Environmental Toxicology and Pharmacology 87 (2021) 103704

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Toxicity assessment of urban marine sediments from Western Norway using a battery of stress-activated receptors and cell-based bioassays from fish

Siri Øfsthus Goksøyr [a], Helene Sørensen [a], Bjørn Einar Grøsvik [b], Daniela M. Pampanin [c], Anders Goksøyr [a], Odd André Karlsen [a,*]

[a] Department of Biological Sciences, University of Bergen, Norway
[b] Institute of Marine Research, Bergen, Norway
[c] Department of Mathematics and Natural Science, University of Stavanger, Norway

ARTICLE INFO

Edited by Dr. M.D. Coleman

Keywords:
Toxicity testing
Nuclear receptors
Atlantic cod
Risk assessment

ABSTRACT

A luciferase reporter gene-based bioassay battery consisting of stress-activated receptors from fish, complemented with traditional fish cell-based bioassays, were used to assess the toxicity of marine sediment samples from the Byfjorden area around the city of Bergen (Norway). The reporter assays covered a wide range of cellular signalling and metabolic pathways, representing different molecular initiating events in the adverse outcome pathway framework. Cytotoxicity, generation of reactive oxygen-species, and induction of 7-ethoxyresorufin-O-deethylase activity were analysed using fish liver and gill cell lines. Chemical analyses of the sediment extracts revealed complex contamination profiles, especially at the innermost stations, which contained a wide array of persistent organic pollutants, polycyclic aromatic hydrocarbons, and metals. Sediment extracts from these sites were more potent in activating the stress-activated receptors than the other extracts, reflecting their toxicant profiles. Importantly, receptor- and cell-based bioassays complemented the chemical analyses and provided important data for future environmental risk assessments of urban marine sediments.

1. Introduction

The marine environment has been a sink for environmental pollutants originating from anthropogenic activities for generations. When the pollution load in marine sediments increases, they also become a source of contaminants that can be further transferred into aquatic food chains via bottom-dwelling organisms (Kosmehl et al., 2007). Approaches to assess sediment quality are therefore essential in order to monitor the health status of aquatic environments and to take the necessary measures to reduce threats and prevent adverse effects on aquatic species and ecosystems. Different programmes and conventions have been initiated to monitor and manage the pollution load in the environment, including the European Water Framework Directive (WFD) (European Commission, 2000), the Marine Strategy Framework Directive (European Commission, 2008), and the Oslo-Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 1992).

Chemical analyses are commonly applied to reveal the presence of contaminants in sediments, and environmental quality standards (EQS) are used for referring to acceptable levels of contaminants in environmental samples (European Commission, 2000; Lepper, 2004, 2005; Gardner et al., 2008). However, chemical analyses cannot be used as the only strategy to evaluate environmental quality as current methodologies are not able to identify every single chemical present, nor the bioavailability of pollutants, and importantly, possible biological effects, including additive, synergistic, potentiating, or antagonistic interactions that may occur when organisms are exposed to mixtures of compounds (Chapman, 2007). In addition, the most abundant chemicals are not necessarily the ones producing the highest biological impact in aquatic organisms (Brack et al., 2005). As described in the adverse outcome pathway (AOP) framework, the interaction of chemicals with organisms initially takes place at the molecular and cellular levels, denoted as the molecular initiating events (MIE) (Ankley et al., 2010). Responses taking place at these levels are considered the first manifestation of toxicity. They often involve stress-activated transcription factors such as the aryl hydrocarbon receptor (AHR) or nuclear receptors (NRs), which can be used in the development of molecular tools that can be applied for the early and sensitive detection of chemical exposures

* Corresponding author.
E-mail address: odd.karlsen@uib.no (O.A. Karlsen).

https://doi.org/10.1016/j.etap.2021.103704
Received 29 March 2021; Received in revised form 8 July 2021; Accepted 12 July 2021
Available online 14 July 2021
1382-6689/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

(Fent, 2001). Different receptor and cell-based bioassays, using fish and human cell models, have previously been successfully used to estimate the biological activity of sediment-bound pollutants, integrating their interactions and covering endpoints such as acute and long-term toxicity, oxidative-stress, bioaccumulation, and endocrine disrupting effects (Creusot et al., 2010; Schnell et al., 2013; Fernandes et al., 2014; Pérez-Albaladejo et al., 2016; Blanco et al., 2018).

Atlantic cod (*Gadus morhua*) is an ecologically and economically important teleost widely distributed in the North Atlantic Ocean. Atlantic cod has commonly been used as a bioindicator species in marine pollution monitoring programs and field studies, and has increasingly been used as a model in environmental toxicological studies (OSPAR, 1992; Goksøyr et al., 1994; Beyer et al., 1996; Hylland et al., 2008; Sundt et al., 2012; Bagi et al., 2018; Dale et al., 2019), which has been facilitated by its sequenced genome (Star et al., 2011). Based on the genomic sequence, the chemical defensome of Atlantic cod, including members of the NR superfamily, was recently characterized (Eide et al., 2018, 2021; Goldstone et al., 2006). Notably, a well-known NR regarding xenobiotic sensing and response, the pregnane X receptor (Pxr), is lacking in the Atlantic cod genome, and also in most other gadiform species (Eide et al., 2018). However, the remaining complement of members in the NR superfamily are present in the Atlantic cod genome, as well as the recently characterized basic helix-loop-helix/Per-Arnt-Sim proteins, the two aryl hydrocarbon receptors (Ahrs), Ahr1a and Ahr2a (Arangur-en-Abadia et al., 2020). In this study, we have used a battery of such ligand- and stress-activated receptors from Atlantic cod to assess the environmental quality of sediment samples collected from different sites in the fjord and harbours surrounding Bergen, the largest city on the Western coast of Norway, and the second largest city in the country (approx. population 285,000). Bergen has historically been an important harbour for ship traffic and maritime industries, and its surrounding environment has for decades received discharges from shipyards, factories, sewage and harbour activities. This has resulted in elevated contaminant levels in both the seabed and biota, including mussels and fish in the rather closed fjord systems around Bergen (Skei et al., 1994; Andersen et al., 1996).

The receptor battery selected in our study includes the androgen receptor alpha (Ara), the estrogen receptor alpha (Era), the peroxisome proliferator-activated receptors alpha 1 and 2 (Ppara1, Ppara2), the vitamin D receptors alpha and beta (Vdra, Vdrb), as well as Ahr1a and Ahr2a, representing key regulators of a diverse set of cellular signalling pathways and physiological processes, such as reproductive functions, energy metabolism, vitamin D signalling, and xenobiotic sensing and response. In addition, although absent in Atlantic cod, the pregnane X receptor (Pxr) from zebrafish was included as an important xenosensor present in most vertebrate species (Lille-Langøy et al., 2019). The battery of stress-activated receptors was complemented with more traditional fish cell-based bioassays. The topminnow (*Poeciliopsis lucida*) PLHC-1 liver cell line and the rainbow trout (*Oncorhynchus mykiss*) RTgill-W1 cell line were used for measuring toxicological endpoints related to oxidative stress, 7-ethoxyresorufin-O-deethylase (EROD) activity, and cell viability.

The aim of this study was to assess how a battery of bioassays based on a collection of stress-activated receptors from an indicator species like Atlantic cod, combined with fish cell-based bioassays, can complement chemical analyses in toxicity assessment of sediment samples. Such comparison can provide insights into mechanisms of toxicity and provide improved tools for risk assessments.

2. Materials and methods

2.1. Sediment collection and extraction

Sediment samples were collected from six different locations within the Bergen Byfjorden area (Station (St.) 1–6), in addition to a more pristine location at Korsfjorden (St. 7) (Fig. 1, Table S1) in January

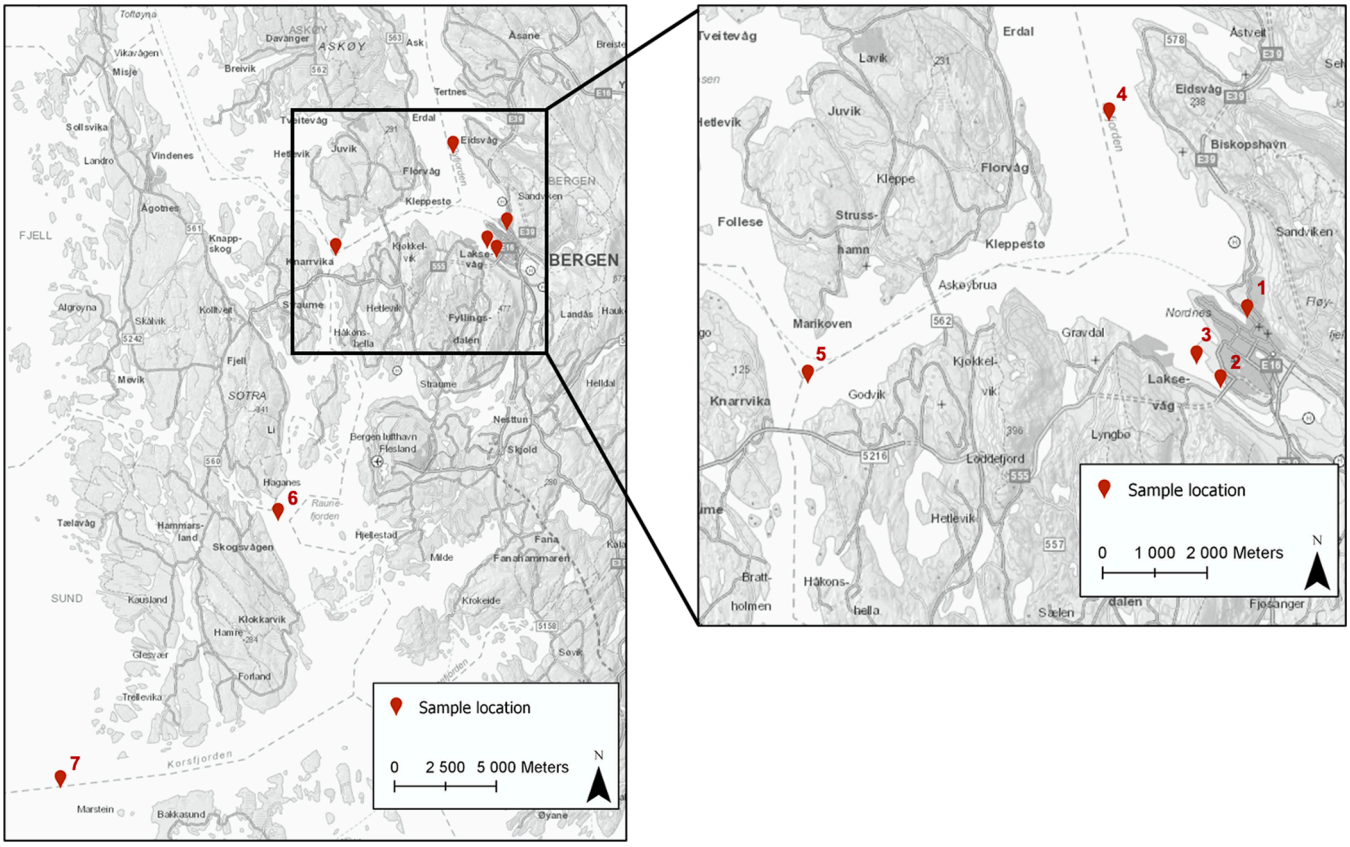

Fig. 1. Locations of sediment sampling in the Bergen Byfjorden area. Station coordinates are listed in Supplementary Table S1.

2018. The sediments were collected using a van Veen grab (KC Denmark, Silkeborg, Denmark). The upper 1 cm was collected to analyse the most recent sedimented material. The samples were frozen, and later divided into three parts; one part was used for chemical analyses performed by Eurofins Environment Testing Norway AS, the second part was dedicated to chemical analyses carried out at the Institute of Marine Research (IMR), Bergen, Norway, and the third part was utilised for the solvent extraction of environmental pollutants for bioassay studies. Sediments to be analysed for organic contaminants at IMR or for bioassays were airdried before 10 g were extracted by accelerated solvent extraction (ASE™, Dionex™ 300, ThermoFisher™). This was performed in two cycles with the following conditions; 100 °C, 1500 psi, 60 % "flush volume", and a solvent of 1:1 hexane:dichloromethane. The 35 mL ASE cells were filled with a mix of sediment and 10 %-deactivated alumina and refilled with Diatomaceous Earth. Sediment extracts were evaporated to a volume of 0.5 mL and active copper was added over night to remove sulfur residues. Extracts were purified and fractionated with hexane and a 1:1 hexane:dichloromethane solution by PowerPrep (Fluid Management Systems, Inc.) using 7.5 g silica columns. Fractions were pooled, and further evaporated until only a small amount of liquid was present, before being dissolved in either DMSO for cell based assays, or in isooctane for analyses of polychlorinated biphenyls (PCB), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDE). The sediment extracts reconstituted in DMSO were equivalent to 12 g dry weight sediment (eQsed)/mL, which was further serially diluted in DMSO to the desired concentrations used for cell-based assays. St. 1 was reanalysed for organochlorines and PBDE by extracting from only 1 g sediment due to the high levels of contaminants present at this site.

2.2. Chemical analysis

Chemical analyses of metals and perfluoroalkyl chemicals (PFAS) in the sediments were performed by Eurofins, Norway (accredited by Norwegian Accreditation, NS-EN ISO/IEC 17,025). Analyses of polycyclic aromatic hydrocarbons (PAHs), OCPs, PCBs, and PBDEs were performed at IMR. Analyses of PAHs were performed using the analytical method accredited by the official Norwegian accreditation body (Norwegian Accreditation) according to the European quality assurance (QA) standard NS-EN ISO/IEC-17,025 using a GC HP-6890 coupled to an Agilent N-5975 mass spectrometry (MS) (EI-SIM) instrument as described by Boitsov et al. (Boitsov et al., 2020). Limit of quantifications (LOQs) for PAHs were 0.5 μg/kg dry weight. Analyses of organochlorines were performed using a gas chromatography (GC) (Agilent 7890)/MS (triple quadropole MS 7010) system. The GC column was an Agilent DB-5 ms (15 cm length, 0.25 mm inner diameter). ^{13}C labelled internal standards were used for quantifications. LOQs for organochlorines were 0.02 μg/kg dry weight. Analyses of PBDE were performed on GC (Agilent 6890) coupled to a triple quadrupole MS 7010 equipped with a GC column (Agilent DB-5 ms, 15 m length, 0.25 mm inner diameter). LOQs for PBDEs were 0.03 μg/kg dry weight.

2.3. Cell cultures

Topminnow PLHC-1 liver cells were cultured using a supplemented Eagles's minimum essential medium in a 5% carbon dioxide (CO_2) humidified incubator at 30 °C (Blanco et al., 2018). RTgill-W1 gill cells from rainbow trout were cultured at 18 °C using the Leibovitz's L-15 medium supplemented with fetal bovine serum (Biowest), L-glutamine (Corning), penicillin and streptomycin (Biowest), according to protocols from ATCC®. Both cell lines were seeded with a cell density of 40,000 cells/mL in 96 well plates, which was determined using the Muse® Cell Analyzer from an 90 % confluent culture flask. After 24 h incubation, culture medium was replaced with appropriate medium containing sediment extracts at selected concentrations.

2.4. Cell viability

Cytotoxic effects produced by the sediment extracts were assessed by monitoring the metabolic activity of exposed PLHC-1 liver cells and RTgill-W1 gill cells using the fluorescent dye Alamar Blue (Alfa Aesar) according to Dayeh et al. (2003). The fluorescence was recorded at 530/590 nm using a SpectraMax Paradigm Multi-Mode plate reader. H_2O_2 was used as positive control for cytotoxicity. Results were expressed as percentage viability (mean ± standard deviation adjusted to 100 %) in comparison to solvent exposed cells from four replicate experiments. Four replicates were prepared for each concentration of the sediment extract, solvent control (DMSO, 0 mg eQsed/mL), and 0.1 μM H_2O_2 as a positive control.

2.5. Reactive oxygen species

The generation of reactive oxygen species (ROS) in PLHC-1 liver cells and RTgill-W1 gill cells exposed to sediment extracts was determined by measuring the fluorescence of the oxidized probe (2′7′-dichlorodihydrofluorescein diacetate, Invitrogen) at 485/528 nm as described in (Blanco et al., 2018). The exposure to H_2O_2 was used as a positive control of cellular ROS production. Results were expressed as percentage change in fluorescence relative to solvent exposed cells (mean ± standard deviation adjusted to 100 %) from four replicate experiments. Four replicates were prepared for each concentration of the sediment extract, solvent control (DMSO, 0 mg eQsed/mL), and 0.1 μM H_2O_2 as a positive control.

2.6. Ethoxyresorufin-O-deethylase activity

The ethoxyresorufin-O-deethylase activity (EROD) assay was performed using PLHC-1 liver cells as reported in Fernandes et al. (2014), with some modifications (Blanco et al., 2018). β-naphthoflavone (BNF, Alfa Aesar) was used as a positive control for induction of EROD activity. The formation of the fluorescent resorufin was recorded at 537/583 nm, and the BCA kit (Pierce™ BCA Protein Assay Kit) was used to determine total cellular protein content in the samples. Results were expressed as pmol of resorufin formed per minute per milligram of protein (pmol/min/mg protein). Three replicates were prepared for each concentration of the sediment extract, solvent control (DMSO, 0 mg eQsed/mL), and a positive control (1 μM BNF).

2.7. Luciferase reporter gene assay

Luciferase reporter gene assays were used to study ligand activation of stress-activated receptors, using transiently transfected COS-7 simian kidney cells. The cells were maintained at 37 °C in a humidified incubator with 5% CO_2 in phenol red Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1 mM sodium pyruvate, 4 mM L-glutamate, and 100 U/mL penicillin and streptavidin (Sigma Aldrich). Cells were subcultured when 80–90 % confluency was reached by dissociating the cells with 0.05 % trypsin-EDTA. The cells were then seeded into 96-well plates (5000 cells/well) and incubated for 24 h before the cells were co-transfected with the plasmid mixes (100 ng/well).

Transfection mixes were prepared in two different ways. For the gmAhr assay, full length Ahr1a (acc. MN329012) and Ahr2a (acc. MN329013) were used by co-transfecting pcDNA3.1/Zeo(+)-based gmARNT1 plasmids with pcDNA3.1/Zeo(+)-based gmAhr1a or gmAhr2a, the luciferase reporter plasmid (pGudLuc6.1), and β-galactosidase normalization plasmids (pCMV-βGAL) as described previously (Aranguren-Abadia et al., 2020). For the NRs, including Atlantic cod gmEra, gmAra, gmPpara1, gmPpara2, gmVdra, gmVdrb, and zebrafish drPXR, only constructs with the ligand binding domains (LBDs) fused to the GAL4-DNA binding domain (DBD) were used essentially as described in (Lille-Langøy et al., 2015). A GAL4-UAS luciferase reporter plasmid

(tk-(MH100)x4 luc) was co-transfected with the pCMX-GAL4(DBD)-NR (LBD) receptor plasmid (2:1 reporter:receptor plasmid weight ratio), and pCMV-βGAL (TransIT®-LT1 Transfection Reagent, Mirus). Twenty-four hours post-transfection the medium was removed, and the cells exposed to sediment extracts in phenol red-free DMEM. The following day the cells were lysed, and luciferase activity and β-galactosidase activity were recorded for determining receptor activation and transfection efficiency, respectively, on a Perkin Elmer Enspire plate reader. Ethynylestradiol, testosterone, WY-14643, calcitriol, 6-formylindolo(3,2-b)carbazole, and clotrimazole were used as model agonists for gmEra, gmAra, gmPpars, gmVdrs, gmAhrs, and drPxr, respectively (Fig. S1). The Atlantic cod Era, Ara, Ppara1, Ppara2, Vdra, and Vdrb LBD sequences used in the receptor plasmids have been deposited in GenBank with the following accession numbers, MW690580, MW690581, MW690582, MW690583, MT344110 and MT344111, respectively.

The viability of the COS-7 cells was assessed after exposure to sediment extracts by measuring mitochondrial metabolic activity with the resazurin assay (Pérez-Albaladejo et al., 2016). None of the sediment extracts produced any cytotoxic effects on the COS-7 cells at the concentrations used in the luciferase assay (Supplementary Fig. S2).

2.8. Principal component analysis

Principal component analysis (PCA) was used to identify patterns in the multivariate data sets. Two PCA plots were generated based on the results from the bioassays (luciferase, cytotoxicity, ROS, and EROD), and chemical analysis data using the PCA tool in GraphPad Prism 9 (Graphpad Software, San Diego, Ca, USA). The data were standardized (scaled to have a mean of 0 and a SD of 1), and PCA plots were generated based on eigenvalues.

2.9. Statistics

For all results presented, Graphpad Prism 9 was used for statistical analyses. Normality was assessed with the Shapiro Wilks test. Normally distributed data were analysed using one-way ANOVA. If the data were not normally distributed, the non-parametric Kruskal Wallis test was used.

3. Results and discussion

In this study, traditional fish cell-based bioassays were complemented with luciferase-based reporter gene assays using a battery of stress-activated receptors from Atlantic cod and zebrafish to investigate the presence of bio-active pollutants in sediments from contaminated sites within the Bergen Byfjorden area. Comprehensive chemistry data of the sediment samples were acquired in parallel. A range of contaminant and toxicity profiles were reported in sediments from the innermost harbour sites of Byfjorden, indicating both general cytotoxicity, ROS-production, Ahr and NR agonist activity.

3.1. Chemicals

Previous analyses have demonstrated that Bergen Byfjorden contains high levels of PAHs, PCBs, and heavy metals such as lead, mercury and cadmium, and is thus regarded as a polluted fjord according to the WFD (Jartun et al., 2009; Jartun and Pettersen, 2010). Priority substances under the WFD (European Commission, 2000) have been assigned EQS, which is used for monitoring environmental pollutant status and prevent potential negative effects in aquatic ecosystems. EQS have been divided into AA-EQS (annual allowance) and MAC-EQS (maximum allowable concentration), where the concentration of a particular pollutant or group of pollutants in water, sediment, or biota is set to protect against long-term (chronic) exposure and acute effects of short-term exposure, respectively. Chemical analyses of the sediment extracts obtained from the individual stations in the Byfjorden area revealed distinct differences in the content of PAHs, metals, PCBs, PBDEs and OCPs (Fig. 2 and Supplementary Table S3 and Table S6). The total PAH content (50 individual PAHs quantified) of St. 1 exceeded a concentration of 100,000 μg/kg dw, which was more than 5-fold higher than the second most PAH abundant station (St. 3). Although it is debated whether it is sufficient for environmental monitoring of PAH exposure (Andersson and Achten, 2015), a set of 16 PAHs (PAH-16, Supplementary Table S2) have previously been added to the list of priority substances for predicting toxicity towards mammals and aquatic organisms. All of the PAH congeners that are included in PAH-16 exceeded their respective EQS-levels at St.1 (89,000 μg/kg dw, 36-fold higher than AA-EQS), which was 6–10 fold higher than the PAH-16 levels measured at St. 2–5, and 200-fold higher than the reference station, St. 7 (Fig. 2 and Supplementary Table S2). At St. 2, 3, 4, and 5, all PAH-16 congeners exceeded AA-EQS, except for acenapthene, fluorene, and phenanthrene. At St. 6 anthracene, indeno[1,2,3-cd]pyrene, dibenzo[ah]anthracene, and benzo[ghi]perylene exceeded the AA-EQS-values, while none of the PAH-16 exceeded AA-EQS at the reference station (St. 7).

PCBs were detected at all stations, with highest levels in St. 1–4 (Fig. 2 and Supplementary Table S5). PCB_7 exceeded AA-EQS at St. 1–5, with concentrations ranging from 8 to 112 μg/kg. In general, the levels of PCB_7 were in the same range to what Jartun et al. (2009) reported in their previous study of Bergen Byfjorden. PCBs have historically been used in a variety of different products and applications such as paint, hydraulic oils, electrical transformers and capacitators, double framed glazing windows, concrete constructions, and sealants (Jartun et al., 2009). Due to environmental concerns, the use of PCBs was restricted and banned globally in the late 1970s. However, owing to their persistence in nature, they are still detected ubiquitously in the environment, including urban fjords. In Bergen, high levels of PCBs have been found in outdoor paint used on concrete facades on buildings and bridges, representing a potential source of contamination for Byfjorden sediments through the stormwater system (Jartun et al., 2009; Andersson et al., 2004). A local point-source of PCB is known from a WWII submarine bunker at Laksevåg, located approx. 1250 m from St. 3. Low-chlorinated PCBs were more prominent than high-chlorinated PCBs at St. 3. Since lower-chlorinated PCBs are more easily degraded, this may indicate a more recent input of PCBs in this area.

Sixteen PBDE congeners were analysed in the sediment extracts. None of the samples had concentrations exceeding the AA-EQS level of $PBDE_6$. BDE209 was the most abundant congener, with St. 2 and 4 possessing higher levels than St. 1 and 3. PBDEs are commonly used as brominated flame retardants in electrical products, textiles, and plastics. In particular, BDE209 is a high-production-volume flame retardant and is one of the most prominent flame retardants present in the environment (Abbasi et al., 2019), which was also in agreement with its prevalent abundance in the Byfjorden sediment samples (Fig. 2 and Supplementary Table S6).

The OCPs, p,p'-DDT and its metabolites (p,p'-DDE, p,p'-DDD), as well as HCB, were detected at all stations, with the highest concentrations found at St. 1, and with high levels at St. 2–4 in comparison to the outer stations (Fig. 2, Supplementary Table S5). Trans-nonachlor (TNC) was also detected in high levels at St. 1–4, but was below LOQ at the reference station (St.7). a−HCH, b−HCH, and g−HCH were only detected above LOQ at St. 1, 2 (only a−HCH) and 4. ∑HCH were exceeding AA-EQS at St. 1 and 4. The observed OCP gradient, with higher levels in the inner harbour, may indicate urban runoff as transport routes for some of these pesticides (Zhang et al., 2010).

Most PFAS congeners were below LOQ in the sediment extracts (Supplementary Table S3). Sum PFAS (∑PFAS, Fig. 2), representing all 17 PFASs analysed, displayed the highest levels at St. 4. However, it should be noted that these levels are calculated based on ½ LOQ for compounds with concentrations below LOQ. Only perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) have been assigned AA-EQS values. PFOS was detected at levels exceeding AA-EQS at St. 4 and notably at the reference station (St. 7), and at levels just

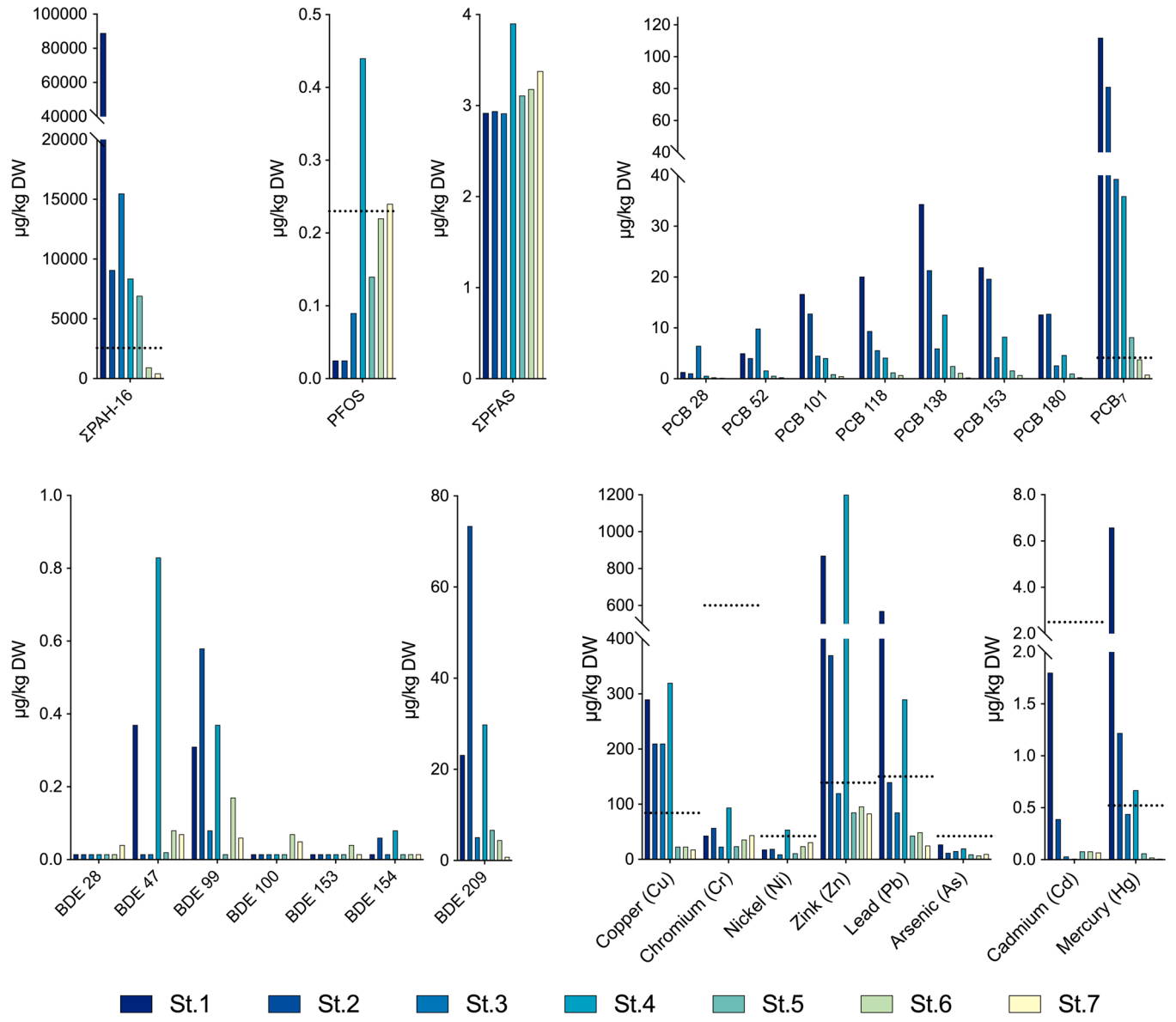

Fig. 2. Levels of ΣPAH-16, PFOS, ΣPFAS, PCBs, PBDEs, and metals measured in sediment samples collected at the seven stations in Bergen Byfjorden. AA-EQS is indicated with a dotted line. The analysis is based on 10 g of extracted sediments, while analysis of PCBs and PBDEs in St. 1 is based on 1 g of sediment extract. Concentrations below LOQ are reported as LOQ/2 in the figure.

below AA-EQS at St. 6, located in Raunefjorden. A possible source of PFOS at the reference station and St. 6 is discharges from PFAS-containing firefighting foam used at Bergen Airport Flesland. PFAS was commonly used in this area from 1970 to 2011, while the use of PFOS was phased out in 2001 (Kaasa et al., 2016).

Metals were detected at all stations, with highest concentrations of most elements observed at St.1–4. St. 1 showed highest levels of Pb, Cd, and Hg, while St. 4 possessed highest levels of Ni, Zn, Cu, and Cr. AA-EQS were exceeded for Cu (St. 1–4), Ni (St. 4), Zn (St. 1, 2, and 4), As (St. 1 and 4), Pb (St. 1 and 4), and Hg (St. 1, 2, and 4) (Fig. 2 and Supplementary Table S4).

The contaminant situation in Bergen Byfjorden was also assessed in 1993, when a similar pattern with high levels of Hg, Pb, Cu, PAH and PCB in sediments from the innermost areas were reported. PCB contamination was linked to point sources such as the submarine bunker near St. 3 and more diffuse sources, while PAH contamination possibly originates from oil spills in the inner harbour area where several shipyards were located and ship traffic used to be high (Skei et al., 1994). The lack of large river inputs to the Bergen Byfjorden area, resulting in only slow accumulation of new sediment layers, is probably causing the continued presence of these legacy pollutants in the upper parts of the sediment. As the stations were collected in a gradient from the inner Byfjorden to the coastal current, there are variations in sedimentation rates. Sedimentation rate of 0.04 cm/year was previously reported from the Byfjorden area (Kuvås, 2015). In Byfjorden, we have observed sediment rates at 340 g dry sediment m^{-2} $year^{-1}$, and in Korsfjorden (St. 7) at 195 g dry sediment m^{-2} $year^{-1}$. Differences in sedimentation rates will naturally affect the period of accumulation of pollutants.

3.2. Cytotoxicity and oxidative stress in PLHC-1 and RTgill-W1 cells

PLHC-1 and RTgill-W1 cells were exposed to the sediment extracts for 24 h at concentrations ranging from 5 to 120 mg eQsed/mL. Cellular metabolic activity was monitored with the Alamar Blue assay, and the sediment extract from St. 1 affected the metabolic activity in both PLHC-1 and RTgill-W1 cells at the highest concentrations used, although with significantly stronger effects on the PLHC-1 cells. Significantly lowered viability of the PLHC-1 and RTgill-W1 cells were observed in a dose-

dependent manner when exposed to 40, 60, and 120 mg eQsed/mL of St.1 extract. The cell viability in PLHC-1 cells exposed to 120 mg eQsed/mL from St. 1 was similar to cells exposed to the positive control (0.1 μM H_2O_2), i.e., approx. 5% (Fig. 3A). Slight reductions in RTgill-W1 cell viability were also observed with sediment extracts from St. 2, 5, 6, and 7. Notably, a dose-dependent increase in metabolic activity was observed with some of the extracts on both the PLHC-1 and RTgill-W1 cell lines, possibly indicating adaptive responses to cellular stress.

The formation of ROS was monitored after exposing PLHC-1 and RTgill-W1 cells to the sediment extracts (5−120 mg eQsed/mL) for 60 min. Significant induction of ROS activity was observed after exposure of the PLHC-1 cells to extracts from St. 1, 2, and 4. In the RTgill-W1 cells, all the innermost stations (St. 1–5) significantly induced ROS formation, with an increasing ROS production from St. 5 to St. 1 (Fig. 3B). Differences in sensitivity between the cell types were also observed, with the RTgill-W1 cells being more sensitive than the PLHC-1 cells. Metals, as well as many other environmental pollutants, are known to promote ROS formation. Notably, elevated levels of Cu, Zn, Pb, Cd, and Hg were found in one or more of the innermost stations (St. 1–4, Fig. 2 and Supplementary Table S5). The St. 1 sediment extract produced the highest levels of ROS activity in both RTgill-W1 and PLHC-1 cells, which agrees with this location possessing the most abundant levels of Cd, Hg, and Pb.

3.3. Induction of EROD activity

The EROD enzymatic assay is used to measure induction of Cyp1a through Ahr activation (Goksøyr, 1995; van der Oost et al., 2020). Compounds that are known to induce Cyp1a are certain PAHs and some persistent organic pollutants such as dioxins and dioxin-like PCBs. PLHC-1 liver cells are commonly used to measure induction of EROD activity and were exposed to sediment extracts at increasing concentrations.

A significant induction of EROD activity was observed in cells exposed to sediment extracts from St. 2, 3, and 4, while no, or only low, EROD activity was measured in cells exposed to extracts from the outer stations (St. 5–7). Intriguingly, only low EROD activity was observed after exposure of PLHC-1 cells to extract from St. 1, which contained the highest levels of PAHs (Fig. 3C). Due to the strong cytotoxicity recorded in the PLHC-1 cells at St. 1 (Fig. 3A), the EROD analysis was also performed with a lower dose range from 0.3 to 10 mg eQsed/mL, i.e., below the levels causing cytotoxicity. However, only low or no EROD activity was still observed. Notably, inhibition of EROD activity has been shown in organisms exposed to metals, such as Cd and Hg, as well as some PCBs (Bozcaarmutlu and Arinç, 2004; Whyte et al., 2000; Beyer et al., 1997). This could be linked to the compromised EROD activation observed for St. 1, which contained the highest levels of these compounds. Although PAHs generally are regarded as strong inducers of Cyp1a and EROD activity (Whyte et al., 2000), a few studies have shown that some PAHs and oxygenated PAHs, e.g. benz[a]anthracene-7,12-quinone and benzo[a]fluorenone, at the same time can act as Cyp1a inhibitors (Wincent et al., 2016). Although these compounds were not analysed in our sediment extracts, it is possible that such compounds also can contribute to the lack of correlation between PAH levels and EROD activity at St. 1.

3.4. Activation of stress-activated receptors

A battery of stress-activated receptors was used in luciferase-based reporter gene assays for assessing the ability of the sediment extracts to activate cellular signalling pathways *in vitro*. COS-7 cells were transfected with either Atlantic cod gmAhr1a, gmAhr2a, gmEra, gmAra, gmPpara1, gmPpara2, gmVdra, gmVdrb, or zebrafish drPxr, and exposed to sediment extracts at increasing concentrations (0−60 mg eQsed/mL and 0−5 mg eQsed/mL for Ahr). The receptor activation was reported as fold-induction relative to solvent control (DMSO). Sediment extracts from all sample stations, including the reference sample (St. 7), activated gmAhr1a and gmAhr2a. Accordingly, some known AHR agonists have been detected in the sediment samples, such as benzo[a]pyrene, chrysene, benzo[b]fluoranthene, and benzo[k]fluoranthene (Pieterse et al., 2013). Furthermore, a gradient in potencies was observed with lower EC_{50} values at the innermost stations in comparison to the outer stations for both gmAhr1a and gmAhr2a. St. 1 extract exhibited the lowest EC_{50}-values, corresponding to 0.008 mg eQsed/mL (gmAhr1a) and 0.006 mg eQsed/mL (gmAhr2a), while the sediment extract from the reference station (St. 7) was the least potent sample for both gmAhr subtypes (1.026 mg eQsed/mL (gmAhr1a) and 0.480 mg eQsed/mL (gmAhr2a)) (Table 1). Differences were also observed in efficacies, where St. 1 produced the highest E_{max} for both gmAhr1a and gmAhr2a.

A similar pattern was observed with the zebrafish drPxr reporter assay, where sediment extracts from St. 1–4, significantly activated drPxr, producing efficacies in the 2- to 6-fold range (Fig. 4 and Table 1). PXR is a promiscuous receptor that is known to be activated by a large range of different chemicals, including pesticides, pharmaceuticals, plasticizers and industrial compounds. A study performed on human and polar bear PXR showed that it can be activated by BPA, PCBs, brominated flame retardants, and PFNA, among others (Lille-Langøy et al., 2015). The zebrafish drPxr bioassay has previously been shown to be activated by sediment extracts from city harbours and river mouths in the Mediterranean (Pérez-Albaladejo et al., 2016; Blanco et al., 2018). Similarly, a human PXR-based bioassay was demonstrated to respond to extracts from water and sediments from French rivers (Creusot et al., 2010; Kinani et al., 2010). The wide range of ligands able to activate Pxr makes it difficult to identify which chemicals that are responsible for the response observed with the complex Bergen harbour extracts, and further analyses, using effect-directed analysis (EDA), could be used to achieve this goal (Li et al., 2018; Houtman et al., 2020).

With the Atlantic cod nuclear receptor assays, including gmEra, gmAra, gmPpara1, gmPpara2, gmVdra, and gmVdrb, the strongest activation was generated with the St. 1 extracts, and with varying activation by the other sediment extracts (dose-response curves shown in Supplementary Fig. S3). A summary of the receptor data is presented as a radar plot in Fig. 4. An interesting observation was the activation of the gmVdrs. The literature on exogenous compounds that can modulate Vdr is sparse, especially in fish. However, we have previously demonstrated that binary exposures of the endogenous ligand of Vdr, calcitriol, and certain PAHs, can modulate the activity of the gmVdrs. Specifically, co-exposure with calcitriol and either chrysene or benzo[a]pyrene potentiated the calcitriol-mediated activation of the gmVdrs (Goksøyr et al., 2021). Similarly, when co-exposing the Bergen Byfjorden sediment extracts to a fixed concentration of calcitriol, we observed potentiation of gmVdra and gmVdrb activities, with highest efficacy produced by St. 1 (Supplementary Fig. S4), which also contains the highest levels of PAHs.

3.5. Integrative analyses and implications

Chemical analyses are normally used in environmental monitoring programs for specifically identifying individual or groups of compounds, while more advanced non-targeted analyses are needed to cover a wider range of contaminants (Hollender et al., 2017). Cell-based bioassays have increasingly been used to complement chemical analyses, providing additional information about the biological activity of environmental samples.

Mostly, these applications have been focusing on one or a few MIEs or receptor targets. For example, Vigano et al. demonstrated the use of the yeast-based estrogen screen (YES) assay as a suitable method to identify estrogenic extracts from the Lambro River in Italy (Viganò et al., 2008). Pérez-Albaladejo et al. (2016), and Blanco et al. (2018) used a combination of traditional measurements of cytotoxicity and EROD induction in PLHC-1 cells, as well as the drPXR receptor assay to characterize polluted harbour sediments from the Mediterranean region. In

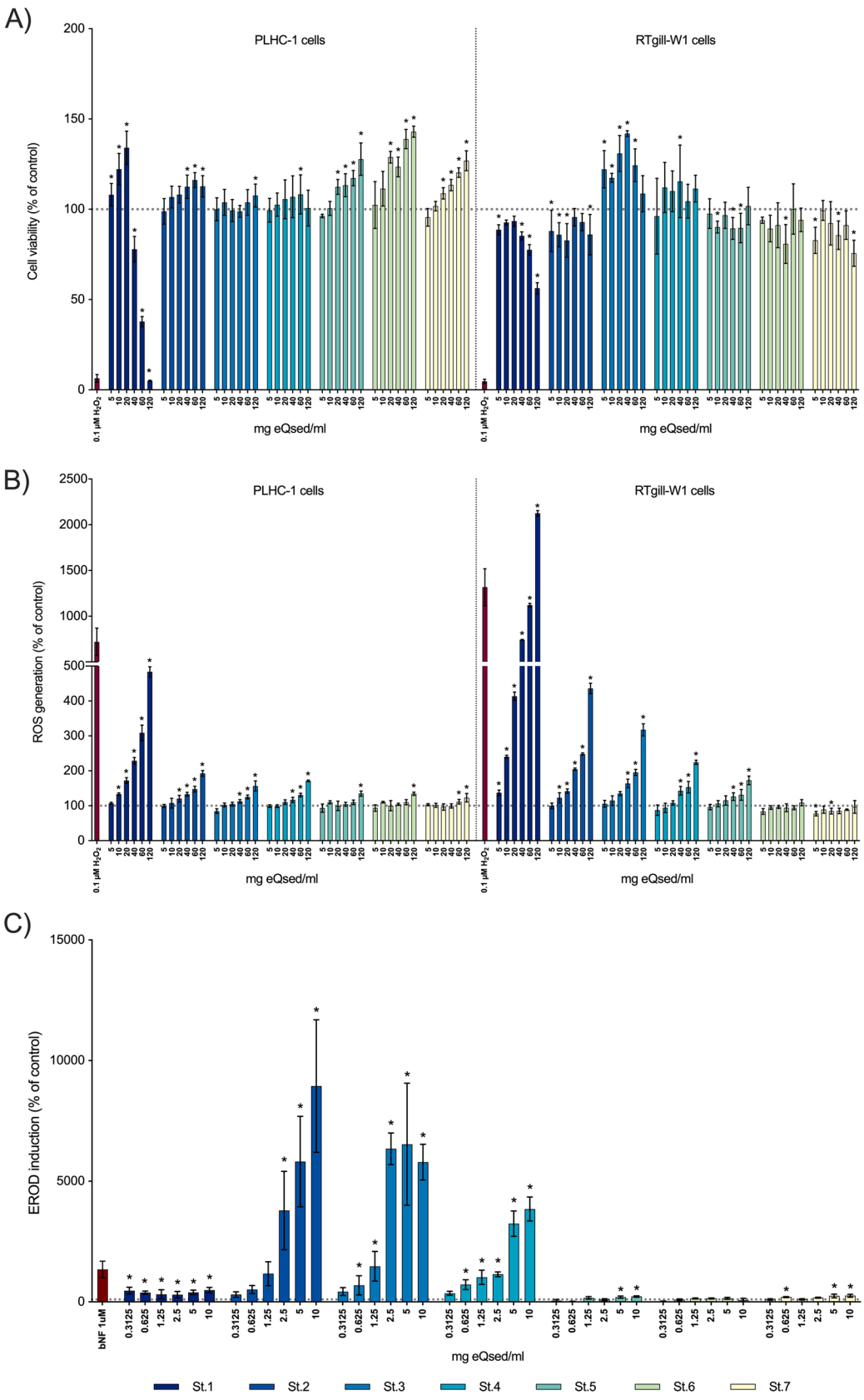

Fig. 3. The effect of sediment extracts (St. 1-7) from Bergen Byfjorden on cell viability, reactive oxygen species (ROS) formation, and EROD induction in fish liver and gill cell cultures. A) Cell viability of PLHC-1 (right) and RTgill-W1 (left) cells after 24 h exposure to sediment extracts (0-120 mg eQsed/mL). The graph describes the cell viability as the percentage of fluorescent response from each dose compared to cells exposed to solvent control (100 %). 0.1 μM H_2O_2 was used as a positive control of reduced viability (red) B) ROS generation measured in PLHC-1 (right) and RTgill-W1 (left) cells after 60 min exposure to sediment extracts (0-120 mg eQsed/mL). The graph describes the cell viability as the percentage of fluorescent response from each dose compared to control cells (100 %), with 0.1 μM H_2O_2 used as a positive control of ROS formation (red). C) EROD activity was measured in PLHC-1 cells after 24 h exposure to sediment extracts (0-10 mg eQsed/mL), solvent control (5.0 ± 3.0 pmol/mg/min), or BNF (1μM). The latter was used as positive control for inducing EROD activity (1345 %) (red). The graph is presented as mean ± SD, n = 4 (n = 3 for St.1 RTgill-W1 cell viability, and EROD measurement). * (p-value < 0.05) represents statistical significance compared to control cells (0 mg eQsed/mL = 100 %), determined using one-way ANOVA.

Table 1
E_{max} and EC_{50} values from the receptor-based luciferase reporter gene assays. E_{max} and EC_{50} was determined by nonlinear regression of dose response in Prism if plateau was reached. When the curve did not reach a plateau, the E_{max} was defined as the highest activation. EC_{50} was then derived from the dose-response curve at half E_{max}. EC_{50} values are mg eQsed/mL.

	gmAhr1a		gmAhr2a		gmAra		gmEra		gmPpara1		gmPpara2		gmVdra		gmVdrb		drPxr	
	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}
St. 1	4.18	0.008	5.24	0.006	1.72	nd	2.40	12.7	4.63	20.0	2.36	2.53	1.71	nd	2.61	3.54	5.69	6.33
St. 2	4.13	0.076	5.28	0.052	1.31	nd	1.52	nd	1.23	nd	1.56	nd	1.12	nd	1.90	nd	4.33	29.5
St. 3	4.15	0.063	5.12	0.045	1.48	nd	1.27	nd	1.16	nd	1.32	nd	1.39	nd	1.81	nd	5.18	20.0
St. 4	3.20	0.054	5.27	0.092	1.01	nd	0.85	nd	1.20	nd	1.29	nd	1.16	nd	1.58	nd	2.74	22.4
St. 5	3.61	0.135	4.88	0.147	1.27	nd	0.98	nd	1.20	nd	1.29	nd	1.17	nd	1.32	nd	1.91	nd
St. 6	3.63	0.267	4.42	0.197	0.82	nd	0.91	nd	1.35	nd	1.06	nd	0.99	nd	1.32	nd	1.62	nd
St. 7	2.83	1.026	4.14	0.480	0.77	nd	0.85	nd	0.94	nd	0.86	nd	1.18	nd	1.21	nd	1.62	nd

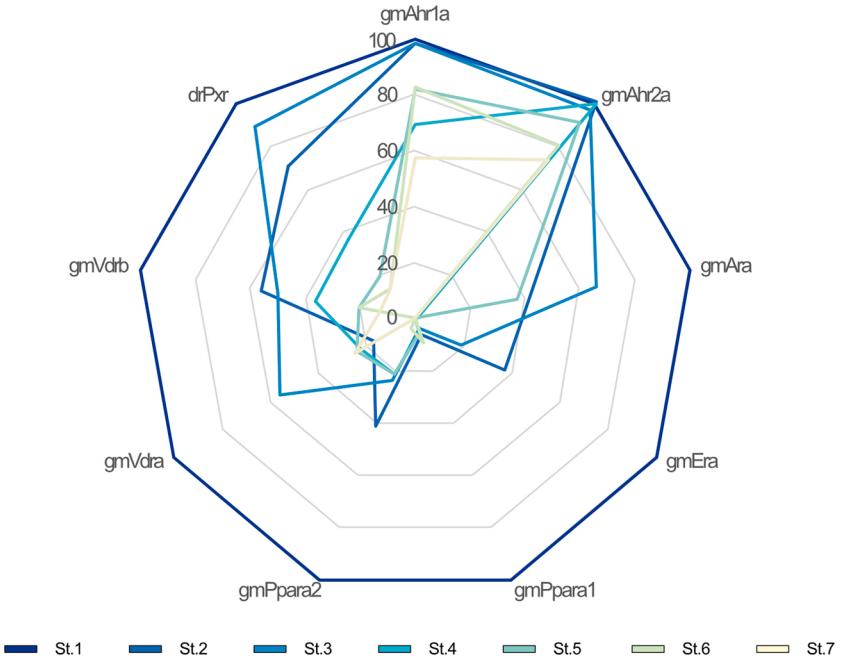

Fig. 4. Radar plot of relative E_{max} values observed in the stress-activated receptor-based luciferase reporter gene assays normalized to the response observed at St. 1 (= 100). Receptor abbreviations: gmAhr1a and gmAhr2a (Atlantic cod aryl hydrocarbon receptor 1a and 2a), gmAra (cod androgen receptor alpha), gmEra (cod estrogen receptor alpha), gmPpara1 and gmPpara2 (cod peroxisome proliferator activated receptor alpha1 and alpha2), gmvdra and gmVdrb (cod vitamin D receptor alpha and beta), and drPxr (zebrafish pregnane X receptor). Station colour codes shown in legend. Data shown in Table 1, with dose-response curves used to derive the data presented in Supplementary Fig. S3.

general, the bioassays were in good agreement with sediment chemistry. Similar to our study, Kinani et al. used a battery consisting of human receptors (ER, AR, and PXR) as well as EROD measurements in PLHC-1 cells to study sediment toxicity from small rivers in France, demonstrating the presence of a wide range of pollutants capable of activating this set of cell-based bioassays (Kinani et al., 2010).

In order to extract patterns in the complex datasets obtained in our study, two PCA analyses, one including all the bioassay data (Fig. 5A) and one containing only the chemistry data (Fig. 5B), were performed. Both PCA plots showed a clear separation of St. 1 from the other stations. In addition, a gradient from St. 7 (least similar to St. 1) to St. 2 and 3 were observed in both plots. In Fig. 5A, the gradient is visible in both components (PC1 and PC2), providing a refined separation of the samples by using exclusively the bioassay data. Furthermore, the bioassay

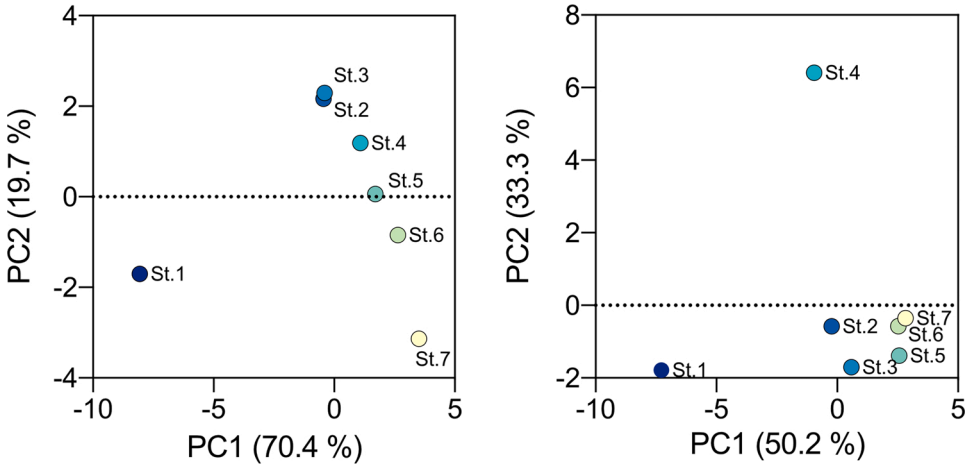

Fig. 5. Multivariate statistical analyses of biological and chemical data. (A) Principal component analysis (PCA) plot with biological data (reporter gene assays, EROD activity, cytotoxicity, and ROS formation). The first two principal components explained 90.1 % of the data set variance, with cytotoxicity (PLHC-1 cells), and Ppar, Vdr, and Pxr responses contributing mostly to PC1, and EROD, Ahr, and cytotoxicity (RTgill-W1 cells) to PC2. (B) PCA plot with chemical data. The first two PCs explained 83.4 % of the data set variance. PFASs were the main positive PC1 and PC2 components. Eigenvectors in supplementary Tables S7 and S8.

data alone discriminates better between the reference station (St. 7) and the other stations in the gradient, than the chemistry data alone. The bioassay data were therefore capable of providing a corresponding and also more comprehensive pattern compared to the chemical analyses, being able in this case to represent the biological effects of the complex mixtures of contaminants better than the chemical analyses. According to the distribution of the variables in the PCA plot of Fig. 5A, the complete array of bioassays would be needed for the best sample station separation.

Other NRs, e.g., glucocorticoid receptor (GR), thyroid hormone receptor (TR), progesterone receptor (PR), are also known to be sensitive to chemicals of emerging concern and could be included in such a battery to provide an even broader coverage of potential ecotoxicity. However, luciferase reporter gene assays using transiently transfected cells are time-consuming, and rather labour-intensive. This approach could be made more efficient by using stably transfected cell lines or by using the receptors in a cell-free and high-throughput system. Another consideration is that luciferase reporter gene assays using only the LBD of receptors, as done here, may not accurately reflect the organismal response to contaminants *in vivo*. Factors such as transcription co-activators and modulators, cross-reactivity from other receptors, and biotransformation of exogenous compounds can have an impact on the responses. However, *in vitro* activation bioassays are useful tools for investigating the relative binding potencies of contaminants to NRs.

The identification of sediment toxicity using effect-based bioassays provides information on complex chemical mixtures and their effects on important MIEs, which could be combined with a TIE (toxicity identification and evaluation) approach to diagnose key toxicity drivers (Li et al., 2018). In combination with an AOP framework, such studies could provide predictive data for environmental risk assessment and the regulation of toxicants in marine ecosystems under pressure (Brockmeier et al., 2017; van der Oost et al., 2020).

4. Conclusion

This study has shown the usefulness of applying a variety of bioassays from fish to assess the toxicity of sediments collected in the Bergen city harbour and urban fjord. The sediment extract from the inner harbour (St. 1) was able to transactivate the entire battery of receptors, promote oxidative stress, and induce cytotoxicity in fish liver and gill cell lines, which also correlated with higher levels of toxicants and the more complex contaminant situation at this site. The gmAhr-receptors were transactivated by all the sediment samples, including the reference station, indicating a wide occurrence of gmAhr agonist at various levels. Notably, the induction of Cyp1a in PLHC-1 cells did not reflect the PAH levels in the sediments as EROD activity was not strongly induced by the St.1 extract, suggesting inhibitory effects caused by other chemicals present in this mixture. The PCA plot showed that the combinations of bioassays represented the spatial separation of the sediment stations from inner parts of Byfjorden to outer parts in a consistent way. This demonstrates that the use of a battery of reporter gene bioassays based on a collection of stress-activated receptors from an indicator fish species like Atlantic cod combined with fish cell-based bioassays can complement chemical analyses and provide important insights into sediment toxicity, which are of great relevance for environmental risk assessment.

CRediT authorship contribution statement

Siri Øfsthus Goksøyr: Writing - original draft, Methodology, Investigation, Formal analysis, Visualization. **Helene Sørensen:** Investigation. **Bjørn Einar Grøsvik:** Resources, Investigation, Writing - review & editing. **Daniela M. Pampanin:** Resources, Investigation, Writing - review & editing. **Anders Goksøyr:** Conceptualization, Methodology, Writing - review & editing. **Odd André Karlsen:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

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

Acknowledgments

We thank the Research Council of Norway for funding through the dCod 1.0: decoding systems toxicology of Atlantic cod project (Center for Digital Life Norway project no. 248840) and the iCod 2.0: integrative environmental genomics of Atlantic cod project (project no. 244564). Sampling and part of the chemical analyses was funded by the IMR project Other contaminants (project no 15445). We are grateful to the personnel at R/V Hans Brattström for assistance in the field and to Grethe Tveit, Guri Nesje, Anna Ersland and Aasim Ali (IMR) for help with chemical analyses.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.etap.2021.103704.

References

Abbasi, G., Li, L., Breivik, K., 2019. Global historical stocks and emissions of PBDEs. Environ. Sci. Technol. 53, 6330–6340. https://doi.org/10.1021/acs.est.8b07032.

Andersson, J.T., Achten, C., 2015. Time to say goodbye to the 16 EPA PAHs? Toward an up-to-date use of PACs for environmental purposes. Polycyclic Aromat. Compd. 35 (2–4), 330–354. https://doi.org/10.1080/10406638.2014.991042.

Andersen, V., Maage, A., Johannessen, P.J., 1996. Heavy metals in blue mussels (Mytilus edulis) in the Bergen Harbor Area, Western Norway. Bull. Environ. Contam. Toxicol. 57, 589–596. https://doi.org/10.1007/s001289900231.

Andersson, M., Ottesen, R., Volden, T., 2004. Building materials as a source of PCB pollution in Bergen, Norway. Sci. Total Environ. 325, 139–144. https://doi.org/10.1016/j.scitotenv.2003.11.014.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J. E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741. https://doi.org/10.1002/etc.34.

Aranguren-Abadia, L., Lille-Langoy, R., Madsen, A.K., Karchner, S.I., Franks, D.G., Yadetie, F., Hahn, M.E., Goksoyr, A., Karlsen, O.A., 2020. Molecular and functional properties of the atlantic cod (Gadus morhua) aryl hydrocarbon receptors Ahr1a and Ahr2a. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.9b05312.

Bagi, A., Riiser, E.S., Molland, H.S., Star, B., Haverkamp, T.H.A., Sydnes, M.O., Pampanin, D.M., 2018. Gastrointestinal microbial community changes in Atlantic cod (Gadus morhua) exposed to crude oil. BMC Microbiol. 18 https://doi.org/10.1186/s12866-018-1171-2.

Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skåre, J.U., Goksøyr, A., 1996. Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sørfjorden, Norway. Aquat. Toxicol. 36, 75–98.

Beyer, J., Sandvik, M., Skåre, J.U., Egaas, E., Hylland, K., Waagbø, R., Goksøyr, A., 1997. Time- and dose-dependent biomarker responses in flounder (Platichthys flesus L.) exposed to benzo[a]pyrene, 2,3,3',4,4',5-hexachlorobiphenyl (PCB-156) and cadmium. Biomarkers 2, 35–44. https://doi.org/10.1080/135475097231959.

Blanco, M., Pérez-Albaladejo, E., Piña, B., Kušpilić, G., Milun, V., Lille-Langøy, R., Karlsen, O.A., Goksøyr, A., Porte, C., 2018. Assessing the environmental quality of sediments from Split coastal area (Croatia) with a battery of cell-based bioassays. Sci. Total Environ. 624, 1640–1648. https://doi.org/10.1016/j.scitotenv.2017.10.055.

Boitsov, S., Klungsøyr, J., Jensen, H.K.B., 2020. Background concentrations of polycyclic aromatic hydrocarbons (PAHs) in deep core sediments from the Norwegian Sea and the Barents Sea: a proposed update of the OSPAR commission background values for these sea areas. Chemosphere 251, 126344. https://doi.org/10.1016/j.chemosphere.2020.126344.

Bozcaarmutlu, A., Arinç, E., 2004. Inhibitory effects of divalent metal ions on liver microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet. Mar. Environ. Res. 58, 521–524. https://doi.org/10.1016/j.marenvres.2004.03.040.

Brack, W., Schirmer, K., Erdinger, L., Hollert, H., 2005. Effect-directed analysis of Mutagens and ethoxyresorufin-O-deethylase inducers in aquatic sediments. Environ. Toxicol. Chem. 24, 2445. https://doi.org/10.1897/05-078r.1.

Brockmeier, E.K., Hodges, G., Hutchinson, T.H., Butler, E., Hecker, M., Tollefsen, K.E., Garcia-Reyero, N., Kille, P., Becker, D., Chipman, K., Colbourne, J., Collette, T.W., Cossins, A., Cronin, M., Graystock, P., Gutsell, S., Knapen, D., Katsiadaki, I., Lange, A., Marshall, S., Owen, S.F., Perkins, E.J., Plaistow, S., Schroeder, A., Taylor, D., Viant, M., Ankley, G., Falciani, F., 2017. The role of omics in the

application of adverse outcome pathways for chemical risk assessment. Toxicol. Sci. 158, 252–262. https://doi.org/10.1093/toxsci/kfx097.
Chapman, P.M., 2007. Determining when contamination is pollution - weight of evidence determinations for sediments and effluents. Environ. Int. 33, 492–501. https://doi.org/10.1016/j.envint.2006.09.001.
Creusot, N., Kinani, S., Balaguer, P., Tapie, N., Lemenach, K., Maillot-Maréchal, E., Porcher, J.-M., Budzinski, H., Aït-Aïssa, S., 2010. Evaluation of an hPXR reporter gene assay for the detection of aquatic emerging pollutants: screening of chemicals and application to water samples. Anal. Bioanal. Chem. 396, 569–583. https://doi.org/10.1007/s00216-009-3310-y.
Dale, K., Müller, M.B., Tairova, Z., Khan, E.A., Hatlen, K., Grung, M., Yadetie, F., Lille-Langøy, R., Blaser, N., Skaug, H.J., Lyche, J.L., Arukwe, A., Hylland, K., Karlsen, O. A., Goksøyr, A., 2019. Contaminant accumulation and biological responses in Atlantic cod (Gadus morhua) caged at a capped waste disposal site in Kollevåg, Western Norway. Mar. Environ. Res. 145, 39–51. https://doi.org/10.1016/j.marenvres.2019.02.003.
Dayeh, V.R., Schirmer, K., Lee, L.E.J., Bols, N.C., 2003. The Use of Fish-Derived Cell Lines for Investigation of Environmental Contaminants, In Current Protocols in Toxicology. John Wiley & Sons, New York, NY, USA, pp. 1.5.1–1.5.17.
Eide, M., Rydbeck, H., Tørresen, O.K., Lille-Langøy, R., Puntervoll, P., Goldstone, J.V., Jakobsen, K.S., Stegeman, J., Goksøyr, A., Karlsen, O.A., 2018. Independent losses of a xenobiotic receptor across teleost evolution. Sci. Rep. 8, 10404. https://doi.org/10.1038/s41598-018-28498-4.
Eide, M., Zhang, X., Karlsen, O.A., Goldstone, J.V., Stegeman, J., Jonassen, I., Goksøyr, A., 2021. The chemical defensome of fish: conservation and divergence of genes involved in sensing and responding to pollutants among five model teleosts. Res. Square.
European Commission, 2000. Establishing a framework for Community action in the field of water policy, 2000/60/EC. In: COMMISSION, E. (Ed.), Official Journal of the European Communities. European Commission.
European Commission, 2008. Establishing a Framework for Community Action in the Field of Marine Envireonmental Policy (Marine Strategy Framework Directive). 2008/56/EC. Official Journal of the European Union.
Fent, K., 2001. Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. Toxicologi Vitro 15, 477–488.
Fernandes, D., Pujol, S., Perez-Albaladejo, E., Tauler, R., Bebianno, M.J., Porte, C., 2014. Characterization of the environmental quality of sediments from two estuarine systems based on different in-vitro bioassays. Mar. Environ. Res. 96, 127–135. https://doi.org/10.1016/j.marenvres.2013.09.019.
Gardner, M.J., Brown, B., Whitehouse, P., Birch, M., 2008. Towards the establishment of an environmental quality standard for aluminium in surface waters. J. Environ. Monit. 10, 877. https://doi.org/10.1039/b717812c.
Goksøyr, A., 1995. Use of cytochrome P450 1A (CYP1A) in fish as a biomarker of aquatic pollution. Toxicology in Transition. Springer.
Goksøyr, A., Beyer, J., Husøy, A.-M., Larsen, H.E., Westrheim, K., Wilhelmsen, S., Klungsøyr, J., 1994. Accumulation and effects of aromatic and chlorinated hydrocarbons in juvenile Atlantic cod (*Gadus morhua*) caged in a polluted fjord (Sørfjorden, Norway). Aquat. Toxicol. 29, 21–35.
Goksøyr, S.Ø., Goldstone, J., Lille-Langøy, R., Lock, E.-J., Olsvik, P.A., Goksøyr, A., Karlsen, O.A., 2021. Polycyclic aromatic hydrocarbons modulate the activity of Atlantic cod (Gadus morhua) vitamin D receptor paralogs in vitro. Aquat. Toxicol. https://doi.org/10.1016/j.aquatox.2021.105914. In press.
Goldstone, J.V., Hamdoun, A., Cole, B.J., Howard-Ashby, M., Nebert, D.W., Scally, M., Dean, M., Epel, D., Hahn, M.E., Stegeman, J.J., 2006. The chemical defensome: environmental sensing and response genes in the Strongylocentrotus purpuratus genome. Dev. Biol. 300, 366–384. https://doi.org/10.1016/j.ydbio.2006.08.066.
Hollender, J., Schymanski, E.L., Singer, H.P., Ferguson, P.L., 2017. Nontarget screening with high resolution mass spectrometry in the environment: ready to go? Environ. Sci. Technol. 51, 11505–11512. https://doi.org/10.1021/acs.est.7b02184.
Houtman, C.J., Ten Broek, R., Van Oorschot, Y., Kloes, D., Van Der Oost, R., Rosielle, M., Lamoree, M.H., 2020. High resolution effect-directed analysis of steroid hormone (ant)agonists in surface and wastewater quality monitoring. Environ. Toxicol. Pharmacol. 80, 103460 https://doi.org/10.1016/j.etap.2020.103460.
Hylland, K., Tollefsen, K.-E., Ruus, A., Jonsson, G., Sundt, R.C., Sanni, S., Utvik, T., Johnsen, S., Nilssen, I., Pinturier, L., Balk, L., Baršienė, J., Marigòmez, I., Feist, S.W., Børseth, J., 2008. Water column monitoring near oil installations in the North Sea 2001–2004. Mar. Pollut. Bull. 56, 414–429. https://doi.org/10.1016/j.marpolbul.2007.11.004.
Jartun, M., Pettersen, A., 2010. Contaminants in urban runoff to Norwegian fjords. J. Soils Sediments 10, 155–161. https://doi.org/10.1007/s11368-009-0181-y.
Jartun, M., Ottesen, R.T., Steinnes, E., Volden, T., 2009. Painted surfaces – important sources of polychlorinated biphenyls (PCBs) contamination to the urban and marine environment. Environ. Pollut. 157, 295–302. https://doi.org/10.1016/j.envpol.2008.06.036.
Kaasa, H., Kvisle, V., Gravem, F.R., Breyholtz, B., Været, L., Jensen, J.G.B., Halvorsen, E., Gregersen, H., 2016. Undersøkelser Av PFAS I Jord, Vann Og Biota Med Risikovurdering. Bergen lufthavn, Flesland (in Norwegian).
Kinani, S., Bouchonnet, S., Creusot, N., Bourcier, S., Balaguer, P., Porcher, J.-M., Aït-Aïssa, S., 2010. Bioanalytical characterisation of multiple endocrine- and dioxin-like activities in sediments from reference and impacted small rivers. Environ. Pollut. 158, 74–83. https://doi.org/10.1016/j.envpol.2009.07.041.
Kosmehl, T., Krebs, F., Manz, W., Braunbeck, T., Hollert, H., 2007. Differentiation between bioavailable and total hazard potential of sediment-induced DNA fragmentation as measured by the comet assay with Zebrafish embryos. J. Soils Sediments 7, 377–387. https://doi.org/10.1065/jss2007.11.261.
Kuvås, J., 2015. Sedimentære Prosesser Og Avsetningsmiljø I Byfjorden, Vest-norge. University of Bergen, Norway, p. 142 (In Norwegian) M. Sc. Master thesis.
Lepper, P., 2004. Manual of the Methodological Framework Used to Derive Quality Standards for Priority Substances of the Water Framework Directive.
Lepper, P., 2005. Manual on the Methodological Framework to Derive Environmental Quality Standards for Priority Substances in Accordance With Article 16 of the Water Framework Directive (2000/60/EC).
Li, H., Zhang, J., You, J., 2018. Diagnosis of complex mixture toxicity in sediments: application of toxicity identification evaluation (TIE) and effect-directed analysis (EDA). Environ. Pollut. 237, 944–954. https://doi.org/10.1016/j.envpol.2017.11.005.
Lille-Langøy, R., Goldstone, J.V., Rusten, M., Milnes, M.R., Male, R., Stegeman, J.J., Blumberg, B., Goksoyr, A., 2015. Environmental contaminants activate human and polar bear (Ursus maritimus) pregnane X receptors (PXR, NR1I2) differently. Toxicol. Appl. Pharmacol. 284, 54–64. https://doi.org/10.1016/j.taap.2015.02.001.
Lille-Langøy, R., Karlsen, O.A., Myklebust, L.M., Goldstone, J.V., Mork-Jansson, A., Male, R., Blumberg, B., Stegeman, J.J., Goksøyr, A., 2019. Sequence variations in pxr (nr1i2) from zebrafish (Danio rerio) strains affect nuclear receptor function. Toxicol. Sci. 168, 28–39. https://doi.org/10.1093/toxsci/kfy269.
OSPAR, 1992. OSPAR Commission - Convention Text [Online]. Available: https://www.ospar.org/convention/text [Accessed].
Pérez-Albaladejo, E., Rizzi, J., Fernandes, D., Lille-Langøy, R., Karlsen, O.A., Goksøyr, A., Oros, A., Spagnoli, F., Porte, C., 2016. Assessment of the environmental quality of coastal sediments by using a combination of in vitro bioassays. Mar. Pollut. Bull. 108, 53–61. https://doi.org/10.1016/j.marpolbul.2016.04.063.
Pieterse, B., Felzel, E., Winter, R., Van Der Burg, B., Brouwer, A., 2013. PAH-CALUX, an optimized bioassay for AhR-Mediated hazard identification of polycyclic aromatic hydrocarbons (PAHs) as individual compounds and in complex mixtures. Environ. Sci. Technol. 47, 11651–11659. https://doi.org/10.1021/es403810w.
Schnell, S., Olivares, A., Pina, B., Echavarri-Erasun, B., Lacorte, S., Porte, C., 2013. The combined use of the PLHC-1 cell line and the recombinant yeast assay to assess the environmental quality of estuarine and coastal sediments. Mar. Pollut. Bull. 77, 282–289. https://doi.org/10.1016/j.marpolbul.2013.09.047.
Skei, J., Knutsen, J., Klungsøyr, J., 1994. Miljøgiftundersøkelser I Bergen Havneområde Og Byfjorden 1993. FASE 1. Miljøgifter I Spiselige Organismer Og Bunnsedimenter (in Norwegian). NIVA Rapport.
Star, B., Nederbragt, A.J., Jentoft, S., Grimholt, U., Malmstrom, M., Gregers, T.F., Rounge, T.B., Paulsen, J., Solbakken, M.H., Sharma, A., Wetten, O.F., Lanzen, A., Winer, R., Knight, J., Vogel, J.-H., Aken, B., Andersen, O., Lagesen, K., Tooming-Klunderud, A., Edvardsen, R.B., Tina, K.G., Espelund, M., Nepal, C., Previti, C., Karlsen, B.O., Moum, T., Skage, M., Berg, P.R., Gjoen, T., Kuhl, H., Thorsen, J., Malde, K., Reinhardt, R., Du, L., Johansen, S.D., Searle, S., Lien, S., Nilsen, F., Jonassen, I., Omholt, S.W., Stenseth, N.C., Jakobsen, K.S., 2011. The genome sequence of Atlantic cod reveals a unique immune system. Nature 477, 207–210. https://doi.org/10.1038/nature10342.
Sundt, R.C., Ruus, A., Jonsson, H., Skarphéðinsdóttir, H., Meier, S., Grung, M., Beyer, J., Pampanin, D.M., 2012. Biomarker responses in Atlantic cod (Gadus morhua) exposed to produced water from a North Sea oil field: laboratory and field assessments. Mar. Pollut. Bull. 64, 144–152.
van der Oost, R., McKenzie, D.J., Verweij, F., Satumalay, C., Van Der Molen, N., Winter, M.J., Chipman, J.K., 2020. Identifying adverse outcome pathways (AOP) for Amsterdam city fish by integrated field monitoring. Environ. Toxicol. Pharmacol. 74, 103301 https://doi.org/10.1016/j.etap.2019.103301.
Viganò, L., Benfenati, E., Cauwenberge, A.V., Eidem, J.K., Erratico, C., Goksøyr, A., Kloas, W., Maggioni, S., Mandich, A., Urbatzka, R., 2008. Estrogenicity profile and estrogenic compounds determined in river sediments by chemical analysis, ELISA and yeast assays. Chemosphere 73, 1078–1089. https://doi.org/10.1016/j.chemosphere.2008.07.057.
Whyte, J.J., Jung, R., Schmitt, C., Tillitt, D., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30, 347–570.
Wincent, E., Le Bihanic, F., Dreij, K., 2016. Induction and inhibition of human cytochrome P4501 by oxygenated polycyclic aromatic hydrocarbons. Toxicol. Res. 5, 788–799. https://doi.org/10.1039/c6tx00004e.
Zhang, W., Ye, Y., Hu, D., Ou, L., Wang, X., 2010. Characteristics and transport of organochlorine pesticides in urban environment: air, dust, rain, canopy throughfall, and runoff. J. Environ. Monit. 12, 2153. https://doi.org/10.1039/c0em00110d.