



Research Paper

Water reuse for aquaculture: Comparative removal efficacy and aquatic hazard reduction of pharmaceuticals by a pond treatment system during a one year study

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ARTICLE INFO

Editor: Dr. R Sara

Keywords:

Pharmaceutical fate

Tertiary treatment

POCIS

Probabilistic hazard assessment

Biological pond

ABSTRACT

Aquaculture is increasing at the global scale, and beneficial reuse of wastewater is becoming crucial in some regions. Here we selected a unique tertiary treatment system for study over a one-year period. This experimental ecosystem-based approach to effluent management included a treated wastewater pond (TWP), which receives 100% effluent from a wastewater treatment plant, and an aquaculture pond (AP) that receives treated water from the TWP for fish production. We examined the fate of a wide range of pharmaceutically active compounds (PhACs) in this TWP-AP system and a control pond fed by river water using traditional grab sampling and passive samplers. We then employed probabilistic approaches to examine exposure hazards. Telmisartan, carbamazepine, diclofenac and venlafaxine, exceeded ecotoxicological predicted no effect concentrations in influent wastewater to the TWP, but these water quality hazards were consistently reduced following treatment in the TWP-AP system. In addition, both grab and passive sampling approaches resulted in similar occurrence patterns of studied compounds, which highlights the potential of POCIS use for water monitoring. Based on the approach taken here, the TWP-AP system appears useful as a tertiary treatment step to reduce PhACs and decrease ecotoxicological and antibiotic resistance water quality hazards prior to beneficial reuse in aquaculture.

1. Introduction

Current pharmaceutical production is increasing to meet the growing demand for human and animal care, reaching more than 4000 pharmaceutically active compounds (PhACs) being produced and marketed globally (Ur Rehman et al., 2015). PhACs are an important group of environmental contaminants of emerging concern, particularly in urban areas. PhACs are ubiquitously found in aquatic environments due to their production, use and disposal. In developed countries, the primary source of PhACs is wide population consumption and consequent discharge via wastewater treatment plants (WWTPs). These compounds usually enter waterways via urban wastewater after being excreted. Studies from around the world have reported the occurrence of PhACs in waste- and surface water in the concentration range from low ng/L to µg/L (Huber et al., 2016; Loganathan et al., 2009; Metcalfe et al., 2003; Sun et al., 2016; Watkinson et al., 2009).

Nevertheless, much higher levels can be detected in industrial and hospital effluents. For example, ciprofloxacin has been detected in effluent from ~ 90 bulk drug manufacturers up to 31 mg/L, which is higher than the maximal therapeutic human plasma levels (Larsson et al., 2007). It exceeds an antibiotic resistance development threshold (100 ng/L) 58% of the time in global effluent discharges (Kelly and Brooks, 2018).

Water reuse is also becoming an increasingly important consideration for aquaculture in many regions. By 2030, the FAO (<http://www.fao.org/home/en/>) projects a 50% increase in food production will be required to meet global food needs. Aquaculture, often called the “Blue Revolution,” represents an important strategy to support growing food demand. Yet, food safety concerns exist for water reuse in aquaculture (Brooks and Conkle, 2019) because ~80% of sewage produced worldwide still goes untreated (<https://www.unwater.org/publications/world-water-development-report-2017/>). Further, with the increasing

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<https://doi.org/10.1016/j.jhazmat.2021.126712>

Received 13 April 2021; Received in revised form 19 July 2021; Accepted 19 July 2021

Available online 27 July 2021

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intensification of aquaculture, identifying sustainable production practices that minimize ecological impacts has been identified as a priority research need, particularly in urbanizing regions (Leung et al., 2020). Herein, PhACs represent important considerations because they can adversely affect aquatic life well below acutely toxic concentrations. For example, numerous studies have documented adverse effects of chronic exposure to low environmental concentrations of PhACs, including endocrine disruption, reproductive disorders, behavioural changes, antibiotic resistance (Cervený et al., 2020; Klaminder et al., 2019; Kvarnryd et al., 2011; Xu et al., 2019). Moreover, once released to the environment, PhACs can enter the food chain due to the uptake by aquatic organisms (Grabicova et al., 2015; Nowakowska et al., 2020; Tang et al., 2020), which again has been identified as a priority research question to achieve more sustainable environmental quality (Furley et al., 2018).

Though numerous efforts have examined PhACs fate during traditional wastewater treatment and subsequent occurrence, fate and transport in receiving systems (Altmann et al., 2014; Ghosh et al., 2009; Golovko et al., 2014; Nakada et al., 2007), studies examining the efficacy of less resource-intensive treatment approaches to reduce ecological hazards during water reuse applications in aquaculture are limited. In the current study, we employed an ecosystem-based treatment system, specifically employing a treated wastewater pond (TWP), which receives 100% treated WWTP effluent as a tertiary treatment component. We selected a model system for a unique 12-month study in which water treated by the TWP is then transferred to an aquaculture pond (AP) for common carp production. This general scenario is common in some parts of Europe. It could be highly relevant in low- and middle-income countries as an ecosystem-based management approach for water reuse and aquaculture. Yet, there remain major knowledge gaps for treatment efficacy of PhACs in this type of system (Zaibel et al., 2016). Here we examined the occurrence of a wide range of PhACs within the different locations of the study system, compared different approaches for temporal monitoring of PhACs, and then performed probabilistic environmental hazard assessments to identify the efficacy of different treatment components over one year. While more common water quality parameters are often monitored, there is no work carried out on the assessment of ecotoxicological and antibiotic resistance hazards associated with PhACs residues in wastewater intended for aquaculture.

2. Materials and methods

2.1. Experimental set-up

The study ecosystem is located in Vodnany, Czech Republic, and includes the TWP Cezarka biological pond (2.6 ha), which retains and treats effluent water from a WWTP (average discharge 2151 m³/day) serving 7000 inhabitants that provides primary mechanical filtration, sedimentation and activated sludge treatment. Thus, the Cezarka pond can be considered a tertiary treatment step because it only receives wastewater effluent and precipitation. Effluent from Cezarka is discharged to AP Dremliny (75 ha), a large pond that is used for fish production. It is estimated that water from Cezarka contributes about 80% of Dremliny's water. The map of studied system is shown in the [Supplementary Materials \(Fig. SM1\)](#).

A control pond – CP (0.12 ha) was also selected for study from the pond system of the Faculty of Fisheries and Protection of Water (FFPW), University of South Bohemia, Vodnany, Czech Republic. This pond was chosen as an ecological representative of an aquaculture pond not receiving effluent, but instead receives source water from the Blanice River upstream from the town. The control pond is located 2 km away from Cezarka pond and thus experiences the same climate conditions as the TWP. Hydraulic retention times for the studied ponds were estimated as follows: TWP – 13 days, AP – 288 days, CP – 4 days.

2.2. Sampling and analysis

Water samples were collected over a one year period at three sites in the TWP, specifically near the wastewater influent to the pond, in the middle of the pond and near the effluent outflow from the pond. In addition, we sampled one location from AP near the effluent discharge from the pond, and one location from the CP at the outflow. Two approaches for water sampling were used: grab sampling and passive sampling using polar organic integrative sampler (POCIS). POCIS (pesticide configuration, purchased from Nya Exposmeter AB, Tavelsjö, Sweden) were deployed for a period of 14 days as we previously identified this time period to be appropriate for most of PhACs in terms of linear uptake by POCIS (Fedorova et al., 2014). LC/MS/MS analysis was determined with methods already published in the literature (Fedorova et al., 2013; Grabic et al., 2012; Lindberg et al., 2015). Details on the sample preparation and analysis are reported in [SM \(SM 2.1–2.3\)](#).

2.3. Compounds of interest

We specifically selected a list of 70 PhACs based on their previous occurrence in wastewaters and receiving systems, their potential to bioconcentrate in fish based on water/plasma model, and existing ecotoxicology and health information. These target analytes include 11 antihypertension drugs, 9 antibiotics, 8 antidepressants, 6 antihistamines, 2 antidiabetics, 2 antifungals, 2 anti-Alzheimer drugs, 2 anti-Parkinsons medicines, 2 tranquilizers, 2 fibrates drugs, 13 medicines from other classes and 11 drug metabolites (list of the studied PhACs is presented in the [Table SM1](#)).

2.4. Principal component analysis

To express qualitative differences among different sites and sampling time we used normalized data sets for principal component analysis (PCA). We normalized individual compound values to the corresponding sum of PhACs to avoid distortion via different concentrations during sampling events. Results below limit of quantification (LOQ) were replaced with 0. PCA was performed in Statistica software version 13.5.0.17 (TIBCO Software Inc., Palo Alto, California, USA).

2.5. Probabilistic environmental hazard assessment (PEHA)

2.5.1. Environmental exposure distributions

Data on PhACs concentrations in TWP, AP and CP were fitted to log-normal distribution to create probabilistic environmental exposure distributions (EEDs). All graphs were prepared with Sigma Plot 14.0 software (Systat Software, Inc., San Jose, California, USA). Concentration data were ranked in ascending order and a percent rank for each concentration was calculated using the Weibull formula:

$$j = i * 100 / (n + 1),$$

where i is numerical rank assigned for each concentration, n is the number of sampling events.

The EEDs were constructed by plotting concentrations (log-normal scale) of certain compounds in water against their respective percent rank (probability scale). Linear regression analysis was performed and centile values were calculated following:

$$\text{Centile value} = \text{NORMDIST}((a * \log_{10}(x)) + y_0),$$

where NORMDIST returns the probability of getting less than or equal to a particular value in a normal distribution, a and y_0 represent the slope and intercept, respectively. More details can be found elsewhere (Brain et al., 2006; Kristofco and Brooks, 2017).

2.5.2. Predicted no-effect concentrations (PNECs)

To identify whether PhACs concentration found in the system TWP-

AP-CP can potentially pose adverse effects in aquatic ecosystems, we employed aquatic predicted no-effect concentrations (PNECs) to perform probabilistic environmental hazard assessment (PEHA). Toxicological data for algae, crustaceans and fish were used for the calculation of PNECs using appropriate assessment factors (Toxicity data was mainly obtained from the United States EPA ECOTOX database <https://cfpub.epa.gov/ecotox/index.cfm>) (Zhou et al., 2019). In addition, development of antibiotic resistance (ABR) was examined for antibiotics using ABR PNEC levels (Bengtsson-Palme and Larsson, 2016).

3. Results and discussion

3.1. PhAC occurrence in the TWP

We identified 52 out of 70 PhACs in water from the Cezarka pond (samples from all sampling locations) above LOQ levels. Though some compounds were only occasionally detected, 37 PhACs were present in all collected samples over the year-long sampling period. Concentrations ranged from sub-ng/L to several $\mu\text{g/L}$, with the highest levels for irbesartan, telmisartan and valsartan (medications used to treat hypertension). We applied PCA to visualize spatial and seasonal differences among PhACs patterns in the TWP. Fig. 1A shows PCA projection of cases (samples from different sites and time) to factor 1 vs 2 plane, where each sampling time is represented by a specific color; influent samples are marked with triangles, middle – with circles, effluent – with squares; all sampling points corresponding to one sampling time are connected with triangles for easier reading. Samples from the influent to pond, middle pond and pond effluent are connected by triangles. Factor 1 represents the difference between influent and effluent levels of PhACs; influent samples are consistently located left of the effluent. Increasing distance between influent and effluent corresponds to the increasing proportion of stable compounds in total PhACs concentration. We clearly observed separation of the sampling events related to sampling time by factor 2, which appears to be a “seasonal factor”. Cold (February, March, April; water temperature ranged from 6.1 to 12.8 °C; mean temperature was 10 °C) and warm (May, July, August, September, October; temperature ranged from 15.8 to 19.5 °C; mean temperature

was 17.5 °C) season samplings are located in different parts of the plot.

The difference between influent and effluent data points appears to be higher during the warm season, suggesting that PhACs are more efficiently removed by the pond during the summer, which may be related to higher water temperature, longer sunlight for potential photolysis, and higher bacterial metabolic activity. Removal was more effective during summertime for the majority of the studied compounds, and these results are in agreement with our previous findings (Golovko et al., 2014). However, we observed some exceptions where PhAC levels were almost identical among sites within one day (e.g., May 2015, October 2015 and April 2016). This observation may be attributed to day to day variability of influent flow, dependent on PhAC consumption patterns and WWTP operational performance.

When we examined changes of individual PhAC concentrations and patterns throughout the year, these partitioned into 4 different groups: stable, seasonal, degradable and seasonal+degradable. The stable pattern may be attributed to 7 PhACs with RSD of all measured concentrations lower than 30%. For example, epoxy-carbamazepine is relatively stable among the influent to pond, middle pond and pond effluent samples for all sampling events (Fig. 2A). Similar patterns were also observed for carbamazepine, dihydro-carbamazepine, trans-10,11-dihydro-10,11-dihydroxy-carbamazepine, cetirizine, glimepiride, and metoprolol acid. However, a seasonal pattern was typical for the antibiotics sulfamethoxazole, its metabolite N4-acetylsulfamethoxazole and sulfamethazine, and also for α 1-blocker alfuzosin. Human consumption of these medications is usually higher in winter and early spring, which is reflected in almost an order of magnitude higher levels during this time period, while differences between influent and effluent concentrations were less pronounced. Such an observation identifies a notably low degradation of this compound in the pond system (Fig. 2B).

Two PhACs, oxazepam and telmisartan, are representative of a degradation occurrence pattern (Fig. 2C). Herein, there was a noticeable difference in levels between pond influent and effluent samples, for which concentrations decreased up to 2 fold during most sampling events. However, most of the detected PhACs, 24 out of 37 compounds (atenolol, azithromycin, bezafibrate, bisoprolol, citalopram, N-desmethylycitalopram, clarithromycin, clindamycin, clindamycin sulfoxide,

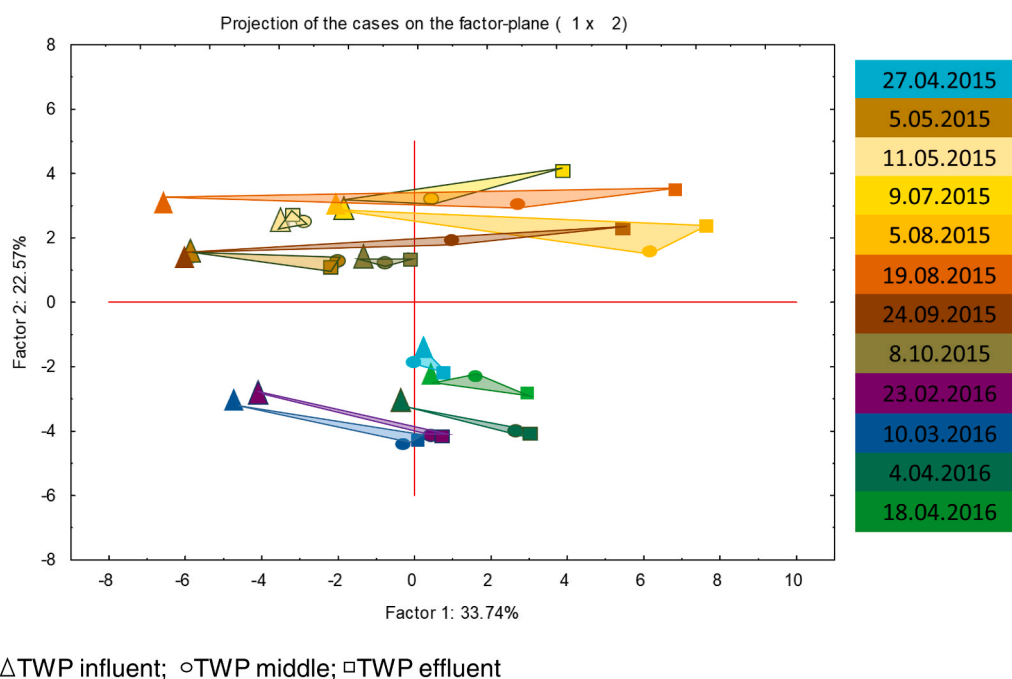


Fig. 1. Principal component analysis (PCA). A. Projection of cases on factor 1 vs 2. Variables are relative proportions of positive pharmaceutically active compounds (PhACs) in grab water samples from the Cezarka treated wastewater pond in all sampling events. Factor 1 represents the difference between influent and effluent levels of PhACs; factor 2 is a “seasonal factor”. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

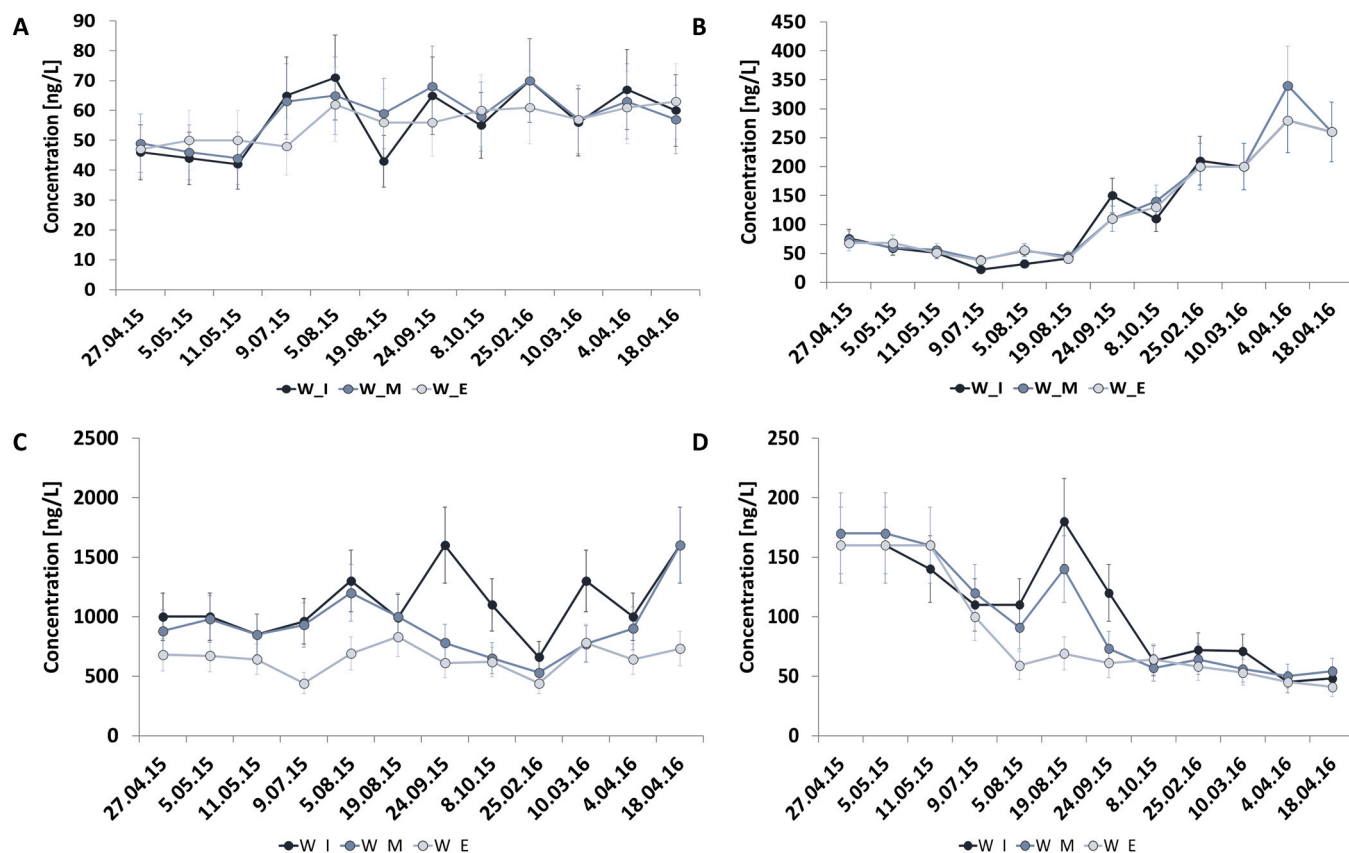


Fig. 2. Concentrations of epoxy-carbamazepine (A), sulfamethoxazole (B), telmisartan (C) and fexofenadine (D) in grab water samples from the Cezarka treated wastewater pond. (W_I – concentration in influent samples, W_M – concentration in samples from the middle of the pond, W_E – concentration in effluent samples). Error bars (20%) represent estimated uncertainty.

diclofenac, erythromycin, fexofenadine, irbesartan, memantine, metoprolol, mirtazapine, oxcabazepine, sotalol, tramadol, trimethoprim, valsartan, venlafaxine, O-desmethylvenlafaxine, verapamil), can be generally characterized by a combination of seasonal and degradation patterns. For example, higher concentrations of the antihistamine fexofenadine were observed during the spring-summer period, which correlates with higher allergen appearance (Golovko et al., 2014). In addition, a marked difference between the influent and effluent concentrations was observed for fexofenadine during summer months indicating elevated degradation of the compound (Fig. 2D).

More detailed insight to PhAC degradation in the pond was possible by examining data for their metabolites. As mentioned above, carbamazepine metabolites epoxy-carbamazepine and trans-10,11-dihydro-10,11-dihydroxy-carbamazepine were stable in the pond throughout the year along with their parent compound carbamazepine. Removal efficiencies of these compounds in the pond, presented here as the ratio of the compound in the effluent to the influent (Fig. 3A), were very similar for the parent compound and both metabolites with the removal efficiency ratio of parent/metabolite close to 1 in all sampling points. Such observations suggest that carbamazepine metabolites in the

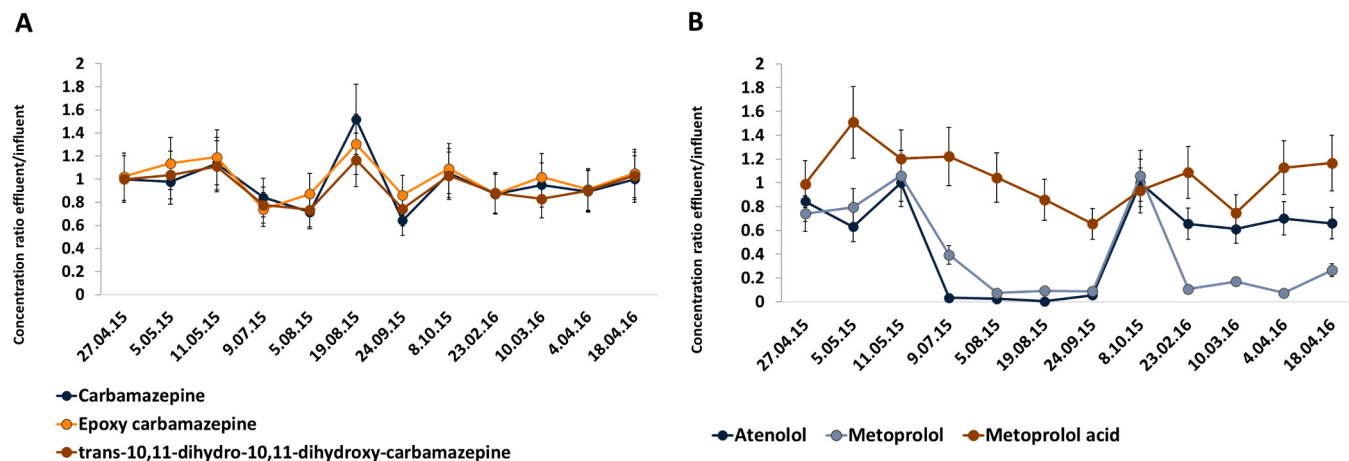


Fig. 3. Seasonal changes in the Cezarka treated wastewater pond removal efficiency of carbamazepine and its metabolites (A), atenolol, metoprolol and their metabolite (B). Error bars (20%) represent estimated uncertainty.

present study originated from human metabolism, not transformation processes in the treatment pond.

A different behavior was observed for metoprolol acid, a metabolite of beta-blockers metoprolol and atenolol (Fig. 3B). Removal efficiencies of both parent compounds showed a similar trend, while another pattern was observed for their metabolite. A high removal rate was noticed for both atenolol and metoprolol (summertime) but metoprolol acid was still rather stable in the pond water. This may indicate that, in addition to being formed as a result of human metabolism, metoprolol acid was formed in the pond due to rapid bacterial transformation of metoprolol and atenolol similar to our observation in soils (Koba et al., 2016). This observations gives a clear evidence that only monitoring parent compounds fails to account for the actual environmental exposure, and the occurrence, fate and effects of metabolites and transformation products should receive more attention.

3.2. Comparison of grab and POCIS sampling

It is well known that passive sampling can overcome limitations of conventional grab sampling at one time point and thus provide data on time-weighted mean concentrations derived from integrative sampling. A further advantage of passive sampling is the ability to potentially mimic biological uptake, minimizing the use of aquatic organisms for biomonitoring (Alvarez et al., 2005; Kot et al., 2000), though ongoing work continues to examine the utility of various passive samplers for diverse chemical contaminants classes. Moreover, comparing concentrations in passive samplers and those in biota promises to help differentiate bioconcentration from bioaccumulation of specifically studied compounds. POCIS have been successfully used for sampling of PhACs in different types of waters (Amdany et al., 2014; Arditoglou and Voutsas, 2008; Bartelt-Hunt et al., 2009; Fedorova et al., 2014).

In the present study, the number of compounds detected in POCIS and grab samples was very similar with 38 PhACs identified in POCIS above the LOQ level. The only difference was propranolol, which was not detected in grab samples, but was found in POCIS at trace concentrations from 0.11 to 0.68 ng/POCIS. Concentration patterns in POCIS reflected those observations from grab samples for most of the studied compounds. Obvious separation of sampling events depended on the season, in addition to greater differences between influent and effluent samples during warmer months were observed in PCA of POCIS data (Fig. SM2). Fig. 4A presents a comparison of amount of O-desmethylvenlafaxine, a main metabolite of antidepressant drug venlafaxine, detected in POCIS and its corresponding concentrations in grab water samples from the same locations. Though it is difficult to compare the concentrations in POCIS and grab water samples directly, the patterns are notably similar. For example, a decrease in concentration and an

elevated difference in influent to pond, middle pond and pond effluent concentrations during the summertime were apparent from both sampling methods.

A slightly different pattern between sampling approaches was observed for tramadol (Fig. 4B). In general, the patterns were similar, except the high difference among influent to pond, middle pond and pond effluent concentrations during wintertime, which was not reflected in POCIS. This may have resulted from varied human consumption and a sporadic increase in the influent, which was not observed in POCIS due to the time integrative sampling. In this case, POCIS provided useful information over longer-term exposure conditions.

3.3. Occurrence of PhACs in AP and CP

While 34 PhACs were identified in effluent from the TWP, only 19 PhACs were observed above LOQ in the AP Dremliny, which receives this treated water from TWP Cezarka. In addition, 28 PhACs were present in the CP indicating contamination of source waters from the Blаницe River. A comparable concentration range of PhAC individuals was observed for both ponds with 0.24–630 ng/L and 0.25–780 ng/L in AP and CP, respectively. The antimycotic drug miconazole, which was not detected in the TWP or the AP, but was present in the CP. This antifungal agent is most likely removed during the WWTP treatment, but may enter the river and consequently be introduced to the CP from onsite wastewater (e.g., septic tanks) associated with summer houses located adjacent to the Blаницe River because these residences are not connected to the WWTP.

Though we estimated input of PhACs from TWP to AP was around 80%, we noticed different concentration ratios for most of the study compounds. For example, the mean level of anti-epileptic drug carbamazepine, which is an environmentally stable compound, in AP was ~2 times lower than the mean concentration of this compound in effluent following treatment by the TWP. This observation supports evidence of such processes as transformation, sorption, bioaccumulation taking place during water displacement from the TWP effluent to the AP and also within the AP, which further contributes to PhAC removal by this ecosystem-based approach for wastewater management. This may be particularly attributed to a much longer retention time of water and higher amount of biomass in AP, among other factors. For example, two representative major patterns are shown in Fig. 5 for the relatively stable occurrence of carbamazepine throughout the year and a seasonal pattern for sulfamethoxazole between the TWP-AP system.

3.4. Probabilistic environmental hazard assessments (PEHA)

Because effluent from TWPs is usually discharged directly to surface

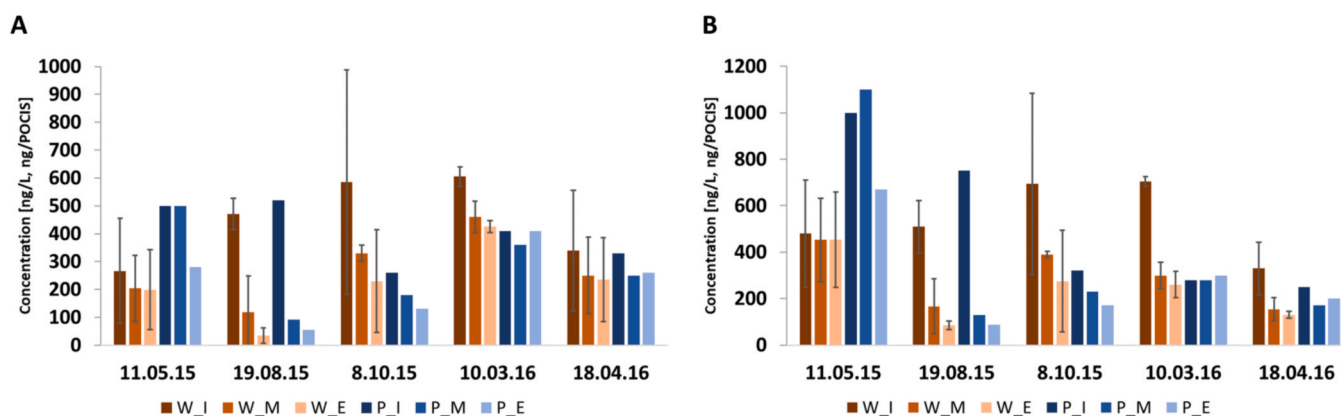


Fig. 4. Concentration of O-desmethylvenlafaxine (A) and tramadol (B) in water (ng/L) and POCIS from the Cezarka treated wastewater pond (ng/POCIS). W_I – influent water samples, W_M – water samples from the middle of the pond, W_E – effluent water samples; P_I – POCIS from the influent, P_M – POCIS from the middle of the pond, P_E – POCIS from the effluent. Mean water concentration during the POCIS deployment period is presented.

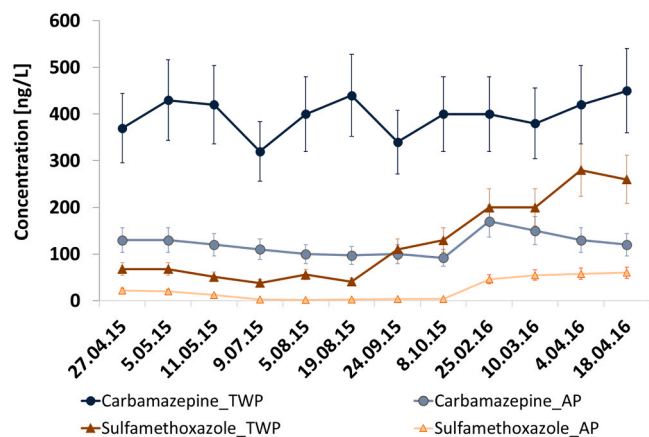


Fig. 5. Occurrence patterns of carbamazepine and sulfamethoxazole in the Cezarka treated wastewater pond and then subsequently in the aquaculture pond. TWP – treated wastewater pond, AP – aquaculture pond. Error bars (20%) represent estimated uncertainty.

water receiving systems or may be reused for aquaculture or agriculture purposes, it remains important to understand ecological hazards and risks presented by PhACs and other CECs to aquatic organisms, including fish, following this tertiary treatment approach. Currently ecological risk assessment for PhACs most commonly relies on a deterministic approach using hazard quotients in which a single exposure level is included to account for exposure. In contrast, probabilistic approaches, including EEDs, are advantaged because a distribution of exposure values can be used to predict the likelihood of encountering specific concentrations of interest in the environment. Consequently, EEDs offer more useful outputs for decision-making than does a deterministic conservative approach, because it provides a more comprehensive characterization of variability in exposure estimates.

In the present study, we constructed probabilistic EEDs for 20 detected PhACs in the TWP-AP system, for which ecotoxicological data are available (regression parameters are given in Table SM3). If water concentration at certain sampling point was below LOQ, it was replaced with LOQ value to avoid input data gaps and thus considered the worst case scenario. We specifically then compared probabilities of these EEDs by study compound among different locations of the TWP-AP system.

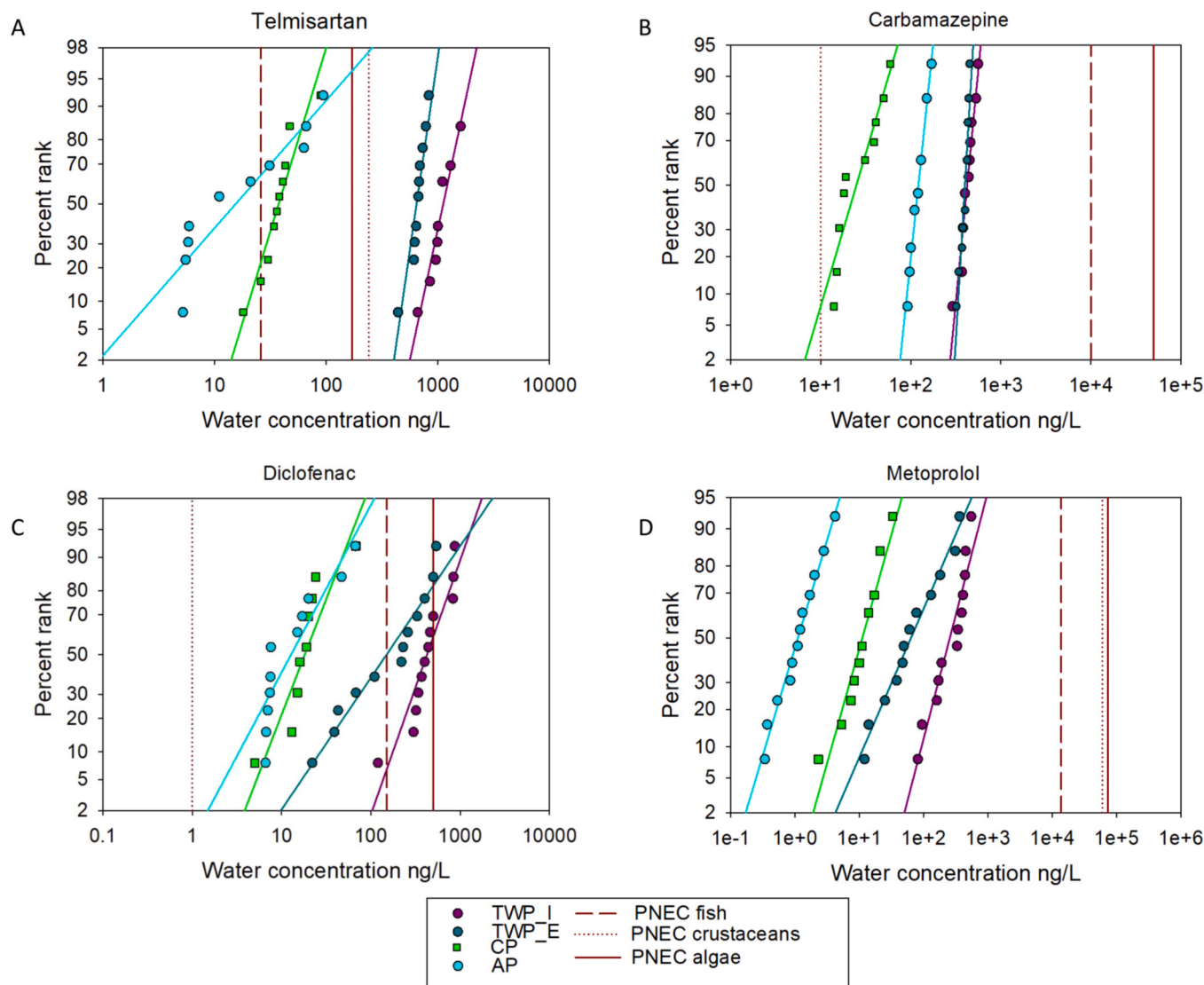


Fig. 6. Environmental exposure distributions (EEDs) for telmisartan (A), carbamazepine (B), diclofenac (C) and metoprolol (D) in the Cezarka treated wastewater pond (TWP) and then subsequently in the aquaculture pond (AP). Vertical reference lines represent corresponding predicted no effect concentrations for algae, invertebrates and fish, which were employed for comparative probabilistic ecological hazard assessments among components of the TWP-AP system and the control pond (CP).

We identified the likelihood of observing stable compounds as carbamazepine (Fig. 6B), oxazepam (Fig. 7B), clindamycin and sulfamethoxazole (Fig. 8B and C) to be similar in both the influent to and effluent from the TWP, while probability of occurrence for most of the compounds (e.g., diclofenac, metoprolol (Fig. 6C, D)); citalopram, tramadol, venlafaxine (Fig. 7A, C, D) was markedly lower for the effluent following treatment by the TWP. It is important to note that the probability of elevated exposure to all studied PhACs was lower in the AP and CP compared to the TWP. Interestingly, the probability of occurrence of some compounds, including metoprolol (Fig. 6D) and venlafaxine (Fig. 7D), was higher in the CP, which again receives water from a local river and thus appears to be affected by onsite wastewater, than in the AP following polishing by the TWP. EEDs for other PhACs are presented in Supplementary Material (Figs. SM3 and SM4).

In this study POCIS showed comparable results with grab sampling regarding occurrence patterns of selected PhACs, so we explored potential use of such information for exposure estimation. A major current limitation in the application of POCIS for monitoring and subsequent exposure, hazard and risk assessment efforts is the recalculation of sampled amount of pollutants to water concentration units, i.e. ng/L,

despite the increasing number of studies trying to advance this work (Bailly et al., 2013; Buzier et al., 2019; Gravell et al., 2020; McKay et al., 2020). As an initial exercise, we used available calibration data for POCIS (Vrana et al., 2021) to back calculate PhAC concentrations in POCIS to water concentrations, and then constructed EEDs based on those estimated water concentrations. EEDs for telmisartan, carbamazepine, diclofenac and metoprolol are given in Fig. SM5. In general, EEDs from actually observed water levels (Fig. 8) and estimated water concentrations based on POCIS measures (Fig. SM5) were very similar with minor differences. Though these comparative results are very promising, future availability of calibration data for a wide range of compounds across environmental gradients will be necessary to support potential use of POCIS for exposure, hazard and risk assessments.

After developing EEDs for detected PhACs at each sampling location within the TWP-AP system, we then performed PEHAs to examine the likelihood of encountering exceedances of ecotoxicology PNECs for three trophic levels (algae, crustaceans, fish). This aquatic hazard assessment approach has previously been employed to examine global aquatic hazards of specific PhAC classes, including antihistamines (Kristofco and Brooks, 2017), calcium channel blockers (Saari et al.,

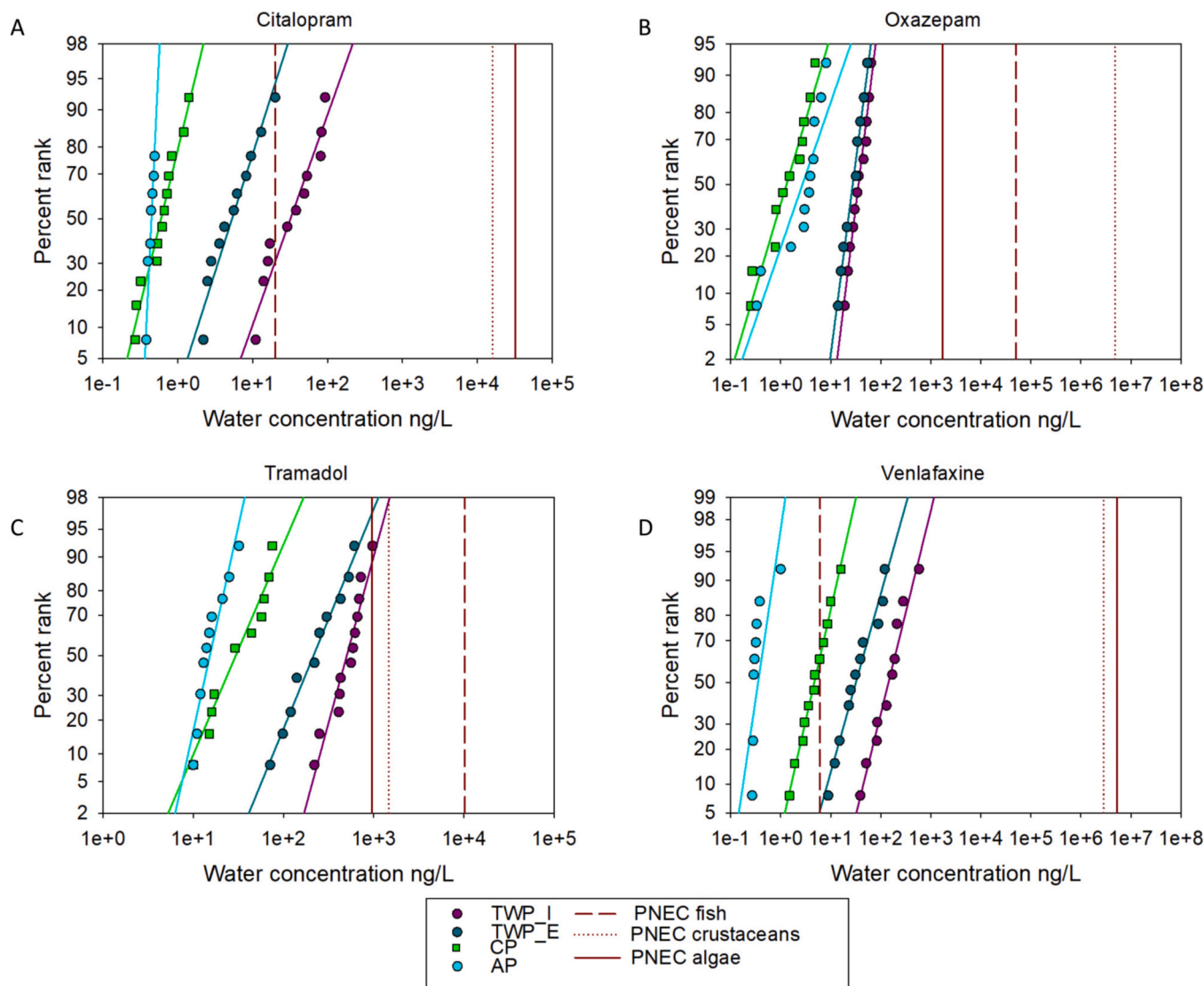


Fig. 7. Environmental exposure distributions (EEDs) for the psychoactive compounds citalopram (A), oxazepam (B), tramadol (C) and venlafaxine (D) in the Cezarka treated wastewater pond (TWP) and then subsequently in the aquaculture pond (AP). Vertical reference lines represent corresponding predicted no effect concentrations for algae, invertebrates and fish, which were employed for comparative probabilistic ecological hazard assessments among components of the TWP-AP system and the control pond (CP).

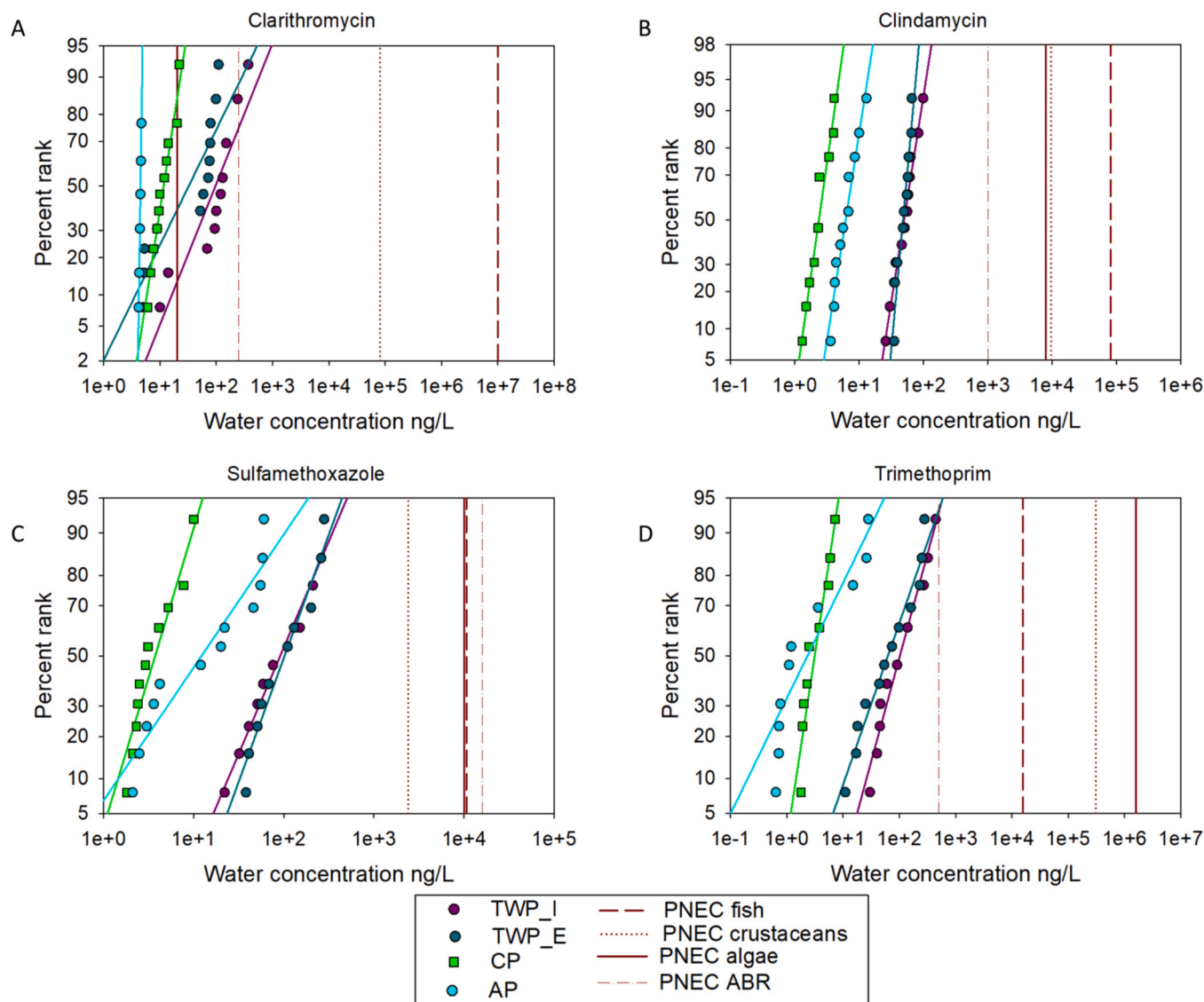


Fig. 8. Environmental exposure distributions (EEDs) for the antibiotics clarithromycin (A), clindamycin (B), sulfamethoxazole (C) and trimethoprim (D) in the Cezarka treated wastewater pond (TWP) and then subsequently in the aquaculture pond (AP). Vertical reference lines represent corresponding predicted no effect concentrations for algae, invertebrates, fish and antibiotic resistance development, which were employed for comparative probabilistic ecological hazard assessments among components of the TWP-AP system and the control pond (CP).

2017) and selective serotonin reuptake inhibitors (Mole and Brooks, 2019). Such applications of PEHA have supported identification of key data gaps, geographic locations, aquatic systems and substances requiring additional study, and efficacy of various traditional WWTP technologies to reduce PhAC discharges. For the antibiotics observed in the TWP-AP system, we performed additional PEHAs, which similarly followed previously reported approaches for ciprofloxacin (Kelly and Brooks, 2018) and erythromycin (Schathausen et al., 2018), to understand exceedance probabilities of PNECs for ABR development, again at each sampling location.

Most of the targeted PhACs in the present study (metoprolol, clindamycin, sulfamethoxazole, atenolol, bisoprolol, sotalol, azithromycin, bezafibrate, cetirizine, erythromycin, oxazepam) did not exceed any PNEC at any of the sampling locations. However, 7 compounds (carbamazepine, citalopram, tramadol, venlafaxine, clarithromycin, trimethoprim and valsartan) exceeded at least 1 PNEC on at least 1 sampling site, while telmisartan and diclofenac exceeded all PNEC values in at least one study location (Figs. 6–8 and SM3 and 4). For example, 100% exceedances of all ecological PNEC values for telmisartan were observed both in TWP influent and effluent and the AP, though the PNEC

exceedance for fish was reduced to 33% and PNECs for algae and invertebrates were further not exceeded in the AP (Fig. 6; Table SM4). Though the TWP-AP system did not effectively reduce aquatic hazards of diclofenac to invertebrates, PNEC exceedances for algae and fish were reduced by the TWP from 33% to 17% and from 92% to 58% in pond influent and effluent, respectively (Table SM4). Following additional treatment by the AP, PNEC exceedances for algae and fish were not observed (Table SM4). Such aquatic hazard reductions by the TWP-AP system is noteworthy because diclofenac has been included on a dynamic “watch list” of EU Water Framework Directive due to potential adverse effects in the aquatic environment (Loos et al., 2018).

When ecological hazards of psychoactive compounds to fish are considered (Fig. 7), PNEC exceedances were observed for citalopram and venlafaxine but not for tramadol and oxazepam. However, these water quality hazards were effectively removed by the TWP-AP system. Specifically, PNEC exceedances for fish were reduced from 58% and 100% in TWP influent for citalopram and venlafaxine, respectively, to 0% exceedances following AP treatment. Similarly, an 8% exceedance for the ABR PNEC for clarithromycin was only observed in TWP influent, but this aquatic hazard was reduced to zero in TWP effluent (Fig. 8,

Table SM4). In addition, we also observed 42% exceedance of the venlafaxine PNEC for fish in the CP, which indicates the need to further examine aquatic hazards of this antidepressant in the source stream. It is also important to note that our work here with PNECs during PEHAs were inherently limited by the available ecotoxicological data. For example, in the case of oxazepam, we employed EC50 values, which underestimate ecologically important adverse sublethal responses following chronic exposure, especially considering evidence for fish behavioural effects of this compound at environmentally relevant concentrations (Brodin et al., 2013). Collectively, these results identify the utility of the TWP-AP system as a tertiary treatment step to effectively reduce PhACs and decrease ecotoxicological and ABR water quality hazards prior to further aquaculture use. Moreover, earlier it was shown that PhACs are not bioaccumulated in the muscle of fish cultured in the wastewater treatment pond (Grabicova et al., 2020).

4. Conclusions

In the present study, we examined the fate of targeted PhACs and associated water quality hazards in the TWP-AP system, an ecosystem-based approach for wastewater management, and a CP over a year study period. We observed similar patterns between grab and passive sampling of water for most of the studied compounds, which suggest potential utility of POCIS as a very valuable tool for PhACs monitoring. Of the 20 PhAC for which aquatic hazards were examined in the TWP, AP and CP, most of the compounds presented low hazards to aquatic organisms, based on the PNECs employed for PEHAs. However, several PhACs, including telmisartan, carbamazepine, diclofenac and venlafaxine, exceeded ecotoxicological PNECs in influent wastewater to the TWP, but these water quality hazards were consistently reduced or ameliorated following treatment in the TWP-AP system. We observed good agreement between water and passive sampling results for risk estimation. Based on the approach taken here, the TWP-AP system appears useful as a tertiary treatment step to reduce PhACs and decrease ecotoxicological and ABR water quality hazards prior to beneficial reuse in aquaculture. These observations can be further refined as additional toxicological information becomes available for PhACs, and their metabolites and degradates. Additional studies are necessary to understand treatment capacity of the TWP-AP system for other chemicals of concern.

CRedit authorship contribution statement

Ganna Fedorova: Formal analysis, Visualization, Writing – original draft; **Roman Grabic:** Methodology, Writing – review & editing, Conceptualization; **Katerina Grabicová:** Formal analysis, Investigation, Writing – review & editing; **Jan Turek:** Resources, Investigation; **Tuyen Van Nguyen:** Resources; **Tomas Randak:** Conceptualization; **Bryan W. Brooks:** Supervision, Writing – review & editing; **Vladimir Zlabek:** Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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

Acknowledgements

The study was financially supported by the Czech Science Foundation [project No. 18-15802S], the Ministry of Education, Youth and Sports of the Czech Republic - project “CENAKVA” [No. LM2018099]. BWB acknowledges support from United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) (#20166900725093) during preparation of this publication. The authors would also like to thank Vodnany town and CEVAK a.s. for

supporting this work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126712.

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