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Artificial infiltration in drinking water production: Addressing chemical hazards using effect-based methods

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ABSTRACT

Artificial infiltration is an established managed aquifer recharge method that is commonly incorporated into drinking water processes. However, groundwater sourced from this type of purification method is prone to contamination with chemical hazards. Such an instance was previously shown at a Swedish DWTP where the river water was contaminated by hazardous chemicals during artificial infiltration. Further, there remains a paucity of research studying the quality of drinking water following this type of treatment from an effect-based bioanalytical perspective. In the current study, an effect-based assessment for chemical hazards was conducted for a Swedish drinking water system comprised of two DWTPs fed artificially-infiltrated river water. In this system, artificial infiltration of the river water takes approximately six to eight months. A sampling event was conducted in the autumn season and the samples were enriched by solid phase extraction. A panel of cell-based reporter gene assays representing several toxicity pathways was selected: oxidative stress response (Nrf2 activity), aryl hydrocarbon receptor (AhR) activation, and hormone receptor-mediated effects (estrogen receptor [ER], androgen receptor [AR]). AhR and ER bioactivities were detected in samples collected from the river intake and in the open-air infiltration basins prior to artificial infiltration. However, the AhR activity decreased and ER activity was effectively removed following artificial infiltration. In the Nrf2 and AR assays, no bioactivities above cut-off levels were detected in any samples collected along the entire treatment process of the drinking water production from source to tap. Using a suite of bioassays, the current study highlighted the effectiveness of artificial infiltration in reducing bioactive compounds in this raw river water. Although artificial infiltration is a common purification method in drinking water production, the limited number of effect-based studies evaluating the effectiveness of this method emphasizes the need for further research to better understand the risks and benefits of this water treatment process.

1. Introduction

Globally, groundwater is commonly used as a freshwater supply for drinking purposes. To sustainably manage this resource, groundwater can be replenished through a process of managed aquifer recharge (MAR) (Balke and Zhu, 2008) wherein the aquifer is artificially recharged with surface water (US National Research Council, 1994). This can be done by various methods such as via infiltration basins, irrigation pits, redirection of the surface water across land surfaces, or via injection wells into the subsurface. In Europe alone, more than 200 different MAR schemes, specifically riverbank filtration, are used in the production of drinking water (Sprenger et al., 2017). In Sweden, for instance, approximately 25% of the public drinking water is sourced from surface waters via artificial infiltration, a method of MAR (Svenskt Vatten, 2021).

However, artificially recharged groundwater can become contaminated by many of the same pollutants that enter surface waters including toxic metals, pesticides, industrial chemicals, microorganisms, natural toxins, and a variety of micropollutants (MPs) via diffuse (non-point) sources (Albergamo et al., 2019; Böhlke, 2002; Díaz-Cruz and Barceló, 2008; Maeng et al., 2011; Sasakova et al., 2018). In a previous study using *in vitro* bioassays, we detected a contamination scenario in the

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artificially infiltrated source water of a Swedish drinking water treatment plant (DWTP) (Oskarsson et al., 2021). In that study, high oxidative-stress activity and anti-androgenic effects were detected in the outlet of the DWTP but not in the raw river water samples collected. Further, a chemical analysis of the samples revealed that the detected organic MPs did not contribute to the observed effects. The results of that study highlighted that further effect-based research into the artificial infiltration process and the associated risks due to chemical contamination is clearly needed.

Effect-based methods using *in vitro* bioassays provide useful information on the total effect and moreover, the toxic potential of a sample for a specific toxicity pathway, integrating both known and unknown chemicals as well as mixture effects (Brack et al., 2019; Escher et al., 2020). The application of such methods in the assessment of drinking water production is not new and has been used in hazard identification as well as in assessing the efficacies of drinking water treatment technologies. However, very few studies have investigated the artificial infiltration process in drinking water production using such a bioanalytical approach.

The current study thus aimed to perform an effect-based assessment of chemical hazards of another Swedish drinking water system comprised of two DWTPs fed artificially-infiltrated groundwater. A panel of *in vitro* reporter gene assays was used representing several common toxicity pathways relevant to human health, such as oxidative stress (Nrf2 activation), aryl hydrocarbon receptor (AhR) activation, and hormone-mediated effects. The selection of bioassays followed what is generally recommended to be comprehensive of effects commonly detected in water samples (Escher et al., 2021). This study also monitored the bioactivities across all subsequent treatment steps in two conventional DWTPs fed the infiltrated groundwater as well as in their respective distribution networks.

2. Materials and methods

2.1. Drinking water production in Uppsala, Sweden

In Uppsala, the drinking water supply is sourced primarily from groundwater extracted from the Uppsala esker. To compensate for water abstraction, a managed aquifer recharge system has been utilized since 1966 to infiltrate surface water from the Fyris River (and additionally from Lake Tämnaren during the summer months). At the source water intake, the raw river first undergoes rapid sand filtration and then is pumped uphill to multiple infiltration basins situated north of the Greater Uppsala area in a nature area (referred to as Tunåsen). The water from the basins percolates into the subsurface and mixes with the naturally formed groundwater as it flows through the aquifer. It takes approximately six to eight months for the infiltrated water to travel to four wellfields that supply two DWTPs (Gränby and Bäcklösa). The groundwater abstracted at the respective four wellfields vary in the proportion of infiltrated water from 15–20%, 40–45%, 45–50%, to 80–90%. At both DWTPs the incoming infiltrated water undergoes similar treatments including: aeration, hardness removal (pellet reactors), sand filtration, and then disinfection via chlorination (with sodium hypochlorite). However, ten granular activated carbon (GAC) filters are also installed at the Bäcklösa DWTP between the sand filtration and chlorination treatment steps. The finished drinking water is stored in underground reservoirs at the DWTPs before entering the distribution network which consists of two municipal water towers and 440 km of pipelines that serve residential, commercial, and industrial water users. An average of 48,300 m^3 of finished drinking water per day was distributed from these two DWTPs in 2021 to serve approximately 190,000 consumers in the city of Uppsala. A more detailed explanation of the drinking water production process is provided in the Supplementary Information (S1).

2.2. Sample collection and preparation

Water samples were collected in late September and early October 2020. Grab samples (2 L) were collected from 22 sampling sites representing the full treatment cycle of the drinking water production process from source to tap (Table 1, Fig. 1). The water samples were collected in two 1-L sterile PET bottles (VWR® collection) and transported immediately to the laboratory where they were stored at -20 °C until sample preparation within 45 days. This specific type of bottle has previously been demonstrated not to contaminate water samples with any activity in the assays assessed in this study (Lundqvist et al., 2021). Procedural controls of ultrapure water (Milli-Q®) sourced from the laboratory were also included.

The samples collected from the three basins sampled at Tunåsen were first filtered using 0.45 μm PES filters under vacuum due to their turbid nature and the presence of visible debris (e.g., dead vegetation). All water samples (2 L) were extracted via solid-phase extraction (SPE) using a SPE-03 8-Channel Automated SPE System (PromoChrom Technologies) and 6-mL HLB cartridges (6cc Oasis Prime HLB cartridge, sorbent weight 200 mg, Waters Corporation). The sample extraction process consisted of: preconditioning with ethanol, loading of water volume, extraction with ethanol, followed by rinsing and evaporation. All samples were re-suspended with ethanol to obtain a final extract volume of 0.4 mL. Each water sample was thus enriched by a factor of 5000. Additional information regarding the sample preparations is provided in the supplementary information (S2.1).

2.3. Bioassays

The concentrated water samples along with procedural (Milli-Q®) controls, vehicle negative (1% ethanol) controls, positive controls, and reference compounds were tested in luciferase reporter gene assays. The assays were selected based on their relevance to effects commonly detected in drinking water extracts and representation of different cellular toxicity pathways relevant to human health. The following endpoints were thus assessed: oxidative stress response (Nrf2 activation), aryl hydrocarbon receptor (AhR) activation, estrogen receptor (ER) activation, androgen receptor (AR) activation and inhibition. Cytotoxicity was initially assessed in all cell lines with cell viability assays (MTS for all assays except ER activity, where the ATP assay was used). The main purpose of the cell viability testing was to ensure that the bioanalytical assessment of specific parameters was performed

Table 1
Description of sampling locations and sample IDs.

Sample ID	Treatment/Location Description
FS	Fyris River pump station before infiltration
T-FW	Tunåsen pre-infiltration water
B1	Tunåsen basin 1
B2	Tunåsen basin 2
B3	Tunåsen basin 3
GWF	Galgbacken wellfield
G-IW	Gränby - incoming water from Galgbacken wellfield
G-A	Gränby - after aeration
G-SRL	Gränby - after softening reactor line 1
G-SF	Gränby - after sand filters 1 to 3 (of 6)
G-C	Gränby - before chlorination (composite sample of all 6 sand filters)
G-OW	Gränby - outgoing water
G-TAP	Gränby - tap water location approximately 2.6 km from DWTP
B-IW1	Bäcklösa - incoming water from Sunnersta wellfield
B-IW2	Bäcklösa - incoming water from Stadsträdgården wellfield
B-A	Bäcklösa - after aeration
B-SRL	Bäcklösa - after softening reactor line 1
B-SF	Bäcklösa - after sandfilter 1-3
B-CF	Bäcklösa - after active carbon filters
B-C	Bäcklösa - before chlorination
B-OW	Bäcklösa - outgoing water
B-TAP	Bäcklösa - tap water location approximately 2.4 km from DWTP

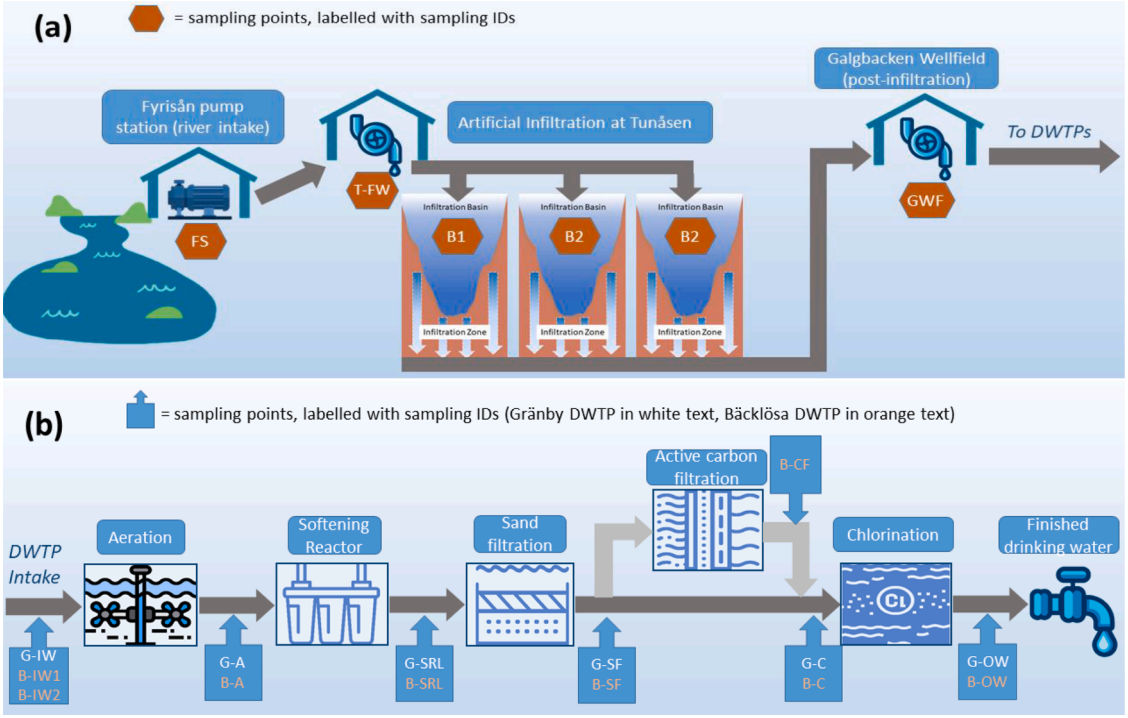

Fig. 1. Simplified diagram of Uppsala Vatten's artificial infiltration pre-treatment process from the raw water source to one of the wellfields (a) prior to downstream water purification in the two DWTPs (b). Note that activated carbon filtration treatment is utilized at Bäcklösa (sampling IDs denoted with "B"), but not at Gränby (sampling IDs denoted with "G"). Refer to Table 1 for sampling location descriptions.

under non-cytotoxic conditions. Cell viability of < 0.80 of the vehicle control was defined as cytotoxic.

The concentrations of the tested samples were expressed in units of relative enrichment factor (REF). When incubated with the cells, the 5000-fold enriched samples and controls were diluted 100-fold with cell medium to attain a final well concentration of 1% ethanol and a REF of 50 (as well as 200 for some samples) in all bioassays. The enrichment and dilution of the samples together constitute the REF (Escher et al., 2014). A REF of 1 is interpreted as the unconcentrated native sample while a REF of 50, for instance, indicates that the sample was enriched 50 times in the bioassay.

Detailed descriptions of the bioanalytical methods are provided in the supplementary information (Sections S2.2 to 2.5). For all bioassays, the concentrated water samples and controls were analyzed in quadruplicate. All bioassays were repeated at least once to prove biological reproducibility. In brief, all activity experiments were conducted in white-walled 384-well cell culture plates with transparent bottoms (Corning Incorporated). Cells were seeded in the plates and incubated for 24 h. The cells were then exposed to the concentrated water samples for another 24 h. On the third day, bioactivity (i.e., luminescence) was measured on a TECAN Spark® Multimode Microplate Reader using the Luciferase® Reporter Assay System (Promega), according to the manufacturer's instructions. Vehicle controls and a dilution series of reference compounds were included on every experimental plate for each assay. For the ER assay, a weak positive control (p,p'-methoxychlor) was also included. A summary of the bioassays and concentration ranges of the reference compounds are provided in Table 2.

Table 2
Summary of the applied bioassays.

Target Cellular Endpoint	Cell Line	Reference Compound & Conc. range
Oxidative stress response (Nrf2 activity)	MCF7AREc32	tBHQ (0.78-50 μM)
Aryl hydrocarbon receptor activation	DR-EcoScreen	TCDD (0.5-1000 pM)
Estrogen receptor agonism	VM7Luc4E2	17ß-estradiol (E2) (0.36- 370 pM)
Androgen receptor agonism	AR-EcoScreen GR KO M1	DHT (0.001-1000 nM)
Androgen receptor antagonism	AR-EcoScreen GR KO M1	OHF (0.01-10000 nM)

2.4. Data evaluation

All concentrated water samples were initially analyzed for bioactivity at a concentration of relative enrichment factor (REF) 50 in all bioassays. Bioactivities in each sample were expressed as the mean fold change normalized to the mean fold change in the vehicle controls, set to 1. For Nrf2 activity, where no maximum effect can be reached, the standard curve for the reference compound was based on a linear regression of activities normalized to the mean activity of the vehicle control. For AhR, AR, and ER, the standard curves for the reference compounds were obtained by fitting data (x-axes were log-transformed) to a four-parameter sigmoidal curve.

Cut-off levels for a positive response in bioactivity were determined as follows: for Nrf2, a fold ratio of 1.5 compared to the normalized vehicle control was used as the cut-off level for bioactivity, as recommended by Escher et al. (2012). For AhR, AR, and ER, cut-off levels for bioactivity were based on the limit of detection (LOD) for that assay, which was defined as 1 plus 3 times the standard deviation (SD) of the normalized vehicle control. A cut-off level for a positive response was then set for each assay as a value exceeding the LOD value. In instances when the LOD was below 1.5, the cut-off level was set to 1.5, and if the LOD was between 1.5 and 2, the cut-off level was set at 2. For AR antagonist activity, the LOD was calculated as 1 min 3 times the SD of the normalized vehicle control, and a cut-off level of 0.7 was set. For some samples, differences in bioactivities at REF50 were statistically evaluated using a one-way ANOVA followed by a Šidák's multiple comparisons test, performed in GraphPad Prism (v. 9.3.1). Statistical

significance was defined as $p < 0.05$.

Samples collected from the inlets, outlets, and distribution networks of Gränby and Bäcklösa were tested further in dilutions series up to REF 200 to calculate bioanalytical equivalent (BEQ) concentrations. In one of the dilution series, a Mann-Whitney test was performed to circumvent a lack of sample volume. Further details of this instance are provided in Section 3.2.2. Mean activities were normalized first to the vehicle control then to the assay maximum, defined as the highest concentration of the reference compound of the respective assay. The normalized data were then fit to four-parameter sigmoidal curves to generate concentration-effect curves (CECs) and analyzed via non-linear regression. The concentrations causing a 10% effect (EC10), expressed as REF, were then interpolated from the curves. The EC values were further translated into BEQ concentrations in units of ng/L or µg/L, using the EC_{10} values of the sample ($EC_{10,\ sample}$) and the reference compounds ($EC_{10,\ ref}$) of the particular assay using Equation (a). A more detailed explanation of the selection of the bioassays and samples for the dilutions series is provided in Section 3.2.2. All statistical analyses as well as graphical presentations were performed using GraphPad Prism (v. 9.3.1).

$$BEQ_{bio} = \frac{(EC_{10}\ or\ EC_{IR1.5})_{ref}}{(EC_{10}\ or\ EC_{IR1.5})_{sample}} \quad \text{(a)}$$

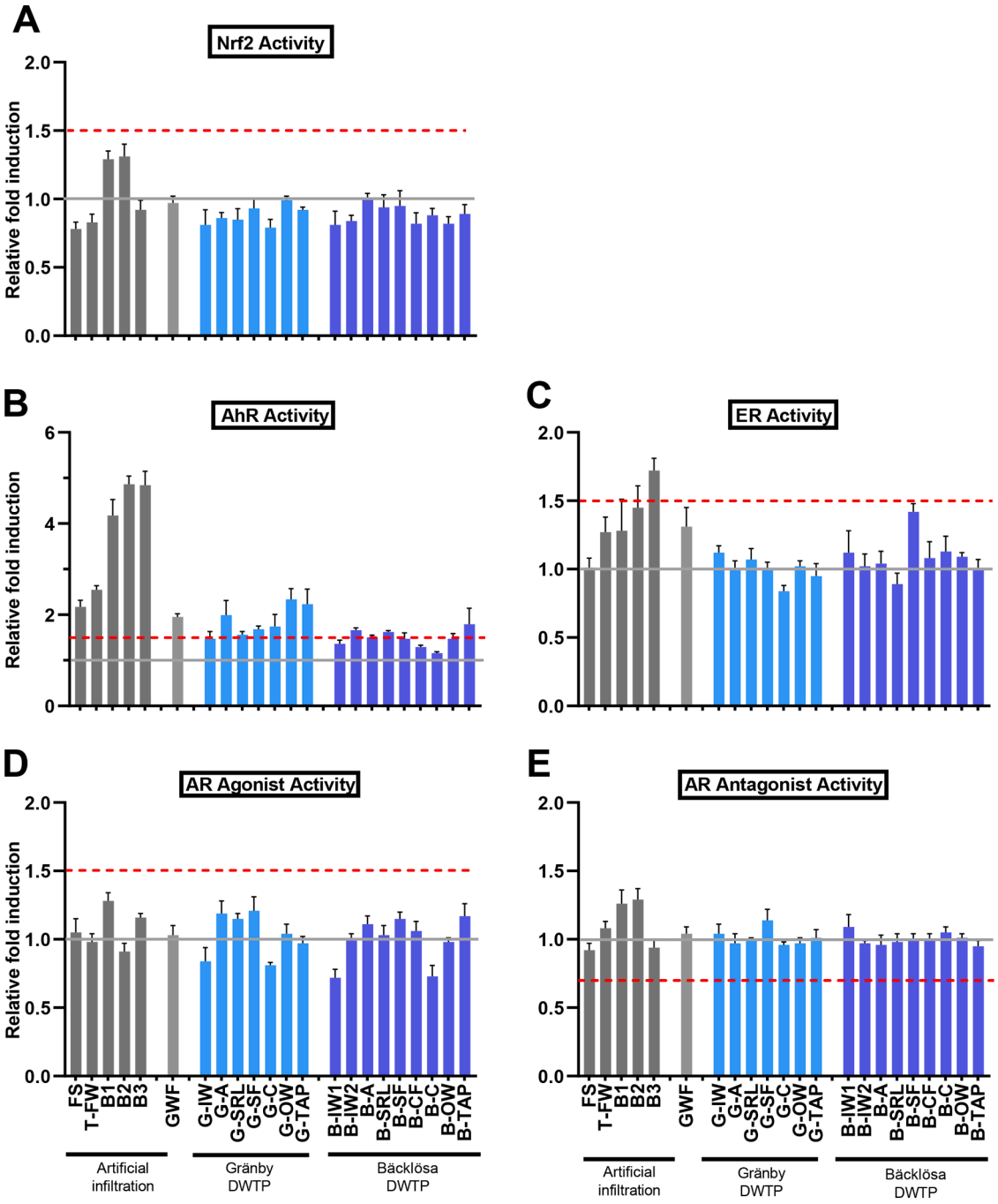

Fig. 2. Relative fold inductions (vs. vehicle control) observed at REF 50 for Nrf2 (A), AhR (B), ER (C), and AR agonist (D) and antagonist (E) activities. Treatment groups (n = 4) were normalized to vehicle controls (n = 8) set to 1 (grey line). The dotted red lines represent the respective cut-off levels. Data presented as mean ± SD. Refer to Table 1 for sampling location descriptions.

3. Results and discussion

3.1. Cytotoxicity

All samples were initially tested for cytotoxicity at REF 50 in all assays. Thereafter, samples that were to be assessed further in dilutions series were tested for cytotoxicity up to REF 200. In all assays, none of the water samples exerted cytotoxicity (Supplementary Information, Fig. S1) which demonstrated that the bioassays were conducted under conditions where the cell viability was not compromised.

3.2. Initial screening of bioactivities

Initially, all samples were analyzed at REF 50 in bioassays for oxidative stress (Nrf2 activity), AhR, and ER agonistic activities, as well as AR agonistic and antagonistic activities (Fig. 2). In general, the majority of the samples were inactive for most of the studied endpoints at REF 50. None of the samples exerted oxidative stress (Fig. 2A) or AR agonist (Fig. 2D) or antagonistic activity (Fig. 2E) above cut-off levels and only one sample showed estrogenicity at REF 50 (Fig. 2C). For AhR, however, several raw water samples taken in the basins prior to infiltration exerted relatively high activities at REF 50 (Fig. 2B). AhR activity was lower in the post-infiltration wellfield sample and significantly different than the activities detected in the basin samples ($p<0.0001$, Šídák's multiple comparisons test, $\alpha= 0.05$). The AhR activity remained at or just above the detection limit in most post-infiltration samples collected downstream in the DWTPs and distribution networks.

3.2.1. Treatment effects of artificial infiltration

In the soil subsurface, chemical contaminants can undergo biodegradation and attenuation over time via various biotic and abiotic processes during migration. They may also be removed from the aqueous environment by adherence to soils. Many studies have reported on the fate and degradation of various chemical contaminants in wastewater effluents treated by natural attenuation in the soil subsurface (Cordy et al., 2004; Drewes et al., 2003; Hoon et al., 2007). Further to this, artificial recharge through infiltration basins has been reported to improve recharged water quality by eliminating various pesticides, pharmaceuticals, and pathogens (Dragon et al., 2018; Maeng et al., 2011; Nagy-Kovács et al., 2018; Tröger et al., 2020; Valhondo et al., 2020; Verstraeten et al., 2003). Moreover, biological toxicity assays have been used to evaluate the safety of reclaimed wastewater and recycled water quality (Leusch and Snyder, 2015; Xu et al., 2020). However, there appears to be a paucity of bioanalytical studies investigating the effectiveness of artificial infiltration processes in drinking water production. Nevertheless, the results of the current study are discussed below in the context of a limited number of studies relevant to the treatment efficiency of artificial infiltration processes in water purification.

In the Nrf2 and AR assays, no bioactivities above cut-off levels (or below in the case of AR antagonist activity) were detected at REF 50 at any of the sampling points between the river source, the Galgbacken wellfield, and the infiltrated water intakes at the two DWTPs. This is in contrast to the findings of Oskarsson et al. (2021)'s study wherein high Nrf2 and anti-androgenic activities were detected in samples collected from abstraction wells and the outlet of the DWTP over different seasons (Oskarsson et al., 2021). That particular DWTP draws artificially infiltrated water from a large river source which receives treated wastewater, storm water discharges, and effluents from industries. The artificial infiltration in that study had been in place since the 1950s. The Nrf2 and anti-androgenic activities in the raw water source to be infiltrated did not show any detectable Nrf2 or anti-androgenic activities, so contamination of the water occurred during the infiltration process. Targeted chemical analysis of the infiltrated water samples detected 17 of 163 analysed MPs (Tröger et al., 2020). A mixture of all the analysed MPs (each at a concentration of 1 μg/L, which was far higher than the concentrations of the 17 detected chemicals) did not induce Nrf2 or anti-AR activities. Thus, it was concluded that the detected MPs were not responsible for the bioactivities observed in the infiltrated water samples. Possible explanations of the observed effects included: the release of contaminants into the infiltrated water retained in the infiltration soil in the past, and/or the release of natural bioactive compounds (toxins) formed by microorganisms present in the infiltration environment. Still, the effectiveness of artificial infiltration in reducing reactive oxygen species (ROS) and anti-androgenic activity has been demonstrated elsewhere in another bioanalytical study that assessed infiltrated wastewater effluent (Jia et al., 2015). The authors reported that infiltration attenuated mutagenic and oxidative stress effects with BEQ reductions up to >97% and >93%, respectively. On another note, the presence of plants as a filtering layer in natural water purification systems has been demonstrated to biodegrade some pollutants. In such an example wherein a drinking water source was purified through a large-scale constructed wetland, decreases in ROS levels as well as in cytotoxicity and anti-androgen activity following purification was reported (Xu et al., 2019). Other studies using bioassays also reported a lack of AR activation or inhibition in finished drinking water samples (Jones et al., 2020; Leusch et al., 2018; Neale et al., 2020; Valcárcel et al., 2018). In contrast to AR activity, Nrf2 activity is often detected in river waters in other parts of the world (Neale et al., 2017; Wang et al., 2013). Further, the oxidative stress response is quite commonly detected in a variety of water types and therefore a highly relevant parameter in water quality assessments (Escher et al., 2014). Overall, the lack of AR and Nrf2 activities detected in any of the samples in the current study, particularly in the source river water, suggests the low presence of bioactive compounds during this sampling event for these two endpoints.

In contrast to the non-detectable bioactivities in the other bioassays tested, water samples collected before the artificial infiltration were above the cut-off level for AhR activity. In particular, bioactivities were higher in the samples collected from the three open-air infiltration basins compared to the source river water even though there is no treatment in between these sampling points. This may be attributed to the fact that the water in the open-air infiltration basins may undergo physical, chemical, or biochemical changes while exposed to sunlight, temperature fluctuations, and other ambient conditions which may affect water quality before entering the groundwater. Natural sunlight irradiation, for instance, plays an important role in transforming MPs and dissolved organic matter in other water environments such as open storage of reclaimed water and in natural surface waters due to natural processes (e.g., photolysis) (Bahnmüller et al., 2014; Tixier et al., 2002; Wang et al., 2021). At the same time, phototoxic products may also be formed during the photolysis of organic contaminants such as dioxin-like bromocarbazoles and chlorocarbazoles (Mumbo et al., 2017) and pharmaceutical mixtures (Wang and Lin, 2014). In short, while identification of the compounds inducing the AhR activity was not in the scope of the current study, the higher AhR bioactivities detected in the basin samples compared to the preceding sampling points provide interesting insight into the presence of AhR-inducing chemical hazards in the basins.

In contrast to the elevated AhR bioactivities detected in the infiltration basin samples, the bioactivity in the sample collected from the subsequent Galgbacken wellfield (GWF) was much lower, with a 2.5-fold decrease compared to the highest bioactivities measured in the basin samples, albeit still above the cut-off level. The lower bioactivities measured in the wellfield sample following infiltration may be due to several explanations. First, the infiltration basins contain approximately one meter of sand directly in contact with the underlying natural esker formation. Water from the basins undergoes infiltration at a rate of approximately 3.5-4.5 m^3/m^2 per day. A biological growth or "schmutzdecke" typically occurs on the sand surface and is a key factor in the treatment process. Physical, chemical, or biochemical changes in the water matrix can occur within the schmultzdecke and the

unsaturated and saturated natural esker material underlying the infiltration basins. The schmultzdecke functions as a biologically active filter and may account for the AhR activity due to the adsorption of contaminants during this process. Next, natural attenuation due to dilution and mixing with the natural groundwater as well as adsorption occurs within the esker material during the transit time from infiltration to extraction which is approximately 6–8 months. Oskarsson et al. (2021) also reported removal of AhR activity detected in raw river water following the artificial infiltration treatment (Oskarsson et al., 2021). Still, in another study of a DWTP-fed riverbank filtrate, AhR-mediated effects at an EC_{10} value of approximately REF 8 (Albergamo et al., 2020) were detected in the filtrate. The raw anaerobic riverbank filtrate in that study had an average infiltration time of 30 years. Taking into account the results from these other studies of the infiltration process, the transit or residence time of the infiltrated water seems to be a notable factor. On the whole, compared to the positive AhR responses in the less treated basin samples, the clear lower response following artificial infiltration is a compelling observation of the current study.

In the ER assay, bioactivity above the cut-off level was detected in only one sample (Basin 3), albeit marginally. Furthermore, no estrogenic activity above the cutoff level was detected in the sample collected from the wellfield (following infiltration) nor in the two DWTP's inlet samples. Riverbank filtration piloted for water supply systems has been demonstrated to remove thyroid-disrupting chemicals as well in the recombinant thyroid hormone receptor (TR) gene yeast assay (Valcárcel et al., 2018). While there appears to be a lack of toxicological effect-based studies investigating the degradation of estrogenic compounds in artificially infiltrated drinking water sources, other studies using chemical analyses have investigated the occurrence and elimination of endocrine-disrupting compounds in groundwater recharge systems in Germany. For instance, a study that investigated the removal of steroids during two different groundwater recharge systems (riverbank infiltration and artificial groundwater replenishment) observed significant decreases in the selected estrogenic compounds following these two processes (Zuehlke et al., 2004). Similarly, a study that monitored the concentrations of 10 natural and synthetic estrogens and progestogens in water samples collected from two artificial recharge plants located in Sweden and Denmark detected only one compound (estrone-3-sulfate) following the recharge processes (Kuster et al., 2010). As such, while no to low estrogenic effects were detected in the current study, the presence of hormones in MAR systems has been observed in other non-bioanalytical studies.

In brief, the lower AhR bioactivities and lack of ER bioactivities in the wellfield and DWTP intake samples compared to the preceding raw water samples (river and basins) where activities were detected above the respective cut-off levels would suggest that artificial infiltration is an effective natural purification method in this study. This is in contrast to the findings of our previous study at another Swedish DWTP utilizing artificial infiltration wherein the artificial infiltration process appeared to be a source of contamination (Oskarsson et al., 2021). As mentioned previously, the contrasting findings may be attributed to several factors regarding the removal efficiency of the artificial infiltration process. One explanation may be due to differences in the residence (or travel) time of the raw water in the subsurface. The infiltrated water in the current study takes approximately six to eight months to reach the wellfields supplying the two DWTPs. The infiltrated water in the previous study takes seven to thirty days to percolate through the subsurface from the infiltration basins. A longer travel time could, therefore, result in greater removal or dilution of bioactivity compounds to undetectable concentrations. An alternative explanation could be due to the accumulation of contaminants in the subsurface in the case of the DWTP in our previous study (Oskarsson et al., 2021). As revealed by Oskarsson et al. (2021) and elsewhere, the artificial infiltration of aquifers may lead to the eventual mobilization of toxic, naturally occurring contaminants into the water, thereby compromising the water quality (Fakhreddine et al., 2021; Oskarsson et al., 2021). Further, certain classes of hydrophilic organics that enter riverbank filtration systems can persist and migrate over prolonger time scales (e.g., decades) (Albergamo et al., 2019).

The fact that bioactivities above cut-off levels following artificial infiltration were detected in the AhR assay, but not in any of the other bioassays in this study may suggest that certain compounds present in infiltrated water cannot be as effectively removed during subsurface attenuation as others due to their resistance to biodegradation and the hydrophilic nature of the compounds, even at low concentrations in the groundwater. This has, for instance, been demonstrated for certain pharmaceuticals (e.g., carbamazepine and primidone), personal care products (PPCPs), and endocrine-disrupting compounds (Benotti et al., 2012; Debroux et al., 2012; Heberer et al., 2004; Hrkal et al., 2018). Compound-specific characteristics such as hydrophilicity and recalcitrance may limit the amount of compound that will adsorb to soils or that can be biodegraded by the soil microbial community (Maeng et al., 2011). Also, mobility during subsurface flow/riverbank filtration depends on the polarity of the MPs (Mishra et al., 2021). Next, the fate of organic compounds and degree of attenuation during artificial recharge is influenced by multiple factors such as the retardation factor, the distance and time spent in travel, depth to water table, sediment porosity and permeability, groundwater flow, and the hydrogeologic characteristics of the aquifer (Mishra et al., 2021; Petrovic et al., 2009). Such characteristics of the aquifer include its lithology, hydraulic and textural properties of the soil, temperature, and the microbial environment. Among these factors, redox conditions of the aquifer play a significant role in that certain pollutants are preferably removed under some particular redox conditions (Barbieri et al., 2011; Valhondo et al., 2015).

Finally, there is the temporal aspect of the sampling strategy in the current study. Given that all samples were collected on the same day in this study, it is likely that the composition of the water samples collected along each step of the total treatment process from the river water source would differ from each other. Further to this, only one sampling event was conducted for this study. Seasonal differences in the quality and chemical profile of the raw water were therefore not assessed. As described by Jokela et al. (2017), fluctuations in commonly monitored water quality parameters alone related to the organic matter content of river waters are typical (Jokela et al., 2017).

Overall, artificial filtration as a natural water purification method has been shown to have its benefits as well as limitations, mainly in that it does not result in the complete removal of all bioactive MPs, and that it may be a cause of contamination of drinking water. This treatment method, therefore, can serve as an effective pre-treatment of raw water but should include some water quality monitoring, with additional purification required thereafter, in drinking water production.

3.2.2. *Water purification at Gränby and Bäcklösa DWTPs*

Following artificial infiltration, the surface water will have mixed with the groundwater (refer to Section 2.1) and then fed into the Gränby and Bäcklösa DWTPs for further purification. A secondary objective of the current study was to monitor the bioactivities across all treatment steps in the two DWTPs fed the infiltrated groundwater. In general, consistent with the lack of Nrf2, AR, and ER bioactivities detected in almost all samples collected between the river and wellfield locations, samples from both DWTPs were below cut-off levels at REF 50 in all assays tested, except in the AhR assay. Low AhR bioactivities either at or slightly above the cut-off level were detected at REF 50 in several samples collected from both DWTPs. It is important to mention that these AhR activities were either lower or similar to what was observed in the raw water in the basins prior to artificial infiltration. However, that AhR activities above cut-off were detected in some samples collected at the DWTPs suggests the limited removal effect of AhR-inducing compounds during the treatment processes utilized at the DWTPs.

As mentioned in Section 2.4, samples collected from the inlets, outlets, and distribution networks of both DWTPs were further analyzed in dilution series to obtain CECs. EC values and BEQs were then

determined to compare to other effect-based studies on DWTPs. This was done for the AhR and ER bioassays only based on the initial screening results, as these two assays showed more frequent bioactivities above cut-off levels at REF 50 compared to the Nrf2 and AR bioassays. However, given the low levels of bioactivities detected in some of the samples at REF 50, it was necessary to increase the sensitivity in the assays by increasing the highest tested concentration to REF 200. The CECs and calculated results are presented in Fig. 3 and Table 3, respectively. For the Gränby DWTP, dilution series were possible to study for the inlet sample but not for the outlet sample due to a lack of sample volume. However, a Mann-Whitney test of the REF 50 results for the outlet sample and the subsequent tap water sample yielded no significant differences in the AhR assay (p=0.686) or the ER assay (p=0.343). Consequently, dilution series of the tap water sample were completed instead. At the Bäcklösa DWTP, it should be pointed out that this plant receives water from two wellfields.

As mentioned previously, low AhR was detected overall in all samples collected from both DWTPs in the current study. Other effect-based studies on river water-sourced DWTPs utilizing similar conventional treatment methods have reported higher activities. Escher et al. (2014), for instance, reported an EC_{10} value of REF 8.6 in the 24 h AhR-CAFLUX assay in finished drinking water samples collected from a river water-sourced DWTP that utilized coagulation and filtration followed by chlorination and finishing with chloramination (Escher et al., 2014). A previous study at that same Australian DWTP reported AhR activity at 0.17 ng TCDD/L in the finished drinking water (Macova et al., 2011). In the ER bioassay, low estrogenic activities were detected in the current study in the finished drinking water samples collected from Gränby and Bäcklösa. Another effect-based study investigating river water-sourced DWTPs reported reduced estrogenic activity to below the limit of detection (EEQ_{bio} $<3.00 \times 10^{-2}$ ng E2/L) in finished water samples following conventional treatments (Neale et al., 2020). Similarly, ten DWTPs sourced from surface stream water, alluvial groundwater, and deeper groundwater in an area of high agricultural use in the USA reported a low prevalence in the detection of ER activity in the finished drinking water (Jones et al., 2020). Still, much higher activities have been reported at other DWTPs elsewhere, such as 0.035–1.51 ng EEQ/L in the E-screen assay in tap water samples collected from ten DWTPs located throughout Taiwan (Gou et al., 2016) and an EC_{10} value greater than REF 30 in the E-SCREEN assay in finished drinking water samples collected from a river water-sourced DWTP in Australia (Escher et al., 2014). Furthermore, the observed estrogenicity in the finished drinking water samples collected from both DWTPs in the current study were at concentrations far below the suggested threshold of concern of 1 ng estradiol/L recommended by the World Health Organization (World Health Organisation Europe, 2017) and included in the 2022 watch list (Council of the European Union, 2020) in the EU drinking water directive (Drinking Water Parameter Cooperation Project. Support to the Revision of Annex I Council Directive 98/83/EC on the Quality of Water Intended for Human Consumption (Drinking Water Directive), 2017).

Table 3
Summary of effect concentrations (EC10), in units of REF, and BEQs expressed as TCDD-eq and E2-eq obtained from the concentration-effect curves for select samples. BEQ values are presented both as molar concentrations (pM) and as pg/L (in parentheses).

Sampling point	AhR activity		Estrogen receptor activation	
	EC10 (REF)	TCDD-EQ (pM)	EC10 (REF)	E2-EQ (pM)
Gränby inlet (G-IW)	$9.81 \times 10^{+01}$	4.33×10^{-02} (13.9 pg/L)	$3.45 \times 10^{+01}$	4.10×10^{-02} (11.2 pg/L)
Gränby outlet (G-OW)	*(insufficient sample vol.)*		*(insufficient sample vol.)*	
Tap water location in distribution network (G-TAP)	$8.00 \times 10^{+01}$	5.31×10^{-02} (17.1 pg/L)	$8.53 \times 10^{+01}$	1.66×10^{-02} (4.52 pg/L)
Bäcklösa inlet 1 (B-IW1)	$1.16 \times 10^{+02}$	3.65×10^{-02} (11.8 pg/L)	$4.93 \times 10^{+01}$	2.86×10^{-02} (7.79 pg/L)
Bäcklösa inlet 2 (B-IW2)	$9.88 \times 10^{+01}$	4.30×10^{-02} (13.8 pg/L)	$7.06 \times 10^{+01}$	2.00×10^{-02} (5.45 pg/L)
Bäcklösa outlet (B-OW)	$1.20 \times 10^{+02}$	3.53×10^{-02} (11.4 pg/L)	$6.05 \times 10^{+01}$	2.33×10^{-02} (6.35 pg/L)
Tap water location in distribution network (B-TAP)	$1.11 \times 10^{+02}$	3.84×10^{-02} (12.4 pg/L)	$4.92 \times 10^{+01}$	2.87×10^{-02} (7.82 pg/L)

4. Conclusions

MAR techniques, such as artificial infiltration, are commonly utilized around the world to improve water quality and increase groundwater storage (Stefan and Ansems, 2018). However, groundwater aquifers are susceptible to contamination by many of the same MPs found in surface waters. Such an instance was previously shown at a Swedish DWTP where the river water source was contaminated by hazardous chemicals during artificial infiltration (Oskarsson et al., 2021). The current study

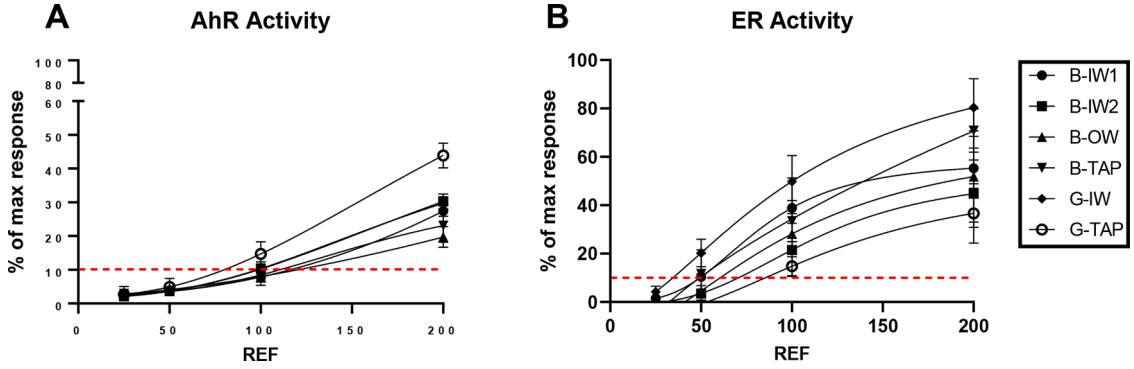

Fig. 3. Concentration-effect curves of AhR activity (A) and ER activity (B) for inlet, outlet, and tap water samples for Gränby and Bäcklösa DWTPs. The symbols denoting each sample are provided in the legend. Treatment groups (n = 4) were normalized to the vehicle control (n = 8), then to the maximum experimental response of the reference compound (TCDD for AhR, E2 for ER), set to 100. Data was fitted to four-parameter sigmoidal regression models. The dotted line indicates 10% activity of assay max. Data presented as mean±SD.

involved an effect-based evaluation of another Swedish DWTP that utilizes artificial infiltration in its drinking water production. In this case, the artificial infiltration process seemed effective in reducing AhR and ER bioactivities. What is important to highlight is that there are still a very limited number of relevant effect-based studies evaluating the effectiveness of artificial infiltration in removing chemical hazards. Given that artificial infiltration is commonly utilized around the world in drinking water production, further research, particularly using effect-based methods, is urgently needed to gain further understanding of the risks and benefits of this water treatment process.

Future work with the current study could include additional sampling to observe any temporal differences along the artificial infiltration process. It would also be worthwhile to investigate operational factors related to the infiltration process such as loading rates, basin material, and pre-treatment which may optimize the reduction of bioactivities.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J. L. and A.O. are the founders and owners of BioCell Analytica Uppsala AB, a company providing effect-based testing services to the water sector. E.L is employed by BioCell Analytica Uppsala AB.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118776.

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