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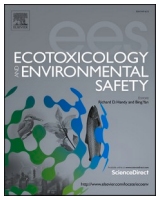

Toxic effects of a mixture of five pharmaceutical drugs assessed using *Fontinalis antipyretica* Hedw.

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ABSTRACT

The potential health risks associated with the pharmaceuticals released into the environment through effluents from sewage treatment plants have become a major cause for concern. Owing to the lack of effective indicators, monitoring the concentration of these pollutants in the aquatic environment is challenging. The aim of this study was to assess the toxicity of a mixture of five pharmaceutical drugs (paracetamol, carbamazepine, diclofenac, irbesartan, and naproxen) using the aquatic moss *Fontinalis antipyretica* as a bioindicator and bioaccumulator. We examined the effects of the drug mixture on the cellular antioxidant system, chlorophyll content, and morphological traits of *F. antipyretica*. The plant was exposed for 5 months to three concentrations of the mixture, including the environmental concentration (MX1), and 10- (MX10) and 100-times (MX100) the environmental concentration. The results showed that only carbamazepine and irbesartan were accumulated by the species. The bioconcentration level increased with exposure time, with the maximum uptake at the 4th month of exposure. The increase in bioaccumulation with exposure time was more evident in plants exposed to MX100. Analysis of the activity of antioxidant enzymes showed that superoxide dismutase (SOD, EC 1.15.1.1.) and catalase (EC 1.11.1.6.) were highly sensitive to the drug mixture. The activity of the enzymes was significantly higher in plants exposed to MX100; however, the activity of guaiacol peroxidase (GPX, EC 1.11.1.7.) was not significantly affected. Plants exposed to MX10 and MX100 had significantly lower total chlorophyll content and chlorophyll a/b ratio compared with those of plants in the control group; however, photosynthetic activity was restored after 5 months of exposure. The morphological characteristics of *F. antipyretica* were less sensitive to the treatment conditions.

1. Introduction

Owing to the increase in the quantity of drugs used in modern societies, several studies have been conducted to determine the presence of pharmacological substances in aquatic environments (Delépée et al., 2004). Studies have shown that substances of pharmaceutical origin are often not removed during waste water treatment and are also not biodegraded in aquatic environments (Daughton and Ternes, 1999; Ternes, 1998; Zwiener and Frimmel, 2000). These substances are present in surface, ground, and drinking water (Heberer, 2002; Jørgensen et al., 2000) at concentrations capable of causing detrimental effects to aquatic organisms (Ebele et al., 2017). Among pharmaceuticals, carbamazepine and diclofenac were detected at the highest frequency in aquatic environments (Zhang et al., 2008).

Wastewater treatment plants (WWTPs) are a gateway through which human pharmaceuticals enter water bodies, while most veterinary pharmaceutical residues are discharged directly into the ecosystem (Zhang et al., 2008). There are limited studies on the effects of the

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potential impacts of these compounds on non-target aquatic organisms (Pisa et al., 2015). Recently, the European Commission established regulatory guidelines to assess the presence of pharmaceuticals in aquatic environments (Directive 2013/39/EU). In 2007, PPCPs such as diclofenac, iopamidol, musks, and carbamazepine were identified as emerging pollutants. In 2018, ibuprofen, clofibric acid, triclosan, phthalates, and bisphenol A were added to this list (EU Decision 2018/840). Over the years, scientist have relied heavily on spot sampling followed by instrumental analytical measurements to determine water quality and the type and concentration of pollutants (Allan et al., 2006). These methods provide information about the concentrations of different pollutants, such as heavy metals and organic compounds, in water samples. However, data generated using this method reflect the concentration of pollutants at the sampling time, but cannot be used to determine the concentration of pollutants resulting from occasional or intermittent pollution events (Greenwood and Roig, 2006). Moreover, the detection of micropollutants in freshwater environment is difficult because of the large number of contaminants and their rapid dilution (Luo et al., 2014). However, occurrence and toxicity analyses of contaminants are difficult to perform (Soares et al., 2008). Occurrence studies are limited by the large numbers of pollutants, most of which are biologically degraded into active metabolites (Scheurer et al., 2012). Sediments are also largely used to assess pollutants in aquatic ecosystems, and it has been reported the adsorption and accumulation of some contaminants in sediments (Ingerslev et al., 2001; Orliński, 2002). The major challenge in sediment monitoring is that adsorption and degradation of pollutants differ according on the structure of deposits (Pouliquen et al., 1996). To overcome this challenge, several biological models, including. algae, bryophytes, fish, mollusks, and macro-invertebrates have been used to assess water quality. Autotrophs, including phytoplankton and macrophytes, account for a large proportion of the total biomass in freshwater environments (Greenberg et al., 1992). Autotrophs are considered effective bioindicators of organic and inorganic pollutants in aquatic environments (Brain et al., 2004; Ravera and Riccardi, 1997; Sossey Alaoui and Rosillon, 2013). Among aquatic plants, bryophytes, especially moss, are frequently used for freshwater biomonitoring (Dazy et al., 2008; Figueira and Ribeiro, 2005). Compared to vascular plants, bryophytes lack water-impermeable cuticles and absorb nutriments through the leaf surface and not through the roots (CENCI, 2001). Owing to the absence of stomata and tracheids, and the presence of stratified leaf lamina, bryophytes are excellent bioaccumulators of pollutants. Bryophytes can be used for pollution biomonitoring by determining the concentrations of pollutants in the tissues (Markert et al., 1999; Samecka-Cymerman and Kempers, 2000), and by examining the activities and concentrations of certain enzymes in the bryophytes (Christmas and Whitton, 1998; Roy et al., 1992). Additionally, the effects of pollutants on the photosynthetic activities of bryophytes, including chlorophyll content, could be used in pollution biomonitoring (Dazy et al., 2008). The bryophyte *Fontinalis. antipyretica* Hedw is widely distributed in Europe (Martins et al., 2004), non-invasive, and has a long life-cycle (Debén et al., 2017), making it suitable for biomonitoring over a long duration of time. Additionally, *F. antipyretica* can easily be identified, sampled, and transplanted, and is resistant to adverse environmental conditions (Siebert et al., 1996). Several researches have demonstrated the suitability of *F. antipyretica* for bioaccumulation of pharmaceuticals, including oxytetracycline, flumequine, and oxolinic acid (Delépée et al., 2004; Le Bris and Pouliquen, 2004), and bioaccumulation of metals in freshwater (Martínez-Carballo et al., 2007; Roy et al., 1992, 1996).

This study aimed to examine the toxicity of five pharmaceutical drug mixtures using *F. antipyretica* as a bioindicator and bioaccumulator under experimental river (mesocosm) conditions.

In order to calibrate and validate predictive population level models by combined chemical and biomarker analyses of some caged organisms (a crustacean Gammarus fossarum a mollusk Dreissena polymorpha a moss Fontinalis antipyretica and a fish species Gasterosteus aculeatus), a lotic mesocosm experiment was set up by French National Institute for Industrial Environment and Risks (INERIS) (David et al., 2020). The study was carried out in twelve 20 m long lotic channels.

Ecotoxicity studies under ecosystem conditions, such as those that can be performed in mesocosms, are of considerable ecological relevance, as complex inter-species interactions, interactions between organisms and environmental conditions can be examined. Thus, the present study was developed to investigate the effect of the pharmaceuticals on the oxidative status, chlorophyll content and morphological traits of F. antipyretica and to assess the possible use of these features as biomarkers for freshwater quality assessment.

2. Materials and methods

2.1. Pharmaceutical residues

Five emerging pharmaceutical substances were selected according to their high concentrations in the Meuse river (Wallonia) (Nott and Ronkart, 2018), persistence, diversity of action, and toxicity for this study. These pharmaceutical substances were carbamazepine 98% (CAS 298464, alpha-aesar) (neuroleptic), acetaminophen (4-acetamidophenol) 98% (CAS 103902, VWR), diclofenac sodium salt (CAS 15307796, Sigma), naproxen sodium (CAS 26159342, VWR) (anti-inflammatory), and irbesartan (CAS 138402116, Sigma) (anti-hypertensive). *Fontinalis antipyretica* was exposed to three concentrations of the mixture of five pharmaceutical drugs, including the normal environmental concentration (MX1), and 10- (MX10) and 100-times (MX100) the environmental concentration. The normal environment concentration (Mx1) of the pharmaceuticals were based on the median concentrations of the test drugs in the Meuse River (Wallonia) (Nott and Ronkart, 2018). This mixture contained 25 ng/L of carbamazepine, 25 ng/L of diclofenac, 25 ng/L of naproxen, 50 ng/L of irbesartan, and 100 ng/L of acetaminophen. The MX10 and MX100 mixtures contained 10 and 100 times the concentrations of MX1. The inlet of the mesocosm was contaminated continuously throughout the study period. We monitored the concentration of the mixture in each channel monthly at several points (0, 5, and 19 m from the inlet of the water). The dosing system and the analytical methods used in this study were according to the procedures described by David et al. (2020).

2.2. Mesocosm experiment

The twelve channels (Fig. 1) consist of three replicates per treatment (MX1, MX10, MX100) and control (Table 1). The mesocosms were set up with artificial sediments, macrophytes, periphyton, and benthic and pelagic invertebrates. More information on the mesocosm experiment can be found in (David et al., 2020). We monitored the water temperature every 10 min using two temperature sensors in each channel at 5 and 15 m from the inlet water at the surface and at 60 cm depth. The study period was between March to October 2017. Physicochemical parameters, such as pH, conductivity, and dissolved oxygen, were measured weekly at 10 m from the water inlet and 35 cm depth in each channel.

2.3. Bryophyte species

2.3.1. Collection and preparation

Fontinalis antipyretica is one of the most recognizable genera of aquatic mosses, with large leaves. *Fontinalis antipyretica* occurs in both still and flowing waters. The *F. antipyretica* plants used in this study were collected from the Meuse River catchment basin in Wallonia (Galoux et al., 2015; Sossey Alaoui and Rosillon, 2013). The sampling site is located in environments with low anthropic pressure, without the effects of major industrialization, urbanization, or intensive agriculture (Sossey Alaoui and Rosillon, 2013). The physicochemical, hydromorphological, and biological characteristics of the sampling site is stable, with limited

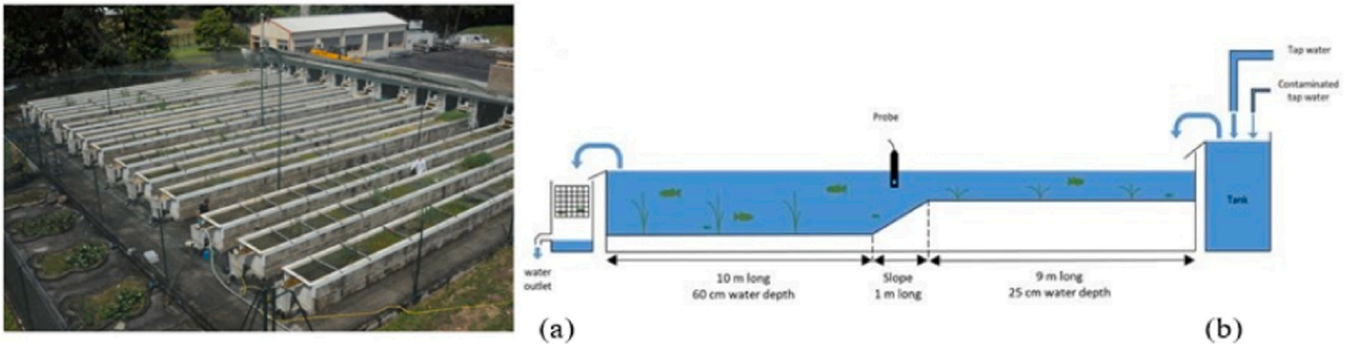

Fig. 1. Overview of the lotic mesocosms (INERIS in France) (a) and lateral view of each channel(b) (INERIS, 2020).

Table 1
(a) Pharmaceutical parameters**:** Concentrations and average measured in situ for each pharmaceutical and mixture treatment averaged over exposure period. And **b)** physicochemical parameters: Temperature °C (T), pH (PH), Dissolved oxygen (mg/L) (DO), Conductivity (**µS/cm**) (Con). Results are presented as mean ± standard deviation for three replicates taken from samples collected each month from May to September.

Mixture	Mx1			Mx10			Mx100		
Substances	[Nominal] (ng L^{-1})	[Measured] (ng L^{-1})	%	[Nominal] (ng L^{-1})	[Measured] (ng L^{-1})	%	[Nominal] (µg L^{-1})	[Measured] (µg L^{-1})	%
a) Pharmaceutical parameters									
Carbamazepine	25	26.5	105.9	250	229.5	91.8	2.5	2.2	88.1
Irbesartan	50	50.4	100.7	500	470.5	94.1	5	4.3	85.1
Diclofenac	25	20.6	82.3	250	179.1	71.6	2.5	1.7	69.2
Naproxen	25	21.1	84.4	250	191.9	76.8	2.5	1.9	78.0
Acetaminophen	100	51.6	51.6	1000	497.5	49.7	10	4.9	49.2
b) Physicochemical parameters measured									
	Control		MX1		MX10			MX100	
	Mean	SD	Mean	SD	Mean	SD		Mean	SD
T	19.03	1.68	18.52	1.83	18.718.74	1.88		18.37	3.77
PH	6.99	0.52	7.02	0.25	7.31	0.13		7.44	0.21
DO	9.06	1.75	8.76	1.93	9.49	1.72		9.2	1.47
Cond	521	23	555	66	557	71		602	102

human disturbance (Galoux et al., 2015). The bryophytes were cleaned in tap water filtered through 5 mm filters and activated charcoal cartridges and then exposed to the pharmaceutical mixtures for 5 months in the mesocosms.

2.4. Oxidative stress analysis

To determine the stress response of *F. antipyretica* to the three mixtures, we examined the superoxidase dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC1.11.1.6), and guaiacol peroxidase (GPX, EC1.11.1.7) activity of the plants. These enzymes are usually the first line of defense against oxidative stress (Bleuel et al., 2005). We weighted 2 g of *F. antipyretica* (green parts) samples and crushed in a mortar placed on ice. Thereafter, the samples were suspended in 10 mL 80% (v/v) phosphate buffer, at solid to solvent ratio of 1:10 (w/v). After 24 h of maceration the supernatants were filtered through a Whatman GF/F glass microfiber filter (0.7 µm). The resulting suspension was centrifuged at 15,000 × *g* for 10 min at 4 °C and supernatant stored at − 80 °C until enzymes analyses.

All methods presented in this section were optimized (with regard to experimental conditions) before being assigned to our samples. Hence, amended protocols are presented below with references to the original papers.

SOD (EC 1.15.1.1) activity was measured based on the inhibition of nitro blue tetrazolium photoreduction in the presence of riboflavin (Dhindsa et al., 1981). Determination of the activity and purification of the enzyme were according to the methods of (Dazy et al., 2008; Qiu-Fang et al., 2005). The reaction medium (final volume: 1 mL) contained 100 µL of methionine (130 mM), 100 µL of riboflavin (600 lM), 100 µL of NBT (22.5 mM), 100 µL of plant extract and 600 µL containing 125 mM potassium phosphate buffer (pH 7.8; containing 3 mM $MgSO_4$, 3.1 mM EDTA and 2% polyvinylpolypyrrolidone). The reaction was initiated by placing the tubes below two 15 W fluorescent lamps for 15 min. The reaction was terminated by keeping the tubes in dark for 10 min. A control reaction was performed without plant extract and replaced with an equal volume of buffer and the reaction was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as the quantity of enzyme that reduced the A560 of control by 50%.

Catalase (CAT, EC1.11.1.6) was determined according to the methods of (Claiborne, 1985), using H_2O_2 as substrate. The reaction mixture (final volume: 1 mL) contained 100 µL of H_2O_2 (100 mM), 100 µL of plant extract, and 125 mM potassium phosphate buffer (pH 7.0). The disappearance of H_2O_2 was evaluated by measuring the decrease in absorbance at 240 nm (molar extinction coefficient: $s = 36$/mM per cm). CAT activity was determined by calculating the quantity of H_2O_2 dissociated and reported in enzyme units per gram of fresh weight of the moss species.

To determine the GPX (Guaiacol: H_2O_2 oxidoreductase, EC 1.11.1.7) activity of the plants, we used 1 mL of final mixture containing 100 µL plant extract, 100 µL guaiacol (22 mM), 100 µL H_2O_2 (100 mM) and 125 mM potassium phosphate buffer (pH 7.0). The enzyme activity was measured by monitoring the increase in absorbance at 470 nm (extinction coefficient of 26.6/mM per cm) during polymerization of guaiacol (Fielding and Hall, 1978). Three replicates per treatment were used for the oxidative assay.

Regarding ascorbate peroxidase (APX) and (E) glutathione reductase (GR) and polyphenol oxidase (PPO) enzymes, some samples related to 100X concentration were tested and no effect was found.

2.5. Photosynthetic pigments

To assess the impact of the three mixtures on the vitality and physiology of the species, we measured the chlorophyll content of the moss samples. Chlorophyll was extracted according to method of Holden (1975), which involved maceration of the plant in acetone. The processing of the samples was done as follows: 1 g of moss samples were cold ground in a mortar with 20 mL of 80% acetone and approximately 100 mg of calcium bicarbonate ($CaCO_3$). Thereafter, the resulting solution was filtered and placed in black boxes to avoid oxidation of the chlorophylls by light. The absorbance was then measured spectrophotometrically using an Ultrospec 7000 UV–visible dual beam spectrophotometer (GEHealthcare, Chicago, IL, USA) at 663 and 645 nm after calibration of the machine with the 80% acetone control solution. The chlorophyll content of plants exposed to the water channels was calculated using the equation (Arnon, 1949).

2.6. Bioaccumulation

2.6.1. Sample preparation

Homogenized moss samples (2 g) were transferred to 15 mL polypropylene centrifuge tubes containing 50 μL of Irbesartan-d4 (internal standard) (IS) solution, and allowed to stand for 15 min. Thereafter, 5 mL of acetonitrile was used to extract the pharmaceuticals and precipitate proteins. The sample was then shaken at room temperature for 15 min and centrifuged at 4650 × *g* for 5 min

The extract was loaded into an Oasis HLB cartridge (preconditioned with 3 mL of methanol and 3 mL of water) under vacuum to obtain a flow rate of about 1 mL/min.

The cartridge was then rinsed with 3 mL ultra-pure water and vacuum-dried to remove excess water. Finally, the retained components were eluted with 3 mL of methanol. The eluate was evaporated to dryness under a gentle stream of nitrogen in a water bath set at 40 °C. The pellet was then resuspended in 1 mL ACN/water (10:90, v/v). The extract was centrifuged at 11,500 × *g* for 5 min at 20 °C. The clear supernatant was stored in a vial prior to UHPLC-MS/MS analysis.

2.6.2. Liquid chromatography–mass spectrometry analysis

Liquid chromatography and mass spectrometry analysis were performed according the procedures described by Schmitz et al. (2018) using Acquity UHPLC system (Waters, Milford, MA, USA). Chromatographic separation was done by injecting 20 μL of reconstituted extract on an Acquity UPLC HSS T3 column (150 × 2.1 mm, 1.7 μm particle size, Waters). Detection was carried out with a Waters Acquity TQ mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization source operating in the positive (ESI+) ionization mode. We used MassLynx 4.1 and TargetLynx 4.1 (Waters) for data acquisition. Matrix matched calibration curves ranging from 1 μg/kg to 100 μg/kg were constructed using fresh moss. For residue determination, their corresponding deduced similar were used as internal standards.

2.6.3. Bioaccumulation factor

We calculated the bioaccumulation factor (BAF) (L/kg) of the mixtures for chemical risk assessment. This is the concentration of pollutants in the tissues of an organism (mg/kg) divided by the concentration of pollutants in the surrounding medium (mg/l) (Fu et al., 2009). The dosing system and the analytical methods used in this study were according to the procedures described by David et al. (2020).

Based on the European risk assessment framework, compounds with BAF > 2000 are considered bioaccumulative, while compounds with BAF > 5000 are considered highly bioaccumulative (EC TGD, 2004). The same criterion (BAF > 5000) is also used by other governments (USEPA, 1976).

2.7. Measurement of morphological traits

During the 5-month exposure period (May to September), ten stems were randomly sampled from each channel monthly for morphological assay. We measured the following morphological traits for each sample: stem length (SL), leaf length (LL), leaf width (LW), and number of branches (NB).

2.8. Statistical analysis

Data obtained from the study were analyzed using SAS v8.2 software. Three replicates of each sample were performed with the exception of the morphological features where the measurements were carried out on ten plants for each sample. The data were subjected to two-way analysis of variance (ANOVA), and means were compared using Tukey's HSD test ($p < 0.05$). Residual normality and homogeneous variance were assessed and confirmed using Levene's test and by residual plots (residual normal quantile plot, residual by row plot, residual by predicted plot and studentized residuals plot). Nonparametric analysis was performed if the results of Levene's test showed that the variance was not equal ($p > 0.05$). The means of each tested concentration was also compared to the control using Dunnett's test (alpha = 0.05). The effect of exposure duration (5 months) of the mixtures on the morphology and oxidative stress status of the plants was examined using generalized linear model (GLM) (McCullagh, 2018). The response variable modeled using GLM followed a normal distribution pattern.

In some instances, a large variability was observed among channels of the same test condition. A linear mixed model (LMM) was then used to analyze the effect of the channel and study duration on the response variables. Compared with the fixed-effects models, LMM takes into account that there could more than one source of random variability in data (Bentsen and Klemetsdal, 1991). In addition to test conditions, random variability may exist across the channel for each tested condition. The proliferation of plants may vary between channels, which could affect the physicochemical conditions. Therefore, we considered variation between channels for more effective analysis. Owing to a high variability of chlorophyll content during the study period, we used the random coefficients model (RC) (Muthén and Curran, 1997) to examine the effect of the mixtures and study duration on the chlorophyll content of the plants. This was done by including the measurement time as a covariate in the model, with a corresponding slope (De Leeuw and Kreft, 1986). It is plausible that the slope may vary with the channel; therefore, it might be useful to model a separate intercept and slope for each channel in the study. Akaike's information criterion AIC (Akaike, 1973) and the Bayesian information criterion BIC (Schwarz et al., 1978) were used to select the best model.

3. Results

3.1. Pharmaceutical and physicochemical concentrations in water

The concentrations of carbamazepine, naproxen, diclofenac, and irbesartan were within the normal levels in freshwater habitats; however, the concentration of acetaminophen was not within the normal level. The concentrations of the five pharmaceuticals at 5 m and 19 m depths along each channel and for each condition ranged between 60% and 100% of the target concentrations throughout the study period. However, the concentration of acetaminophen ranged between 0% and 50% (Table 1a). Time and water inlet distance significantly affected the concentrations of carbamazepine, acetaminophen, and naproxen. There was a significant decrease in the concentrations of carbamazepine, acetaminophen, and naproxen with an increase in water inlet distance. The concentration of carbamazepine in water was mostly below the detection level or at 5% of the target level.

There were no significant differences ($p > 0.05$) in the physicochemical characteristics of the channels (Table 1b). The mean

temperature was approximately 18.4 ℃, with a standard deviation of 0.64 ℃, while the mean conductivity was 552.5 μS/cm, with a standard deviation of 31 μS/cm. The mean concentration of dissolved oxygen (DO) 9.2 mg/l, with a standard deviation of 0.33 mg/l.

3.2. Effects of the pharmaceutical mixtures on oxidative stress indices of *F. antipyretica*

The oxidative stress response of *F. antipyretica* to the drug mixtures are shown in Fig. 2. There was a significant increase in the activities of CAT, SOD and GPX, with an increase in the concentration of the mixture compared with that of the control (Fig. 2a–c). However, CAT and SOD were more sensitive to the mixtures than did GPX, exhibiting a dose dependent response.

The maximum concentration was recorded in the third month and decreased thereafter. The responses of CAT and SOD were strongly correlated ($r > 0.8$; $p < 0.05$). The R^2 values for catalase and SOD were 71% and 92%, respectively. The preliminary ANOVA showed that the effects of both treatment and study duration were significant ($p < 0.05$).

3.3. Effects of the pharmaceutical mixtures on chlorophyll content

There was a significant decrease ($p < 0.05$) in the chlorophyll content and chlorophyll a/b ratio of plants exposed to the three treatments compared with that of plants in the control group (Fig. 3 and Table 2). However, there was an increase in photosynthetic activity of the mosses after the first 3 months of exposure (Fig. 3a-b, and Table 2). The result of the Levene's test showed that the variances of total chlorophyll and chlorophyll a/b ratio were not significantly different ($p > 0.05$).

The RC model was most suitable for our data. The effects of the treatments and study duration were significant (Table 2), and the residuals of the models follow a normal distribution.

However, the interaction (concentration*time) was not significant ($p > 0.05$), hence it was removed from the analysis. The three treatments had a negative effect on the chlorophyll content of the plants (Table 2). These findings were confirmed by Dunnett's test and Tukey's

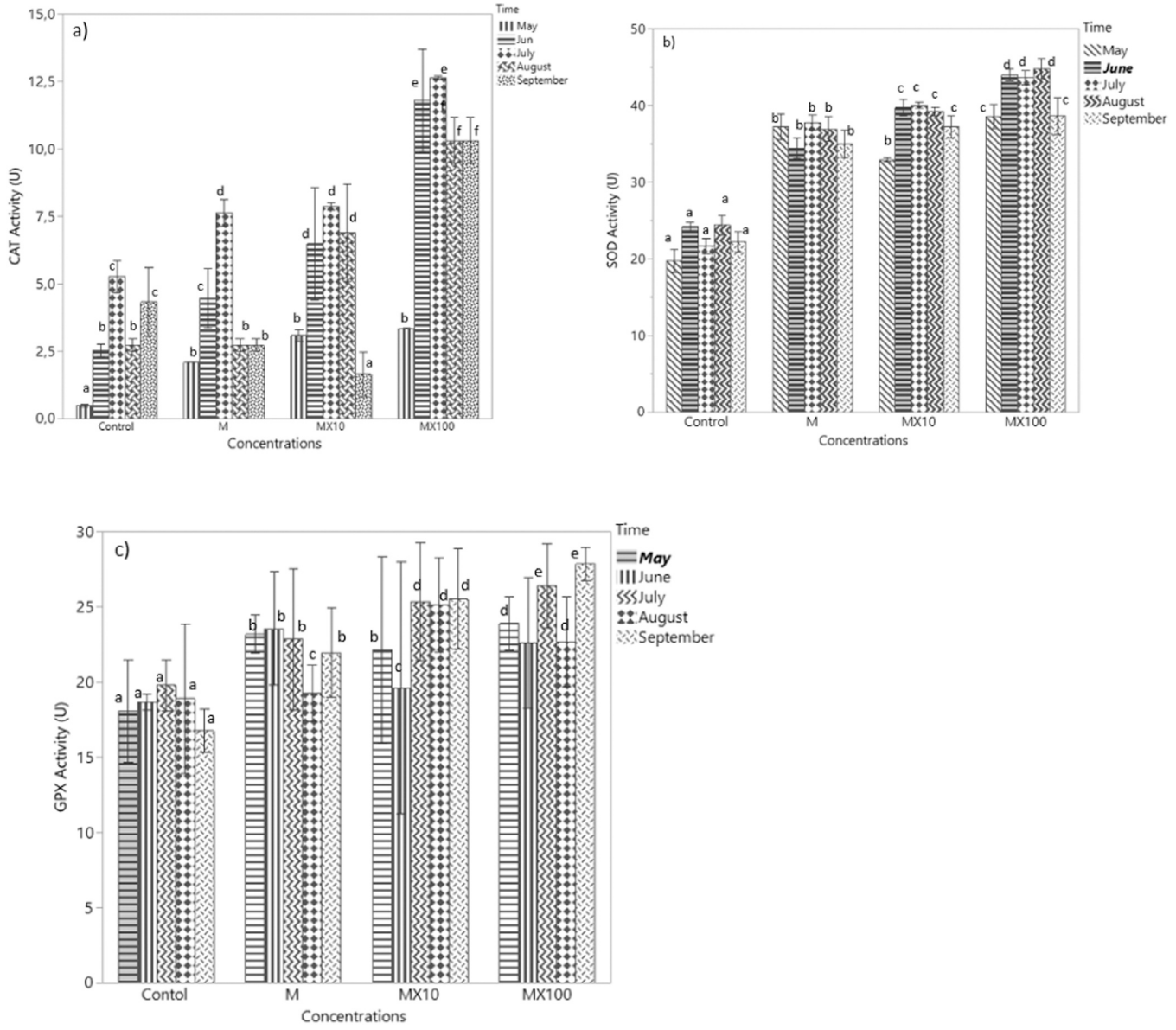

Fig. 2. Antioxidant enzyme activities of *F. antipyretica* at various pharmaceutical mixture concentrations and throughout time. CAT: catalase(a), SOD: superoxide dismutase(b), GPX: guaiacol peroxidase(c), Results are presented as mean ± standard deviation for three replicates taken from samples collected each month from May to September. Values with different letters are statistically different (ANOVA followed by Tukey's HSD test, $p < 0.05$).

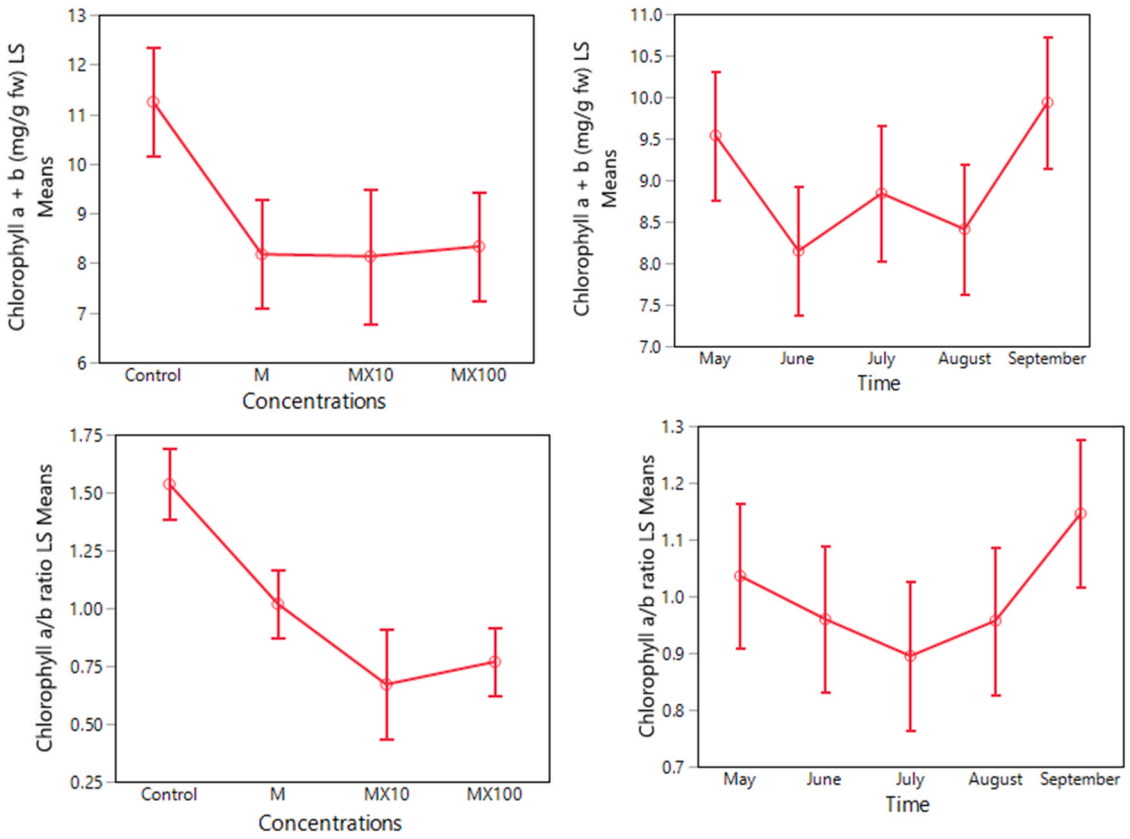

Fig. 3. Least squares means of total chlorophyll content (a,b) and chlorophyll a/b ratios(c, d) measured in F. antipyretica under various pharmaceutical mixture conditions and throughout time.

Table 2
Results of the random coefficient model (RC) applied to Total chlorophyll content and chlorophyll a/b ratios in *Fontinalis antipyretica* exposed to the different mixture concentrations.

	Random coefficient model (RC)	
Criteria	Chlorophyll a/b ratio	Total chlorophyll
-2 Res Log Likelihood	4.9	**205.63**
BIC	9.7	**209.93**
AIC	8.9	**209.5**
Solution for Fixed Effects		
Concentration	**Estimate**	**Estimate**
Intercept	1.3883***	10.2484***
MX1	-0.5286***	-3.2560***
MX10	-0.8775***	-3.2029***
MX100	-0.7803***	-3.1067***
Control	**0**	0
Time	0.05228*	0.3792*
Type 3 Tests of Fixed Effects		
	F Value	F Value
Concentration	14.29***	14.50***
Time	7.63*	8.64*
Covariance Parameter Estimates		
Canal	0.002690	
Canal*Time	0.000246*	

*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$.
AIC: Akaike's information criterion, BIC: Bayesian information criterion.

HSD test. However, the positive time coefficient confirmed the recovery of photosynthetic activity during the study period.

The component of variance explained by the channel (water inlet conditions) was 0.0027 and 0.23, for chlorophyll ratio and total chlorophyll, respectively. The channel explained a significant proportion of the variability.

3.4. Bioaccumulation

Among the five substances, only carbamazepine and irbesartan were accumulated by *F. antipyretica*. The plants displayed higher bioaccumulation of carbamazepine than that of irbesartan. The concentration of carbamazepine and irbesartan in the plant tissues increased with an increase in the concentration of the pharmaceuticals. Levene's test showed that the variance was not equal ($p > 0.05$). Comparison for each pair using Wilcoxon method showed that the bioaccumulation under the different treatment were significantly different. The Dunnett's test for joint ranking showed significant differences between the three treatments and the control. Irbesartan was accumulated only by plants exposed to MX100, and the concentration was significantly higher ($p \leq 0.05$) than that of plants in the control group (Fig. 4). The results of the bioaccumulation factor calculation showed that *F. antipyretica* was highly bioaccumulative (BAF > 5000 L/kg). The species largely bioaccumulated carbamazepine (BAFs between 6 and 79). However, bioaccumulation of irbesartan (BAFs = 4) was low.

3.5. Effects of the pharmaceutical mixture on morphological traits

The results of the effects of the three treatments (MX1, MX10, and MX100) on the morphological traits of the plants are shown in Fig. 5. The treatments did not significantly affect ($p > 0.05$) the morphological traits of the plants. However, during the study period, the growth rate of the plants decreased slightly in the fourth month of exposure and increased slightly in the fifth month (Fig. 5). Slight variations were observed in the leaf width and stem length of plants in the MX1 and MX100 (Fig. 5). The interaction between exposure duration and channel was significant and the channels could explain part of the variation in plant growth.

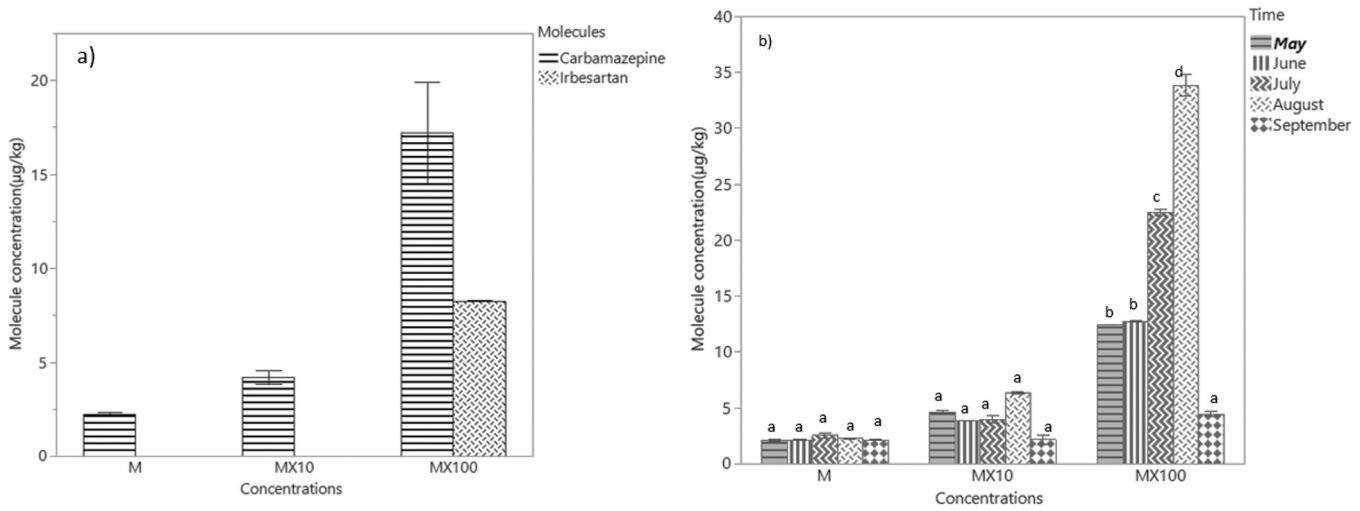

Fig. 4. Carbamazepine and irbesartan concentrations in F. antipyretica exposed to different mixture concentrations of the five pharmaceuticals (a) and carbamazepine concentration along 5 months of exposure (b). Results are presented as mean ± standard deviation for three replicates. Values with a different letter are significantly different (Wilcoxon test followed by Wilcoxon Each Pair test, $p < 0.05$).

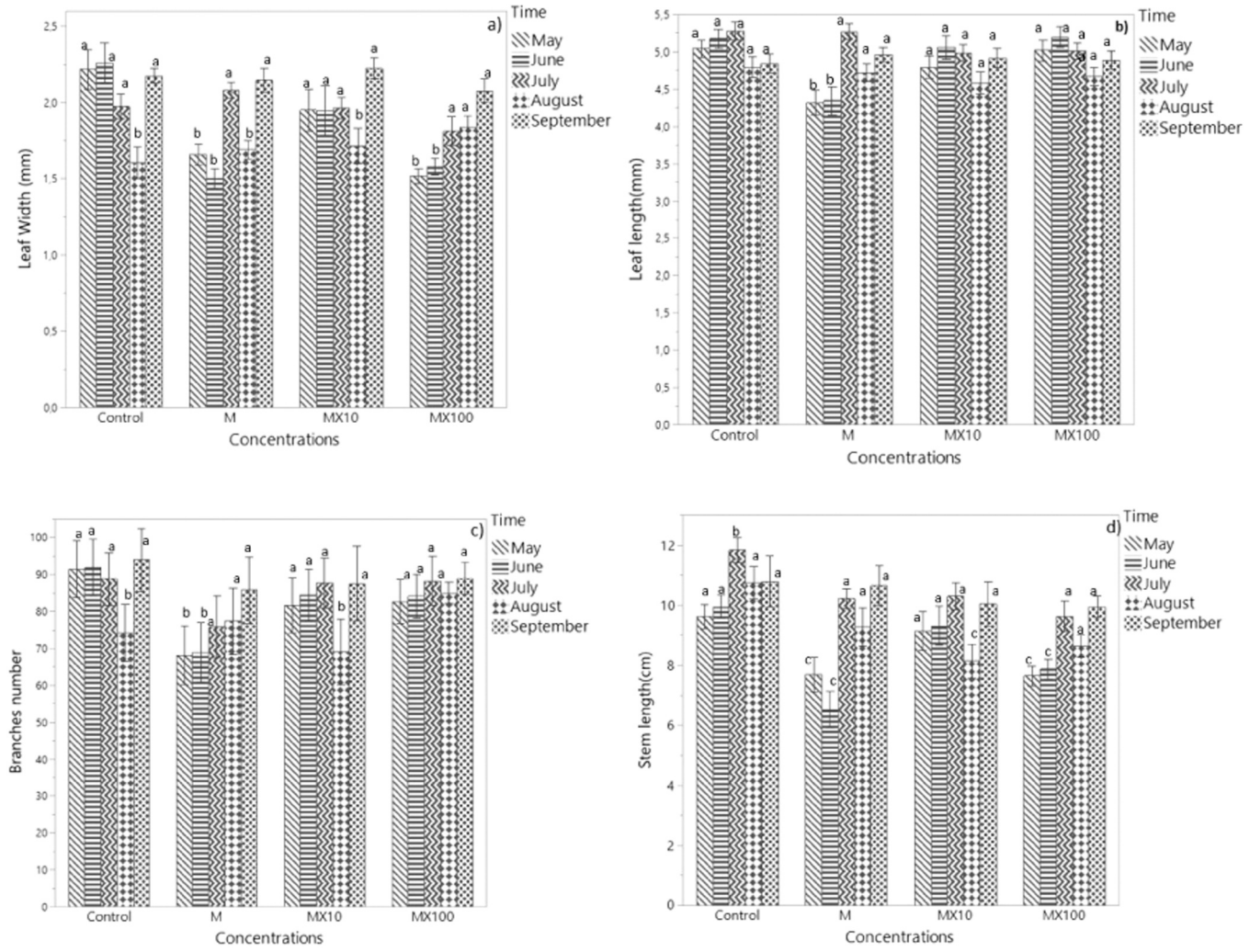

Fig. 5. Morphological characteristics measured in F. antipyretica exposed to mixture concentrations of the five pharmaceuticals. Results are presented as mean ± standard deviation for three replicates. The different values with different letters are statistically significant (ANOVA followed by Tukey's HSD test, $p < 0.05$). a: Leaf width (mm), b:Leaf length (mm), c: Number of branches and d:Stem length (cm).

4. Discussion

Numerous abiotic stressors, such as pharmaceutical substances, can cause damages to plant cells either directly or indirectly through the activities of ROS (Radotić et al., 2000; Shah et al., 2001). Plant cells respond to high concentrations of reactive oxygen species (ROS) by activating their antioxidant defense mechanisms (Bleuel et al., 2005; Sun et al., 2009). The effects of stressors on the oxidative status of plants is well documented in moss and phanerogam species (Chen et al., 2015; Dazy et al., 2009;). However, most studies (96%) have focused on inorganic contamination, with limited studies (4%) on the effects of organic contaminants (Debén et al., 2017).

The present study examined the effects of organic pollutants on the morphology and oxidative stress response of aquatic moss. a significant increase in the SOD and catalase activity of *F. antipyretica* with an increase in the concentration of the mixture, peaking at the third month of the study (July). This indicated that *F. antipyretica* responded to oxidative stress by increasing SOD activity. The effective scavenging of O_2^- by SOD is an essential defense mechanism against cellular oxidative damage, as O_2^- acts as a precursor of more cytotoxic and highly reactive oxygen derivatives, such as peroxynitric and OH (Duman et al., 2010). Therefore, SOD is usually considered the main line of protection against oxidative stress (Figueira and Ribeiro, 2005). The lower SOD activity in September (the last month of exposure) could be attributed to the downregulation of SOD activity or inactivation of certain isoenzymes. Similar observations have been reported in a few studies, including in *Holcus lanatus* exposed to arsenic (Hartley-Whitaker et al., 2001), *F. antipyretica* exposed to 10 M copper (Dazy et al., 2009), and *Nasturtium officinale* exposed to 5 mg/L of nickel (Duman et al., 2010). Dietz et al. (1999) attributed this phenomenon to the sensitivity of the enzymes to excess H_2O_2 produced in the cells. However, it has been reported that the sensitivity of the enzymes varies with species (Chen et al., 2015). The antioxidant enzymatic response alone is insufficient to determine the oxidative stress response of plants. Therefore, more specific biochemical parameters, such as lipid peroxide content, and redox state of cell membranes should be employed in future studies.

The treatments significantly decreased the chlorophyll content of the plants. However, after 3 months of exposure, the chlorophyll content was restored to normal, indicating that the effects of the treatments were possibly acute and that the photosynthetic system of *F. antipyretica* could acclimatize to the presence of different substances. This latter phenomenon was consistent with the report of Wang et al. (2012), who demonstrated that the photosystem II (PSII) reactive center of *Chlorella vulgaris* and *Haematococcus pluvialis* could produce a certain resistance to cadmium content. Similar responses have also been reported in the study of Wang et al. (2012) conducted on *Microcystis aeruginosa*. The effects of heavy metals on chlorophyll content have been reported by several authors (Monnet et al., 2001; Scoccianti et al., 2006). However, discrepancies in the results have been observed concerning chlorophyll a/b ratios (Mysliwa-Kurdziel et al., 2004). These results appeared to depend on the biological model and the metal used for the study. To confirm these results, further studies on photosynthetic performance, especially chlorophyll fluorescence and rate coefficient of photoinactivation (Jpi) test, need to be conducted.

In the present study, carbamazepine and irbesartan were accumulated by *F. antipyretica*; however, the plant had a higher bioaccumulation rate for carbamazepine than irbesartan. Besse and Garric (2008) have assigned these two components to category IB, which includes compounds that are potentially hazardous to aquatic environments. According to these authors, these substances also have risk ratios (predicted environmental concentration (PEC) higher than 100 ng/L). This accumulation capacity suggests that *F. antipyretica* could be suitable for environmental studies. In the present study, except for acetaminophen, which was only 50% of the expected concentration in the microcosm, the concentrations of the other four pharmaceuticals were within the normal concentration. This probably limited the bioaccumulation of acetaminophen by the species. To the best of our knowledge, this is the first study to investigate the bioconcentration of a mixture of five pharmaceuticals in *F. antipyretica*. Our findings on the bioaccumulation potential of *F. antipyretica* are consistent with those of previous studies. *Pseudokirchneriella subcapitata* (algae) and *Thamnocephalus platyurus* (crustacean) had bioaccumulation factors of 2.2 and 12.6, respectively, for carbamazepine (Vernouillet et al., 2010). Delépée et al. (2004) reported that at the end of a 10 d exposure period to three pharmacological substances, (oxolinic acid (OA), flumequine (FLU), and oxytetracycline (OTC)), *F. antipyretica* had a higher concentration of OA, FLU, and OTC compared to the microcosm. Gallissot (1988) reported a rapid and significant accumulation of PCBs by *F. antipyretica* and *Rhynchostegium riparioides*, in vitro and 50% of the steady-state concentrations were attained in 24 h. The same phenomenon has also been observed for heavy metals. Studies have reported a rapid accumulation (from a few hours to a few days) of several micropollutants and decontaminants (or depurate) by bryophytes after a few weeks or a few months (Claveri et al., 1994; Mersch and Kass, 1994; Vray et al., 1992). However, data on the cumulative concentration per unit time are often lacking in these studies, which limits comparison with our results. In the present study, a large variation was observed in the oxidative stress response of *F. antipyretica*, and the bioaccumulation of the pharmaceuticals. This phenomenon has been observed in several species. López-Gómez et al. (1997) reported that *F. antipyretica* exhibited intermediate potential for metal accumulation but showed a broader range of variation in tissue concentration compared to similar contamination levels.

In the present study, carbamazepine persisted in the plant tissue for 4 months and there was no decrease in its concentration, which is similar to previous findings (Delépée et al., 2004; Roy et al., 1992). Delépée et al. (2004) reported that the concentrations of OA, FLU and OTC ranged between 0.19 and 3.04 ng/g/day, with mean residence time ranging between 18 and 59 days. Studies suggests that organic pollutants appear to accumulate in the cell wall of bryophytes through negatively charged surface sites (Gallissot, 1988). Furthermore, during intracellular uptake, the less polar pollutants accumulate in the lipid compartments of the cells (Mouvet, 1986). The lipid content of *F. antipyretica* represents approximately 3.5% of its dry matter (Frisque et al., 1983; Maiss, 1988), which offers a real potential for the accumulation of hydrophobic substances. Therefore, it can be inferred that the concentration of these micropollutants is generally proportional to the lipid concentration of the organism. Aquatic mosses of the genera *Fontinalis*, *Cinclidotus*, and *Rhynchostegium* rapidly accumulate large concentrations of pollutants and release them slowly (Mouvet, 1984a, 1984b; Wehr and Whitton, 1983). The removal of organic pollutants by *F. antipyretica* was generally slow and decreased with time. There was a decrease in the removal of Benzo (a) anthracene (BaA) and Benzo (a) pyrene (BaP) after 24 h and 48 h, respectively (Roy et al., 1992). This slow removal of organic pollutants from plant tissues seems to be related to their low solubility in water (Steen and Karickhoff, 1981; Smith et al., 1991). The elimination of pollutants through this method is never total and complete. A residual fraction of heavy metals is maintained after a certain period of decontamination (Gallissot, 1988). Pollutants may be trapped in cells in non-toxic forms at low concentration levels, thereby increasing the elimination period of these pollutants by the plants.

Regarding pharmaceutical, it should be noted, that the MX1 concentrations are more or less within the range measured in other regions and are well below the 100X tested in this work.

The maximum concentrations of carbamazepine and diclofenac in surface waters are reported as 1075 ng L^{-1} and 1030 ng L^{-1} respectively detected in Berlin by Heberer et al. (2002). These are higher than the MX1 concentrations measured on the Meuse in Wallonia, which are respectively around 25 ng/l but much lower than the maximum concentrations (100X = 2.5 μg/l) tested in this work.

However, the maximum concentrations of carbamazepine and diclofenac detected in WWTP effluents are reported respectively as

6300 ng L^{-1} and 1600 ng L^{-1} (Ternes, 1998; Paxeus, 2004).

Ferrari et al. (2003) has studied the toxic effects of these two substances on bacteria, algae, microcrustaceans, and fish and he has shown that concentrations that cause 50% of effect (EC50) were 13,800 μg L^{-1} for carbamazepine on D.magna over 48 h and 11,454 μg L^{-1} for diclofenac on the Microtox system over 30 min

The concentrations of Naproxen in surface waters reported by Wang et al. (2010) are about 1.3 ng/l which is less than that measured in the Meuse River (25 ng/l).

For acetaminophen, the highest concentration detected in the river water was (346.3 ng L^{-1}) (Al-Odaini et al., 2013). This one is higher than that measured in the Meuse River and lower than 100X tested in this work. For Irbesartan however, Oosterhuis et al. (2013) indicates, limited literature data on its occurrence.

In the present study, despite the negative effects of the treatments on the oxidative status, chlorophyll content, and chlorophyll a/b ratio of the plants, the growth of the plant was not significantly affected. However, small changes were observed in plants exposed to the control condition and this may be attributed to the conditions of the mesocosms, which were not optimal for the species. Additionally, the study was conducted during summer, whereas the growth period of mosses is usually during the autumn-winter period. Ecotoxicity studies under ecosystem conditions are of considerable ecological relevance. Future ecotoxicity studies should take into consideration the interaction between and impact of different factors, for example the availability of the added pharmaceuticals with regard to different aquatic species.

5. Conclusion

Exposure to the mixture of the five pharmaceuticals at environmentally realistic concentrations adversely affected the oxidative status, chlorophyll content, and chlorophyll a/b ratio in *F. antipyretica* during the first 3 months of exposure. The treatments significantly affected the catalase, SOD, and GPX activity of *F. antipyretica*, suggesting that exposure to the treatments triggered the production of ROS. However, the chlorophyll content of the plants was restored to normal after 3 months of exposure to the three treatments. This indicates that the effects of the treatments were possibly acute and that the photosynthetic system of *F. antipyretica* could adapt to the occurrence of the different substances. However, oxidative response and chlorophyll content alone are insufficient to determine the oxidative stress response of plants. Therefore, complementary analyses, such as chlorophyll fluorescence, Jpi test, lipid peroxide content, and oxidative stress response of cell membranes, should be incorporated in future studies. The findings of this study showed that *F. antipyretica* bioaccumulated two (carbamazepine and irbesartan) of the five pharmaceuticals investigated. Carbamazepine persisted for 4 months in the plant tissue; its release or transformation was observed only after 5 months of exposure. Despite the negative effects of the three treatments on the oxidative status and chlorophyll content of the species, the morphological traits were not significantly affected by the treatments. The higher accumulation capacity and resistance to pollution, wider distribution, and higher abundance of *F. antipyretica* compared with some species, render this species suitable for environmental studies, as it meets all the criteria. However, the lack of standardized methods limits comparison between studies and the use of this species by environmental authorities as a tool for routine environmental assessment.

CRediT authorship contribution statement

Khadija Sossey Alaoui: Conceptualization, Methodology, Visualization, Software, Writing – original draft, Writing – review & editing. **Bernard Tychon**: Conceptualization, Supervision, Project administration. **Sandrine Joachim**: Methodology, Investigation. **Alain Geffard**: Conceptualization, Supervision, Project administration. **Katherine Nott**: Investigation. **Sébastien Ronkart**: Investigation. **Jean-Marc Porcher**: Methodology, Supervision. **Rémy Beaudouin**: Methodology, Supervision. **Christelle Robert**: Investigation. **Marie-Laure Fauconnier**: Investigation. **Matthew Saive**: Investigation.

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.

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References

Akaike, H., 1973. Maximum likelihood identification of gaussian autoregressive moving average models. Biometrika 60 (2), 255–265.
Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., Abdulghani, M., 2013. The occurrence of humanpharmaceuticals in wastewater effluents and surface water of langat river and its tributaries, Malaysia. Int. J. Environ. Anal. Chem. 93 (3), 245–264.
Allan, I.J., Vrana, B., Greenwood, R., Mills, G.A., Roig, B., Gonzalez, C., 2006. A toolbox" for biological and chemical monitoring requirements for the European union's water framework directive. Talanta 69 (2), 302–322.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in Beta vulgaris. Plant. Physiol. 24 (1), 1–15.
Bentsen, H., Klemetsdal, G., 1991. The use of fixed effect models and mixed models to estimate singlegene associated effects on polygenic traits. Genet. Sel. Evol. 23 (5), 1–13.
Besse, J.P., Garric, J., 2008. Human pharmaceuticals in surface waters: implementation of a prioritization methodology and application to the french situation. Toxicol. Lett. 176 (2), 104–123.
Bleuel, C., Wesenberg, D., Sutter, K., Miersch, J., Braha, B., Bärlocher, F., Krauss, G.J., 2005. The use of the aquatic moss Fontinalis antipyretica l. ex hedw. as a bioindicator for heavy metals: 3. Cd2+ accumulation capacities and biochemical stress response of two fontinalis species. Sci. Total Environ. 345 (1–3), 13–21.
Brain, R.A., Johnson, D.J., Richards, S.M., Sanderson, H., Sibley, P.K., Solomon, K.R., 2004. Effects of 25 pharmaceutical compounds to Lemna gibba using a seven-day static-renewal test. Environ. Toxicol. Chem. 23 (2), 371–382.
CENCI, R.M., 2001. The use of aquatic moss (Fontinalis antipyretica) as monitor of contamination in standing and running waters: limits and advantages. J. Limnol. 60 (1s), 53–61.
Chen, Y.E., Cui, J.M., Yang, J.C., Zhang, Z.W., Yuan, M., Song, C., Yang, H., Liu, H.M., Wang, C.Q., Zhang, H.Y., Zeng, X.Y., Yuan, S., 2015. Biomonitoring heavy metal contaminations by moss visible parameters. J. Hazard. Mater. 296, 201–209.
Christmas, M., Whitton, B.A., 1998. Phosphorus and aquatic bryophytes in the swale–ouse river system, North-East England. 2. Phosphomonoesterase and phosphodiesterase activities of Fontinalis antipyretica. Sci. Total Environ. 210, 401–409.
Claiborne, A., 1985. Handbook of Methods for Oxygen Radical Research. Catalase Activity.
Claveri, B., Morhain, E., Mouvet, C., 1994. A methodology for the assessment of accidental copper pollution using the aquatic moss rhynchostegium riparioides. Chemosphere 28 (11), 2001–2010.
Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107 (Suppl 6), 907–938.
David, V., Joachim, S., al, 2020. Effects of chronic exposure to a pharmaceutical mixture on the three-spined stickleback (Gasterosteus aculeatus) population dynamics in lotic mesocosms. Aquat. Toxicol. 224, 2020–105499.
Dazy, M., Béraud, E., Cotelle, S., Meux, E., Masfaraud, J.F., Férard, J.F., 2008. Antioxidant enzyme activities as affected by trivalent and hexavalent chromium species in Fontinalis antipyretica hedw. Chemosphere 73 (3), 281–290.
Dazy, M., Masfaraud, J.F., Férard, J.F., 2009. Induction of oxidative stress biomarkers associated with heavy metal stress in Fontinalis antipyretica hedw. Chemosphere 75 (3), 297–302.

De Leeuw,, J, Kreft, I.., 1986. Random coefficient models for multilevel analysis. J. educ. stat 11(1), 57–85.
Debén, S., Aboal, J., Carballeira, A., Cesa, M., Fernández, J., 2017. Monitoring river water quality with transplanted bryophytes: a methodological review. Ecol. Indic. 81, 461–470.
Delépée, R., Pouliquen, H., Le Bris, H., 2004. The bryophyte Fontinalis antipyretica hedw. bioaccumulates oxytetracycline, flumequine and oxolinic acid in the freshwater environment. Sci. Total Environ. 322 (1–3), 243–253.
Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32 (1), 93–101.
Dietz, K.J., Baier, M., Krämer, U., 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. Heavy Metal Stress in Plants. Springer, pp. 73–97.
Duman, F., Ozturk, F., Aydin, Z., 2010. Biological responses of duckweed (Lemna minor l.) exposed to the inorganic arsenic species as (iii) and as (v): effects of concentration and duration of exposure. Ecotoxicology 19 (5), 983–993.
Ebele, A.J., Abdallah, M.A.E., Harrad, S., 2017. Pharmaceuticals and personal care products (ppcps) in the freshwater aquatic environment. Emerg. Contam. 3 (1), 1–16.
EC TGD, 2004. European Commission. 1996. 2004. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, EUR 20418/EN/3, 2nd ed. 2003. Luxembourg, Luxembourg.
Ferrari, B., Paxéus, N., Giudice, R.L., Pollio, A., Garric, J., 2003. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol. Environ. Saf. 55, 359–370.
Fielding, J.L., Hall, J., 1978. A biochemical and cytochemical study of peroxidase activity in roots of Pisum sativum: Ii. distribution of enzymes in relation to root development. J. Exp. Biol. 29 (4), 983–991.
Figueira, R., Ribeiro, T., 2005. Transplants of aquatic mosses as biomonitors of metals released by a mine effluent. Environ. Pollut. 136 (2), 293–301.
Frisque, G., Galoux, M., Bernes, A., 1983. Accumulation de deux micropolluants (les plychlorobiphényles et le gammahch) par des bryophytes aquatiques de la meuse. Med. Fac. Landbouw Rijksuniv. Gent. 48 (4), 971–983.
Fu, W., Franco, A., Trapp, S., 2009. 'Methods for estimating the bioconcentration factor of ionizableorganic chemicals'. Environ. Toxicol. Chem. Int. J. 28 (7), 1372–1379.
Gallissot, B., 1988. Mise en évidence du potentiel bioaccumulateur des bryophytes aquatiques vis-à-vis des pcb et des pesticides organochlorés', Etudes in situ et en laboratoire, Proposition de grilles d'fvaluation de la contamination par les organo-chlorfs, Th¯se de doctorat en Ecotoxicologie, Universit6 de Metz.
Galoux, D., Chérot, F., Rosillon, F., Sossey-Alaoui, K., 2015. Contribution to the macrophytic typology of Belgian reference watercourses. Adv. Bot. 2015, 1–13.
Greenberg, B.M., Huang, X.D., Dixon, D.G., 1992. Applications of the aquatic higher plant Lemna gibba for ecotoxicological assessment. J. Aquat. Ecosyst. Health 1 (2), 147–155.
Greenwood, R., Roig, B., 2006. Directory of 'screening tools'—a toolbox of existing and emerging methods for chemical and ecological status monitoring under the wfd. SWIFT-WFD, Screening methods for Water data InFormaTion in support of the implementation of the Water Framework Directive. Armines, France/Portsmouth, pp. 71–72.
Hartley-Whitaker, J., Ainsworth, G., Meharg, A.A., 2001. Copper-and arsenate-induced oxidative stress in *Holcus lanatus* l. clones with differential sensitivity. Plant Cell Environ. 24 (7), 713–722.
Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol. Lett. 131 (1–2), 5–17.
Heberer, T., Reddersen, K., Mechlinski, A., 2002. From municipal sewage to drinking water: fateand removal of pharmaceutical residues in the aquatic environment in urban areas. Water Sci. Technol. 46 (3), 81–88.
INERIS, 2020. Overview of the lotic mesocosms'. URL: ⟨https://www.ineris.fr/en/node/160⟩.
Ingerslev, F., Toräng, L., Loke, M.L., Halling-Sørensen, B., Nyholm, N., 2001. Primary biodegradation of veterinary antibiotics in aerobic and anaerobic surface water simulation systems. Chemosphere 44 (4), 865–872.
Jørgensen, S.E., Halling-Sørensen, B., 2000. Drugs in the environment. Chemosphere 40 (7), 691–699.
Le Bris, H., Pouliquen, H., 2004. Experimental study on the bioaccumulation of oxytetracycline andoxolinic acid by the blue mussel (Mytilus edulis). An evaluation of its ability to bio-monitor antibiotics inthe marine environment'. Mar. Pollut. Bull. 48 (5–6), 434–440.
López-Gómez, R., Campbell, A., Dong, J.G., Yang, S.F., Gómez-Lim, M.A., 1997. Ethylene biosynthesis in banana fruit: isolation of a genomic clone to acc oxidase and expression studies. Plant Sci. 123 (1–2), 123–131.
Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. Areview on the occurrence of micropollutants in the aquatic environment and their fate and removal duringwastewater treatment. Sci. Total. Environ. 473, 619–641.
Maiss, M., 1988. Mousses Aquatiques Comme Bioindicateurs de la Pollution par les Pesticides et les pcbs dans les cours d'eau Luxembourgeois ainsi que dans les eaux de Percolation d'une Décharge Sarroise. Fondation Universitaire Luxembourgeoise, Arlon, Belgique.
Markert, B., Wappelhorst, O., Weckert, V., Herpin, U., Siewers, U., Friese, K., Breulmann, G., 1999. The use of bioindicators for monitoring the heavy-metal status of the environment. J. Radioanal. Nucl. Chem. 240 (2), 425–429.
Martínez-Carballo, E., González-Barreiro, C., Scharf, S., Gans, O., 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ. Pollut. 148 (2), 570–579.
Martins, R.J., Pardo, R., Boaventura, R.A., 2004. 'Cadmium (ii) and zinc (ii) adsorption by the aquaticmoss Fontinalis antipyretica: effect of temperature, ph and water hardness'. Water Res. 38 (3), 693–699.
McCullagh, P., 2018. Generalized Linear Models. Routledge.
Mersch, J., Kass, M., 1994. La mousse aquatique Fontinalis antipyretica comme traceur de la contamination radioactive de la moselle en aval de la centrale nucléaire de cattenom. Bull. Soc. Nat. Luxemb. 95, 109–117.
Monnet, F., Vaillant, N., Vernay, P., Coudret, A., Sallanon, H., Hitmi, A., 2001. Relationship between psii activity, Co2 fixation, and Zn, Mn and Mg contents of Lolium perenne under zinc stress. J. Plant Physiol. 158 (9), 1137–1144.
Mouvet, C., 1984a. Accumulation of chromium and copper by the aquatic moss Fontinalis antipyretica l. ex hedw transplanted in a metal-contaminated river. Environ. Technol. 5 (12), 541–548.
Mouvet, C., 1984b. Métaux lourds et mousses aquatiques, spéciation physico-chimique, bioaccumulation et toxicité. Th. Doct. Sc. Bot:⟨Univ.Liège@elgique⟩.
Mouvet, C., 1986. Métaux Lourds et Mousses Aquatiques: Synthèse Méthodologique. Agence de l'eau Rhin, Meuse.
Muthén, B.O., Curran, P.J., 1997. General longitudinal modeling of individual differences inexperimental designs: a latent variable framework for analysis and power estimation. Psychol. Methods 2 (4), 371–402.
Mysliwa-Kurdziel, B., Prasad, M., Strzalka, K., 2004. Photosynthesis in heavy metal stressed plants, Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, pp. 146–181.
Nott, K., Ronkart, S., 2018. Recherche des substances émergentes dans les eaux et intéressant la santé publique et l'environnement, Technical report. P. d. R. I. I. d. M. H. e. O. e. T. d. les and E. P. e. Potabilisables.
Orliński, R., 2002. Multipoint moss passive samplers assessment of urban airborne polycyclic aromatic hydrocarbons: concentrations profile and distribution along warsaw main streets. Chemosphere 48 (2), 181–186.
Oosterhuis, M., Sacher, F., Ter Laak, T.L., 2013. Prediction of concentration levels of metformin andother high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Sci. Total Environ. 442, 380–388.
Paxeus, N., 2004. Removal of selected non-steroidal anti-inflammatory drugs (nsaids), gemfibrozil,carbamazepine, b-blockers, trimethoprim and triclosan in conventional wastewater treatment plants in fiveeu countries and their discharge to the aquatic environment. Water Sci. Technol. 50 (5), 253–260.
Pouliquen, H.Le, Bris, H., Buchet, V., Pinault, L., 1996. Comparative study on the contamination and decontamination of japanese oyster Crassostrea gigas and blue mussel Mytilus edulis by oxytetracycline and oxolinic acid. Mar. Ecol. Prog. Ser. 133, 143–148.
Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.-M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M., McField, M., Morrissey, C.A., Noome, D.A., Settele, J., Simon-Delso, N., Stark, J.D., Van der Sluijs, J.P., Van Dyck, H., Wiemers, M., 2015. Effects of neonicotinoids and fipronil on non-targetinvertebrates. Environ. Sci. Pollut. Res. 22 (1), 68–102.
Qiu-Fang, Z., Yuan-Yuan, L., Cai-Hong, P., Cong-Ming, L., Bao-Shan, W., 2005. Nacl enhances thylakoid-bound sod activity in the leaves of c3 halophyte suaeda salsa l. Plant Sci. 168 (2), 423–430.
Radotić, K., Dučić, T., Mutavdžić, D., 2000. Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. Environ. Exp. Bot. 44 (2), 105–113.
Ravera, O., Riccardi, N., 1997. Biological monitoring with organisms accumulator of pollutants. Mar. Chem. 58 (3–4), 313–318.
Roy, S., Ihantola, R., Hänninen, O., 1992. Peroxidase activity in lake macrophytes and its relation to pollution tolerance. Environ. Exp. Bot. 32 (4), 457–464.
Roy, S., Sen, C.K., Hänninen, O., 1996. Monitoring of polycyclic aromatic hydrocarbons using 'moss bags': bioaccumulation and responses of antioxidant enzymes in Fontinalis antipyretica hedw. Chemosphere 32 (12), 2305–2315.
Samecka-Cymerman, A., Kempers, A.J., 2000. Bioindication of heavy metals with aquatic macrophytes: the case of a stream polluted with power plant sewages in Poland. J. Environ. Sci. Health Part A 62 (1), 57–67.
Scheurer, M., Michel, A., Brauch, H.-J., Ruck, W., Sacher, F., 2012. Occurrence and fate of theantidiabetic drug metformin and its metabolite guanylurea in the environment and during drinking watertreatment. Water Res. 46 (15), 4790–4802.
Schmitz, M., Beghin, M., Mandiki, S.N., Nott, K., Gillet, M., Ronkart, S., Robert, C., Baekelandt, S., Kestemont, P., 2018. Environmentally-relevant mixture of pharmaceutical drugs stimulates sex-steroid hormone production and modulates the expression of candidate genes in the ovary of juvenile female rainbow trout. Aquat. Toxicol. 205, 89–99.
Schwarz, G., 1978. Estimating the dimension of a model. Ann. Stat. 6 (2), 461–464.
Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., Lester, J., 2008. Nonylphenol in the environment:a critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ. Int. 34 (7), 1033–1049.
Scoccianti, V., Crinelli, R., Tirillini, B., Mancinelli, V., Speranza, A., 2006. Uptake and toxicity of cr(iii) in celery seedlings. Chemosphere 64 (10), 1695–1703.
Shah, K., Kumar, R.G., Verma, S., Dubey, R., 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci. 161 (6), 1135–1144.
Siebert, A., Bruns, I., Krauss, G.-J., Miersch, J., Markert, B., 1996. The use of the aquatic mossFontinalis antipyretica l. ex hedw. as a bioindicator for heavy metals: 1.

fundamental investigations intoheavy metal accumulation in Fontinalis antipyretica l. ex hedw. Sci. Total Environ. 177 (1–3), 137–144.
Smith, J.D., Bagg, J., Wrigley, I., 1991. Extractable polycyclic hydrocarbons in waters from rivers in south-eastern Australia. Water Res. 25 (9), 1145–1150.
Sossey Alaoui, Rosillon, F., 2013. Macrophytic distribution and trophic state of some natural andimpacted watercourses-belgium wallonia. Int. j. water sci. 2 (3), 1–11.
Steen, W.C., Karickhoff, S.W., 1981. Biosorption of hydrophobic organic pollutants by mixed microbial populations. Chemosphere 10 (1), 27–32.
Sun, S.Q., He, M., Cao, T., Zhang, Y.C., Han, W., 2009. Response mechanisms of antioxidants in bryophyte (hypnum plumaeforme) under the stress of single or combined pb and/or ni. Environ. Monit. Assess. 149 (1–4), 291–302.
Ternes, T.A., 1998. Occurrence of drugs in german sewage treatment plants and rivers1. Water Res. 32 (11), 3245–3260.
USEPA, 1976. U.S. Environmental Protection Agency. 1976. Toxic substances control act. U.S. Government Printing Office, Washington, DC.
Vernouillet, G., Eullaffroy, P., Lajeunesse, A., Blaise, C., Gagné, F., Juneau, P., 2010. Toxic effects and bioaccumulation of carbamazepine evaluated by biomarkers measured in organisms of different trophic levels. Chemosphere 80 (9), 1062–1068.
Vray, F., Baudin, J.P., Svadlenková, M., 1992. Effects of some factors on uptake and release of 106 ru by a freshwater moss, plathypnidium riparioïdes. Arch. Environ. Contam. Toxicol. 23 (2), 190–197.
Wang, L., Ying, G.-G., Zhao, J.-L., Yang, X.-B., Chen, F., Tao, R., Liu, S., Zhou, L.-J., 2010. Occurrenceand risk assessment of acidic pharmaceuticals in the yellow river, hai river and liao river of north china. Sci. Total Environ. 408 (16), 3139–3147.
Wang, S., Zhang, D., Pan, X., 2012. Effects of arsenic on growth and photosystem ii (psii) activity of Microcystis aeruginosa. Ecotoxicol. Environ. Saf. 84, 104–111.
Wehr, J., Whitton, B., 1983. Accumulation of heavy metals by aquatic mosses. 3: Seasonal changes. Hydrobiologia 100 (1), 285–291.
Zhang, Y., Geißen, S.U., Gal, C., 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere 73 (8), 1151–1161.
Zwiener, C., Frimmel, F., 2000. Oxidative treatment of pharmaceuticals in water. Water Res. 34 (6), 1881–1885.