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Article

Effect-Based Assessment of Runoff Water Streams in Stormwater Manufactured Barriers

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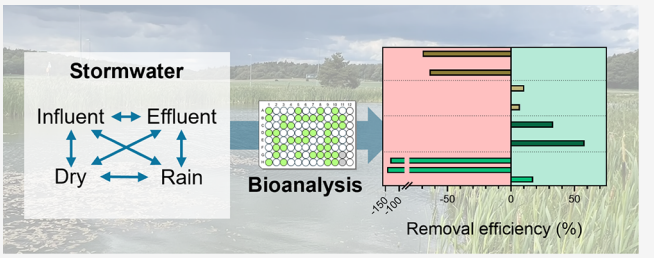
ABSTRACT: Stormwater and urban runoff have been identified as one of the major sources of chemical pollution in the aquatic environment. Although traditionally treated with man-made stormwater ponds to prevent flooding as well as to foster the remediation of some pollutants, the biological activities and removal efficiencies of toxic micropollutants are largely unknown. In this study, two stormwater ponds were studied during different hydrological conditions by means of a battery (n = 6) of cell-based bioassays, whereby the toxic pressure of the inlet and outlet water could be assessed. While no activities were observed for the oxidative stress reporter gene or androgenic activation or inhibition, clear agonistic and antagonistic estrogenic as well as aryl hydrocarbon activation responses were observed. Our observations further indicate that the efficiency of the ponds' ability to lower this bioactivity from inlet to outlet was highly variable, with several cases where higher activity was observed in the outgoing water than in the ingoing water, indicating poor management of the stormwater and the need for improved treatment approaches before the stormwater is discharged into recipient water bodies.



KEYWORDS: *stormwater ponds, influent, effluent, water quality evaluation*

1. INTRODUCTION

The cocktail of natural and anthropogenic chemicals present in the environment is still incalculable. While several million chemicals are expected to occur in the environment, only a small fraction of them have hitherto been chemically identified and reported.[1] Thus, monitoring the potential impact of this chemical cocktail on affected ecosystems with complementary tools is of utmost importance.

Stormwater and street runoff (from now on, stormwater) has shown to be a relevant source of chemical pollution for the aquatic environment.[2−5] Generally, stormwater is traditionally treated with rudimentary processes, such as natural or constructed barriers, e.g., ponds and wetlands,[5−8] or even left untreated[9−11] before being released into the aquatic environment. Additionally, with the current drift toward more extreme weather with longer drought periods followed by intense hydrological events,[12] pollutants accumulate on surfaces, land, streets, etc., for longer periods before getting flushed with heavy rain events, putting recipient water bodies quality at large risk.[13−15] Thus, thorough evaluation of potential toxic pollution associated with stormwater in both dry and rainy events, before and after treatment in stormwater ponds, is of paramount importance to ensuring a sustainable aquatic environment.

Over recent decades, chemical analysis has been widely used for the detection of contaminants of emerging concern (CECs) in aquatic samples.[16−18] Nevertheless, even wide-scope chemical analyses cover a limited fraction of the chemical cocktail[19] and lack the potential to detect most of the toxicants present in the sample or provide information on the potential toxic effects of mixtures of natural and anthropogenic chemicals.[20−22] As complementary analyses, effect-based methodologies can help unravel biological activities relevant to freshwater organisms[20,23−25] narrowing the gap between chemical analysis and the real environmental status.[21,26] As an example, it is estimated that 90−99% of the oxidative stress response activated by aquatic samples cannot be explained by chemical analysis and may account for cocktail effects or unknown chemicals.[27]

The selection of the appropriate and relevant toxicity end points to measure is, as a consequence, key for meaningful water quality evaluation.[28] While the number of available bioassays is largely expanding, the most relevant effect-based methods for environmental samples can be narrowed to a limited number of toxicity end points with the potential to detect cocktail effects of organic and inorganic pollutants.[29] The aryl hydrocarbon receptor (AhR), for example, has various

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physiological functions in relation to chemical and microbial defense, reproduction, immunity, inflammation, development, and energy metabolism in several organisms[30] and AhR activation is often triggered in aquatic samples with elevated content of aromatic hydroalkyl substances.[31] Additionally, the presence of endocrine-disruptive chemicals as well as natural and synthetic hormones can be evaluated by means of activation or inhibition of the androgen receptor (AR) and estrogen receptor (ER). Both androgenic and estrogenic hormones are highly relevant for the function of several systems such as immune, reproductive, neural, and cardiovascular systems in exposed organisms[32,33] and, thus, their effect is largely relevant for environmental samples. Finally, measuring the oxidative stress response, often triggered by the presence of organic micropollutants, is a good hint on the overall water quality.[34,35]

In this work, a set of stormwater samples from two different ponds has been evaluated in both dry and rainy periods for a panel of 6 environmentally relevant toxicity end points. Both influent and effluent stormwater have been analyzed for a comprehensive evaluation of the potential risks associated with the discharge of stormwater into recipient water bodies. In this regard, toxicity toward AhR activity, oxidative stress, and activation and inhibition of AR and ER has been evaluated.

2. MATERIALS AND METHODS

2.1. The Tibble and Gottsunda Ponds. The selection of outdoor stormwater ponds for this study was based on three different criteria. On one hand, man-made barriers with a continuous inflow and outflow of street runoff water were prioritized so that dry and rainy periods could be compared. On the other hand, both old and recently constructed ponds were of interest so that pond age could be assessed as a potential difference in their performance in pollution remediation. Finally, pond outflow streams impacting bigger and relevant water bodies were also of utmost importance for the selection of the sampling locations. Consequently, 2 stormwater ponds in the Stockholm−Uppsala region (Sweden) were studied.

The Tibble pond in Upplands-Bro municipality (Sweden) is a stormwater pond that has been active for almost 65 years with only particle sedimentation as the stormwater treatment process. With a catchment area of approximately 649 ha, a surface area of 5 700 m^2 and an average depth of 1.5 m, its vast majority (97%) consists of residential areas, woodland, and meadowland; while the remaining 3% consists of industrial areas, motorways, and parking spaces.[36] The pond occasionally receives untreated wastewater that is redirected to the inlet of the pond from a nearby sewage pump station. However, such incidents are rare, and none occurred during the study time. The effluent water stream from the Tibble pond discharges into a small natural wetland and then directly into Görväln Bay in Lake Mälaren, which is Sweden's most important water source, serving drinking water for nearly 2 million people.[37]

Differently, the Gottsunda pond in Uppsala municipality (Sweden), with a catchment area of approximately 104 ha, a surface area of 5 860 m^2 and an average depth of 1−1.2 m, is a recently built stormwater pond consisting of several presedimentation ponds as well as a large lagoon also converted into a stormwater park for educational purposes.[38,39] While Tibble pond has some industrial area and motorway impact, the Gottsunda pond is limited to residential and green areas. Thus, the pollution profiles of both ponds could differ. The effluent water stream from the Gottsunda pond reaches River Hågaån, which eventually meets Lake Mälaren via Lake Ekoln, thus also impacting the biggest source of drinking water in the area.

2.2. Sampling Strategy. Samples were collected during periods with no hydrological events (hereafter referred to as dry samples) as well as during hydrological events (hereafter referred to as rain samples). With the collection of both dry and rain samples, we aimed to investigate the potential differences in water quality between the distinct hydrological conditions. Additionally, samples were collected at the inlet (influent stream) and outlet (effluent stream) of the ponds to investigate the potential impact of retention time, such as the loss of micropollutants through sedimentation, volatilization, and biotransformation processes. For the simplicity of the text, samples are coded according to G (Gottsunda), T (Tibble), I (influent), E (effluent), D (dry conditions), and R (rain conditions). As an example, GER refers to Gottsunda Effluent under Rain conditions.

During dry periods, both stormwater ponds showed a relatively constant inflow and outflow of water. This basal flow (approximately 300 m^3 h^{-1} for Tibble and 9 m^3 h^{-1} for Gottsunda) was sampled to evaluate the influx of contaminants into the ponds. During this period, time-integrated composite samples were collected for a period of 72 h (total sample volume: 10 L). In this sense, TID and TED samples were collected in September 2022 and GID and GED in August 2023. Additionally, hydrological events represent a relevant change in the influent and effluent flow rates and, as a consequence, time-integrated sampling would not be representative.[40] Thus, volume-proportional composite and flow-triggered sample collection methods were employed . In this sense, a significant increase in the influent flow to the stormwater ponds as a result of rain precipitation automatically triggered the start of sample collection. For Tibble, 40 mL of stormwater was automatically collected for every 100 m^3 entered/exited the pond, covering approximately 36 h of rain events. For the Gottsunda pond, 40 mL of stormwater was automatically collected for every 40 m^3 entered/exited the pond, covering approximately 11 days of consecutive rain events. TIR and TER samples were collected in March 2023 and GIR and GER in September 2023. Detailed flow profiles as well as aliquot collection events are shown in Figure S1.

All samples were collected and stored in prerinsed HDPE bottles and kept at −20 °C upon reception at the laboratory until analysis.

2.3. Sample Extraction. In brief, samples were filtered through 0.45 μm regenerated cellulose filters (0.7 μm pore size, Whatman, China) and extracted by means of solid-phase extraction (SPE) with Oasis PRiME HLB 6 cc 200 mg cartridges (Waters) in an 8-channel automated SPE system (SPE-03 system, PromoChrom Technologies). Cartridges were previously conditioned with 5 mL of methanol (VWR, Sweden; HPLC grade; 5 mL/min) followed by 5 mL of ethanol (Solveco, Sweden; analytical grade; 5 mL/min) and finally with 5 mL of Milli-Q water (5 mL/min). Then, 400 mL of sample were loaded with a flow rate of 20 mL/min, followed by air drying of the column by two times 5 mL volumes of air. Samples were then eluted with 2 × 5 mL of ethanol (Solveco, Sweden; analytical grade). The aggregated eluate was evaporated under vacuum and adjusted to 200 μL with 99% ethanol. More details about the solvents and Milli-Q water can be found in the Supporting Information. Overall, the

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Table 1. Panel of Reporter Gene Assays Applied[a]

Effect-based method	Cell line	Reference compound for positive control	Cytotoxicity assay	%-effect level above which sample is considered active
Aryl hydrocarbon receptor activation (AhR)	DR Ecoscreen	2,3,7,8-Tetrachloro-*p*-dibenzodioxin (TCDD)	MTS	EC_{10}
Androgen receptor agonist (AR ago)	AR-EcoScreen	Dihydrotestosterone (DHT)	MTS	EC_{20}
Androgen receptor antagonism (AR anta)	AR-EcoScreen	Hydroxyflutamide (OHF) (Stimulant: DHT)	MTS	IC_{30}
Estrogen receptor agonism (ER ago)	VM7Luc4E2	17β-estradiol (E2)	CellTiter-Glo Luminescent Cell Viability Assay	EC_{20}
Estrogen receptor antagonism (ER anta)	VM7Luc4E2	Raloxifene (Ral) (Stimulant: E2)	CellTiter-Glo Luminescent Cell Viability Assay	IC_{30}
Nuclear factor erythroid 2-related factor 2 (Nrf2). Oxidative stress activity	MCF7C32ARE	Tert-butylhydroquinone (tBHQ)	MTS	$EC_{IR1.5}$

[a]Cell lines used , cytotoxicity assay method , reference compound for positive control , and %-effect level chosen for BEQ .

preconcentration factor achieved was 2000. The extracts were then diluted in a cell culture medium at least 100 times. The concentrations of the water samples in the cell cultures are expressed as the relative enrichment factor (REF), which is calculated by dividing the concentration factor of the SPE by the dilution factor in the cell culture medium. The highest tested REF for all samples was 20. Sample extracts were kept at −20 °C until analysis. The sample extraction method was slightly modified from Lundqvist et al.[25]

2.4. Effect-Based Methods. Influent and effluent stormwater samples from both dry and hydrological events and vehicle controls were evaluated for a panel of six bioassays consisting of aryl hydrocarbon receptor activation (AhR), androgen receptor agonism (AR ago) and antagonism (AR anta), estrogen receptor agonism (ER ago) and antagonism (ER anta), and oxidative stress activation (Nrf2) (Table 1). Cytotoxicity was evaluated in all cell lines using cell viability assays (CellTiter-Glo luminescent cell viability assay and MTS-based colorimetric assay). The threshold for cytotoxicity was set as ≤80% of the activity of the vehicle control for all the cell lines tested.

Transcriptional activation of AhR was evaluated in a stably transfected mouse hepatoma cell line (DR-EcoScreen), and agonistic and antagonistic AR activity was studied in a stably transfected Chinese Hamster Ovary cell line with a GR knockout gene, AR-EcoScreen GR-KO-M1 cells, both purchased from Hiro Biotech via the Japanese Collection of Research Bioresources (JCRB), National Institutes of Biomedical Innovation, Health and Nutrition (Ibaraki city, Osaka, Japan). Agonistic and antagonistic ER activities were evaluated in a variant of the human breast carcinoma MCF7 cell line, VM7Luc4E2 (donated by Prof. Michael Denison, University of California, Davis, USA), which contains a stably integrated ER-responsive luciferase reporter plasmid.[41] AR and ER activities were analyzed mainly according to OECD guidelines.[41−43] The stably transfected human breast adenocarcinoma cell line, MCF7 ARE c32, was used to measure oxidative stress corresponding to Nrf2 activity and was kindly provided by R. Wolf (University of Dundee, Nethergate, Scotland).[44] Further details and an expanded description of activity and cell viability assays are available in Section S1.

Positive controls (Table 1) were analyzed alongside stormwater samples. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and *tert*-butylhydroquinone (tBHQ) were used as positive controls for AhR and Nrf2 reporter gene assays, respectively. In the ER reporter gene assay, 17β-estradiol (E2) was used as a control for agonistic activity and raloxifene (Ral) for antagonistic activity. For the AR bioassay, dihydrotestosterone (DHT) was used as a positive control for agonistic activity and hydroxyflutamide (OHF) for antagonistic activity. For antagonistic effects, cells were cotreated with an agonistic stimulator as a negative control test at a concentration corresponding to approximately EC_{80}. The positive controls were analyzed in 6−12 concentration levels to obtain standard calibration curves. No specific blanks for this study were performed as the research group holds a history of no blanks inducing any activity of the bioassay panel. Thus, it was deemed not necessary to include additional tests.

All water samples were tested for cell viability and bioactivity in concentration−response relationships (REF = 2.5, 5, 10, and 20) with 4 replicates for each concentration, as previously proposed by Mehinto et al.[45] In all experiments, vehicle controls were included, consisting of 1% ethanol, equivalent to the water sample's ethanol content. Vehicle controls were tested in 8 replicates. Effect-based methods' performance was evaluated by including controls as well as positive controls in each experimental batch.

2.5. Data Processing. Measured bioactivities were normalized to vehicle controls on each plate. Additionally, the observed activity was normalized to the maximum (for agonistic) measured activity from the positive control. Sample bioactivity was then expressed as % of the maximum response of the positive control. Standard curves for AhR, AR, and ER (nuclear receptor bioassays) were drawn by fitting data to a four-parameter sigmoidal curve. For antagonist activity, the maximum activity was normalized to vehicle control with DHT or E2.[46] For Nrf2, since no maximum effect can be reached, the activity was normalized to vehicle control, and standard data were fitted to a liner regression.

The classification of samples as active was based on the effect concentration of 10% (EC_{10}) for AhR, EC_{20} for agonistic AR and ER, inhibition concentration of 30% (IC_{30}) for antagonistic AR and ER, and effect concentration for 50% induction in signal ($EC_{IR1.5}$) for Nrf2. Stormwater samples were analyzed at 4 different REF values (2.5, 5, 10, and 20) to enable the calculation of effect concentration (EC) values by means of statistical analysis. Bioequivalent concentration (BEQ) values were calculated by means of eq 1 where EC_x refers to the effect concentration (either as the concentration value for the reference compound or the REF value for samples) for the corresponding sample and assay. For this purpose, the dose−response curve of each sample was adjusted to a sigmoidal model (except for Nrf2 where linear regression was used).[47] Statistical analysis and graphical presentation

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Table 2. Interpolated Bioequivalent Concentrations (BEQs) for the Panel of Effect-Based Methods[a] Sample coding as T: Tibble pond; G: Gottsunda pond; I: influent; E:effluent; D: dry conditions; R: rain conditions.

		Bioequivalent concentrations (BEQs) in samples							
Bioassay	Reference compound	TID	TED	TIR	TER	GID	GED	GIR	GER
AhR	TCDD (pM)	0.59	1.01	1.36	1.22	1.82	1.21	0.87	2.04
AR ago	DHT (pM)	-	-	-	-	-	-	-	-
AR anta	OHF (μM)	-	-	-	-	-	-	-	-
ER ago	E2 (pM)	d[b]	d[b]	-	-	-	0.61	0.51	1.24
ER anta	Ral (nM)	1.24	2.03	2.48	2.31	12.44	5.24	2.48	2.05
Nrf2	tBHQ (μM)	-	-	-	-	-	-	-	-

[a]Concentration values do not indicate the measured concentration of the chemical but an observed toxicity equivalent to that concentration of the reference compound . [b]d: No BEQ could be derived although signal was clearly above cutoff value; -: no activity detected.

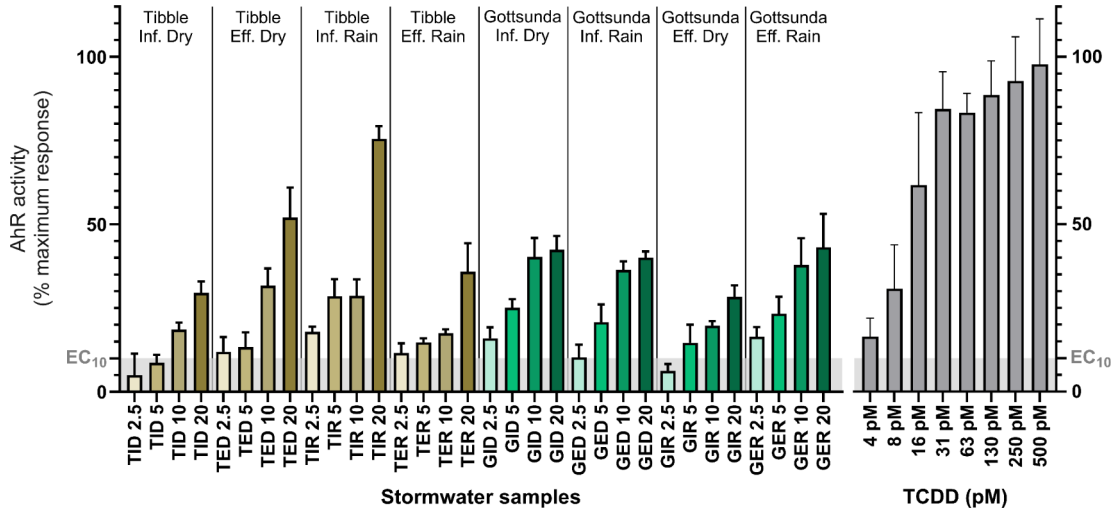

Figure 1. AhR bioactivities for Tibble (brown-colored) and Gottsunda (green-colored) stormwater samples at REF 2.5, 5, 10, and 20 (n = 4 at each REF value) and the positive control TCDD (gray-colored). Observations below EC_{10} (gray-shaded) are considered nonactive.

were performed using GraphPad Prism version 10.3.1. It is worth noting that the BEQ does not refer to a measured concentration of the chemical but rather to the necessary concentration of the positive control to infer the observed toxicity. Thus, they serve as a means to permit comparison between samples, as well as other analysis batches.

$$\mathrm{BEQ}_i = \frac{(\mathrm{EC}_x)_{\mathrm{Ref.\ comp.}}}{(\mathrm{EC}_x)_{\mathrm{sample}\ i}} \quad (1)$$

Additionally, toxicity removal efficiency (RE in %) in the ponds was estimated by means of eq 2 as an indication of the percentage change in toxicity from inlet to outlet, where $\mathrm{BEQ}_{i,j}^{\mathrm{Effluent}}$ refers to the calculated BEQ for location i in the rain/dry event j for the effluent, and $\mathrm{BEQ}_{i,j}^{\mathrm{Influent}}$ refers to the calculated BEQ for location i in the rain/dry event j for the influent.

$$\mathrm{RE}_{i,j}(\%) = \frac{\mathrm{BEQ}_{i,j}^{\mathrm{Influent}} - \mathrm{BEQ}_{i,j}^{\mathrm{Effluent}}}{\mathrm{BEQ}_{i,j}^{\mathrm{Influent}}} \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Cell Viability. Cell viability was evaluated in all cell lines used for the bioassays, showing that for most of the tested REF values no cytotoxicity was observed (Figure S2). For the cell lines Vm7LucE4 and MCF7c32ARE, corresponding to ER and Nrf2 activities, no cytotoxicity was observed even at the highest REF values tested. However, cell viability was compromised at high REF values in the cell lines DR Ecoscreen and AR Ecoscreen, which might have some implications for the measurement of AhR and AR activities. For the DR Ecoscreen cell line, samples at REF 20 systematically showed cytotoxicity and even in some cases such as GED and GIR at REF 10. This indicates that the maximum trustable REF value for AhR activation analysis needed to be limited to REF 5; otherwise, the BEQ could be underestimated. For AR Ecoscreen, it is worth noting that samples collected during rain events in Gottsunda (both GIR and GER) showed limited cell viability at most of the tested REF values (including REF 2.5). Thus, the detection of androgenic activities in those samples could potentially be hindered.

3.2. Bioactivity in Manufactured Stormwater Barriers. Table 2 summarizes the bioequivalent (BEQ) concentrations per bioassay and sample. In general, only AhR, ER ago, and ER anta were activated by the sample extracts, demonstrating that the stormwater composition in both Tibble and Gottsunda did not trigger oxidative stress or androgenic effects. In the following sections, the observed activities are discussed.

3.2.1. Aryl Hydrocarbon Receptor Bioactivity. Figure 1 depicts the bioactivities observed for AhR in the stormwater samples, as well as the positive control. With a cutoff for AhR activity at EC_{10}, all samples caused AhR activity at least in one concentration. In general, good dose−response curves were observed for all samples and the positive control, although

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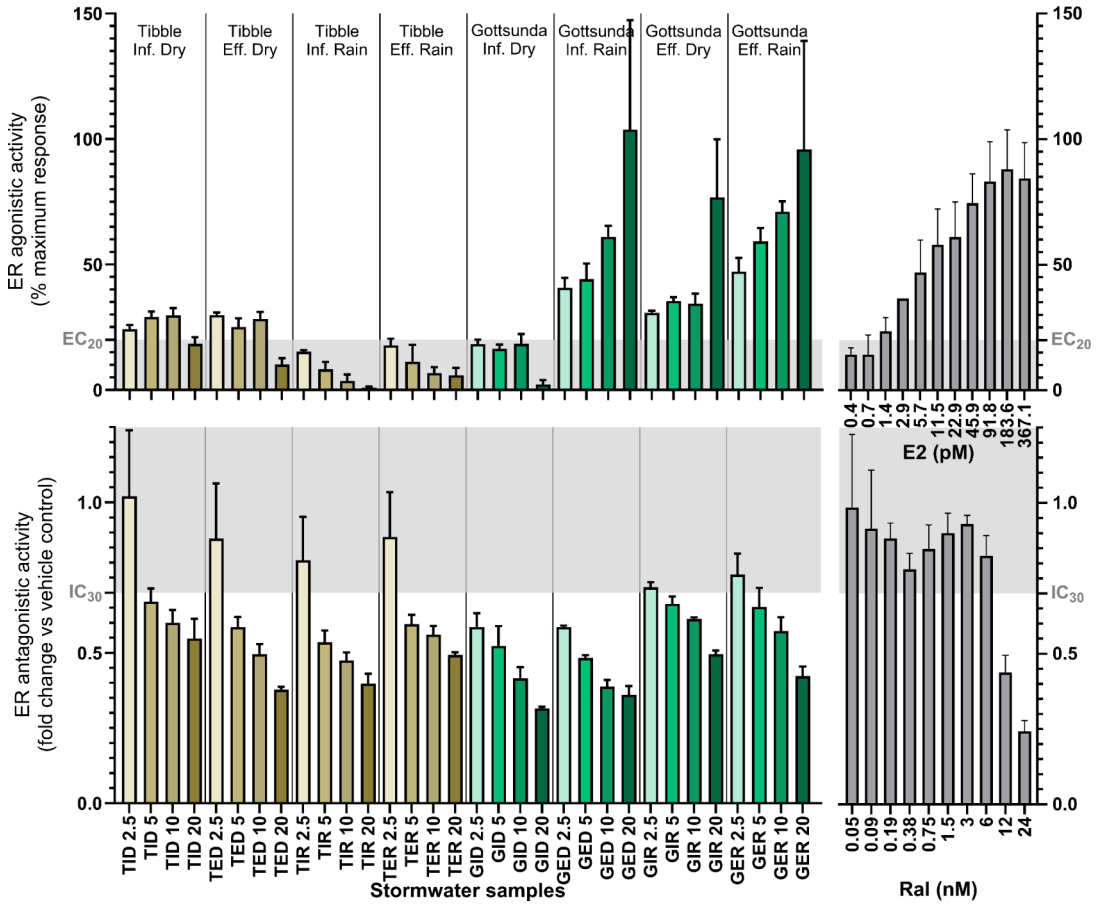

Figure 2. ER agonistic (top) and antagonistic (bottom) bioactivities for Tibble (brown-colored) and Gottsunda (green-colored) stormwater samples at REF 2.5, 5, 10, and 20 (n = 4 at each REF value) and positive control E2 (agonistic) and Ral (antagonistic) (gray-colored). Gray-shaded areas indicate regions in which the sample response cannot be categorized as active (below EC20 for agonistic and above IC30 for antagonistic assays).

some degree of cytotoxicity was observed for high REF values in all samples (Figure S2—MTS_DR Ecoscreen). This cytotoxicity was not reflected in the AhR activity calculation, and good nonlinear regression curves could be interpolated for all samples, permitting extrapolation of TCDD-eq in the range of 0.59−2.04 pM TCDD-eq (Table 2).

While no big differences were observed between the different samples, the biggest variation was observed in the Rain Influent samples, either as an increase in the TCDD-eq or as a decrease compared to the other samples from the sample pond. The TID, TED, and TER showed activities in the range of 0.59−1.22 pM TCDD-eq with small variations; however, the TIR sample showed an increase to 1.36 pM TCDD-eq, which indicates a higher concentration of AhR-activating chemicals. This could be the result of a flush of chemicals present on the surfaces and roads that serve the stormwater pond and, thus, an increase in their impact on water quality. Contrarily, the GIR sample showed lower AhR activation values compared to GID, GED, and GER (1.21−2.04 pM TCDD-eq) to 0.87 pM TCDD-eq. While it is also expected that there is influence of flushing surfaces and roads in the GIR sample, the dilution factor of the high flow of rain could be responsible for such a decrease in activity compared to the other Gottsunda pond samples.

This systematic activation of AhR highlights the fact that there might be some potential harm to the surrounding environment, since both the influent and effluent streams can impact multiple physiological functions involving energy metabolism, chemical and microbial defense, reproduction, development, immunity, and inflammation in aquatic organisms.[30] While the identity of the chemical drivers for such toxicity and their likely origin (either natural or anthropogenic) is still unknown, the results indicate the presence of organic chemicals with aryl hydrocarbon groups. AhR activation by environmental water samples has been repeatedly reported[35,48,49] at similar BEQ levels, including in stormwater matrices.[50,51] In addition, there is evidence of human activities fostering the activation of the AhR receptor in environmental samples such as agricultural practices,[52] industrial areas as well as wastewater influence.[48] None of these activities can be excluded from impacting either the Tibble or Gottsunda pond.

3.2.2. Androgen Receptor (Ant)agonistic Bioactivity. In general, no bioactivity was observed for the AR reporter gene assay in the stormwater samples. In the case of AR ago, no appropriate dose−response effect was observed in the samples, with all the responses below the cutoff value of the assay (Figure S3). However, slight cytotoxicity could be derived from the analyses since the responses in the AR agonistic test decreased with increasing REF, even to negative values (when normalized to the vehicle control). Although some cytotoxic effects were also observed in the cell viability test (Figure S2), they were not as pronounced as those observed in the AR agonistic evaluation. For AR antagonism, similar data was observed. With all the responses above the cutoff value, no AR

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anta activity was identified (Figure S4). Additionally, a reinforced activity (over a 1-fold change vs the vehicle control) was observed for all samples at low REF values, which decreased down to 1 at higher REF values. While there is no clear explanation for this behavior, it could potentially be produced by a cocktail effect of the sample's endogenous compound together with the DHT stimulant added to stimulate the AR, suggesting some potential but uncharacterizable agonistic effect in the samples.[49] It is also noteworthy that previous studies identified androgenic activities in stormwater[53] or river water,[48] highlighting the relevance of the absence of (anti)androgenicity in the samples analyzed herein.

3.2.3. Nuclear Factor Erythroid 2-Related Factor 2 Bioactivity. Neither sample caused oxidative stress (Figure S5) indicating that no oxidative stress-inducing chemicals were present in the stormwater samples, or if present, their concentration was not high enough to trigger any oxidative stress effect. Contrarily to AhR, which is regularly activated by environmental surface water samples, for oxidative stress several studies have reported the nondetection of any triggering.[35,49,52] However, other studies have identified oxidative stress effects in stormwater[50,51] although implementing similar sample preparation methodologies (solid-phase extraction by means of HLB sorbent) as done in the present study. Consequently, it was unexpected that no oxidative stress was detected in the analyzed samples.

3.2.4. Estrogen Receptor (Ant)agonistic Bioactivity. Estrogenicity and antiestrogenicity in the analyzed stormwater samples yielded more complex results than the other reporter gene assays. The evaluation of ERago showed a pronounced and appropriate dose−response curve for samples GED, GIR, and GER, clearly above the cutoff value. For these samples, E2-eq ranging from 0.51 to 1.24 pM could be extrapolated (Figure 2 top). Similarly to the AhR case, there was a clear increase in the BEQ calculated for GED and GER samples compared to the corresponding influent stream samples from Gottsunda (GER), which might be an indication of some estrogenicity induction in the pond. Thus, detecting E2-eq in GED and GER indicates poor treatment efficiency of the pond for removing compounds triggering ERago and a risk to the recipient water quality. On the other hand, the observations for TID, TED, TIR, TER, and GID samples were not as straightforward since a decrease in agonistic activity was observed for high REF values. While the cell viability evaluation did not detect cytotoxicity for these samples, the ERago assay indicates some kind of impact on cell viability. With the goal of identifying whether lower REF values would shed some light on this, 4 additional dilution series were analyzed for TID, TED, TIR, TER, and GID (REF 0.2, 0.3, 0.6, and 1.2) (Figure S6). However, no additional information could be gathered since no dose−response effect was observed for the extra REF values analyzed. Nevertheless, TID and TED showed activity above the cutoff value, yet no dose−response was observed and, as a consequence, no E2-eq could be extrapolated for them. Thus, their activity could only be detected (Table 2). Estrogenic activity has been previously detected in environmental water samples[35,48,49,54] as well as stormwater samples.[50,55,56] Rauert et al. detected estrogenicity at levels ranging from E2-eq 0.3 to 3 pM, while Shuliakevich et al. observed E2-eq within 0.6−8 pM.[50,56] Additionally, Tang et al. observations were in 95% of cases, E2-eq levels were below 3 pM, with some outliers up to 40 pM.[55] In this light, our observations align with previously measured estrogenicity in stormwater systems.

For ERanta, intense antiestrogenic effects were observed in several samples. With all of them clearly below the cutoff value, they showed a clear dose−response curve for the inhibition of estrogenic activity (Figure 2 bottom) with Ral-eq values ranging from 1.24 to 12.44 nM (Table 2). Additionally, no relevant differences could be observed between influent or effluent, rain, or dry periods, except for GID and GED, where the pond was able to decrease the antiestrogenicity of the effluent stream at dry conditions. In any case, this indicates a relatively stable and consistent presence of antiestrogenic chemicals in the stormwater streams. While scarce research has been conducted on the antiestrogenic effects of stormwater samples, a previous study on surface water identified much higher Ral-eq values in Spanish protected wetlands.[49] This highlights the relevance of this rather low ERanta detection in stormwater, which is usually considered a much more complex matrix than surface water.

Mainly, estrogenicity is triggered by both natural and synthetic hormones and/or phytoestrogens,[57] while antiestrogenicity results from the presence of planar organic compounds, such as dioxin-like substances.[58] Thus, the identification of (anti)estrogenic activities in stormwater samples could be an indicator of the potential presence of such types of chemicals and the potential impact of the recipient water body quality and living organisms.

3.3. Stormwater Pond Water Quality and Its Potential Implications. The predicted no-effect concentration (PNEC) value indicates the lowest concentration at which a certain chemical is not expected to induce any toxicity in the surrounding ecosystem.[59] In this sense, a comparison of PNEC with calculated BEQ could help estimate the relevance of the observed toxicities. However, one should bear in mind that calculated BEQ values are most likely the result of a combination of multiple compounds, meaning that, in practicality, the actual concentration of the specific reference compound is lower than the calculated BEQ level. Yet, it gives an estimation of the overall level of toxicants in the sample, and thus, their comparison against PNEC should be performed vigilantly.

The NORMAN Ecotoxicological Database[60] serves as a repository of the lowest PNEC values reported in the scientific literature for different environments. Therein, the lowest PNEC in freshwater for TCDD is 0.026 μg/L (8.07 pM),[61] for E2 0.0004 μg/L (1.47 pM),[62] and for Ral 0.033 μg/L (0.069 nM).[60] When comparing PNEC values against the calculated BEQ in the analyzed stormwater samples, for both AhR and ERago, the activities observed in the samples do not appear to pose an important concern for the surrounding ecosystems as TCDD-eq and E2-eq are below $PNEC_{TCDD}$ and $PNEC_{E2}$ values, respectively. However, for antiestrogenic activities, calculated Ral-eq largely exceeded the $PNEC_{Ral}$. This draws attention to the importance of evaluating water quality to establish appropriate treatment or remediation techniques in these particular stormwater ponds.

As a means to estimate the efficiency of these passive stormwater treatments to remove observed bioactivities, toxicity removal efficiency (RE) was estimated by eq 2, i.e., by comparing observed activities in influent and effluent streams of the ponds during the same sampling event. Overall, the observations did not indicate systematic behavior (Figure 3) as there was no single activated bioassay showing the same

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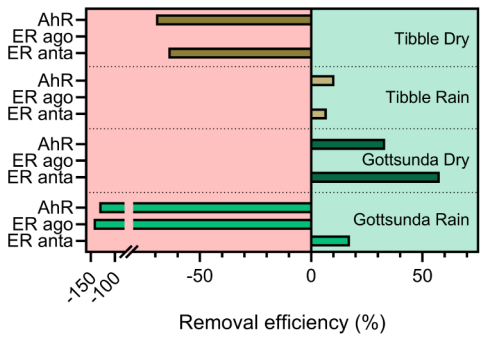

Figure 3. Estimated removal efficiencies for the detected effects (AhR, agonistic ER, and antagonistic ER) in Tibble and Gottsunda under rain and dry conditions.

trend for all sampling events. As an example, AhR activity increased from inflowing water to outflowing water in the pond for Tibble Dry and Gottsunda Rain samples, particularly in the latter case, while it was partially removed for Tibble Rain and Gottsunda Dry. Similar observations were found for the pond's impact on ERanta, with Tibble Dry increasing the antiestrogenic effects, while Tibble Rain, Gottsunda Dry, and Gottsunda Rain reduced antiestrogenicity in the outlet streams. It is also worth noting the large increase in AhR and ERago effects in Gottsunda Rain effluent samples, reaching an increase of up to ∼150% in the pond. This could potentially be due to remobilization or redissolution of already present contaminants through particle−water partitioning reactions with the bottom sediment or resuspended particulate matter.

Overall, as mentioned above, the observed bioactivities of some of the studied end points in both in- and outlet streams and the substantially increased bioactivities for outlet streams during some sampling events (rain and dry) suggest that toxic pollutants enter the stormwater pond systems but that the ponds' treatment capacity with respect to reducing the bioactivity is limited, in some cases even to the extent that water with higher bioactivity leaves the pond. Considering the higher flow and therefore higher fluxes of pollutants during rain events, the increase in the observed activities during rain events is even more remarkable.

4. CONCLUSIONS

A set of stormwater samples from both dry and rainy periods, at both the inlet and outlet of man-made stormwater barriers, has been evaluated for a panel of 6 toxicity end points. While no activities were observed for the oxidative stress reporter gene nor androgen activation or inhibition, the analyzed samples showed clear agonistic and antagonistic estrogenic responses as well as AhR activation. With AhR and ERago BEQ values not raising concern toward potential adverse effects, the BEQ estimated for antagonistic ER arose concerning values. From another perspective, the ponds' efficiency to remove the observed toxicities was evaluated, highlighting the poor treatment performance of these man-made stormwater barriers. While in some cases the toxicity observed was lower in the outlet than in the inlet, it is also quite relevant that in several cases the observed toxicity in the outlet stream was substantially higher. With these outlet streams eventually reaching recipient waterbodies serving as a source for drinking water, the need for deeper evaluation of the efficacy and improvement of these passive treatments for the pollutants' remediation is of paramount importance.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.5c00256.

The effect-based methodological details, flow profiles at both Tibble and Gottsunda ponds, cell viability of stormwater samples for the different cell lines used as well as AR agonistic, AR antagonistic, Nrf2 and ER agonistic bioactivity graphs (PDF)

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Author Contributions

A.C.: conceptualization, investigation, formal analysis, data curation, visualization, writing—original draft; G.M.: investigation, formal analysis, data curation, writing—review and editing; K.W.: conceptualization, funding acquisition, writing—review and editing; J.L.: conceptualization, data curation, funding acquisition, writing—review and editing.

Notes

The authors declare the following competing financial interest(s): Johan Lundqvist is a co-founder and co-owner of BioCell Analytica Uppsala AB, a company providing effect-based testing services to the water sector. Further, he is one of the inventors on the Swedish granted patent SE 546 454 C2 (Effect-based biosensor comprising reporter cells for water analysis).

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