

Impact of inorganic and organic pollutants from a Belgian wastewater treatment plant on adjacent surface and groundwaters

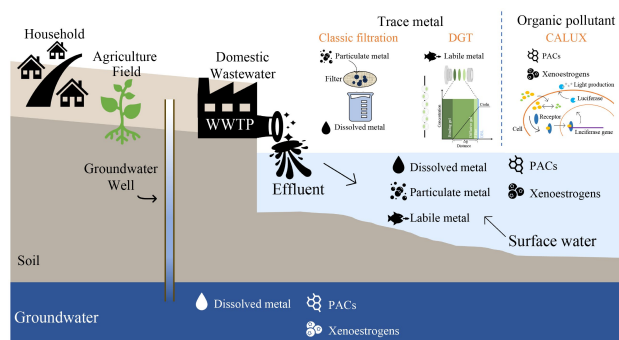
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HIGHLIGHTS

- Wastewater reuse for groundwater replenishment and agricultural irrigation.
- Assessment of (in)organic pollutants in WWTP effluent and adjacent water systems.
- Measurement of bioavailable inorganic pollutants using DGT technique.
- Calculation of lability of inorganic pollutants based on the DGT results.

GRAPHIC ABSTRACT



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ABSTRACT

Under the pressure of global droughts and water shortage, it is essential to evolve toward a sustainable and robust water system. One possible avenue is the maximum reuse of treated wastewater, but the quality of which determines its reuse. Therefore, inorganic (Cd, Pb, Cr, Ni, Cu, and As) and organic (xenoestrogens and polycyclic aromatic contaminants, PACs) contaminants were monthly monitored in an effluent of the wastewater treatment plant (WWTP), the surrounding surface waters and the local groundwater in Belgium. Dissolved and particulate concentrations of inorganic contaminants in these water bodies were analyzed. In addition, Diffusive Gradients in Thin-films (DGT) was used *in situ* to obtain bioavailable metal fractions. In the WWTP effluent and surface waters, only Ni exceeds the Annual Average-Environmental Quality Standard (AA-EQS), while in the groundwater, dissolved As was the predominant element. Moreover, in the surface and effluent waters the highest lability degrees were observed for Cd and Ni. The concentrations of these metal species in the effluent water were lower than in the other water bodies. Micro-organic pollutants, xenoestrogens and PACs were analyzed by dual Estrogen and Aryl hydrocarbon Receptor - Chemical Activated Luciferase gene eXpression (ER & AhR-CALUX) assays. Since the annual averaged (AA) bioequivalent concentration of E2 (0.18 ng/L) is below the AA-EQS standard (0.4 ng/L), and the bioequivalent concentration of benzo[a]pyrene never exceeded the maximum admissible concentration (MAC), the reclamation and reuse of treated wastewater for groundwater replenishment and agricultural irrigation should pose no environmental problems, at least in a short-term.

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1 Introductions

Climate factors such as an increasing temperature and droughts are threats to the water availability for drinking water and food production, leading to over-reliance on irrigation to meet food demand (Manabe et al., 2004; Dupont and Van Eetvelde, 2013; Gellens et al., 2018). As droughts will be a continuous problem due to climate change, human beings try to find other water sources to relieve the water-shortage pressure. A possible solution to this issue might be water-reuse and reclamation of treated domestic wastewater. However, the application of treated wastewater has some concerns, including public perception and also its environmental impact since treated wastewater can still contain a diversity of contaminants like bacteria, viruses, pesticides, inorganic and organic pollutants reducing the water quality and posing a threat to the ecosystem and human health (Brion et al., 2015). Therefore, without reassuring water quality regarding diverse pollutants, the reuse of treated wastewater for irrigation will not be accepted by environmental agencies and the public. Based on these two concerns, GROW project was initiated under the collaboration of Vrije Universiteit Brussel, Aquafin, Soil Service of Belgium (BDB) and local farmers, aiming to assess and monitor the water quality of the effluent of a wastewater treatment plant (WWTP) at a pilot site located in Kinrooi, Belgium and to evaluate its impact on the surrounding surface and groundwater.

In this study, besides monitoring of the physico-chemical parameters, the dissolved and particulate fractions of inorganic trace constituents (Cd, Pb, Cr, Ni, Cu and As, which will all be called trace metals hereafter) were collected by a classic sampling and filtration method and analyses of the contaminants by Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICPMS). In addition, a passive sampling technique of Diffusive Gradients in Thin-films (DGT) was applied *in situ* in the WWTP effluent (RWZI Molenbeersel), and the surrounding surface waters to assess labile metal fractions (Davison, 2016) including free metal ions and weakly bound metal complexes (Zhang and Davison, 2015; Baeyens et al., 2018; Gao et al., 2019). This technique is based on a molecular controlled diffusion of analytes from the bulk solution into the DGT device, which is equipped with a Chelex-100 binding gel and a diffusive gel (polyacrylamide hydrogel, pore size $\approx 10\text{nm}$) capped by a filter membrane (0.45 μm pore size, 0.125 mm thickness). Knowledge of labile metal fractions becomes more and more important when studying the potential toxicity and impact of trace metals on water quality (Leermakers et al., 2005; Gaulier et al., 2019; Zhou et al., 2020).

As part of the Water Framework Directive (WFD), environmental quality standards (EQS) have been established for a number of chemicals that pose risks to

the environment. Xenoestrogens and PACs were included in the first watch list by the Commission Implementing Decision (Commission Decision/2015/495EU). For this reason, the biological activity of xenoestrogens and PACs was also determined by a dual *in vitro* assay system for Chemical Activated Luciferase gene eXpression (CALUX) using an estrogenic receptor (ER) and an aryl hydrocarbon receptor (AhR). The method is used here as a screening tool for compliance monitoring. Responses exceeding EQS levels for xenoestrogens and PACs have to be confirmed by instrumental analysis. The development and evaluation of effects-based monitoring tools are an important goal for achieving efficient chemical analysis and monitoring strategies. These tools offer several advantages, including the reduction of expenses on monitoring and analysis, and the ability to prioritize targeted chemical analyses based on effects assessment, which directly addresses the impact of chemicals on the environment and wildlife. Over the last decade, the need for effects-based monitoring tools to measure estrogenic activity and persistent organic pollutants has become very important (Altenburger et al., 2015; Könemann et al., 2018; Elskens et al., 2023).

The objectives of this study are (1) to determine baseline concentrations of metal contaminants and agonistic activity of xenoestrogens and PACs in the surface water connected to the WWTP effluent and groundwater at the pilot site where the artificial irrigation systems were installed; (2) to measure labile fractions of metal contaminants in the same water bodies using the DGT technique, for a better understanding of metal bioavailability and ecological risk to local aquatic systems; (3) to compare the concentrations of trace metals and bioequivalent concentrations of xenoestrogens and PACs at the pilot site with those in other river systems and with legal standard values.

2 Materials and methods

2.1 Study sites

The pilot site of this project is in Kinrooi, a north-east town in Limburg province of Belgium, with a sparse population of 12000 and a total area of 54.76 km². The main economic activity in the region is agriculture and 77% of the landscape is dominated by agriculture land and nature reserves. Our study site is in a large open agricultural area (9 ha) including a WWTP (RWZI Molenbeersel), which is 1 km far from the residential area. The WWTP is a relatively small scaled unit with only primary (mechanical), secondary (biological) and sludge treatment procedures. The effluent from the WWTP is believed to have a better water quality than the bigger scaled plants since the influent only gathers the domestic wastewater from a low number of households

(around 5000), and no industrial wastewater is involved. However, the water quality of the effluent and its impact on the receiving aquatic systems (river and groundwater) is unknown. The effluent water is discharged into the Abeek river, one of the pilot sites. There are two rivers flowing adjacent to this site, rivers Abeek and Itterbeek, which are artificially intersected at the mechanical distributor Abeek-Itterbeek (Vlaamse Milieu Maatschappij, VMM).

Several sampling sites were selected at Kinrooi, including surface water sites (upstream of Abeek-Itterbeek river, and downstream of Abeek river), groundwater wells (PB01A and PB03A) and RWZI Molenbeersel WWTP effluent. The upstream site was over the mechanical distributor Abeek-Itterbeek. The downstream site was on the road bridge over the Abeek river, with a distance of 50 m from the discharge pump of the WWTP effluent. A monthly campaign was carried out at the pilot sites from June 2020 to September 2021 besides the downstream site of the the WWTP effluent in Abeek river. This site was sampled from November 2020 on. The groundwater wells were installed in the field close to the bank of two rivers (PB01A), and at the road few meters away from the WWTP (PB03A). The coordinates of each sampling station are presented in Table 1. The field work was always carried out following the same pattern on a monthly schedule: Abeek upstream, PB01A, WWTP effluent, PB03A and Abeek downstream, at 10 am, 12 pm, 2 pm, 3 pm and 5 pm, respectively.

2.2 Physical-chemical parameters

Physical-chemical parameters including pH, conductivity, oxygen concentration, and temperature were measured in situ by a multimeter (VWR[®] MD 8000 H) equipped with a pH electrode (VWR[®] pHenomenal IDP 711), a conductivity electrode (VWR[®] pHenomenal IDP 761-C) and an oxygen probe (VWR[®] OPOX 11-3).

2.3 Trace metal sampling and analysis

2.3.1 Sampling and sample treatment for suspended particulate matter (SPM), dissolved and particulate metal fractions

All bottles (250 mL, Pyrex[®]), tweezers, cylinders and

Table 1 Identification and coordinates of the sampling stations

Sample station	Water type	Latitude (N)	Longitude (E)
WWTP effluent	Effluent	51° 08.476'	5° 45.460'
Abeek upstream	Surface water	51° 08.545'	5° 45.054'
Abeek downstream		51° 08.450'	5° 45.498'
PB01A	Groundwater	51° 08.587'	5° 45.308'
PB03A		51° 08.523'	5° 45.583'

filtration systems for water sample collection were all precleaned with 10% HNO₃, and rinsed three times with Milli-Q water in the laboratory prior to use. Upon arrival in the field, the plastic bucket, the syringe (30 mL, Terumo[®]) and the bottles mentioned above were rinsed three times with bulk water before sample collection.

200 to 500 mL water sample was filtered by the Battery-operated field electric vacuum pump (SDEC, France) via the cleaned filtration system. The filtration was performed in triplicate to obtain 3 filters with SPM for all sampling sites. The filters were dried in the laminar hood in the clean laboratory and the mass of SPM was obtained by the mass difference of filters before and after filtration (dry weight). The filtrate was collected and acidified to 1% HNO₃ for dissolved metal analysis. Also, blank filters were prepared in the same way, but with Milli-Q water passing through. The groundwater samples were directly filtered by connecting a syringe filter with the tubing from the groundwater wells, and immediately acidified to 1% HNO₃.

To get particulate metal concentrations, the dry filters with SPM were acid digested according to [Gaulier et al. \(2019\)](#) (detailed information can be found in supplementary information (SI)). To validate this digestion method, a blank and 0.15 g of certified reference material ERM-CC144 (sewage sludge, European Reference Materials) were treated in the same way in duplicate (recovery can be found in Table S1). In this study, we only present the results of SPM and particulate metal concentrations in surface water and WWTP effluent, but not those in groundwater for the following reasons ([Puls and Barcelona, 1989](#)): first, filtration of anoxic or suboxic groundwater samples is very difficult without causing iron oxidation and colloid formation; second, clogging of pores with fine particles may occur, reducing the nominal size of the filter ([Danielsson, 1982](#)); third, it is known that the particulate metal in a groundwater sample is not always a natural part of the groundwater but a side-product of establishing wells and the method used for groundwater sampling ([USGS, 2018](#)).

2.3.2 Sampling and sample treatment of labile metal fraction

2.3.2.1 DGT preparation, deployment, and treatment

The DGT piston is a round plastic molding, tightly enclosing three layers in order, which are: a binding gel (Chelex[®]-100 resin embedded in polyacrylamide hydrogel, 0.4 mm thickness) and a diffusive gel (polyacrylamide hydrogel, 0.8 mm thickness) capped by a membrane filter (0.45 µm pore size, 0.125 mm thickness). Gel preparation and DGT assembly were conducted under a laminar flow hood in a clean laboratory room according to the method reported by [Zhang and Davison \(1995\)](#).

Details related to the preparation of diffusive gel and Chelex®-100 gel are reported in the SI.

In this study, the DGT pistons were used to measure Cd, Pb, Ni and Cu and additionally for Cr (III), which is the most toxic species of Cr and can be selectively sampled by Chelex-100 DGT (Ernstberger et al., 2002). For reasons of uniformity with the other trace metals, Cr (III) species will be mentioned as Cr hereafter. Moreover, arsenic cannot be sampled by the classic DGT due to the low affinity of the Chelex-100 resin for as species. Prior to deployment, six DGT pistons were connected by an acid-cleaned fishing line with 2 cm interval and enclosed in a plastic holder which was placed inside a plastic cage. The cage was immersed in the water and anchored by a 5 kg weight settled at the riverbed. A nylon rope connected the cage with the bridge railing. The deployment time for each sampling was around three weeks, but the exact time of deployment and retrieval were recorded. Besides, the water temperature was also measured at DGT deployment and retrieval, due to its impact on the diffusion rate of analytes into the DGT pistons. After retrieval, the DGT pistons were cleaned thoroughly by Milli-Q water to remove dirt and particles attached to the membrane filters. The diffusive gels and membrane filters were peeled off and the binding gels were eluted in 1 mL of 1

mol/L HNO₃ for 24 h to obtain the accumulated labile metals. Also, six blank DGT pistons were treated by the same procedure except for the deployment. The elute was diluted 10 times, prior to analysis. DGT measured concentration (C_{DGT}) represents the time averaged labile concentration of the analyte and can be calculated according to Fick's law (Zhang and Davison, 1995):

$$C_{DGT} = \frac{M \times (\Delta g + \delta)}{A \times D \times t}, \quad (1)$$

where M is the mass of target analyte accumulated on the binding gel (g), Δg is the thickness of the diffusive layer (diffusive gel + filter membrane, 0.094 cm), δ is the thickness of diffusive boundary layer (DBL, see SI for details), D is the diffusion coefficient of target analyte in the diffusive layer (cm²/s), A is the surface area of each gel (cm²), and t (s) is the deployment time of DGT piston.

2.3.2.2 Lability and dissociation calculation of trace metals

The lability of trace metals is expressed as the DGT measured concentration (C_{DGT}) divided by the dissolved concentration. A theoretical derivation (Mongin et al., 2011) of the lability relates this variable to the dissociation rate constant (k_d) of the metal complex (Eq. (2)).

$$\xi = 1 - \frac{(1 + \varepsilon K')}{\varepsilon K' + \frac{\delta^s}{\sqrt{\frac{D_{ML}}{k_d(1 + \varepsilon K')}}} \coth \left(\frac{\delta^s}{\sqrt{\frac{D_{ML}}{k_d(1 + \varepsilon K')}}} \right) + \frac{\delta^s}{\sqrt{\frac{D_{ML}}{k_d}}} (1 + \varepsilon K') \tanh \left(\frac{\delta^r}{\sqrt{\frac{D_{ML}}{k_d}}} \right)}, \quad (2)$$

where ξ is the lability degree of the metal complex and equals to $C_{DGT}/C_{dissolved}$ (1 is full labile and 0 is inert), D_{ML} and D_M are respectively the diffusion coefficients of the free ion and the metal complex, $\varepsilon = D_{ML}/D_M$, δ^s and δ^r are respectively the thickness of the diffusive and binding gel and K' is the conditional stability constant of the complex (i.e., $K' = c_{ML}/c_M$). The latter one is calculated via Visual MINTEQ (VMINTEQ, (Gustafsson, 2013)). Speciation software can be used to estimate the equilibrium concentrations of the metal species present in solution. These calculations rely on the database of thermodynamic magnitudes for the working temperature as well as on the consideration of all the reactions taking place in the solution. We are interested in inorganic and organic metal complexes. VMINTEQ includes for the speciation with natural organic matter the NICA-Donnan model with a set of constants derived for generic fulvic or humic acids (Milne et al., 2003). The result of the VMINTEQ calculation is the species distribution at equilibrium which allows us to divide the metal complex concentration by the free ion concentration and to calculate K' . Once the lability of the metal complex (ξ) as well as the other parameters in the Eq. (2) are known, k_d

will be calculated via an iteration procedure, starting with a default k_d and minimizing the difference between calculated and measured ξ values.

2.3.3 Sample analysis and limits of detection (LODs)

All treated samples including dissolved water samples, acid digested particulate samples, and DGT gel solutions were analyzed by using a Sector Field Inductively Coupled Plasma Mass Spectrometer (SF-ICPMS; ThermoFisher Scientific™, Thermo Finnigan Element II, instrumental detection limit equal to or below 1 ng/L). Quantification of trace metal concentrations was based on calibration curves resulting from external calibration by using appropriate dilutions of an acidified multi-element stock solution (Merck, ICPMS standard XIII) and internal standardization with Indium to compensate for instrumental drift. For each measurement, blanks, and CRMs (ERM-CC144 and SLRS-6) were included to verify accuracy and precision. Results of recoveries are displayed in Table S1. Limits of detection (LODs) are shown in Table 2.

Table 2 Calculated limits of detection (LODs) for each performed measurement

LODs	Unit	Element					
		Cd	Pb	Cr	Ni	Cu	As
ICPMS	µg/L	0.002	0.002	0.004	0.045	0.002	0.003
SPM	µg/L	0.016	0.047	0.495	0.144	0.107	0.015
DGT	µg/L	0.002	0.006	0.002	0.268	0.217	0.002

2.4 Sampling, sample treatment and measurement of organic pollutants

2.4.1 Sampling and sample extraction

Water samples were collected in 1 L brown glass bottles pre-cleaned with detergent, rinsed three times with Milli-Q water, dried naturally, and baked at 450 °C for 8 h. Upon arrival in the field, these bottles were rinsed three times with bulk water before sample collection. After the collection, the water samples were transported as soon as possible to the laboratory and stored at 4 °C. Then the water samples were filtered using glass fiber filters type F (GF/F) with a 0.7 µm pore size (Sartorius Stedim) within 24 h after collection.

Extraction of xenoestrogens was performed by solid phase extraction (SPE) with Oasis HLB cartridges (5 mL, 200 mg, glass cartridge, Waters, USA), according to Vandermarken et al. (2018). Liquid-liquid extraction (LLE) was used to obtain the crude water sample extract (i.e., PACs): first, the water sample was mixed with n-hexane (2:1, v/v) and vortexed; second the mixture was shaken for 15 min and centrifuged for 10 min; third the n-hexane layer was collected by using a glass pipet and transferred to a new glass bottle (repeated this step once more); fourth, the water layer was extracted with n-hexane (1:1, v/v) for the last time; fifth, the extract was collected, evaporated to dryness, and dissolved in 5 mL n-hexane.

2.4.2 Measurement of organic pollutants

ER ligand binding activity—The recombinant human breast cancer cell line VM7Luc4E2 (variant MCF7, formerly known as BG1Luc4E2) was used to determine estrogenic related activities. These cell lines express ER α endogenously but lack functional ER β (Rogers and Denison, 2000; Brennan et al., 2016). The bioanalytical procedure is described in Elskens et al. (2023).

AhR ligand binding activity—A mouse hepatoma cell

line H1L7.5c1 which contains a stable transfected AhR-responsive luciferase reporter gene plasmid, pGudLuc7.5 (He et al., 2011) was used to determine the aryl hydrocarbon related activities. The bioanalytical procedure is described in Boonen et al. (2021).

Data analysis and Bio-Equivalent (BEQ) E2 or BaP concentration—A four-parameter logistic function was fitted to the data points using a weighted least squares regression (Elskens et al., 2011):

$$y = a + \frac{(d-a)}{1 + (x/c)^b}, \quad (3)$$

where a , b , c , and d are the parameters of the model, and where the concentration x corresponds to the explanatory variable and y to the measured response activity (in Relative Light Unit - RLU); a and d respectively represent the lower and upper asymptote, b the slope or Hill coefficient, and c is the half-effective concentration or EC_{50} .

The BEQ concentration is used to express the potency of the sample compared to a reference compound (E2 for ER and BaP for AhR) and is obtained by dividing the $EC_{25/50/75}$ concentrations of the standard by the $EC_{25/50/75}$ concentration of the sample.

$$BEQ_{E2 \text{ or BaP}} = \frac{1}{3} \sum_{i=1}^3 \frac{EC_{x_i(\text{standard})}}{EC_{x_i(\text{sample})}}, \quad (4)$$

where $x_i = 25, 50$ and/or 75 , respectively.

Control and quality assurance (QC/QA) - The best-fit parameters for the concentration response-curves were obtained by minimizing the least squares residuals corresponding to Eq. (2) using a Levenberg-Marquardt algorithm in R version 4.1.1-2021-08-10. For the standard curves ($n = 20$), the parameter-values under repeatable conditions and the goodness of fit criteria are summarized in Table 3.

Quality control samples were systematically performed in triplicate on the 96-well plates. They consisted of standard at the half maximal effective concentration (EC_{50}). The recovery rates were 89%–120% for E2 and 70%–134% for BaP, results which can be considered as satisfactory. The root mean square error (RMSE) indicates the absolute fit of the model to the data, i.e. how close the observed data points are to the values predicted by the model. As the square root of a variance, the RMSE is interpreted as the standard deviation of the unexplained variance. It is therefore a robust measure of the accuracy with which the model predicts the response. RSQ indicates the percentage of the variance explained by the logistic model.

Table 3 Parameter values (95% CI) and goodness of fit criteria min-max for ER and AhR-CALUX

Assay	STD	LoD (pg/well)	Efficacy (%)	EC_{50} (pg/well)	Slope	RMSE	RSQ
ER	E2	0.02 (0.01)	95 (4)	0.50 (0.03)	1.4 (0.13)	1.4–3.3	0.992–0.998
AhR	BaP	0.03 (0.02)	97(1)	35 (6)	0.9 (0.06)	1.8–9.3	0.928–0.998

3 Results and discussion

3.1 Physical-chemical parameters

Physical-chemical parameters at all stations from June 2020 to September 2021 are presented in Fig. 1. Temperature shows a seasonal variation as expected, but this variation is smaller in the WWTP effluent and the groundwater than in the surface water. Generally, the pH profiles of surface water and WWTP effluent are correlated and show an average value of 7.3. The pH profile of groundwater (station PB01A) follows a similar trend as surface and effluent water, but with a slightly lower pH value (7.1). This is probably due to the production and decomposition of organic matter in the soil adjacent to the well, as PB01A is located close to a horse ranch. According to (Burkholder et al., 2007), animal waste or application of animal waste As fertilizer may impact groundwater quality because the decomposition of high amounts of organic matter can decrease the pH value as a result of CO₂ production. The pH in the groundwater at PB03A differs a lot from those of surface and effluent water, with an average value of 6.6, which is much lower than those in the surface and effluent water. This slight acidic condition is possibly caused by the off gas produced from the cars passing right beside the well (Fondriest-Environmental-Inc., 2013). The emitted nitrogen and sulfur oxides and other

acidic compounds are deposited at the PB03A area, resulting in a lower pH. Overall, our pH values are in a normal range found in freshwater bodies and within the limits of natural values that allow aquatic life (~6.5–8.5 by the World Health Organization) (WHO/SDE/WSH/07.01/1). Generally, the conductivity of WWTP effluent and surface water follows a similar trend, but with higher values in the WWTP effluent. In the WWTP effluent and surface water, the conductivity increases to higher values in winter and summer and decreases to lower values in spring and autumn. However, the conductivity in the groundwater remains stable, except for an obvious decrease in February 2021. Overall, the conductivity is higher in the groundwater and WWTP effluent than in the surface water. Dissolved oxygen in the surface water shows a higher value in spring 2021, while the peak in the groundwater occurs in winter 2020. However, dissolved oxygen concentrations at the two groundwater stations are generally lower than in the surface and effluent waters, which is a normal phenomenon for groundwater samples. The lower oxygen concentration observed in the surface water in summer is caused by the degradation of phytoplankton detritus and the consumption of oxygen by heterotrophic bacteria. Dissolved oxygen levels in the WWTP effluent remain stable around 7 mg/L, which is due to a permanent and constant addition of air in the WWTP.

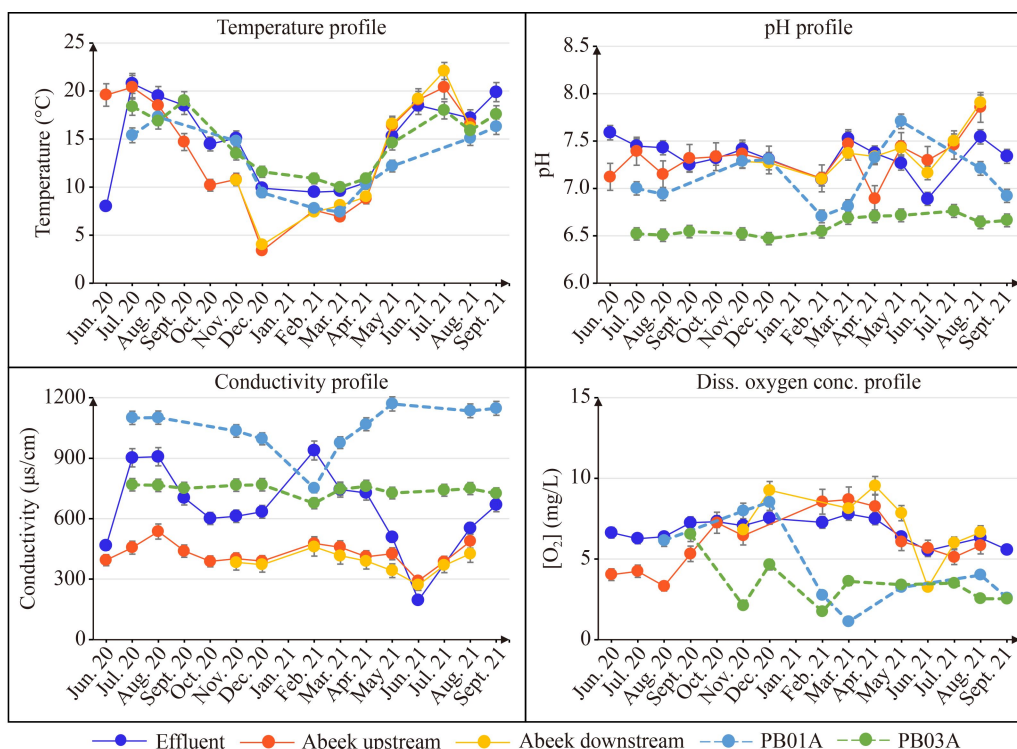


Fig. 1 Temperature, pH, conductivity, and oxygen concentration profiles in the pilot sampling sites from June 2020 to September 2021.

3.2 Suspended particulate matter (SPM)

The amounts of SPM in the WWTP effluent and surface waters are shown in Fig. 2. Generally, the SPM amounts in surface waters are 2 to even 10 times higher than in the WWTP effluent. The lower SPM values can be attributed to the treatment process in the WWTP, which largely removes the SPM from the inlet water. Seasonal variation is also observed for these water bodies. Two obvious peaks of SPM ranging between 15 and 20 mg/L are found in two summer seasons for the surface waters, however an elevated level of SPM is also found between November 2020 and February 2021 with more pronounced values in the surface waters than in the effluent. SPM fluctuations may impact the partitioning of the pollutants between dissolved and particulate fractions, and further influence their bioavailability.

3.3 Dissolved trace metal concentrations

No clear seasonal pattern of dissolved metals can be distinguished, nevertheless they can be divided into two groups based on their seasonal variations (Fig. 3, the first group includes Cd, Cr, and Cu, and the second group one of Pb, Ni and As). The highest concentrations are found in February or March 2021 at most stations beside Cr at PB03A, where it is 8 times higher than at other stations. Generally, dissolved Cd, Cr and Cu show a less fluctuating pattern in all surface and ground water samples than the other metals and in the WWTP effluent their patterns are even more constant. Looking at the other metals, dissolved Pb and Ni are higher in surface and WWTP effluent water samples than in groundwater samples, whereas As presents the opposite trend. This is not surprising since As and Cr are two oxygen sensitive elements and they become more mobile when the conditions in the groundwater change to more reducible (Fig. 1). In general, the dissolved concentrations of these metals in the WWTP effluent are lower than in the river

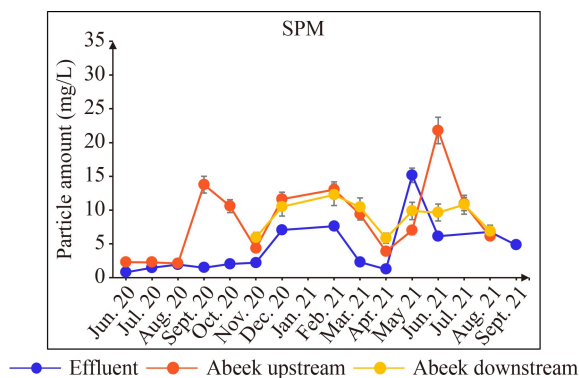


Fig. 2 Seasonal variations of suspended particulate matter (SPM) from June 2020 to September 2021 in Effluent, Abeek upstream and Abeek downstream.

and the groundwater sampling sites, which assures us that regarding inorganic pollutants infiltration of effluent water in groundwater has no consequences for agricultural irrigation.

According to Table 4, dissolved Cd, Cr, Cu and As are below the guideline values of dissolved trace metals in freshwater at all stations. In addition, all the dissolved metal concentrations in our sampling sites are far below the Maximum Allowable Concentration (MAC) values (DIRECTIVE/2013/39/EU). Only dissolved Ni exceeds the Annual Average (AA) (DIRECTIVE/2013/39/EU) value at all stations, but also high Ni levels are found in soil samples in the same area, which indicates a wide and chronic exposure of Ni at our pilot site. Even an updated MAC value for dissolved Ni of 8.2 $\mu\text{g/L}$, which was proposed by the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) in 2022 (SCHEER, 2022a), is only half of the concentration found in the Abeek river.

We also compared our results with the levels found in other fresh river systems in Western Europe: Zenne river in Belgium and Garonne river in France are also connected to WWTPs treating domestic wastewater (Deycard et al., 2014; Ma et al., 2023), however the WWTPs are of a bigger scale treating wastewater of much more residents. Besides these two rivers, another urban river, the Deûle (Lesven et al., 2009) in France is also included in the comparison because it flows through a former heavily industrialized region (Auby, Courcelles-Lens and Noyelles-Godault). Ranking the rivers from low to high flow rate: Abeek river (0.3–1 m^3/s), Zenne river (6–10 m^3/s), Deûle river (8 m^3/s) and Garonne river (40–60 m^3/s) (Jalón-Rojas et al., 2015; Superville et al., 2015; Ma et al., 2023). As shown in Table S2, the lowest dissolved metal concentrations, besides in Abeek river, occur in Garonne river. In our study, all the dissolved metal concentrations are close to/less than those in Garonne river, except for dissolved Ni and Cr. The most striking result is that dissolved Pb is 100 times lower in Abeek river than in Deûle river, which was heavily polluted by the Metaleurop smelter. Dissolved Ni concentration is comparable to/ slightly higher than in Zenne and Deûle rivers. The dissolved metal results indicate that our pilot site has a better water quality than other rivers due to a limited industrial activity and a low number of inhabitants.

3.4 Labile trace metal fraction and lability calculation

3.4.1 Labile trace metal concentrations

Labile concentrations of Cd, Pb, Cr, Ni and Cu were measured by the DGT technique in surface and WWTP effluent waters (Fig. 4), but not in the groundwater samples due to very slow water flow and narrow width of the wells, under which conditions, the DGT principle

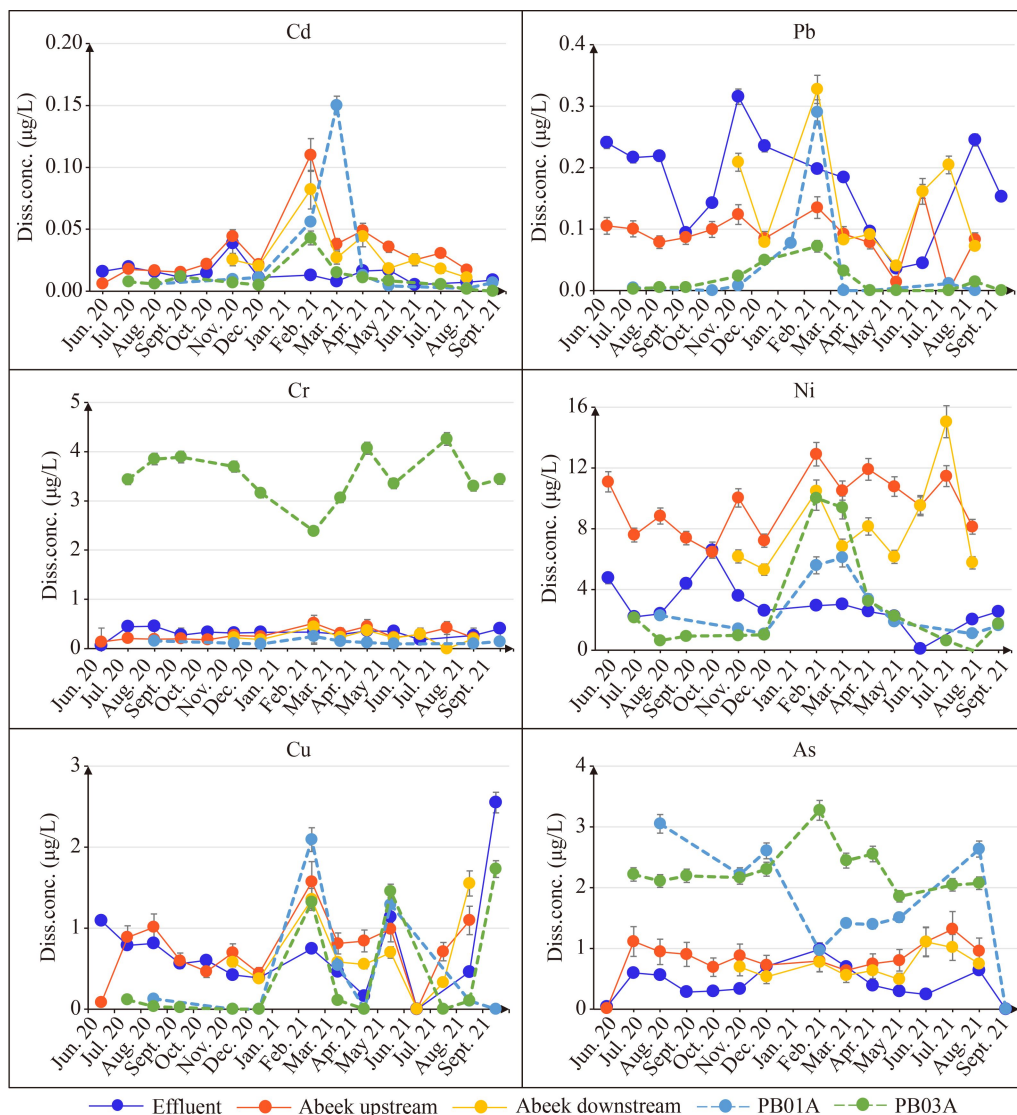


Fig. 3 Seasonal variations of the measured dissolved trace metal concentrations from June 2020 to September 2021 in Effluent, Abeek upstream, Abeek downstream, PB01A and PB03A.

Table 4 Water quality guidelines for dissolved trace elements in fresh water

Guideline values	Unit	Cd	Pb	Cr	Ni	Cu	As
AA*	µg/L	0.2 ^a	1.2 ^a	/	4 ^a	/	/
MAC**	µg/L	0.45 ^l	14 ^l	100 ^b	34 ^l	8.2 ^c	10 ^d
BC ^e	µg/L	0.08	0.2	0.2	3.3	0.4	0.8

* Annual Average value; ** Maximum Allowable Concentration value. ^a DIRECTIVE/2013/39/EU; ^b Current Chromium Drinking Water Standard according to the Safe Drinking Water Act, United States Environmental Protection Agency (USEPA); ^c DIRECTIVE/2000/60/EC; ^d Guideline value for arsenic in drinking-water, World Health Organization (WHO, 2022); ^e Natural background concentrations (BC) used in Dutch water policy (Osté et al., 2011).

does not apply. Different from the dissolved metal fraction, the labile one is similar to the bioavailable fraction and can be directly assimilated by some organisms such as phytoplankton (Macoustra et al., 2019;

Macoustra et al., 2021; Price et al., 2022). Therefore, it is important to understand the kinetics and mobility of this fraction so that this concept can be applied when metal toxicity is considered.

Generally, labile Cd, Ni and Cu show lower values in the WWTP effluent than in the surface water bodies, while the reverse is true for labile Pb. Labile Cd and Ni present similar trends in surface water, with maximum values in March 2021. Labile Cu presents an increase from November 2020 and reaches the highest value of 0.4 µg/L in September 2021 in surface water.

Since labile metal concentrations have never been included in environmental regulations, an intrinsic comparison with existing allowed levels for dissolved trace metals (water guideline values of dissolved trace metals in inland surface waters, Table 4) is the only solution. Labile metal levels are all below the AA values,

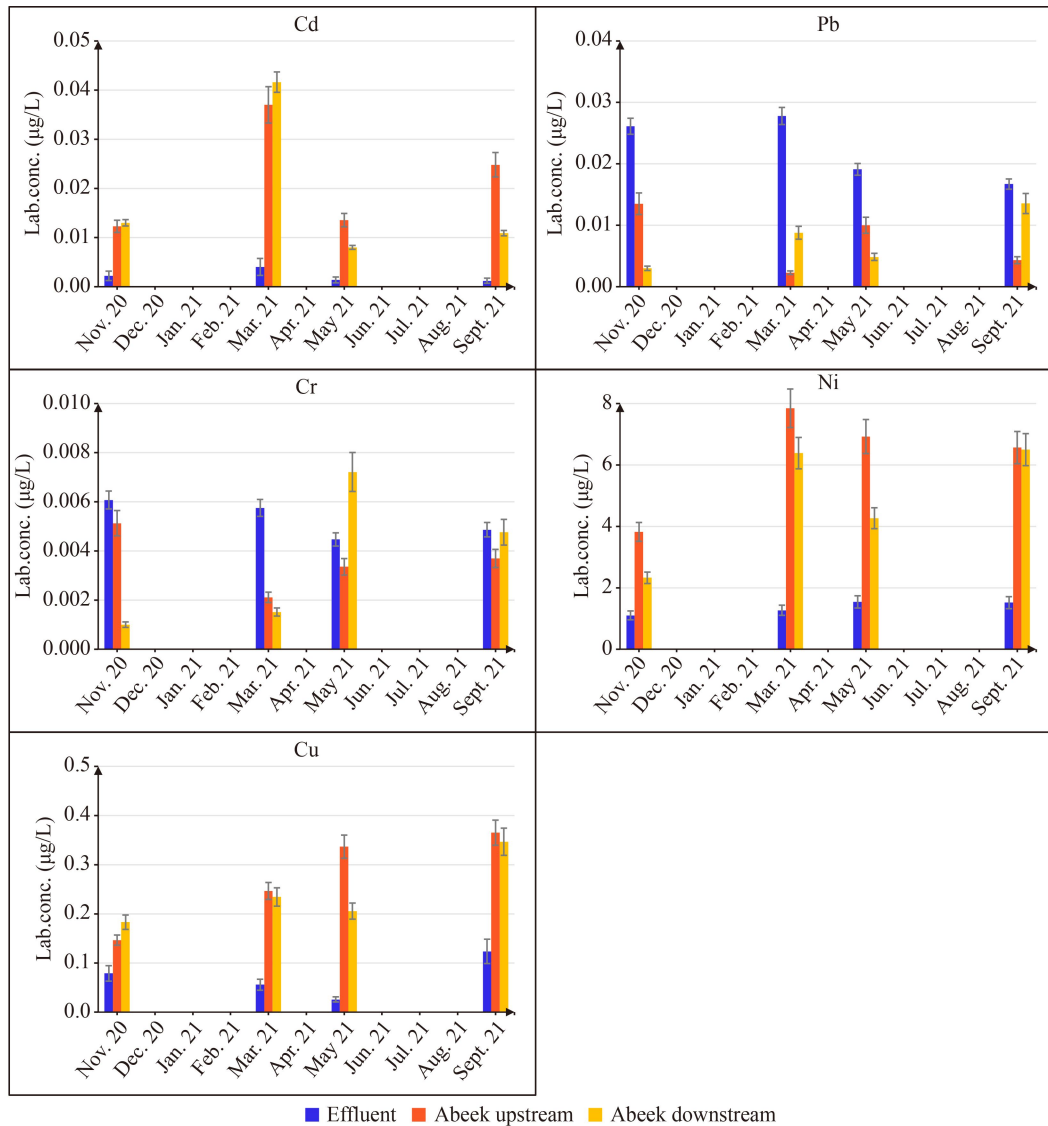


Fig. 4 Labile trace metal concentrations in Effluent, Abeek upstream, Abeek downstream. (Samples were collected in November 2020, March 2021, May 2021, September 2021 by DGT device).

except for Ni whose concentrations are higher than AA values in the surface water in 2021 but are below the MAC values (Table 4). Even though these labile metal concentrations are much lower than the dissolved ones, it is difficult to conclude that these labile fractions are at the safe side considering their direct consumption by aquatic organisms. Recently, SCHEER proposed an updated bioavailable standard for Ni ($AA-QS_{\text{bioavailable, fw}} = 1.9 \mu\text{g/L}$) (SCHEER, 2022a) based on the biotic ligand model (BLM). When we compared the labile concentrations of Ni measured in this study with this updated standard (BLM simulated), we found that only labile Ni in the WWTP effluent is lower than this value. However, we have to notice that DGT and BML are based on different principles, since DGTs are perfect sinks for analytes while the BML model considers equilibrium between the free ion and ligands (the more and the stronger the

ligands, the less free ion). This is probably the reason that the labile Ni concentration obtained from the DGT technique exceeded the SCHEER value. In addition, several studies jointly measuring labile trace metals with the DGT technique and metal toxicity (Cu, Ni and Zn) to *Chlorella* sp. proved that the DGT can mostly provide accurate results close to the metal levels causing direct toxicity to this specific phytoplankton species (Macoustra et al., 2019; Macoustra et al., 2021; Price et al., 2022).

It is, however, noticed that the current legislation, implementing the dissolved concentrations as a criterion, becomes out of date for several reasons: first, it is well known that using a filtration on 0.45 or 0.20 μm filter, may result in significant underestimations of metal mobility or lability, due to colloidal associations; second, the filtrate includes metal ions, metal inorganic complexes and low molecular weight organic complexes,

which all have a different degree of dissociation (also meaning mobility and lability) and hence bioavailability; third, the assimilation of labile metal species by phytoplankton is governed by both phytoplankton characteristics and size and form of the metal species. Therefore, it is essential to measure all metal fractions and understand their lability and hence toxicity for the environment and humans.

3.4.2 Metal complexes in the Abeek river: lability and dissociation kinetics

The lability of the metal complexes in the Abeek river is highest for Ni and Cd (0.60 and 0.57) and lowest for Pb (0.07) (Table 5). This means that Ni and Cd bioavailability for phytoplankton species living in that river is much higher than for Pb. We have also calculated the dissociation rates of the Cd, Cu, Ni and Pb complexes, because they also inform us how fast free ions of these metals (the toxic form) can be produced. For that calculation we need the conditional stability constants of the metal complexes (K'), the diffusion coefficients of the free ion and the complexes, the thicknesses of diffusion and binding gel and the measured lability (Table 5). First the metal speciation in the river at in situ parameter conditions were calculated using VMINTEQ software. Typical values of the general parameters in Abeek river are: $I = 0.003$, $pH = 7.25$, $Eh = 400$ mV and for the NICA-DONNAN module, $DOC = 12$ mg-DOC/L (from vmm.be/data/waterkwaliteit) and DOC is assumed 50% Fulvic and 50% Humic acid. The speciation calculations for each of the metals indicated that the inorganic complexes are negligible compared to complexes with organic matter. The concentrations of free ion and metal

complexes with organic matter, as well as the derived K' , are presented in Table 5. Cd and Ni show the lowest stability constants, Cu and Pb have the highest ones (Osté et al., 2011).

An iterative model was then used to solve k_d in Eq. (2). After each run the k_d was adapted to give a lability value that approaches better the measured lability. Dissociation constants (k_{ds}) derived for the various metal-OM complexes (Table 5) are in decreasing order: $1.7 \times 10^{-3} s^{-1}$ for Ni, $10^{-3} /s$ for Cd, $3.3 \times 10^{-4} s^{-1}$ for Cu and $4.7 \times 10^{-5} s^{-1}$ for Pb. To give an idea what these dissociation constants mean, we estimated the time necessary to dissociate half of the initial metal complex concentrations assuming no equilibrium will be reached. Half of the Cd complexes are dissociated after 11.5 min and of Ni after 6.8 min, but this would take 4 h before half of the Pb complexes are dissociated. For Cu this time equals 35 min. The consequence of the much faster dissociation rate of the Cd complexes compared to that of the Pb complexes is that for a similar concentration of Cd and Pb, Cd is by far more toxic for phytoplankton than Pb. The reason is that the free ionic Cd can be much easier internalized by phytoplankton species.

Pb and Cu form the most stable complexes and have also the highest affinity for the particulate fraction, while Cd and Ni form the least stable complexes and have the lowest affinity for the particulate fraction. This is logic since the particulate fraction consists of a high amount of organic matter (POM) interacting stronger with the stable Pb-OM or Cu-OM complexes than with the free ions of Cd or Ni.

We did a similar exercise in the effluent of the WWTP as we did for the Abeek river (Table 5). While we cannot really speak about a natural ecosystem in WWTP

Table 5 Dissociation constants of metal complexes in Abeek river and WWTP (Kinrooi) and parameters used for their calculation

	$C_{dissolved}^a$ ($\mu g/L$)	C_{DGT}^b ($\mu g/L$)	I^c	pH^d	DOC^e (mg/L)	K'^f	D_M^g ($E-06 cm^2/s$)	D_{ML}^h ($E-06 cm^2/s$)	ε^i	ξ^j	k_d^k (s^{-1})
Abeek river											
Cd	0.035	0.02	0.003	7.25	12	1.21 E + 09	4.57	2.3	0.5	0.57	1.0 E-03
Cu	0.0785	0.022	0.003	7.25	12	3.16 E + 10	4.67	2.3	0.49	0.33	3.30 E-04
Ni	9.21	4.76	0.003	7.25	12	6.46 E + 08	4.33	2.3	0.53	0.6	1.7 E-03
Pb	0.119	0.0104	0.003	7.25	12	9.08 E + 09	6.03	2.3	0.38	0.07	4.7 E-05
WWTP Effluent											
Cd	0.014	0.002	0.0045	7.3	17	3.26 E + 09	4.57	2.3	0.5	0.152	1.13 E-04
Cu	0.784	0.071	0.0045	7.3	17	8.48 E + 10	4.67	2.3	0.49	0.091	6.3 E-05
Ni	2.997	1.356	0.0045	7.3	17	1.33 E + 09	4.33	2.3	0.53	0.452	5.8 E-04
Pb	0.173	0.022	0.0045	7.3	17	1.78 E + 10	6.03	2.3	0.38	0.13	9.5 E-05

^a $C_{dissolved}$: the averaged dissolved metal concentrations during the sampling period (Jun. 2020–Sept. 2021); ^b C_{DGT} : the averaged labile metal concentrations during the sampling period (Nov. 2020–Dec. 2020, Mar. 2021–Apr. 2021, May 2021–Jun. 2021, and Sept. 2021–Oct. 2021); ^c I : Ionic strength; ^d pH : the average value during the sampling period (Jun.2020-Sept.2021); ^e DOC : Dissolved Organic Carbon; ^f K' : conditional stability constant of the complex as calculated by Visual MINTEQ ($K' = C_{ML}/C_M$); ^g D_M : diffusion coefficient of the free metal ion. D_M is retrieved from the DGT research website (www.dgtresearch.com); ^h D_{ML} : diffusion coefficient of the metal complex (ML is Metal-Humic acid complex). D_{ML} is the diffusion coefficient of Humic acid ($2.30 \times 10^{-6} cm^2/s$); ⁱ ε : ratio of the diffusion coefficients of the free ions and the metal complex, $\varepsilon = D_{ML}/D_M$; ^j ξ : lability of the metal complex; ^k k_d : dissociation rate constant.

effluents, the risk exerted by those effluents is that they can contaminate either the Abeek river or the ground water reservoir that is used for drinking water. The lability degrees in the WWTP effluent at Kinrooi are very low except for Ni (0.45). This means that Cd, Cu and Pb form stable complexes with organic ligands and are thus less harmful to some organisms or humans in case they use ground water as drinking water. Also, the dissociation rate constants are low for Cd, Cu and Pb while the k_d for Ni is the highest with $5.8 \times 10^{-4} \text{ s}^{-1}$. In this case it takes almost 20 min for half of the Ni complexes to dissociate and more than 1h 40 min for the other metal complexes.

3.5 Particulate trace metal concentrations

Particulate metal concentrations are only reported in

surface waters and WWTP effluent (Fig. 5) and those of Cd, Pb and Ni in the effluent are all lower than those in the surface waters, while those of Cu, Cr and As show similar levels in both aquatic systems. Due to the lower or similar particulate metal concentrations in the effluent, there is no impact on the receiving surface water bodies: the particulate concentrations upstream and downstream the discharge point in Abeek river are quite similar.

Our particulate metal concentrations are compared with those obtained in the Zenne (Ma et al., 2023), Garonne (Deycard et al., 2014) and Deûle (Lesven et al., 2009) (Table S3) too. Zenne river presents the highest particulate concentrations of Pb, Cr and Cu, and Deule river shows the highest concentration of particulate Cd. The particulate metal concentrations in our pilot WWTP are the lowest beside Pb in the Deule river and As in the

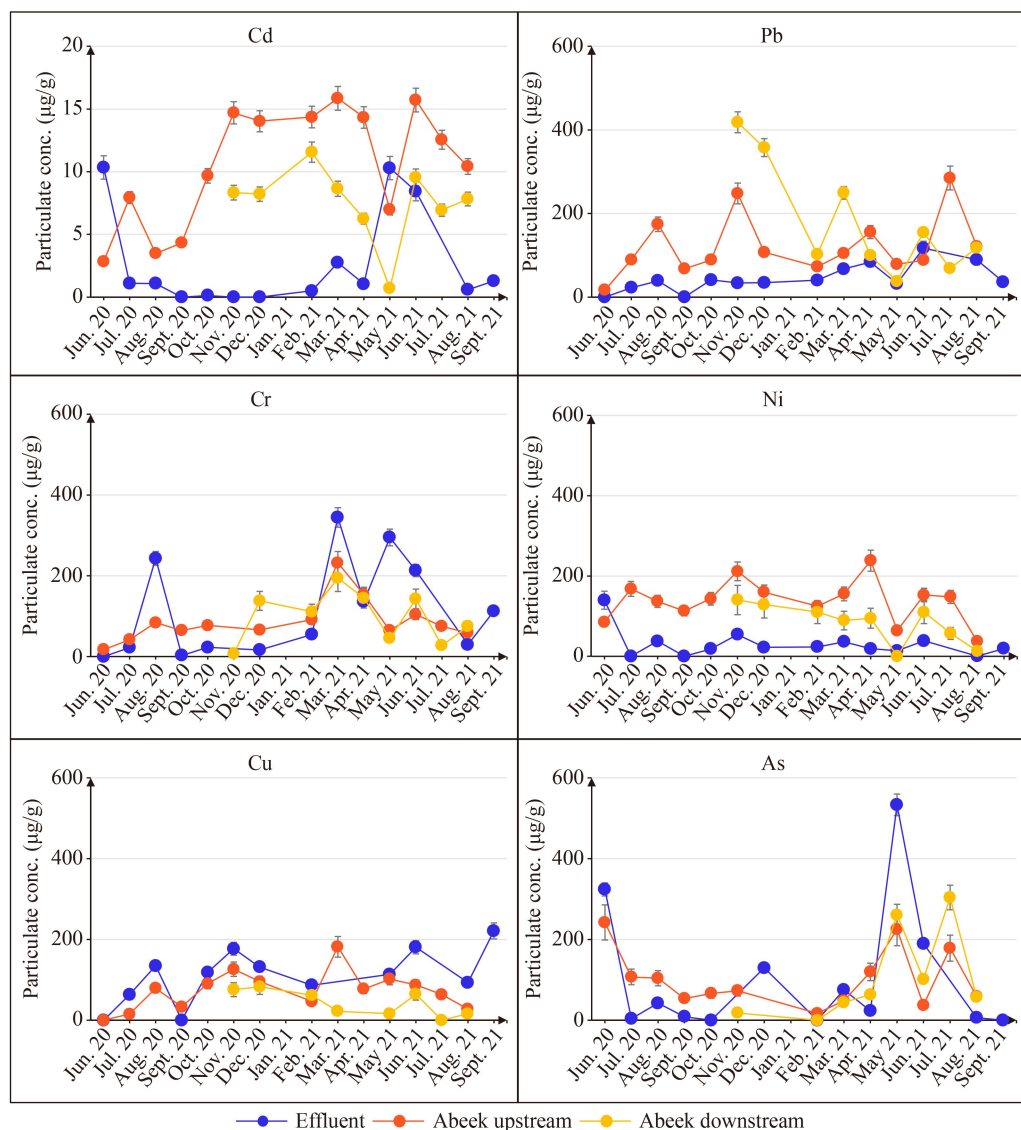


Fig. 5 Seasonal variations of particulate metal concentrations from June 2020 to September 2021 in Effluent, Abeek upstream and Abeek downstream.

Garonne river. Particulate metal concentrations in Abeek river are comparable to those in the Garonne and Deule rivers (beside Cd and Pb), but lower than in Zenne river. Abeek river shows the highest particulate concentrations of Ni too. Due to the impact from high urbanization and extensive industrial activities (Brion et al., 2015; Smolíková et al., 2022), Zenne river and its turbidities are considered to be historically heavily contaminated. Regarding the particulate metal levels in our pilot sites, we can also conclude that the Abeek waters have a relatively good water quality.

3.6 Trace metal partitioning between the dissolved and particulate fraction

In a river, metal contaminants partition between dissolved and particulate phases (Allison and Allison, 2005). To determine the affinity of a trace metal for each of these phases, the partition coefficient K_d of the metals was calculated according to the following equation:

$$K_d(\text{L/kg}) = \frac{\text{Metal}_p (\mu\text{g/kg})}{\text{Metal}_D (\mu\text{g/L})}, \quad (5)$$

where K_d is the partition coefficient, Metal_p is the concentration of the trace metals in particulate fraction, and Metal_D is the concentration of the trace metals in dissolved fraction. Thus, when K_d is low, metals have a high affinity for the dissolved fraction meaning they are more available for phytoplankton species. The logarithm of K_d ($\log K_d$) varies between 4 and 6.2 for Cd, 5.0–6.8 for Pb, 4.0–6 for Cr, 2.4–5.7 for Ni, 4–5.6 for Cu, and 2.4–7.2 for As (Fig. S2). The seasonal variation of $\log K_d$ of Cd, Pb, Cr, Ni in the effluent follows a similar trend as the SPM in the effluent, with elevated levels in the two summers and also in the period of February–March. However, these pronounced variations are less obvious for the surface water bodies. Generally, the $\log K_d$ values of the selected trace metals are around the average values found in the literature (Allison and Allison, 2005), except $\log K_d$ values of Cr and As are above those average range, indicating Cr and As in our pilot sites have higher affinity for the particulate phase. However, caution is still required in future monitoring as $\log K_d^{\text{As}}$ presents occasionally quite low values in the effluent, indicating that a higher dissolved fraction exists in the water column, which may increase the availability of As to some aquatic organisms. Similarly, lower $\log K_d^{\text{Ni}}$ values are also found in the effluent compared to the minimum values reported in literature, indicating that Ni prevails in the dissolved phase during the season transition from summer to autumn. According to previous studies (Nabelkova and Kominkova, 2012; Sedeño-Díaz et al., 2019), values of $\log K_d < 3$ characterize aquatic systems where chemicals are less associated with the particulate phase.

We made a ranking of the $\log K_d$ per metal in the

different water bodies included in this study (Table S4) and find:

WWTP Effluent: $\log K_d^{\text{Pb}} > \log K_d^{\text{Cu}} > \log K_d^{\text{Cr}} > \log K_d^{\text{Cd}} > \log K_d^{\text{As}} > \log K_d^{\text{Ni}}$

Surface water:

Upstream: $\log K_d^{\text{Pb}} > \log K_d^{\text{Cd}} > \log K_d^{\text{Cr}} > \log K_d^{\text{As}} > \log K_d^{\text{Cu}} > \log K_d^{\text{Ni}}$

Downstream: $\log K_d^{\text{Pb}} > \log K_d^{\text{Cr}} > \log K_d^{\text{Cd}} > \log K_d^{\text{As}} > \log K_d^{\text{Cu}} > \log K_d^{\text{Ni}}$

Overall: $\log K_d^{\text{Surfacewater}} > \log K_d^{\text{WWTPEffluent}}$

The overall ranking shows that $\log K_d$ in the surface water is higher than that in the WWTP effluent except for Cu. This signifies that in the surface water the metals show a higher affinity for the particulate fraction and are thus more bound to or adsorbed by Fe/Mn oxides, silicates, organic matter or other substrates, compared to the effluent. Lead has the highest $\log K_d$ values in all the stations which means that Pb is the least soluble trace metal while the opposite is true for Ni which has the highest solubility. In most of the cases $\log K_d$ values of Cr and Cd are next to those of Pb in the ranking. We also compared the $\log K_d$ values obtained in the Kinrooi aquatic systems with those in the effluent of the largest WWTP in Belgium (Brussels North) and the receiving water (Zenne river) (unpublished data) (Table S4). We observed similar trends for all metals that the $\log K_d$ values in the surface water are higher than those in the effluent of the WWTP.

3.7 Xenoestrogens and PACs

3.7.1 Seasonal variation of agonistic ligands for ER in the WWTP effluent and groundwater

To gather information on seasonal variations of xenoestrogens and PACs in effluents and groundwaters at the pilot sites, the mediated BEQ-E2 and -BaP (Eq. (4)) from all sampling stations were calculated monthly. The uncertainty on BEQ is then represented by the standard error on the averaged value. We felt that this was a robust method for dealing with variations in EC-values between samples and the standard when response curves were not parallel (Gottschalk and Dunn, 2005). The seasonal trend in BEQ-E2 concentrations in all water samples is shown in Fig. 6. ER-mediated agonist activity in effluent ranged from the lower limit to 3.4 ng/L BEQ-E2, while activity measured in groundwater ranged from the lower limit to 1.3 ng/L BEQ-E2. Most of these results are inferior to 2 ng/L BEQ-E2 reported as a background in the Zenne river upstream of Brussels (Elskens et al., 2023). There was no significant difference at level $\alpha = 0.05$ between effluents and groundwater (upper-tailed Wilcoxon signed-rank test, $p = 0.378$), and there was a significant correlation between the two water bodies (Pearson = 0.783, $p = 0.002$), suggesting that effluents could be a direct source of groundwater contamination. A drastic

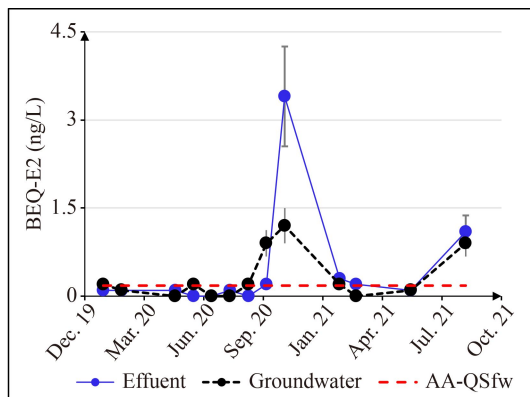


Fig. 6 Overview of the seasonal trend of BEQ-E2 in effluents and groundwater at Kinrooi. The dotted red line indicates the value of AA-QSfw for E2 as endorsed by SCHEER (2022b).

increase of BEQ-E2 is observed in October 2020, with values raising up to 3.4 ng/L BEQ-E2, followed by a subsequent decrease to 0.3 ng/L BEQ-E2 in March 2021. This could indicate that xenoestrogen levels are lower during summer than autumn. Similar results were also reported by Nie et al. (2012), who found that xenoestrogens were eliminated more efficiently during summertime but accumulated due to higher applications of detergents and medicine in cold days. It is important to note that the peak of 3.4 ng/L BEQ-E2 remains comparable to levels recorded in the downstream Zenne river, and in effluents of the Brussels North WWTP (Elskens et al., 2023). The question on EQS for estrogenic compounds was reviewed by SCHEER (2022b). The SCHEER endorsed that the maximum allowable concentration (MAC-QS_{fw,eco}) for estrogens cannot be derived because of the chronic exposure pattern and the large acute to chronic ratios observed. However, the committee has approved a value of 0.18 ng/L E2 for the annual average (AA-QS_{fw,eco}) in fresh water, qualifying the chemical status of the water body as “good.” We can show that the median values of the effluent and groundwater samples are not significantly higher than the AA-QS_{fw,eco} value throughout the year 2020–2021 (Upper tailed Wilcoxon signed rank test, $p = 0.759$). This observation contrasts with that reported in the urban Zenne river, where the BEQ-E2 concentration consistently exceeds the AA-QS_{fw,eco} in all sections of the river, regardless of the season (Elskens et al., 2023).

3.7.2 Seasonal variation of agonistic ligands for AhR in the WWTP effluent and groundwater

The seasonal trend in BEQ-BaP concentrations in all water samples is shown in Fig. 7. AhR-mediated agonist activity in effluent ranged from 9 to 165 ng/L BEQ-BaP, while activity measured in groundwater ranged from 9 to 26 ng/L BEQ-BaP. There is a significant difference at the $\alpha = 0.05$ level between effluent and groundwater (Wilcoxon signed rank test, $p = 0.001$), with higher

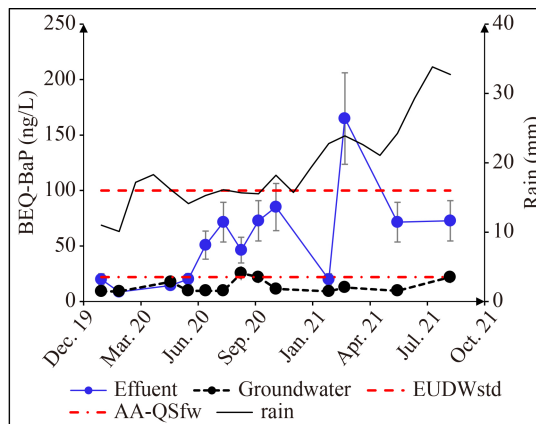


Fig. 7 Overview of seasonal trend of BEQ-BaP in effluent and groundwater at Kinrooi. The dotted red line indicates the value of AA-QSfw for BaP as approved by SCHEER (2023) and the European standard for drinking water (DIRECTIVE/2020/2184/EU). The solid black line indicates the level of precipitation smoothed over the period considered.

values in effluents and no significant correlation between the two water bodies. The lowest levels of AhR-mediated activity are often monitored in groundwater samples which appear to be relatively constant (at most a factor of 2), whereas levels in effluents are much more variable (a factor of 20), suggesting that there is a decoupling between these two water bodies in terms of PAC contamination. In the effluents, the BEQ-BaP concentration increases from June 2020 to reach a peak of 165 ng/L in March 2021 and follows the smoothed precipitation curve over the same period (Spearman = 0.643, $p = 0.028$). A previous study conducted in rural areas in Germany also reported a positive correlation between the amount of precipitation and the concentration of PACs (Gocht et al., 2007), suggesting that wet deposition may be a source of PACs for water systems. Ribeiro et al. (2012) moreover demonstrated that the wet trapping of atmospheric PACs was quite complex (see also Baek et al. (1991)), and that the correlation between these variables was generally not enough to assess the sources and levels of contamination. The BEQ-BaP in the Kinrooi water system is comparable to that reported in the Zenne river (Brion et al., 2018) for the $\Sigma(4)$ PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene) with values ranging between 10 and 100 ng/L. Similar concentrations are also found in the Tiber for the $\Sigma(6)$ PAHs (fluoranthene benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene), which varied between 24 and 72 ng/L (Patrolecco et al., 2010). For PAH mixtures, the Joint Research Center chose an eight-component mixture (benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and chrysene) (SCHEER, 2023). In this study, BaP is considered as a suitable

indicator for the assessment of this mixture (Pieterse et al., 2013; Boonen et al., 2020). The European drinking water standard for the sum of PAHs is equal to 100 ng/L (DIRECTIVE/2020/2184/EU), while for BaP an AA-QS_{fw,eco} of 22 ng/L is endorsed by SCHEER (2023). Figure 7 shows that most groundwater samples comply with the limit suggested by SCHEER, while most effluent water samples are conform with the directive (DIRECTIVE/2020/2184/EU).

4 Conclusions

In this baseline survey, the dissolved and labile metal concentrations were monitored over a year in the Kinrooi WWTP effluent water, Abeek river and the surrounding groundwater before infiltration of the effluent into the groundwater. SPM and particulate trace concentrations were also studied and the metal partitioning coefficient $\log K_d$ was calculated in the effluent water and Abeek river. The dissolved, labile and particulate metal concentrations did not show a clear seasonal trend and almost all metal concentrations in the effluent were lower than or equal to those obtained in the river and groundwater. Dissolved and labile metal concentrations are all far below the MAC (Maximum Allowable Concentration) values except Ni, which issues a positive sign for the infiltration of effluent water to groundwater for further irrigation. In addition, up to date, all regulations for inorganic contamination only consider dissolved metal concentrations, which could overestimate the potential risk of these contaminants to organisms such as phytoplankton, since the dissolved fraction includes also a non-labile fraction which is not bioavailable to phytoplankton. The labile fraction includes free metal ions and weakly bound metal complexes, which can be dissociated in a short period and release metal ions that can be used by organisms such as phytoplankton. In Abeek river, Pb and Cu form the most stable complexes and have also the highest affinity for the particulate fraction, while Cd and Ni form the least stable complexes and have the lowest affinity for the particulate fraction. Half of the Ni and Cd complexes are dissociated in less than 12 min, but this would take 4 h before half of the Pb complexes is dissociated. The toxicity of Ni and Cd complexes in Abeek river is thus by far higher than those of Pb. The actual legislation does not consider the bioavailable metal fraction, however, since this is the best proxy for the toxic fraction in solution it should be included in any future monitoring program.

In addition to monitoring inorganic contaminants, this study also provides an overview of variations in ER and AhR agonist activity in effluent and groundwater samples throughout the year. In general, estrogenic activities in groundwater and effluents were low and close to the AA-QS for E2 (fw,eco) endorsed by SCHEER, except in

winter. The results obtained by AhR-CALUX suggest that effluents may contain higher levels of AhR agonists than groundwater, and that seasonal changes in environmental conditions may affect the AhR activity in these samples. In terms of health, environmental and emerging risks, AhR activities in groundwater were always below the EU drinking water standard for the sum of 16 PAHs (100 g/L), and mostly below the AA-QS for BaP (fw,eco) approved by SCHEER.

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Conflict of Interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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