of activation of gene expression by the AhR described in Figure 2.6b.

A MVLN bioassay was used to evaluate ER-mediated potencies in organic extracts of sediments (Khim et al., 1999a, 1999b). Trypsinized cells from a culture plate were diluted to a concentration of approximately 1.25 × 10^5 cells mL^{-1} in the 60 interior wells of a 96-well plate with 250 mL medium per well. After 24 h incubation, test and control wells were dosed with 0.25 mL per well. Luciferase activity was determined after 72 h of exposure using methods described previously (Villeneuve et al., 2002). Estrogenic compounds induced expression of the *luc*-gene in the basal cellular assay (Figure 2.6b). Details of culturing conditions of two cell-lines for bioassays were provided in Table 2.3

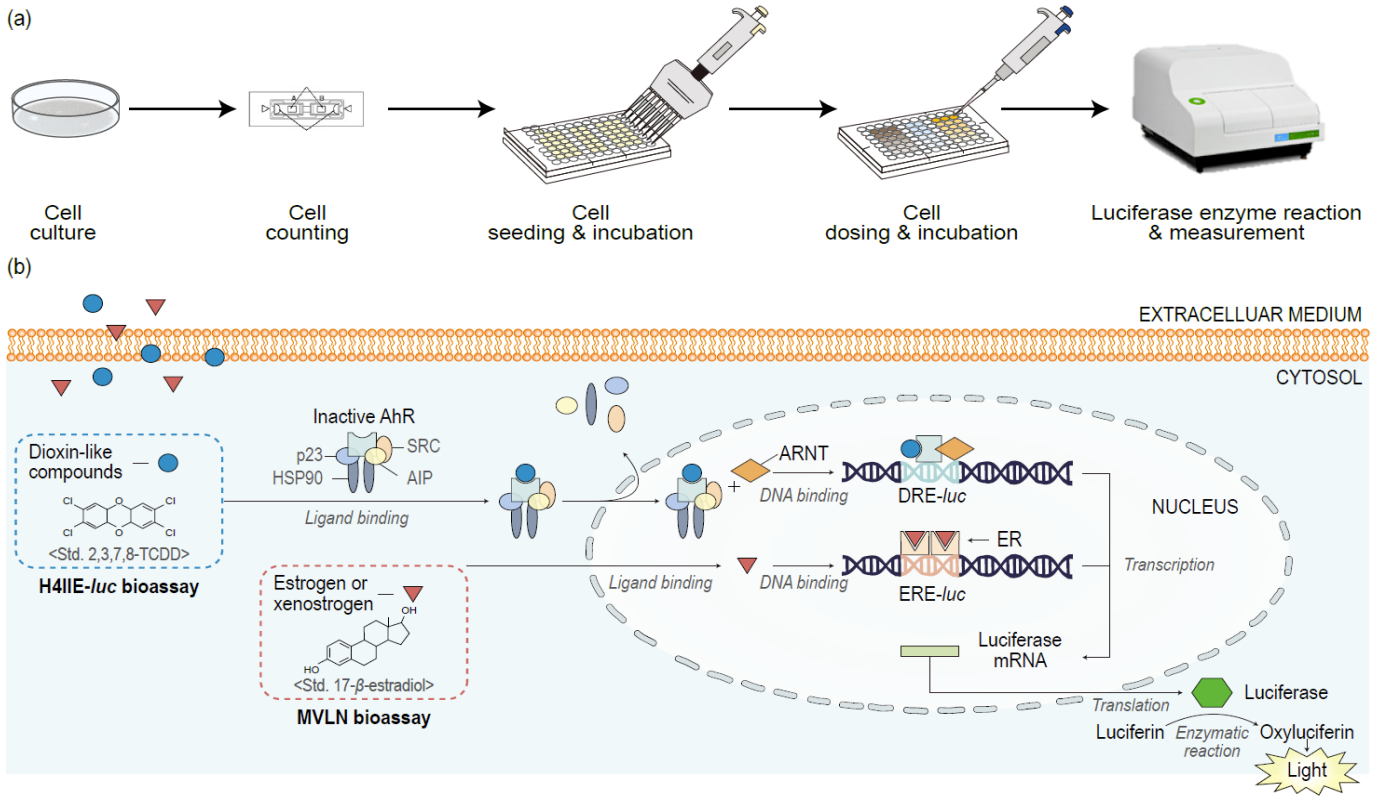


Figure 2.6.

(a) Graphic summary of the recombinant-cell lines bioassay's procedure. (b) The molecular mechanisms of activation of gene expression by the aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)- mediated responses in cell. 2,3,7,8-TCDD; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; HSP90; 90 kDa heat shock protein, AIP; AhR-interaction protein (also known as XAP2), ARNT; AhR nuclear translocator, DRE; dioxin responsive element, ERE; estrogen responsive element.

Table 2.3.
Culturing conditions of two recombinant cells (H4IIE-*luc* and MVLN).

Cell type	H4IIE-*luc*	MVLN
ATCC number	CRL-1548	HTB-22
Organism	Rat	Human
Tissue	Hepatoma; liver	Breast cancer
Growth properties	Epithelial	
Sub-culturing	Remove medium → add fresh 025% trypsin solution for 3 min. → remove trypsin → add fresh medium and dispense into a new petri dish	
Split ratio [a]	A sub cultivation ratio of 1:4	
Fluid renewal [a]	Every 2 to 3 days	
Growth environment [b]	37 ℃, 5% CO_2	
Culture duration [c]	Initiate new culture from frozen cells after approximately 10 passages to reduce the risk of intraspecies cross-contamination, phenotypic drift, or senescence	

[a] Described by ATCC — http://www.atcc.org/.
[b] White et al. (2010).
[c] Freshney (1992).

2.2.4 Potency balance analysis

Responses of the H4IIE-*luc* assay expressed as mean relative luminescence units were converted to percentages of maximum response (% BaP_{max} or % $TCDD_{max}$) for a standard containing 50 nM BaP (100% BaP_{max}) for 4 h and 300 pM 2,3,7,8-tetrachlorodibenzop- dioxin (TCDD) (100% $TCDD_{max}$) for 72 h. Responses of the MVLN bioassay were converted to relative response units expressed as the percentage of the maximum response (% $E2_{max}$) observed for 1235 nM 17β-estradiol (E2). Significant responses were defined as those that were three times the standard deviation of the mean of the solvent controls.

AhR-mediated potencies at 72 h exposure were expressed as 2,3,7,8-TCDD equivalent concentrations (pg TCDD-EQ g^{-1} dm). These concentrations were determined directly from the sample dose-response relationships generated by testing samples at multiple levels of dilution. The E2 standard equivalent concentration (pg E2-EQ g^{-1} dm) was also calculated using the same method. Effective concentrations (EC50s) of BaP and TCDD in H4IIE-*luc* cells and E2 in MVLN cells were 2.91, 0.72, and 4.06 log fmol $well^{-1}$, respectively (Figure 2.7). Relative potencies (RePs) were determined directly from the sample dose-response relationships for each sample and standard curve generated from the range of standard materials (TCDD and E2) dilutions. ReP50s were determined at dilutions of samples for which responses were equivalent to 50% response levels of maximum concentrations of TCDD or E2 on standard curves, respectively (Hong et al., 2012; Lee et al., 2013). All samples were performed in triplicates.

A potency balance analysis between bioassay-derived concentrations (for TCDD-EQs and E2-EQs) and instrument-derived concentrations (TCDD equivalent concentrations [TEQs] and E2 equivalent concentrations [EEQs]) in sediments were conducted to evaluate how individual chemicals contributed to total dioxin-like and estrogenic potencies. Concentrations of TEQs were calculated as the sum of the TEQs, by multiplying the concentration of individual PAHs by their respective ReP values obtained from previous studies (Villeneuve et al., 2002) (Table 2.4). EEQ values of APs were summed from concentrations of NPs and 4-t-OP multiplied by their respective RePs, which were previously reported (Villeneuve et al., 1998).

Principal component analysis (PCA) was performed using the normalized values of the chemical analysis data (individual chemicals) and the bioassay data (bioassay-derived equivalents). SPSS 23.0 (SPSS INC., Chicago, IL) was used for the statistical analysis.

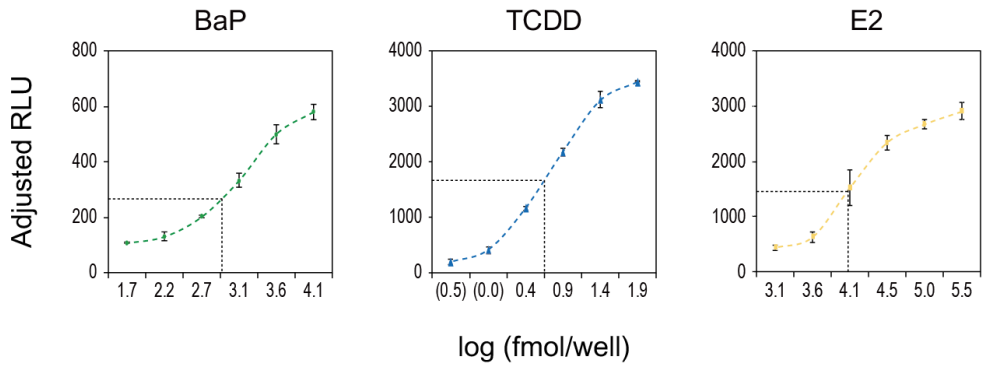


Figure 2.7.
Dose-response curves for all tested standard materials in the present study. Each data point is the Mean ± SD (n = 3).

Table 2.4.
Relative potency values of PAHs for the AhR-mediated potencies used in this study.

Target compounds	ReP values of H4IIE-*luc* cells [a] (72 h)
Benzo[*a*]anthracene (BaA)	1.9×10^{-6}
Chrysene (Chr)	2.3×10^{-6}
Benzo[*b*]fluoranthene (BbF)	5.1×10^{-6}
Benzo[*k*]flouranthene (BkF)	1.4×10^{-4}
Benzo[*a*]pyrene (BaP)	1.6×10^{-6}
Indeno[*1,2,3*-cd]pyrene (IcdP)	1.5×10^{-5}
Dibenz[*a,h*]anthracene (DBahA)	4.6×10^{-6}

[a] Villeneuve et al. (2002).

2.3. Results and Discussion

2.3.1. Long term changes in distributions of PTS in sediments

PAHs, APs, and SOs were detected in all sediment samples (Figure 2.8). Total concentrations of PAHs ranged from 11.3 to 1900 ng g^{-1} dm (mean: 231 ng g^{-1} dm). Concentrations of APs and SOs ranged from 1.24 to 5024 ng g^{-1} dm (mean: 965 ng g^{-1} dm) and 10.1 to 740 ng g^{-1} (mean: 227 ng g^{-1} dm), respectively. In general, concentrations of target chemicals varied among locations and when samples were collected, with an increasing trend for PAHs and a decreasing trend for APs and SOs.

Concentrations of PAHs extracted from sediment in inland creeks ranged from 12.8 to 643 ng g^{-1} dm (mean: 226 ng g^{-1} dm, n = 8) in 2001 (Koh et al., 2005) and from 210 to 1900 ng g^{-1} dm (mean: 693 ng g^{-1} dm, n = 6) in 2015. Mean concentrations of PAHs in sediments from inland creeks increased by nearly three-fold over the ~15-year period between the two collections (Figure 2.8a). These changes might be due to releases of pyrogenic (combustion) and petrogenic (petroleum) sources between 2001 and 2015 (Valavanidis et al., 2008). In lake sediments, concentrations of PAHs were similar in 1998 (mean: 18.9 ng g^{-1} dm) and 2015 (mean: 25.1 ng g^{-1} dm). Detectable concentrations of PAHs in sediments from the lake observed in sediments collected in 1998 (Khim et al., 1999a) were similar to those in sediments collected in 2015. In general, concentrations of PAHs were greater in sediments from inland creeks than in those from the lake. These results were consistent with the results of a previous study, in which concentrations of PAHs were greater near urban centers (Juhasz and Naidu, 2000; Lee et al., 2016).

Similar to PAHs, concentrations of APs were also greater in sediments from inland creeks than those from the lake (Khim et al., 1999a; Li et al., 2004a; Imran et al., 2005). Thus, it can be concluded that most PAHs and APs originated from surrounding industrial complexes and cities. Mean concentrations of APs in sediments from the lake were 410 ng g^{-1} dm (n = 8) in 1998 and 3 ng g^{-1} dm (n = 5) in 2015. SOs are emerging pollutants resulting from the degradation of plastics in marine environments. However, during the last decade, there are no reports are available on historical distributions of SOs in coastal environments.

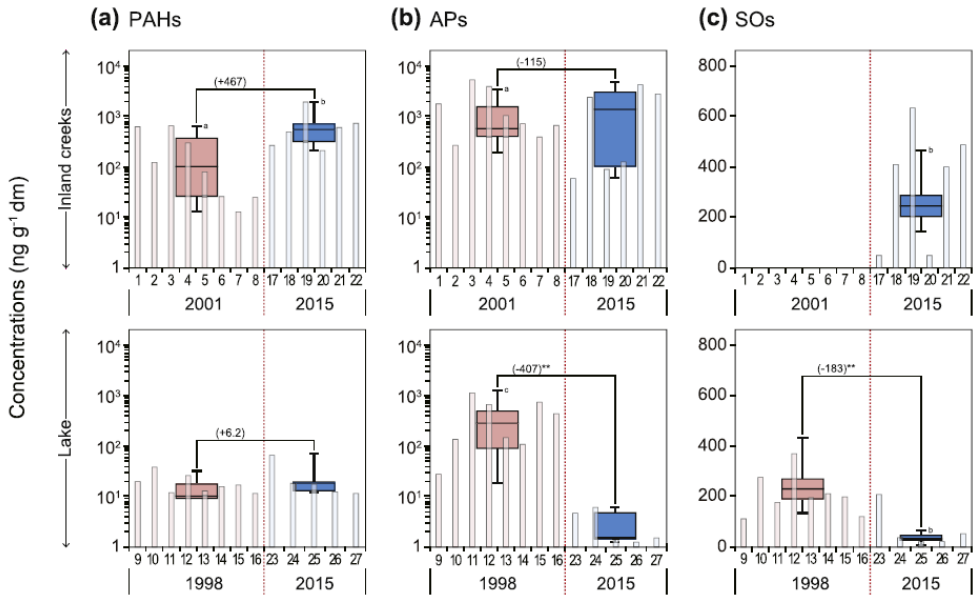


Figure 2.8.

Spatio-temporal distributions of (a) polycyclic aromatic hydrocarbons (PAHs), (b) alkylphenols (APs), and (c) styrene oligomers (SOs) in the sediment samples from the inland creeks and inner side of dam of Lake Sihwa in the past and current studies. Box plots represent the minimum, 25%, median, 75%, and maximum values of data from same period and region. Data refer to [a] Koh et al. (2005), [b] Hong et al. (2016b), [c] Khim et al. (1999a). ** Significant declines in concentration are indicated for each chemical group ($p < 0.05$).

Thus, to address the historical occurrence as well as long-term changes, in this study, archived samples from 1998 were re-analyzed together with samples collected in 2015. Concentrations of SOs in sediments from the lake were ranged from 132 to 324 ng g^{-1} dm (mean: 217 ng g^{-1} dm) in 1998 and from 10.1 to 62.6 ng g^{-1} dm (mean: 34.2 ng g^{-1} dm) in 2015 (Hong et al., 2016b). These results indicated that, although only recently recognized as emerging PTSs, SOs have been present in the Lake Sihwa aquatic system for several decades. The major reason for the dramatic decrease concentrations of SOs in sediments between the two sampling years was likely, not due to dilution, but rather, changes in multiple sources of SOs contamination in the given area. Limited information is available on the occurrences of SOs in sediments; thus, more complementary studies would be necessary to identify their sources and fate.

Concentrations of PAHs, APs, and SOs in sediments from inland creeks were greater than those from the lake (Figure 2.8). This spatial distribution of PTSs indicates that chemicals tend to accumulate in sediments near the sources, because of anthropogenic activities in municipal and industrial areas. PTSs that are related to industrialization, urbanization, and hydrophobic compounds, rather than hydrophilic contaminants, can contribute significantly to toxicities of sediments (Schulze-Sylvester et al., 2016). Because PTSs could not be transported to distant regions due to the hydrodynamic conditions of the lake, greater concentrations of PAHs, APs, and SOs observed in sediments from inland creeks suggest that the region is still affected by industrial complex in nearby cities.

Since the late 1990s, sediments from the lake, which had been contaminated previously could have been purified by occasional circulation of seawater through the water gate (Lee et al., 2014). In addition, construction of the tidal power station in 2012 could have reduced sediment contamination in the lake due to circulation of seawater into the lake.

Relative compositions of target PTSs in sediments collected in 2015 were compared to profiles of the same chemicals in sediments collected in 1998 and 2001 (Figure 2.9). Profiles of relative concentrations of PAHs, APs, and SOs varied among locations and in particular between the lake and inland creeks and between

when sediments were collected. These results indicated that spatio-temporal changes in sources of contaminants with possible recent input(s) (Yunker et al., 2002). When compared to PAHs collected in 1998 and 2001, PAHs observed in 2015 were dominated by higher-molecular weight (HMW) PAHs that had five to six rings (Figure 2.10). These patterns were especially predominant in the sediments from the inland creeks. On average, HMW PAHs (such as BkF, BaP, DBahA, and BghiP) from the inland creeks represented 23.4% of total PAHs in 2001; however, by 2015, this percentage had increased to 41.1%. Over time relative contributions of HMW PAHs in sediments from inland creeks increased by more than two-fold, while those of lower-molecular weight (LMW) PAHs (such as Ant, Py, and Chr) declined. These results confirmed that industrial waste (combustion of heavy-duty diesel engines and gasoline engines) contributes to the concentrations of HMW PAHs (Rogge et al., 1993) in sediments of inland creeks. This phenomenon occurred because HMW PAHs are derived from combustion of gasoline and diesel in vehicles, whereas LMW PAHs in the environment are derived from incomplete combustion of wood, coal, and biomass (Li and Duan, 2015). It is also possible that due to lesser hydrophobicity LMW PAHs they can degrade more rapidly, which would cause a decline in relative contributions of LMW PAHs to total concentrations of PAHs. Ratios of IcdP / (IcdP + BghiP) against BaA / (BaA +Chr) and Fl / (Fl + Py) as a function of Ant / (Ant + Phe) in sediments were calculated to verify sources of PAHs contributed from petroleum and combustion, respectively (Figure 2.11) (Yunker et al., 2002). Results showed that PAHs in sediments of inland creeks originated primarily from combustion of petroleum, whereas those in sediments in the lake originated from combustion of grass, wood, and coal (Hong et al., 2012; Jiao et al., 2012).

In 2015, relative compositions of APs varied between sediments from inland creeks and the lake (Figure 2.9b). In sediments from inland creeks, among APs, NP (72.7%) was the predominant constituent, followed by NP1EO (15.2%). Similarly, relative proportions of 4-*t*-OP were greater than those of OP1EO and OP2EO in sediments from inland creeks. The pattern observed in sediments from the lake was different. For example, NP2EO (40.3%) and NP1EO (37.6%) were the primary APs in sediments from the lake. It is possible that, because NP and 4-*t*-OP are

hydrophobic chemicals that tend to adsorb to sediments, the chemicals do not break down well in water columns (Ying et al., 2002). These results collectively suggest that current regulations, which prohibit their use, along with more efficient treatment of wastes have been effective in reducing inputs of APs into the inland creeks and subsequently into the lake.

In 2015, of the 10 SOs, ST1 (43.6%) was the predominant constituent in sediments from inland creek sediments, followed by SD3 (22.1%). In contrast, also in 2015, in sediments from the lake, SD3 (47.0%) was the predominant chemical (Figure 2.9c). These results indicate that SD3 is widely distributed in the coastal area, and might be transported to areas relatively far from land. Also, the composition of STs (71.0%) in sediments collected from the lake in 1998 was greater compared to that in sediments (37.0%) collected during 2015. Greater concentrations of STs compared to SDs indicate that the relationship between polystyrene pollution and the human population is affected by direct inputs from local sources (Kwon et al., 2014; Hong et al., 2016b). Because, after mechanical breakdown, ST1 is first detected from decomposition of polystyrene, relatively large contributions of STs might indicate recent inputs of fresh materials. Results of a previous study showed that ST1 adsorbs to surfaces of sediments and can persist for some time (Saido et al., 2014). However, the results of the present study indicated that concentrations of STs were not maintained over the 15 years. Few studies have been conducted on distributions and relative compositions of SOs in coastal marine sediments (Kwon et al., 2014, 2015; Saido et al., 2014; Hong et al., 2016b), thus more studies are required on sedimentary SOs.

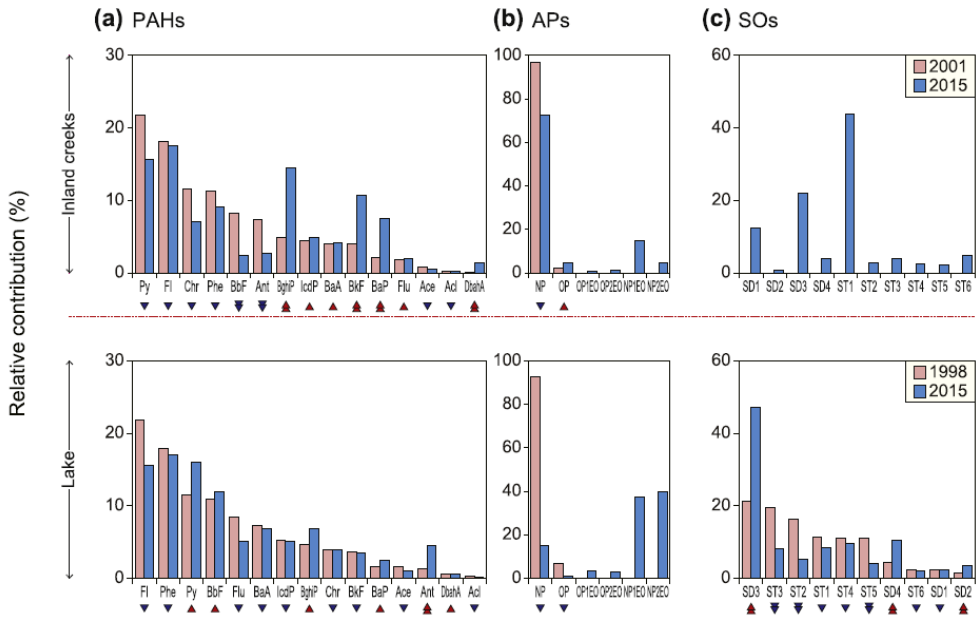


Figure 2.9.

Comparison of profiles of relative concentrations of (a) 15 individual PAHs to total concentrations of PAHs, (b) two APs (4-*t*-OP + NP) and four APEOs to total APs for the past and recent samples, and (c) 10 individual SO to total SOs concentrations in the sediments from the inland creeks and inside of dam in the past (1999 and 2001) and recent (2015) years.

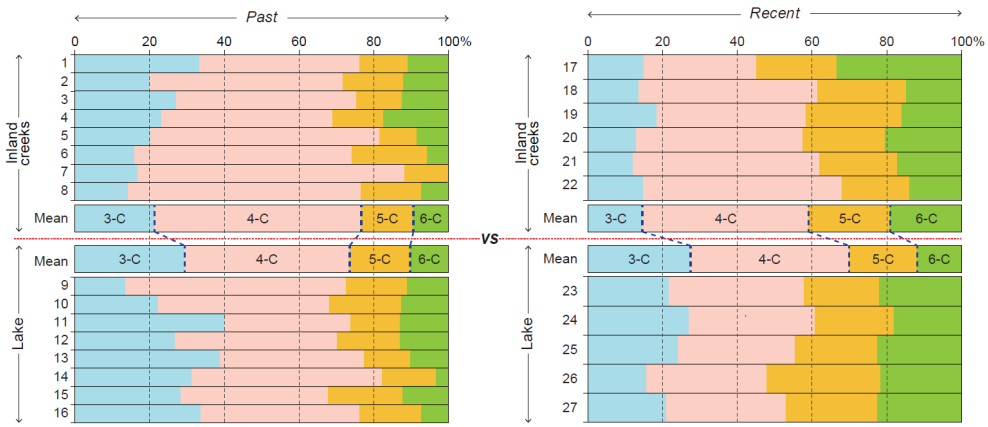


Figure 2.10.
Mean composition of PAH ring number to total PAHs from the inland creeks and inside of dam sediments in the past and current studies.

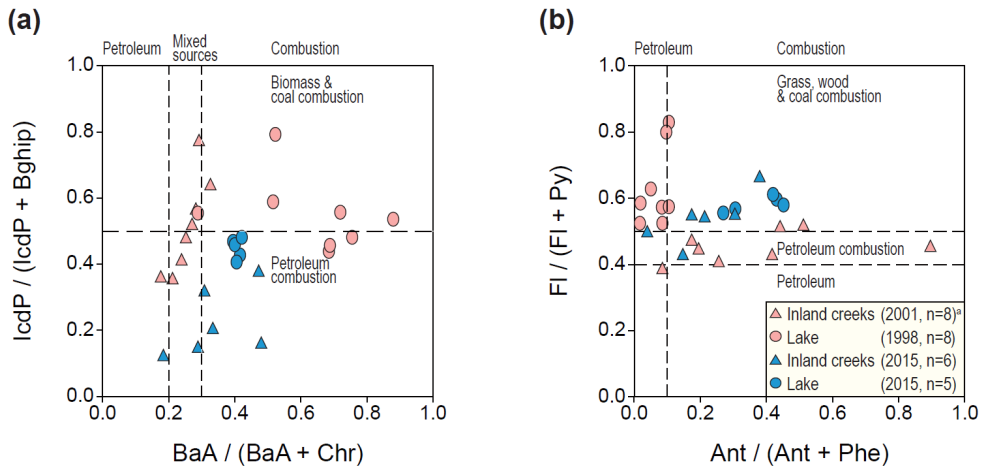


Figure 2.11.
Results of the diagnostic ratios for the source identification of the PAHs. (a) Cross plots for the ratios between BaA / (BaA + Chr) with IcdP / (IcdP + BghiP) and (b) the ratios between Ant / (Ant + Phe) with Fl / (Fl + Py) for source identifications of PAHs in the sediments.

2.3.2. AhR- and ER-mediated potencies

To assess potential AhR-mediated potencies of sediments from Lake Sihwa (Figure 2.12) and differentiate between labile and more recalcitrant compounds, H4IIE-*luc* bioassays were performed after both 4 h and 72 h. All extracts contained AhR-mediated potency that was dependent on duration of exposure as well as location. In sediments collected from the lake in 2015, mean concentrations of AhR-mediated potency for 4 h exposure were 60 to 127% BaP_{max} (mean = 103% BaP_{max}). In comparison, mean concentrations of AhR-mediated potency after 72 h exposure ranged from 2 to 11% $TCDD_{max}$ (mean = 6% $TCDD_{max}$) (Figure 2.12). These results suggest that the chemicals responsible for activation are rapidly metabolized by the cells. This finding supports the hypothesis that PAH-like chemicals are important in observed AhR-mediated activity (Louiz et al., 2008; Kinani et al., 2010). Also, greater AhR-mediated potency was detected in sediments from inland creeks compared to that in sediments from the lake. Dioxin-like activity and chemical analysis showed large variation across sites, which might be related to industrial and urban activities in the vicinities of inland creeks (Figure 2. 13a). Thus, results of the H4llE-*luc* bioassay indicated that various actions implemented to reduce releases of these compounds to the creeks have been effective, but concentrations of dioxin-like compounds in sediments of Lake Sihwa remain high.

The 19 sediment extracts were screened for their ability to promote ER-mediated gene expression in MVLN cells (Figure 2.13a; inland creek 2001 sediment data were obtained from Koh et al., 2005). All of the tested samples elicited a significant increase in luciferase expression. The sediments that exhibited estrogenicity were collected from rivers that receive sewage discharges. Sources of various ER agonists in sediments are hypothesized to originate from sewage effluents and industrial discharges (Luo et al., 2011). Over time, ER-mediated potency in sediments from the lake has decreased significantly. Mean estrogenic potencies were 211 pg E2-EQ g^{-1} dm and 128 pg E2-EQ g^{-1} dm in sediments collected 1998 and 2015, respectively (Table 2.5 and Figure 2.13a).

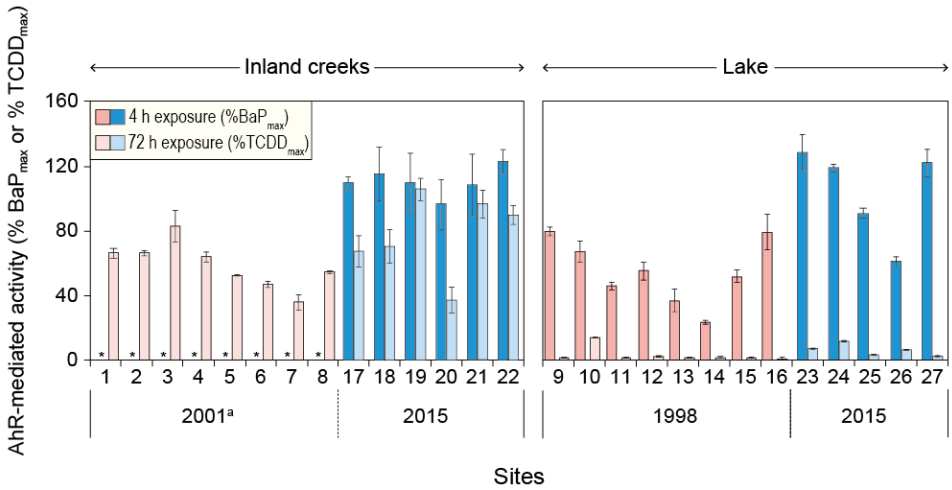


Figure 2.12.

AhR-mediated potencies of the sediment raw extracts from the inland creeks and lake in the past and current years at 4 h and 72 h exposure durations in the H4IIE-*luc* bioassay. [a] Data from Koh et al. (2005). * Data were not available.

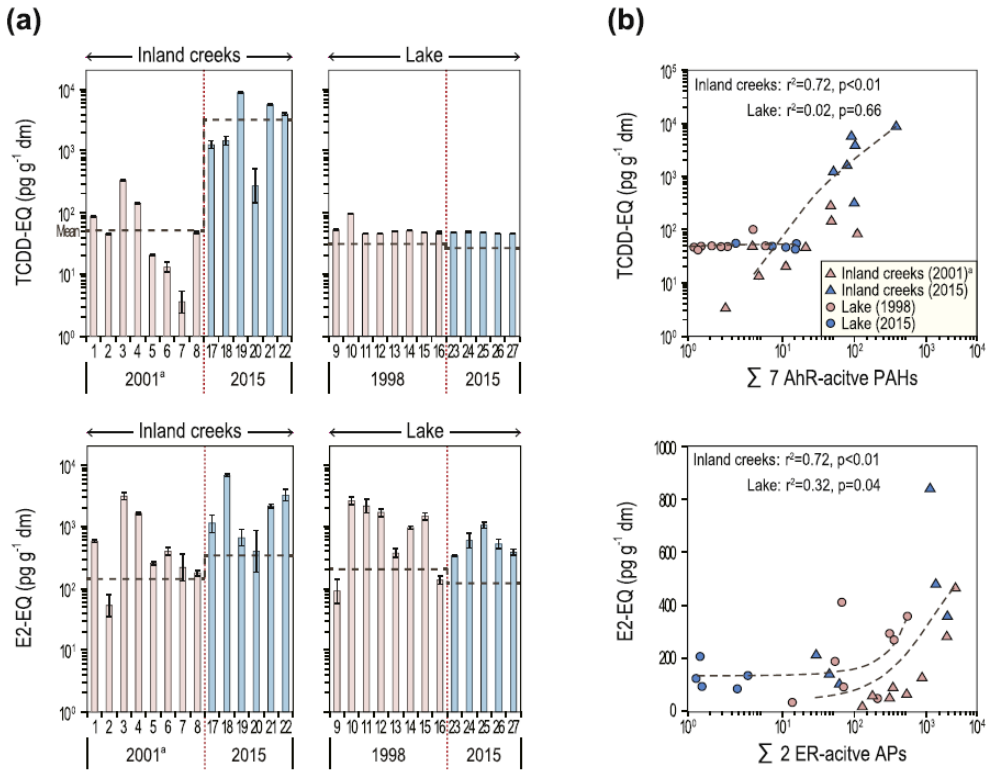


Figure 2.13.

Spatiotemporal distributions of (a) TCDD-EQs and E2-EQs in sediments from inland creeks and Lake Sihwa in 1998 and 2001 or 2015 (Mean ± SD (n = 3)).

(b) Scatter plots showing the dose-response relationships between the chemical analyses and biological responses in the inland creeks and lake sediments of Lake Sihwa. [a] Data refer to Koh et al. (2005).

Table 2.5.

Overview of the results for the instrument-derived equivalents and bioassay-derived equivalents of sediments collected from Lake Sihwa, Korea.

Region	Sampling year	# of sites	Instrument-derived equivalents		[a] Bioassay-derived equivalents		[b] Potency balance analysis	
			[c] TEQ	[d] EEQ	TCDD-EQ	E2-EQ	TEQ/TCDD-EQ	EEQ/E2-EQ
			Min.–Max. (Mean)	Min.–Max. (Mean)	Mean	Mean	Min.–Max. (Mean)	
			(pg g^{-1} dm)		(pg g^{-1} dm)		(%)	
Inland creeks	2001	[e] 8	0.1–4.7 (1.7)	3.4–65.9 (22.0)	86.3	148	0.6–5.6 (2.6)	8.1–18.5 (14.6)
	2015	6	0.6–5.9 (2.1)	[f] n.d.–13.5 (2.9)	3630	211	0.0–0.2 (0.1)	0.02–3.8 (0.8)
Lake	1998	8	n.d.–0.3 (0.1)	0.4–14.4 (5.4)	54.7	356	0.1–0.4 (0.2)	0.4–12.9 (3.5)
	2015	5	0.3–0.9 (0.6)	0.0–0.1 (0.03)	47.0	128	0.6–2.0 (1.3)	0.0–0.1 (0.03)

[a] Bioassay- derived values were obtained from sample dose-response relationships generated by testing samples at multiple levels of dilution.
[b] Potency balance values were obtained from the percentage of instrument-derived values to the bioassay-derived values.
[c] TEQ values of PAHs were summed from the chemical concentrations of BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA multiplied by the ReP values obtained in a previous study (Villeneuve et al., 2002).
[d] EEQ values of APs were summed from the chemical concentrations of NPs and 4-*t*-OP multiplied by the ReP values obtained in a previous study (Villeneuve et al., 1998).
[e] Data from Koh et al. (2005).
[f] n.d.: Below detection limits.

These results indicated that concentrations of major ER-active compounds in sediments from the lake have decreased over the last 15 years. This change indicates that efforts directed towards the improvement of the Lake Sihwa environment, including the ban in 2002 on the use of ER-active compounds in household products, have been effective (Hong et al., 2010).

Although concentrations of ER-active compounds declined over time, spatial patterns of estrogenic potencies in sediments did not change. ER-mediated potencies in the past and present sediment samples from the inland creeks were greater those in sediments from the lake. This difference might be because the sediments in the inland creeks have been more contaminated by industrial, agricultural, and domestic wastewaters from the nearby cities. Several studies have reported that the sources of various ER agonists in sediments originate from industrial discharge and sewage effluent. Examples of such agonists include NPs, 4-*t*-OP, estrone (E1), and estradiol (E2) (White et al., 1994; Kinani et al., 2010; Luo et al., 2011). In our study, APs concentrations (such as NPs and 4-*t*-OP) were consistently detected over time in the sampled sediments. Thus, these results support the conclusion that even though APs are relatively weak ER agonists, their concentrations are sufficient to contribute a significant portion of the ER-mediated potency measured in the bioassay.

2.3.3. Potency balance

To determine the proportion of *in vitro* potencies observed in samples, that could be explained by concentrations of known agonists, a potency balance analysis was conducted (Khim et al., 2001; Giesy et al., 2002; Hong et al., 2012). TEQs contributed by Ah-active PAHs explained only a small portion of total concentrations of TCDD-EQ. Proportions explained by PAHs ranged from 0.2 to 2.6% in sediments from 1998–2001 and from 0.1 to 1.3% in sediments collected in 2015 (Table 2.5 and Figure 2.14). By examining the relationship between TCDD-EQs and AhR-active PAHs of the dataset in a scatterplot (Figure 2.13b), TCDD-EQs were found to be correlated ($r^2 = 0.75$, $p < 0.01$) with AhR-active PAHs in sediments from inland creeks. Known AhR-active compounds accounted for only a small portion of total induced AhR-mediated potencies in sediments but significant correlations between some AhR-active PAHs and dioxin-like activities suggested some compounds similar to PAHs were acting as agonists. Overall, the results of the potency balance suggest that unknown AhR-mediated compounds are broadly distributed in the sediments.

NP and 4-*t*-OP contributed 14.6% of the concentration of EEQs in sediments collected from inland creeks in 2001 and 0.8% of EEQs in 2015. In comparison, in sediments from the lake, NP and 4-*t*-OP contributed only 3.5% of concentrations of EEQs in 1998 and 0.03% of concentrations of EEQs in 2015 (Table 2.5). Contributions of known ER agonists, such as NP, decreased over 15 years. Target APs did not explain much of the overall estrogenic activities, particularly in sediments collected more recently. Thus, studies on unidentified ER agonists in sediments are needed. For example, to better characterize sources of contamination of these sites, use of EDA, based on sample fractionation and identification of fractions causing significant potencies in bioassays followed by detailed untargeted instrumental analyses (Brack, 2003; Hong et al., 2016c). In sediments from inland creeks, ER-mediated potencies (E2-EQs) were correlated ($r^2 = 0.50$, $p < 0.01$ and $r^2 = 0.32$, $p = 0.04$, respectively) with concentrations of $\sum$ 2 ER-active APs (Figure 2.13b). These results indicate that total concentrations of ER-mediated potencies in sediments from Lake Sihwa were relatively well explained by concentrations of APs.

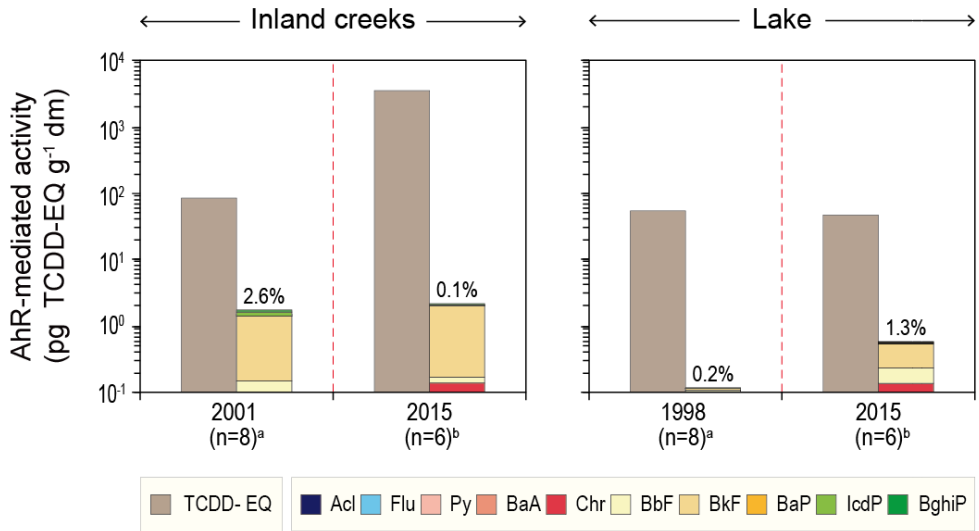


Figure 2.14.
Potency balance between bioassay-derived (TCDD-EQs) and instrument-derived TEQs in sediments collected from Lake Sihwa and the relative contributions of identified TEQs in the sediment samples.

To characterize the bioassay-EQ in the sediments further, a principal component analysis (PCA), based on the concentrations of residues, was performed (Figure 2.15). The PCA produced two major components that collectively accounted for 56.0% of the total variance. PC1 (explaining 30.0% of total variance) was positively correlated with PAHs, APs, and E2-EQ, but was negatively correlated with TCDD-EQ. When sites were ordinated based on PCA scores, they were classified into three groups. The first group contained sediments collected in 2015 from inland creeks. These sites exhibited greater concentrations (top 10%) of target PTSs and contained greater concentrations of TCDD-EQ and E2-EQ. The second group was characterized by sites with relatively great concentrations of PAHs and APs. These sites might have accumulated these compounds from independent sources and/or due to different conditions along the creeks to the lake. The lesser correlation between concentrations of TCDD-EQ and PAHs indicates that AhR-active PAHs explain only a small proportion of concentrations of TCDD-EQ. Overall, the PCA analysis demonstrated that the majority of variance was observed among locations but generally supported the time-dependent aggregation.

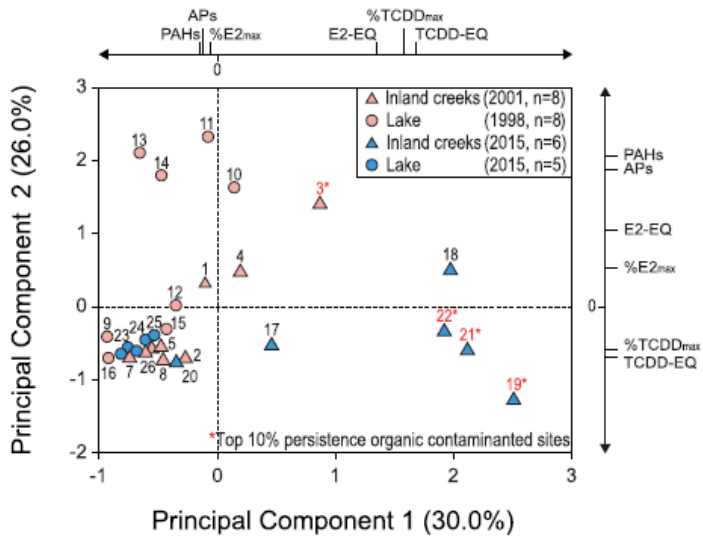
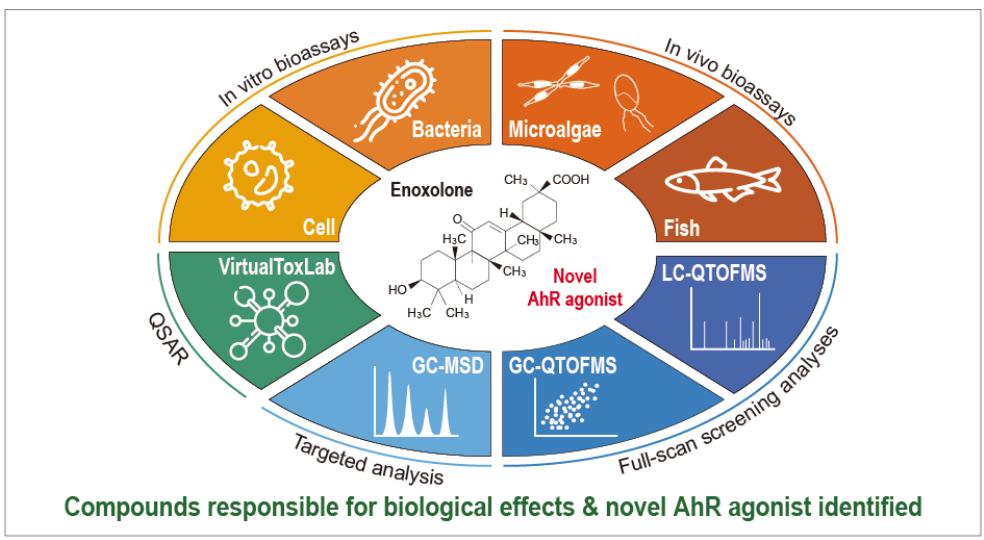


Figure 2.15.
Principal component analysis of PAHs, APs, AhR-mediated potencies (% $TCDD_{max}$), ER-mediated potencies (% $E2_{max}$), and bioassay-derived equivalents associated with the sediments collected from the 27 locations in Lake Sihwa. Clusters are represented as locations (inland creeks and lake), in which principal components 1 and 2 accounted for 30.0% and 26.0% of the variability of the dataset, respectively.

CHAPTER 3.

MULTIPLE BIOASSAYS AND TARGETED AND NONTARGETED ANALYSES TO CHARACTERIZE POTENTIAL TOXICOLOGICAL EFFECTS ASSOCIATED WITH SEDIMENTS OF MASAN BAY: FOCUSING ON AhR-MEDIATED POTENCY



This chapter has been published in Environmental Science and Technology.
Lee, J., Hong, S., Kim, T., Lee, C., An, S.-A., Kwon, B.-O., Lee, S., Moon, H.-B., Giesy, J.P., Khim, J. S. Multiple bioassays and targeted and nontargeted analyses to characterize potential toxicological effects associated with sediments of Masan Bay: focusing on AhR-mediated potency. *Environ. Sci. Technol.* 2020, **54**, 4443–4454. https://doi.org/10.1021/acs.est.9b07390

3.1. Introduction

Assessing chemical contamination of sediments is complicated because chemicals occur as complex mixtures and undergo both biotic and abiotic transformations (Brack, 2003). The development of highly sensitive target analyses allows precise and accurate quantification of chemical contaminations in sediments (Seoane et al., 2017). However, due to technical limitations, such analyses often provide little information about toxicity of chemical mixtures and/or bioavailability (Xiao et al., 2016; Brack et al., 2019). More importantly, chemical concentrations are not sufficient to demonstrate biological effects, because they do not provide information about the potential adverse effects on aquatic organisms (Brack, 2003; Wang et al., 2014; Lee et al., 2018).

In the present study, EDA was conducted to identify the toxicity profile of contaminants in sediments by use of multiple bioassays. Based on the results of previous pollution studies in Korean coastal waters, Masan Bay was selected as a study area from which to obtain contaminated sediments. Masan Bay, located on the southeastern coast of South Korea, is a semi-enclosed bay with restricted water exchange. It is subject to numerous anthropogenic impacts, including urbanization, industrialization, and intensive shipping activity (Figure 3.1). Severe pollution of sediments and associated significant toxic effects have been reported for Masan Bay since the 1990s (Figure 3.1) (Khim et al., 1999b; Jeon et al., 2017; Lee et al., 2018). In particular, over the past few decades, riverine and bay sediments from the study area are reported to be contaminated with various environmental pollutants (Hong et al., 2009; Yim et al., 2014; Lee et al., 2016).

Bioassays utilized in the present study cover three mechanisms of actions including specific-, baseline-, and reactive-toxicity (Jia et al., 2019). *In vitro* bioassays comprise receptor gene assays for measuring “specific toxicity” of AhR agonists, such as polycyclic aromatic hydrocarbons (PAHs) (H4IIE-*luc* bioassay) and the bacterial test (*Vibrio fischeri* assay), which is related to the energy metabolism of a bacterium, was used for “baseline toxicity” measurements (Brack et al., 1999; ISO, 2007; Jia et al., 2019). Inhibition of growth of microalgae and cell

viability tests and the fish embryo toxicity test (FET) were conducted to assess the acute toxicity with multiple endpoints on the primary producers and vertebrate species, respectively (Hollert et al., 2003). Each bioassay exhibits different sensitivities to chemical contaminations in sediments; thus, the combination of a battery of bioassays provides a better assessment of the sediment contaminations (Maltby et al., 2005; Brack et al., 2019).

The present study aimed to (i) assess potential toxicological effects of polluted sediments using a battery of bioassays with various organisms and endpoints, (ii) measure concentrations and compositions of target PTSs (PAHs, styrene oligomers (SOs), and alkylphenols (APs)) in sediments using GC-MSD, (iii) identify untargeted AhR agonists in some potent fractions using GC-QTOFMS and LC-QTOFMS, (iv) evaluate relative potencies (RePs) of tentative AhR agonists, if any, using the H4IIE-*luc* bioassay, and finally (v) determine relationships between chemical concentrations and/or compositions in sediments and observed *in vitro* and *in vivo* biological effects. A schematic representation of the stepwise procedures of the present study is shown in Figure 3.2 (details description of Figure 3.2 in materials and methods section).

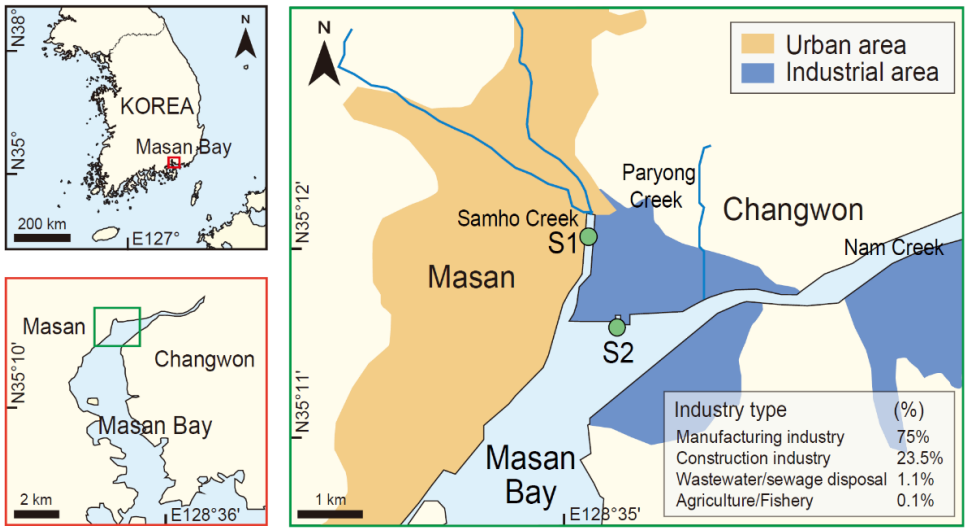


Figure 3.1.
Sampling sites (S1 and S2) of surface sediments from the vicinity of inland creeks of Masan Bay, Korea (March 2016). The type of business in the industrial complex was referred from the website of Changwon City (https://www.changwon.go.kr).

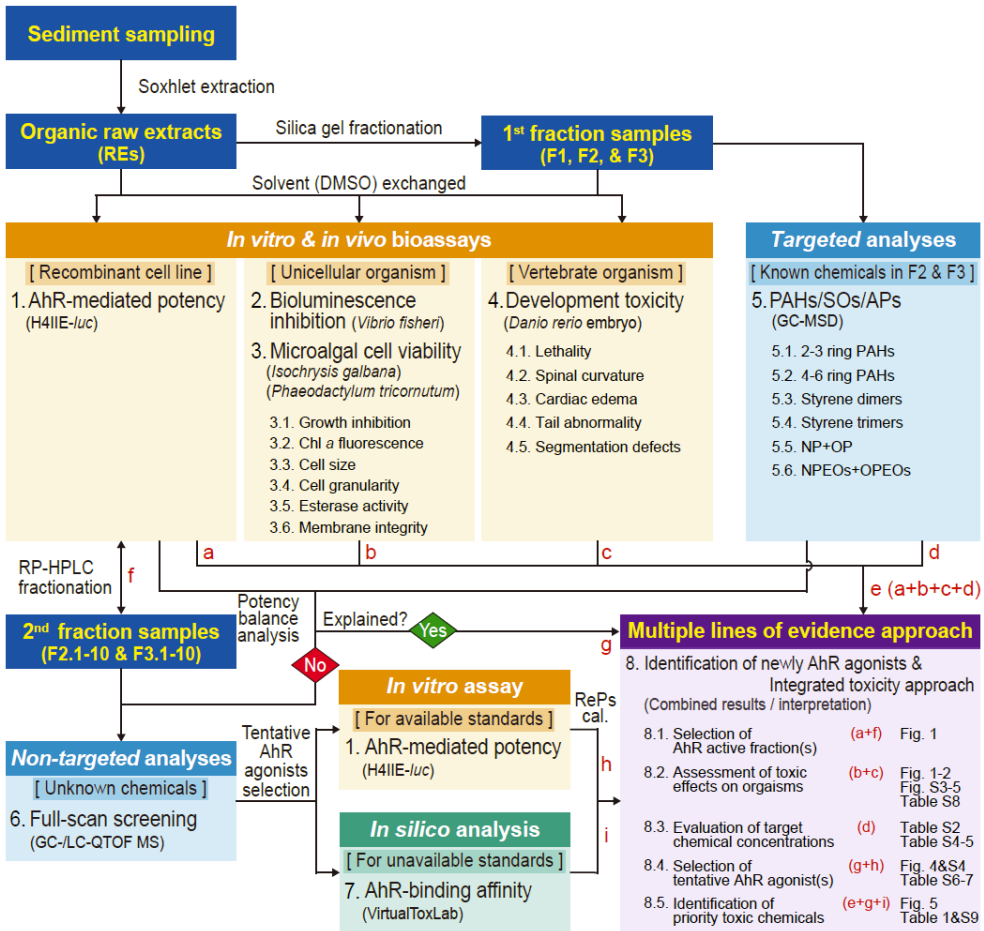


Figure 3.2.
Workflow overview of the fractionation strategy, bioassays, and chemical analyses used to identify priority substances in the sediments from Masan Bay, Korea.

3.2. Materials and Methods

3.2.1. Sampling and sample preparations

Sites S1 and S2 were located near Samho Creek and Nam Creek, respectively, which are two major rivers flowing into the Masan Bay (Figure 3.1). In March 2016, surface sediments (~3 cm) were collected by use of a hand shovel. Sample preparation for bioassays and chemical analyses was conducted, with minor modifications to previously published methods (Hong et al., 2015). In brief, sediments were freeze-dried, passed through a 1-mm sieve, and homogenized. Sediments (60 g) were extracted with 350 mL dichloromethane (DCM, J.T Baker, Phillipsburg, NJ) in a Soxhlet extractor for 16 h. Raw organic extracts were concentrated to 6 mL with a rotary evaporator and N_2 gas flow (~10 g sediment equivalent (SEq) mL^{-1}). For bioassays, the aliquot of raw extracts was exchanged into dimethyl sulfoxide (DMSO, Sigma-Aldrich, Saint Louis, MO) to obtain a final concentration of 0.1% DMSO in the test solution (Table 3.1).

REs were fractionated in a two-step procedure (Figure 3.2). Step one fractionation was conducted with 4 mL raw extracts using 8 g activated silica gel (70−230 mesh, Sigma-Aldrich) in a packed glass column based on differences in polarity (F1 to F3) (Hong et al., 2015). The first fraction (F1) was collected by elution with 40 mL hexane. The second fraction (F2) was eluted with 50 mL of 20% DCM in hexane (v/v). The third fraction (F3) was eluted in 50 mL of 60% DCM in acetone (J.T Baker).

If the primary fractions were significantly toxic, a secondary fractionation step was applied using reverse-phase (RP)-HPLC (Agilent 1260 HPLC; Agilent Technologies, Santa Clara, CA) (Figure 3.2). Instrumental conditions of RP-HPLC, including sampling times and log K_{ow} intervals, were presented (Table 3.2). Separation conditions of RP-HPLC were optimized previously through several tests with GC-MSD confirmation using 34 PCBs, 16 PAHs, 7 APs, and 5 phthalates (with log K_{ow} values) (Hong et al., 2015). Acceptable elution efficiency with all compounds was achieved (> 85%).

Table 3.1.

Description of the experimental design for the bioassays examined in this study.

Bioassays	***In vitro* assays**		***In vivo* assays**	
Test organism	**H4IIE-*luc***	***Vibrio fischeri***	***Isochrysis galbana* /*Pheodactylum tricornutum***	***Danio rerio***
Specific purpose	Measurement of AhR-mediated potencies	Evaluation of inhibition of bioluminescence	Reproduction inhibition of microalgae	Measurement of developmental toxicity
Test samples	Raw, F1–F3, F2.1–F2.10 for S1 and S2 F3.1–F3.10 for S2	S.T [a]: Raw, F1–F3 E.T [b]: Raw, F1–F3 for S1 Raw, F1–F2 for S2	Raw, F1–F3	Raw, F1–F3
Experimental conditions				
Test chamber	96-well plates	96-well plates	250 mL culture flasks	24-well plates
Solvent carrier	0.1% DMSO	0.1% DMSO	0.1% DMSO	0.1% DMSO
Temperature (°C)	37	15	15	26 ± 1
Test duration (hours)	4	0.5	96	96
Initial concentrations	7.0×10^4 cells mL^{-1}	S.T: 200 µL of samples with 25 µL of bacterial solution E.T [c]: 100 µL of samples with 100 µL of bacterial solution	6.0×10^4 cells mL^{-1} / 3.0×10^4 cells mL^{-1}	5 organisms
Replicates	3	4	3	3
Positive control	Benzo[*a*]pyrene	Zinc sulfate solution	-	-
Endpoint	AhR-mediated potency	Bioluminescence inhibition	Growth inhibition Cell size Cell granularity Chlorophyll *a* Esterase activity Membrane integrity	Lethality Spinal curvature Cardiac edema Tail abnormality Segmentation defects
Data presented in	Figure 3.7a	Figure 3.7b and Table 3.9	Figures 3.8, 3.9a and b	Figure 3.9c

[a] S.T: Screening test.

[b] E.T: EC50 test.

[c] Tested with eight concentrations of 50% serial dilution.

Table 3.2.

Instrumental conditions of reverse phase-HPLC for fractionation of organic raw extracts. Retention times of various organic chemicals (test standards, $n = 62$) given as a function of the log K_{ow} values of chemicals.

RP-HPLC system	Agilent 1260 HPLC system (Preparative scale)
Detector	1260 Multiple wavelength detector
Column	PrepHT XDB-C18 reverse phase column (250 mm × 21.2 mm × 7 μm)
Mobile phase	Water (A) : MeoH (B) (40 : 60, v/v), Isocratic elution
Injection volume	1 mL
Flow rate	10 mL min^{-1}

Gradient conditions [a]

Time (min.)	Solvent	
	A	B
0	40	60
40	0	100
65	0	100
66	0	60
70	40	60

Test standards [a]

34 polychlorinated biphenyls
16 polycyclic aromatic hydrocarbons
7 alkylphenols
5 phthalates

Fractions collected [a]

RP-HPLC Sub-fraction	Starting–End sampling time (min.)	Log K_{ow}
1	1.81–8.30	< 1
2	8.30–14.78	1–2
3	14.78–21.27	2–3
4	21.27–27.75	3–4
5	27.75–34.24	4–5
6	34.24–40.73	5–6
7	40.73–47.21	6–7
8	47.21–53.70	7–8
9	53.70–60.18	8–9
10	60.18–66.67	> 9

[a] Hong et al. (2016).

3.2.2. *In vitro* bioassays

To measure AhR-mediated potencies in REs, silica-gel fractions, and RP-HPLC fractions, a panel of established *in vitro* reporter gene cell lines (H4IIE-*luc*) were used (Khim et al., 1999) (Figure 3.2). The bioassay methods were previously described in detail (Figure 2.6 and Table 3.1) (Hong et al., 2016). Luminescence of luciferase was quantified using a Victor X3 multi-label plate reader. The responses were converted to the percentage of the maximum response according to 50 nM BaP, viz., % BaP_{max}.

A bioluminescence test with the marine bacterium, gram-negative bacteria, *V. fischeri* (NRRL B-11177) was conducted by use of the luminescent bacteria toxicity measurement apparatus (N-TOX model 200; NeoEnBiz Inc., Bucheon, Korea), following standard methods of the Ministry of Maritime Affairs and Fisheries of South Korea (MOMAF, 2005) and Lee et al. (2019). The screening test was performed following the methods described in Table 3.1 and Figure 3.3a. When the inhibition of luminescence was detected in the screening test, a serial dilution test was performed. Each experiment consisted of four controls and four replicates. A Quality Assurance/Quality Control (QA/QC) test to maintain the validity of the test method was also conducted with the reference standard using zinc sulfate solution for each fresh vial of bacteria that was opened (ISO, 1998).

Bacterial physiological processes of luminescence are regulated by quorum sensing (QS) systems (Schaefer et al., 1996) including the two genes (*lux*I and *lux*R) and two like-proteins (LuxI and LuxR) which control expression of the lux operon (*lux*ICDABE) (Figure 3.3b) (Engebrecht et al., 1983). The genes *lux*ICDABE are common to all of them and code for the luciferase and the fatty-acid-reductase polypeptides (Meighen, 1993). *lux*I gene directs the synthesis of *N*-acylhomoserine lactone (AHLs) which is extracellular signaling molecules to monitor their population density in QS control of gene expression. It can diffuse in and out of the cell membrane and increases in concentration with increasing cell density. The *lux*R gene is located upstream of the lux operon and encoded LuxR.

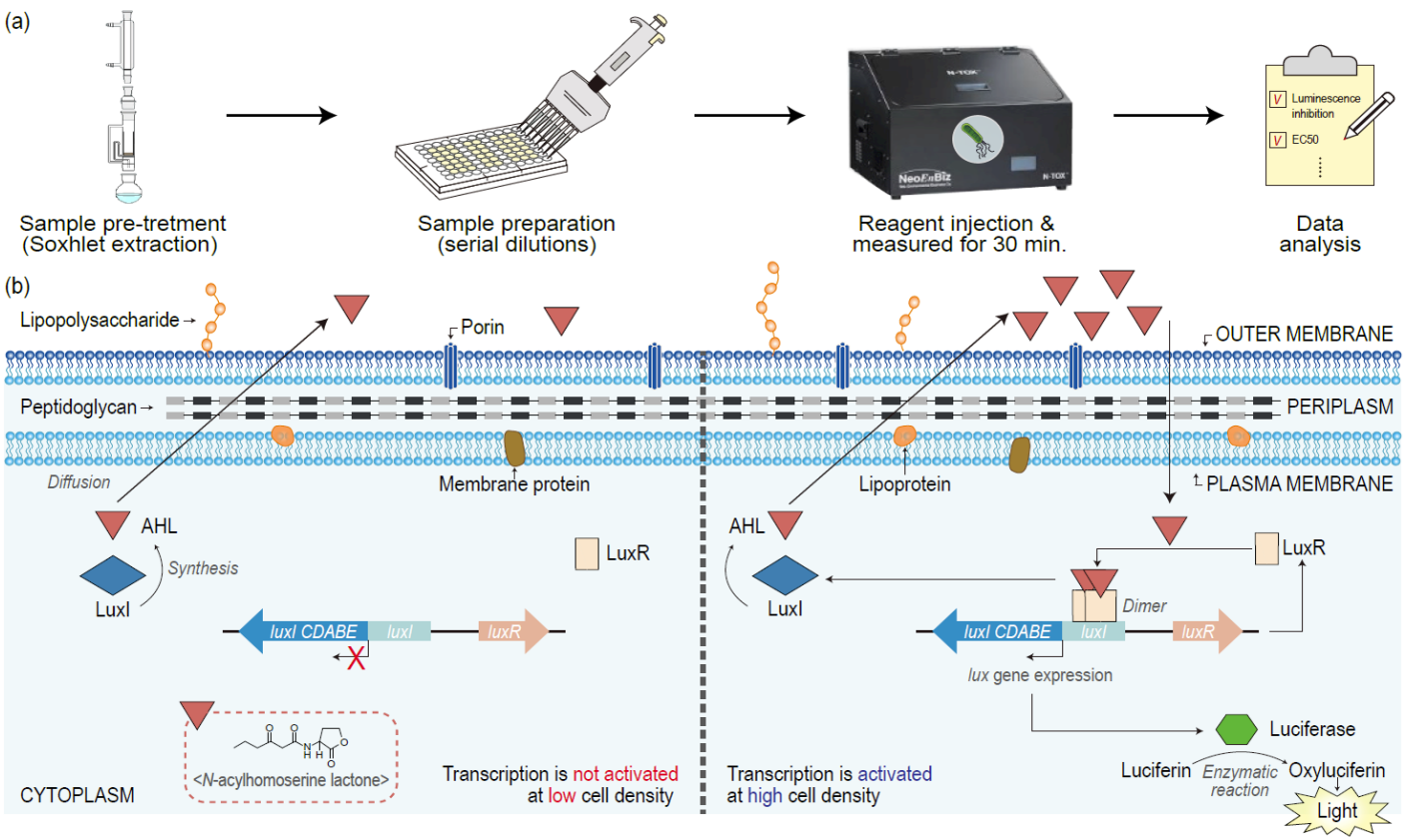


Figure 3.3.
(a) Graphic summary of *Vibrio fischeri* bioassay. (b) A simplified view of *V. fischeri* quorum-sensing system and chemical structures of *N*-acylhomoserine lactone (AHL) autoinducer.

3.2.3. *In vivo* bioassays

To investigate possible adverse effects of sediments on primary producers, inhibition of growth of algae was investigated, with some modifications to ISO (2006) and Lee et al. (2015) (Figure 3.2 and Table 3.1). The marine algal strains *Isochrysis galbana* and *Pheodactylum tricornutum* were obtained from the Korea Marine Microalgae Culture Center (KMMCC) and cultured (Table 3.3). After 96 h of culture, inhibition growth of microalgae was determined by counting the number of cells in each treatment by use of a disposable hemocytometer (In CYTO, Chungcheongnam-do, Korea) under a light microscope (Optical Microscope: CHS, Olympus, Japan) (Figure 3.4a). Growth inhibition rate (μ) was calculated using ISO (ISO, 2006).

The viability of the microalgae population after exposure to samples were assessed by flow cytometry (FCM) with specific fluorescent dyes, which is described in Figure 3.4b. Sub-samples (1 mL) for the FCM analysis of microalgae (*I. galbana* and *P. tricornutum*) were taken after 96 h from the same culture flasks as those measured microscopically. FCM analysis of microalgae was performed on a BD FACS Canto II flow cytometer equipped with 405 nm laser exciting SYTOX blue (Thermo Fisher Scientific) and propidium iodide (PI; Invitrogen, Ltd. UK), 488 nm laser exciting fluorescein diacetate (FDA; Sigma), and 633 nm laser exciting chlorophyll *a* (Chl *a*) (Table 3.3). The microalga population was quantified as the Pacific Blue (450/50), FITC (530/30), and APC (660/20) filters related to fluorescence from SYTOX blue, FDA, and Chl *a*, respectively (Shapiro, 2005). For *I. galbana*, dual staining was performed with FDA and SYTOX blue.

Esterase activity of microalgae exposed to the samples was evaluated using non-fluorescent lipophilic dye FDA (Hadjoudja et al., 2009). FDA, which is taken up by live cells and converted to its fluorescent derivative fluorescein by cellular esterase, was applied at a final concentration of 5 μM mL^{-1} (15 min., room temperature, and darkness). After FDA staining, samples were stained with SYTOX blue at a final concentration of 2 μM mL^{-1} and incubated in darkness for 10 min. at room temperature. Membrane integrity was assessed by SYTOX blue, which penetrates damaged membrane cells, becoming stuck to nucleic acid structures (Olsen et al., 2016). Esterase activity and membrane integrity changes can indicate

either the preliminary stage of toxic action or display the adaptation ability of organisms to influence toxic substances in samples (Li et al., 2011). The FCM signals from *I. galbana*, dual stained with FDA and SYTOX blue were presented as dot plots and separated into quadrants (Q1 to Q4) (Figure 3.5).

For *P. tricornutum*, staining was conducted with FDA (30 μM mL^{-1}) and PI (5 μM mL^{-1}) (Cid et al., 1996). Thermal shock dead cells were obtained using a 60 ℃ water bath for 1 h heating and were used as a positive control. FCM dot plots of *I. galbana* and *P. tricornutum* cell signals were plotted as coordinates of FDA, SYTOX blue, and PI fluorescence intensity for unstained, stained, and boiled cells, respectively (Figure 3.5). Cell viability was expressed as the percentage of metabolically active cells with respect to the cells of solvent control treatment (Figure 3.5). Samples were measured in triplicate using the counting cells function of the flow cytometery. In addition, cell size and granularity (intracellular complexity) were measured by forward scatter detector and side scatter (SS), respectively (Mullaney et al., 1969; Tousova et al., 2017). The data were analyzed with the software FlowJo™ ver.10.0, which is independent of the flow cytometer used.

Maintenance and breeding of zebrafish, *Danio rerio*, followed the previously described method (Kim et al., 2016). The fish embryo testing (FET) was performed according to the OECD Test No. 236 (OECD, 2013) using a wild-type zebrafish strain. In brief, five freshly fertilized zebrafish embryos were randomly placed in 24-well plates, with one embryo per well in 2 mL of test media (i.e., solvent control or sample extract) (Abdel-Shafy and Mansour, 2016). Three replicates were tested per treatment (total n = 15 embryos per treatment) (Table 3.1). The 24-well plates were then covered with self-adhesive foil and incubated at 26 ± 1 °C. Lethal and sub-lethal effects were monitored every 24 h after the start of exposure until the end of the test, at 96 h.

Table 3.3.
Culture condition of three microalgae and test condition of each microalgae species for measured cell viability of algae cells using flow cytometry.

Scientific name	***Isochrysis galbana***	***Phaeodactylum tricornutum***
Class	Prymnesiophyceae	Bacillariophyceae
Cell size	5−6 μm	5−6 μm × 10−14 μm
Culture condition	20 ℃, 12:12 (Light : Dark)	20 ℃, 12:12 (Light : Dark)
Initial cell density	6×10^4 cells mL^{-1}	3×10^4 cells mL^{-1}
Staining protocol	Double staining	Single staining
Dye	FDA, SYTOX blue	FDA, PI (propidium iodide)
Ex/Em (nm)	488/530, 444/480	488/530, 488/582
Endpoint	Growth inhibition (72 h) Esterase activity (FDA) Cell membrane intensity (SYTOX blue) Chlorophyll-*a* Cell size (FS) Intracellular complexity (SS)	Growth inhibition (72 h) Esterase activity (FDA) Cell membrane intensity (PI) (PI) Chlorophyll-*a* Cell size (FS) Intracellular complexity (SS)

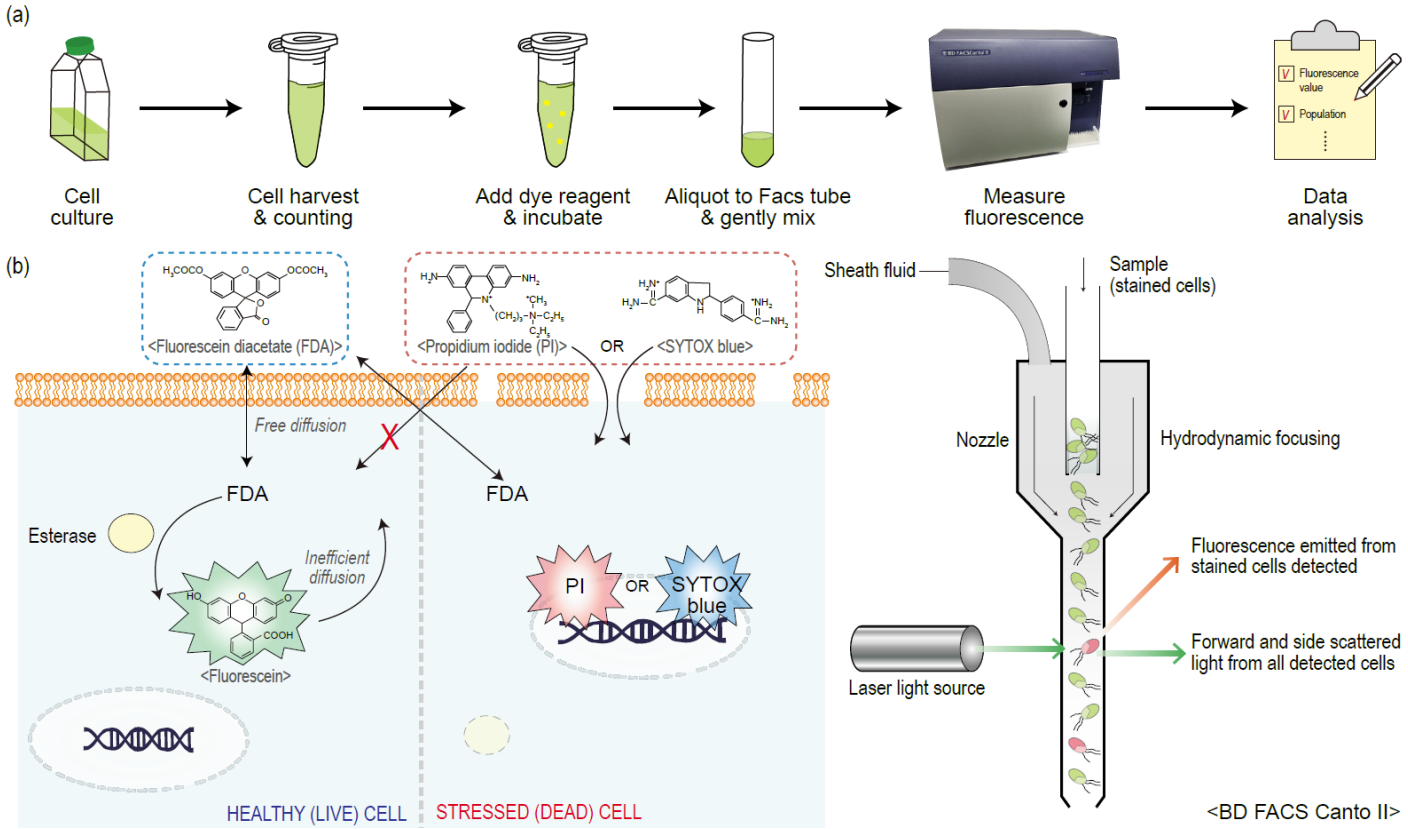


Figure 3.4.
(a) Graphic summary of microalgae bioassay using flow cytometry. (b) A simplified view of stain mechanism using in this study to determine healthy cells and stressed cells.

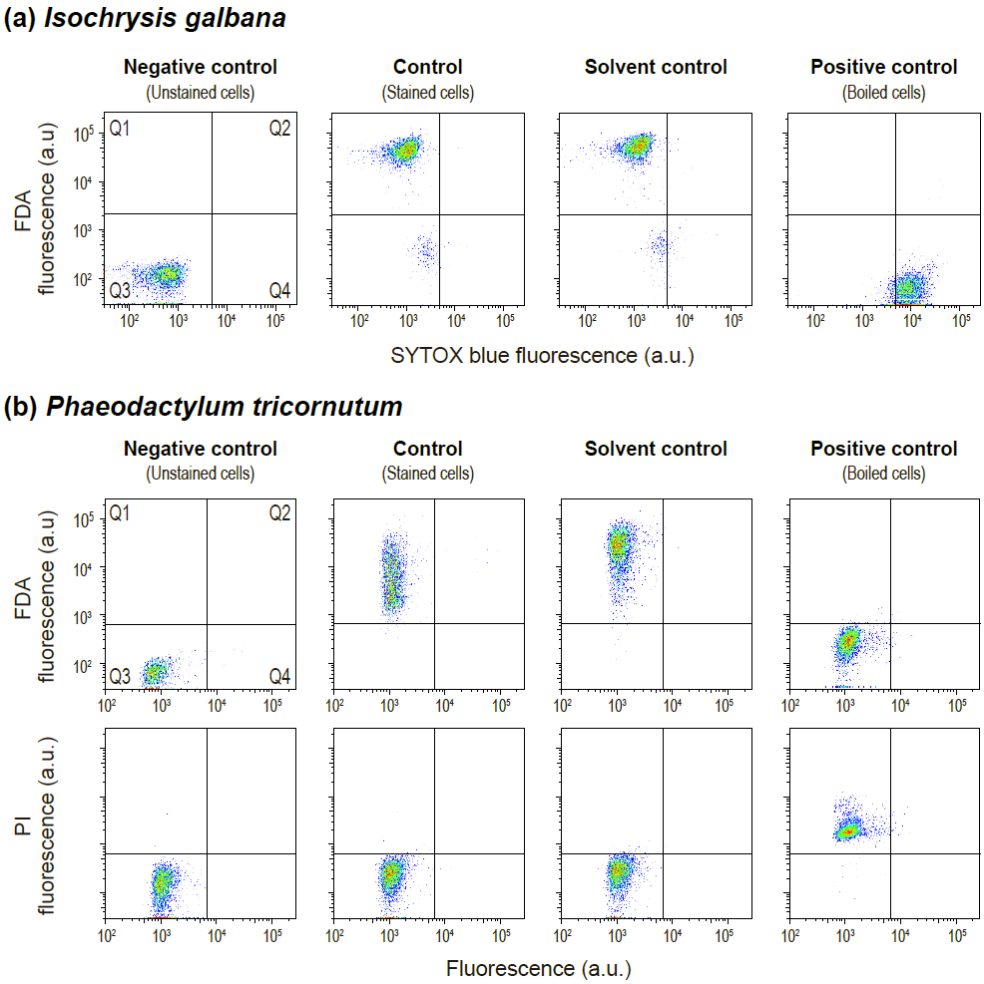


Figure 3.5.

Dot plots of flow cytometry analysis of (a) *Isochrysis galbana* and (b) *Phaeodactylum tricornutum*. Mean values were obtained in logarithmic scale and represented in arbitrary units (a.u.). Q1 (upper left quadrant, healthy cells) presented active esterase and intact membrane, Q2 (upper right quadrant, membrane-damaged cells) presented active esterase and membrane minimally damaged, Q3 (lower left quadrant, inactive cells) presented esterase activity not detectable and intact membrane, and only stained with SYTOX blue presented in Q4 (lower right quadrant, dead cells; esterase activity not detectable and membrane compromised).

3.2.4. Targeted chemical analyses

Thirty-one PTSs (including 15 PAHs, 10 SOs, and 6 APs) were analyzed in sediments, using the methods adapted from Hong et al., 2015, and 2016b (Figure 3.2). The full names of the target compounds and results of QA/QC test were provided in Tables 3.4–3.5. Detailed information on instrumental conditions was presented in Table 2.1 (see Chapter 2).

Table 3.4.

Concentrations and relative potency of AhR-mediated activity for PAHs reported previously and potency balance between instrument-derived BEQs and bioassay-derived BaP-EQs in the Soxhlet fraction (F2) of sediment samples from Masan Bay, South Korea.

Target compounds	Abbreviations	ReP [a]	Sites	
PAHs concentrations (ng g^{-1} dm)			S1	S2
Acenaphthene	Ace		0.5	0.3
Acenaphthylene	Acl		1.4	0.7
Fluorene	Flu		6.3	2.4
Phenanthrene	Phe		78	13
Antracene	Ant		5.0	1.6
Fluoranthene	Fl		17	27
Pyrene	Py		67	19
Benzo[*a*]anthracene	BaA	3.2×10^{-1}	36	22
Chrysene	Chr	8.5×10^{-1}	5.3	6.0
Benzo[*b*]fluoranthene	BbF	5.0×10^{-1}	22	33
Benzo[*k*]fluoranthene	BkF	4.8×10^{-1}	2.3	4.0
Benzo[*a*]pyrene	BaP	1.0	5.4	8.7
Dibenz[*a,h*]anthracene	DbahA	6.6×10^{-1}	3.3	3.6
Indeno[*1,2,3*-cd]pyrene	IcdP	5.8×10^{-1}	5.9	11
Benzo[*g,h,i*]perylene	BghiP		23	29
Sum of PAHs	Σ PAHs		280	180
BEQ concentrations (Instrument-derived equivalents, ng BEQ g^{-1} dm)	Σ BEQs		39	48
Magnitude-based BaP-EQ concentrations [b] (Bioassay-derived equivalents, ng BaP-EQ g^{-1} dm)			3.3×10^4	4.5×10^4
Potency balance analysis (BEQ/BaP-EQ × 100 (%))			0.12	0.11

[a] Kim et al. (2019).

[b] Bioassay-derived BaP-EQs were calculated as a percentage of the maximum response observed for a 50 nM BaP standard elicited by 100% sediment organic extracts.

Table 3.5.

Concentrations of styrene oligomers (SOs) and alkylphenols (APs) in the sediments of Masan Bay, South Korea.

Compounds / Sites	[a] Abb.	Concentrations (ng g^{-1} dm)		Detection limit (ng g^{-1} dm, n = 7)
		S1	S2	
Styrene Oligomers				
1,3-diphenylpropane	SD1	0.6	0.0	0.34
cis-*1,2*-diphenylcyclobutane	SD2	0.7	0.0	0.65
2,4-diphenyl-1-butene	SD3	7.3	49	0.94
trans-*1,2*-diphenylcyclobutane	SD4	0.7	0.0	0.28
4,6-triphenyl-1-hexene	ST1	5.4	1.6	0.57
1e-phenyl-4e-(1-phenylethyl)-tetralin	ST2	2.7	1.5	0.53
1a-phenyl-4e-(1-phenylethyl)-tetralin	ST3	4.2	1.0	0.30
1a-phenyl-4a-(1-phenylethyl)-tetralin	ST4	4.3	2.8	0.49
1e-phenyl-4a-(1-phenylethyl)-tetralin	ST5	1.1	0.7	0.32
1,3,5-triphenylcyclohexane (isomer mix)	ST6	7.6	0.0	0.34
Sum of SOs		35	56	
Alkylphenols				
4-*tert*-octylphenol	4-*t*-OP	30	5.5	0.09
Nonylphenols	NP	540	140	0.97
4-*tert*-octylphenol-monoethoxylate	OP1EO	20	1.2	0.10
Nonylphenol-monoethoxylates	NP1EO	310	21	0.49
4-*tert*-octylphenol-diethoxylate	OP2EO	40	2.3	0.10
Nonylphenol-diethoxylates	NP2EO	170	5.4	0.88
Sum of APs		1100	180	

[a] Abb.: Abbreviations.

3.2.5. Full-scan screening analyses

FSA using GC-QTOFMS was performed on F2.7 and F2.8 of S2, on which AhR-mediated potencies of samples were great. Instrumental conditions and process of tuning are presented in Table 3.6. LC-QTOFMS analysis was performed with a Kintex Core-Shell C18 column to screen F3 sub-fractions (F3.6 and F3.7). Information on the instruments and analytical conditions are presented in Table 3.7. Peak View SoftwareTM v.2.2 (AB SCIEX, Foster City, CA) was used to detect peaks, and peak lists were obtained from full-scan chromatograms of the samples, solvent, and processed blanks (Xie et al., 2012). GC-QTOFMS and LC-QTOF MS were performed in the laboratory of Human & Ecology analytical in Hanyang University and National Instrumentation Center for Environmental Management in Seoul National University, respectively.

Five criteria were used to select candidates for AhR agonists based on the LC-QTOFMS analysis and Figure 3.6a. From the FSA, four commercially available compounds (such as 1,2-di(*p*-tolyl)ethane, flucofuron, niflumic acid, and enoxolone) were selected to confirm their AhR-mediated potencies using the H4IIE-*luc* bioassay (Figure 3.6b). The first filtering step involved elemental composition matching of the compounds with a mass tolerance of 20.0 ppm to the NIST library (ver. 2017) (Figure 3.6a) (Zedda and Zwiener, 2012). The matching score (mass accuracy score) indicated how close the observed mass is to the theoretically expected mass. It is a function of the user's specific Parent Mass Tolerance. Intermediate matches are scored based on the following linear equation (Equation 1).

$$\text{Matching Score (mass accuracy score)} = \frac{\text{-0.5 Delta Mass (AMU)}}{\text{Precursor Mass Tolerance (AMU)}} + 1 \quad (1)$$

The second step involved removing noise peaks. If blank samples showed the intensity, they were removed (Cui et al., 2018). The number of candidates was greatly reduced for most peaks by using a cutoff value of the MS/MS matching score (in all cases ≥ 90) in the third step (Kind and Fiehn, 2010). The fourth step involved selecting only compounds with a score of ≥ 90 by isotopic distribution (Cui et al.,

2018). Finally, the chemicals were chosen that had an aromatic ring because a previous study showed that compounds with aromatic ring structures had AhR binding affinity.

Table 3.6.
Instrumental conditions of GC-QTOFMS for full-scan screening analysis.

Instrument	GC: Agilent Technologies 7890B QTOFMS: Agilent Technologies 7200
Samples	F2.7 and F2.8 RP-HPLC fractions from Site 2
Column	DB-5MS UI (30 m × 0.25 mm i.d. × 0.25 μm film)
Carrier gas	He
Flow rate	1.2 mL $min.^{-1}$
Injection volume	2 μL
Mass range	50–600 *m/z*
Ion source temperature	230 °C
Ionization mode	EI mode (70 eV)
Tuning condition	- Instruments are tuned before use each day batches are initiated. - Tuned with a compound of known mass spectrum; perfluorotributyl-amine (PFTBA) - Ions from the PFTBA spectrum for its tuning: *m/z* 68.9947, 130.9915, 218.9851, 413.9770, 463.9738 and 501.9706 - Mass accuracy and correct mass errors to within 5 ppm
Software	Qualitative analysis B.08.01 MassHunter Quantitative analysis Unknown analysis B.08.01 NIST Library (ver. 2014)
Criteria of candidate AhR compounds	Minimum number of ion peaks > 5 Peak shape quality > 60% Matching factor scores > 70 - Matching factor score (the accurate mass pattern match score) = Contribution of Mass Abundance Score, Mass Accuracy Score, Mass Spacing Score - Mass Abundance Score records how well the abundance pattern of the measured isotope cluster compared with values predicted from the proposed formula - Mass Accuracy Score records how well the measured mass (or *m/z*) compared with the value predicted from the proposed formula - Mass Spacing Score records how the *m/z* spacing between the lowest *m/z* ion and the A+1 and A+2 ions compared with the values predicted from the proposed formula

Table 3.7.
Instrumental conditions of LC-QTOFMS for full-scan screening analysis.

Instrument	LC: Thermo Scientific Ultimate 3000 QTOFMS: Triple time-of-flight (TripleTOF®) 5600 mass spectrometer (Sciex, Foster City, CA, USA)
Samples	F3.6 and F3.7 RP-HPLC fractions from Site 2
Analytical column	Phenomenex Kinetex XB-C18 column (2.1 mm × 100 mm i.d. × 1.7 µm film)
Column temperature	40 °C
Injection volume	3 µL
Flow rate	0.4 mL $min.^{-1}$
Mobile phase	A: 0.1% Formic acid and 10 mM ammonium formate in water, B: 0.1% Formic acid in acetonitrile
Mobile phase gradient	Time (min.) / Solvent A / Solvent B 0 / 90 / 10 1 / 90 / 10 15 / 0 / 100 24 / 0 / 100 25 / 90 / 10 30 / 90 / 10
Ionization mode	Electro spray ionization (ESI) Positive and Negative mode
Mass scan type	Full scan and Information Dependent Acquisition (IDA) Scanning
Mass scan range	50–2000 *m/z*
MS/MS scan range	50–2000 *m/z*
Nebulizing gas	50 psi
Heating gas	50 psi
Curtain gas	25 psi
Desolation temperature	500 °C
Ion spray voltage	Positive: 5.5 kV, Negative: 4.5 kV
Collision voltage	Positive: 35 ± 15 eV, Negative: -35 ± 15 eV
Collision gas	Nitrogen
Software	PeakView 2.2 Scafford Elements version 2.2.1 NIST Library (ver. 2017 for Elements) HMDB Library MoNA export LC-MS, MS-MS Library

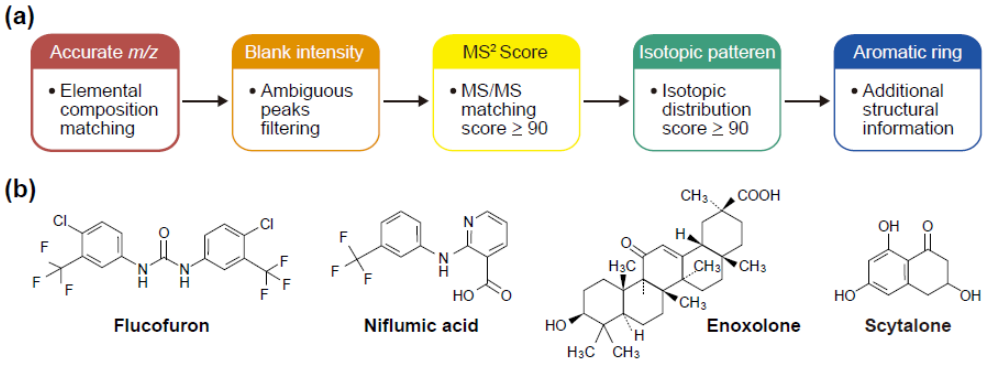


Figure 3.6.
(a) A stepwise approach of LC-QTOFMS data analysis to select AhR agonist candidates and (b) the molecular structure of tentative AhR agonists.

3.2.6. Toxicological confirmation

The RePs for the AhR-mediated potencies of the four candidate compounds were determined. Each compound was prepared at six concentrations (*viz*., 1000, 200, 40, 8.0, 1.6, and 0.32 μg mL^{-1}), and was analyzed using the method of *in vitro* bioassay that described above (see Chapter 2). RePs were measured based on the previous study with minor modifications (Villeneuve et al., 2000; Kim et al., 2019). Potency balnce analysis of targeted chemicals was performed between instrument-derived BEQs and bioassay-derived BaP-EQs to determine how each compound contributed to total induced AhR-mediated potency (see Chapter 2).

3.2.7. VirtualToxLab *in silico* analysis

For compounds without reference compounds, tentative identification was completed using QSAR modeling (Figure 3.2). The AhR binding affinities of tentative compounds, which were identified from FSA, were simulated by VirtualToxLab. It combines automated, flexible docking with multi-dimensional QSAR to simulate and quantify the toxic potential and the binding of chemicals toward a series of currently implemented proteins that trigger adverse effects (Vedani et al., 2015). Univariate analyses were carried out using SPSS 23.0.

The difference among treatments and control was analyzed by Kruskal–Wallis test, followed by Dunnett's test for microalgae bioassay. And the difference between sites of the response of treatments was analyzed by *t*-test for *Danio rerio* bioassay. In all statistical analyses, *p* values less than 0.05 were considered to be statistically significant. Multivariate data analyses were performed using the PRIMER 6 statistical software (PRIMER-E Ltd, Plymouth, UK) with the PERMANOVA+ add-on package. The data for each endpoint (for microalgae cell viability is only data from *I. galbana* and except for SOs because of less concentration) were standardized and were used to construct a Bray-Curtis similarity matrix. This matrix was then subjected to canonical analysis of principal coordinates (CAP) and principal component analysis (PCA) to visualize the similarity between fractionation and bioassays (Anderson and Willis, 2003).

3.3. Results and Discussion

3.3.1. AhR-mediated potencies

Raw extracts of sediments from both sites, S1 and S2, reached saturation efficiency (≥ 100% BaP_{max}) exhibited strong AhR-mediated potencies in the H4IIE-*luc* bioassay (Figure 3.5A). Among the three silica gel fractions (F1–F3) of raw extracts, AhR-mediated potencies were greater in F2 than those of F1 and F3. Typical AhR-active compounds in F2 reported include planar hydrophobic contaminants, such as PAHs, PCBs, and dioxins. These chemicals have been repeatedly reported as major groups of sedimentary PTSs in the study area and elsewhere in the Korean coastal waters (Hong et al., 2012; Kinani et al., 2010; Lee et al., 2017; Louiz et al., 2008). Although co-eluting chemicals in F1 include some active AhR agonists, such as hexachlorobenzene and *p,p′*-1,1-*dichloro*-2,2-bis(*p*-chlorophenyl)ethylene, these chemicals likely occur at small concentrations in sediments of Masan Bay (Hong et al., 2003; Koh et al., 2005) (Table 3.8).

The F2 of S1 and F2 and F3 of S2 exhibited greater AhR-mediated potencies compared to those of raw extracts. Thus, these F2 and F3 were further fractionated into 10 sub-fractions using RP-HPLC. Among the 10 RP-HPLC sub-fractions of F2 from both sampling sites, the greatest AhR-mediated potency was observed in the F2.7, followed by F2.8 and F2.6 (Figure 3.7a). The results indicated that these fractions contained major AhR active chemicals, which seemed to be aromatics with 5–8 log K_{ow} values. In addition, significant AhR-mediated potencies were shown in the super-hydrophobic fractions such as F2.8–F2.10 of S1, of which fractions contained the 7−9 ring PAHs (≥ C24-PAH) (Thiäner et al., 2019). Meanwhile, S2 sediment had greater AhR-mediated potency of the F3 (Figure 3.7a), which indicated that polar AhR agonists (e.g., dinitro-, hydroxyl-PAHs, and N-heterocycles) were present (Lübcke-von Varel et al., 2011; Xiao et al., 2016). For the 10 RP-HPLC sub-fractions of F3 from S2, considerable AhR-mediated potencies were evidenced in the F3.5−F3.7 fractions.

Table 3.8.

Profile of eluted compounds in fractions from different polarities of the solvent.

Elution solvent from other study	Sample type	Fractions in this study			Eluted compounds	References
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane	Sediments	○			PAHs with two aromatic rings (DDE, PCBs) & Bexachlorobenzene	Khim et al., 1999a
Hexane:DCM (80:20, v/v)			○		PAHs with two aromatic rings (Ace, Flu, Na) PAHs with four aromatic rings (BaA, Chr, Fl, Py) PAHs with five aromatic rings (BaP, BbF, BkF, DbahA) PAHs with six aromatic rings (BghiP)	
DCM:Methanol (50:50, v/v)				△	PAHs with two aromatic rings (Bisphenol A) & NP, OP	
Toluene	Sediments	△			PAHs with two aromatic rings (3,3',4,4',5,5'-hexachlorobiphenyl, 3,3',4,4',5-Pentachlorobiphenyl, PCN, 3,3',4,4'-Tetrachlorobiphenyl)	Khim et al., 1999b
Hexane:DCM (80:20, v/v)	Fish		○		PAHs with two aromatic rings (*p,p* -DDD, *p,p* -DDT) Chlordanes, HCHs	Kannan et al., 2000
Toluene	Sediments	△			PAHs with two aromatic rings (PCDFs)	Yamashita et al., 2000
Hexane	Sediments	○			PAHs with two aromatic rings (23478-PeCDF, 2,4,4'-Trichlorobiphenyl, 2,2',5,5'-Tetrachlorobiphenyl, 2,2',4,5,5'-Pentachlorobiphenyl, 2,3',4,4',5-Pentachlorobiphenyl, 2,2',3,4,4',5'-Hexachlorobiphenyl, 2,2',4,4',5,5'-Hexachlorobiphenyl, 2,2',3,4,4',5,5'-Heptachlorobiphenyl)	Hilscherova et al., 2001
Hexane:DCM (80:20, v/v)			○		OC pesticides	
DCM				△	Polar metabolites steroid compounds	
Hexane	Sediments	○			Non-polar aliphatic compounds	Brack et al., 2003

Table 3.8.

Profile of eluted compounds in fractions from different polarities of the solvent (continued).

Elution solvent from other study	Sample type	Fractions in this study			Eluted compounds	References
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane:DCM (90:10, v/v)	Sediments		△		PAHs	Grote et al., 2005
Hexane:DCM (95:5, v/v)	Sediments		△		PAHs with two aromatic rings (Anthraquinone)	Varel et al., 2008
					PAHs with three aromatic rings (Benzo[*b*]naphtho[2,1-*d*] thiophene, 4H-Cyclopenta[*def*]phenanthrene-4-one,2,2-naphthalenylbenzothiophene, 9-nitroanthracene)	
DCM				△	PAHs with four aromatic rings (4H-Cyclopenta[*cd*]pyrene-3[4*H*]-one, 1-Hydroxypyrene)	
					PAHs with five aromatic rings (Dibenz[*a,j*]acridine)	
Acetonitrile				△	PAHs with two aromatic rings (2-Hydroxyanthraquinone)	
Hexane:DCM (50:50, v/v)	Sediments		△		BHT, Phthalate compounds	Kaisarevic et al., 2009
DCM	Sediments			△	Androgenic compounds	Weiss et al., 2009
Hexane		○			Aliphatic hydrocarbons	
Hexane	Soils	○			Non-polar aliphatic compounds	WÖlz et al., 2010
Hexane:DCM (90:10, v/v)			△		Non-polar PAHs	
DCM				△	Polar compounds	
Hexane:DCM (90:10, v/v)	Sediments	△			PAHs with two aromatic rings (PBDEs)	Qu et al., 2011
Hexane:DCM (80:20, v/v)		△			PAHs with two aromatic rings (TBBPA) & HBCDs	

Table 3.8.

Profile of eluted compounds in fractions from different polarities of the solvent (continued).

Elution solvent from other study	Sample type	Fractions in this study			Eluted compounds	References
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane:DCM (90:10, v/v)	Sediments	△			PAHs with two aromatic rings (PBDEs)	Qu et al., 2011
Hexane:DCM (80:20, v/v)		△			PAHs with two aromatic rings (TBBPA) & HBCDs	
Hexane	Worm, sediments, crude oil	○			Saturate hydrocarbons	Vrabie et al., 2012
DCM				△	Aromatic compounds	
Heptane	Sediments	△			PAHs with two aromatic rings (2,4′-DDT, TCDD)	Creusot et al., 2013
Heptane:DCM (50:50, v/v)			△		PAHs with two aromatic rings (BP3, Cypermethrin, Fenofibrate) PAHs with three aromatic rings (Clotrimazole, Fenvalerate, TPP)	
Ethyl acetate				△	PAHs with two aromatic rings (*n*-Benzylparaben) Dexamethasone, *β*-Estradiol, Isoproturon, Prednisolone, Spironolactone, and *α*-Zearalanol	
Hexane:Ace (97:3, v/v)	Sediments		△		PAHs with two aromatic rings (C6-Na, Diclofenac, Methyltriclosan, Triclosan, Benzophenone, Ketoprofen, Naproxen) PAHs with three aromatic rings (Carbamazepine) Butylparaben, Clofibric acid, Mecoprop, Methyl chlorophenoxy acetic acid, Methyl dihydrojasmonate, Propylparaben, Tertbuthylazine, Tris(2-chloroethyl) phosphate	Regueiro et al., 2013
Hexane:DCM (50:50, v/v)	Porewater		△		PAHs with two aromatic rings (Carbazole) PAHs with three aromatic rings (Retene)	Fang et al., 2014

Table 3.8.

Profile of eluted compounds in fractions from different polarities of the solvent (continued).

Elution solvent from other study	Sample type	Fractions in this study			Eluted compounds	References
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane	Crude oil	○			Aliphatic compounds	Radović et al., 2014
DCM				△	Aromatic compounds	
Methanol					Resins and polar compounds	
Hexane:DCM (80:20, v/v)	Crude oil		○		PAHs with two aromatic rings (Dbthio, C1-Dbthio, C2-Dbthio, C3-Dbthio, C1-Flu, C2-Flu, C1-Na, C2-Na, C3-Na, C4-Na) PAHs with three aromatic rings (C1-Phe, C2-Phe, C3-Phe, C4-Phe) PAHs with four aromatic rings (BeP, C1-Chr, C2-Chr, C3-Chr)	Hong et al., 2015
Hexane:DCM (80:20, v/v)	Sediments		○		PAHs with two aromatic rings (Styrene dimers) PAHs with three aromatic rings (Styrene trimers)	Hong et al., 2016
Hexane	Sediments	○			PCBs, Co-planar PCBs without chlorination in ortho-position, PCNs with 3-6 Cl	Xiao et al., 2016
Hexane: DCM (95:5, v/v)			△		PAHs with two rings to seven aromatic rings Monoitro-PAHs	
DCM				△	(Hydroxy-)quinones, keto-, dinitro-, hydroxyl-PAHs and N-Heterocycles with rising polarity	
Acetonitrile				△	2-Hydroxyanthraquinone	
Hexane	Oiled sediments	○			Saturate hydrocarbons	Kim et al., 2017
Ace:DCM (40:60, v/v)				○	Resins and polar compounds	

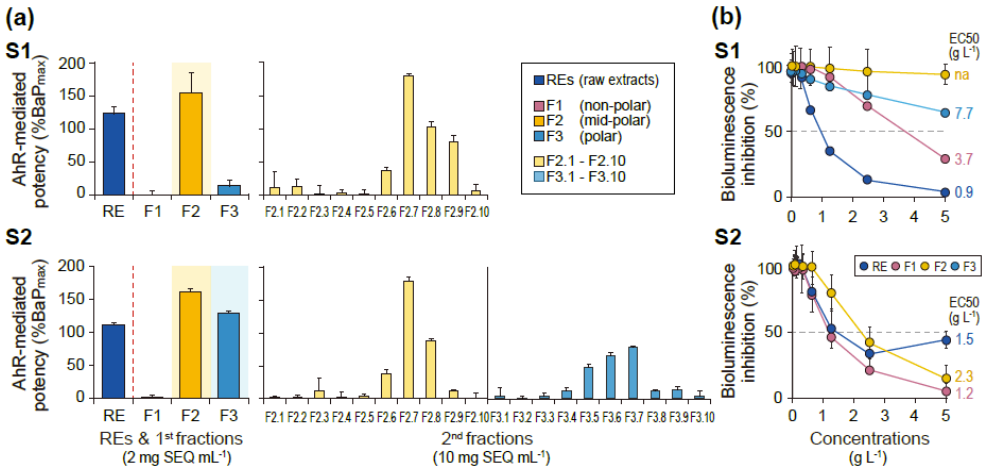


Figure 3.7.

(a) AhR-mediated potencies of raw extracts (RE), silica gel fractions, and RP-HPLC fractions (F2.1–F2.10 and F3.1–F3.10; sub-fractions of F2 and F3, respectively) of S1 and S2 sediments from Masan Bay, Korea determined at 4 h exposures in the H4IIE-*luc* bioassay (Error bar: Mean ± SD; n = 3). (b) Bioluminescence inhibition of *Vibrio fischeri* for the effective concentration (EC50) test of raw extracts and fraction samples in the sediments of Masan Bay over 30 min. period.

3.3.2. Inhibitions of bioluminescence

In the screening test, nearly all fractions were acutely toxic to the bacterium, *V. fischeri* (Table 3.9). Only one sample, F3 of sediment extract of S2, showed a hormetic effect with bacterial inhibition rate of −4%. Thus, the EC50 testing was performed excluding F3 of S2. Bioluminescence of *V. fischeri* was inhibited by both raw extracts of S1 and S2; however, raw extracts from S1 (EC50 = 0.9 g SEq L^{-1}) showed greater inhibition compared to raw extracts from S2 (EC50 = 1.5 g SEq L^{-1}) (Figure 3.7b). Of the three silica gel fractions, the least EC50s were observed in F1 for both sites. F2 of S2 also showed significant acute toxicity (EC50 = 2.3 g SEq L^{-1}), while the F2 of S1 did not cause inhibition.

The greater inhibition of *V. fischeri* in F1 was likely associated with some non-polar compounds, such as *n*-alkanes (C11–C29), and 4,4′-dichlorodiphenylsulphide being eluted in the fraction (Table 3.10) (Brack et al., 1999). In addition, the toxicity of F1 was suspected to be caused by aliphatic hydrocarbons containing sulfur, which are known as major toxicants to *V. fischeri* in raw extracts of sediments (Svenson et al., 1998). Even if elemental sulfur is removed in the pre-treatment procedure, the sulfide compounds in sediments might have been partially removed. In the previous study, toxicity to *V. fischeri* completely disappeared after removal of sulfur (Brack et al., 1999). Some chinoidic PAH metabolites, which frequently occur in environmental samples, are suspected as contributors to the potential toxicity in F2. Possible toxicants contributing to the effects of F3 are polar compounds, such as endosulfan, dimethoate, atrazine, and *n*-tributyltin (Table 3.10) (Brack et al., 1999; Kammann et al., 2005). In particular, NPs of known toxic chemicals to *V. fischeri* are present in the F3 can be suspected to be responsible at least for certain parts of the observed adverse effects shown in S1 (Stasinakis, 2008).

Table 3.9.

Bioluminescence inhibition of *Vibrio fischeri* for the screening test and effective concentration (EC50) test of organic raw extracts (REs) and fraction samples in the sediments of Masan Bay over 30 min. period.

Sites	Samples	Screening test			EC50 test
		Control (%)	Treatment (%)	Inhibition rate (%)	EC 50 (g L^{-1})
S1	REs	100	29.8	70.2	0.9
	F1	95	31.5	63.5	3.7
	F2	95.8	42.3	53.5	n.c.[a]
	F3	95.3	63.0	32.3	7.7
S2	REs	100	29.0	71	1.5
	F1	95.0	27.5	67.5	1.2
	F2	95.8	21.8	74.0	2.3
	F3	95.3	99.3	n.c.	n.a.[b]

[a] n.c.: Not calculated.

[b] n.a.: Not analyzed.

Table 3.10.

Mini reviews on the application of bioassays for toxicity assessment of various chemicals.

Assay	Endpoint	Compounds	Uses/origins	References
In vitro bioassays				
H4IIE-*luc*	AhR-mediated potency	*1,2*-benzanthracene	Urban particulate matters and tobacco smoke	USEPA, 2007
		1,2-benzofluorene	Tar and naturally synthesized	USEPA, 2007
		2-(methylthio)benzothiazole (MTBT)	Rubber additive	Xiao et al., 2016
		2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)	Waste incineration, metal production, and fossil-fuel and wood combustion	Khim et al., 1999
		2-mercaptobenzothiazole (MBT)	Rubber additive	Xiao et al., 2016
		4,5-methanochrysene	Urban particulate matters and tobacco smoke	Cha et al., 2019
		5-methylbenz[*a*]anthracene (5MBA)	Petrogenic origin	Kim et al., 2019
		11H-benzo[*a*]fluorene	Gasoline engines and tobacco smoke	Cha et al., 2019
		11H-benzo[*b*]fluorene (11BF)	Petrogenic origin	Kim et al., 2019
		Alkylbenzenes	Derivatives of benzene	Vrabie et al., 2012
		Benzothiazole	Rubber additive	Xiao et al., 2016
		Benz[*b*]anthracene	Film layer of OFETs and OLEDs	Cha et al., 2019
		Dinaphthofurans (DNFs)		Lübcke-von Varel et al., 2011
		Halogenated aromatic hydrocarbons (HAHs)	Emissions from diesel vehicles, coal burning stoves, wood burning	Floehr et al., 2015
		Methylated PAHs [methylated benzo[*a*]pyrenes, 1-methylchrysene (1MC), 3-methylchrysene (3MC), 3-methylphenanthrene (3MP)]	Petrogenic origin	Trilecova et al., 2011
		Nitro-PAHs	Emissions from diesel vehicles, coal burning stoves, wood burning	Jung et al., 2012
		Organochlorine pesticides OCPs)	Agricultural/ Produced in the high temperature environment of forest fires	Wang et al., 2014

Table 3.10.

Mini reviews on the application of bioassays for toxicity assessment of various chemicals (continued).

Assay	Endpoint	Compounds	Uses/origins	References
H4IIE-*luc*		Polychlorinated biphenyl (PCBs) and Polychlorinated naphthalenes (PCNs)	Electrical apparatus, carbonless copy paper and in heat transfer fluids	Luo et al., 2009; Qiao et al., 2006
		Polychlorinated dibenzo-*p*-dioxins (PCDD/F)	By-products in the manufacture of some organochlorides	Khim et al., 1999,25, Wang et al., 2014-34
		Polycyclic aromatic hydrocarbons (PAHs)	Anthropogenic origin gas work and coke	Floehr et al., 2015
		β-naphthoflavone	Putative chemopreventive agent.	Nannelli et al., 2009
Vibiro fischeri	Bioluminescence inhibition	-) -Z) 2,6-dimethylocta-5,7-diene-2,3-diol	Plant-derived pesticides	Pino-Otín et al., 2019
		4-nonylphenol (4-NP)	Non-ionic surfactants, / wastewater disposal	Stasinakis et al., 2008
		Atrazine	Agriculture practices, golf courses	Hernando et al., 2007
		Azaarene	Anthropogenic origin gas work and coke manufacturing sites, timber and asphalt treatment facilities	Neuwoehner et al., 2009
		Carbamazepine, ibuprofen, fluoxetine, 17*α*-ethynylestradiol, propranolol, and caffeine	Pharmaceutical products	Maranho et al., 2015
		Chinoidic PAHs, brominated phenols and indoles	Combustion and natural origin	Kammann et al., 2005
		Dexketoprofen	Pharmaceutical compounds	Mennillo et al., 2018
		Dimethoate	Organophosphate insecticide and acaricide	Farré et al., 2002
		Endosulfan	Chlorinated insecticide	Palma et al., 2008
		Heavy metals	Fishing, mining, industry, wastewaters, transport and/or recreational activities	Garcia-Ordiales et al., 2019,Olajire et al., 2005
		Imidazolium ionic liquids (ILs) with alkyl chain from C4 to C10	Accidental spills, containers washing operations, leaching from waste disposal sites	Diaz et al., 2018, Jafari et al., 2019
		n-alkanes, *n*-tributyltin, sulfur, *4,4′*-dichlorodiphenylsulphide	Antifouling agents in paints for boats, ships, and docks (*n*-tributyltin)	Brack et al., 1999

Table 3.10.
Mini reviews on the application of bioassays for toxicity assessment of various chemicals (continued).

Assay	Endpoint	Compounds	Uses/origins	References
Vibiro fischeri	Bioluminescence inhibition	Phenolic pollutants	Natural substance degradation, industrial activities pulp and paper industry, petrochemical works)	Kahru, 2002
		Polycyclic aromatic hydrocarbons (PAHs)	Combustion	Liehr, 2013
		Simazine	Nonselective weed control in industrial areas	Hernando et al., 2007
		Triclosan	Antimicrobial agent; Soaps, toothpaste, mouthwash, and cosmetics as well as in textiles	Gorenoglu et al., 2018
In vivo bioassays				
Isochrysis galbana	Growth inhibition	4-nonylphenol (4-NP)	Non-ionic surfactants/wastewater disposal	Tato et al., 2018
		Aniline	Polyurethane and other industrial chemicals	Wang et al., 2019
		Bisphenol A (BPA)	Polycarbonate and epoxy	Tato et al., 2018
		Dibromochloromethane	Disinfection byproducts	Fisher et al., 2014
		Ethylhexyl dimethyl p-aminobenzoic acid		Giraldo et al., 2017
		Octocrylene (OC)	Ingredient in sunscreens and cosmetics	Giraldo et al., 2017
		Oil tar mat and MC252	Accidental spills and industry	Garr et al., 2014
		Pentachlorophenol	Organochlorine biocide	Beiras and Tato, 2018
		PAH (pyrene)	Thermal decomposition of organic matter	Petersen and Dahllof, 2007
		PAHs benz[*a*]anthracene and fluoranthene)	Thermal decomposition of organic matter	Othman et al., 2012
		Petroleum hydrocarbon	Accidental spills and industry	Wang et al., 2016
		Propranolol carbamazepine, ibuprofen, fluoxetine, 17α-ethynylestradiol	Pharmaceutical products	Maranho et al., 2015
		Triclosan	Antimicrobial agent; Soaps, toothpaste, mouthwash, and cosmetics as well as in textiles	Tato et al., 2018

Table 3.10.
Mini review on the application of bioassays for toxicity assessment of various chemicals (continued).

Assay	Endpoint	Compounds	Uses/origins	References
Danio rerio	Embryo toxicity	*2,3*-benzofuran	Component of coal tar	Kais et al., 2015
		4-nonylphenol (4-NP)	Detergents, paints, pesticides, personal care products, and plastics	Kammann et al., 2009
		BPA	Polycarbonate and epoxy	Kais et al., 2015
		Chlorpyrifos	Pesticides and insecticide	Kais et al., 2015
		Heterocyclic aromatic compounds (Acridine and carbazole)	Acridine dye, pesticides, and atebrin	Peddinghaus et al., 2012
		Hexachlorobenzene	Fungicide	Kosmehl et al., 2012
		Metals	Construction and home appliances.	LeFauve and Connaughton, 2017
		Methyl mercury chloride	Inorganic mercury	Kais et al., 2015
		PAHs	Combustion	Kosmehl et al., 2012, Schiwy et al., 2015
		Paraoxon-methyl	Insecticide parathion	Kais et al., 2015
		PCBs	Heat transfer agent	Di Paolo et al., 2015
		Phthalate	Plasticizers	Mu et al., 2018
		Quinoline	Alkaloid and quinoline dye	Kais et al., 2015
	Embryo toxicity Gene expression	Polychlorinated dibenzo-*p*-dioxins (PCDD/F)	By-products in the manufacture of some organochlorides	Boulanger-Weill and Sumbre, 2019; Peddinghaus et al., 2012
	Embryo toxicity EROD activity Gene expression Teratogenicity	*β*-naphthoflavone	Drug products	Schiwy et al., 2015

Overall, *V. fischeri* bioassay showed another aspect of acute *in vitro* response to environmental fraction samples, compared to the H4IIE-*luc* bioassay. That is, inhibition of bioluminescence was sensitive with increasing chain length among non-polar molecules, whilst insignificant acute toxicity was observed in the H4IIE-*luc* cells (Diaz et al., 2018). This is because the gram-negative luminescent bacteria have a multi-layered structure, and thus transmittance of polar molecules is likely small (Park et al., 2017).

3.3.3. Inhibition of growth and viability of cells

Rates of inhibition of growth were 68% and 39% in the F3 of S1 and S2, respectively (Figure 3.8). More toxicants that affected *I. galbana* were present in S1 compared to S2. In contrast, the numbers of algal cells of the F1 increased for both sites (Figure 3.8). This hormesis is a phenomenon that is defined as a stimulatory beneficial effect at low concentrations of toxic chemicals (Calabreseci, 1999). Rates of growth of *I. galbana* were significantly inhibited ($p < 0.05$), mainly by F3 from both sites, of which fraction contain aromatic esters, such as alkylsulfonic acid phenylesters, phthalates, and aromatic amine such as *n*-phenyl-*b*-naphthalene amine (Brack et al., 1999; Schwab et al., 2009).

Previous studies analyzing the toxicities of environmental samples to microalgae mostly focused on inhibition of growth (Brack, 2003; de Castro-Català et al., 2016; Schwab et al., 2009). In this study, inherent properties and viability of microalgae exposed to polarity-based fraction samples were assessed by flow cytometry. *I. galbana* cultured in the presence of the F3 from S1 exhibited lesser forward scatter signals, which were related to a significant decrease in sizes of cells ($p < 0.05$) (Figure 3.8) (Mullaney et al., 1969). This sample also exhibited significantly ($p < 0.05$) less chlorophyll-*a* (Chl *a*) fluorescence, whereas the other samples exhibited 14% greater amounts of Chl *a* fluorescence in comparison to control cultures. However, this second result was not statistically significant ($p > 0.05$) (Figure 3.8). Cell granularity responded similarly to Chl *a* fluorescence.

In *I. galbana*, the least percentages of living cells (healthy cells; % solvent control gate) were observed in F2 for both sites (Figure 3.9a). Cells exposed to F2 samples; effects were observed for esterase activity rather than membrane integrity. This result was more pronounced in S1. In other words, compounds that existed in F2 caused toxicity to algae, affecting enzyme activity, but did not cause lethality. Variations in sensitivities of microglial enzyme activity between samples could be explained by chemical compositions since total concentrations of PAHs in S1 and S2 did not greatly differ but corresponding homologs were different between these two samples. For example, the high proportion of phenanthrene (28%) and pyrene (24%) contributing to total concentrations of PAHs in S1 might result in significantly

greater enzyme activity compared to that of S2 (both explained 17% to total PAHs) (Figure 3.9a, b and Table 3.4). This result is consistent with the previous finding that phenanthrene and pyrene adversely affect the growth and abundance of marine phytoplankton (Echeveste et al., 2010a). Especially, pyrene has strong phytotoxic effects on algae (average LC10 values for algae 2–6 μg L^{-1}) (Echeveste et al., 2010b; Kottuparambil and Agusti, 2018) affecting nutrient uptake (ammonium, nitrate, and silicate) and incorporation carbon by algal communities (Petersen and Dahllof, 2007).

Relatively high populations of *I. galbana* were observed in Q3/Q4 quadrants (by a vertical and a horizontal line based on the FDA- and SYTOX blue-fluorescence intensity from the cells) for F3 for both S1 and S2 (stressed and dead cell; details in SI) especially S1, which indicated the effect of F3 might be associated with nonylphenols (NP). The concentration of NP was 5.4 μg L^{-1} in the sample exposed to microalgae. Although the exposed NP concentration was relatively low considering the effective NP concentration reported in the previous study (EC50 = 24.1 μg L^{-1}; calculated by growth inhibition), NP could be one potential environmental pollutant being related to membrane damage of *I. galbana* (Figure 3.9a). Results of previous studies have shown that NPs as well as *n*-phenyl-b-naphthalene amine, which elutes in F3, damaged cell membranes of algae and invertebrates, by causing oxidative stress (Table 3.10) (Mullaney et al., 1969; Echeveste et al., 2010 a, b). It is necessary to confirm the toxicity contributions of NPs by spiking toxicity tests, which will provide a better understanding of the ecologically relevant predictions of NP's risk in the contaminated sediments.

For *P. tricornutum*, no significant effects were observed on growth, Chl *a* fluorescence, cell size, and cell granularity for all of the samples (Figure 3.8 and Figure 3.9b). However, lesser esterase activity was identified by displacement of FDA fluorescence on the *x*-axis in the histogram (shift to the left) (Figure 3.9b). Strong inhibition of esterase activity was detected in F3 for both sites and F2 from S1 (Figure 3.9b). The percentage of nonviable cells of *P. tricornutum* (measured using PI staining) was not significantly different from that of control cultures. Because *P. tricornutum* has a thicker cell layer than does *I. galbana*, effects on enzymatic activity were more pronounced than was damage to the cell membrane.

In general, toxicological responses and sensitivities of Masan Bay sediments to the two species of microalgae, *P. tricornutum* and *I. galbana*, generally indicated both species- and endpoint-dependent toxic responses to environmental mixtures (Lee et al., 2019).

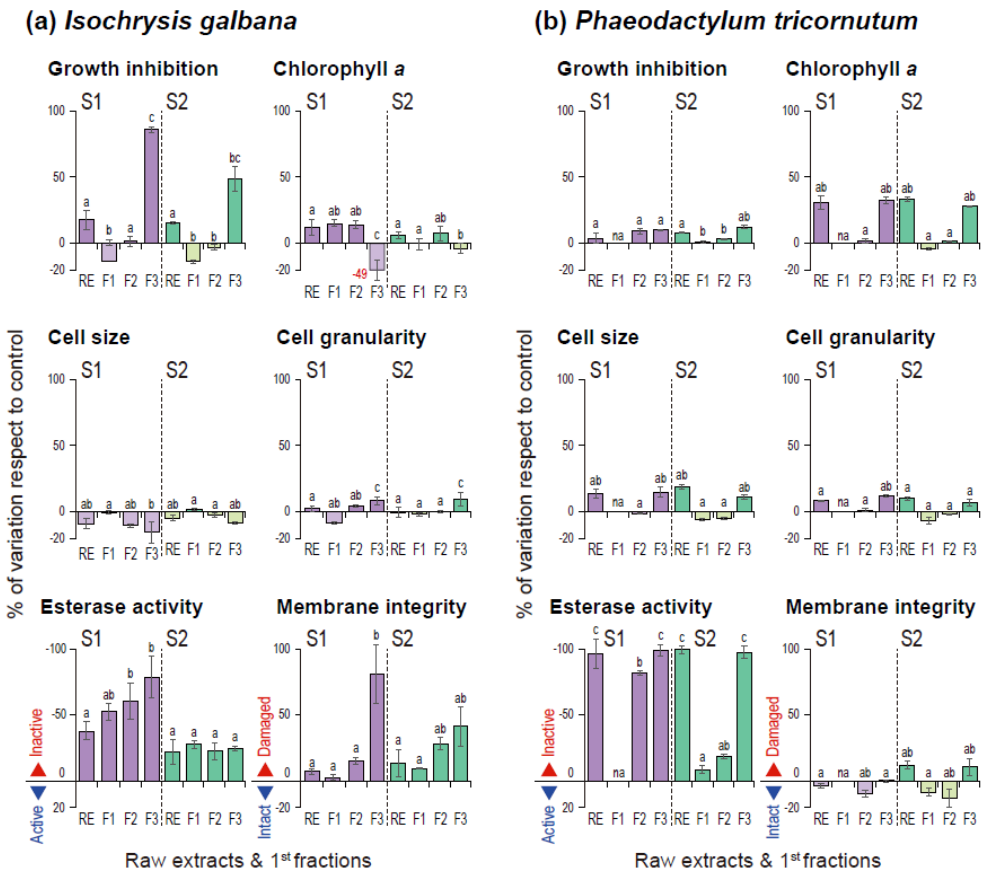


Figure 3.8.

Variation in growth inhibition, inherent cell properties (chlorophyll *a*; autofluorescence, cell size, and cell granularity), and cell viability (esterase activity and membrane integrity) of (a) *Isochrysis galbana* and (b) *Phaeodactylum tricornutum* exposed to raw extract (RE) and fraction samples (F1 to F3). Significant differences with respect to control are shown using lower case letters (Error bar: Mean ± SD; $n = 3$).

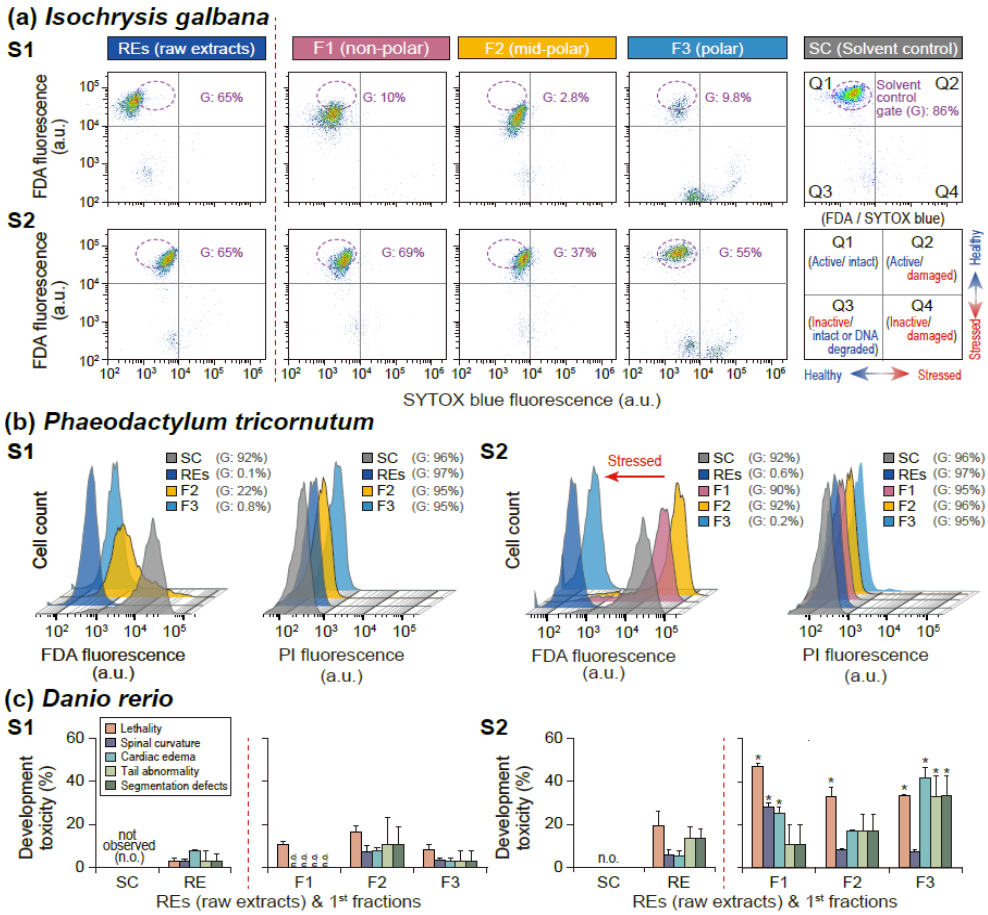


Figure 3.9.

(a) Two-dimensional flow cytometry dot plots of *I. galbana* cell signals plotted as coordinates of FDA- and SYTOX blue-fluorescence intensity treated for 96 h with solvent control, raw extracts (REs), and silica gel fractions (F1–F3). Each dot represents one cell; colors denote relative particle density in each population. All cells were placed in four categories and were easily differentiated: Viable FDA-unstained SYTOX blue cells (Q1; upper left quadrant, healthy cells), viable fluorescein diacetate (FDA)- stained SYTOX blue cells (Q2; upper right quadrant, membrane-damaged cells), unstained cells (Q3; lower left quadrant, inactive cells), and only stained with SYTOX blue (Q4; lower right quadrant, dead cells). (b) Fluorescence histograms of *P. tricornutum* stained with FDA or PI following exposure to solvent control, raw extracts, and F1–F3. F1 fraction of Site 1 was not analyzed. Mean values were obtained in the logarithmic scale and represented in arbitrary units (a.u.). Microalgae of solvent control exposed gated. (c) Results of *in vivo* bioassay with zebrafish embryo (*Danio rerio*) exposed to solvent controls, raw extract, and fraction samples (Error bar: Mean ± SD; $n = 3$).

3.3.4. Embryo developmental toxicity

REs from both sites caused significant lethality, after 96 h exposures, compared to the solvent control (Figure 3.9c). Exposures to fractions also caused significant developmental toxicities, with varying responses among samples and sites. Results of a previous study demonstrated significant toxic effects on this species are mainly associated with the presence of PAHs, e.g., naphthalene, phenanthrene, pyrene, and benzo[*a*]pyrene, all of which eluted in F2 (Sogbanmu et al., 2016). However, in contrast to the greater concentration of PAHs in S1, toxic effects were not significantly different between sampling sites (Figure 3.9c). Results indicated that the PAHs concentration in sediments from the Masan Bay deposit is below the level that is sensitive to FET. Further study is necessary to clarify the evaluation of PAHs (dioxin-like compounds) in sediments, the analyses of CYP1A at the transcriptional and protein levels, and use of a transgenic along with for developmental toxicity (Dong et al., 2019).

However, F1 and F3 in S2 caused significantly greater lethality and developmental toxicities than those in S1. Specifically, among fractions of S2, the greatest lethality (47%) was observed in embryos exposed to F1, followed by similar levels for F3 (34%) and F2 (33%). The incidence of spinal curvature followed a similar trend to lethality; however, sub-lethal effects were greatest in embryos that exposed to F3. Many lipophilic neurotoxins accumulating in sediments primarily target membrane sodium channels and cause adverse effects on *D. rerio* (Silver et al., 2010; Wang and Wang, 2003). In the previous study, brominated phenols and indoles showed a sensitivity of the *D. rerio* embryo test (Kammann et al., 2005). Thus, it is plausible that these chemicals, which are predominant in F3, contribute to the observed effects. Polar organic toxicants including NP and phthalates were detected in the FSA and those are well known to cause toxicity to fish embryos (Table 3.10). However, NP concentration in S1 was greater than that of S2, indicating that NP might not be responsible for the observed effects (Figure 3.10a).

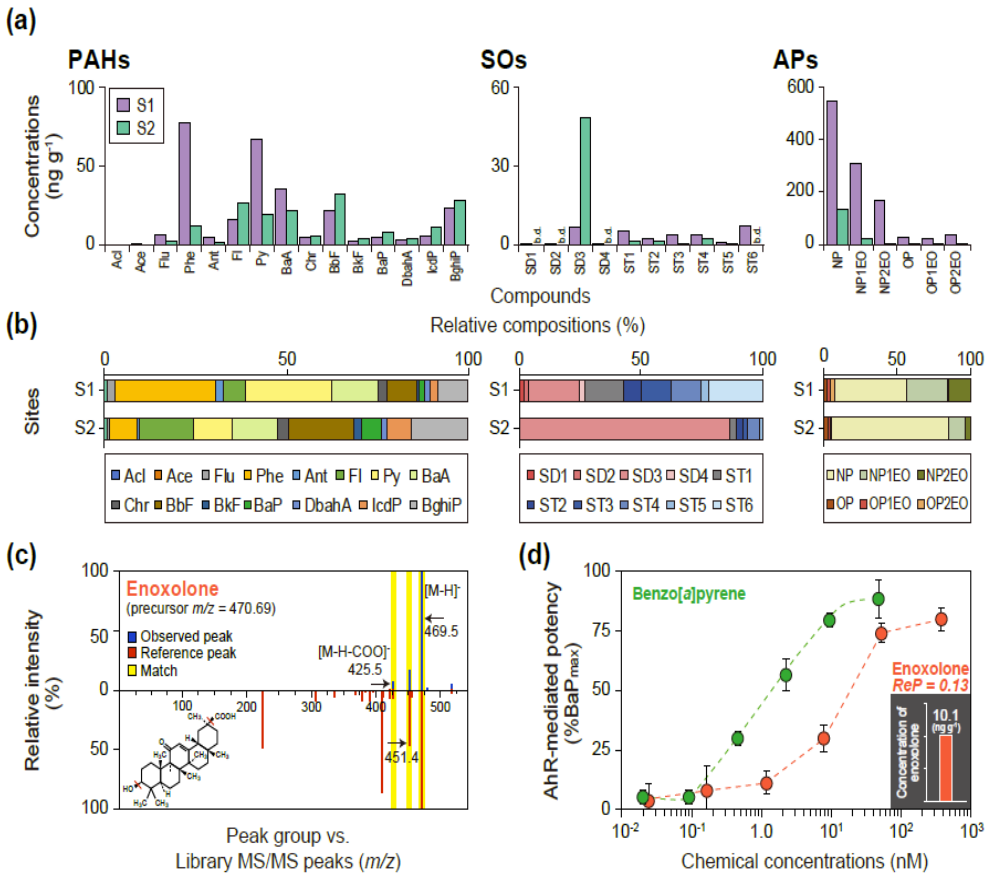


Figure 3.10.

(a) Concentrations of targeted polycyclic aromatic hydrocarbons (PAHs), styrene oligomers (SOs), and alkylphenols and their ethoxylates (APs) in sediments from Masan Bay, Korea. Abbreviations of target compounds were shown in Tables 3.4 and 3.5. (b) Comparison of the relative composition of PAHs, SOs, and APs in sediments. (c) Molecular structure of tentative AhR agonist for toxicological confirmation and butterfly plot comparing the observed MS/MS annotated spectrum of enoxolone (*m/z* 469.5 → 425.5) to the library spectrum. (d) Dose-response relationships for AhR-mediated potency of enoxolne and benzo[*a*]pyrene in the H4IIE-*luc* bioassay (Error bar: Mean ± SD; $n = 3$) and concentrations of enoxolone.

3.3.5. Occurrence, distributions, and sources of targeted analytes

All of the targeted chemicals, including PAHs, SOs, and APs were detected in sediments from both sites in Masan Bay. Concentrations of PAHs were moderate to low with 280 ng g^{-1} and 180 ng g^{-1} dry mass (dm) at S1 and S2, respectively (Table 3.4). Phenanthrene was the most dominant PAH at S1, followed by pyrene and benzo[*g,h,i*]perylene (Figure 3.10a and Table 3.4). Phenanthrene and pyrene are widely used in industries, including the manufacture of resins, pesticides, and pigments some of which are highly developed in the Masan industrial region (Abdel-Shafy and Mansour, 2016; Jiao et al., 2012; Kim et al., 2007). Concentrations of PAHs in sediments from both sites did not exceed the interim sediment quality guidelines (ISQGs) suggested by the Canadian Council of Ministers of the Environment (CCME, 2002). Results of recent studies have shown that concentrations of PAHs have been decreasing in sediments of Masan Bay for the past decade. This is in response to control of pollution by implementation of the Total Pollution Load Management System, thus the lesser concentrations of PAHs observed in 2016 samples were reasonable (Lee et al., 2016).

Concentrations of SOs were also low with 35 and 56 ng g^{-1} dm, at S1 and S2, respectively (Table 3.5). It was recently reported the historical pollution of SOs in Masan Bay sediments by analysis of 1998 archived and 2014 collected samples. A drastic decrease in sedimentary concentrations of 10 SOs in the region from 1998 (mean = 4940 ng g^{-1} dm; n = 7) to 2014 (mean = 128 ng g^{-1} dm; n = 7). SOs found in the present study reflected the relatively low concentrations of recently reported SOs in Masan Bay, thus continuing input would be minimal for this group of emerging chemicals of concern at the moment.

Concentrations of APs in sediments from S2 (180 ng g^{-1} dm) were relatively low, but an elevated concentration of APs was found in the upper creek site of S1 (1100 ng g^{-1} dm), which exceeded the ISQG (Figure 3.10 and Table 3.5) (CCME, 2002). Indeed, Masan Bay area has long been contaminated by APs with maximum reported NP + 4-*t*-OP concentrations of 4070 ng g^{-1} dm in the lower reach of Samho creek in 1998 (Khim et al., 1999a). Thus, the relatively great concentration of APs detected in S1 sediment reflected the continuing input of land-driven municipal

sources such as surfactants into the bay.

Concentrations of PAHs did not greatly differ between S1 and S2, but S1 sample showed relatively greater concentration (280 ng g^{-1} dm) compared to that of S2 (180 ng g^{-1} dm). While, proportions of PAHs with 4−6 rings (higher molecular mass PAHs), which include relatively strong AhR agonists, prevailed in S2. These compositional characteristics of PAHs apparently influenced great BEQs in S2 than that in S1, however, targeted PAHs can explain as much as 0.1% of total AhR-mediated potency in corresponding samples and fractions. Collectively, these results indicated presence of more unknown AhR agonists in S2. Thus, FSA was further conducted on fraction samples of S2.

There was a difference in SOs composition between S1 and S2. Among SOs, styrene trimers (STs) mainly dominated in S1, while styrene dimers (SDs), especially 2,4-diphenyl-1-butene (SD3), dominated in S2 (Figure 3.10b). However, concentrations of SOs were relatively small compared to other targeted chemicals. Thus, interpretation of direct association to varied responses of multiple bioassays was limited. APs showed both great variations in concentrations and compositions between S1 and S2 samples. Among 6 APs, NP dominated concentrations in sediments at both sites, but other APs showed varied proportion to total APs. Nonylphenol ethoxylates (NPEOs) dominated in S1 compared to S2 (Figure 3.10b). Differences in sensitivity for APs enriched fraction sample, viz., F3 from S1, to *in vivo* bioassays were observed for both inhibition of growth of algae and embryo developmental toxicity. Differences in homologue compositions within a group of targeted analytes justified use of multiple bioassays and endpoints in risk assessment.

3.3.6. Full-scan screening analyses

In order to address possible contributions of unidentified AhR agonists in samples to the observed potencies as determined by the H4IIE-*luc* bioassay, FSA with GC-QTOFMS was performed on some of the most active fractions; F2.7 and F2.8 of raw extracts of S2 (Figure 3.7a). First, formulas derived from accurate mass were compared to those in the NIST library (Booij et al., 2014). Following two-step fractionations of sediment raw extracts, 142 and 264 compounds were detected in F2.7 and F2.8, respectively. Overall, 34 and 118 compounds in F2.7 and F2.8 had matching factor scores of > 70 (Muz et al., 2017). Next, 11 and 28 aromatic compounds were selected from F2.7 and F2.8, respectively (Mekenyan et al., 1996). Of these chemicals, no compounds with more than 3-rings, which is the main feature of AhR agonists, were detected (Xiao et al., 2016). However, five compounds with more than two benzene rings were detected in F2.7 and F2.8. These chemicals were selected as tentative AhR-active compounds (Table 3.10).

From F3 sub-fractions, F3.6 and F3.7 of raw extracts of S2 sediment exhibited significant AhR-mediated potencies (Figure 3.7a). Thus, this study also focused on characterizing the AhR agonists in these two fractions using LC-QTOFMS. After peaks were detected, elemental compositions were matched exhaustively using accurate *m/z* (Zedda and Zwiener, 2012). Overall, 1732 and 2402 compounds were detected in F3.6 and F3.7, respectively. Second, after removing noise peaks, 1229 and 1888 compounds were retained (Cui et al., 2018). Based on accurate masses and isotope distribution scores, flucofuron, niflumic acid, and enoxolone in F3.6 and scytalone in F3.7 were finally identified as tentative AhR active compounds (Figure 3.6b and Table 3.10).

Overall, 14 candidate compounds were identified including xenobiotics, antibiotics, and insecticide (Kind and Fiehn, 2010; Cui et al., 2018). Among these chemicals, flucofuron is an insecticide and known to be highly toxic to fish and invertebrates (Cole, 1999). Enoxolone is a pentacyclic triterpenoid derivative of the beta-amyrin type obtained from the hydrolysis of glycyrrhizic acid, and has some additional pharmacological properties, with possible antiviral, antifungal, antiprotozoal, and antibacterial activity (Badam, 1997; Salari and Kadkhoda, 2003).

3.3.7. Toxicological confirmation

During biological characterization, AhR-mediated potencies of four tentative compounds including 1,2-di(*p*-tolyl)ethane, flucofuron, niflumic acid, and enoxolone, for which authentic standards were available, were assessed by use of the H4IIE-*luc* bioassay (Table 3.11). Among these chemicals, only enoxolone (18*β*-glycyrrhetinic acid) showed significant AhR-mediated potency. Results of previous studies suggested that enoxolone increases CYP gene activity, but, to our knowledge, none have been identified as AhR agonists (Zhao et al., 2012; Lv et al., 2015). The ReP value of enoxolone for the AhR-mediated potency compared to that of BaP was determined for the first time during this study, by use of the dose-response relationship obtained with the H4IIE-*luc* bioassay. AhR-mediated potency of the identified AhR agonist was sufficiently great (4 − 80% BaP_{max}), and the calculated ReP value was 0.13 (Figure 3.10d). The enoxolone peak was confirmed in the LC-QTOFMS by use of authentic standards and was detected in F3.6 from S2 (~10 ng g^{-1} dm) (Figure 3.10d).

Binding affinities with AhR and other potential toxicities (mutagenicity, carcinogenicity, developmental toxicity, and estrogen activity) of 14 candidates, including 10 unavailable standard materials, were tested by use of *in silico* method such as VirtualToxLab. Among 14 candidates, seven compounds were found to be able to bind to the AhR (Table 3.10). Interestingly, it was found that enoxolone, which showed AhR potencies by *in vitro* assay (H4IIE-*luc*), did not bind to the AhR in the VirtualToxLab. Because VirtualToxLab is based solely on thermodynamic considerations, i.e. ignoring mechanisms influencing the availability of a compound at the site of action, there could be many anti-empirical examples that are not consistent with toxicity results (de Lima Ribeiro and Ferreira, 2005; Zvinavashe et al., 2008). This result shows that the use of QSAR models has also a certain limitation and thus prediction needs careful caution with empirical confirmations by exercising multiple bioassays. Data on co-occurrences of several toxicological endpoints generated by the same sample represent a clearer structure when reduced by a comprehensive understanding by statistical tools. The canonical analysis of principal coordinates (CAP) for raw extracts and fraction samples of both sites and

all endpoints, such as AhR-mediated potency, bioluminescence inhibition, microalgae cell viability of *I. galbana*, development toxicity, and concentrations of PAHs and APs, identified that each endpoint could be grouped with ecotoxicologically-relevant fractions (Figure 3.11).

Of the five bioassays used as detectors for sediment toxicity in the raw extracts and fractions, the inhibition of bioluminescence of *V. fischeri* and lethality of FET showed remarkable sensitivities to non-polar fraction of F1. The AhR-mediated potency was associated mainly with the mid-polar fraction of F2. Overall, the effect of compounds eluting in the most polar fraction of F3 was reflected by membrane integrity of microalgae cell viability, not by cell size or granularity. Strict regulations on releases of NPs have been implemented, so concentrations of NPs in F3 are less. In all bioassays (except luminescent bacteria), the biological effects associated with fractions were greater than those in the raw extracts. For the sediments of Masan Bay investigated here, antagonistic reaction in mixture raw extracts seems to play an important role as highlighted by the previous study (Kammann et al., 2005). Such antagonistic effects might be the result of false-negative results in bioassays exposed to raw extracts (Weiss et al., 2009; Wang et al., 2014). For example, the presence of polymeric PAHs, such as dibenz[*a,j*]anthracene, dibenz[*a,c*]anthracene, and picene inhibited BaP-induced AhR toxic potency (Pushparajah and Ioannides, 2018). Another possible explanation for antagonism lies in the natural environment, compounds might be mixed; resulting in a delayed uptake as well as a reduced accumulation (Schwab et al., 2009; Pushparajah and Ioannides, 2018). Future studies are necessary to confirm this effect such as testing of the remixing the separated samples (Khim et al., 2000).

Table 3.11.

List of candidates for AhR-active compounds in the fraction samples (F2.7, F2.8, F3.6, and F3.7) of organic raw extracts of S2 sediment using GC-QTOFMS (F2.7 and F2.8 fractions) and LC-QTOFMS (F3.6 and F3.7 fractions) and binding affinity to AhR estimated by VirtualToxLab.

Fraction and compounds	CAS Number	Molecular formula	Molecular weight (g mol^{-1})	Matching factor/ Mass error [a]	AhR binding affinity [b]
F2.7 fraction					
2,5-Dimethyl-8-(*propan-2-yl*)-1,2,3,4,4a,7,8,8a-octahydronaphthalen-2-ol	254182 [c]	$C_{15}H_{26}O$	222.37	76.5	Not binding
1,2-di(*p-tolyl*)ethane*	61558 [c]	$C_{16}H_{18}$	210.31	71.3	Binding
Methyl 1-(*2,3,4-trifluorobenzoyl*)prolinate	462819 [c]	$C_{13}H_{12}F_3NO_3$	287.23	72.5	Not binding
2-(*4-biphenylyl*)-2-propanol	34352-74-4	$C_{15}H_{16}O$	212.29	72.8	Binding
4-*tert*-butylbiphenyl	1625-92-9	$C_{16}H_{18}$	210.31	74.0	Binding
F2.8 fraction					
2,6-Di-isopropylnaphthalene	24157-81-1	$C_{16}H_{20}$	212.33	83.3	Binding
cis-Calamenene	72937-55-4	$C_{15}H_{22}$	202.33	72.5	Binding
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.26	75.0	Binding
2-[(*5-methylpyridin-2-yl*)amino]-1-phenylethanol	14140 [b]	$C_{14}H_{16}N_2O$	228.29	71.9	Not binding
5,7-Difluoro-3,4-dihydro-2(*1H*)-napthalenone	280105 [c]	$C_{10}H_8F_2O$	182.17	71.0	Binding
F3.6 fraction					
Flucofuron*	370-50-3	$C_{15}H_8Cl_2F_6N_2O$	417.13	1.996	Failed
Niflumic acid*	4394-00-7	$C_{13}H_9F_3N_2O_2$	282.22	1.248	Not binding
Enoxolone*	471-53-4	$C_{30}H_{46}O_4$	470.69	0.507	Not binding
F3.7 fraction					
Scytalone	49598-85-8	$C_{10}H_{10}O_4$	194.18	0.611	Not binding

[a] Mass error expressed in ppm for LC-based results.
[b] Data from VirtualToxLab.
[c] Chemspider ID.
* These chemicals were confirmed for AhR-mediated potency by use of H4IIE-*luc* bioassay.

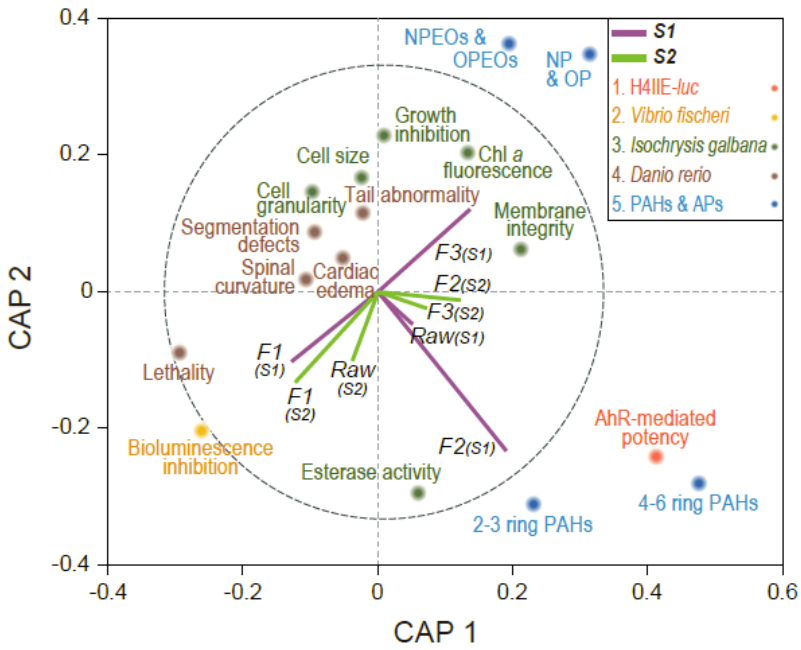
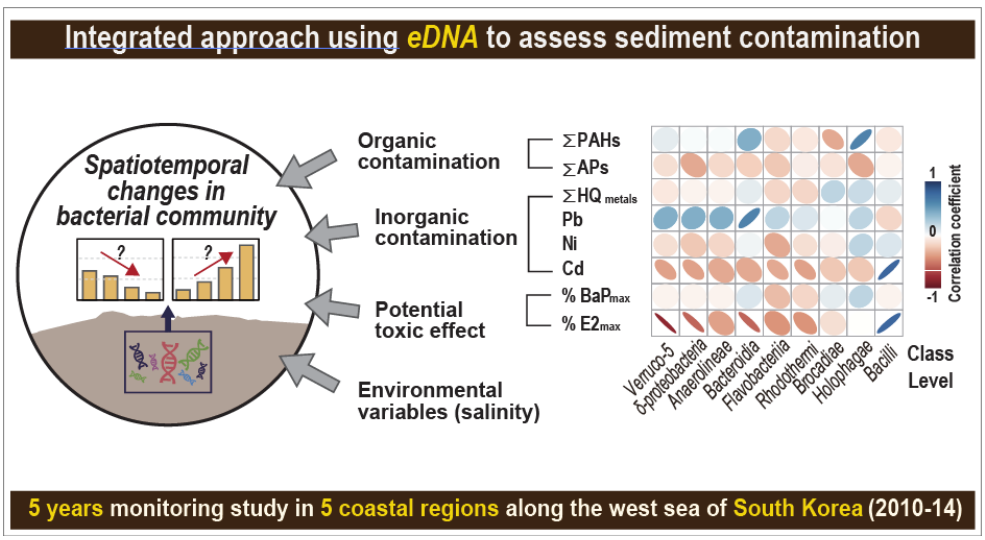


Figure 3.11.
Scatter diagram of canonical analysis of principal coordinates (CAP) between the bioassay data and treated samples. CAP was significant with $p = 0.05$. Purple and green vectors (Spearman pairwise correlations) point in the direction of the increased values for any given variable.

CHAPTER 4.

INTEGRATED ASSESSMENT OF WEST COAST OF SOUTH KOREA BY USE OF BENTHIC BACTERIAL COMMUNITY STRUCTURE AS DETERMINED BY eDNA, CONCENTRATIONS OF CONTAMINANTS, AND IN VITRO BIOASSAYS



This chapter has been published in Environment International.

Lee, A. H[1]., Lee, J[1]., Hong, S., Kwon, B.-O., Xie, Y., Giesy, J. P., Zhang, X., Khim, J. S. Integrated assessment of west coast of South Korea by use of benthic bacterial community structure as determined by eDNA, concentrations of contaminants, and *in vitro* bioassays. *Environ. Int.,* 2020, **137**, 105569. https://doi.org/10.1016/j.envint.2020.105569

4.1. Introduction

The west coast of South Korea, along with nearby rivers/estuaries, is part of the Yellow Sea region, a region that has become highly urbanized and industrialized (Jeon et al., 2017). Various types of anthropogenically-produced contaminants from agricultural, industrial, and domestic activities have been discharged into coastal sediments in the region, which is the final sink for those contaminants (Khim and Hong, 2014). Based on results of previous toxicological studies, concentrations of PTSs in sediments off the west coast of South Korea, were deemed to pose risks to biota (Khim et al., 1999; Hong et al., 2012; Jeon et al., 2017). PTSs, such as PAHs, APs, metals, and metalloids, accumulating in coastal sediments can adversely affect aquatic and/or benthic ecosystems at all levels of biological organization from molecules to communities (Fent, 2001).

To evaluate of quality of sediments contaminated by PTSs, conventional sediment assessments use chemical analyses combined with a determination of toxicological effects. However, this approach focuses on a limited suite of ecotoxicological effects and often lacks ecological relevance. In addition, the conventional approach cannot capture effects on populations and communities in natural ecosystems (de Castro-Català et al., 2016). Therefore, to comprehensively evaluate sediment quality, a triad approach was applied, wherein physicochemical data, such as salinity, pH, and concentrations of contaminants, toxicological data, and benthic community structure data are concurrently examined (Lee et al., 2018). However, few studies have addressed the ecological association of concentrations of PTSs and benthic communities (Lee et al., 2018; Yoon et al., 2017). Especially, characteristics of *in situ* bacterial communities have been neglected in conventional sediment quality assessment approaches (Torsvik et al., 2002).

More than 99% of microorganisms that have been observed in nature cannot generally be cultivated or phenotypically identified with standard culture techniques. However, with environmental DNA (eDNA) metabarcoding, most microbial communities can be identified (Amann et al., 1995). Thus, approaches that use metagenomic level analysis to characterize the complexity of microbial ecosystems

in sediments could provide a rapid and efficient way to identify and monitor benthic microbial communities and enumerate individual taxa (Sharmin et al., 2013; Gibbons et al., 2014). Improvements in methods for metabarcoding eDNA, in concert with advances in bioinformatics analysis, could provide a promising approach for improving ecological risk assessments (Zhang, 2019).

From the 1970s to the early 1990s, many sea dikes (estuary dams) and landfill projects occurred along the west coast of South Korea (Figure 4.1). As a result, most major rivers and estuaries along the west coast have been disconnected from the open sea, leading to different salinity regimes on the two sides of the dams. Salinity is thought to be the major factor affecting bacterial community composition (Wu et al., 2006; Campbell and Kirchman, 2013), more important than the influence of temperature or pH (Lozupone and Knight, 2007). Several studies on shifts in bacterial community composition along aquatic salinity gradients have substantiated the influence of salinity on bacterial composition (Kirchman et al., 2005; Kan et al., 2008; Campbell and Kirchman, 2013). However, most of these gradient studies were conducted in one region and/or over a short duration. To bridge the multiple environmental variables including salinity and chemical contaminants, *in vitro* toxicological test, and bacterial communities in the assessment of sediment quality, a comprehensive five-year field study on the west coast of South Korea had been conducted. The present study investigated the effects of salinity and concentrations of toxic chemicals on bacterial composition in sediments. The toxic chemicals included dioxin-like and estrogenic chemical pollutants, measured as aryl hydrocarbon receptor (AhR)-mediated potencies and estrogen receptor (ER)-mediated potencies because those endpoints represent a large spectrum of dioxin-like and estrogenic chemicals that might exist in contaminated sediments (Hong et al., 2016; Lee et al., 2017).

Specific objectives of the present study were to: (1) characterize spatio-temporal distributions of benthic bacterial communities in sediments along the west coast of South Korea; (2) compare *in situ* bacterial communities and endpoints of chemical analysis and *in vitro* bioassays; and (3) identify bacterial taxa indicative of specific environmental variables.

4.2. Materials and Methods

4.2.1. Study area and sediment collection

Surface sediments were collected during May, annually between 2010 and 2014 from 15 sites (11 seawater sites and 4 freshwater sites) in five coastal regions from rivers, estuaries, and open sea along the west coast of South Korea. The five regions include Lake Sihwa (Region A), Asan & Sapgyo (Region B), as well as the Taean Coast (Region C), and estuaries of the Geum River (Region D) and Yeongsan Rivers (Region E) (Figure 4.1 and Table 4.1). The inside of the estuary dams or sea dikes (i.e., landward) is composed of freshwater except for Lake Sihwa, which has a tidal power plant that has allowed tides to pass through since its construction in 2011 (Figure 4.1 and Table 4.1). In contrast, seaward sides of dikes are saline. Sediments were immediately transported, at 4 °C, to the laboratory and stored at –20 °C until analysis. Samples were freeze-dried and ground with a mortar and pestle prior to analyses.

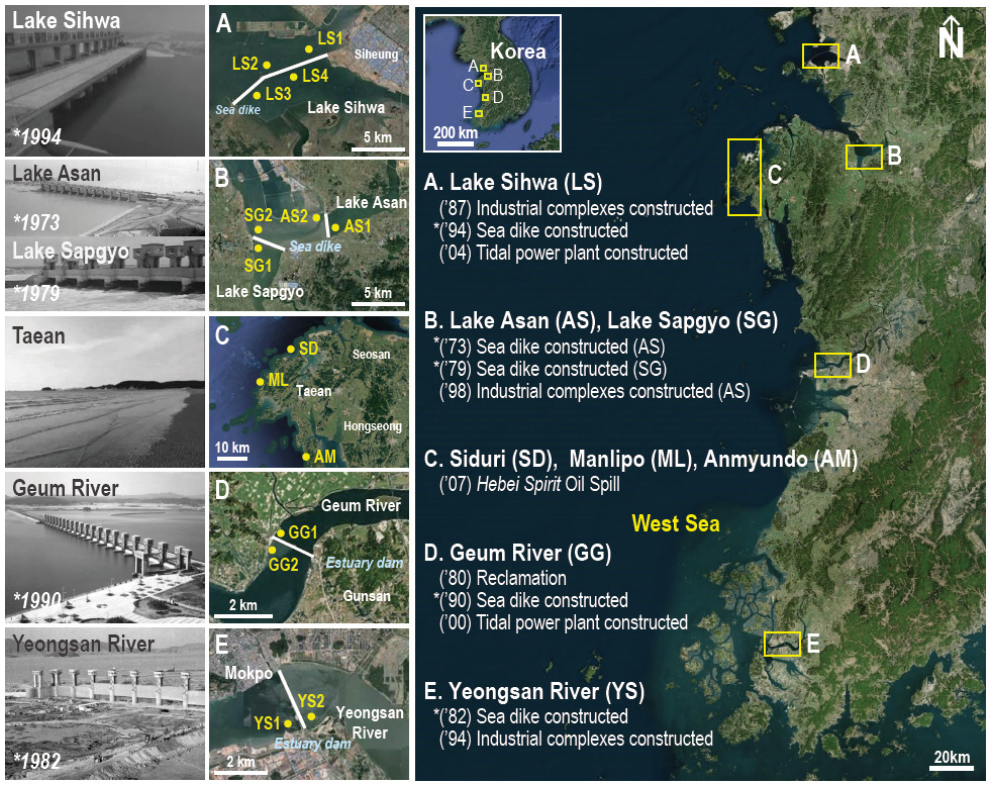


Figure 4.1.
Pictures and satellite images of sampled sites from five regions of the west coast of South Korea and brief summary of activities related to sampling each region. Panels: (a) Lake Sihwa (LS), (b) Lakes Sapgyo and Asan (SG and AS), (c) Taean coast, including Sinduri (SD), Manlipo (ML), Anmyundo (AM), (d) Geum River Estuary (GG), and (e) Youngsan River Estuary (YS).

Table 4.1.

Latitude, longitude, and region information for each sampling site along the west coast of South Korea.

Province	Region	Sites	Latitude	Longitude	Year					Remark	Salinity on May
					2010	2011	2012	2013	2014	Geographical description	
Gyeonggi	Lake Sihwa (A)	LS1	N37° 20.093’	E126° 41.370’	√	√	√	√	√	Coastal area, outside of sea dike	30.9 ± 1.8
		LS2	N37° 19.543’	E126° 39.427’			√			Coastal area, outside of sea dike	32.0 ± 2.0
		LS3	N37° 18.657’	E126° 36.618’	√				√	Inside of sea dike	32.4 ± 2.2
		LS4	N37° 19.494’	E126° 39.340’	√				√	Inside of sea dike	31.9 ±2.5
Chungnam	Asan (B)	AS1	N36° 53.600’	E126° 54.742’	√		√		√	Inside of sea dike	0.5 ± 0.1
		AS2	N36° 54.929’	E126° 54.317’	√	√	√	√	√	Coastal area, outside of sea dike	29.0 ± 4.3
	Sapgyo (B)	SG1	N36° 52.728’	E126° 49.633’	√	√	√	√	√	Inside of sea dike	0.5 ± 0.0
		SG2	N36° 53.704’	E126° 49.148’	√	√	√	√	√	Coastal area, outside of sea dike	29.1 ± 1.7
	Taean (C)	SD	N36° 50.312’	E126° 11.004’	√	√	√	√	√	Coastal area (beach; Sinduri)	32.3 ± 0.1
		ML	N36° 47.027’	E126° 08.185’	√		√		√	Coastal area (beach; Manlipo)	32.0 ± 0.3
		AM	N36° 32.403’	E126° 19.588’	√	√	√	√	√	Coastal area (beach; Anmyundo)	32.4 ± 0.7
Jeonbuk	Geum River (D)	GG1	N36° 01.347’	E126° 44.532’	√	√	√	√	√	River, inside of dam	0.2 ± 0.0
		GG2	N36° 00.510’	E126° 44.117’	√	√	√	√	√	Coastal area, outside of dam	29.8 ± 4.1
Jeonnam	Yeongsan River (E)	YS1	N36° 46.930’	E126° 26.648’	√	√	√	√	√	Coastal area, outside of dam	27.9 ± 2.3
		YS2	N36° 47.198’	E126° 27.767’	√	√	√	√	√	River, inside of dam	0.6 ± 0.3

4.2.2. Next-generation sequencing and bioinformatics analyses

Total DNA was extracted from 0.25 g aliquots of each homogenized surface sediment by use of a Power Soil DNA Kit (MoBio Laboratories Inc., CA, USA). Detailed descriptions for amplifying bacterial 16S rRNA genes (V3 fragment) have been previously published (Xie et al., 2016). Triplicate PCR reactions were performed for each sample to minimize potential PCR bias. Products of PCR were checked, purified, and quantified (Figure 4.2). All purified products of PCR were pooled equally for subsequent sequencing. Sequencing adapters were linked to purified DNA fragments with the ION proton sequencer (Life Technologies, CA, USA) following the manufacturer's instructions. These processes were conducted by the research laboratory of Ecotoxicology and Health Risk in Nanjing University.

Low quality raw reads (mean quality score < 20, scanning window = 50) and sequences which contained ambiguous 'N' and were shorter than 100 bp but longer than 180 bp in length were discarded by using the Quantitative Insights into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010). Chimeras were removed and clustered operational taxonomic units (OTUs) with a similarity cutoff of 97% following the UPARSE pipeline method (Edgar, 2013). Taxonomy of bacterial OTUs was assigned to representative sequences by use of the Ribosomal Database Project (RDP) classifier against the Greengenes database (DeSantis et al., 2006; Wang et al., 2007). Observed OTUs were rarefied at equal sequencing depth to reduce biases resulting from differences in sequencing depth. Alpha-diversity (Shannon indices) was calculated on all twenty equal-depth rarefactions and then averaged them.

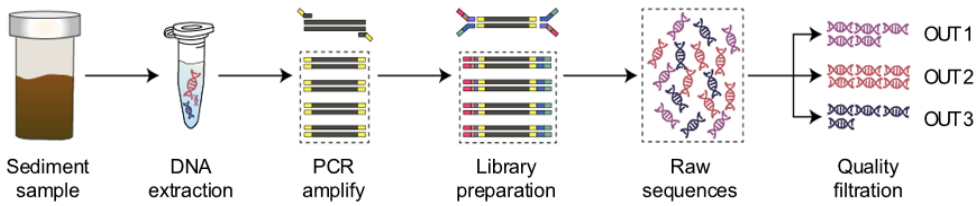


Figure 4.2.
The experimental procedure used for bacterial community analysis in this study.

4.2.3. Analyses of chemicals and toxicological tests

Concentrations of 15 PAHs and six alkylphenols (APs) and *in vitro* ecotoxicological assays [H4IIE-*luc* bioassays for determining AhR-mediated potencies (presented as % BaP_{max}) and MVLN bioassays for determining ER-mediated potencies (presented as % $E2_{max}$)] were obtained for sediments collected from the same locations during 2010 to 2014, Jeon et al. (2017). Concentrations of eight metals (Cd, Cr, Cu, Hg, Li, Ni, Pb, and Zn) and one metalloid (As) were also reported for the sediments in a previous study by Kim et al. (2020). Metals and metalloids were expressed in sum of hazard quotients (Σ HQ_{metals}) by Ryu et al. (2016). All prior chemical measurements and toxicological results are summarized in Figure 4.3.

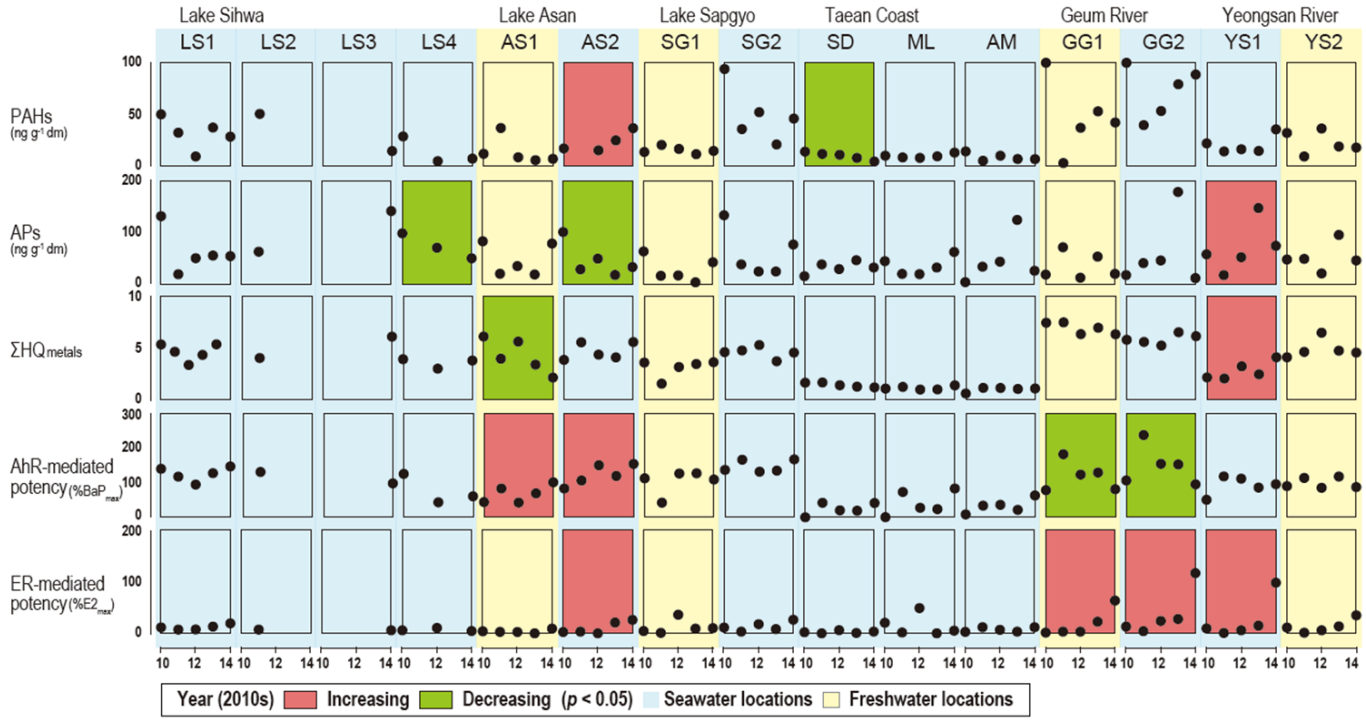


Figure 4.3.
Spatial and temporal distributions of persistent toxic substances (PTSs) and potential toxicities in sediments along the west coast of South Korea from 2010 to 2014. PTSs include organic pollutants, metals, and metalloid arsenic. Potential toxicities include AhR- and ER-mediated potencies.

4.2.4. Statistics analyses

SPSS 23.0 and PRIMER 6 statistical software (PRIMER-E Ltd, Plymouth, UK) with the PERMANOVA+ add-on package (Lozupone and Knight, 2005; Clarke and Gorley, 2006) were used to perform statistical analyses. Statistical significance was set at $p < 0.05$. The bacterial community with phyla data was reduced by eliminating species that contributed < 1% of total abundance. Abundance was log-transformed [ln (x+1)] and normalized to balance it across the recorded taxa in the measure of similarity (Clarke and Warwick, 2001). The nonparametric Mann-Whitney U (M-W) and Kruskal-Wallis (K-W) with Bonferroni correction were used to detect significant differential features between the salinity, sampled regions, and years. Subsequently, to perform categorize of bacterial assemblage composition, cluster analysis (CA) was performed using a Bray-Curtis similarity matrix (Legendre and Legendre, 2012). This study used the nonparametric Mann-Whitney U (M-W) test and Kruskal-Wallis (K-W) test with Bonferroni correction to detect significant differential features between the salinity, sampled regions, and years. Subsequently, to categorize (differentiate) bacterial assemblages, cluster analysis (CA) using a Bray-Curtis similarity matrix was performed (Legendre and Legendre, 2012).

All environmental variables including Σ PAHs, Σ APs, metals, and metalloid (including Σ HQ_{metals}), and toxicological results were log-transformed and normalized following procedures by Xie et al., 2017. Discriminating chemical and toxicological results by sampled year was confirmed by canonical analysis of principal coordinates (CAP) with Euclidean distance matrix. Next, principal coordinate analysis (PCoA) applied to the distance matrix to enable comparisons of biota among sedimentary environments. This allowed assessment of potential interactions between environmental variables and compositions of bacterial communities, at the phylum taxonomic level, based on the Bray-Curtis similarity matrix and Spearman's rank correlation coefficients (Lozupone and Knight, 2005). PCoA is useful for reducing and representing patterns present in distance matrices by displaying dissimilarities among objects (Gower, 1996).

Finally, to identify specific bacterial taxa that may have responded to the various variables measured, Spearman's rank correlation coefficients were developed for associations between results of chemical and toxicological data and the abundance of bacteria with phylum/class data.

4.3. Results and Discussion

4.3.1. Description of next-generation sequencing data

A total of 4,634,661 bacterial 16S, V3 sequences were obtained for sediments along the west coast of South Korea. Sequences with low quality, short lengths, PCR bias, lack of annotated references, or lineage filtering were discarded (Bragg et al., 2013). Due to low sequencing depth, samples collected in 2012 were discarded. After our quality check of raw reads, there remained a total of 2,464,770 reads for further analyses. From those remaining filtered raw reads, the number of 5,001 bacterial OTUs which represented 49 phyla, were discovered. According to rarefaction curves for bacterial communities, most of the abundant bacterial OTUs were saturated from more than 80,000 sequences per sample (Figure 4.4).

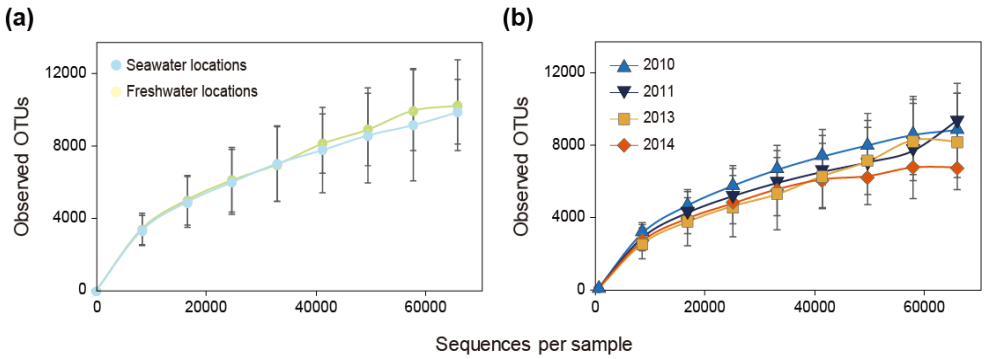


Figure 4.4.
Rarefaction curves of the observed OTU numbers of bacterial communities (a) by salinity and (b) sampling years in sediments.

4.3.2. Spatial distributions of bacterial communities with phylum taxonomic level

At the level of phyla, compositions of bacterial communities varied among sediments and were dominated by Proteobacteria (mean 28.7%), Actinobacteria (13.1%), Firmicutes (12.7%), Chloroflexi (12.5%), Planctomycetes (8.7%), Bacteroidetes (8.2%), Verrucomicrobia (4.2%), Acidobacteria (3.7%), and Gemmatimonadetes (1.5%) (Figure 4.5a). As expected, Proteobacteria were the most abundant bacterial phylum from all sediments collected from along the west coast of South Korea between 2010 and 2014. A similar pattern of compositions for bacterial communities in sediments has been observed by several previous studies conducted around the world (Gibbons et al., 2014; King et al., 2015; Xie et al., 2016). These dominant phyla are free-living and also include some nitrogen-fixing bacteria (Li et al., 2009; Sharmin et al., 2013).

Relationships between mean, relative abundance salinity, sampled region, and year were observed (Figure 4.5b to 4.5e). Although salinity and/or spatial variability are known to be major contributors to microbial community structure and function (Lozupone and Knight, 2007; Campbell and Kirchman, 2013; Xie et al., 2017), in this study mean compositions of bacteria, as determined at the level of phyla, exhibited no statistically significant, correlation with salinity (M-W test) or sampled region (K-W test) (Bonferroni-corrected, $p > 0.05$) (Figure 4.5b and 4.5c). This unexpected result might be due to the fact that salinity gradients are sometimes temporarily diluted in response to an input of freshwater from temporally irregular discharges of freshwater through the sea dikes in our study regions (Noh et al., 2019). Therefore, a better understanding of patterns in spatial proximity and shared environment characteristics will require intensive sediment sampling from along transects that reflect a longer gradient in physicochemical parameters, such as salinity, pH, and contaminant composition.

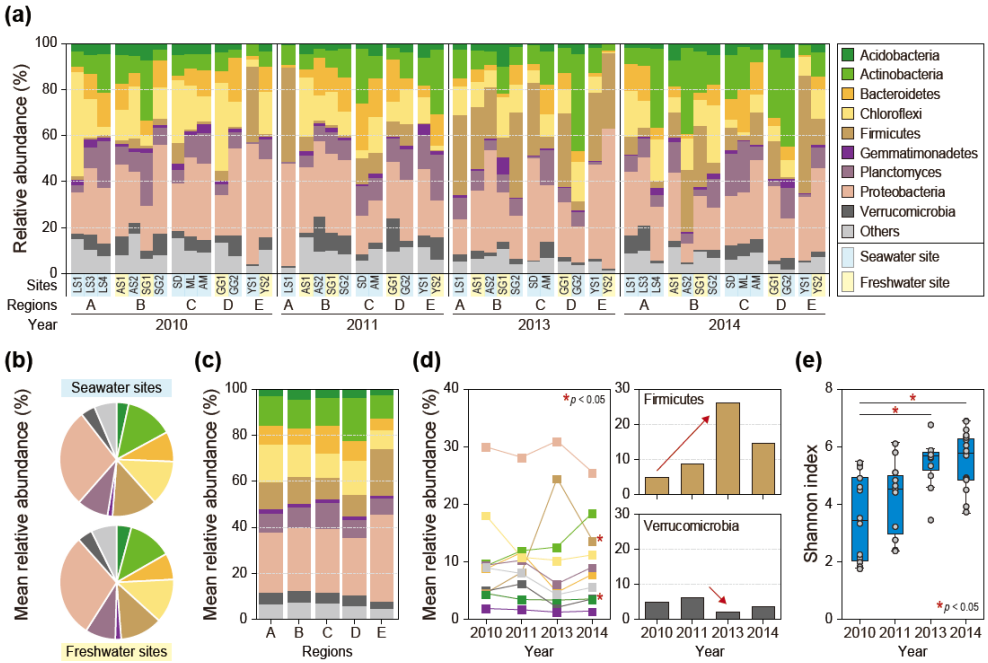


Figure 4.5.

The structures of the bacterial communities at phylum taxonomic level in sediments along the west coast of South Korea. Panels: (a) relative abundance of the dominant bacteria at the phylum level across all samples, statistical comparisons for mean relative abundances at the phylum taxonomic level between (b) salinity, (c) sampling region, (d) sampling years, and (e) alpha-diversity estimated with Shannon indices of all sediment samples. Low (< 1%) abundant phyla and unresolved taxa were indicated as "Others" in panel (a). Mann-Whitney U test for panel (b) and Kruskal-Wallis test for panels (c−e) was obtained with Bonferroni corrections. Significance was determined at $p < 0.05$ (*).

Among the dominant phyla, Firmicutes and Verrucomicrobia showed significant differences in mean relative abundance across sampling year (K-W test, Bonferroni-corrected, $p < 0.05$) (Figure 4.5d). In 2010, Firmicutes and Verrucomicrobia were distributed at similar level, but Firmicutes abundance gradually increased over time, whereas Verrucomicrobia abundance declined over time, with the Firmicutes eventually becoming 20 times more abundant than Verrucomicrobia by 2013. Firmicutes is quite rare phylum in natural sediments but is dominant taxa in sugar cane processing sites (Sharmin et al., 2013). However, none of the sites which sampled in this study were near sugar cane processing areas. Verrucomicrobia was related to Planctomycetes and was known to exist in majority of which are eutrophic or even heavily polluted (Spring et al., 2016), and were particularly abundant occurrence in marine environments (Cardman et al., 2014). Several reports of Verrucomicrobia occurring in extreme environments, such as in sulfide-rich water and sediments, have been published (Freitas et al., 2012; Spring et al., 2016).

Mean values of alpha-diversity (Shannon indices) from all sites bacterial communities were significantly greater in 2013 and 2014 than in the 2010 (K-W test, Bonferroni-corrected, $p < 0.05$) (Figure 4.5e). Our results indicate that although only one bacterial community showed a significant increase in diversity over time at the phylum taxonomic level, population change could become even more significant if lower level taxa are examined. Hierarchical clustering of our surface sediment samples also showed a separation of bacterial communities by sampling year (2010–11 and 2013–14). High bootstrap values indicated changing environmental influences on the bacterial community over time (Figure 4.6a). Changes in patterns of diversity could have any number of explanations. However, assuming that sediment contamination favors pollution-tolerant species over non-tolerant species, then complex communities will respond to chemical contamination in a magnitude-dependent effect with respect to toxic pollutants so that over time microbial communities will increase in diversity as pollution increases (Gillan et al., 2005).

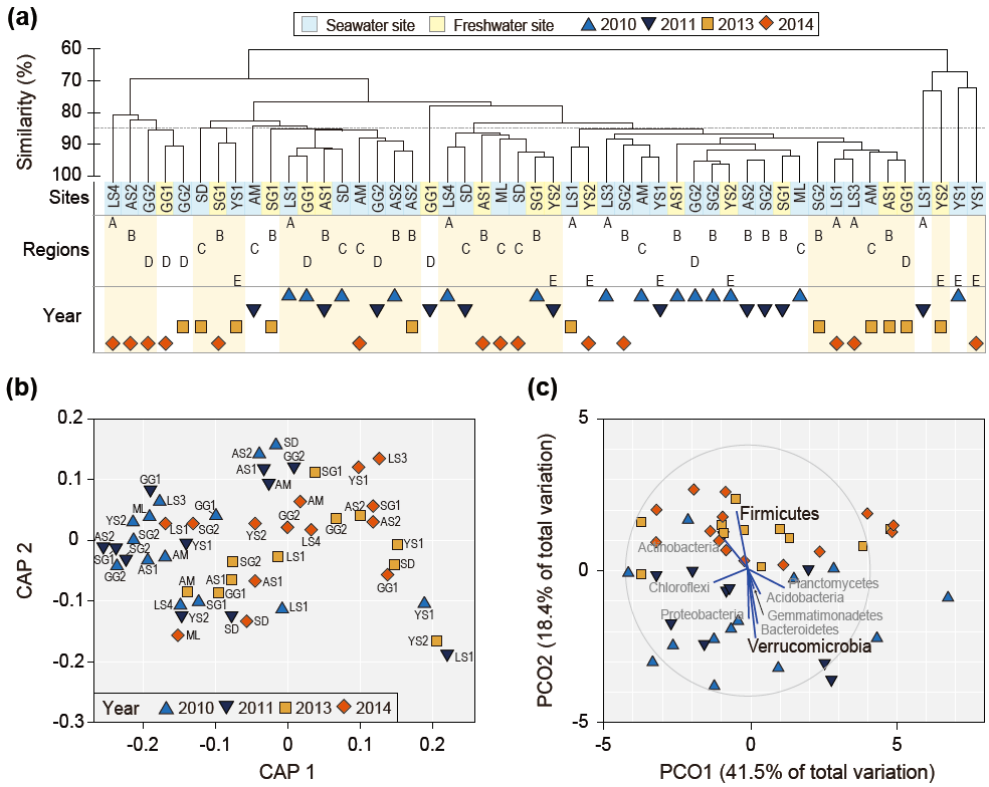


Figure 4.6.

Classifications of sampled sites based on environmental conditions and biotic composition. Panels: (a) bacterial composition along the west coast of South Korea based on a Bray-Curtis dendrogram of 16S rRNA sequences clustered into OTUs at 97% similarity, followed by orange-colored background bars indicating the designated group, (b) scatter diagram of canonical analysis of principal coordinates (CAP) with Euclidean distance matrix, including results of 15 PAHs, 6 APs, and 9 metals and metalloid, and AhR- and ER-mediated potencies grouped by year, (c) Principal coordinates analysis (PCoA) ordinations (first two principal coordinates are displayed) based on Bray-Curtis dissimilarity, showing similarity in community composition between samples. Data are Phylum taxonomic level and have been log-transformed [ln(x+1)]. Blue vectors (Spearman correlation test) point in the direction of the increased values for any given variable. Sediments with similar environmental profiles or bacterial compositions are located near each other on the diagram.

The scatter diagram of CAP of environmental variables (including chemical contaminants and toxicological parameters) show a pattern similar to that observed for bacterial communities (Figure 4.6b). Our CAP analysis could partition effects of 'sampling year' for the various environmental variables. This study determined temporal trends (which increased, decreased, or did not change) of PTSs and potential toxicity in sediments along the west coast of South Korea by examining linear correlations between concentrations (or toxicities) and sampling years (Figure 4.3) (Jeon et al., 2017; Kim et al., 2020). From 2010 to 2014, temporal distributions of PAHs and APs declined from overtime, whereas concentrations of heavy metals Cd, Cr, and Hg increased. According to a previous study, PAHs can contribute to reductions in freshwater and marine biotic diversity (Malaj et al., 2014); thus, variations in of chemical concentrations might indicate variations in the composition of bacterial communities. In addition, the adverse effects on biota of high concentrations of some metals are due to the metals' abilities to block and inactivate sulfhydryl groups of proteins (Valls and De Lorenzo, 2002). In this study, AhR-mediated potency in sediments declined over time, whereas ER-mediated potency showed a slightly increase from 2010 to 2014 (Jeon et al., 2017). Overall, the concentration of metals (Cd, Cr, and Hg) and ER-mediated activity was relatively higher during the 2013–2014 period than in the 2010–2011 period (Kim et al., 2020).

Despite the spatial differences, the temporal variability of contaminants and toxicological results showed the dominant role contaminants have in shaping the structures of bacterial communities in sediments on west coast of South Korea. When integrated results from chemical, toxicological, and microbiological data were plotted with PCoA (Figure 4.6c), the results showed that 59.9% of the variability in the composition of the bacterial assemblages in sediments could be explained by the first two principal component axes (PCoA1 and PCoA2) (Figure 4.6c). Bacterial phyla were divided into two groups in the distribution by the PCoA diagram (years 2010–2011 and years 2013–2014). Distributions of Firmicutes were correlated with profiles of contaminants/toxicological measured during 2013–14, whereas Verrucomicrobia were greatly correlated with 2010–11 samples, which corresponds to the above results. Observed correlations cannot be constructed to indicate

causation of either the effects of factors or dissimilarities between individual sample points, but they do provide insight into where additional work can be focused to determine causation (Clarke and Gorley, 2006).

Due to heterogeneity of estuarine and coastal environments, sediment characteristics and rate of discharge of the river, which correlated most strongly with bacterial communities (Crump and Hobbie, 2005), can vary slightly from year to year, even if the same sites are monitored in the same season each year. However, according to the previous study, TOC and TN did not show a large difference over the years (Kim et al., 2020), the effect of changes of PTSs is more effective than that from the differences in sediment properties in terms of spatiotemporal heterogeneity of samples. Due to the inherent heterogeneity of sediments in estuaries and coastal environments, sediment characteristics and river-flow rates, which correlated most strongly with bacterial community compositions (Crump and Hobbie, 2005), can vary slightly from year to year, even at the same in the same season each year. However, according to a recent study by Kim et al. (2020), the values of TOC and TN do not show significant differences among years and changes over time in coastal sediments are due more to differences in spatiotemporal heterogeneity of PTSs than to differences in the heterogeneity of sediment properties.

4.3.3. Temporal variation in the bacterial communities at the class and family taxonomic levels

Analysis of data presented here at the class and family taxonomic levels provided deeper understanding of factors related to the composition of the dominant fraction of bacterial communities in sediments. For all sampled years, the relative abundances of the top five classes at each site were presented in Figure 4.7a. Although the four most frequently occurring classes were Gammaproteobacteria, followed by Anaerolineae, Alphaproteobacteria, and Deltaproteobacteria, the highest relative abundances for all sampled years were Bacilli (24.1%), followed by Gammaproteobacteria (17.5%), Acidimicrobiia (13.6%), and Anaerolineae (13.3%) (Figure 4.7a). This result is consistent with several previous studies that characterized microbial communities in sediments (Hullar et al., 2006; Crump and Hobbie, 2005). Although the relative abundance of Gammaproteobacteia was highest in 2013, with no significant difference among sampled years and steadily dominant. However, at the family taxonomic level, OM60, Halomonadaceae, Piscirickettsiaceae, and Thiotrichaceae were significantly different relative to abundance among sampled years.

Among these four families, only Halomonadaceae abundance increased over time, while OM60, Piscirickettsiaceae, and Thiotrichaceae decreased over time. A study by Feris et al. (2003) suggested that the presence of Gammaproteobacteria in sediments is positively associated with heavy metal concentrations, whereas Gaboyer et al. (2014) suggested that Halomonadaceae may be tolerant to metals such as Cd, Cr, Cu, and Ag. Among the environmental factors, the concentrations of heavy metals (Cd, Cr, and Hg) increased in sediments over time, especially Cd (significantly increased) and Halomonadaceae, which is tolerant of Cd have become more abundant.

Classes Bacilli and Clostridia in the Phylum Firmicutes exhibited significantly different abundances by year (Figure 4.7b). Within the Phylum Verrucomicrobia, the dominant classes were Verrucomicrobiae and Verruco-5, followed by Spartobacteria and Pedosphaerae (Figure 4.7b). Opitutae and Methylacidiphilae were present but occurred at relatively lower abundances than the other classes (Figure 4.7b).

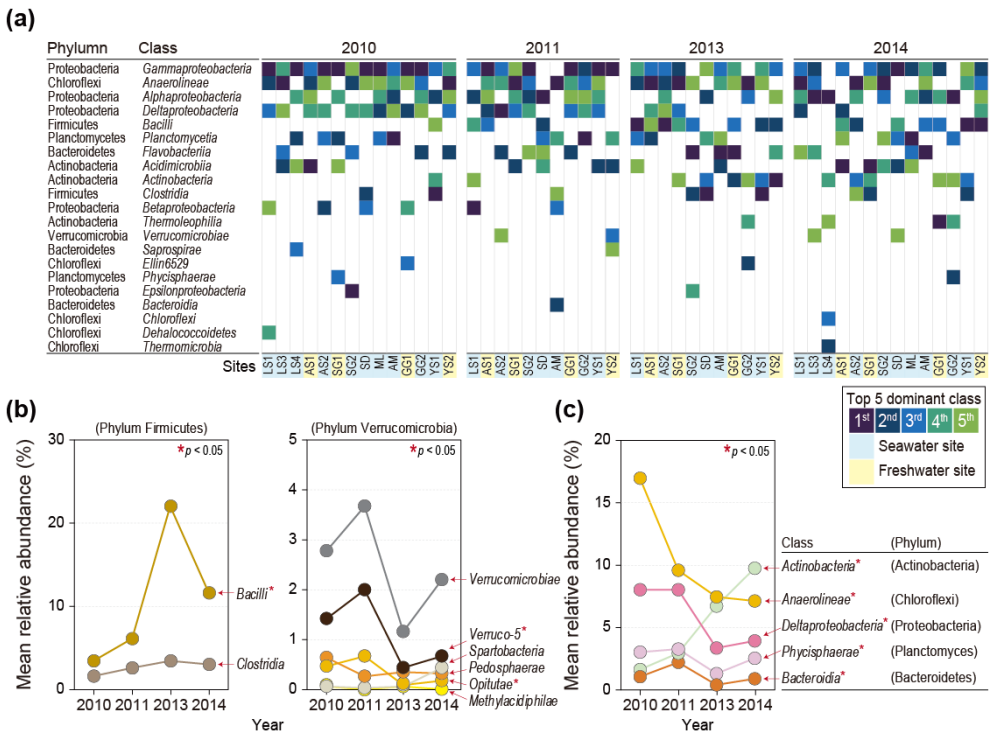


Figure 4.7.

Structures of bacterial communities (at class taxonomic level) in sediments along the west coast of South Korea. Panels: (a) the five most abundant bacteria at each site were marked for all sampled years, (b) mean relative abundance of bacteria (at the class taxonomic level) that showed differences at phylum taxonomic level, (c) mean relative abundance of bacteria (at the class taxonomic level) that showed no difference at the phylum taxonomic level. The Kruskal-Wallis test, followed by a Bonferroni correction, was performed on data in by panels (b)–(c). Significance was determined at $p < 0.05$ (*).

According to previous studies and consistent with our results, Opitutae is significantly more frequent in the water column, whereas Verrucomicrobiae is more common in marine sediments and to some extent in lakes (Allgaier and Grossart, 2006; Arnds et al., 2010; Freitas et al., 2012). Among the classes of bacteria that exhibited a significant difference in abundance by year, the relative abundances of Bacilli and Actinobacteria showed relatively increasing pattern over time, while abundances of Anaerolineae, Deltaproteobacteria, Phycisphaera, Verruco-5, and Bacterodia showed relatively decreasing pattern over time (Figure 4.7b and 4.7c).

Desulfobacteraceae and Syntrophobacteraceae, which include Deltaproteobacteia, were significantly different in abundance between year 2010 and 2014 ($p < 0.05$). The relative abundance of these two bacterial communities is shown to decrease, which was consistent with the temporal pattern in PAHs concentrations. In previous studies, Deltaproteobacteia has been mostly observed in sites highly contaminated by PAHs and is crucial in the anaerobic degradation of organic contaminants and the cycling of sulfur compounds (Sun et al., 2013; Quero et al., 2015).

Overall, in this study, four families of bacteria (Anaerolinaceae, Desulfobacteraceae, Piscirickettsiaceae, and Spirochaetaceae) were more concentrated in sediments during the 2010–11 period that in the 2013–14 period (Table 4.2). Anaerolinaceae, Desulfobacteraceae, and Piscirickettsiaceae have been found to be positively associated with residual oils in sediments, which indicates relatively great concentrations of PAHs being associated (Xie et al., 2018). Furthermore, presence of Anaerolinaceae and Desulfobacteraceae indicates the biodegradation of petroleum hydrocarbons (Xie et al., 2018). In the presence of PTSs, these two bacterial families might metabolize PAHs, APs, and metals, resulting in the possible removal of these chemical species from sediments (Chariton et al., 2010; Zhang et al., 2017; Genderjahn et al., 2018; Lee et al., 2019).

Table 4.2.

The mean relative abundances of the bacterial communities at family level which showed significant difference by sampling years in sediments along the west coast of South Korea.

Phylum	Class	Order	Family	Relative abundance (%)				Bonferroni-corrected *p* value					
				2010	2011	2013	2014	10–11	10–13	10–14	11–13	11–14	13–14
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	0.08	0.58	0.28	0.20		0.00	0.02	0.02	0.05	
			Frankiaceae	0.07	0.08	0.14	0.50						
			Geodermatophilaceae	0.02	0.02	0.06	0.46		0.03				
			Microbacteriaceae	0.12	0.09	0.48	0.84				0.02	0.01	
			Micrococcaceae	0.03	0.08	0.91	0.24		0.01		0.01		
			Micromonosporaceae	0.06	0.07	0.23	1.59					0.04	
			Mycobacteriaceae	0.36	0.82	1.05	0.80				0.02		
			Nocardioidaceae	0.05	0.10	0.30	0.22						
			Sporichthyaceae	0.01	0.03	0.04	0.62						
			Streptomycetaceae	0.03	0.04	0.19	0.22	0.02	0.00	0.01	0.01		
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	1.03	0.68	0.44	0.30			0.03			
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	0.04	0.03	0.10	0.43			0.05		0.04	
			Bacillaceae	0.83	1.41	3.95	4.62		0.01	0.00	0.04	0.01	
			Exiguobacteraceae	0.02	0.03	1.02	0.07		0.00	0.01	0.00		
			Paenibacillaceae	0.05	0.02	0.14	0.55			0.05		0.01	
			Planococcaceae	0.95	1.79	7.53	2.68		0.00	0.02	0.00		
		Lactobacillales	Carnobacteriaceae	0.01	0.02	2.95	0.09		0.00		0.00		
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	0.06	0.15	0.37	0.22		0.05		0.04		
			Isosphaeraceae	0.03	0.08	0.12	0.55			0.02			
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	0.03	0.13	0.15	0.34		0.02	0.00			
	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	2.11	1.74	0.59	0.84			0.04			
		Syntrophobacterales	Syntrophobacteraceae	1.04	0.70	0.31	0.31			0.03			
	Gammaproteobacteria	Alteromonadales	OM60	0.69	0.54	0.17	0.16			0.01			
		Oceanospirillales	Halomonadaceae	3.88	4.66	15.88	8.67		0.01		0.02		
		Thiotrichales	Piscirickettsiaceae	2.27	2.38	0.62	0.86			0.05			
			Thiotrichaceae	0.41	0.08	0.12	0.07			0.03			
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	0.71	0.62	0.16	0.26		0.05	0.04			

4.3.4. Correlation between bacterial communities and environmental variables

Significant Spearman's rank correlation between the relative abundance of bacterial individual taxa and the concentration/value of environmental variables facilitated our detection of potential bioindicators of environmental contaminants. Among the measured environmental variables, each bacterial abundance based on phyla were more significantly related to concentrations of Σ PAHs, Σ APs, and heavy metals (i.e., Cd and Hg) than were AhR- and ER-mediated potencies (Figure 4.8a). The Σ PAHs negatively correlated with the abundance of Planctomycetes. Abundance of Firmicutes was positively correlated with concentrations of Cd, whereas abundances of Planctomycetes and Verrucomicrobia were negatively correlated with concentrations of Cd. In addition, abundances of Chloroflexi were positively correlated with concentrations of Hg, but negatively correlated with abundances of Planctomycetes.

Overall, metals have greater influences on shaping structures of microbial communities in sediments than do other variables measured in this study. Responses to changes in concentrations of metals can reduce abundances of less metal-tolerant species in sediments and thus allow other, more-tolerant species to dominate, which in turn reduces biodiversity. In particular, adverse effects on biota of high concentrations of some metals are due to their abilities to block and inactivate sulfhydryl groups of proteins (Valls and de Lorenzo, 2002). Otherwise, non-linear correlations showed between abundant phyla Actinobacteria and Proteobacteria and environmental variables. Because these two phyla were the most abundant in the sediments, the variance at lower bacterial levels is offset, making it difficult to determine relationships between bacterial abundances and environmental variables.

The effect of Cd on bacteria was statistically discernible at both the phylum and class taxonomic levels, whereas concentrations of APs and % $E2_{max}$, which indicate the presence of estrogenic compounds, were only discernible at the phylum and class taxonomic levels, respectively. The abundances of various bacterial taxa were associated with ER-mediated potencies, whereas associations were not observed relative to AhR-mediated potencies.

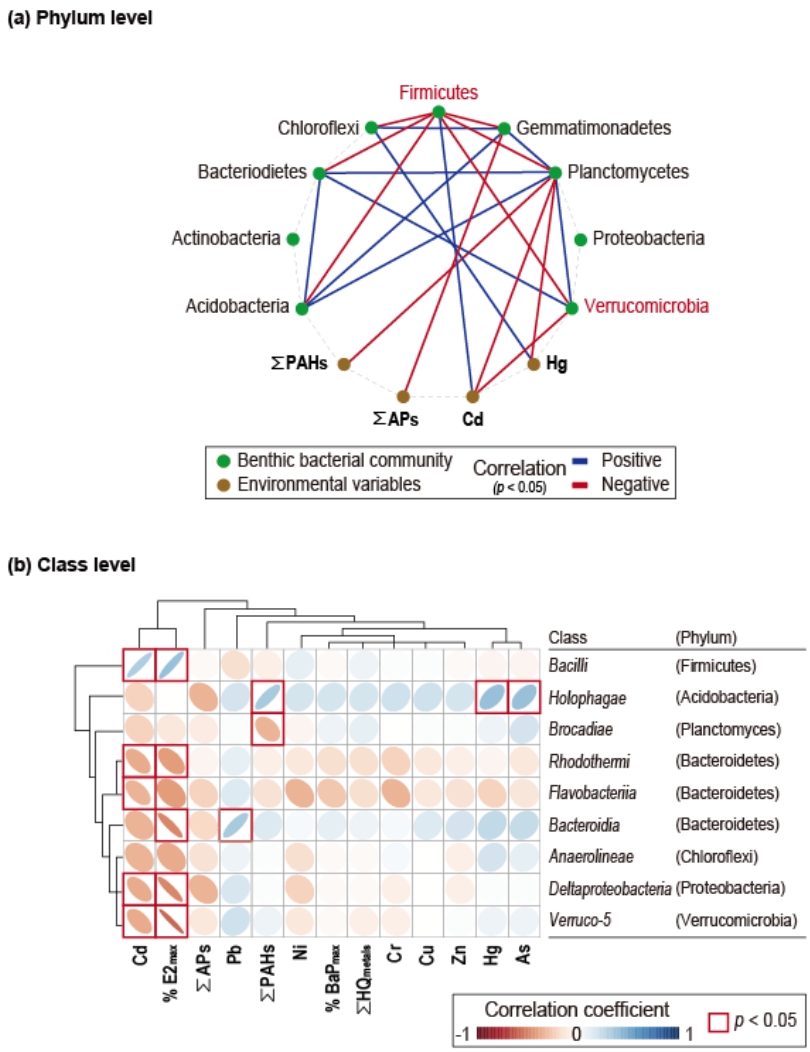


Figure 4.8.

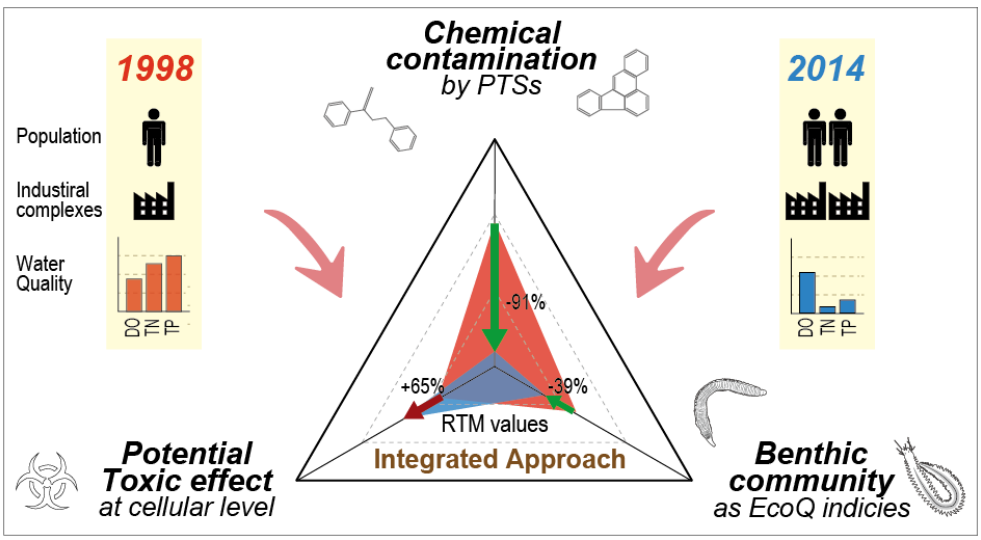
Correlations and classifications of bacterial assemblages in sediments along the west coast of South Korea at phyla and class taxonomic levels relative to tested environmental variables. Panels: (a) correlation results between environmental variables and bacterial communities of phyla taxonomic levels within the depicted phyla (two phyla that were significantly different among years are depicted in red), (b) pairwise comparisons of environmental variables with a color gradient denoting Spearman's rank correlation coefficient with bacterial community structure based on taxonomic classes. The network of panel (a) was filtered to include only a 'two-tailed' $p < 0.05$.

These results are consistent with those of previous studies (Xie et al., 2017). The phyla Acidobacteria and Planctomycetes both showed a positive correlation with Σ PAHs concentrations, whereas at the class taxonomic level, Holophagae (Phylum Acidobacteria) and Brocadiae (Phylum Planctomycetes) exhibited a negative association with Σ PAHs concentrations. Like metals, Σ PAHs can shape the structure of bacterial communities by increasing the relative abundance of Holophagae in a bacterial community. Thus, the relative abundances of Holophagae and Brocadiae could be used as potential indicators of the concentrations of PAHs in sediments.

Patterns of response to contaminants varied among bacterial classes. Bacilli with their ability to reduce soluble and amorphous ferric iron and other oxidized metal species were resistant to Cd.

CHAPTER 5.

INTEGRATED ASSESSMENT OF PERSISTENT TOXIC SUBSTANCES IN SEDIMENTS FROM MASAN BAY, SOUTH KOREA: COMPARISON BETWEEN 1998 AND 2014



This chapter has been published in Environmental Pollution.

Lee, J., Hong, S., Kwon, B.-O., Cha, S. A., Jeong, H.-D., Chang, W. K., Ryu, J., Giesy, J. P., Khim, J. S. Integrated assessment of persistent toxic substances in sediments from Masan Bay, South Korea: Comparison between 1998 and 2014. *Environ. Pollut.* 2018, **238**, 317–325. https://doi.org/10.1016/j.envpol.2018.02.064

5.1. Introduction

Integrated evaluations of benthic community structure together with chemistry and tests of toxic potencies of sediments can provide a more comprehensive assessment of PTS-induced contamination than the integration of only chemistry and toxicity data (McPherson et al., 2008). An adaptation of the ratio-to-reference (Long and Chapman, 1985) and ratio-to-maximum values (DelValls and Chapman, 1998) methods known as the ratio-to-mean (RTM) values method can be used to combine the chemical, toxicological, and ecological data (Chapman, 1990). The RTM values method is appreciated for its simplicity and visual presentation of data, despite simplification and loss of detail during reduction of data into a single index. The RTM values method is also useful for time-series monitoring, particularly in terms of enabling changes to be summarized by time and location (Cesar et al., 2009).

Masan Bay, located on the southern coast of Korea, is a semi-enclosed bay with restricted water exchange (Figure 5.1). In the 1970s, large amounts of contaminants from nearby industrial complexes were discharged without appropriate treatment into Masan Bay. Thus, Masan Bay has been identified as a hot spot for coastal pollution from loading of land-driven pollutants. A doubling of the human population in the region from the 1990s to 2014 has further contributed to the pollution of Masan Bay (Figure 5.1). With the goal of improving local water quality, in 1982, the South Korean government designated Masan Bay as a special management area, dredged out contaminated sediments, and implemented the Total Pollution Load Management System (TPLMS) in 2007 (Figure 5.1) (Lee et al., 2016). As a result, water quality of Masan Bay has improved, but studies continue to report severe contamination with metals and PTSs, including polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and polychlorinated biphenyls (Hong et al., 2009; Yim et al., 2014; Lee et al., 2016). In particular, PAHs and APs have long been identified as prominent contaminants in Masan Bay and considered as priority PTSs in coastal sediments to cause adverse effects on marine benthic organisms (Neff, 2002; Khim and Hong, 2014; Lee et al., 2016).

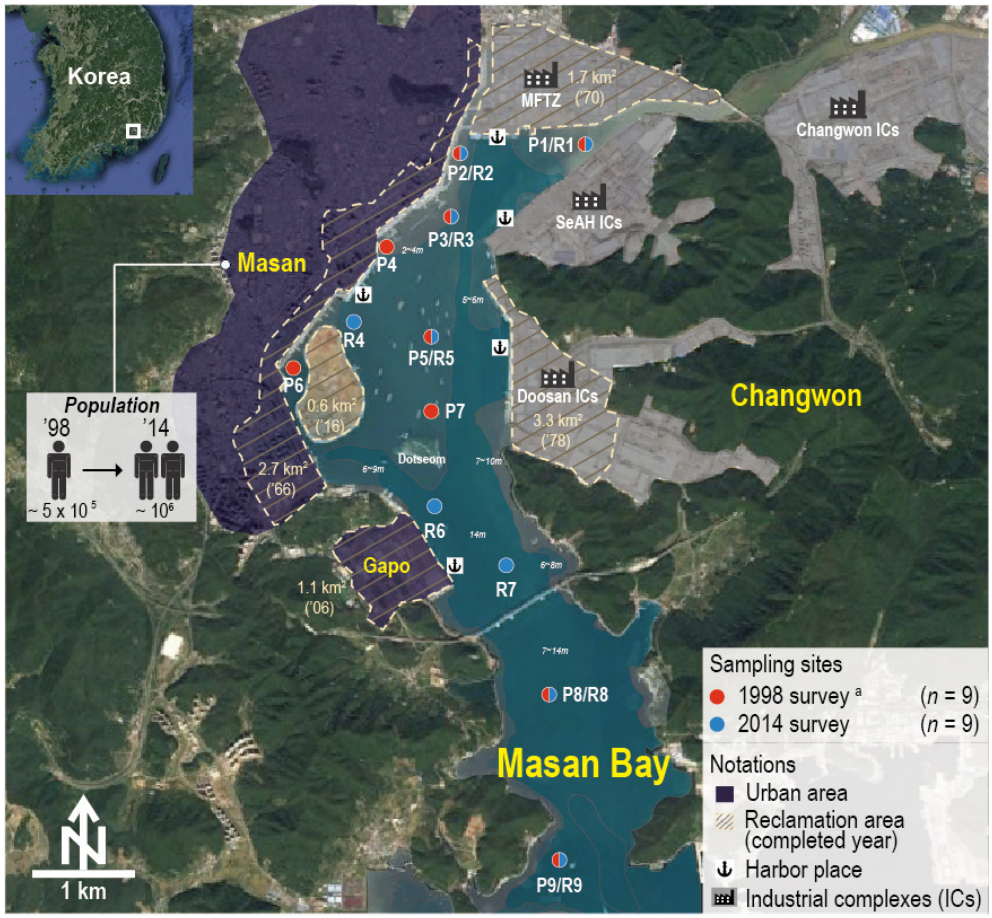


Figure 5.1.
Map showing locations from which sediments were collected in the inner- (P1–P4) and outer- (P5–P9) region sediment sampling sites in 1998 and the inner- (R1–R3) and outer- (R4–R9) region sediment sampling sites in 2014 ([a] Data from Khim et al., 1999a for chemical contamination, Khim et al., 1999b for toxic effects, and Ryu et al., 2016 for benthic community quality).

Prior studies of contamination of sediments by PTSs in Masan Bay have been focused on the diagnosis of causes of toxicity and monitoring of status and trends (Tables 5.1–5.3). Although there is some evidence of significant reductions in concentrations of PTS in sediments from Masan Bay (Jin et al., 2016), there has yet to be a comprehensive report on emerging PTSs and their potential adverse effects on organisms. For example, styrene oligomers (SOs) are emerging pollutants resulting from plastic degradation in marine environments and the usage of plastics has been increasing, bringing attention to SO pollutants (GBI, 2012). SOs have been reported to cause adverse effects on aquatic organisms (Tatarazako et al., 2002), while their occurrence and distribution in coastal sediments are seldom documented. In Chapter 2, the results demonstrated that SOs were widely distributed in sediments of coastal environments, with relatively great concentrations that comparable to PAHs (Hong et al., 2016). Accordingly, the historical occurrence and distributions of these chemicals remain question and would be of significant concern.

The purpose of the present study was to assess ecotoxicological effects of PTSs in sediments from Masan Bay by use of an integrated approach, combining chemical analyses, *in vitro* bioassays, and *in situ* investigations of benthic communities. Specific aims were to: (1) investigate spatio-temporal changes in classic PTSs, namely PAHs and APs, and emerging chemicals, namely SOs, in the sediments from Masan Bay; (2) evaluate the aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)-mediated potencies associated with sediment extracts; (3) assess the risk for the benthic community being associated with residual contamination in the Masan Bay; and (4) seek common trends in integrated sediment assessment data over a recent 16-year period.

Table 5.1.

Summary of reported PAH, AP, and SO concentrations in Masan Bay sediments.

Region	Sampling year	# of sites	Target chemicals	Concentrations (ng g^{-1} dm)				References
				Min.	Max.	Mean	Median	
In land	2000	8	16 PAHs	33.4	481	216	157	Koh et al., 2005
	2000	8	NP	84.7	1070	395	315	Koh et al., 2005
Bay	1997	20	16 PAHs	207	2670	680	[a] -	Yim et al., 2005
	1998	28	16 PAHs	41.5	1100	354	312	Khim et al., 1999a
	1998	28	NP+4-*t*-OP	122	4070	527	346	Khim et al., 1999a
	1998	7	10 SOs	3110	10200	4940	3810	***This study***
	2000	1	16 PAHs	-	-	55.4	-	Moon et al., 2001
	2004	18	NPs	131	2810	581	421	Li et al., 2008
	2005	20	16 PAHs	123	1670	928	-	Moon et al., 2008
	2005	20	NP	39.7	1208	411	-	Moon et al., 2008
	2006	5	NP	24	504	248	206	Hong et al., 2009
	2006-2007	5	NPs	49.4	124	71.1	63.0	Choi et al., 2009
	2010	9	NP	28.4	589	245	157	Al-Odaini et al., 2015
	2012	21	16 PAHs	171	707	309	292	Jung et al., 2012
	2012	21	NPs	142	2190	574	483	Jung et al., 2012
	2013	20	16 PAHs	47.9	151	83.7	-	Jin et al., 2016
	2014	29	16 PAHs	-	-	175	-	Yim et al., 2014
	2014	7	16 PAHs	58.1	191	90.7	80.2	***This study***
	2014	7	APs	17.8	71.7	39.3	32.9	***This study***
	2014	7	10 SOs	73.1	353	128	100	***This study***

[a] No data.

Table 5.2.

Summary of reported AhR- and ER-mediated potencies in Masan Bay sediments.

Region	Sampling year	# of sites	Endpoint	AhR- or ER-mediated potencies			References
				Min.	Max.	Mean	
In land	2000	8	$\%TCDD_{max}$	37.8	93.5	64.6	Koh et al., 2005
		8	$\%E2_{max}$	9.3	113	44.9	Koh et al., 2005
	2003	15	$\%TCDD_{max}$	0.1	93	35	Yoo et al., 2006
Bay	1998	9	$\%TCDD_{max}$	39.4	2080	594	Khim et al., 1999b
		9	$\%E2_{max}$	51.4	221	99.7	Khim et al., 1999b
	2014	7	TCDD-EQ	49.8	161	75.1	***This study***
		7	E2-EQ	486	17000	3880	***This study***

Table 5.3.

Summary of reported temporal occurrence of macrobenthos species found in Masan Bay.

Phylum	Species	Year					
		1980–1981	1987–1990	1998	2004	2010–2012	2014
Annelida	*Paraprionospio patiens*	√	√	√	√	√	√
	Nectoneanthes latipoda	√					
	Chone sp.	√		√			
	Nephtys sp.	√					
	Capitella capitata		√		√	√	√
	Chaetozone setosa		√	√	√		
	Lumbrineris longifolia		√	√	√	√	√
	Cirratulus cirratus			√			
	Glycinde sp.			√			
	Sigambra tentaculata			√			
	Tharyx sp.			√	√		√
	Euchone analis				√		
	Glycera chirori				√		
	Heteromastus filiformis				√	√	√
	Prionospio cirrifera				√		
	Magelona japonica						√
	Polydora ligni						√
	Spiochaetopterus koreana						√
	Sternaspis scutata						√
Mollusca	*Theora lata*						√
	Raetellops pulchella						
	Macoma tokyoensis						
Amphipoda	*Corophium sp.*						
Urochordata	*Ciona intestinalis*						
Reference		a	b	c	d	e	f

[a] Hong and Lee., 1983, [b] Lim and Hong., 1997, [c] Paik and Yun., 2000; Lim et al., 2007; Ryu et al., 2016, [d] Choi et al., 2005, [e] KORDI., 2010; Seo et al., 2015, [f] ***This study***.

5.2. Materials and Methods

5.2.1. Sampling and sample preparation

Two surveys of quality of sediments in Masan Bay were conducted 16 years apart; sediment samples were collected in May 1998 and in May 2014 at almost the same locations (Figure 5.1). At each time point, samples were collected from nine, including seven benthic community sites, termed P1–P7 and R1–R7, respectively. These sites represent both inner (P1–P4 and R1–R3; average water depth, ≤ 5 m) and outer (P5–P9 and R4–R9; average water depth, ≥ 5 m) regions of the bay.

Results of analysis to identify and quantify chemicals (except for SOs), *in vitro* bioassay data, and benthic community data from 1998 were obtained from previous studies (Khim et al., 1999a and 1999b; Ryu et al., 2016). Although sampling sites were not exactly the same and bay dynamics can shift sediment and associated contaminants over time, it is useful to evaluate these data with respect to changes in chemical concentrations over time. To address historical occurrences of SOs as well as long-term changes in concentrations of SOs in this study, archived samples from 1998 were re-analyzed for SOs levels together with samples collected in 2014.

All samples were transferred immediately to the laboratory, freeze-dried, and stored at - 20 °C until analyzed. Freeze-dried samples of 1988 were kept at - 20 °C in a freezer in our laboratory for 16 years. To avoid technical and/or methodological errors in chemical analyses for the use of archived samples collected in 1998, the 1998 samples were newly extracted and analyzed together with the 2014 samples. Detailed descriptions of sample preparation for chemical and bioassay analyses have been published previously (Hong et al., 2012, 2015). In brief, 10 g of freeze-dried sediment samples were extracted by dissolving them in 350 mL of dichloromethane (Burdick & Jackson, Muskegon, MI) in a Soxhlet extractor for 16 h (see Chapter 2). To remove elemental sulfur, the extracts were treated with activated copper powder (Sigma Aldrich, Saint Louis, MO) and concentrated into 1 mL. For the *in vitro* assays, the aliquot of the extract was exchanged in dimethyl sulfoxide (DMSO, Sigma-Aldrich) using differential volatilization.

5.2.2. Instrumental analysis

Concentrations of PAHs, APs, and SOs in extracts of sediments were quantified by use of an Agilent 7890 gas chromatograph equipped with a 5975C mass-selective detector (MSD, Agilent Technologies, Santa Clara, CA); instrument settings used for the detection of PAHs, APs, and SOs are provided in Chapter 2. A total of 16 PAHs, 6 APs, 4 styrene dimers (SDs), and 6 styrene trimers (STs) (full chemical names and abbreviations are provided in Table 2.1) were analyzed according to previously reported methods (Hong et al., 2016a) (see Chapter 2).

For source appointment of sedimentary PAHs and SOs, principal component analysis (PCA) was performed based on the concentrations of 16 PAHs and 10 SOs, respectively. PCA was performed with normalized chemical concentrations of individual chemicals. The statistical analyses were conducted in SPSS 23.0.

5.2.3. *In vitro* analyses

H4IIE-*luc* bioassays were performed to detect AhR-mediated potencies according to previously reported methods (Hong et al., 2012). H4IIE-*luc* bioassay results (expressed as mean relative luminescence units) were converted to percentages of the maximum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin response (% $TCDD_{max}$), where 300 pM TCDD was considered 100% $TCDD_{max}$ (see Chapter 2). AhR-mediated potencies were expressed as a TCDD equivalent concentration (pg TCDD-EQ g^{-1} dm) for direct comparison to instrumentally-derived TCDD equivalent concentrations (TEQs). All samples were assayed in triplicate.

An MVLN bioassay was used to evaluate ER-mediated potencies in organic extracts of the sediments (Khim et al., 1999b). Luciferase activity was determined after 72 h of exposure as described previously (Villeneuve et al., 2002). MVLN bioassay responses were converted to relative response units expressed as the percentage of the maximum response (% $E2_{max}$) observed for 1235 nM 17β-estradiol (E2). Significant responses were defined as those that were at least three times the standard deviation of the mean of the solvent controls. The E2 standard equivalent concentration (pg E2-EQ g^{-1} dm) was also calculated using the same method. All samples were assayed in triplicate (see Chapter 2).

5.2.4. Ecological quality (EcoQ) indices of macrobenthic community

Duplicate sediment samples were collected with a van Veen grab (surface area, 0.1 m^2). Of note, pooled samples were used for species identification and individual organism counting to minimize site-specific variation or possible technical errors in grab sampling. Identification of macrobenthos was performed in Anyang University. For ecological quality (EcoQ) assessment, this study employed one simple index, the Shannon-Wiener diversity index (H′), and four multivariate indices, namely the Ecological Quality Ratio (EQR), Benthic Quality Index (BQI), Azti Marine Biotic Index (AMBI), and Multivariate-AMBI (M-AMBI). More details about these multivariate indices are provided in Table 5.4 and previous publications (Blanchet et al., 2008; Ryu et al., 2016). Pearson's correlation coefficients were determined to assess potential correlations between PTS contamination and toxic effects on the benthic community. Statistical analyses were performed in SPSS 23.0.

5.2.5. Integrated approach: Ratio-to-mean values method

The RTM values method is an integrative approach wherein a data matrix is used to convert values for each variable of interest in each LOE to non-dimensional values by dividing the value obtained by the arithmetic mean obtained for all stations (Cesar et al., 2009). RTM values were combined through the calculation of a mean, thus producing a single new value for each LOE. These single new values were plotted in three-axis graphs to producing a triangular pyramid reflecting each class at each survey time point, providing a visual representation of sediment quality.

Table 5.4.

Definition of benthic community quality index levels.

Biotic indices	Abbreviation	Ecological status				
		Bad	Poor	Moderate	Good	Excellent
Ecological index						
Shannon-Wiener diversity index	H′[a]	< 1	1–2	2–3	3–4	> 4
Multivariate index						
Ecological Quality Ratio	EQR	< 0.2	0.2–0.43	0.43–0.65	0.65-0.80	> 0.80
AZTI Marine Biotic Index	AMBI	> 5.5	4.3–5.5	3.3–4.3	1.2–3.3	0–1.2
Multivariate-AMBI	M-AMBI[b]	< 0.2	0.20–0.41	0.41–0.62	0.62–0.83	> 0.83
Benthic Quality Index	BQI[c]	< 3.6	3.6–7.2	7.2–10.8	10.8–14.4	> 14.4

[a] Blanchet et al. (2008).
[b] Muxika et al. (2007).
[c] Rosenberg et al. (2004).

5.3. Results and Discussion

5.3.1. Distributions of PTSs in sediments

Mean concentrations of PAHs were 4.6×10^2 ng g^{-1} dm (range, 901.1×10^3 ng g^{-1} dm) in 1998 and 91 ng g^{-1} dm (range, 58–1.9×10^2 ng g^{-1} dm) in 2014 (Figure 5.2a), demonstrating an 80.1% reduction and a statistically significant decline ($p < 0.05$). Reductions in concentrations of PAHs in sediments from outer regions (P5–P9 and R4–R9) were significantly more pronounced than those of the inner regions (P1–P4 and R1–R3) ($p < 0.05$, Figure 5.2a). These decreasing trends seemed to be associated with South Korea's implementation of pollution control measures and management of toxic substances. For example, new environmental quality and dioxin emission standards were established in 1998, waste incinerator flue gas has been regulated since 1999, and TPLMS has been implemented since 2007 (Chang et al., 2012; Lee et al., 2016). Although these environmental regulations and chemical controls do not manage PAHs levels directly, they control land-derived pollutants, which are major causes of coastal and marine pollution. The environmental regulations and pollution controls were apparently more effective for reducing contamination of outer regions, relative to inner regions of the bay. Greater concentration of PAHs was observed at both sampling time points, presumably due to the geographical characteristics (i.e., a long, narrow inlet) limiting flushing, thereby resulting in localized PAHs sedimentation in the inner regions (Li et al., 2008).

Based on loadings of the 16 PAHs analyzed, the total variances of principal components (PCs) 1 and 2 were 67% and 16%, respectively (Figure 5.2d). PC 1 had a high positive loading for 4–6 ring PAHs [benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), BaP, indeno[*1,2,3*-cd]pyrene (IcdP), and benzo[*g,h,i*]perylene)] and was thus selected to represent vehicular emission sources (Li et al., 2015). PC 2 was heavily loaded with compounds related to coal combustion, including phenanthrene, fluoranthene, and pyrene (Li et al., 2015; Figure 5.3). The compositional profiles of PAHs were in principle similar for both sampling years but showed greater inner region variance in 1998. The predominant PAHs found in inner region sediments in 1998 were

consistent with coal combustion derivatives. Overall, industrial waste (combustion of diesel and gasoline engines) can be considered the major origin of PAHs in sediments from Masan Bay (Rogge et al., 1993).

Patterns of distribution reductions in concentrations of APs in sediments of Masan Bay were similar to those of PAHs; and mean concentrations of APs in sediment likewise decreased from 1998 (5.8×10^2 ng g^{-1} dm; range, 1.8×10^2–1.1×10^3 ng g^{-1} dm) to 2014 (39 ng g^{-1} dm; range, 18–72 ng g^{-1} dm) (Figure 5.2b). NPs were used as detergents, wetting agents, dispersing agents, and emulsifiers in various industrial, domestic, and household applications throughout the latter half of the twentieth century (Hong and Shin, 2011). The South Korean government named NPs as priority chemicals in 2001, banning their use in kitchen cleaners in 2002, and then designating them as restricted chemicals and prohibiting their use for all domestic applications in 2007. The use of NPs in paints and ink binders was banned in 2010 (MOE, 2007). Our observation which means concentrations of APs declined by 93% between 1998 and 2014 (Figure 5.2b, $p < 0.01$) indicates that these regulations appear to have been quite effective. The mean concentrations of APs declined significantly between 1998 and 2014 in both inner and outer bay regions. Direct comparison of APs data with past levels was difficult because historical alkylphenol ethoxylate data are lacking. Nonetheless, the presence of NP precursors in sediments collected from inner regions in 2014 suggests that fresh APs input to the bay is still occurring.

A dramatic reduction (97%) in mean concentrations of SOs in sediments was observed between 1998 (4.9×10^3 ng g^{-1} dm; range, 0.3×10^3–1.0×10^4 ng g^{-1} dm) and 2014 (1.3×10^2 ng g^{-1} dm; range, 73–3.5×10^2 ng g^{-1} dm) (Figure 5.2c). Concentrations of SOs in sediments from Masan Bay in the presently reported 1998 and 2014 are greater than respective mean values from similar time points (1998 mean, 2.2×10^2 ng g^{-1}; 2015 mean, 34 ng g^{-1} dm) for sediments from Lake Sihwa in South Korea (Hong et al., 2016a; Lee et al., 2017).

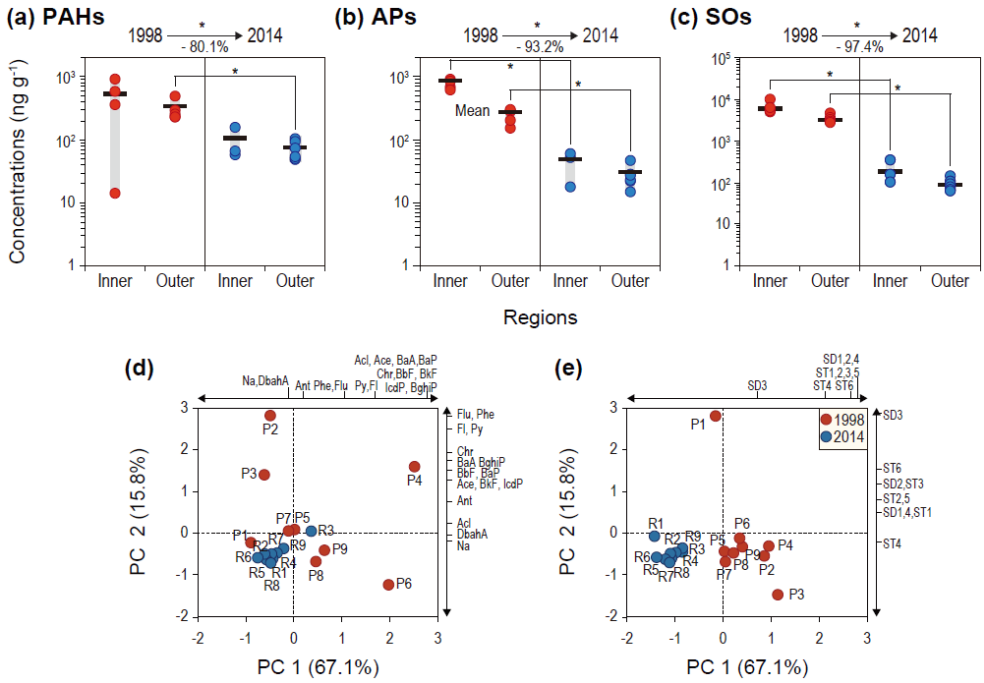


Figure 5.2.

Concentrations of (a) PAHs, (b) APs, and (c) SOs in sediment from Masan Bay in 1998 and 2014. Cluster results from PCA of (d) PAH and (e) SO concentrations in sediments collected from 18 locations in Masan Bay. Percentages of variability in each data accounted for by PC 1 and PC 2 are shown in the graph (* $p < 0.05$).

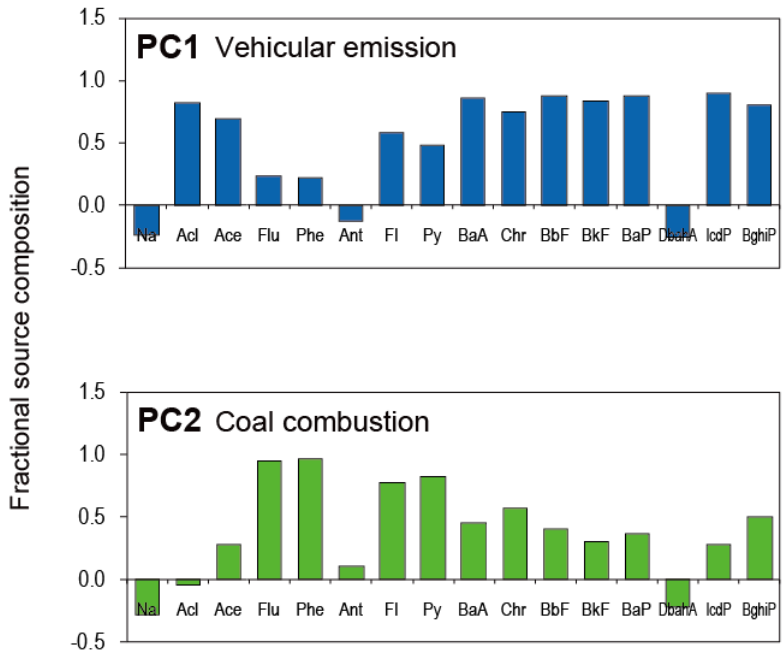


Figure 5.3.
PCA after Varimax rotation for selected PAHs in Masan Bay sediments.

Results of PCA indicated that environmental management practices, such as TPLMS, have been successful in reducing land-based pollution loads. PC 1 and PC 2 accounted for 67.1% and 15.8% of the dataset variability, respectively (Figure 5.2e), and when sites were ordinated based on PCA scores, all samples except P1 were divided into sampling years 1998 and 2014. SD3 was the predominant (70%) chemical pollutant at P1, whereas STs were the predominant pollutants at other sites (Figure 5.4). Relatively large contributions of STs might indicate recent inputs of fresh materials because ST1 emerges early in polystyrene decomposition after mechanical breakdown (Saido et al., 2014). However, our understanding of SO compound is limited because few studies have been conducted on the distributions and relative compositions of SOs in coastal marine sediments (Kwon et al., 2014). Additional complementary studies are needed to identify the origins and fates of these compounds. Furthermore, continuous pollutant monitoring is needed and regulation of new materials should be considered.

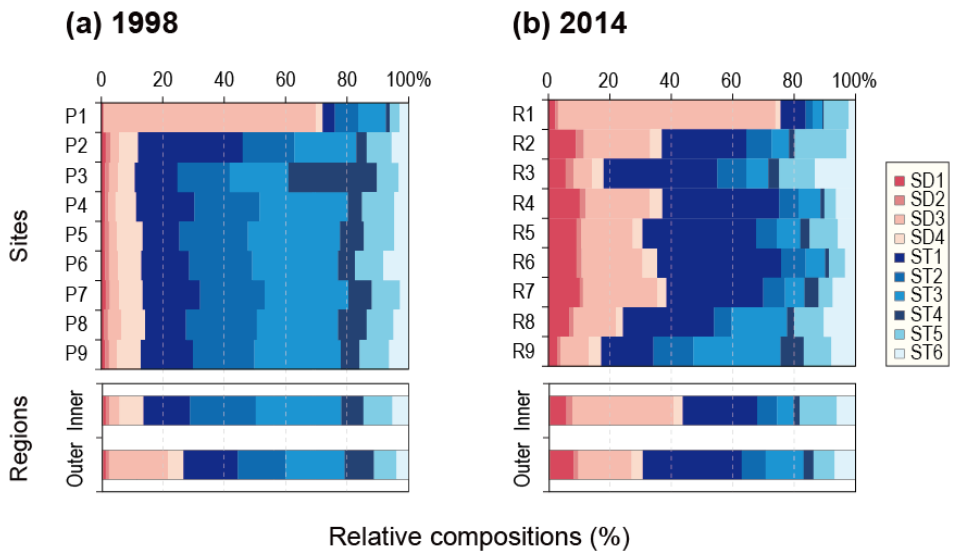


Figure 5.4.
Comparison of the relative SOs compositions (10 component SOs) at each sampling site and inner-region versus outer-region sites within Masan Bay.

5.3.2. *In vitro* potencies in sediments

Mean concentrations of TCDD-EQ in sediments of the Masan Bay decreased from 5.9×10^2 pg g^{-1} dm (range, 39–2.1×10^3 pg g^{-1} dm) in 1998 to 75 pg g^{-1} dm (range, 50–1.6×10^2 pg g^{-1} dm) in 2014 (Figure 5.5a); mean concentrations of AhR-mediated potencies in sediments also decreased significantly between 1998 and 2014 ($p < 0.05$). These TCDD-EQs exceeded both US (< 25 pg g^{-1} dm, possible-effect level; USEPA, 1993) and Canadian (< 0.85 pg g^{-1} dm; CCME, 2002) sediment quality guidelines for dioxin-like compounds at both time points. In contrast to concentration distribution patterns of PAHs, AhR-mediated potencies in 1998 were greater in sediments from outer regions than in sediments from inner regions. Except for outer region samples in 1998, AhR-mediated potencies were generally well correlated with PAHs concentrations in sediments (Figure 5.5b). These results suggested that the aforementioned actions implemented to reduce the release of chemicals (including AhR agonists) into the bay have been effective. Indeed, dioxin emissions decreased by 88% from 2001 to 2011 (MOE, 2012).

The results of a direct comparison between bioassay-derived TCDD-EQ and instrument-derived TEQ potency balance analyses conducted to identify chemical-specific contributions to total induced AhR-mediated potencies in sediments are presented in Table 5.5. Known AhR agonists such as PAHs and SOs explained only a small portion of TCDD-EQs, ~ 2.7% in 1998, and ~ 2.2% in 2014 (Table 5.5), revealing the apparent presence of possible unknown AhR agonists in sediments. For instance, several untargeted Ah-R active agonists such as dioxins and furans, some co-planar PCBs and PCNs, four- to five-ring PAHs and/or their derivatives (e.g., oxy-, nitro-, sulfur-, alkyl-, cyano-, amino-, or methylated PAHs) in sediments of Masan Bay might explain the unidentified proportion of dioxin-like activities (Khim et al., 1999a; Kannan et al., 2000; Barron et al., 2004; Horii et al., 2009; Trilecova et al., 2011).

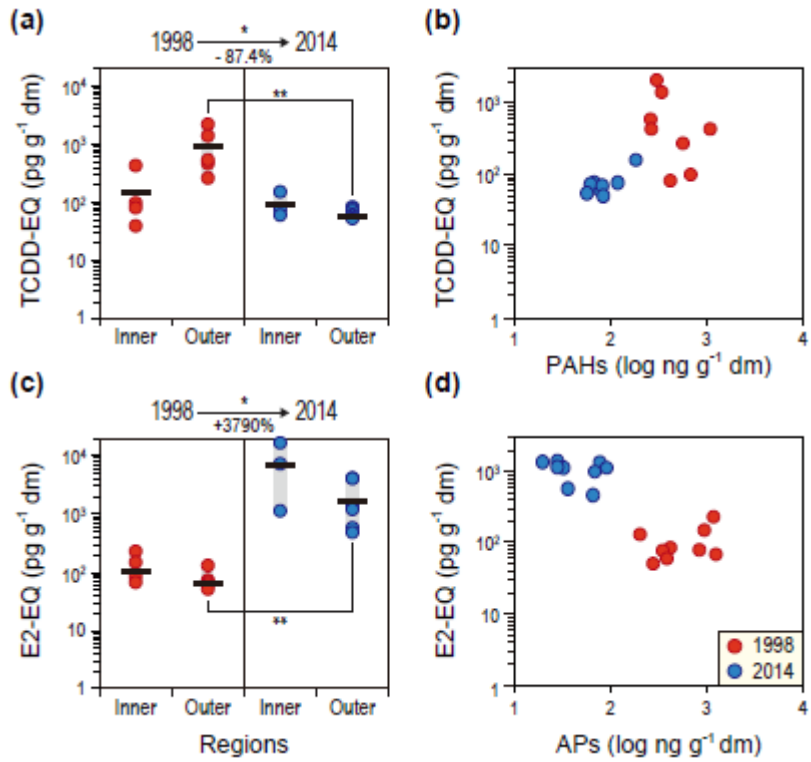


Figure 5.5.
Spatiotemporal distributions of (a) TCDD-EQ and (c) E2-EQ biological responses in inner- and outer-region Masan Bay sediment samples for 1998 and 2014. (b) and (d) Scatter plots showing dose-response relationships between chemical contaminant levels and biological responses in sediment samples collected from Masan Bay (* $p < 0.05$).

Table 5.5.

Comparison of instrument-derived equivalents and bioassay-derived equivalents in 1998 and 2014 sediment samples from Masan Bay, South Korea.

Sampling year	Region	# of sites	Instrument-derived equivalents		[a] Bioassay-derived equivalents		[b] Potency balance analysis	
			[c] TEQ	[d] EEQ	TCDD-EQ	E2-EQ	TEQ/TCDD-EQ	EEQ/E2-EQ
			Min.–Max. (Mean)		Mean		Min.–Max. (Mean)	
			(pg g^{-1} dm)		(pg g^{-1} dm)		(%)	
[e] 1998	Inner	7	0.3–5.7 (2.6)	9.8–14.8 (12.3)	159	127	0.9–2.7 (1.7)	6.1–21.9 (12.1)
	Outer	7	1.2–4.3 (2.1)	2.5–4.8 (4.0)	942	78.3	0.1–0.9 (0.4)	1.9–7.0 (5.7)
2014	Inner	7	0.6–2.9 (1.5)	0.2–0.8 (0.4)	100	8,580	0.8–1.8 (1.4)	[f] n.d.–0.1 (0.04)
	Outer	7	0.6–1.4 (0.8)	0.2–0.7 (0.4)	62.6	1,530	0.8–2.2 (1.4)	n.d.–0.1 (0.02)

[a] Bioassay-derived values obtained from sample dose-response relationships generated by testing samples at multiple dilution levels.

[b] Values are the percentages of instrument-derived values relative to bioassay-derived values.

[c] TEQ values of PAHs were summed from concentrations of BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA multiplied by the ReP values reported in Villeneuve et al. (2002).

[d] EEQ values of APs were summed from concentrations of NPs and 4-*t*-OP multiplied by the ReP values reported in Villeneuve et al. (1998).

[e] Data from Khim et al. (1999a) and (1999b).

[f] n.d.: Below detection limits.

Mean concentrations of E2-EQs in sediments were 1.0×10^2 pg g^{-1} dm (range, 51–2.2×10^2 pg g^{-1} dm) in 1998 and 3.9×10^3 pg g^{-1} dm (range, 4.9×10^2–1.7×10^4 pg g^{-1} dm) in 2014 (Figure 5.5c), evidencing an approximately 40-fold increase over about 16 years. Although concentrations of APs known to be ER agonists decreased, overall ER-mediated potencies increased between 1998 and 2014. Thus, the correlation between ER-mediated potencies and concentrations of APs showed an opposite tendency relative to AhR activity (Figure 5.5d). ER-mediated potencies expressed as instrument-derived EEQs were at 22% in 1998, but only 0.1% in 2014 (Table 5.5). These results indicated that other untargeted ER agonists might exist in sediments of Masan Bay. For example, several unmeasured chemicals such as pesticides (DDT, *o*,*p′*-DDD, and *o*,*p′*-DDE), kepone, and parabens have been reported to show ER binding affinity, which might be present in sediments of Masan Bay (Gadio et al., 1997; Legler et al., 1998; Routledge et al., 1998). In other words, APs were not a major ER agonist in Masan Bay sediments. Overall, the portion of unknown AhR agonists and ER agonists increased over the sampling interval. Unknown toxic chemicals may contribute to the total induced toxicity in coastal sediments. Thus, complementary research, such as effect-directed analysis, may lead to a better understanding of new substances and the identification of appropriate countermeasures (Hong et al., 2016b).

5.3.3. Macrobenthic community

The number of species (taxa) and species density differed between the two sampling years ($p < 0.05$, Figures 5.6 and 5.7). In 1998, a total of 14 taxa were found with a mean density of 241 ind. m^{-2}, whereas in 2014, 45 taxa were found with a mean density of 1,800 ind. m^{-2}. Few samples were collected in some sites and the range of mean densities in the inner region varied broadly in 1998 (Table 5.6). It seemed that contamination of PTSs was serious in sediments from 1998, potentially leading to deterioration of some inner-region sites. Outer regions presented greater diversity than inner regions, indicating that benthic biodiversity reflected the geographical and contamination gradients of the semi-enclosed Masan Bay system (Khim et al., 1999a; Khim and Hong, 2014). The aforementioned numbers of species observed in Masan Bay sediments were lower than values previously recorded for the southeastern coastal area (means in 1998 and 2014 were 43 and 123, respectively; MOF, 2014; Ryu et al., 2016), suggesting that the benthic community in Masan Bay had not yet fully recovered by 2014. Indeed, some polychaete species such as *Capitella capitate* and *Lumbrineris longifolia*, which are well-known opportunistic species and organic pollution indicators were dominant across our sampling sites (Tables 5.3, 5.6, and Figure 5.7) (Bae et al., 2017).

The five EcoQ indices calculated based on our benthic community data showed some improvement in ecological quality over the study time interval. The H′ index results for all sites were moderate or bad in 1998, but good or poor in 2014 (Figure 5.7c). Likewise, EQRs for the sites shifted from bad or poor in 1998 to poor or moderate in 2014 (Figure 5.7d). Among the five indices, these two indices best-reflected contamination of PTSs in sediment (Figures 5.7 and 5.8). The AMBI, by comparison, seemed to be less sensitive than the other multivariate indicators to sedimentary pollution, yielding bad to good values in 1998, where other indicators indicated poor or bad outputs (Figure 5.8). Averaging of index grading indicated that the ecological qualities of the inner and outer regions in 1998 were 4.45 (poor) and 3.87 (moderate), respectively, improving to 3.2 (moderate) and 2.54 (good), respectively, by 2014. These EcoQ assessment-based results indicated that Masan Bay sediment quality improved over time, albeit under the constraints of the

geographical setting and PTSs contamination of the bay. This conclusion is further reinforced by the increased biodiversity observed over the study time interval.

Scatter plots and Pearson correlation analyses demonstrated a multitude of significant correlations among the presently developed chemical analysis, toxic effect, and benthic community quality variables (see Table 5.7 for coefficient values and significance levels) (Figure 5.9). Notably, all of the chemical concentration values correlated significantly with EQR and BQI ($p < 0.05$, Table 5.7), suggesting that these indices of benthic community composition are responsive to chemical concentration changes. However, the results of neither of the toxicity bioassays were correlated with any of the EcoQ indices. This pattern of greater sensitivity to sedimentary toxicants in resident benthos than *in vitro* toxicity bioassays is consistent with similar analyses of US estuaries (Hyland et al., 1999). Overall, the EcoQ results were useful for demonstrating general pollution of sediment.

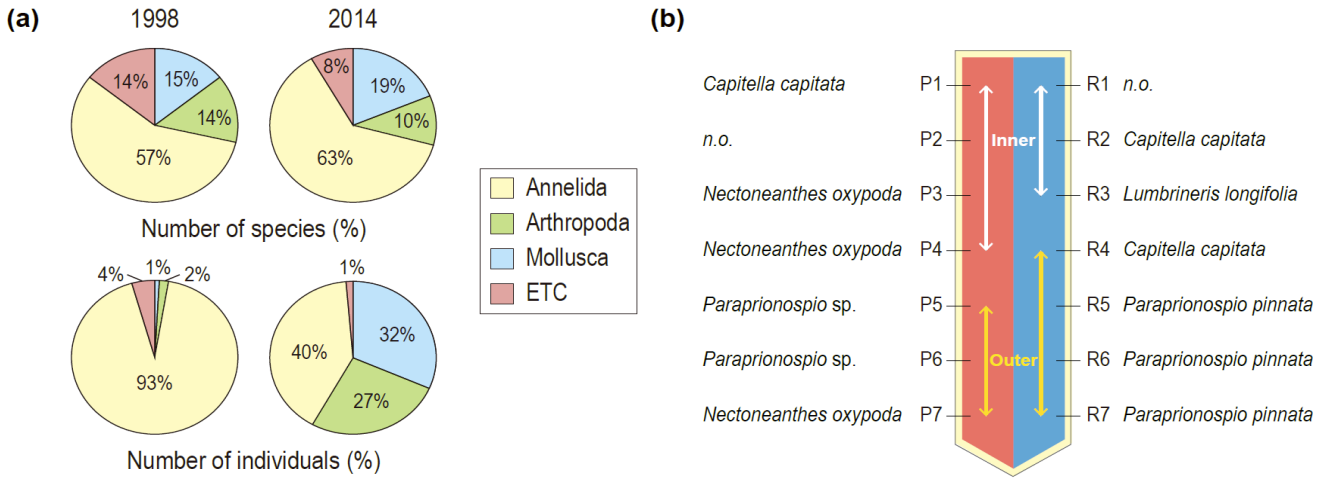


Figure 5.6.
(a) Compositions each taxon of numbers of species and individuals and (b) dominant species in Annelida of benthic communities in Masan Bay between 1998 and 2014. n.o.: Not observed.

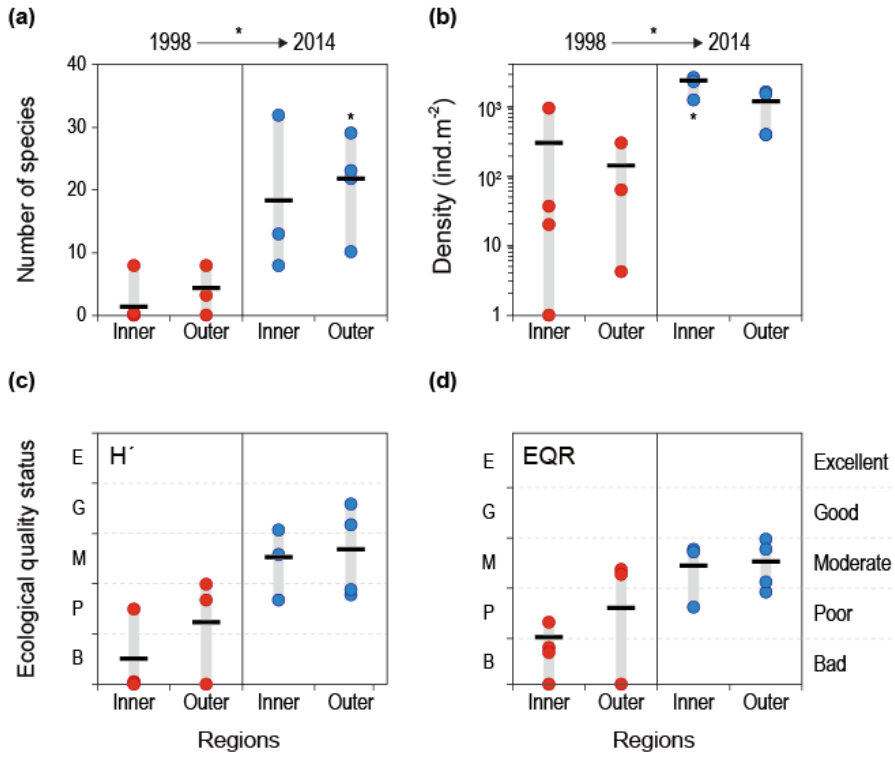


Figure 5.7.
Recovery of benthic community health in Masan Bay. Comparisons of (a) numbers of taxa (species) and (b) distributions (density, ind. m^{-2}) of benthic communities in Masan Bay between 1998 and 2014. Comparisons of EcoQ status as represented by (c) the Shannon-Wiener (H′) index and (d) the EQR in Masan Bay between 1998 and 2014 (* $p < 0.05$).

Table 5.6.
Faunal list of macrobenthos species with abundance found in Masan Bay.

Pylum	Scientific name	1998							2014						
		P1	P2	P3	P4	P5	P6	P7	R1	R2	R3	R4	R5	R6	R7
Annelida	*Amage* sp.										12			1	9
	Aricidea simplex										3		2		
	Capitella capitata	159				5		2		190	11	15			
	Chone teres													1	
	Cirratulus cirratus										1			1	1
	Dorvillea sp.									4	15	1		4	1
	Eteone sp.									1	6	1	1		1
	Euchone sp.														5
	Glycera chirori										1				1
	Glycera convoluta														3
	Glycera onomichensis										1				
	Glycinde sp.														
	Goniada sp.										3		1	1	6
	Heteromastus filiformis														1
	Lumbrineris heteropoda												1		
	Lumbrineris longifolia					1				1	152		16	10	31
	Magelona japonica														
	Neanthes succinea					1				2	1				
	Nectoneanthes multignatha									5	5	1	6	2	9
	Nectoneanthes oxypoda	12		9	5	10		8							
	Nephtys polybranchia					1					1				
	Nereis longior									1	4		2	1	6

Table 5.6.
Faunal list of macrobenthos species with abundance found in Masan Bay (continued).

Pylum	Scientific name	1998							2014						
		P1	P2	P3	P4	P5	P6	P7	R1	R2	R3	R4	R5	R6	R7
Annelida	*Ophiodromus* sp.									1	10	1	15	7	8
	Paraprionospio pinnata	1				15		3					5	11	117
	Paraprionospio sp.	7				40	1	3			2		1	1	4
	Parougia caeca	30													
	Polydora sp.													1	
	Polynoidae indet.													1	
	Prionospio elegantula									65	15	2	1	1	1
	Prionospio membranacea										1			1	29
	Scolelepis sp.										1				1
	Sigambra tentaculata									1	5		31	14	5
	Spiochaetopterus koreana									12	60	7	13		3
	Thelepus sp.												1	2	1
	indet.								2		3	1			
Sipunculida	indet.								6		4	2		2	3
Nemertina	*Lineus* sp. 1					1					3	2		2	2
Cnidaria	*Anthopleura kurogane*	14													
Mollusca	*Arcidae* indet.										1				1
	Hastula sp.								4						
	Hydatina albocincta										1	1			
	Macoma sp.								39	2	10	4	1		3
	Musculus senhausia								18		13	9		1	
	Philine orientalis	2													

Table 5.6.
Faunal list of macrobenthos species with abundance found in Masan Bay (continued).

Pylum	Scientific name	1998							2014						
		P1	P2	P3	P4	P5	P6	P7	R1	R2	R3	R4	R5	R6	R7
Mollusca	*Raetella pulchella*														1
	Ruditapes philippinarum								13						
	Scapharca broughtonii												1		
	Theora lata					1			20	12	184	276	61	18	100
Arthropoda	*Chionidae* indet.										2				
	Corophium sp.								457	7	123		3		26
	Gaprella sp.								1						
	Grandidierella sp.										13		5	12	9
	indet.												1		
Arthropoda	*Nebalia bipes*	5									4	5		1	1
	Oratosquilla oratoria	1													
	Number of species	9	0	1	1	9	1	4	9	14	33	15	20	23	30
	Total species	231	0	9	5	75	1	16	560	304	671	328	168	96	389
	Mean density (ind. m^{-2})	1155	0	45	25	375	5	80	2800	1520	3355	1640	840	480	1945

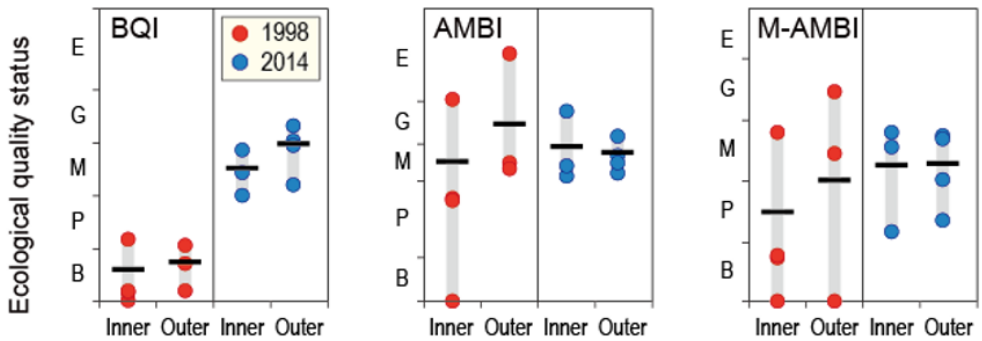


Figure 5.8.
Ecological quality (EcoQ) status as indicated by Benthic Quality Index (BQI), Azti Marine Biotic Index (AMBI), and Multivariate-AMBI (M-AMBI) indices.

Table 5.7.

Pearson correlation analysis results for associations among chemical contamination levels, toxicity bioassay outcomes, and environmental quality parameters for Masan Bay benthic macrobenthic community.

Component		Chemical			Bioassay		Macrobenthic Community						
Chemicals		PAH	APs	Sos	TCDD-EQ	E2-EQ	# of species	Density	H'	BQI	AMBI	M-AMBI	EQR
Chemical	PAH		0.54	0.48	0.17	-0.26	-0.63	-0.56	0.72	0.74	-0.05	0.74	0.77
	APs	+		0.95	-0.08	-0.38	-0.64	-0.50	0.74	0.74	0.21	0.55	0.74
	SOs		++		0.09	-0.42	-0.64	-0.50	0.70	0.75	0.32	0.39	0.65
Bioassay	TCDD-EQ					-0.16	-0.19	-0.27	0.00	0.26	-0.15	-0.36	-0.16
	E2-EQ						0.63	0.64	-0.41	-0.47	-0.07	-0.17	-0.27
Macrobenthic Community	# of species	+	+	+		+		0.82	-0.83	-0.89	-0.19	-0.58	-0.69
	Density	+				+	++		-0.58	-0.69	-0.09	-0.46	-0.55
	H'	++	++	++			++	+		0.91	0.28	0.80	0.89
	BQI	++	++	++			++	++	++		0.20	0.62	0.75
	AMBI											0.16	0.29
	M-AMBI	++	+				+		++	+			0.94
	EQR	++	++	+			++	+	++	++		++	

+ $p < 0.05$, ++ $p < 0.01$, two-tailed correlation.

Acronyms for corresponding all parameters and criteria are as in Table 5.4.

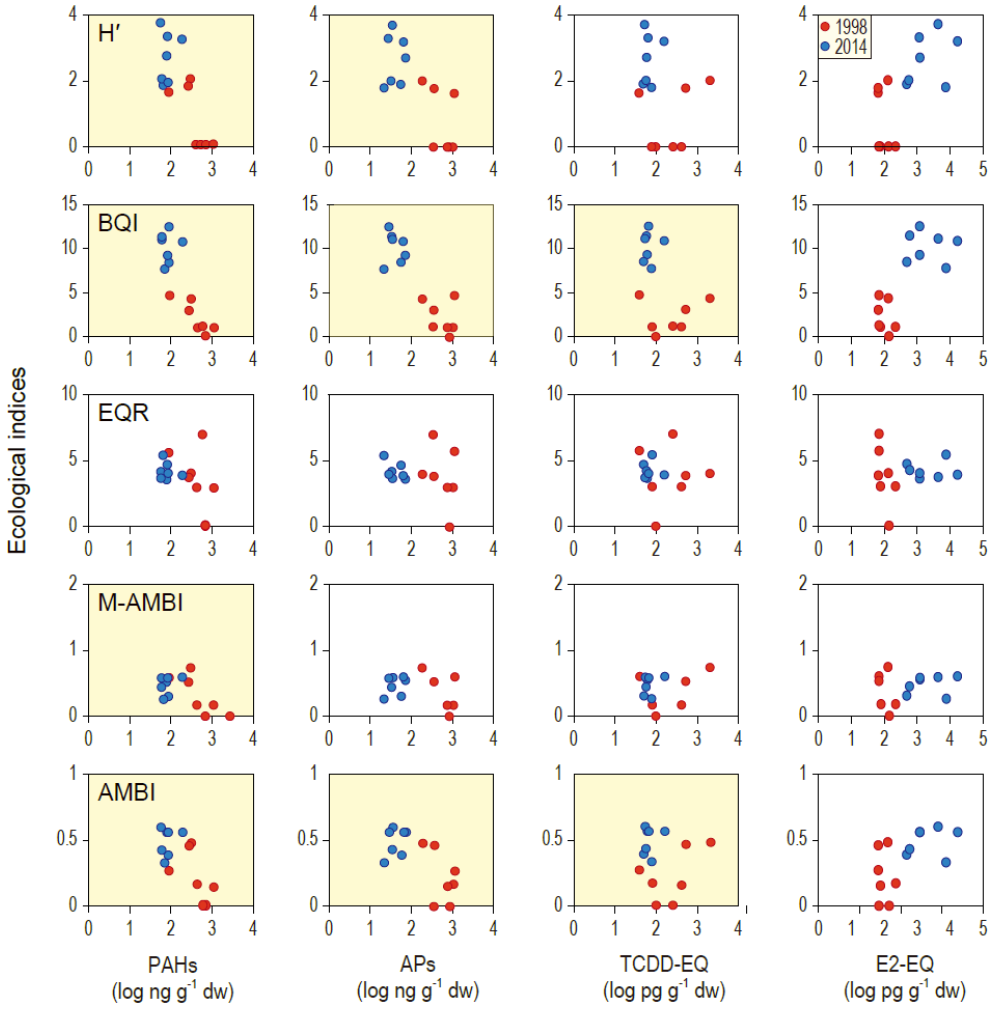


Figure 5.9.

Scatter plots of five EcoQ indices vs. concentrations of two persistent toxic substances (PAHs and APs) and AhR-, ER-mediated potencies in sediments of the Masan Bay, Korea. Yellow box indicated that $p < 0.05$.

5.3.4. Integrated approach: Ratio-to-mean values

The data obtained from chemical contamination, toxicity effect, and benthic community (EQR and BQI) data were integrated by use of the RTM values method to identify how conditions within the sampled sites changed over the study time interval. Comparing RTM values calculated for samples with respect to time (1998 vs. 2014) and region (inner vs. outer) revealed an overall decrease of RTM values from 1.34 in 1998 to 0.78 in 2014, a pronounced outer-region RTM values decrease from 1.28 in 1998 to 0.44 in 2014 ($p < 0.01$), and a no significant inner-region decrease from 1.39 in 1998 to 1.12 in 2014. It was demonstrating better and more improved conditions in the outer regions than in the inner regions. Chemical contamination, toxic potency, and benthic community index RTM values each decreased in the outer regions from 1998 to 2014; meanwhile, during the same time interval, only the chemical contamination and benthic community index RTM values decreased in the inner regions, while the inner-region toxicity RTM values rose sharply (Figure 5.8).

Presumably, because of the geographical features of the semi-closed bay, it is taking more time for sediment quality to recover in the inner regions than in the outer regions. In the inner regions, chemical contamination was the most pressing aspect of bay pollution in 1998, whereas toxic effect had emerged as a more important concern in 2014. Meanwhile, in the outer regions, the values of three-factor were of similar magnitude in 1998, whereas benthic community quality and toxic effect RTM values were of notably greater magnitude than the chemical contamination RTM values in 2014. The inner-region and outer-region chemical contamination RTM values fell dramatically from 1998 (2.59 and 1.23, respectively) to 2014 (0.22 and 0.14, respectively). The APs and SOs components of the chemical contamination RTM values showed more pronounced reductions than did the PAHs component in both the inner and outer regions (Figure 5.10), perhaps because PAHs continue to be generated and transported unceasingly through the atmosphere, whereas APs are now strictly controlled. Previous studies on PAHs and APs in sediments of Masan Bay also revealed that the RTM values exhibited a generally decreasing trend in both inner and outer regions during the last decade (Figure 5.11). Overall, these reduced

RTM values for chemical contamination reflect effective legislative actions. RTM values of toxic effect increased from 0.86 in 1998 to 1.40 in 2014, but opposite tendencies were observed for the inner versus outer regions.

The RTM values obtained for sediments from inner regions increased about eight-fold over the ~16-year period, while those from outer regions decreased by almost two thirds. RTM values for E2-EQs were increased in both inner and outer regions. However, these increasing RTM values were offset by decreasing RTM values for TCDD-EQs in outer regions. These results indicate that unknown ER agonists have been, and likely are still, accumulating more in inner regions than in outer regions and/or that there are ER agonist sources near inner-region sites. The RTM values pattern of the benthic indices was similar to that observed for our chemical analysis, although less dramatic changes in the study time interval. These results show that while chemical concentrations can be reduced rapidly, it takes more time for benthic communities to recover. Other conditions such as metal contaminations and/or hypoxia in bottom water were considered as anthropogenic pressures on the benthic community. Results of a previous study conducted in Masan Bay indicated that benthic ecological quality generally reflected the pollution gradient of metals (Ryu et al., 2016). Similar to the temporal decrease of target organic PTSs, some metal concentrations, such as Cu, Zn, and Pb, reported in sediments of Masan Bay showed a decreasing trend during the last decade (Table 5.8). Thus, in addition to the important work of regulating the use and emission of chemicals, ongoing assessments of environmental impact are also needed. Overall, RTM values of chemical contamination and benthic community were decreased while the toxicity RTM values increased, indicating that there are unmeasured chemicals or conditions with the potential to cause degradation of inner-region in Masan Bay ecology (Chapman, 1990).

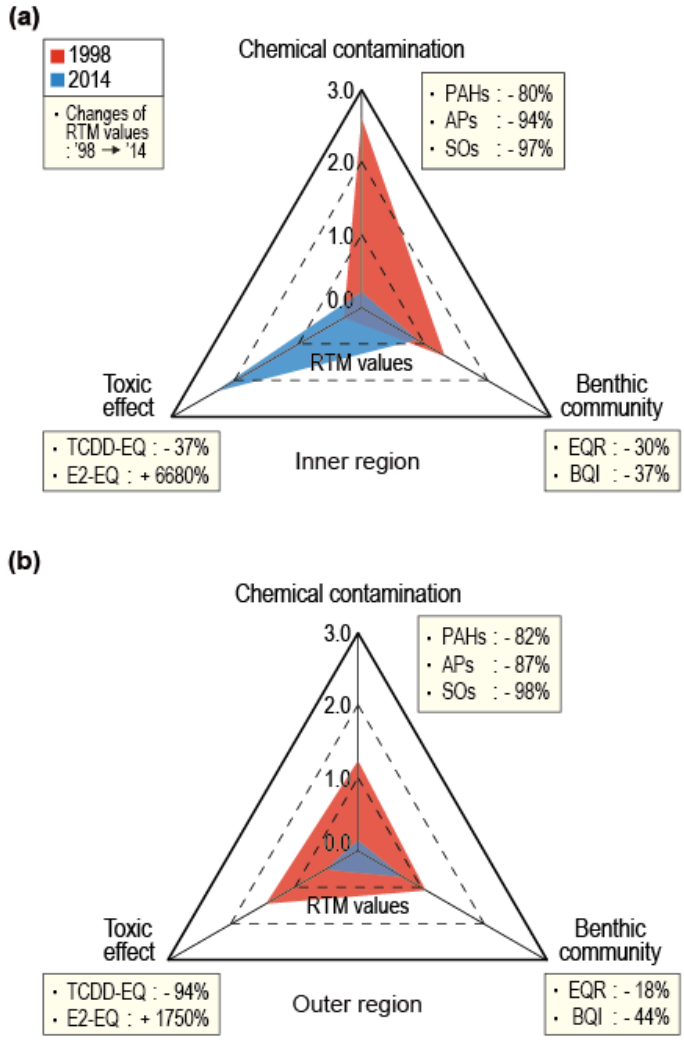


Figure 5.10.
Sediment quality triads of normalized to Ratio-to-mean (RTM) values obtained for chemical contamination, toxic effects, and benthic community quality of sediment samples from (a) inner and (b) outer regions in 1998 and 2014.

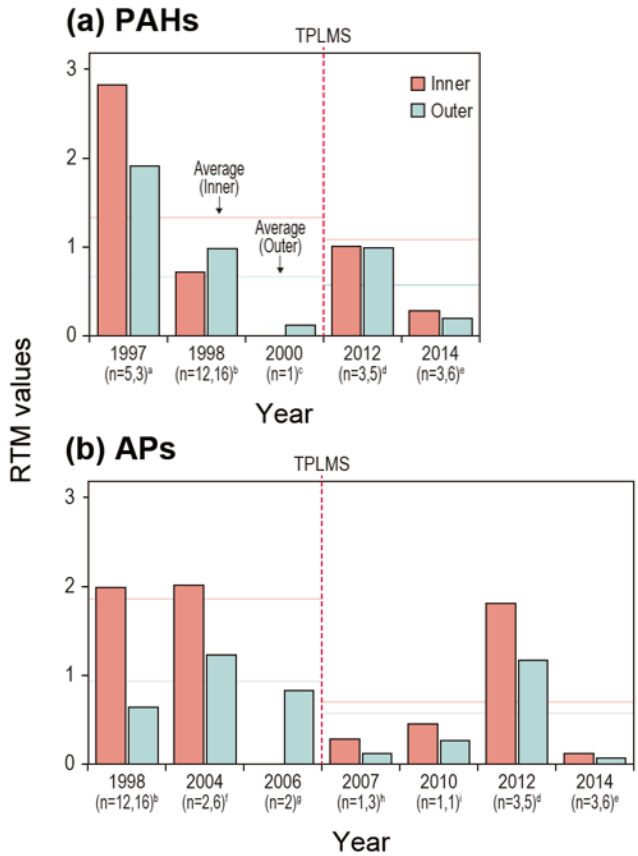


Figure 5.11.
Comparisons of normalized Ratio-to-mean (RTM) values for (a) PAHs and (b) APs in sediments of inner and outer regions of Masan Bay obtained from this study and previous studies. Data from [a] Yim et al. (2005), [b] Khim et al. (1999b), [c] Moon et al. (2001), [d] Jung et al. (2012), [e] This study, [f] Li et al. (2008), [g] Hong et al. (2009), [h] Choi et al. (2009), and [i] Al-Odaini et al. (2005).

Table 5.8.

Summary of concentrations of metals in sediments of Masan Bay reported previously.

Sampling year	Cr	Co	Ni	Cu	Zn	Pb	Hg	References
	(mg kg $^{-1}$)			(ug kg^{-1})				
1998	68.0	14.0	32.0	97.0	360.0	67.0		Ryu et al., 2016
2002	73.3			60.3	263.7	51.3		Woo et al., 2007
2005	67.1	11.5	28.8	43.4	206.3	44.0		Hyun et al., 2007
2006	79.8		29.6	75.1	314.5	66.5		Cho et al., 2015
2010		14.8	32.4	25.3	218.3	48.4	109.1	Lim et al., 2013
2010	71.4	13.4	32.5	39.4	232.0	55.4		Ra et al., 2013

CHAPTER 6.

CONCLUSIONS

6.1. Summary

In the present study, the multiple lines of evidence approach including triad approach with advanced EDA was adopted to address the ecotoxicological effects through comprehensive sediment assessments (Figure 6.1). One of the greatest challenges of present study was related to use of archived samples of sediments to assess long-term changes (or differences) in distributions of absolute and relative concentrations of target chemicals and their ecotoxicological effects. Major findings of the present study are summarized below:

In the **Chapter 2**, *in vitro* H4IIE-*luc*, MVLN bioassays and quantitative chemical analyses were performed to assess the current contamination and temporal changes in dioxin-like and estrogenic contaminants in sediments from Lake Sihwa. The results showed that: 1) sediment PAHs were most persistent toxic chemicals over 15-year period, particularly in inland, 2) sediment alkylphenols (APs) and styrene oligomers (SOs) generally declined over the past 15 years but hotspot still found, 3) SOs, recently reported emerging pollutants, were found to be significantly high in concentrations 15 years ago (Figure 6.1), and 4) *in vitro* activities were still high in sediments after 15 years, indicating continuing input of toxic chemicals.

In the **Chapter 3**, five *in vitro* and *in vivo* bioassays with total of 13 endpoints to address potential toxicological effects associated with polluted sediments. In addition, sediment mixture samples were comprehensively assessed using targeted and non-targeted full-scan screening analyses in aspects of exposure and effect characterization. The results revealed that: 1) a novel AhR agonist, namely enoxolone, was found in the environmental samples containing chemical mixture, and it presents a new relative potency value (ReP) in the H4IIE-*luc* bioassay (Figure 6.1), 2) enoxolone has a relative potency of 0.13 compared to benzo[*a*]pyrene (1.0) in the H4IIE-*luc* bioassay, 3) a sensitive novel microalgal bioassay using flow cytometry analysis was developed for assessment of potential physiological effects for fractionated samples in place of traditional algal population-based endpoint, 4) nonylphenols associated with membrane damage that influenced the viability of the microalgae were also observed, and 5) inhibitions of bioluminescence of *V. fischeri*

and lethality of *D. rerio* embryos were strongly related to nonpolar compounds (Figure 6.1).

In the **Chapter 4**, microbial community structure as determined by eDNA was used along with classic assessments of exposure, such as chemistry and *in vitro* bioassays, to evaluate overall status of the benthic community. Two perspectives were highlighted in this work, strengthening logistics of a comprehensive assessment in time (long-term; 2010–14) and space (inland vs. coastal comparison; for 5 regions). The key findings included: 1) bacterial assemblages did not greatly vary over 4 years and along the 5 coastal regions (Figure 6.1), 2) some bacterial assemblages were explained by chemical and toxicological data, 3) Planctomycetes was indicative and/or sensitive taxa to PAHs and Hg pollution, and 4) ER-mediated potency was correlated with bacterial communities at Class level.

In the **Chapter 5**, an integrated assessment was performed by, combining chemical analysis, *in vitro* bioassays, and *in situ* investigations of benthic communities, highlighting the relatively long-term changes in quality of sediments of Masan Bay. The results indicated that: 1) concentrations of target PTSs (PAHs, APs, and SOs) decreased over 16 years, 2) recently increased ER-mediated potency indicates ongoing inputs of unknown PTSs, 3) benthic community has been affected by sedimentary contamination history in Masan Bay, and 4) sediment quality of Masan Bay has been generally improved in association to reduced PTSs.

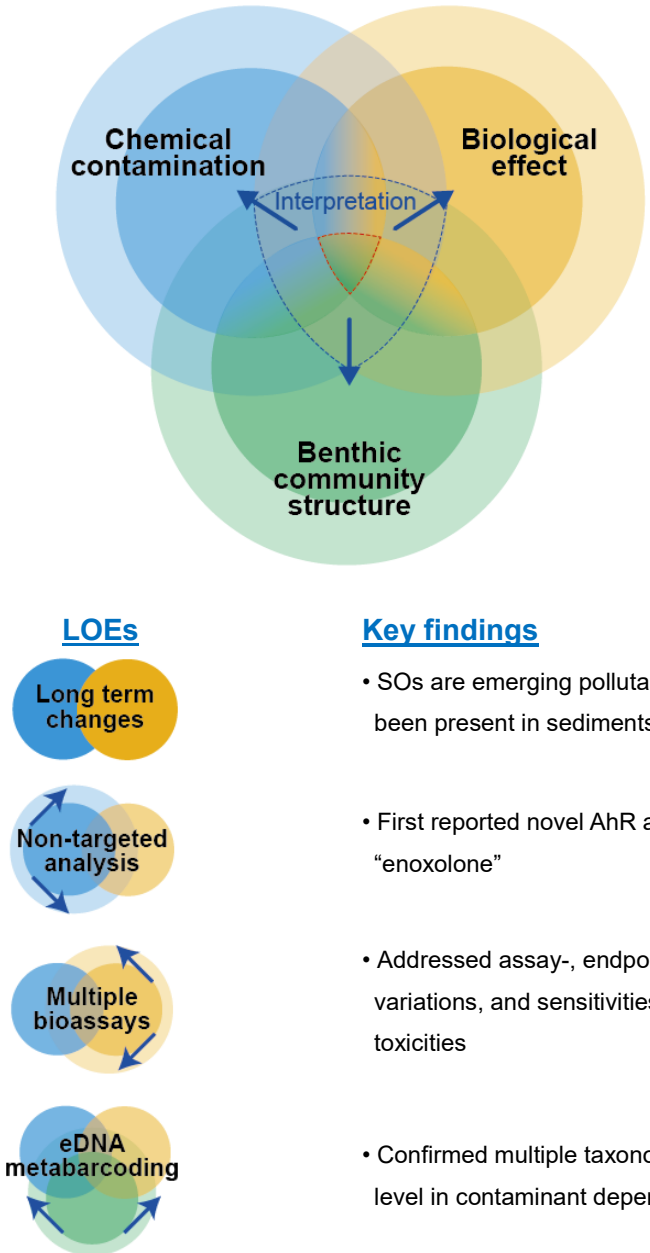


Figure 6.1.
Multiple lines of evidence (LOEs) approach for the integrated sediment assessment used in this study: for representative LOEs are presented by the selection of multiple data set from 1) chemical contamination (concentrations and composition), 2) biological effect (*in vitro* and *in vivo* bioassays), and 3) benthic community structure (*in situ* studies). Key findings for each LOE are highlighted.

To summarize the results of the above studies, sediment data from the Korean coasts were analyzed of which data collectively includes three LOEs of chemical, toxicological, and ecological measures. First, the results of chemistry data enhanced understanding of the long-term spatiotemporal differences of several PTSs of interest, including new PTSs such as SOs, which could be measured in archived samples of sediments from Lake Sihwa, Masan Bay, and west coast of Korea. Spatial distributions data indicated that presence of the multiple independent sources of PTSs, with hotspot areas (e.g., Lake Sihwa and Masan Bay) producing high pressures of certain PTSs. Temporal trends of sedimentary PTSs show decreases in recent years which seem to be associated with chemical controls and regulations.

In addition, to improve the bioassay-based strategies (with five bioassays and 13 end points involving a 2-step fractionation procedure) combined with target analysis and nontarget analyses to identify major AhR agonists. Such a multiple lines of evidence approach would enhance the accuracy in evaluation of the potential sediment toxicity in sediments. In parallel, quantification of selected PTSs was undertaken, thus allowing one to investigate the correlation between identified substances and observed biological effects. The target chemicals partly caused the effects, but mostly they were caused by a wide spectrum of untargeted substances present in the environmental samples. These results provided a better understanding of the toxicological signatures of contaminant mixtures that commonly exist in Masan Bay and elsewhere, showing different patterns of potential toxicity to the sediment raw extracts and fraction samples.

The benthic community responses given by species occurrence and diversity also reflected the type and degree of sediment contamination, however, could not be fully explained by the known target chemicals. Overall, the triad assessment of PTSs in Korean coastal sediments seemed to be useful and much powerful when all the components are fully addressed.

6.2. Environmental implications and Limitations

One of the greatest challenges of this study was related to the use of archived samples of sediments to assess long-term changes (or differences) in the distributions of absolute and relative concentrations of target PTSs. The results showed that, despite support by recent efforts from the Korean governmental pollution control, PTSs remain a major threat to the local ecosystem, especially in Sihwa Bay and Masan Bay. In particular, NP, which was demonstrated to adversely affect organisms in this study, was detected at high concentrations in the sediment. NP was banned from kitchen cleaners in 2002, and was designated a restricted-use chemical that was prohibited from domestic applications in 2007. The use of NP in paints and ink binders has been banned in Korea since 2010 (MOE, 2012). However, NP continues to be used in industrial cleaning agents, textile, and leather processing; consequently, it was detected in the coastal waters of Korea, and elsewhere (MOA, 2016). Thus, further restrictions, regulations, and monitoring of NP are immediately required. Of note, certain bioassays detecting NP exposure in the sediments were demonstrated in the present work; thus, rapid biological monitoring could be implemented to screen NP in the coastal environment.

The highlight of this study was integrating multiple LOEs for ERA. In particular, one newly identified AhR agonist (enoxolone) was determined by using EDA combined with FSA. This approach improved the explaining power of AhR-mediated potency; however, a large portion of potency could not be explained. Thus, it is necessary to investigate other unknown AhR agonists that potentially exist in sediments. Evaluation of cell viability using flow cytometry analysis demonstrated the benefits of incorporating more sensitive and high-resolution toxicity screening of samples in the integrative sediment assessment. In addition, this study demonstrated that the sensitivity of toxicity tests is not only species and contaminant-specific, but that it also varies depending on the endpoint of the measurement. It is important to assess the sensitivity of a biotest to evaluate responses and translate them into management decisions. If a biotest shows slight sensitivity, only the strongest effects could be determined. In contrast, the use of a highly sensitive test

system might be overprotective. Information on the responsiveness of a test system is, thus, highly important to interpret biotest responses and the degree of confidence in resulting management decisions. Assay- and end point-specific variations and sensitivities of the potential toxicity of mixed samples provide useful information for the selection and management of priority substances in coastal environments. Overall, the results of the present study are expected to provide baseline information for future monitoring studies and coastal management.

Nevertheless, this study had some limitations. First, a common response to varying physical factors was not considered, such as sediment grain size, water depth, redox potential, and biotic factors (including competition and predation). For advanced ERAs, both chemical and non-chemical stressors should be evaluated. However, ERAs rarely evaluate potential interaction among various non-contaminants (physical or biological) or indirect effects (USEPA, 1998; Chariton et al., 2016). Whole-organism *in vivo* bioassays were used to evaluate the toxicity of the sediment; however, such bioassays tend to have limited sample throughput, and cannot distinguish the effects of pollutants from matrix components, such as salinity, and pH.

Second, the sediment EDA performed in this study was based on organic extracts, which might be limited with respect to bioavailability. To estimate the eco-toxicological relevance of the identified compounds in respective sediments, additional data on their bioavailability are required in future studies (Brack, 2009; You and Li, 2017). Third, various statistical analyses were used to integrate the LOEs in this study. However, more site-specific diagnostic approaches and criteria are required, particularly with respect to identifying and confirming stressors that cause toxicity to sediment biota (Table 6.1). To generate ecological and environmentally relevant ERA, it is important to determine predicted no-effect concentrations (PNECs) and sediment quality guidelines (SQGs) of toxic chemicals in marine sediment. For these approaches, large data sets, constructed from data assimilated over a long period are required. Future research directions of the sediment assessment are suggested in the next section based on the current knowledge and limitations.

Table 6.1.

Employed lines of evidence in this study and other approaches identified in the literature with strengths and weaknesses for ecological risk assessment (related in 1.1).

Lines of evidence	Ecological risk assessment (√ can be used for this purpose)				Applied years	Interpretation	
	Problem Formulation	Exposure Assessment	Effects Assessment	Causation		Strength	Weakness
TIE				√	1989 [a]	To identify quickly and cheaply those chemicals causing toxicity	Multiple toxicants are present, the proportion of the overall toxicity due to each toxicant often varies significantly over time and matrix will also changed
Multivariate analyses				√	1992 [b]	To identify key stressors, which triggered the observed biological responses	Not accept any missing data, and both biological response and stressor data could be concurrently obtained at the same time and space
EDA				√	2003 [c]	To identify organic contaminants causing sediment toxicity	Less consider the bioavailability and ecological relevance
CBRs				√	2005 [d]	Tissue concentrations of a contaminant associated with toxic effects are the same irrespective of exposure pathways or bioavailability	Ignores reactivity of contaminants once they are taken up by organisms
Field based SQGs with f-SSD				√	2005 [e]	Environmentally relevant and realistic measurement of the biological community response to a specific chemical in the presence of other chemicals in the sediment	Only work when at least some species have been impacted and cannot be used for predicting acceptable exposures for new chemicals
Habitat morphology				√	2005 [f]	To distinguish between toxicity-induced and habitat morphology-induced alterations in the field	Non-chemical stressors, both biotic and abiotic also need to be considered
Grey TOPSIS				√	2007 [g]	Providing complete ranking results; more suitable to be combined with stochastic analysis	Not being able to handle vague assessments

TIE; Toxicity identification and evaluation, EDA; Effect-directed analysis, CBRs; Critical body residues, SQGs; sediment quality guidelines, f-SSD; field-based species sensitivity distributions, TOPSIS; Technique for order preference by similarity, [a] Burkhard and Ankley (1989), [b] Bocard et al. (1992), [c] Brack (2003), [d] Gust and Fleeger (2005), [e] Leung et al. (2005), [f] Burckhardt-Holm et al. (2005). [g] Critto et al. (2007). Orange box indicated that used approaches in this study.

6.3. Future research directions

In this study, the multiple LOEs approach was implemented as an advanced assessment, including EDA with non-target analyses, multiple bioassays, and eDNA metabarcoding. The approach performed well at addressing ecotoxicological effects associated with contaminated coastal sediments. However, many areas require further research to extend current sediment assessments.

Future studies should investigate non-contaminant parameters, such as sediment oxygen demand, redox potential, total organic carbon, and particle size distribution. These parameters might cause the structure of benthic communities to change through regulating chemical bioavailability. Consequently, it is important to evaluate the risk these parameters present to sediment (Xu et al., 2015). Different species interact with each other in biological communities, with chemical contaminants and non-contaminant parameters generally affecting these interactions differently in natural habitats (Leung et al., 2014).

Furthermore, weighting multiple bioassays could provide useful information, such as effect-based trigger values, which represent acceptable risks for complex mixtures (Escher et al., 2018, 2020). Scores on the relevance and feasibility of various methods should be considered (Martin et al., 2018). In particular, overlooking the bioavailability of EDA could lead to biased, and even erroneous, identification of causative toxicants in mixed samples. It is important to consider bioavailability in the EDA approach to improve the accurate identification of key toxicants and to prevent overestimates of toxicity in the environments. Recently, various approaches were introduced to address these limitations, and to improve the integration of bioavailability in the EDA of abiotic samples, such as bioaccessibility-based extraction with XAD resin and adverse outcomes pathway-directed EDA techniques and their combined application (Li et al., 2019; Cheng et al., 2020).

Moreover, SQGs were derived by concurrently collecting field data on benthic communities and contaminants loading with measurements of sediment samples from the Korean coastal waters (Figure 6.2). For example, it is important to discriminate the risks of the interaction between environmental stressors and biota from different sites with site-specific sediment quality guidelines. Such information

could be used to delineate ecological stress and identify the relative importance of causal stressors. The multiple LOEs approach in combination with the grey TOPSIS (technique for order preference by similarity) can be used to discriminate the relative magnitude of risks at different sites, and rate them as having high, moderate, or low ecological risk. This information guides us to take pertinent measures to combat polluted sediment (Jiang et al., 2015) (Table 6.1). In addition, species-sensitivity distribution approaches that are field-based allow the generation of sediment quality guidelines, for which the criteria are more environmentally relevant and realistic for use as site-specific guidelines (Leung et al., 2005) (Table 6.1). Although it is difficult to evaluate the biological effects of chemicals on various living organisms and species with differing sensitivity, it is necessary to evaluate the biological impacts of benthic organisms and to focus on marine ecosystem research (Figure 6.2).

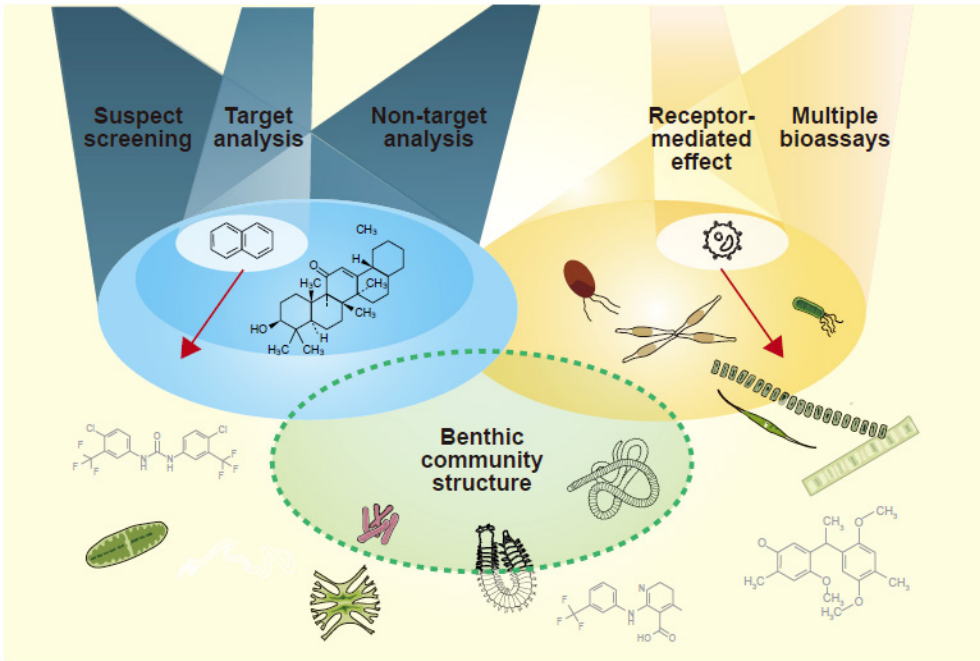


Figure 6.2.
Future strategies for multiple lines of evidence approach to address the ecotoxicological effects associated with contaminated coastal sediments.

BIBLIOGRAPHY

Abdel-Shafy, H. I., Mansour, M. S. M. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egypt. J. Pet*. 2016, **25** (1), 107–123.

Allgaier, M., Grossart, H.-P. Seasonal dynamics and phylogenetic diversity of free-living and particle-associated bacterial communities in four lakes in northeastern Germany. *Aquat. Microb. Ecol*. 2006, **45**, 115–128.

Al-Odaini, N. A., Shim, W. J., Han, G. M., Jang, M., Hong, S. H. Enrichment of hexabromocyclododecanes in coastal sediments near aquaculture areas and a wastewater treatment plant in a semi-enclosed bay in South Korea. *Sci. Total Environ*. 2015, **505**, 290–298.

Amann, R. I., Ludwig, W., Schleifer, K.-H. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Mol. Biol. Rev*. 1995, **59**, 143–169.

Anderson, M. J., Willis, T. J. Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology* 2003, **84** (2), 511–525.

Arnds, J., Knittel, K., Buck, U., Winkel, M., Amann, R. Development of a 16S rRNA-targeted probe set for Verrucomicrobia and its application for fluorescence *in situ* hybridization in a humic lake. *Syst. Appl. Microbiol*. 2010, **33**, 139–148.

Babić, S., Barišić, J., Stipaničev, D., Repec, S., Lovrić, M., Malev, O., Martinović-Weigelt, D., Čož-Rakovac, R., Klobučar, G. Assessment of river sediment toxicity: Combining empirical zebrafish embryotoxicity testing with *in silico* toxicity characterization. *Sci. Total Environ*. 2018, **643**, 435–450.

Badam, L. *In vitro* antiviral activity of indigenous glycyrrhizin, licorice and glycyrrhizic acid (Sigma) on Japanese encephalitis virus. *J. Commun. Dis.* 1997, **29** (2), 91–99.

Bae, H., Lee, J. H., Song, S. J., Park, J., Kwon, B.-O., Hong, S., Ryu, J., Choi, K., Khim, J. S. Impacts of environmental and anthropogenic stresses on macrozoobenthic communities in Jinhae Bay, Korea. *Chemosphere* 2017, **171**, 681–691.

Barra, R., Popp, P., Quiroz, R., Bauer, C., Cid, H., von Tümpling, W. Persistent toxic substances in soils and waters along an altitudinal gradient in the Laja River basin, central southern Chile. *Chemosphere* 2015, **58**, 905–915.

Barron, M. G., Heintz, R., Rice, S. D. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. *Mar. Environ. Res*. 2004, **58**, 95–100.

Behnisch, P. A., Hosoe, K., Sakai, S.-I. Bioanalytical screening methods for dioxins and dioxin-like compounds — a review of bioassay/biomarker technology. *Environ. Int*. 2001, **27**, 413–439.

Beiras, R., Tato, T. Marine environmental risk assessment and acute water quality criterion for pentachlorophenol in coastal waters. *Ecotoxicology* 2018, **27** (7), 803–808.

Blanchet, H., Lavesque, N., Ruellet, T., Dauvin, J., Sauriau, P.-G., Desroy, N., Desclaux, C., Leconte, M., Bachelet, G., Janson, A. L. Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats — implications for the implementation of the European Water Framework Directive. *Ecol. Indic*. 2008, **8**, 360–372.

Borcard, D., Legendre, P., and Drapeau, P. Partialling out the spatial component of ecological variation. *Ecology*, 1992, **73**, 1045–1055.

Booij, P., Vethaak, A. D., Leonards, P. E., Sjollema, S. B., Kool, J., de Voogt, P., Lamoree, M. H. Identification of photosynthesis inhibitors of pelagic marine algae using 96-well plate microfractionation for enhanced throughput in effect-directed analysis. *Environ. Sci. Technol*. 2014, **48** (14), 8003–8011.

Boulanger-Weill, J., Sumbre, G., Functional integration of newborn neurons in the zebrafish optic tectum. *Front. Cell Dev. Biol*. 2019, **7**, 57.

Brack, W., Aissa, S. A., Backhaus, T., Dulio, V., Escher, B. I., Faust, M., Hilscherova, K., Hollender, J., Hollert, H., Müller, C., Munthe, J., Posthuma, L., Seiler, T.-B., Slobodnik, J., Teodorovic, I., Tindall, A. J., de Aragão Umbuzeiro, G., Zhang, X., Altenburger, R. Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. *Environ. Sci. Eur*. 2019, **31** (1), 10–19.

Brack, W., Ait-Aissa, S., Burgess, R. M., Busch, W., Creusot, N., Di Paolo, C., Escher, B. I., Mark Hewitt, L., Hilscherova, K., Hollender, J., Hollert, H., Jonker, W., Kool, J., Lamoree, M., Muschket, M., Neumann, S., Rostkowski, P., Ruttkies, C., Schollee, J., Schymanski, E. L., Schulze, T., Seiler, T.-B., Tindall, A. J., De Aragão Umbuzeiro, G., Vrana, B., Krauss, M. Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview. *Sci. Total Environ*. 2016, **544**, 1073–1118.

Brack, W., Bandow, N., Schwab, K., Schulze, T., Streck, G. Bioavailability in effect-directed analysis of organic toxicants in sediments. *Trends Anal. Chem.* 2009. **28**, 543–549.

Brack, W. Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? *Anal. Bioanal. Chem*. 2003, **377** (3), 397–407.

Brack, W., Altenburger, R., Ensenbach, U., Möder, M., Segner, H., Schüürmann, G. Bioassay-directed identification of organic toxicants in river sediment in the industrial region of Bitterfeld (Germany) — A contribution to hazard assessment. *Arch. Environ. Contam. Toxicol*. 1999, **37** (2), 164–174.

Bragg, L. M., Stone, G., Butler, M. K., Hugenholtz, P., Tyson, G. W. Shining a light on dark sequencing: characterising errors in ion torrent PGM data. *PLoS Comput. Biol*. 2013, **9**, e1003031.

Burkhardt-Holm, P., Giger, W., Güttinger, H., Ochsenbein, U., Peter, A., Scheurer, K., Suter, M. J. F., Segner, H., Staub, E. Where have all the fish gone? *Environ. Sci. Technol.* 2005, **39**, 441A–447A.

Burkhard, L. P., Ankley, G. T., Identifying toxicants: NETAC's toxicity-based approach. *Environ. Sci. Technol.* 1989, **23**, 1438–1443.

Calabreseci, E. J. Evidence that hormesis represents an "overcompensation" response to a disruption in homeostasis. *Ecotoxicol. Environ. Saf*. 1999, **42** (2), 135–137.

Campbell, B. J., Kirchman, D. L. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *The ISME J*. 2013, **7**, 210–220.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 2010, **7**, 335–336.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A., Teske, A. Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an arctic fjord of Svalbard. *Appl. Environ. Microbiol*. 2014, **80**, 3749–3756.

CCME (Canadian Council of Ministers of the Environment). Canadian sediment quality guidelines for the protection of aquatic life: Nonylphenol and its ethoxylates. *Canadian environmental quality guidelines*, Winnipeg, 2002.

Cesar, A., Abessa, D. M. d. S., Pereira, C. D. S., Santos, A. R., Fernández, N., Choueri, R. B., DelValls, T. A. A simple approach to integrate the ecotoxicological and chemical data for the establishment of environmental risk levels. *Braz. Arch. Biol. Techn*. 2009, **52**, 233–240.

Cha, J., Hong, S., Kim, J., Lee, J., Yoon, S. J., Lee, S., Moon, H.-B., Shin, K.-H., Hur, J., Giesy, J. P., Khim, J. S. Major AhR-active chemicals in sediments of Lake Sihwa, South Korea: Application of effect-directed analysis combined with full-scan screening analysis. *Environ. Int*. 2019, **133**, 105199.

Chang, W. K., Ryu, J., Yi, Y., Lee, W. C., Lee, C. W., Kang, D., Lee, C. H., Hong, S., Nam, J., Khim, J. S. Improved water quality in response to pollution control measures at Masan Bay, Korea. *Mar. Pollut. Bull.* 2012, **64**, 427–435.

Chapman, P. M. Determining when contamination is pollution — Weight of evidence determinations for sediments and effluents. *Environ. Int*. 2007, **33** (4), 492–501.

Chapman, P. M. Presentation and interpretation of sediment quality triad data. *Ecotoxicol.* 1996, **5**, 327–339.

Chapman, P. M. The sediment quality triad approach to determining pollution-induced degradation. *Sci. Total Environ*. 1990, **97**, 815–825.

Chapman, P. M., Wang, F., Caeiro, S. S. Assessing and managing sediment contamination in transitional waters. *Environ. Int.* 2013, **55**, 71–91.

Chariton, A. A., Sun, M. Y., Gibson, J., Webb, J. A., Leung, K. M. Y., Hickey, C. W., Hose, G. C. Emergent technologies and analytical approaches for understanding the effects of multiple stressors in aquatic environments. *Mar. Freshw. Res.* 2016, **67**, 414–428.

Chariton, A. A., Court, L. N., Hartley, D. M., Colloff, M. J., Hardy, C. M. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. *Front. Ecol. Environ.* 2010, **8**, 233–238.

Cheng, F., Li, H., Ma, H., Wu, F., Fu, Z., You, J. Identifying bioaccessible suspect toxicants in sediment using adverse outcome pathway directed analysis. *J. Hazard. Mater*. 2019, 121853.

Cho, J., Hyun, S., Han, J. H., Kim, S., Shin, D. H. Historical trend in heavy metal pollution in core sediments from the Masan Bay, Korea. *Mar. Pollut. Bull*. 2015, **95**, 427–432.

Choi, H. G., Moon, H.-B., Choi, M., Yu, J. Monitoring of organic contaminants in sediments from the Korean coast: spatial distribution and temporal trends (2001–2007). *Mar. Pollut. Bull*. 2011, **62**, 1352–1361.

Choi, J. W., Seo, J. Y., Lee, C. H., Ryu, T. K., Sung, C. G., Han, G. M., Hyun, S. M. Spatial distribution patterns of macrobenthic communities during winter and summer in the Masan Bay special management area, southern coast of Korea. *Oce. Pol. Res*. 2005, **27**, 381–395.

Choi, M., Moon, H.-B., Yu, J., Kim, S. S., Pait, A. S., Choi, H. G. Nationwide monitoring of nonylphenolic compounds and coprostanol in sediments from Korean coastal waters. *Mar. Pollut. Bull*. 2009, **58**, 1086–1092.

Cid, A., Fidalgo, P., Herrero, C., Abalde, J. Toxic action of copper on the membrane system of a marine diatom measured by flow cytometry. *Cytometry* 1996, **25** (1), 32–36.

Clarke, K., Gorley, R. Primer. PRIMER-e, Plymouth. 2006.

Clarke, K., Warwick, R. A. Further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Mar. Ecol. Prog. Ser*. 2001, **216**, 265–278.

Cole, S., Codling, I. D., Parr, W., Zabel, T. Guidelines for managing water quality impacts within UK European marine sites. *UK Marine SACs Project*. 1999, 88.

Critto, A., Torresan, S., Semenzin, E., Giove, S., Mesman, M., Schouten, A. J., Rutgers, M., Marcomini, A. Development of a site-specific ecological risk assessment for contaminated sites: part I. A multi-criteria based system for the selection of ecotoxicological tests and ecological observations. *Sci. Total Environ*. 2007, **379** (1), 16–33.

Crump, B. C., Hobbie, J. E. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. *Limnol. Oceanogr*. 2005, **50**, 1718–1729.

Cui, L., Lu, H., Lee, Y. H. Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases. *Mass Spectrom. Rev*. 2018, **37** (6), 772–792.

Dai, G., Huang, J., Chen, W., Wang, B., Yu, G., Deng, S. Major pharmaceuticals and personal care products (PPCPs) in wastewater treatment plant and receiving water in Beijing, China, and associated ecological risks. *Bull. Environ. Contam. Toxicol*. 2014, **92** (6), 655–661.

Davoren, M., Ni Shuilleabhain, S., Hartl, M. G., Sheehan, D., O' Brien, N. M., O' Halloran, J., Van Pelt, F. N., Mothersill, C. Assessing the potential of fish cell lines as tools for the cytotoxicity testing of estuarine sediment aqueous elutriates. *Toxicol. Vitro.* 2005, **19** (3), 421–431.

de Castro-Català, N., Kuzmanovic, M., Roig, N., Sierra, J., Ginebreda, A., Barceló, D., Pérez, S., Petrovic, M., Picó, Y., Schuhmacher, M., Muñoz, I. Ecotoxicity of sediments in rivers: Invertebrate community, toxicity bioassays and the toxic unit approach as complementary assessment tools. *Sci. Total Environ*. 2016, **540**, 297–306.

de Lima Ribeiro, F. A., Ferreira, M. M. C. QSAR model of the phototoxicity of polycyclic aromatic hydrocarbons. *Theochem-J. Mol. Struct.* 2005, **719** (1–3), 191–200.

DelValls, A. T., Chapman, P. M. Site-specific sediment quality values for the Gulf of Cádiz (Spain) and San Francisco Bay (USA), using the sediment quality triad and multivariate analysis. *Cienc. Mar.* 1998, **24**, 3–13.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G. L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol*. 2006, **72**, 5069–5072.

Di Paolo, C., Groh, K. J., Zennegg, M., Vermeirssen, E. L. M., Murk, A. J., Eggen, R. I. L., Hollert, H., Werner, I., Schirmer, K. Early life exposure to PCB126 results in delayed mortality and growth impairment in the zebrafish larvae. *Aquat. Toxicol*. 2015, **169**, 168–178.

Diaz, E., Monsalvo, V. M., Lopez, J., Mena, I. F., Palomar, J., Rodriguez, J. J., Mohedano, A. F. Assessment the ecotoxicity and inhibition of imidazolium ionic liquids by respiration inhibition assays. *Ecotoxicol. Environ. Saf*. 2018, **162**, 29–34.

Dong, W., Wang, F., Fang, M., Wu, J., Wang, S., Li, M., Yang, J., Chernick, M., Hinton, D. E., Pei, D. S., Chen, H., Zheng, N., Mu, J., Xie, L., Dong, W. Use of biological detection methods to assess dioxin-like compounds in sediments of Bohai Bay, China. *Ecotoxicol. Environ. Saf*. 2019, **173**, 339–346.

Echeveste, P., Agusti, S., Dachs, J. Cell size dependent toxicity thresholds of polycyclic aromatic hydrocarbons to natural and cultured phytoplankton populations. *Environ. Pollut*. 2010, **158** (1), 299–307.

Echeveste, P., Dachs, J., Berrojalbiz, N., Agustí, S. Decrease in the abundance and viability of oceanic phytoplankton due to trace levels of complex mixtures of organic pollutants. *Chemosphere* 2010, **81** (2), 161–168.

Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 2013, **10**, 996–998.

Engebrecht, J., Nealson, K., Silverman, M. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 1983, **32**,773–781.

Escher, B. O., Stapleton, H. M., Schymanski, E. L. Tracking complex mixtures of chemicals in our changing environment. *Science* 2020, **367**, 388–392.

Escher, B. I., Aït-Aïssa, S., Behnisch, P. A., Brack, W., Brion, F., Brouwer, A., Buchinger, S., Crawford, S. E., Pasquier, D. D., Hamers, T., Hilscherova, K., Hollert, H., van der Oost, R., Vermeirssen, E., Neale, P. A. Effect-based trigger values for *in vitro* and *in vivo* bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Sci. Total Environ*. 2018, **628–629**, 748–765.

Farré, M., Gonçalves, C., Lacorte, S., Barceló, D., Alpendurada, M. F. Pesticide toxicity assessment using an electrochemical biosensor with *Pseudomonas putida* and a bioluminescence inhibition assay with *Vibrio fischeri*. *Anal. Bioanal. Chem*. 2002, **373** (8), 696–703.

Fent, K. Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. *Toxicol. Vitro*. 2001, **15**, 477–488.

Feris, K., Ramsey, P., Frazar, C., Moore, J. N., Gannon, J. E., Holben, W. E. Differences in hyporheic-zone microbial community structure along a heavy-metal contamination gradient. *Appl. Environ. Microbiol*. 2003, **69**, 5563–5573.

Fischer, H., Pusch, M. Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river. *Freshwat. Biol*. 2001, **46**, 1335–1348.

Fisher, D., Yonkos, L., Ziegler, G., Friedel, E., Burton, D. Acute and chronic toxicity of selected disinfection byproducts to *Daphnia magna*, *Cyprinodon variegatus*, and *Isochrysis galbana*. *Water Res.* 2014, **55**, 233–244.

Floehr, T., Scholz-Starke, B., Xiao, H., Hercht, H., Wu, L., Hou, J., Schmidt-Posthaus, H., Segner, H., Kammann, U., Yuan, X., Roß-Nickoll, M., Schäffer, A., Hollert, H. Linking Ah receptor mediated effects of sediments and impacts on fish to key pollutants in the Yangtze Three Gorges Reservoir, China — A comprehensive perspective. *Sci. Total Environ*. 2015, **538**, 191–211.

Freitas, S., Hatosy, S., Fuhrman, J. A., Huse, S. M., Mark Welch, D. B., Sogin, M. L., Martiny, A. C. Global distribution and diversity of marine Verrucomicrobia. *The ISME J*. 2012, **6**, 1499–1505.

Freshney, R. I. Animal cell culture. *New York: Oxford University Press* 1992.

Freyberger, A., Schmuck, G. Screening for estrogenicity and anti-estrogenicity: a critical evaluation of an MVLN cell-based transactivation assay. *Toxicol. Lett.* 2005, **155** (1), 1–13.

Gaboyer, F., Vandenabeele-Trambouze, O., Cao, J., Ciobanu, M.-C., Jebbar, M., Le Romancer, M., Alain, K. Physiological features of *Halomonas lionensis* sp. nov., a novel bacterium isolated from a Mediterranean Sea sediment. *Res. Microbiol*. 2014, **165**, 490–500.

Gaido, K. W., McDonnell, D. P., Korach, K. S., Safe, S. H. Estrogenic activity of chemical mixtures: is there synergism? CIIT activities. *Chem. Ind. Instit. Toxicol*. 1997, **2**, 1–7.

Gallampois, C. M. J., Schymanski, E. L., Krauss, M., Ulrich, N., Bataineh, M., Brack, W. Multicriteria approach to select polyaromatic river mutagen candidates. *Environ. Sci. Technol.* 2015, **49** (5), 2959–2968.

Garcia-Ordiales, E., Roqueñí, N., Rico, J. M., Cienfuegos, P., Alvarez, R., Ordoñez, A. Assessment of the toxicity toward *Vibrio fischeri* in sediments of a mining impacted estuary in the north of Spain. *Sci. Total Environ*. 2019, **660**, 826–833.

Garr, A. L., Laramore, S., Krebs, W. Toxic effects of oil and dispersant on marine microalgae. *Bull. Environ. Contam. Toxicol*. 2014, **93** (6), 654–659.

GBI (Global Business Intelligence) Research. Polystyrene (PS) and expandable polystyrene (EPS) global market to 2020 — Continued development in the construction (EPS) and packaging (PS) end-use segments to accelerate future demand. *GBI Research* 2012, GBICH0059MR.

Genderjahn, S., Alawi, M., Mangelsdorf, K., Horn, F., Wagner, D. Desiccation- and saline-tolerant bacteria and archaea in Kalahari Pan sediments. *Front Microbiol.* 2018, **9**, 2082.

Gibbons, S. M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., Hooker, J., Madsen, R., Coleman, M. L., Gilbert, J. A. Human and environmental impacts on river sediment microbial communities. *PloS one* 2014, **9** (5), e97435.

Giesy, J. P., Hilscherova, K., Jones, P., Kannan, K., Machala, M. Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Mar. Pollut. Bull.* 2002, **45**, 3–16.

Gillan, D. C., Danis, B., Pernet, P., Joly, G., Dubois, P. Structure of sediment-associated microbial communities along a heavy-metal contamination gradient in the marine environment. *Appl. Environ. Microbiol*. 2005, **71**, 679–690.

Giraldo, A., Montes, R., Rodil, R., Quintana, J. B., Vidal-Linan, L., Beiras, R. Ecotoxicological evaluation of the UV filters ethylhexyl dimethyl *p*-aminobenzoic acid and octocrylene using marine organisms *Isochrysis galbana*, *Mytilus galloprovincialis* and *Paracentrotus lividus*. *Arch. Environ. Contam. Toxicol*. 2017, **72** (4), 606–611.

Gorenoglu, E., Aydin, E., Topuz, E., Pehlivanoglu-Mantas, E. Effect of triclosan and its photolysis products on marine bacterium *V. fischeri* and freshwater alga *R. subcapitata*. *J. Environ. Manage*. 2018, **211**, 218–224.

Gower, J. C. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika*. 1996, **53**, 325–338.

Gust, K. A., Fleeger, J. W. Exposure-related effects on Cd bioaccumulation explain toxicity of Cd-phenanthrene mixtures in Hyalella azteca. *Environ. Toxicol. Chem.* 2005, **24**, 2918–2926.

Hadjoudja, S., Vignoles, C., Deluchat, V., Lenain, J. F., Le Jeune, A. H., Baudu, M. Short term copper toxicity on *Microcystis aeruginosa* and *Chlorella vulgaris* using flow cytometry. *Aquat. Toxicol*. 2009, **94**, 255–264.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Caterina D'Agrosa, F. M., Bruno, J. F., Casey, K. S., Ebert, C., Fujita, F. F., Heinemann, D., Lenihan, H. S., Madin, M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., Watson, R. A global map of human impact on marine ecosystems. *Science.* 2008, **319** (5865), 948–952.

Herlemann, D. P., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J. J., Andersson, A. F. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME J*. 2011, **5**, 1571–1579.

Hernando, M., de Vettori, S., Bueno, M. M., Fernández-Alba, A. Toxicity evaluation with *Vibrio fischeri* test of organic chemicals used in aquaculture. *Chemosphere* 2007, **68** (4), 724–730.

Hollert, H., Dürr, M., Olsman, H., Halldin, K., van Bavel, B., Brack, W., Tysklind, M., Engwall, M., Braunbeck, T. Biological and chemical determination of dioxin-like compounds in sediments by means of a sediment triad approach in the catchment area of the River Neckar. *Ecotoxicol*. 2002, **11**, 323–336.

Hollert, H., Keiter, S., König, N., Rudolf, M., Ulrich, M., Braunbeck, T. A new sediment contact assay to assess particle-bound pollutants using zebrafish (*Danio rerio*) embryos. *J. Soils Sed*. 2003, **3** (3), 197–208.

Hong, J. S., Lee, J. H. Effects of the pollution on the benthic macrofauna in Masan Bay, Korea. *J. Korean Soc. Oceanogr*. 1983, **18**, 169–179.

Hong, S., Yim, U. H., Ha, S. Y., Shim, W. J., Jeon, S., Lee, S., Kim, C., Choi, K.-H., Jung, J., Giesy, J. P. Bioaccessibility of AhR-active PAHs in sediments contaminated by the *Hebei Spirit* oil spill: application of tenax extraction in effect-directed analysis. *Chemosphere* 2016a, **144**, 706–712.

Hong, S., Lee, J., Lee, C., Yoon, S. J., Jeon, S., Kwon, B.-O., Lee, J.-H., Giesy, J. P., Khim, J. S. Are styrene oligomers in coastal sediments of an industrial area aryl hydrocarbon-receptor agonists*? Environ. Pollut*. 2016b, **213**, 913–921.

Hong, S., Giesy, J. P., Lee, J.-S., Lee, J.-H., Khim, J. S. Effect-directed analysis: Current status and future challenges. *Ocean Sci. J*. 2016c, **51**, 413–433.

Hong, S., Lee, S., Choi, K.-H., Kim, G. B., Ha, S. Y., Kwon, B.-O., Ryu, J., Yim, U. H., Shim, W. J., Jung, J. Effect-directed analysis and mixture effects of AhR-active PAHs in crude oil and coastal sediments contaminated by the *Hebei Spirit* oil spill. *Environ. Pollut*. 2015, **199**, 110–118.

Hong, S., Khim, J. S., Ryu, J., Park, J., Song, S. J., Kwon, B.-O., Choi, K.-H., Ji, K., Seo, J., Lee, S. Two years after the *Hebei Spirit* oil spill: residual crude-derived hydrocarbons and potential AhR-mediated activities in coastal sediments. *Environ. Sci. Technol*. 2012a, **46**, 1406–1414.

Hong, S., Khim, J. S., Naile, J. E., Park, J., Kwon, B.-O., Wang, T., Lu, Y., Shim, W. J., Jones, P. D., Giesy, J. P. AhR-mediated potency of sediments and soils in estuarine and coastal areas of the Yellow Sea region: a comparison between Korea and China. *Environ. Pollut.* 2012b, **171**, 216–225.

Hong, S., Shin, K.-H. Sources, distributions and temporal trends of nonylphenols in South Korea. In: Loganathan BG, LamPKS, editors. Global contamination trends of persistent organic chemicals. *CRC Press* 2011, 259–278.

Hong, S., Won, E. J., Ju, H. J., Kim, M. S., Shin, K.-H. Current nonylphenol pollution and the past 30-years record in an artificial Lake Shihwa, Korea. *Mar. Pollut. Bull*. 2010, **60**, 303–313.

Hong, S. H., Kannan, N., Yim, U. H., Li, D., Kim, M., Shim, W. J. Assessment of sediment contamination by persistent organic pollutants in Gyeonggi Bay, Korea. *Toxicol. Environ. Health. Sci.* 2009a, **1**, 56–63.

Hong, S. H., Munschy, C., Kannan, N., Tixier, C., Tronczynski, J., Héas-Moisan, K., Shim, W. J. PCDD/F, PBDE, and nonylphenol contamination in a semi-enclosed bay (Masan Bay, South Korea) and a Mediterranean lagoon (Thau, France). *Chemosphere* 2009b, **77**, 854–862.

Hong, S. H., Yim, U. H., Shim, W. J., Oh, J. R., Lee, I. S. Horizontal and vertical distribution of PCBs and chlorinated pesticides in sediments from Masan Bay, Korea. *Mar. Pollut. Bull*. 2003, **46** (2), 244–253.

Horii, Y., Khim, J. S., Higley, E. B., Giesy, J. P., Ohura, T., Kannan, K. Relative potencies of individual chlorinated and brominated polycyclic aromatic hydrocarbons for induction of aryl hydrocarbon receptor-mediated responses. *Environ. Sci. Technol*. 2009, **43**, 2159–2165.

Hullar, M. A. J., Kaplan, L. A., Stahl, D. A. Recurring seasonal dynamics of microbial communities in stream habitats. *Appl. Environ. Microbiol*. 2006, **72**, 713–722.

Hunt, J. W., Anderson, B. S., Phillips, B. M., Tjeerdema, R. S., Taberski, K. M., Wilson, C. J., Puckett, H. M., Stephenson, M., Fairey, R., Oakden, J. A. A largescale categorization of sites in San Francisco Bay, USA, based on the sediment quality triad, toxicity identification evaluations, and gradient studies. *Environ. Toxicol. Chem.* 2001, **20**, 1252–1265.

Hyland, J. L., Vandolah, R. F., Snoots, T. R. Predicting stress in benthic communities of southeastern U.S. estuaries in relation to chemical contamination of sediments. *Environ. Toxicol. Chem*. 1999, **18**, 2557–2564.

Hyun, S., Lee, C. H., Lee, T., Choi, J. W. Anthropogenic contributions to heavy metal distributions in the surface sediments of Masan Bay, Korea. *Mar. Pollut. Bull*. 2007, **54**, 1059–1068.

Imran, H., Kim, J. G., Kim, K. S., Park, J. S. Polyaromatic hydrocarbons (PAHs) levels from two industrial zones (Sihwa and Banwal) located in Ansan city of the Korean Peninsula and their influence on lake. *J. Appl. Sci. Environ. Mgt*. 2005, **9**, 63–69.

ISO (International Organization for Standardization) 11348-3. Water Quality — Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent bacteria test). Part 3: methodusing freeze-dried bacteria. Geneva, Switzerland. 2007.

ISO, 10253. Water Quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. Geneva, Switzerland. 2006.

Jafari, M., Keshavarz, M. H., Salek, H. A simple method for assessing chemical toxicity of ionic liquids on *Vibrio fischeri* through the structure of cations with specific anions. *Ecotoxicol. Environ. Saf*. 2019, **182**, 109429.

Jeon, S., Hong, S., Kwon, B.-O., Park, J., Song, S. J., Giesy, J. P., Khim, J. S. Assessment of potential biological activities and distributions of endocrine-disrupting chemicals in sediments of the west coast of South Korea. *Chemosphere* 2017, **168**, 441–449.

Jeong, H. J. The ecological roles of heterotrophic dinoflagellates in marine planktonic community. *J. Eukaryot. Microbiol.* 1999, **46** (4), 390–396.

Jia, Y., Schmid, C., Shuliakevich, A., Hammers-Wirtz, M., Gottschlich, A., der Beek, T. A., Yin, D., Qin, B., Zou, H., Dopp, E., Hollert, H. Toxicological and ecotoxicological evaluation of the water quality in a large and eutrophic freshwater lake of China. *Sci. Total Environ.* 2019, **667**, 809–820.

Jiang, Y. X., Liu, Y. S., Ying, G. G., Wang, H. W., Liang, Y. Q., Chen, X. W. A new tool for assessing sediment quality based on the Weight of Evidence approach and grey TOPSIS. *Sci. Total Environ.* 2015, **537**, 369–376.

Jiao, W., Wang, T., Khim, J. S., Luo, W., Hu, W., Naile, J. E., Giesy, J. P., Lu, Y. PAHs in surface sediments from coastal and estuarine areas of the northern Bohai and Yellow Seas, China. *Environ. Geochem. Health* 2012, **34** (4), 445–456.

Jin, X., Lee, H. K., Badejo, A. C., Lee, S. Y., Shen, A., Lee, S., Jeong, Y., Choi, M., Moon, H.-B. Decline in sediment contamination by persistent toxic substances from the outfall of wastewater treatment plant: Effectiveness of legislative actions in Korea. *Chemosphere* 2016, **153**, 426–435.

Johnson, B. T., Long, E. R. Rapid toxicity assessment of sediments from large estuarine ecosystems: a new tandem *in vitro* testing approach. *Environ. Toxicol. Chem.* 1998, **17**, 1099–1106.

Juhasz, A., Naidu, R. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[*a*]pyrene. *Int. Biodeter. Biodegr.* 2000, **45**, 57–88.

Jung, J. H., Hong, S. H., Yim, U. H., Ha, S. Y., Shim, W. J., Kannan, N. Multiple *in vitro* bioassay approach in sediment toxicity evaluation: Masan Bay, Korea. *Environ. Contam. Tox*. 2012, **89** (1), 32–37.

Kahru, A., Maloverjan, A., Sillak, H., Pollumaa L. The toxicity and fate of phenolic pollutants in the contaminated soils associated with the Oil-Shale industry. *Environ. Sci. Pollut. R.* 2002, **9**, 27–33.

Kais, B., Stengel, D., Batel, A., Braunbeck, T. Acetylcholinesterase in zebrafish embryos as a tool to identify neurotoxic effects in sediments. *Environ. Sci. Pollut. R*. 2015, **22** (21), 16329–16339.

Kammann, U., Biselli, S., Reineke, N., Wosniok, W., Danischewski, D., Hühnerfuss, H., Kinder, A., Sierts-Herrmann, A., Theobald, N., Vahl, H.-H., Vobach, M., Steinhart, H. Bioassay-directed fractionation of organic extracts of marine surface sediments from the North and Baltic Sea-Part II: Results of the biotest battery and development of a biotest index. *J. Soil. Sediment* 2005, **5** (4), 225–232.

Kammann, U., Vobach, M., Wosniok, W., Schaffer, A., Telscher, A. Acute toxicity of NP and its metabolites for zebrafish embryos. *Environ. Sci. Pollut*. *R*. 2009, **16** (2), 227–231.

Kan, J., Evans, S. E., Chen, F., Suzuki, M. T. Novel estuarine bacterioplankton in rRNA operon libraries from the Chesapeake Bay. *Aquat. Microb. Ecol*. 2008, **51**, 55–66.

Kannan, K., Yamashita, N., Imagawa, T., Decoen, W., Khim, J. S., Day, R. M., Summer, C. L., Giesy, J. P. Polychlorinated naphthalenes and polychlorinated biphenyls in fishes from Michigan waters including the Great Lakes. *Environ. Sci. Technol*. 2010, **34**, 566–572.

Karuppiah, M., Gupta, G. Impact of point and nonpoint source pollution on pore waters of two Chesapeake Bay tributaries. *Ecotox. Environ. Safe.* 1996, **35** (1), 81–85.

Khim, J. S., Hong, S. Assessment of trace pollutants in Korean coastal sediments using the triad approach: a review. *Sci. Total Environ*. 2014, **470**, 1450–1462.

Khim, J. S., Lee, K. T., Villeneuve, D. L., Kannan, K., Giesy, J., Koh, C.-H. *In vitro* bioassay determination of dioxin-like and estrogenic activity in sediment and water from Ulsan Bay and its vicinity, Korea. *Arch. Environ. Contam. Toxicol*. 2001, **40**, 151–160.

Khim, J. S., Villeneuve, D. L., Kannan, K., Hu, W. Y., Giesy, J. P., Kang, S.-G., Song, K.-J., Koh, C.-H. Instrumental and bioanalytical measures of persistent organochlorines in blue mussel (*Mytilus edulis*) from Korean coastal waters. *Arch. Environ. Contam. Toxicol.* 2000, **39** (3), 360–368.

Khim, J. S., Villeneuve, D. L., Kannan, K., Koh, C.-H., Giesy, J. P. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 2. *In vitro* gene expression assays. *Environ. Sci. Technol.* 1999a, **33** (23), 4206–4211.

Khim, J. S., Kannan, K., Villeneuve, D. L., Koh, C.-H., Giesy, J. P. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. Instrumental analysis. *Environ. Sci. Technol.* 1999b, **33**, 4199–4205.

Khim, J. S., Villeneuve, D. L., Kannan, K., Lee, K. T., Snyder S. A., Koh, C.-H., Giesy, J. P. Alkylphenols, polycylic aromatic hydrocarbons and organochlorines in sediment from Lake Shihwa, Korea: Instrumental and bioanaytical characterization. *Environ. Toxic*. *Chem*. 1999c, **18**, 2424–2432.

Kim, J., Hong, S., Cha, J., Lee, J., Kim, T., Lee, S., Moon, H.-B., Shin, K.-H., Hur, J., Lee, J.-S., Giesy, J. P., Khim, J. S. Newly identified AhR-active compounds in the sediments of an industrial area using effect-directed analysis. *Environ. Sci. Technol*. 2019, **53** (17), 10043–10052.

Kim, S., Hong, S., Lee, J., Kim, T., Yoon, S. J., Lee, J.-S., Choi, K., Kwon, B.-O., Giesy, J. P., Khim, J. S. Long-term trends of persistent toxic substances and potential toxicities in sediments along the west coast of South Korea. *Mar. Pollut. Bull*. 2020, **151**, 110821.

Kim, S., Sohn, J. H., Ha, S. Y., Kang, H., Yim, U. H., Shim, W. J., Khim, J. S., Jung, D., Choi, K.-H. Thyroid hormone disruption by water-accommodated fractions of crude oil and sediments affected by the *Hebei Spirit* oil spill in zebrafish and GH3 cells. *Environ. Sci. Technol*. 2016, **50** (11), 5972–5980.

Kim, S. K., Khim, J. S., Lee, K.-T., Giesy, J. P., Kannan, K., Lee, D. S., Koh, C.-H. Chapter 2: Emission, contamination and exposure, fate and transport, and national management strategy of POPs in South Korea. In: *Devel. Environ. Sci*., Li, A., Tanabe, S., Jiang, G., Giesy, J. P., Lam, P. K. S. *Elsevier*. 2007, **7**, 31–157.

Kinani, S., Bouchonnet, S., Creusot, N., Bourcier, S., Balaguer, P., Porcher, J. M., Ait-Aissa, S. Bioanalytical characterisation of multiple endocrine- and dioxin-like activities in sediments from reference and impacted small rivers. *Environ. Pollut*. 2010, **158** (1), 74–83.

Kind, T., Fiehn, O. Advances in structure elucidation of small molecules using mass spectrometry. *Bioanal. Rev.* 2010, **2** (1–4), 23–60.

King, G., Kostka, J., Hazen, T., Sobecky, P. Microbial responses to the *Deepwater Horizon* oil spill: from coastal wetlands to the deep sea. *Ann. Rev. Mar. Sci*. 2015, **7**, 377–401.

Kirchman, D. L., Dittel, A. I., Malmstrom, R. R., Cottrell, M. T. Biogeography of major bacterial groups in the Delaware estuary. *Limnol. Oceanogr*. 2005, **50**, 1697–1706.

Kitamura, S., Ohmegi, M., Sanoh, S., Sugihara, K., Yoshihara, S., Fujimoto, N., Ohta, S. Estrogenic activity of styrene oligomers after metabolic activation by rat liver microsomes. *Environ. Health Perspect*. 2003, **111**, 329–334.

Koh, C.-H., Khim, J. S., Villeneuve, D. L., Kannan, K., Johnson, B. G., Giesy, J. P. Instrumental and bioanalytical measures of dioxin-like and estrogenic compounds and activities associated with sediment from the Korean coast. *Ecotoxicol. Environ. Saf*. 2005, **61**, 366–379.

Koniecko, I., Staniszewska, M., Falkowska, L., Burska, D., Kielczewska, J., Jasinska, A. Alkylphenols in surface sediments of the Gulf of Gdansk (Baltic Sea). *Water Air Soil Pollut*. 2014, **225**, 2040.

KORDI (Korea Ocean Research and Development Institute). Development of Marine Ecosystem Health Index (MEHI) in the special management areas of the South Sea. *KORDI: Ansan*. 2010.

Kosmehl, T., Otte, J. C., Yang, L., Legradi, J., Bluhm, K., Zinsmeister, C., Keiter, S. H., Reifferscheid, G., Manz, W., Braunbeck, T., Strähle, U., Hollert, H. A combined DNA-microarray and mechanism-specific toxicity approach with zebrafish embryos to investigate the pollution of river sediments. *Reprod. Toxicol*. 2012, **33** (2), 245–253.

Kottuparambil, S., Agusti, S. PAHs sensitivity of picophytoplankton populations in the Red Sea. *Environ. Pollut*. 2018, **239**, 607–616.

Kwon, B. G., Koizumi, K., Chung, S. Y., Kodera, Y., Kim, J. O., Saido, K. Global styrene oligomers monitoring as new chemical contamination from polystyrene plastic marine pollution. *J. Hazard. Mater*. 2015, **300**, 359–367.

Kwon, B. G., Saido, K., Koizumi, K., Sato, H., Ogawa, N., Chung, S. Y., Kusui, T., Kodera, Y., Kogure, K. Regional distribution of styrene analogues generated from polystyrene degradation along the coastlines of the North-East Pacific Ocean and Hawaii. *Environ. Pollut.* 2014, **188**, 45–49.

Lachmund, C., Köcher, B., Manz, W., Heininger, P. Chemical and microbiological *in situ* characterization of benthic communities in sediments with different contamination levels. *J. Soil. Sediment.* 2003, **3**, 188–196.

Larsson, M., Hagberg, J., Giesy, J. P., Engwall, M. Time-dependent relative potency factors for polycyclic aromatic hydrocarbons and their derivatives in the H4IIE-*luc* bioassay. *Environ. Toxicol. Chem*. 2014, **33**, 943–953.

Lee, C. H., Lee, B. Y., Chang, W. K., Hong, S., Song, S. J., Park, J., Kwon, B.-O., Khim, J. S. Environmental and ecological effects of Lake Shihwa reclamation project in South Korea: a review. *Ocean Coast. Manage*. 2014, **102**, 545–558.

Lee, J., Kim, T., Yoon, S. J., Kim, S., Lee, A. H., Kwon, B.-O., Allam, A. A., Al-khedhairy, A. A., Lee, H., Kim, J.-J., Hong, S., Khim, J. S. Multiple evaluation of the potential toxic effects of sediments and biota collected from an oil-polluted area around Abu Ali Island, Saudi Arabia, Arabian Gulf. *Ecotoxicol. Environ. Saf*. 2019, **183**, 109547.

Lee, J., Hong, S., Kwon, B.-O., Cha, S. A., Jeong, H.-D., Chang, W. K., Ryu, J., Giesy, J. P., Khim, J. S. Integrated assessment of persistent toxic substances in sediments from Masan Bay, South Korea: Comparison between 1998 and 2014. *Environ. Pollut*. 2018, **238**, 317–325.

Lee, J., Hong, S., Yoon, S. J., Kwon, B.-O., Ryu, J., Giesy, J. P., Allam, A. A., Al-khedhairy, A. A., Khim, J. S. Long-term changes in distributions of dioxin-like and estrogenic compounds in sediments of Lake Sihwa, Korea: Revisited mass balance. *Chemosphere* 2017, **181**, 767–777.

Lee, J., Choi, E. J., Rhie, K. Validation of algal viability treated with total residual oxidant and organic matter by flow cytometry. *Mar. Pollut. Bull*. 2015, **97** (1), 95–104.

Lee, J.-S., Hong, S., Lee, J., Choi, T. S., Rhie, K., Khim, J. S. Evaluation of residual toxicity of hypochlorite-treated water using bioluminescent microbes and microalgae: Implications for ballast water management. *Ecotoxicol. Environ. Saf*. 2019, **167**, 130–137.

Lee, K.-T., Hong, S., Lee, J.-S., Chung, K. H., Hilscherová, K., Giesy, J. P., Khim, J. S. Revised relative potency values for PCDDs, PCDFs, and non-ortho-substituted PCBs for the optimized H4IIE-*luc in vitro* bioassay. *Environ. Sci. Pollut. R*. 2013, **20**, 8590–8599.

Lee, Y., Ryu, J., Hong, S., Khim, J. S. Temporal trends of persistent toxic substances and benthic community responses in special management area of Korea: The Masan Bay and Lake Sihwa cases. In Loganathan BG (Ed.), Persistent organic chemicals in the environment: Status and trends in the Pacific Basin Countries. *ACS Publications* 2016, 103–126.

LeFauve, M. K., Connaughton, V. P. Developmental exposure to heavy metals alters visually-guided behaviors in zebrafish. *Curr. Zool*. 2017, **63** (2), 221–227.

Legendre, P., Legendre, L. F. Numerical ecology. *Elsevier*. 2012.

Legler, J., van den Brink, C. E., Brouwer, A., Murk, A. J., van der Saag, P. T., Vethaak, A. D., van der Burg, B. Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47D breast cancer cell line. *Toxicol. Sci.* 1999, **48**, 55–66.

Leung, K. M. Y., Merrington, G., Warne, M. J., Wenning, R. J. Scientific derivation of environmental quality benchmarks for the protection of aquatic ecosystems: challenges and opportunities. *Environ. Sci. Pollut. Res.* 2014, **21**, 1–5.

Leung, K. M. Y., Bjørgesæter, A., Gray, J. S., Li, W. K., Lui, G. C. S., Yuan, W., Lam, P. K. S. Deriving sediment quality guidelines using field-based species sensitivity distributions. *Environ. Sci. Technol*. 2005, **39**, 5148–5156.

Li, D., Dong, M., Shim, W. J., Yim, U. H., Hong, S. H., Kannan, N. Distribution characteristics of nonylphenolic chemicals in Masan Bay environments, Korea. *Chemosphere* 2008, **71**, 1162–1172.

Li, D., Kim, M., Oh, J.-R., Park, J. Distribution characteristics of nonylphenols in the artificial Lake Shihwa, and surrounding creeks in Korea. *Chemosphere* 2004, **56**, 783–790.

Li, H., Yi, X., Cheng, F., Tong, Y., Mehler, W. T., You, J. Identifying organic toxicants in sediment using effect-directed analysis: A combination of bioaccessibility-based extraction and high-throughput midge toxicity testing. *Environ. Sci. Technol*. 2019, **53** (2), 996–1003.

Li, H., Yu, Y., Luo, W., Zeng, Y., Chen, B. Bacterial diversity in surface sediments from the Pacific Arctic Ocean. *Extremophiles* 2009, **13**, 233–246.

Li, J., Dong, H., Zhang, D., Han, B., Zhu, C., Liu, S., Liu, X., Ma, Q., Li, X. Sources and ecological risk assessment of PAHs in surface sediments from Bohai Sea and northern part of the Yellow Sea, China. *Mar. Pollut. Bull.* 2015, **96**, 485–490.

Li, J., Ou, D. Y., Zheng, L. L., Gan, N. Q., Song, L. R. Applicability of the fluorescein diacetate assay for metabolic activity measurement of *Microcystis aeruginosa* (Chroococcales, Cyanobacteria). *Phycol. Res*. 2011, **59**, 200–207.

Li, Y., Duan, X. Polycyclic aromatic hydrocarbons in sediments of China Sea. *Environ. Sci. Pollut. R*. 2015, **22**, 15432–15442.

Li, Z., Li, D., Oh, J.-R., Je, J.-G. Seasonal and spatial distribution of nonylphenol in Shihwa Lake, Korea. *Chemosphere* 2004, **56**, 611–618.

Liehr, G. A., Heise, S., Ahlf, W., Offermann, K., Witt, G. Assessing the risk of a 50-year-old dump site in the Baltic Sea by combining chemical analysis, bioaccumulation, and ecotoxicity. *J. Soil Sedi*. 2013, **13** (7), 1270–1283.

Lim, D. I., Choi, J. W., Shin, H. H., Jeong, D. H., Jung, H. S. Toxicological impact assessment of heavy metal contamination on macrobenthic communities in southern coastal sediments of Korea. *Mar. Pollut. Bull*. 2013, **73**, 362–368.

Lim, H. S., Hong, J. S. Ecology of the macrozoobenthos in Chinhae Bay, Korea 3. Community structure. *Korean J. Fisher. Aqua. Sci.* 1997, **30**, 175–187.

Lim, K. H., Shin, H. C., Yoon, S. M., Koh, C.-H. Assessment of benthic environment based on macrobenthic community analysis in Jinhae Bay, Korea. *J. Korean Soci. Oceanogra*. 2007, **12**, 9–23.

Long, E. R., Chapman, P. M. A sediment quality triad: measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. *Mar. Pollut. Bull*. 1985, **16**, 405–415.

Lotufo, G. R., Fleeger, J. W. Effects of sediment-associated phenanthrene on survival, development and reproduction of two species of meiobenthic copepods. *Mar. Ecol. Prog. Ser.* 1997, **151**, 91–102.

Louiz, I., Kinani, S., Gouze, M. E., Ben-Attia, M., Menif, D., Bouchonnet, S., Porcher, J. M., Ben-Hassine, O. K., Aït-Aïssa, S. Monitoring of dioxin-like, estrogenic and anti-androgenic activities in sediments of the Bizerta lagoon (*Tunisia*) by means of *in vitro* cell-based bioassays: Contribution of low concentrations of PAHs. *Sci. Total Environ*. 2008, **402**, 318–329.

Lozupone, C., Knight, R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol*. 2005, **71**, 8228–8235.

Lübcke-von Varel, U., Machala, M., Ciganek, M., Neca, J., Pencikova, K., Palkova, L., Vondracek, J., Löffler, I., Streck, G., Reifferscheid, G., Flückiger-Isler, S., Weiss, J. M., Lamoree, M., Brack, W. Polar compounds dominate *in vitro* effects of sediment extracts. *Environ. Sci. Technol*. 2011, **45** (6), 2384–2390.

Luo, J., Ma, M., Zha, J., Wang, Z. Characterization of aryl hydrocarbon receptor agonists in sediments of Wenyu River, Beijing, China. *Water Res.* 2009, **43** (9), 2441–2448.

Luo, J. P., Lei, B. L., Ma, M., Zha, J. M., Wang, Z. J. Identification of estrogen receptor agonists in sediments from Wenyu River, Beijing, China. *Water Res.* 2011, **45**, 3908–3914.

Lv, Q. L., Wang, G. H., Chen, S. H., Hu, L., Zhang, X., Ying, G., Zhou, H. H. *In vitro* and *in vivo* inhibitory effects of glycyrrhetinic acid in mice and human cytochrome P450 3A4. *Int. J. Environ. Res. Public Health* 2015, **13** (1), 84–91.

Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C. P., Usseglio-Polatera, P., Brack, W., Schäfer, R. B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci.* 2014, **111**, 9549–9554.

Maltby, L., Blake, N., Brock, T. C., van den Brink, P. J. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. *Environ. Toxicol*. *Chem*. 2005, **24** (2), 379–388.

Maranho, L. A., Garrido-Pérez, M. C., Baena-Nogueras, R. M.; Lara-Martín, P. A., Antón-Martín, R., DelValls, T. A., Martín-Díaz, M. L. Are WWTPs effluents responsible for acute toxicity? Seasonal variations of sediment quality at the Bay of Cádiz (SW, Spain). *Ecotoxicology* 2015, **24** (2), 368–380.

Martin, P., Bladier, C., Meek, B., Bruyere, O., Feinblatt, E., Touvier, M., Watier, L., Makowski, D. Weight of evidence for hazard identification: A critical review of the literature. *Environ. Health Perspect.* 2018, **126** (7), EHP3067

Massei, R., Hollert, H., Krauss, M., von Tümpling, W., Weidauer, C., Haglund, P., Küster, E., Gallampois, C., Tysklind, M., Brack, W. Toxicity and neurotoxicity profiling of contaminated sediments from Gulf of Bothnia (Sweden): a multi-endpoint assay with Zebrafish embryos. *Environ. Sci. Eur*. 2019, **31** (1), 8.

May W. E., Chesler S. N., Cram S. P., Gump B. H., Hertz H. S., Enagonio D. P., Dyszel S. M. Chromatographic analysis of hydrocarbons in marine sediments and seawater. *J. Chromatogr. Sci.* 1975, **13** (11), 535–540.

McLachlan, J. A., Korach, K. S., Newbold, R. R., Degen, G. H. Diethylstilbestrol and other estrogens in the environment. *Fundam. Appl. Toxicol*. 1984, **4**, 686–691.

McPherson, C., Chapman, P. M., Adrian, M., Cooper, L. The importance of benthos in weight of evidence sediment assessments — a case study. *Sci. Total Environ*. 2008, **394**, 252–264.

Meighen, E. A., Bacterial bioluminescence: organization, regulation, and application of the lux genes. *Faseb. J.* 1993, **7**, 1016–1022.

Mekenyan, O. G., Veith, G. D., Call, D. J., Ankley, G. T. A QSAR evaluation of Ah receptor binding of halogenated aromatic xenobiotics. *Environ. Health Perspect*. 1996, **104** (12), 1302–1310.

Meng, J., Hong, S., Wang, T., Li, Q., Yoon, S. J., Lu, Y., Giesy, J. P., Khim, J. S. Traditional and new POPs in environments along the Bohai and Yellow Seas: An overview of China and South Korea. *Chemosphere* 2017, **169**, 503–515.

Mennillo, E., Arukwe, A., Monni, G., Meucci, V., Intorre, L., Pretti, C. Ecotoxicological properties of ketoprofen and the S (+)-enantiomer (dexketoprofen): Bioassays in freshwater model species and biomarkers in fish PLHC-1 cell line. *Environ. Toxicol. Chem*. 2018, **37** (1), 201–212.

MOE (Ministry of Environment). Amendment of notification of designation of toxic and restricted substances administrative notice (in Korean). *Seoul, Environmental policy office, Department of hazardous chemical management*. 2016.

MOE. National emmisions of dioxins in Korea (in Korean). *Seoul, Environmental policy office, Department of hazardous chemical management*. 2012.

MOE. A designation of nonylphenolic compounds as the restricted chemical in Korea (in Korean). *Seoul, Environmental policy office, Department of hazardous chemical management*. 2007.

MOF (Ministry of Oceans and Fisheries). Environment report (in Korean). 2014. 204.

MOMAF (Ministry of Maritime Affairs and Fisheries of South Korea), Establishment of integrative management system for ocean dumping (in Korean). *Seoul, Korea*. 2005.

Moon, H.-B., Choi, H. G., Kim, S. S., Jeong, S. R., Lee, P. Y., Ok, G. Monitoring of polycyclic aromatic hydrocarbons in sediments and organisms from Korean coast. *Fish. Aquat. Sci*. 2001, **4**, 219–228.

Moon, H.-B., Yoon, S. P., Jung, R. H., Choi, M. Wastewater treatment plants (WWTPs) as a source of sediment contamination by toxic organic pollutants and fecal sterols in a semi-enclosed bay in Korea. *Chemosphere* 2008, **73**, 880–889.

Mu, X., Huang, Y., Li, J., Yang, K., Yang, W., Shen, G., Li, X., Lei, Y., Pang, S., Wang, C., Li, X., Li, Y. New insights into the mechanism of phthalate-induced developmental effects. *Environ. Pollut*. 2018, **241**, 674–683.

Mullaney, P. F., Van Dilla, M. A., Coulter, J. R., Dean, P. N. Cell sizing: A light scattering photometer for rapid volume determination. *Rev. Sci. Instrum*. 1969, **40** (8), 1029–1032.

Muxika, I., Borja, A., Bald, J. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Mar. Pollut. Bull*. 2007, **55**, 16–29.

Muz, M., Krauss, M., Kutsarova, S., Schulze, T., Brack, W. Mutagenicity in surface waters: Synergistic effects of carboline alkaloids and aromatic amines. *Environ. Sci. Technol.* 2017, **51** (3), 1830–1839.

Nannelli, A., Rossignolo, F., Tolando, R., Rossato, P., Longo, V., Gervasi, P. G. Effect of beta-naphthoflavone on AhR-regulated genes (CYP1A1, 1A2, 1B1, 2S1, Nrf2, and GST) and antioxidant enzymes in various brain regions of pig. *Toxicol*. 2009, **265** (3), 69–79.

Neff, J. M. Bioaccumulation in marine organisms: Effect of contaminants from Oil Well Produced Water (OSPW). *Elsevier Ltd., Oxford*. 2002.

Neuwoehner, J., Reineke, A. K., Hollender, J., Eisentraeger, A. Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site. *Ecotoxicol. Environ. Saf*. 2009, **72** (3), 819–827.

Noh, J., Yoon, S. J., Kim, H., Lee, C., Kwon, B.-O., Lee, Y., Hong, S., Kim, J., Ryu, J., Khim, J. S. Anthropogenic influences on benthic food web dynamics by interrupted freshwater discharge in a closed Geum River estuary, Korea. *Environ. Int*. 2019, **131**, 104981.

OECD (Organisation for Economic Co-operation and Development). Guidelines for the testing of chemicals. Test No. 236: Fish Embryo Acute Toxicity (FET) test. 2013.

Ohyama, K., Nagai, F., Tsuchiya, Y. Certain styrene oligomers have proliferative activity on MCF-7 human breast tumor cells and binding affinity for human estrogen receptor. *Environ. Health Perspect*. 2001, **109**, 699–703.

Oost, R., Beyer, J., Vermeulen, N. P. E. Fish Bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. *Toxicol. Pharm.* 2003, **13**, 57–149.

Olajire, A. A., Altenburger, R., Kuster, E., Brack, W. Chemical and ecotoxicological assessment of polycyclic aromatic hydrocarbon-contaminated sediments of the Niger Delta, Southern Nigeria. *Sci. Total Environ*. 2005, **340** (1–3), 123–136.

Olsen, R. O., Hess-Erga, O. K., Larsen, A., Hoffmann, F., Thuestad, G., Hoell, I. A. Dual staining with CFDA-AM and SYTOX Blue in flow cytometry analysis of UV-irradiated Tetraselmis suecica to evaluate vitality. *Aqua. Biol.* 2016, **25**, 39–52.

Olson, R. J., Chisholm, S. W., Zettler, E. R., Armbrust, E. V. Pigments, size, and distributions of *Synechococcus* in the North Atlantic and Pacific Oceans. *Limnol. Oceanogr*. 1990, **35**, 45–58.

Othman, H. B., Leboulanger, C., Le Floc'h, E., Hadj Mabrouk, H., Sakka Hlaili, A. Toxicity of benz[*a*]anthracene and fluoranthene to marine phytoplankton in culture: Does cell size really matter? *J. Hazard. Mater*. 2012, **243**, 204–211.

Paik, S. G., Yun, S. G. Community structure of the macrobenthos in Chinhae bay, Korea (in Korean). *J. Fisher. Aqua. Sci*. 2000, **33**, 572–580.

Palma, P., Palma, V. L., Fernandes, R. M., Soares, A. M. V. M., Barbosa, I. R. Acute toxicity of atrazine, endosulfan sulphate and chlorpyrifos to *Vibrio fischeri*, *Thamnocephalus platyurus* and *Daphnia magna*, relative to their concentrations in surface waters from the Alentejo region of Portugal. *Environ. Contam. Toxicol*. 2008, **81** (5), 485–489.

Park, C., Cha, H. G., Lee, J. H., Choi, T. S., Lee, J., Kim, Y. H., Bae, M., Shin, K., Choi, K.-H. The effects of chemical additives on the production of disinfection byproducts and ecotoxicity in simulated ballast water. *J. Sea Res*. 2017, **129**, 80–88.

Peddinghaus, S., Brinkmann, M., Bluhm, K., Sagner, A., Hinger, G., Braunbeck, T., Eisenträger, A., Tiehm, A., Hollert, H., Keiter, S. H. Quantitative assessment of the embryotoxic potential of NSO-heterocyclic compounds using zebrafish (*Danio rerio*). *Reprod. Toxicol*. 2012, **33** (2), 224–232.

Petersen, D. G., Dahllof, I. Combined effects of pyrene and UV-light on algae and bacteria in an arctic sediment. *Ecotoxicology* 2007, **16** (4), 371–377.

Pino-Otín, M. R., Ballestero, D., Navarro, E., González-Coloma, A., Val, J., Mainar, A. M. Ecotoxicity of a novel biopesticide from Artemisia absinthium on non-target aquatic organisms. *Chemosphere* 2019, **216**, 131–146.

Pushparajah, D. S., Ioannides, C. Antagonistic and synergistic interactions during the binding of binary mixtures of polycyclic aromatic hydrocarbons to the aryl hydrocarbon receptor. *Toxicol. Vitro.* 2018, **50**, 54–61.

Qiao, M., Chen, Y., Zhang, Q., Huang, S., Mei, M., Wang, C., Wang, Z. Identification of Ah receptor agonists in sediment of Meiliang Bay, Taihu Lake, China. *Environ. Sci. Technol.* 2006, **40** (5), 1415–1419.

Quero, G. M., Cassin, D., Botter, M., Perini, L., Luna, G. M. Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Front. Microbiol*. 2015, **6**, 1053.

Ra, K., Kim, E. S., Kim, K. T., Kim, J. K., Lee, J. M., Choi, J. Y. Assessment of heavy metal contamination and its ecological risk in the surface sediments along the coast of Korea. *J. Coastal Res*. 2013, 105–110.

Reed, H. E., Martiny, J. B. H. Microbial composition affects the functioning of estuarine sediments. *The ISME J*. 2013, **7**, 868–879.

Richard, C. S., Donald, W. S., George, R. D., Waldemar, A. D., Faith, A. C. Sediment toxicity, contamination, and microbenthic communities near a large sewage outfall. *ASTM special Tech. Pub.* 1985, 152–175.

Rogge, W. F., Hildemann, L. M., Mazurek, M. A., Cass, G. R., Simoneit, B. R. Sources of fine organic aerosol. 2. Noncatalyst and catalyst-equipped automobiles and heavy-duty diesel trucks. *Environ. Sci. Technol.* 1993, **27**, 636–651.

Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H., Dimming, A. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Mar. Pollut. Bull*. 2004, **49**, 728–739.

Rothhammer, V., Quintana, F.J. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat. Rev. Immunol.* 2019, **19**, 184–197.

Routledge, E. J., Parker, J., Odum, J., Ashby, J., Sumpter, J. P. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Applied Pharmacol*. 1998, **153**, 12–19.

Ryu, J., Hong, S., Chang, W. K., Khim, J. S. Performance evaluation and validation of ecological indices toward site-specific application for varying benthic conditions in Korean coasts. *Sci. Total Environ*. 2016, **541**, 1161–1171.

Saido, K., Koizumi, K., Sato, H., Ogawa, N., Kown, B. G., Chung, S.-Y., Kusui, T., Nishimura, M., Kodera, Y. New analytical method for the determination of styrene oligomers formed from polystyrene decomposition and its application at the coastlines of the North-West Pacific Ocean. *Sci. Total Environ*. 2014, **473–474**, 490–495.

Salari, M. H., Kadkhoda, Z. *In vitro* antibacterial effects of glycyrrhetinic acid on periodontopathogenic and capnophilic bacteria isolated from adult periodontitis. *Clin. Microbiol. Infect*. 2003, **9** (9), 987–988.

Schaefer, A. L., Hanzelka, B. L., Eberhard, A. Greenberg, E. P. Quorum sensing in *Vibrio fischeri*: probing autoinducer-LuxR interactions with autoinducer analogs. *J. Bacteriol.* 1996, **178** (10), 2897–2901.

Schiwy, S., Braunig, J., Alert, H., Hollert, H., Keiter, S. H. A novel contact assay for testing aryl hydrocarbon receptor (AhR)-mediated toxicity of chemicals and whole sediments in zebrafish (*Danio rerio*) embryos. *Environ. Sci. Pollut. R*. 2015, **22** (21), 16305–16318.

Schulze-Sylvester, M., Heimann, W., Maletz, S., Seiler, T. B., Brinkmann, M., Zielke, H., Schulz, R., Hollert, H. Are sediments a risk? An ecotoxicological assessment of sediments from a quarry pond of the Upper Rhine River. *J. Soils Sed.* 2016, **16** (3), 1069–1080.

Schwab, K., Altenburger, R., Lubcke-von Varel, U., Streck, G., Brack, W. Effect-directed analysis of sediment-associated algal toxicants at selected hot spots in the river Elbe basin with a special focus on bioaccessibility. *Environ. Toxicol. Chem*. 2009, **28** (7), 1506–1517.

Schymanski, E. L., Singer, H. P., Slobodnik, J., Ipolyi, I. M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., Thomaidis, N. S., Bletsou, A., Zwiener, C., Ibanez, M., Portoles, T., de Boer, R., Reid, M. J., Onghena, M., Kunkel, U., Schulz, W., Guillon, A., Noyon, N., Leroy, G., Bados, P., Bogialli, S., Stipanicev, D., Rostkowski, P., Hollender, J. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. *Anal. Bioanal. Chem*. 2015, **407** (21), 6237–6255.

Seo, J. Y., Lim, H. S., Choi, J. W. Spatio-temporal distribution of macrobenthic communities in Jinhae Bay, Korea. *Ocean Polar Res*. 2015, **37**, 295–315.

Seoane, M., Esperanza, M., Rioboo, C., Herrero, C., Cid, Á. Flow cytometric assay to assess short-term effects of personal care products on the marine microalga *Tetraselmis suecica. Chemosphere* 2017, **171**, 339–347.

Shapiro, H. M. Practical flow cytometry. *John Wiley & Sons* 2005.

Sharmin, F., Wakelin, S., Huygens, F., Hargreaves, M. Firmicutes dominate the bacterial taxa within sugar-cane processing plants. *Sci. Rep*. 2013, **3**, 3107.

Sherr, E. B., Sherr, B. F. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar. Ecol. Prog. Ser.* 2007, **352**, 187–197.

Silver, K. S., Song, W., Nomura, Y., Salgado, V. L., Dong, K., Mechanism of action of sodium channel blocker insecticides (SCBIs) on insect sodium channels. *Pest. Biochem. Physiol.* 2010, **97** (2), 87–92.

Simon, E., Lamoree, M. H., Hamers, T., de Boer, J. Challenges in effect-directed analysis with a focus on biological samples. *TrAC-Trends Anal. Chem.* 2015, **67**, 179–191.

Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., Lester, J. Nonylphenol in the environment: a critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environ. Int.* 2008, **34**, 1033–1049.

Sogbanmu, T. O., Nagy, E., Phillips, D. H., Arlt, V. M., Otitoloju, A. A., Bury, N. R. Lagos lagoon sediment organic extracts and polycyclic aromatic hydrocarbons induce embryotoxic, teratogenic and genotoxic effects in *Danio rerio* (zebrafish) embryos. *Environ. Sci. Pollut. R.* 2016, **23** (14), 14489–14501.

Spring, S., Bunk, B., Spröer, C., Schumann, P., Rohde, M., Tindall, B. J., Klenk, H.-P. Characterization of the first cultured representative of Verrucomicrobia subdivision 5 indicates the proposal of a novel phylum. *The ISME J*. 2016, **10**, 2801–2816.

Stasinakis, A. S., Gatidou, G., Mamais, D., Thomaidis, N. S., Lekkas, T. D. Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. *Water Res*. 2008, **42** (6–7), 1796–1804.

Sun, M. Y., Dafforn, K. A., Johnston, E. L., Brown, M. V. Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. *Environ. Microbiol*. 2013, **15**, 2517–2531.

Suter, G. W., Vaughan, D. S., Gardner, R. H. Risk assessment by analysis of extrapolation error: a demonstration for effects of pollutants on fish. *Environ. Toxicol. Chem.* 1983, **2** (3), 369–378.

Svenson, A., Viktor, T., Remberger, M. Toxicity of elemental sulfur in sediments. *Environ. Toxicol.* 1998, **13** (3), 217–224.

Svenson, A., Edsholt, E., Ricking, M., Remberger, M., Röttorp, J. Sediment contaminants and Microtox toxicity tested in a direct contact exposure test. *Environ. Toxicol. Water Qual. J*, 1996, **11**(4), 293–300.

Tatarazako, N., Takao, Y., Kishi, K., Onikura, N., Arizono, K., Iguchi, T. Styrene dimers and trimers affect reproduction of daphnid (*Ceriodaphnia dubia*). *Chemosphere* 2002, **48**, 597–601.

Tato, T., Salgueiro-González, N., León, V. M., González, S., Beiras, R. Ecotoxicological evaluation of the risk posed by bisphenol A, triclosan, and 4-nonylphenol in coastal waters using early life stages of marine organisms (*Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Acartia clausi*). *Environ. Pollut*. 2018, **232**, 173–182.

Thiäner, J. B., Nett, L., Zhou, S., Preibisch, Y., Hollert, H., Achten, C. Identification of 7–9 ring polycyclic aromatic hydrocarbons in coals and petrol coke using High performance liquid chromatography - Diode array detection coupled to Atmospheric pressure laser ionization - mass spectrometry (HPLC-DAD-APLI-MS). *Environ. Pollut.* 2019, **252**, 723–732.

Torsvik, V., Øvreås, L., Thingstad, T.F. Prokaryotic diversity-magnitude, dynamics, and controlling factors. *Science* 2002, **296**, 1064–1066.

Tousova, Z., Oswald, P., Slobodnik, J., Blaha, L., Muz, M., Hu, M., Brack, W., Krauss, M., Di Paolo, C., Tarcai, Z., Seiler, T.-B., Hollert, H., Koprivica, S., Ahel, M., Schollée, J. E., Hollender, J., Suter, M. J. F., Hidasi, A. O., Schirmer, K., Sonavane, M., Ait-Aissa, S., Creusot, N., Brion, F., Froment, J., Almeida, A. C., Thomas, K., Tollefsen, K. E., Tufi, S., Ouyang, X., Leonards, P., Lamoree, M., Torrens, V. O., Kolkman, A., Schriks, M., Spirhanzlova, P., Tindall, A., Schulze, T. European demonstration program on the effect-based and chemical identification and monitoring of organic pollutants in European surface waters. *Sci. Total Environ*. 2017, **601–602**, 1849–1868.

Trilecova, L., Krckova, S., Marvanova, S., Pencikova, K., Krcmar, P., Neca, J., Hulinkova, P., Palkova, L., Ciganek, M., Milcova, A., Topinka, J., Vondracek, J., Machala, M. Toxic effects of methylated benzo[*a*]pyrenes in rat liver stem-like cells. *Chem. Res. Toxicol*. 2011, **24** (6), 866–876.

USEPA (Environmental Protection Agency). Guide to using the NCP subpart J - Product schedule technical notebook. *United States Environmental Protection Agency*. 2007.

USEPA. Interim report on data and methods for assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin risks to aquatic life and associated wildlife. 1993.

USEPA. Framework for ecological risk assessment. Risk assessment forum. U.S. Envrironmental Protection Agency, Washington, DC (EPA/630/R-950001). 1992.

Valavanidis, A., Iliopoulos, N., Gotsis, G., Fiotakis, K. Persistent free radicals, heavy metals and PAHs generated in particulate soot emissions and residue ash from controlled combustion of common types of plastic. *J. Hazard. Mater*. 2008, **156**, 277–284.

Valls, M., De Lorenzo, V. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS Microbiol. Rev*. 2002, **26**, 327–338.

Vedani, A., Dobler, M., Hu, Z., Smieško, M. OpenVirtualToxLab — A platform for generating and exchanging *in silico* toxicity data. *Toxicol. Lett*. 2015, **232** (2), 519–532.

Villeneuve, D. L., Khim, J., Kannan, K., Giesy, J. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxin-like and estrogenic responses in three cell lines. *Environ. Toxicol*. 2002, **17**, 128–137.

Villeneuve, D. L., Khim, J. S., Kannan, K., Giesy, J. P. *In vitro* response of fish and mammalian cells to complex mixtures of polychlorinated naphthalenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons. *Aquat. Toxicol.* 2001, **54** (1–2), 125–141.

Villeneuve, D. L., Blankenship, A. L., Giesy, J. P. Derivation and application of relative potency estimates based on *in vitro* bioassay results. *Environ. Toxicol. Chem*. 2000, **19** (11), 2835–2843.

Villeneuve, D. L., De Vita, W., Crunkilton, R. L. Identification of cytochrome P4501A inducers in complex mixtures of polycyclic aromatic hydrocarbons. In: *Environmental Toxicology and Risk Assessment: Seventh Volume*, ed. E. Little, B. Greenberg, and A. DeLonay (West Conshohocken, PA: ASTM International). 1998, 92–104.

Vrabie, C. M., Sinnige, T. L., Murk, A. J., Jonker, M. T. Effect-directed assessment of the bioaccumulation potential and chemical nature of Ah receptor agonists in crude and refined oils. *Environ. Sci. Technol*. 2012, **46** (3), 1572–1580.

Wang, C., Jiao, X., Liu, G. A toxic effect at molecular level can be expressed at community level: A case study on toxic hierarchy. *Sci. Total Environ*. 2019, **693**, 133573.

Wang, C., Su, R., Zhang, Y., Liu, G. A method linking the toxic effects at community-level with contaminant concentrations. *Sci. Total Environ*. 2016, **569–570**, 352–360.

Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 2007, **73**, 5261–5267.

Wang, S. Y., Wang, G. K., Voltage-gated sodium channels as primary targets of diverse lipid-soluble neurotoxins. *Cell Signal*. 2003, **15** (2), 151–159.

Wang, Y., Wen, Y., Li, J. J., He, J., Qin, W. C., Su, L. M., Zhao, Y. H. Investigation on the relationship between bioconcentration factor and distribution coefficient based on class-based compounds: The factors that affect bioconcentration. *Environ. Toxicol. Pharmacol*. 2014, **38** (2), 388–396.

Wania, R., Mackay, D. Tracking the distribution of persistent organic pollutants. *Environ. Sci. Technol.* 1996, **30**, 390–396.

Weiss, J. M., Hamers, T., Thomas, K. V., van er Linden, S., Leonards, P. E. G., Lamoree, M. H., Masking effect of anti-androgens on androgenic activity in European river sediment unveiled by effect-directed analysis. *Anal. Bioanal. Chem*. 2009, **394** (5), 1385–1397.

Wernersson, A. S., Carere, M., Maggi, C., Tusil, P., Soldan, P., James, A., Sanchez, W., Dulio, V., Broeg, K., Reifferscheid, G. The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environ. Sci. Europe.* 2015, **27**, 7.

White, J. J., Schmitt, C. J., Tillitt, D. E. The H4IIE cell bioassay as an indicator of dioxin-like chemicals in wildlife and the environment. *Crit. Rev. Toxicol*. 2004, **34** (1), 1–83.

White, R., Jobling, S., Hoare, S., Sumpter, J., Parker, M. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 1994, **135**, 175–182.

Woo, H. J., Cho, J. H., Choi, J. U. Characteristics of heavy metals and benthic foraminifera on surface sediments in Masan Bay and Gadeog Channel, Korea. *Ocean Polar Res*. 2007, **29**, 233–244.

Wu, Q. L., Zwart, G., Schauer, M., Kamst-van Agterveld, M. P., Hahn, M. W. Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. *Appl. Environ. Microbiol*. 2006, **72**, 5478–5485.

Xiao, H., Krauss, M., Floehr, T., Yan, Y., Bahlmann, A., Eichbaum, K., Brinkmann, M., Zhang, X., Yuan, X., Brack, W., Hollert, H. Effect-directed analysis of aryl hydrocarbon receptor agonists in sediments from the Three Gorges Reservoir, China. *Environ. Sci. Technol*. 2016, **50** (20), 11319–11328.

Xie, T., Liang, Y., Hao, H., A, J., Xie, L., Gong, P., Dai, C., Liu, L., Kang, A., Zheng, X., Wang, G. Rapid identification of ophiopogonins and ophiopogonones in Ophiopogon japonicus extract with a practical technique of mass defect filtering based on high resolution mass spectrometry. *J. Chromatogr. A*. 2012, **1227**, 234–244.

Xie, Y., Wang, J., Wu, Y., Ren, C., Song, C., Yang, J., Yu, H., Giesy, J. P., Zhang, X. Using *in situ* bacterial communities to monitor contaminants in river sediments. *Environ. Pollut*. 2016, **212**, 348–357.

Xie, Y., Wang, J., Yang, J., Giesy, J. P., Yu, H., Zhang, X. Environmental DNA metabarcoding reveals primary chemical contaminants in freshwater sediments from different land-use types. *Chemosphere* 2017, **172**, 201–209.

Xie, Y., Zhang, X., Yang, J., Kim, S., Hong, S., Giesy, J. P., Yim, U. H., Shim, W. J., Yu, H., Khim, J. S. eDNA-based bioassessment of coastal sediments impacted by an oil spill. *Environ. Pollut*. 2018, **238**, 739–748.

Xu, E. G., Leung, K. M. Y., Morton, B., Lee, J. H. An integrated environmental risk assessment and management framework for enhancing the sustainability of marine protected areas: The Cape d'Aguilar Marine Reserve case study in Hong Kong. *Sci. Total Environ.* 2015, **505**, 269–281.

Yanagiba, Y., Ito, Y., Yamanoshita, O., Zhang, S.-Y., Watanabe, G., Li, C.M., Kamijima, M., Gonzalez, F. J. Styrene trimer may increase thyroid hormone levels via down-regulation of the aryl hydrocarbon receptor (AhR) target gene UDP-glucuronosyltransferase. *Environ. Health Perspect*. 2008, **116** (6), 740–745.

Yim, U. H., Hong, S. H., Ha, S. Y., Han, G. M., An, J. G., Kim, N. S., Lim, D. I., Choi, H. W., Shim, W. J. Source- and region-specific distribution of polycyclic aromatic hydrocarbons in sediments from Jinhae Bay, Korea. *Sci. Total Environ*. 2014, **470**, 1485–1493.

Yim, U. H., Hong, S. H., Shim, W., Oh, J., Chang, M. Spatio-temporal distribution and characteristics of PAHs in sediments from Masan Bay, Korea. *Mar. Pollut. Bull*. 2005, **50**, 319–326.

Yim, U. H., Hong, S. H., Shim, W. J. Distribution and characteristics of PAHs in sediments from the marine environment of Korea. *Chemosphere* 2007, **68**, 85–92.

Ying, G. G., Williams, B., Kookana, R. Environmental fate of alkylphenols and alkylphenol ethoxylates-a review. *Environ. Int*. 2002, **28**, 215–226.

Yoo, H., Khim, J. S., Giesy, J. P. Receptor-mediated *in vitro* bioassay for characterization of AhR-active compounds and activities in sediment from Korea. *Chemosphere* 2006, **62**, 1261–1271.

Yoon, S. J., Hong, S., Kwon, B.-O., Ryu, J., Lee, C. H., Nam, J., Khim, J. S. Distributions of persistent organic contaminants in sediments and their potential impact on macrobenthic faunal community of the Geum River estuary and Saemangeum Coast, Korea. *Chemosphere* 2017, **173**, 216–226.

You, J., Li, H. Improving the accuracy of effect-directed analysis: the role of bioavailability. *Environ. Sci. Proc. Imp.* 2017, **19**, 1484–1498.

Yunker, M. B., Macdonald, R. W., Vingarzan, R., Mitchell, R. H., Goyette, D., Sylvestre, S. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Org. Geochem*. 2002, **33**, 489–515.

Zedda, M., Zwiener, C. Is nontarget screening of emerging contaminants by LC-HRMS successful? A plea for compound libraries and computer tools. *Anal. Bioanal. Chem*. 2012, **403** (9), 2493–2502.

Zhang, S., Yao, H., Lu, Y., Yu, X., Wang, J., Sun, S., Liu, M., Li, D., Li, Y.-F., Zhang, D. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. *Sci. Rep*. 2017, **7**, 12165.

Zhang, X. Environmental DNA shaping a new era of ecotoxicological research. *Environ. Sci. Technol*. 2019, **53**, 5605–5612.

Zhao, K., Ding, M., Cao, H., Cao, Z. X. *In vitro* metabolism of glycyrrhetinic acid by human and rat liver microsomes and its interactions with six CYP substrates. *J. Pharm. Pharmacol.* 2012, **64** (10), 1445–1451.

Zvinavashe, E., van den Berg, H., Soffers, A. E., Vervoort, J., Freidig, A., Murk, A. J., Rietjens, I. M. QSAR models for predicting *in vivo* aquatic toxicity of chlorinated alkanes to fish. *Chem. Res. Toxicol*. 2008, **21** (3), 739–745.

ABSTRACT (IN KOREAN)

해양 저서퇴적물은 육상기인오염물질의 최종 종착지이며, 퇴적물 내 장기간 잔류하는 오염물질은 다양한 해양생물에게 해로운 영향을 미친다. 특히, 잔류성 유기오염물질은 생물농축 · 확대 등을 통해 궁극적으로 인간의 건강까지 위협한다는 점에서 오염퇴적물 생태위해성 평가는 매우 중요하다. 전통적으로 오염퇴적물 평가는 1) 오염물질의 농도, 2) 생물학적 영향, 3) 저서군집 구조의 세가지 측면에서 개별적으로 이루어졌다. 그러나 미지독성물질 또는 기물질의 혼합 영향 등으로 인해 기존의 단편적 오염평가는 생태위해성을 종합적으로 평가하는데 한계가 있었다. 본 연구에서는 생물영향동정 기반의 다중증거 접근법을 이용하여 국내 연안퇴적물 내 유기오염물질의 생태위해성을 통합 평가하였다. 특히, 유기오염물질의 시 · 공간 변화에 따른 생물영향 특성과 생태위해성을 평가하고, 국내 연안의 우선관리대상물질을 제시하였다. 연구 지역은 육상기인오염물질이 다량으로 유입되는 시화호, 마산만 인근의 특별관리해역과 대도시, 산업단지 등이 밀집된 서해 연안역으로 하였다.

첫째로, 특별관리해역의 저서퇴적물 오염도 평가를 위해 퇴적물 내 다환방향족탄화수소, 알킬페놀류, 스티렌올리고머 화합물의 시 · 공간적인 농도변화와 분포특성을 파악하였다. 1990년대 말 기채취 된 시료와 최근(2014–15)에 채취한 시료를 동시분석한 결과, 다환방향족탄화수소와 알킬페놀류 화합물 농도는 과거에 비해 최근에 현저히 감소한 것으로 나타났다. 또한 2015년 국내 연안퇴적물에서 최초로 검출되었던 스티렌올리고머의 경우, 과거에도 시화호와 마산만 퇴적물 내에 고농도로 존재했음이 확인되었다. 대체로 지난 20년간 국내 연안퇴적물의 오염도는 감소 추세를 보였는데, 이는 2000년대부터 시행한 ‘연안오염총량관리제’와 최근 정착된 ‘유해물질 배출 규제’의 정책적 효과로 여겨진다. 한편, 대부분의 유기오염물질은 육상과 인접한 정점에서 상대적으로 높게 검출되었는데, 이는 저서퇴적물의 오염을 저감하기 위해서 육상기인오염물질의 우선관리가 시급함을 시사한다.

둘째로, 저서퇴적물 내 잔류하는 유기오염물질의 생물학적 영향을 파악하기 위해, 시화호와 마산만 퇴적물을 유기추출하고, 화합물의 극성에 따라 분액화한 후 다양한 생물을 대상으로 노출평가를 수행하였다. 유전자 재조합

세포(H4IIE-*luc*, MVLN)를 이용한 유기추출액(또는 분액)의 생물활성 반응은 퇴적물 내 유기오염물질의 농도와 유의한 상관관계를 보였다. 하지만 두 세포주의 등가농도 분석결과, 검출된 화합물의 등가농도는 전체 발현된 세포 활성치 보다 매우 낮게 나타났다. 이는 퇴적물 시료 내 미지의 아릴 탄화수소 수용체 또는 에스트로겐 수용체 활성 화합물이 다수 혹은 다량으로 존재함을 시사한다. 해양박테리아의 발광저해도와 제브라피쉬 배아의 치사 영향은 퇴적물 내 존재하는 무극성 화합물에 의한 것으로 확인되었다. 한편, 미세조류의 생리 활성 영향 중, 효소활성은 중극성 화합물의 영향을 받는 것으로 나타났고, 세포막 손상은 주로 극성 화합물(노닐페놀 등)에 기인한 것으로 확인되었다. 퇴적물 내 노닐페놀 농도는 과거에 비해 감소했음에도, 여전히 미세조류의 활성에 부정적인 영향을 미치고 있어 향후 지속적인 모니터링이 요구된다. 종합적으로, 생물학적 영향은 화합물질(종류, 농도)-, 생물 종-, 메커니즘-특이적으로 나타났다. 따라서, 오염퇴적물의 생태위해성을 정확히 평가하기 위해서는 적합한 생물검정법을 선택하는 것이 매우 중요하다.

셋째로, 생물영향동정법을 이용하여 아릴 탄화수소 수용체 활성을 매개하는 미지의 화합물질을 검색하여 유의한 세포활성능을 보이는 물질을 선별하였다. 본 실험을 위하여 비표적 기기분석과 H4IIE-*luc* 세포 활성 평가를 함께 수행하였다. 그 결과, 퇴적물 내 잔류하는 에녹솔론(피부치료제 성분)이 아릴 탄화수소수용체 활성에 기여했음을 새롭게 밝혀냈다. 에녹솔론은 아릴 탄화수소 수용체와 가장 결합능이 높은 벤조에이피렌과 비교하여 약 10%의 결합능을 가지는 것으로 확인되었다. 따라서 향후 오염퇴적물의 우선관리대상물질 선정에 있어 에녹솔론류 물질의 포함 여부에 대한 검토가 요구된다.

끝으로, 오염퇴적물의 생태위해성 평가를 위해 미생물과 대형저서동물의 군집구조를 살펴보았다. 서해연안 퇴적물 내 미생물 군집구조의 경우, 환경요인(지리적 위치, 염분) 보다는 오염물질 농도 변화에 따라 우점군이 급변하였다. 대표적으로 Planctomycetes 미생물군의 경우, 카드뮴과 다환방향족 탄화수소의 농도가 높아질수록 개체군 밀도(상대 풍부도)가 유의하게 감소하는 것으로 나타났다. 마산만 퇴적물 내 대형저서동물 중 가장 우점하는 분류군은 다모류였으며, 특히 내측 정점에서 과거(1998년)와 최근(2015년) 모두 유기오염지시종인 등가시버들갯지렁이가 극우점하였다. 이는 오염원에 인접한

지역에서 일부 대형저서동물이 장기간 지속적인 영향을 받고 있음을 시사한다. 한편 생태지수 분석 결과 마산만 저서퇴적물 오염도는 최근에, 특히 외해에서 현저히 감소한 것으로 확인되었다. 그러나 대형저서동물 군집의 건강도는 오염물질 농도의 급격한 감소 또는 생물학적 영향 저감에도 불구하고, 뚜렷한 증가 추세를 보이지 않았다.

이상의 연구결과를 종합 요약하면, 첫째, 국내 연안 저서퇴적물 내 유기오염물질은 최근 전반적으로 감소했으나, 일부 핫스팟 지역에서 여전히 유의한 생물 영향을 보였다. 둘째, 유기오염물질의 종류와 농도, 대상 생물의 종류와 측정 항목에 따라 저서퇴적물의 오염도 및 생태위해성이 상이하게 평가되었다. 본 연구에서 제시한 다중증거접근법은 기존 퇴적물 오염평가법을 대체할 수 있는 효과적인 오염퇴적물 생태위해성 평가법으로 사료된다. 향후, 저서 퇴적물의 오염도가 심각한 연안 해역을 체계적으로 관리하기 위해서는 지속적인 생태위해성 평가 기법의 개발이 요구된다.

주제어: 해양오염,
오염퇴적물,
잔류성오염물질,
생물검정법,
생물영향동정분석,
해양생태위해성 통합평가

학 번: 2015-30986