

One set of sediments, three tiers of bioanalytical tools: can acute, cytotoxic and mechanistic responses serve as indicators of stormwater pond sediment contamination?

Un ensemble de sédiments, trois niveaux d'outils bio-analytiques : peuvent les réponses aiguës, cytotoxiques et mécanistiques servir d'indicateurs de la contamination des sédiments de bassins pluviaux ?

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RÉSUMÉ

Cette étude a examiné si trois niveaux d'outils bio-analytiques—la toxicité aiguë (Microtox[®]), la cytotoxicité *in vitro* et des bioessais fondés sur des récepteurs—peuvent servir d'indicateurs de la charge contaminante dans des sédiments provenant de 17 bassins de rétention d'eaux pluviales en Suède, préalablement analysés pour 259 contaminants organiques, historiques et contemporains. La toxicité aiguë a rarement été observée et les réponses Microtox[®] n'ont montré aucune correspondance claire avec la contamination chimique. En revanche, la cytotoxicité dérivée d'extraits méthanoliques de sédiments s'est fortement alignée avec plusieurs indicateurs de contamination, notamment le nombre de substances quantifiées et les dépassements de seuils environnementaux. Les bioessais à base de récepteurs (récepteur des œstrogènes, récepteur des hydrocarbures aromatiques polycycliques et stress oxydatif) ont révélé des activités biologiques étendues, dépassant fréquemment les niveaux de référence établis à partir des PNEC des composés de référence, et ont mis en évidence des réponses mécanistiques non détectées par les tests apicaux. Dans l'ensemble, les résultats montrent que si Microtox[®] est peu adapté à l'évaluation des risques liés aux sédiments pluviaux, riches en matière organique, la cytotoxicité et les bioessais fondés sur des récepteurs fournissent des informations sensibles et complémentaires sur les effets de mélange et la charge contaminante. Ces résultats soutiennent l'intégration d'outils fondés sur les effets avec le suivi chimique afin d'améliorer l'évaluation de la qualité des sédiments.

ABSTRACT

This study examined whether three tiers of bioanalytical tools, acute toxicity (Microtox[®]), *in-vitro* cytotoxicity, and receptor-specific bioassays, can serve as indicators of contaminant burden in sediments from 17 Swedish stormwater ponds which were previously analysed for 259 legacy and contemporary contaminants. Acute toxicity was rarely observed, and Microtox[®] responses showed no clear correspondence with chemical contamination. In contrast, cytotoxicity derived from methanol-based sediment extracts strongly aligned with chemical contamination indicators, including the number of quantified substances and exceedances of environmental threshold frameworks. Receptor-based assays (estrogenic receptor, aryl hydrocarbon receptor and oxidative stress) revealed widespread biological activities, frequently exceeding benchmark levels derived from reference-compound PNECs, and illustrated mechanistic responses not captured by apical tests. Taken together, the results show that while Microtox[®] is poorly suited for assessing hazards in organic-rich stormwater sediments, cytotoxicity and receptor-based bioassays provide sensitive and complementary insights into mixture effects and contaminant loading. These findings support integrating effect-based tools with chemical monitoring to improve sediment quality assessment.

KEYWORDS

Estrogen, Micropollutant, Modes of action, Oxidative stress, Stormwater sediments

1 INTRODUCTION

Urban stormwater conveys contamination from a wide variety of sources in the urban environment, including materials used in urban surfaces and anthropogenic activities such as traffic, industry and maintenance (Müller et al., 2020). This contamination includes many potentially toxic substances, such as trace metals and organic micropollutants, which accumulate in the sediments of facilities designed to manage stormwater (Crane, 2019; Tedoldi et al., 2024). The nature of this contamination (a large number of known and as-yet unknown substances often present at low concentrations) poses a problem for accurately and robustly evaluating the environmental risks associated with this material during operation or at the time of its removal for facility maintenance or decommissioning. While chemical analysis is useful for identifying specific substances and associated risks as well as identifying a specific need for remediation, these only include the substances that have been targeted in the analysis. Bioassays, including whole-organism toxicity tests and *in-vitro* effect-based methods for evaluating specific modes of action (MOAs), are a complementary method, which can apprehend adverse biological effects of all substances present towards a specific organism or end-point (Escher et al., 2020) In this context, the objective of this paper is to consider the links between chemical contamination and three different assessments of biological effects: acute toxicity with the commonly-used Microtox® test, *in-vitro* bioassays used to evaluate specific MOAs and cytotoxicity to the cells used in the *in-vitro* bioassays.

2 METHODS

2.1 Sediment sampling

Sediment was collected in Autumn 2019 from 17 wet pond facilities, located in four different Swedish municipalities: Örebro (Or), Östersund (Os), Stockholm (S) and Växjö (V). The ponds represent different urban and sub-urban catchments (industrial, residential, road or highway). They were constructed between 1988 and 2010 and were all intended to improve stormwater quality through sedimentation. Both catchment size (ca. 1 to 1490 ha) and facility-to-catchment ratios (0.007 to 2.6%) varied greatly between facilities. Sediment samples were collected at the inlet and outlet of each facility using a Kayak core sampler (KC Denmark) with a stainless-steel liner. At each of these locations, multiple sediment cores were collected and combined to create one 3-L composite sample. The cores were homogenized in a stainless-steel tray and then portioned into aliquots for the various analyses. The sample nomenclature includes both the municipality's name and the sample location, for example, 'Or1-In' and 'Or1-Out' denote inlet and outlet samples, respectively, from one of the facilities in Örebro.

2.2 Sediment analysis

The sediment samples were analysed for 259 organic substances, 13 trace elements and various physico-chemical parameters (Flanagan et al., 2019). The samples further underwent bio-analytical testing to identify their toxicity potential. First, they were analysed for acute toxicity to the bioluminescent bacteria *Aliivibrio fischeri* a using the Microtox® test according to CSN EN ISO 11348–3:2007 following elution in a 1:1 ratio with deionized water. In addition, *in vitro* bioassays were used to evaluate the potential for the cells to cause biological effects through five specific modes of action (MOAs): oxidative stress (Nrf2), aryl hydrocarbon receptor activation (AhR), androgen receptor agonism (AR), androgen receptor antagonism (Anti-AR) and estrogen receptor agonism (ER). Cytotoxicity was assessed using the CellTiter® (MTS) assay on the same cell lines used for evaluating the MOAs. These measurements were carried out following a methanol extraction, followed by a hydrophilic-lipophilic (HLB) solid-phase extraction (SPE) to concentrate organic substances and reduce the sample matrix.

3 RESULTS AND DISCUSSION

Only 5 of the 32 sediment samples produced quantifiable acute toxicity in the Microtox® assay, exceeding the 20 % inhibition threshold (IC20), and of these, three also surpassed 50 % inhibition (IC50). The overall toxicity of the samples with measurable effects followed the order V4-Out>S3-Out>V1-Out>S3-In> Or6-Out (Flanagan et al., 2025). Samples exhibiting at least IC20 were characterised by significantly higher total organic carbon (TOC) and lower dissolved oxygen (DO); however, there was a complete mismatch between toxicity according to this test and concentrations of the extended list of examined contaminants, indicating that the results of this test cannot easily be related to overall chemical contamination in urban stormwater sediments.

The ranking of samples according to the cytotoxicity obtained for the same set of sediments was also very different from that observed with Microtox®. (Figure 1). The cytotoxicity profiles were instead found to align closely with the underlying sediment contamination patterns. For instance, sediments from Örebro were

consistently identified among those with the lowest cytotoxicity, with significantly lower normalised cytotoxicity ranking scores than sediments from Stockholm and Väjö (pairwise Wilcoxon rank-sum tests, $p < 0.01$). This pattern is consistent with earlier findings showing significantly lower concentrations of organic substances in the Örebro ponds, which have been attributed to a greater proportion of natural mineral material and dilution processes in those catchments (Flanagan et al., 2021).

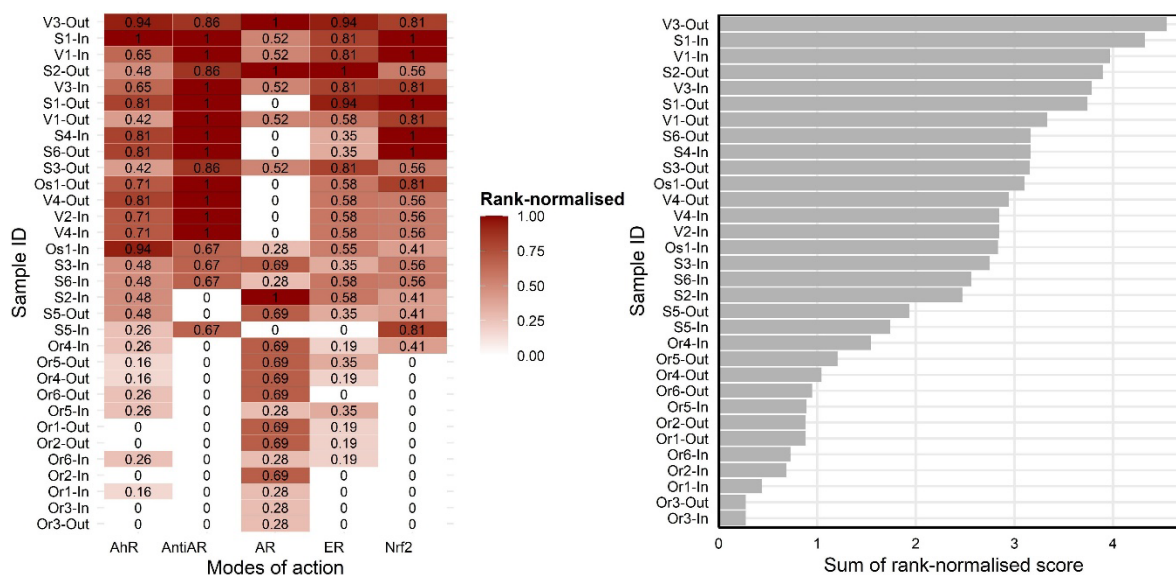


Figure 1. Heat map and sum of rank-normalised score of cytotoxicity across the samples

To evaluate how well cytotoxicity reflects chemically-defined hazards, the summed normalised cytotoxicity scores were compared with the following contamination indicators: the number of quantified substances, the number of exceedances against three environmental threshold frameworks—namely the Norwegian environmental quality standards for sediments, developed under the EU Water Framework Directive, the Swedish contaminated site guidelines for sensitive land use, which focus primarily on legacy contaminants in terrestrial contexts, and the predicted no-effect concentrations (PNECs), representing thresholds derived through a unified approach for a broad range of organic and emerging substances—as well as the cumulative ratios of quantified concentrations to these thresholds. Strong and significant positive correlations were identified for all comparisons (Spearman’s rank-order tests, $p < 0.01$, $\rho = 0.63-0.8$), indicating that cytotoxicity is closely aligned with the overall contaminant burden represented by these chemical hazard metrics.

Receptor-specific bioassays targeting ER, AhR and Nrf2 pathways revealed widespread biological activity across the sediment samples, with BEQs spanning several orders of magnitude and frequently exceeding the PNEC-equivalent benchmarks of the respective reference compounds (Figure 2).

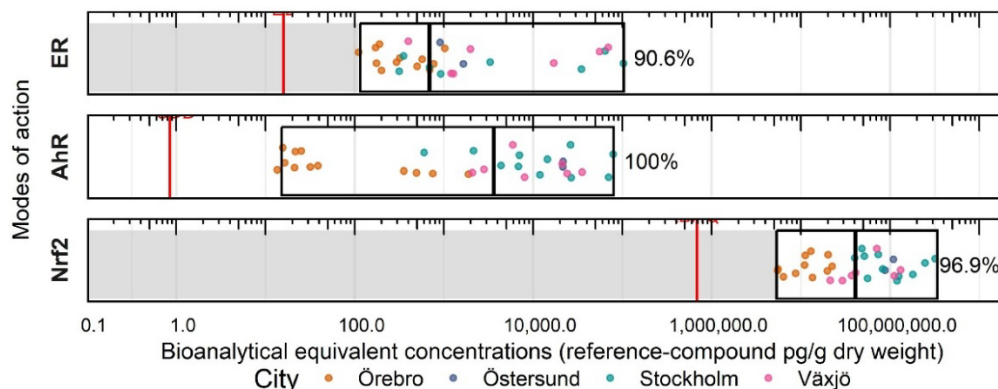


Figure 2. Minimum, median and maximum bioanalytical equivalent concentrations (BEQs) and detection frequencies for each mode of action. BEQs are expressed as equivalent concentrations of the respective reference compounds (E2 for ER, TCDD for AhR and tBHQ for Nrf2). Red lines indicate the PNEC benchmarks of the reference compounds. Where BEQs were not quantified in all samples, medians were estimated using regression on order statistics, shown as extended grey bars. Coloured points represent quantified BEQs by city.

Clear spatial differences were evident: BEQs were significantly lower in sediments from Örebro than in those from the other cities (Peto–Peto one-factor test, $p < 0.01$), consistent with the earlier observation of markedly lower organic contaminant concentrations and lower cytotoxicity (see above) in the Örebro ponds. Correlations between BEQ_{bio} and the concentrations of corresponding agonists were also examined. Although significant positive associations were identified for most substances (Kendall's tau test, $p < 0.01$), these relationships do not necessarily indicate direct causation. Many of the agonists were themselves strongly inter-correlated (Kendall's tau test, $p < 0.01$), suggesting that the observed correlations may primarily reflect shared co-occurrence patterns rather than specific substances acting as dominant drivers of the measured bioactivities.

When the three tiers (Microtox[®], cytotoxicity and *in vitro* receptor-based assays) of bioanalytical assessment are considered together, clear differences in their sensitivity, interpretative value and applicability emerge. Although both Microtox[®] and the cytotoxicity measure apical, non-specific toxicity arising from the “full chemical mixture”, their responses diverged markedly. One major reason for this is likely the difference in elution procedures: Microtox[®] was conducted on water-leachates containing both organic and inorganic constituents, including metals, whereas cytotoxicity testing was undertaken after methanol extraction, followed by SPE, intended to both concentrate the organic contaminants and largely exclude inorganic constituents. As the cytotoxicity was tested following an extraction procedure more similar to that used for the analysis of organic contaminants, it is logical that this type of test is better correlated to the abundance of organic contaminants. On the other hand, extract used for Microtox[®] is more representative of the labile fraction of contaminants under the conditions of the test (i.e. on disturbed sediments with changing oxygen levels). Further, the fact that the extract was highly concentrated prior to the cytotoxicity test makes this type of test much more sensitive. Besides this, differences in the biological sensitivity of the cell lines used to measure cytotoxicity and the marine bacterium *Aliivibrio fischeri* used in Microtox[®] may influence the results. *Aliivibrio fischeri* is a prokaryotic organism known with a limited response to many hydrophobic organic contaminants (Hermens et al., 1985), which is of limited ecological relevance in the stormwater pond context, where exposure predominantly affects benthic freshwater taxa.

Compared with these apical assays, receptor-based *in vitro* bioassays provide mechanistic information linked to specific toxicity pathways, such as xenobiotic metabolism (AhR) or oxidative stress (Nrf2). Although these assays offer high sensitivity and the ability to integrate effects from structurally diverse agonists, their relevance at higher biological levels remains challenging to interpret. As with other *in vitro* systems, cellular responses (including MOAs and cytotoxicity) do not straightforwardly translate to organism- or population-level effects, and their ecological significance requires careful contextualisation. Nonetheless, these assays can function effectively as bioanalytical tools for indicating the overall loading of compounds associated with particular modes of action, and they can reveal mixture effects that targeted chemical screening does not capture.

Taken together, the three tiers provide complementary lines of evidence. Microtox[®] appears poorly suited for providing an indication of the environmental risk due to hydrophobic organic contaminants in organic-rich stormwater sediments; cytotoxicity reflects the broader contaminant burden with substantially greater sensitivity; and receptor-based assays offer mechanistic insight and early-warning capability by detecting cellular perturbations that may precede ecological impacts. While such assays should not be interpreted as direct predictors of ecological risk, their use can support more targeted and efficient monitoring by highlighting contaminant groups of toxicological relevance and identifying samples that warrant further investigation through whole-organism testing or detailed chemical analysis.

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