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Removal of selected pharmaceuticals from aqueous solutions by cyanobacteria and aquatic macrophytes

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Abstract

The aim of the work was to study the bioaccumulation of paracetamol (PRC) and ibuprofen (IBF) by living biomass of cyanobacteria *Synechococcus bigranulatus*, freshwater plant *Egeria densa* or moss *Vesicularia dubyana*. In the case of PRC bioaccumulation by cyanobacterial biomass, it was found that in the first minutes of exposure, rapid, probably sorption processes were subsequently replaced by a slower uptake of PRC. From this point, the bioaccumulation showed a linear dependence, reaching almost 32% removal of PRC ($3.12 \pm 0.03 \text{ mg} \cdot \text{g}^{-1}$) from 10% Hoagland medium with an initial concentration of PRC $50 \text{ mg} \cdot \text{dm}^{-3}$ and after 14 d of exposure. Sorption processes played a minor role in the binding of PRC by *E. densa* biomass. In the first 2 h of exposure, we did not observe the uptake of PRC, and more significant bioaccumulation at the level of 17% removal of PRC ($0.066 \pm 0.010 \text{ mg} \cdot \text{g}^{-1}$) from a medium containing $5 \text{ mg} \cdot \text{dm}^{-3}$ of PRC occurred later and during the 24 h of exposure. Also, it was shown that with increasing concentration of *E. densa* biomass the percentage of PRC binding increased, but the specific uptake of PRC (in $\text{mg} \cdot \text{g}^{-1}$) decreased. Like PRC and *E. densa* biomass, we found that the sorption contributed minimally to IBF uptake by moss biomass. However, further bioaccumulation was characterized by a significant removal of IBF at the level of 22% ($0.052 \pm 0.040 \text{ mg} \cdot \text{g}^{-1}$) from a medium containing $12.5 \text{ mg} \cdot \text{dm}^{-3}$ of IBF.

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Introduction

For decades, many pharmaceutical products have been developed and introduced to meet the needs of an ever-growing world population (Rantanen and Khinast 2015). The presence of pharmaceutical compounds in wastewater is becoming an environmental challenge, particularly because of their adverse effects on human health and on terrestrial and aquatic ecosystems (Sivarajasekar et al. 2017). Pharmaceuticals, such as anti-retroviral

drugs, painkillers, antibiotics, etc., and their derivatives enter water bodies and the environment through the excretion (Zhang et al. 2020; Ortúzar et al. 2022; Samal et al. 2022). Non-steroidal anti-inflammatory drugs, Class IA and IIB pollutants, are excreted unmetabolized into water in the form of urine (Koltsakidou et al. 2021). More than 50 different drugs from these groups are available on the global market and are used by nearly 35 million

people every day (López-Miranda *et al.* 2022). Pharmaceutical residues from a variety of sources (human, agricultural, and industrial) have also been found in almost all environmental matrices, such as surface water, groundwater, wastewater treatment plant effluents, sludge and even ocean water, despite increasing dilution (Fang *et al.* 2012; Bedoya-Ríos *et al.* 2018; Patel *et al.* 2019). However, many of them can cause environmental damage because their maximum permissible concentrations in water have not yet been regulated (Villaescusa *et al.* 2011). The levels at which these compounds have been measured range from the $\text{ng}\cdot\text{dm}^{-3}$ to the $\mu\text{g}\cdot\text{dm}^{-3}$, but in specific cases, the concentrations can reach the values in $\text{mg}\cdot\text{dm}^{-3}$ (Oggier *et al.* 2010; Nunes *et al.* 2014). Many of these compounds can damage ecosystems and their long-term and synergistic effects on human health and other organisms are mostly unknown (Petrie *et al.* 2015). The potential toxicological risks and consequences resulting from human exposure, such as the development of antibiotic-resistant bacteria, feminisation of male fish, and toxicity and genotoxicity in aquatic life, are very often described (Dvořáková Březinová *et al.* 2018). Among the various pharmaceutical compounds frequently detected in water resources are paracetamol and ibuprofen, which are classified as anti-inflammatory and analgesic drugs (Ahmed 2017; Sellaoui *et al.* 2017). Paracetamol (*N*-(4-hydroxyphenyl) acetamide) is one of the most widely used analgesics, antipyretics, and non-steroidal anti-inflammatory drugs available without prescription worldwide, as well as one of the most commonly occurring drugs in various environmental matrices, i.e. in sediments, groundwater or drinking water (Žur *et al.* 2018). This drug belongs to emerging pollutants and is often detected in the waterbodies in a concentration range of $\text{ng}\cdot\text{dm}^{-3}$ to $\mu\text{g}\cdot\text{dm}^{-3}$ (Islam *et al.* 2024). Continuous unintentional recharge of these low levels can have negative impacts on organisms and sub-organisms in the receiving aquatic environment (Žur *et al.* 2018). Due to its high stability, solubility, and hydrophilicity, it has been detected in surface water, wastewater, and drinking water worldwide (Bonnefille *et al.* 2018). The identity, persistence, and toxicity of the degradation products of paracetamol are not widely described in

the literature (Ranieri *et al.* 2011). When secondary effluents are discharged to surface waters, the Predicted Environmental Concentration (PEC) may exceed the predicted no-effect concentration (PNEC), which may lead to ecotoxicity to the aquatic environment (Grung *et al.* 2008). Ibuprofen (2-(4-isobutylphenyl)propanoic acid) is a widely used non-steroidal anti-inflammatory drug for treatment of inflammation, pain, fever, rheumatoid arthritis, and migraines. It is sparingly soluble in polar solvents, such as water, but completely soluble in non-polar solvents, such as methanol or ethanol (Bello *et al.* 2019). The concentration of ibuprofen as the third most consumed drug in wastewater treatment plant effluents is up to $25\text{ mg}\cdot\text{dm}^{-3}$ (Melillo *et al.* 2004; Mestre *et al.* 2007). The World Health Organisation (WHO) found that the concentrations in surface water in Finland, France and Germany were 65, 120, and $530\text{ ng}\cdot\text{dm}^{-3}$, respectively (Mlunguza *et al.* 2019). It is known that ibuprofen can cause changes in the endocrine system of aquatic species (Ding *et al.* 2017). Menicagli *et al.* (2024) found that ibuprofen did not affect plant growth of seagrass *Cymodocea nodosa* but caused physiological alterations which varied in severity depending on its concentration. Concentrations of 0.25 and $2.5\text{ }\mu\text{g}\cdot\text{dm}^{-3}$ caused oxidative stress. Elimination efficiencies of up to 90% were recorded in municipal wastewater treatment plants (López-Miranda *et al.* 2022). Within European Union, ibuprofen is considered by the Priority Substance Directive (EC 2013) and the EU commission (DG Env 2020) as a candidate for the relatively short priority substance list under the Water Framework Directive (WFD, EC 2000).

The removal of pharmaceuticals from wastewaters, water streams, and aquatic systems is a challenge for researchers worldwide in terms of the development of new alternative methods and technological solutions to remove these potential environmental contaminants and hazardous compounds. In addition to traditional methods and techniques for the removal of this group of substances, advanced wastewater treatment methods, such as flotation, filtration, precipitation, chemical oxidation, hydrolysis, chemical reduction, reverse osmosis, physical degradation, chlorination, photochemical reactions, adsorption, and ion exchange, have been modified and developed

(Sharma and Bhattacharya 2017; Singh *et al.* 2021). However, the implementation of these techniques is limited by high operating costs and high energy consumption.

Bioaccumulation and biosorption using living and dead biomass are alternative bioremediation methods designed to remove various environmental contaminants. The potential applications of these bioremediation processes have been explored in several papers and evaluated in terms of their application advantages and disadvantages in the following reviews (Mondal *et al.* 2016; de Jesus Menk *et al.* 2019; Show *et al.* 2021; Thabede *et al.* 2023; Nasir *et al.* 2024). A significant part of the works deals with the use of algal and aquatic plant biomass in the removal of pharmaceutical drugs by bioaccumulation or biosorption processes (Mustafa *et al.* 2021; Couto *et al.* 2022; Mojiri *et al.* 2022; Gómez-Regalado *et al.* 2023; Ramesh *et al.* 2023).

In the last ten years, our research has been focused on the study of bioaccumulation and biosorption of heavy metals, radionuclides or synthetic dyes by living or dead biomass of freshwater microorganisms, plants or mosses (Horník *et al.* 2013; Šušnovská *et al.* 2016; Pipiška *et al.* 2018; Pipiška *et al.* 2020; Machalová *et al.* 2023; Valica *et al.* 2023). The aim of the present work was to study the bioaccumulation of selected pharmaceutical drugs, paracetamol and ibuprofen, by living biomass of cyanobacteria *Synechococcus bigranulatus*, freshwater plant *Egeria densa* or moss *Vesicularia dubyana*. The selection of these biological objects was focused on freshwater photosynthetic organisms forming important components of the biocenosis of freshwater systems. Cyanobacteria of the genus *Synechococcus* represent the prokaryotic autotrophic picoplankton of both freshwater and marine waters. However, the freshwater plant *E. densa*, whose habitat is in South and Central America and Africa, is also found in the rivers of Central Europe as an introduced invasive plant. This fact is confirmed by the work of Hussner and Löscher (2005), who found *E. densa* in the Erft River (Northrhine-Westphalia, Germany), where it was found in the riverbed to a depth of 1 m within 7 m of the bank. The freshwater moss *V. dubyana* is native to Southeast Asia (Philippines, Java, Malaysia).

Experimental

Reagents

Single-component solutions of paracetamol (PRC; *N*-(4-hydroxyphenyl) acetamide, CAS 103-90-2; purity $\geq 98\%$, $M_r = 151.16$, $\lambda_{\max} = 243$ nm; Sigma-Aldrich, USA) or ibuprofen (IBF; 2-(4-isobutylphenyl)propanoic acid, CAS 15687-27-1; purity $\geq 98\%$, $M_r = 206.28$, $\lambda_{\max} = 265$ nm; Sigma-Aldrich, USA) of known concentrations (in $\text{mg} \cdot \text{dm}^{-3}$) were prepared by dissolving appropriate amounts of the pharmaceutical in deionised water (18.2 M Ω .cm at 25°C; prepared by PURELAB® 4 Flex system, Elga Veolia, Great Britain) contained 10% v/v of methanol (CAS 67-56-1; purity 99.9%; Sigma Aldrich, USA) or by appropriate dilution of prepared stock solutions, and used throughout this study. The initial pH of the solutions was adjusted by addition of 0.1 $\text{mol} \cdot \text{dm}^{-3}$ HCl or 0.1 $\text{mol} \cdot \text{dm}^{-3}$ NaOH solution.

Biomass of cyanobacteria, freshwater plant and moss

The strain of cyanobacteria *Synechococcus bigranulatus* (CCALA 187) obtained from the Culture Collection of Autotrophic Organisms (CCALA) (Institute of Botany, Czech Academy of Sciences, Czech Republic) was isolated by Kovrov in 1972 from a thermal spring, Kunashir, Russian Federation. Inoculum for the experiments was cultivated in BG11 medium (sterilized at 121°C during 20 min) according to Rippka *et al.* (1979) in 500 cm^3 Erlenmeyer flasks. Cultures were incubated inside the growth chamber under controlled temperature 27°C, continuous illumination provided by cool-white fluorescent light (4,400 lx, PAR 12 $\text{W} \cdot \text{m}^{-1}$, 48.6 $\mu\text{mol photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and under stationary conditions for 2 months. Before introducing the cyanobacterial culture into the experiments, the biomass was washed 3-times with deionized water to remove any remaining medium.

Commercially available freshwater plant *Egeria densa* and moss *Vesicularia dubyana* were purchased from Rataj aquatic plant farm (Šumperk, Czech Republic). After washing with tap water *E. densa* and *V. dubyana* were acclimatized in 10%

Hoagland medium (Hoagland 1920) for 1 week at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80% which was provided by the plant growth chamber (KBWF 720; Binder, Germany). After acclimation, uniform plants and mosses were selected for experimental purposes and washed 3-times with deionized water to remove residual impurities.

Bioaccumulation of drugs

Into sterilized Erlenmeyer flasks (100 cm³) filled with 20 cm³ of 10% Hoagland medium containing PRC or IBF of known concentration and pH, pre-cultured cyanobacteria *S. bigranulatus*, freshwater plant *E. densa* or freshwater moss *V. dubyana* were transferred. The exposure was carried out on a rotary shaker (150 rpm) at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Evaporation of water from the surface of the solution was prevented by partially sealing the Erlenmeyer flasks with a parafilm or a sterile cellulose plug. At a specified time interval, aliquots of the solution were taken from the flasks and centrifuged (4,500 rpm, 5 min). Then the supernatant was filtered through the syringe filters (13 mm diameter; 0.45 µm permeability) to remove cyanobacterial cells or impurities and the residual PRC or IBF concentration was determined using UV-Vis spectrophotometry. All experiments were carried out in three parallels. The removal efficiencies ($Q\%$) of PRC or IBF accumulation by the biomass of the cyanobacterium *S. bigranulatus*, freshwater plant *E. densa* or moss *V. dubyana* were quantified according to the following equation (Eq. 1):

$$Q\% = \frac{(C_0 - C_t)}{C_0} \times 100 \quad (\text{Eq. 1})$$

where C_0 and C_t are the initial concentration of PRC or IBF in the medium and the concentrations at the end of experiment (mg·dm⁻³), respectively. The specific bioaccumulation (Q ; mg·g⁻¹) of PRC or IBF was calculated using the following equation (Eq. 2):

$$Q = (C_0 - C_t) \times \frac{V}{m_B} \quad (\text{Eq. 2})$$

where C_0 and C_t are the initial concentration of PRC or IBF in the medium and the concentrations at the end of experiment (mg·dm⁻³); V is the medium volume (dm³) and m_B is the amount of the biomass (g).

The bioconcentration factor (BCF) of each drug accumulated in the studied biomasses was also determined by the equation (Eq. 3):

$$BCF = \frac{C_B}{C_M} \quad (\text{Eq. 3})$$

where C_B and C_M are the concentrations of drugs accumulated in the biomass (mg·g⁻¹) and residual concentrations of drugs in medium at the end of exposure (mg·dm⁻³), respectively.

Drug quantification

Spectrophotometric analyses were realized by UV-Vis spectrophotometer Cary 50 (Varian, Australia) with 1 cm quartz cells. The maximum absorption wavelength for PRC and IBF was determined as equal to 246 nm and 222 nm, respectively. At these wavelengths, the spectra of PRC and IBF did not vary in the pH range 1.0 – 10.0. The linear dependencies between absorbance of drugs and their concentration in the solution were confirmed for concentration ranges 0.5 – 20.0 mg·dm⁻³ for PRC and 1.0 – 12.5 mg·dm⁻³ for IBF, respectively. After appropriate dilution of samples of aqueous solutions originating from the experiments and containing PRC or IBF, their absorbance was measured at a given wavelength and the residual concentration of PRC or IBF was determined by obtained calibration curves.

To eliminate the effect of the release of biomolecules (of protein or genetic nature) from the biomass of the cyanobacteria *S. bigranulatus*, freshwater plant *E. densa* or moss *V. dubyana* during the experiments for the determination of the residual concentrations PRC or IBF in the UV wavelength region, parallel experiments were also carried out under identical conditions, but without the addition of the drugs.

Data analysis

For the graphical evaluation of the results and the statistical analysis of the obtained data, the program OriginPro ver. 8.5 (OriginLab Corporation, USA) was used.

Results and discussion

Bioaccumulation of PRC

In the first step of the work, we focused on the evaluation of the effect of contact time on the bioaccumulation of PRC by living biomass of cyanobacteria *S. bigranulatus*. This parameter is important not only from the point of view of the description of the rate of bioaccumulation processes, but also on the basis of the course of kinetics of the bioaccumulation of substances, it is possible to partially identify whether sorption (physico-chemical) processes occurring on the surface of the biomass are involved, or whether it is rather the transport of substances through the membranes into the cytosol (biochemical processes). It is well known that the sorption processes are time-dependent processes, describing the typical reaching or stabilisation the concentration equilibrium of $[\text{substance}]_{\text{biomass}} : [\text{substance}]_{\text{solution}}$ in a relatively short time. In the case of accumulation processes, this is also a time-dependent process, but without reaching the concentration equilibrium mentioned.

Fig. 1 shows the bioaccumulation of PRC by living biomass of cyanobacteria *S. bigranulatus* from a solution containing $50 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium during 14 d of exposure. As can be seen, in the first minutes of exposure of cyanobacterial biomass, there was a partial achievement of concentration equilibrium $[\text{PRC}]_{\text{biomass}} : [\text{PRC}]_{\text{solution}}$ and a relatively rapid but quantitatively low uptake of PRC (2 – 3% of the total amount of PRC in the medium). These rapid processes were subsequently replaced by a slower uptake of PRC, with the course of bioaccumulation processes showing a linear dependence ($R^2 = 0.945$) and reaching almost 32% ($3.12 \pm 0.03 \text{ mg} \cdot \text{g}^{-1}$) removal of PRC after 14 d of exposure (Fig. 1A). A similar PRC removal efficiency of 41% was also found by Escapa *et al.* (2017) when evaluating the

bioaccumulation of PRC by living algal biomass *Chlorella sorokiniana* from solutions containing $25 \text{ mg} \cdot \text{dm}^{-3}$ of PRC during 8 d of exposure. The bioaccumulation of PRC was also described in the form of bioconcentration factor *BCF* defined as the ratio of PRC concentration accumulated in cyanobacterial biomass ($\text{mg} \cdot \text{g}^{-1}$) and residual concentration of PRC in solution ($\text{mg} \cdot \text{dm}^{-3}$) (Fig. 1B).

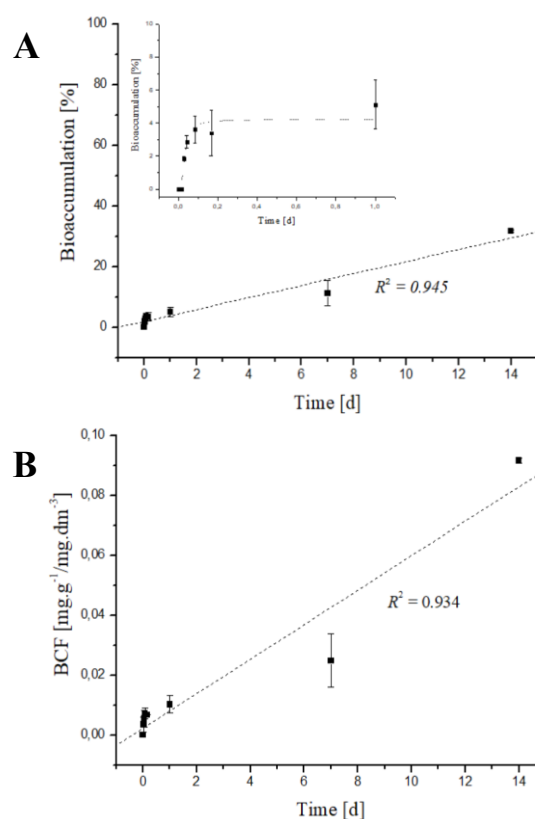


Figure 1: Kinetics of PRC bioaccumulation by living biomass of cyanobacteria *S. bigranulatus* (biomass concentration $C_B = 5 \text{ g} \cdot \text{dm}^{-3}$) from a solution containing $50 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Bioaccumulation of PRC expressed as % of the total amount of PRC in the medium (A) and *BCF* (B; $\text{mg} \cdot \text{g}^{-1} / \text{mg} \cdot \text{dm}^{-3}$). Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm \text{SD}$; $n = 3$).

The bioaccumulation efficiency of PRC uptake was also characterized in the case of the freshwater plant *E. densa*. As we assumed rather lower PRC removal efficiency for this object compared to the cyanobacteria *S. bigranulatus*, we reduced the initial concentration of PRC in solution to the

concentration $5 \text{ mg} \cdot \text{dm}^{-3}$. At the same time, with the uptake of PRC by this type of biological object and considering the data reported in scientific databases, we expected rather the existence of rapid, sorption processes. For this reason, we studied the bioaccumulation of PRC by living biomass of *E. densa* during 24 h of exposure.

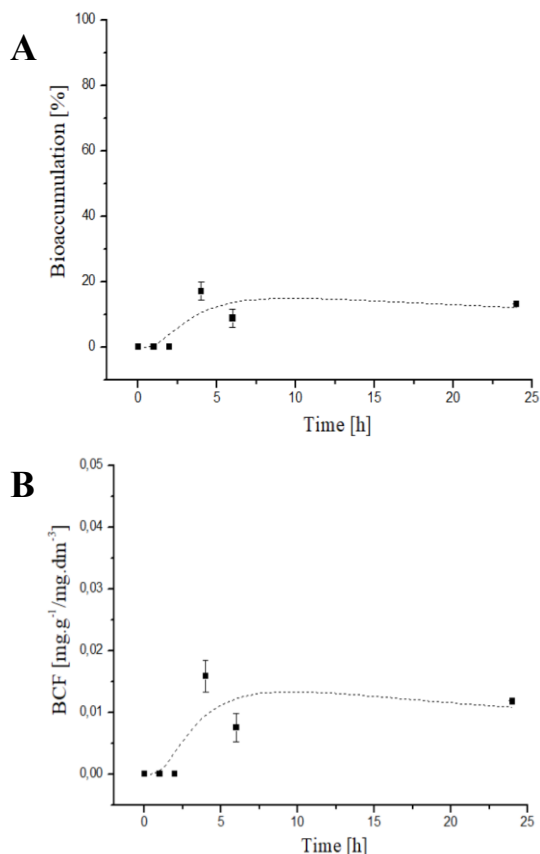


Figure 2: Kinetics of PRC bioaccumulation by living biomass of freshwater plant *E. densa* ($C_B = 5 \text{ g} \cdot \text{dm}^{-3}$) from a solution containing $5 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28 °C (light) / 18 °C (dark) and a relative humidity of 60 – 80 %. Bioaccumulation of PRC expressed as % of the total amount of PRC in the medium (A) and BCF (B; $\text{mg} \cdot \text{g}^{-1} / \text{mg} \cdot \text{dm}^{-3}$). Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm \text{SD}$; $n = 3$).

Our expectations were only partially fulfilled. Based on Fig. 2, it can be claimed that sorption processes played a minor role in the uptake of PRC by living biomass of *E. densa*. Also, we found that no uptake of PRC was observed in the early exposure times (up to 2 h of exposure). More significant bioaccumulation of PRC occurred later during the exposure at the level of 17% removal

of PRC from 10% Hoagland medium with an initial PRC concentration of $5 \text{ mg} \cdot \text{dm}^{-3}$, which represented a specific bioaccumulation of $0.066 \pm 0.010 \text{ mg} \cdot \text{g}^{-1}$.

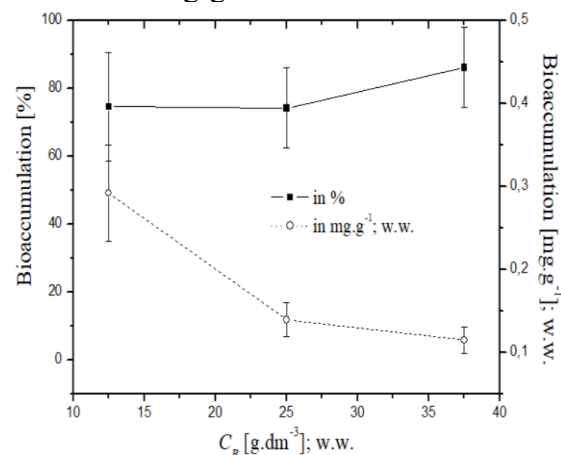


Figure 3: Effect of biomass concentration C_B on bioaccumulation of PRC by living biomass of freshwater plant *E. densa* from a solution containing $5 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) during 24 h and at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Bioaccumulation of PRC expressed as % of the total amount of PRC in the medium and $\text{mg} \cdot \text{g}^{-1}$. Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm \text{SD}$; $n = 3$).

Since in the previous experiment we confirmed only a minimal bioaccumulation of PRC by living biomass of *E. densa*, in the next experiment the effect of the biomass concentration (C_B) on the bioaccumulation of PRC was evaluated. It was found that with increasing concentration of living biomass of *E. densa* ranging from 12.5 to $37.5 \text{ g} \cdot \text{dm}^{-3}$, the percentage of PRC uptake increased from 74.5 to 86.0% (Fig. 3). On the other hand, in terms of specific bioaccumulation (in $\text{mg} \cdot \text{g}^{-1}$), an opposite course of the evaluated dependence was described, where the specific uptake of PRC decreased from 0.292 ± 0.058 to $0.114 \pm 0.016 \text{ mg} \cdot \text{g}^{-1}$ with increasing concentration of living biomass of *E. densa*. Practically the same result was obtained under identical conditions in the case of PRC bioaccumulation by living biomass of freshwater moss *V. dubyana* (Fig. 4). Also in this case, it was confirmed that the percentage of PRC bioaccumulation increased from 34.8 to 45.9% with increasing biomass concentration in the range of 12.5 to $37.5 \text{ g} \cdot \text{dm}^{-3}$, and conversely, the specific

uptake of PRC decreased from the value of 0.136 ± 0.034 to $0.061 \pm 0.003 \text{ mg} \cdot \text{g}^{-1}$.

Some authors explain this fact by biomass aggregation leading to the change of specific surface area of biomass for uptake of substances ($\text{m}^2 \cdot \text{g}^{-1}$) as well as the conditions for efficient mixing of the whole system (Roy and Mondal 2017; Bind *et al.* 2018).

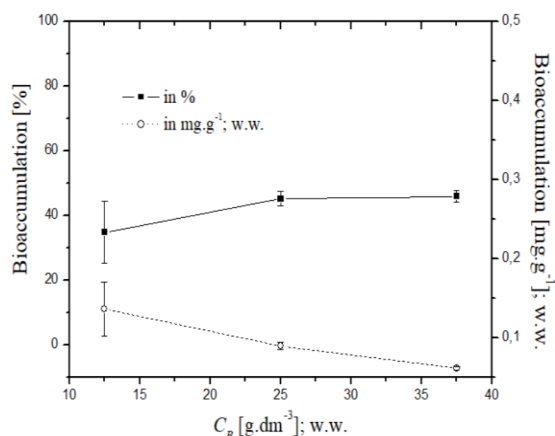


Figure 4: Effect of biomass concentration C_B on bioaccumulation of PRC by living biomass of freshwater moss *V. dubyana* from a solution containing $5 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) during 24 h and at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Bioaccumulation of PRC expressed as % of the total amount of PRC in the medium and $\text{mg} \cdot \text{g}^{-1}$. Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm \text{SD}$; $n = 3$).

An interesting finding is that under identical conditions, the living biomass of the moss *V. dubyana* showed approx. 50% lower bioaccumulation of PRC compared to the living biomass of the freshwater plant *E. densa*. In terms of morphological structure, moss *V. dubyana* exhibits a higher specific surface area compared to the biomass of *E. densa*. Therefore, it should have a certain morphological advantage in the uptake of substances from the aquatic environment. The observed lower bioaccumulation capacity of *V. dubyana* to uptake of PRC is likely to be due to differences at the biochemical and genetic level between these freshwater photosynthetic organisms. However, confirmation of this would require further detailed study.

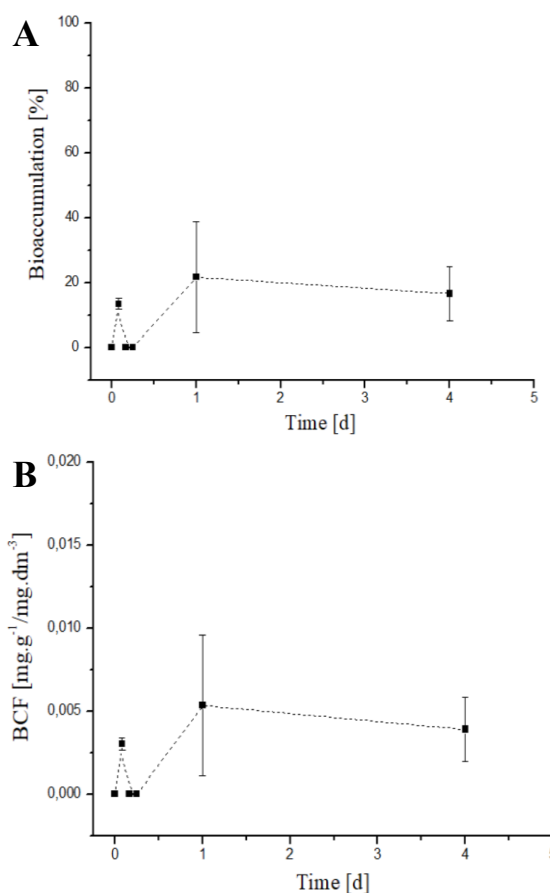


Figure 5: Kinetics of IBF bioaccumulation by living biomass of freshwater moss *V. dubyana* ($C_B = 50 \text{ g} \cdot \text{dm}^{-3}$) from a solution containing $12.5 \text{ mg} \cdot \text{dm}^{-3}$ of IBF in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) at a photoperiod of 16 h light / 8 h dark (maximum light intensity was 11,450 lx), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Bioaccumulation of IBF expressed as % of the total amount of IBF in the medium (A) and BCF (B; $\text{mg} \cdot \text{g}^{-1} / \text{mg} \cdot \text{dm}^{-3}$). Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm \text{SD}$; $n = 3$).

Bioaccumulation of IBF

As in the case of PRC, we started with the evaluation and description of the kinetics of IBF bioaccumulation by living biomass of freshwater moss *V. dubyana* ($C_B = 50 \text{ g} \cdot \text{dm}^{-3}$) from a solution containing $12.5 \text{ mg} \cdot \text{dm}^{-3}$ of IBF in 10% Hoagland medium during 7 d of exposure and at pH = 7.0 (Fig. 5).

Similarly to PRC and living biomass of *E. densa*, we found that the sorption processes had only a minor contribution to the uptake of IBF by living biomass of the moss *V. dubyana* (Fig. 5A). We did not confirm the uptake of IBF in the early exposure

times (up to 6 h of exposure), and in the later course there was a significant bioaccumulation at the level of 22% removal of IBF from the medium with an initial concentration of $12.5 \text{ mg} \cdot \text{dm}^{-3}$, which represented the specific uptake of $0.052 \pm 0.040 \text{ mg} \cdot \text{g}^{-1}$. The kinetics of PRC bioaccumulation is also described in the form of bioconcentration factor BCF (Fig. 5B).

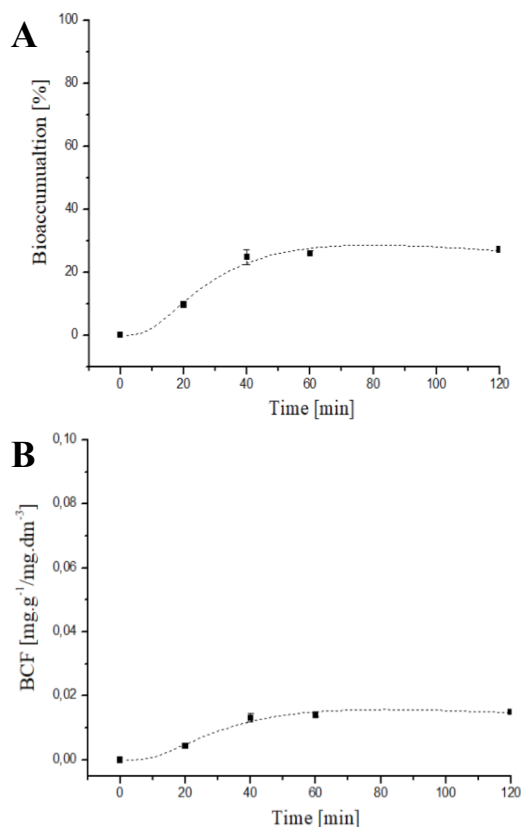


Figure 6: Kinetics of IBF bioaccumulation by living biomass of freshwater plant *E. densa* ($C_B = 25 \text{ g} \cdot \text{dm}^{-3}$) from a solution containing $80 \text{ mg} \cdot \text{dm}^{-3}$ of IBF in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) at a photoperiod of 16 h light / 8 h dark (maximum light intensity was $11,450 \text{ lx}$), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Bioaccumulation of IBF expressed as % of the total amount of IBF in the medium (A) and BCF (B; $\text{mg} \cdot \text{g}^{-1} / \text{mg} \cdot \text{dm}^{-3}$). Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

Since living biomass of *E. densa* showed a high efficiency of PRC bioaccumulation, for IBF bioaccumulation, we focused in detail on the characterization the kinetics of IBF uptake at short exposure times. The description of the kinetics of IBF bioaccumulation by living biomass of freshwater plant *E. densa* ($C_B = 25 \text{ g} \cdot \text{dm}^{-3}$) from a solution containing $80 \text{ mg} \cdot \text{dm}^{-3}$ of IBF in 10%

Hoagland medium (pH = 7.0) during 120 min of exposure is depicted on Fig. 6. We found that bioaccumulation reached the concentration equilibrium $[\text{IBF}]_{\text{biomass}} : [\text{IBF}]_{\text{solution}}$ from 40 min of exposure time, representing the involvement of mainly sorption processes in the removal of this drug. We confirmed a similar result for the bioaccumulation of PRC by cyanobacterial biomass of *S. bigranulatus* (Fig. 1).

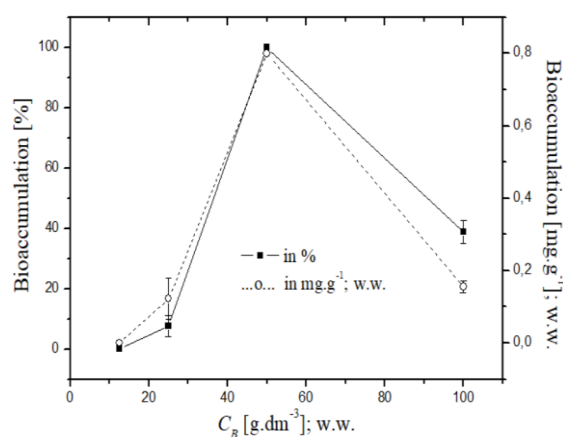


Figure 7: Effect of biomass concentration C_B on bioaccumulation of IBF by living biomass of freshwater plant *E. densa* from a solution containing $80 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium, pH = 7.0. Exposure was carried out on a rotary shaker (150 rpm) during 24 h and at a photoperiod of 16 h light / 8 h dark (maximum light intensity was $11,450 \text{ lx}$), temperature of 28°C (light) / 18°C (dark) and a relative humidity of 60 – 80%. Bioaccumulation of IBF expressed as % of the total amount of IBF in the medium and $\text{mg} \cdot \text{g}^{-1}$. Error bars represent the standard deviations of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

Fig. 7 describes the effect of biomass concentration C_B on bioaccumulation of IBF by living biomass of freshwater plant *E. densa* from a solution containing $80 \text{ mg} \cdot \text{dm}^{-3}$ of PRC in 10% Hoagland medium. In this case, a different dependence was observed than was the case for PRC bioaccumulation by living biomass of freshwater plant *E. densa* or moss *V. dubyana*, respectively. First, the effects of biomass concentration on the efficiency of IBF bioaccumulation (in %) and specific bioaccumulation of IBF (in $\text{mg} \cdot \text{g}^{-1}$) showed similar dependencies. Second, the bioaccumulation of IBF by living biomass of *E. densa* gradually increased with increasing biomass concentration from 12.5 to $50 \text{ g} \cdot \text{dm}^{-3}$, whereby the highest bioaccumulation of IBF was identified at a biomass concentration of $50 \text{ g} \cdot \text{dm}^{-3}$. On the other

hand, at the highest concentration of *E. densa* biomass $C_B = 100 \text{ g} \cdot \text{dm}^{-3}$, there was described a significant decrease in IBF uptake.

Conclusions

The number of pharmaceuticals released showing a steadily increasing trend, suggesting that their negative impacts on ecosystems will also become more pronounced. Since these substances enter the environment mainly in the form of municipal wastewater and thus threaten aquatic systems, it is important to carry out studies to assess the bioaccumulation of pharmaceuticals by the biocenosis of freshwater systems in terms of their toxicological effects, the risks associated with their further distribution through the food chain, and the possibility of application of freshwater organisms to remove them from aqueous solutions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Ahmed MJ (2017) Adsorption of non-steroidal anti-inflammatory drugs from aqueous solution using activated carbons: Review. *J. Environ. Manage.* 190: 274-282.
- Bedoya-Ríos DF, Lara-Borrero JA, Duque-Pardo V, Madera-Parra CA, Jimenez EM, Toro AF (2018) Study of the occurrence and ecosystem danger of selected endocrine disruptors in the urban water cycle of the city of Bogotá, Colombia. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 53(4): 317-325.
- Bello OS, Alao OC, Alagbada TC, Olatunde AM (2019) Biosorption of ibuprofen using functionalized bean husks. *Sustain. Chem. Pharm.* 13: 100151.
- Bind A, Goswami L, Prakash V (2018) Comparative analysis of floating and submerged macrophytes for heavy metal (copper, chromium, arsenic and lead) removal: sorbent preparation, characterization, regeneration and cost estimation. *Geol. Ecol. Landsc.* 2: 61-72.
- Bonnefille B, Gomez E, Courant F, Escande A, Fenet H (2018) Diclofenac in the marine environment: a review of its occurrence and effects. *Mar. Pollut. Bull.* 131: 496-506.
- Couto E, Peixoto Assemany P, Assis Carneiro GC, Ferreira Soares DC (2022) The potential of algae and aquatic macrophytes in the pharmaceutical and personal care products (PPCPs) environmental removal: a review. *Chemosphere* 302: 134808.
- de Jesus Menk J, Soares do Nascimento AI, Gomes Leite F, Angrizani de Oliveira R, Faustino Jozala A, de Oliveira Junior JM, Vinicius Chaud M, Grotto D (2019) Biosorption of pharmaceutical products by mushroom stem waste. *Chemosphere* 237: 124515.
- DG Env (2020) Priority Substances Review-Next steps. [https://circabc.europa.eu/sd/a/97910629-048c-4b2b-90a2-fe520aab2adc/WGChem2020Jan\(7\)PrioritySubstancesReview-nextsteps.pdf](https://circabc.europa.eu/sd/a/97910629-048c-4b2b-90a2-fe520aab2adc/WGChem2020Jan(7)PrioritySubstancesReview-nextsteps.pdf).
- Ding TD, Yang MT, Zhang JM, Yang B, Lin KD, Li JY, Gan J (2017) Toxicity, degradation and metabolic fate of ibuprofen on freshwater diatom *Navicula* sp. *J. Hazard. Mater.* 330: 127-134.
- Dvořáková Březinová T, Vymazal J, Koželuh M, Kule L (2018) Occurrence and removal of ibuprofen and its metabolites in full-scale constructed wetlands treating municipal wastewater. *Ecol. Eng.* 120: 1-5.
- EC (2013) European Commission, European Parliament Legislative Resolution of 2 July 2013 on the Proposal for a Directive of the European Parliament and of the Council Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy.
- Escapa C, Coimbra RN, Paniagua S, García AI, Otero M (2017) Paracetamol and salicylic acid removal from contaminated water by microalgae. *J. Environ. Manage.* 203: 799-806.
- Fang TH, Nan FH, Chin TS, Feng HM (2012) The occurrence and distribution of pharmaceutical compounds in the effluents of a major sewage treatment plant in Northern Taiwan and the receiving coastal waters. *Mar. Pollut. Bull.* 64(7): 1435-1444.
- Gómez-Regalado MC, Martín J, Santos JL, Aparicio I, Alonso E, Zafra-Gómez A (2023) Bioaccumulation/bioconcentration of pharmaceutical active compounds in aquatic organisms: Assessment and factors database. *Sci. Total Environ.* 861: 160638.
- Grung M, Kallqvist T, Sakshaug S, Skurtveit S, Thomas KV (2008) Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. *Ecotoxicol. Environ. Saf.* 71: 328-340.
- Hoagland DR (1920) Optimum nutrient solutions for plants. *Science* 52: 562-564.
- Horník M, Šušnovská A, Partelová D, Pipiška M, Augustín J (2013) Continuous sorption of synthetic dyes on dried biomass of microalga *Chlorella pyrenoidosa*. *Chem. Pap.* 67: 254-264.
- Hussner A, Lösch R (2005) Alien aquatic plants in a thermally abnormal river and their assembly to neophyte-dominated macrophyte stands (River Erft, Northrhine-Westphalia). *Limnologica* 35: 18-38.
- Islam MA, Nazal MK, Sajid M, Suliman MA (2024) Adsorptive removal of paracetamol from aqueous media: a review of adsorbent materials, adsorption mechanisms,

- advancements, and future perspectives. *J. Mol. Liq.* 396: 123976.
- Koltsakidou A, Terzopoulou Z, Liakos EV, Evgenidou E, Lambropoulou DA, Bikiaris DN, Kyzas GZ (2021) Acrylic acid copolymers as adsorbent materials for the removal of anti-inflammatory pharmaceuticals from synthetic biomedical wastewaters. *Coll. Surf. A: Physicochem. Eng. Asp.* 629: 127382.
- López-Miranda JL, Molina GA, Esparza R, González-Reyna MA, Silva R, Estévez M (2022) Ecofriendly and sustainable *Sargassum* spp.-based system for the removal of highly used drugs during the COVID-19 pandemic. *Arab. J. Chem.* 15: 104169.
- Machalová L, Lempochner T, Marková P, Valica M, Adamcová V, Hutárová L, Krajčovič J, Horník M (2023) Biosorption of Zn by dried biomass of *Euglena gracilis* from aqueous solutions. *Nova Biotechnol. Chim.* 22: e1568.
- Melillo M, Gun'ko VM, Tennison SR, Mikhalevska LI, Phillips GJ, Davies JG, Lloyd AW, Kozynchenko OP, Malik DJ, Streat M, Mikhalevsky SV (2004) Structural characteristics of activated carbons and ibuprofen adsorption affected by bovine serum albumin. *Langmuir* 20: 2837-2851.
- Menicagli V, Castiglione MR, Cioni E, Spanò C, Balestri E, De Leo M, Bottega S, Sorce C, Lardicci C (2024) Stress responses of the seagrass *Cymodocea nodosa* to environmentally relevant concentrations of pharmaceutical ibuprofen: Ecological implications. *J. Hazard. Mater.* 476: 135188.
- Mestre AS, Pires J, Nogueira JMF, Carvalho AP (2007) Activated carbons for the adsorption of ibuprofen. *Carbon* 45: 1979-1988.
- Mlunguza NY, Ncube S, Mahlambi NP, Chimuka L, Madikizela LMJ (2019) Adsorbents and removal strategies of non-steroidal anti-inflammatory drugs from contaminated water bodies. *J. Environ. Chem. Eng.* 7: 103142.
- Mojiri A, Zhou JL, Ratnaweera H, Rezaei S, Nazari VM (2022) Pharmaceuticals and personal care products in aquatic environments and their removal by algae-based systems. *Chemosphere* 288: 132580.
- Mondal S, Aikat K, Halder G (2016) Biosorptive uptake of ibuprofen by chemically modified *Parthenium hysterophorus* derived biochar: Equilibrium, kinetics, thermodynamics and modeling. *Ecol. Eng.* 92: 158-172.
- Mustafa S, Nawaz Bhatti H, Maqbool M, Iqbal M (2021) Microalgae biosorption, bioaccumulation and biodegradation efficiency for the remediation of wastewater and carbon dioxide mitigation: Prospects, challenges and opportunities. *J. Water Process Eng.* 41: 102009.
- Nasir A, Saleh M, Tahir Aminzai M, Alary R, Dizge N, Yabalak E (2024) Adverse effects of veterinary drugs, removal processes and mechanisms: A review. *J. Environ. Chem. Eng.* 12: 111880.
- Nunes B, Antunes SC, Santos J, Martins L, Castro BB (2014) Toxic potential of paracetamol to freshwater organisms: A headache to environmental regulators? *Ecotoxicol. Environ. Saf.* 107: 178-185.
- Oggier DM, Weisbrod CJ, Stoller AM, Zenker AK, Fent K (2010) Effects of diazepam on gene expression and link to physiological effects in different life stages in zebrafish *Danio rerio*. *Environ. Sci. Technol.* 44: 7685-7691.
- Ortúzar M, Esterhuizen M, Olicón-Hernández DR, González-López J, Aranda E (2022) Pharmaceutical pollution in aquatic environments: a concise review of environmental impacts and bioremediation systems. *Front. Microbiol.* 13: 869332.
- Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU, Mohan D (2019) Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. *Chem. Rev.* 119(6): 3510-3673.
- Petrie B, Barden R, Kasprzyk-Hordern B (2015) A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* 72: 3-27.
- Pipiška M, Valica M, Partelová D, Horník M, Lesný J, Hostin S (2018) Removal of synthetic dyes by dried biomass of freshwater moss *Vesicularia dubyana*: A batch biosorption study. *Environments* 5: 10.
- Pipiška M, Zarožňanská S, Horník M, Ďuriška L, Holub M, Šafařík I (2020) Magnetically functionalized moss biomass as biosorbent for efficient Co²⁺ ions and thioflavin T removal. *Materials* 13: 3619.
- Ramesh B, Saravanan A, Kumar PS, Yaashikaa PR, Thamarai P, Shaji A, Rangasamy G. (2023) A review on algae biosorption for the removal of hazardous pollutants from wastewater: Limiting factors, prospects and recommendations. *Environ. Pollut.* 327: 121572.
- Ranieri E, Verlicchi P, Young TM (2011) Paracetamol removal in subsurface flow constructed wetlands. *J. Hydrol.* 404: 130-135.
- Rantanen J, Khinast J (2015) The future of pharmaceutical manufacturing sciences. *J. Pharm. Sci.* 104(11): 3612-3638.
- Rippka R, Deruelles J, Waterbury JB, Herdman M, Stainer RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111: 1-61.
- Roy TK, Mondal NK (2017) Biosorption of Congo Red from aqueous solution onto burned root of *Eichhornia crassipes* biomass. *Appl. Water Sci.* 7: 1841-1854.
- Samal K, Mahapatra S, Ali H (2022) Pharmaceutical wastewater as Emerging Contaminants (EC): treatment technologies, impact on environment and human health. *Energy Nexus* 6: 100076.
- Sellaoui L, Lima EC, Dotto GL, Lamine AB (2017) Adsorption of amoxicillin and paracetamol on modified activated carbons: equilibrium and positional entropy studies. *J. Mol. Liq.* 234: 375-381.
- Sharma S, Bhattacharya A (2017) Drinking water contamination and treatment techniques. *Appl. Water Sci.* 7: 1043-1067.
- Show S, Chakraborty P, Karmakar B, Halder G (2021) Sorptive and microbial riddance of micro-pollutant

- ibuprofen from contaminated water: A state of the art review. *Sci. Total Environ.* 786: 147327.
- Singh S, Kumar V, Anil AG, Kapoor D, Khasnabis S, Pavithra SN, Samuel J, Subramanian S, Singh J, Ramamurthy PC (2021) Adsorption and detoxification of pharmaceutical compounds from wastewater using nanomaterials: A review on mechanism, kinetics, valorization and circular economy. *J. Environ. Manage.* 300: 113569.
- Sivarajasekar N, Mohanraj N, Balasubramani K, Prakash Maran J, Ganesh Moorthy I, Karthik V, Karthikeyan K (2017) Optimization, equilibrium and kinetic studies on ibuprofen removal onto microwave assisted – activated *Aegle marmelos* correa fruit shell. *Desalin. Water Treat.* 84: 48-58.
- Šušnovská A, Hasíková V, Horník M, Pipiška M, Hostin S, Lesný J (2016) Removal of Cd by dried biomass of freshwater moss *Vesicularia dubyana*: Batch and column studies. *Desalin. Water Treat.* 57: 2657-2668.
- Thabede PM, Hapiness Khumalo NA, Mahlambi PN, Nyamukamba P, Modise SJ (2023) Sorption of Ibuprofen by chemically treated maize cob. *S. Afr. J. Chem. Eng.* 46: 376-385.
- Valica M, Lempochner T, Machalová L, Adamcová V, Marková P, Hutárová L, Pipiška M, Krajčovič J, Horník M (2023) Removal of cadmium from aqueous solution using dried biomass of *Euglena gracilis* var. *bacillaris*. *Eng. Proc.* 57: 41.
- Villaescusa I, Fiol N, Poch J, Bianchi A, Bazzicalupi C (2011) Mechanism of paracetamol removal by vegetable wastes: The contribution of π - π interactions, hydrogen bonding and hydrophobic effect. *Desalination* 270: 135-142.
- WFD, EC (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *J* 327 (L).
- Zhang X, Yan S, Chen J, Tyagi RD, Li J (2020) Physical, chemical, and biological impact (hazard) of hospital wastewater on environment: presence of pharmaceuticals, pathogens, and antibiotic-resistance genes. In: Tyagi RD, Sellamuthu B, Tiwari B, Yan S, Drogui P, Zhang X, Pandey A (eds) *Current Developments in Biotechnology and Bioengineering*. Elsevier, Cambridge, United States, pp 79-102.
- Žur J, Wojcieszynska D, Hupert-Kocurek K, Marchlewicz A, Guzik U (2018) Paracetamol – toxicity and microbial utilization. *Pseudomonas moorei* KB4 as a case study for exploring degradation pathway. *Chemosphere* 206: 192-202.