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Original paper

The Use of Marine Algae in the Bioremediation of Contaminated Water with Pharmaceutical Products and Persistent Organic Products (POPs)

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Abstract

The presence of pharmaceutical products and persistent organic products (POPs) in surface waters, their high toxicity and the fact that they are difficult to remove by classical water treatment methods has led to the research and development of alternative methods. POPs are extremely hazardous substances; have a high degree of resistance to chemical and biological degradation; can be easily transported to the atmosphere at great distances and deposited away from the emission site; may cause harm to human health and the environment.

The aim of this study is to present a method of bioremediation of contaminated water with pharmaceutical products and POPs using marine brown algae. The results are presented from experiments to remove the Venlafaxine antidepressant and Cypermetrin from aqueous solutions with marine brown alga *Saccorhiza sp*.

Our experiments have led to the conclusion that brown marine alga *Saccorhiza sp*. is suitable for use in bioremediation of polluted water with venlafaxine and cypermetrin.

Keywords Pharmaceutical products, POPs, marine algae, bioremediation, bioadsorbtion.

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Introduction

Pharmaceutical products are persistent substances that represent a threat to environmental stability (SANTOS & al. [1]). Due to increasing consumption of drugs, pharmaceuticals and their metabolites appear more often in the aquatic environment (BOUISSOU-SCHURTZ & al. [2]). Slow decay rates for pharmaceuticals, constant emissions and inefficiency of waste water treatment plants have led to increased concentrations in waters (OSKARSSON & al. [3]).

The relevance of pharmaceutical products in waters is given by their universal use, their physicochemical properties and the mode of action known in aquatic organisms. After administration, most drugs and their transformation products remain in the water to some extent, and from sewage treatment plants enter into the aquatic environment in considerable quantities (ZENKER & al. [5]).

Today, the appearance of pharmaceuticals products in the environment is reported globally. Moreover, the new data on the sources, evolution and effects of pharmaceuticals on the environment seem to indicate their negative impact on different ecosystems and pose a risk to people's health. For this hypothesis, the data from the acute and chronic eco-toxicity tests on species belonging to different trophic levels, such as bacteria, algae, crustaceans and fish, among others, illustrates several adverse effects that environmental exposure to the measured concentrations of these contaminants can have them (SANTOS & al. [1]). There is a risk of long-lasting effects in the aquatic environment on the release of pharmaceuticals into water. Can occur endocrine disorders at moderate concentrations (BOUISSOU-SCHURTZ & al. [2]). Sources of polution are either direct, through discharges from wastewater treatment plants or from manufacturing companies or indirect, through wastewater from the pharmaceutical industry and through excreta human wastewater (OSKARSSON & al. [3]). For this reason, pharmaceuticals do not appear alone in the aquatic environment, but together with other substances. Because a wide range of different substances are used simultaneously in humans, pharmaceuticals are present in combinations in the environment (BOUISSOU-SCHURTZ & al. [2]).

Lately there has been a significant increase in research in the field (ZALOLO & al. [4]; OBUEKWE & al. [8]). This researches mostly referred to the disposal of pharmaceuticals in wastewater(BAXI & al. [7]). These researches have shown that the most frequently available treatment options are not effective in eliminating these compounds (CALISTO & al. [6]). The most detectable pharmaceutical products in waters are psychiatric drugs. Of these, carbamazepine, venlafaxine, lorazepam and citalopram were nominated to be the most representative compounds (SANTOS & al. [9]).

Persistent organic pollutants (POPs) are chemicals that persist in the environment, bioaccumulate in living organisms (via food, water and air inspired) and pose a risk of adverse effects on human health and the environment. These substances enter the environment as a result of anthropogenic activity. The most important persistent organic pollutants are: pesticides (aldrin, clordan, DDT, dieldrin, endrin, heptachlor, mirex, toxafen), industrial chemicals (hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs)) and by-products: dioxins and furans (LOHMANN & al. [10]).

A characteristic of POPs is persistence, which is resistance to degradation by chemical, photochemical and biochemical reactions in the aqueous phase. In the atmosphere and in the soil determines the long life of these substances and their long-term stability (due to this property POPs can exist in regions where they have never been used). Their half-lives are greater than 2 months in water, longer than 6 months in soil, longer than 2 days in the air. Another characteristic of POPs is the toxicity, which is the property of the chemical to cause damage to man and the environment. Acute toxicity is more of a landmark (due to lack of exact knowledge). It has harmful effects on humans and animals, leading to Imbalances of the immune, endocrine, reproductive system. They have carcinogenic and genotoxic effects (https://bibliotecaregielive.ro [11]).

Materials and methods

2.1. Materials

We chose to work with brown marine algae due to the structure of their cell walls, which generally contain three components: cellulose, alginic acid, polymers (eg guluronic acid) complexed with light metals, like sodium, potassium or calcium, and polysaccharides. These compounds contain functional groups, such as amino, carboxyl, sulfate or hydroxyl, that have an important role in bioadsorption.

We have chosen to work with pharmaceutical products and POPs because they have not been practically removed in wastewater treatment plants (PEREIRA & al. [12]). With the current type of pharmaceutical and POPs treatment capacity, the removal is limited, depending on the concentration of the influent and the configuration of the biological reactor (LAGERGREN [13]).

2.2. Bioadsorbtion

Kinetics. To determine the time needed to establish the balance between pharmaceuticals products and POPs and algae, prepare a solution of pharmaceuticals or POPs of a known concentration. From this solution take a sample to measure the initial concentration. Thereafter, the solution is mixed with a known amount of algae and stirred

for 2 hours. During the different time intervals samples are taken from this solution. Each sample is centrifuged for 10 minutes at 9000 rpm. The concentration of the pharmaceutical product or POPs remaining in the aqueous phase is being analyzed using HPLC.

The experimental data obtained are fitted to the kinetic models, pseudo-first kinetic model (ec. (1)) and pseudo-second kinetic model (ec. (2)) (LAGERGREN [13]), respectively, using ORIGIN program, version 9.0.

$$q_t = q_e(1 - e^{-k_1 t}) \quad (1)$$

$$q_t = \frac{q_e(1-e^{-k_1 t})}{1+q_e k_2 t} \quad (2)$$

where t (min) is the adsorbent/solution contact time, q_t (µg mg^{-1}) is the amount of solute adsorbed by mass unit of adsorbent at time t, q_e (µg mg^{-1}) is the amount of solute adsorbed when the equilibrium is attained and k_1 (min^{-1}) and k_2 (mg $\mu g^{-1} min^{-1}$) are the pseudo-first and pseudo-second order rate constants, respectively.

To determine the time needed to reach the balance between cypermethrin and alga *Saccorhiza* sp. we made a solution with an initial concentration of 200 µg/l of cypermethrin to which we added 400 mg of algae. Aqueous solutions were sampled at intervals ranging from 0 to 100 minutes. Liquid-liquid micro-extraction of the samples with hexane was performed, and the obtained extracts were analyzed on a gas chromatograph. The adsorption capacity of each algae was determined over time.

In order to determine the time needed to establish the equilibrium between venlafaxine and algae, a solution of venlafaxine with a concentration of 2000 µg L-1 was prepared. A sample was taken in order to measure the initial concentration. After that, 1 L solution was mixed with 500 mg of algae and stirred (Velp Scientific Arec.x, Europe) for 2 hours. At different time samples were taken and then centrifuged (Sigma 2-16, U.K.) for 10 min at 9000 rpm. The initial pH of the venlafaxine solution was 5.2 and the quantification of venlafaxine after the adsorption was made at pH 6.3. Venlafaxine was quantified in the supernatant by HPLC-FLD.

Equilibrium. Solutions of pharmaceutical products and POPs with different concentrations are shaken with a known amount of algae for a sufficient time to reach equilibrium (2 h). The pharmaceutical products and POPs concentration must vary within fairly large limits. After the solutions are stirred for 2 h, samples are taken which are centrifuged for 10 minutes at 9000 rpm. The concentration of the pharmaceutical product and POPs remaining in the aqueous phase is being analyzed using HPLC. The experimental data needs to be fitted for the two nonlinear isothermal models commonly used to describe the adsorption process, namely the Langmuir model (ec. (3)) (LANGMUIR [14]) and the Freundlich model (ec. (4)) (FREUNDLICH [15]), using ORIGIN program, version 9.0.

$$q_e = \frac{q_m K_1 C_e}{1+K_l C_e} \quad (3)$$

$$q_e = K_1 C_e^{1/N} \quad (4)$$

where q_e is the amount of solute adsorbed at equilibrium (µg mg-1), C_e is the amount of solute in the aqueous phase at equilibrium (µg L-1), q_m is the Langmuir maximum adsorption capacity (µg mg-1) and K_L (L µg-1) the Langmuir affinity coefficient, 1/n and K_F ((µg mg-1) (L µg-1)1/n) are the Freundlich's constants related to adsorption intensity and adsorption capacity, respectively.

For equilibrium experiments, 400 mg of algae was used for each 30 ml aqueous solution of different concentrations. Initial concentrations of cypermethrin solutions were between 100-5000 µg/l. The sampling of each aqueous solution of different concentrations of cypermethrin adsorbed after 120 minutes was performed. Each initial aqueous solution of cypermethrin was also sampled with different concentrations. Liquid-liquid micro-extraction was performed for each sample. Extracts obtained from micro-extraction were analyzed at GC-ECD.

Different solutions of venlafaxine with concentrations ranging from 2.20 to 8.70 mg L-1 were stirred (Velp/ Multistirrer 15, Italy) with 250 mg of algae for 2 h, the time needed to attain equilibrium. Then samples were centrifuged (Sigma 2-16, U.K.) for 10 min at 9000 rpm. The pH was established at 6 and in the end the quantification of venlafaxine was made by HPLC-FLD.

2.3. Chemical analysis

Cypermethrin analysis was performed with the electron capture gas chromatograph (GC-ECD).

Before chromatographic analysis, all the samples were centrifuged (Sigma 2-16, U.K.) at 9000 rpm, 10 min, and the supernatant was injected for the quantification of cypermethrin.

The analysis was carried out with the Shimadzu GC 2010 equipment (Shimadzu Corporation, Kyoto, Japan), consisting of: split / splitless injector, Zelron column, electron capture detector (ECD), HTA autosampler (liquid GC autosampler).

Chromatographic separation is performed on the Zelron chromatographic column (lenght: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 µm) (Phenomenex, USA).

The mobile phase is helium (6.0 purity) and nitrogen make-up gas with a flow rate of 30ml / min (Flow control mode – pressure, total flow: 32.0ml/min, split ratio: 18.7).

Column heating is done in gradient, according to the temperature program.

Total Program Time: 31,33 min.

Quantification of cypermethrin was performed using an external calibration curve generated using linear regression analysis. Linearity was achieved over the concentration range of 50 to 5000 µg L-1 with a good fit (r2 > 0.999).

The analysis of venlafaxine was performed by HPLC-FLD with fluorescence detection (HPLC-FLD). Before chromatographic analysis, all the samples were centrifuged (Sigma 2-16, U.K.) at 9000 rpm, 10 min, and the supernatant was injected for the quantification of venlafaxine.

Chromatographic analysis was performed in a Prominence Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a LC 20AD pump, a DGU-20ASR degasser, a SIL 20AHT autosampler, a CTO-10ASVP column oven, a system controller CBM-20A and a RF-20AXS fluorescence detector (FLD). The chromatographic separation was achieved using a Luna C18 column (4.6 × 150 mm, 5 µm particle size) (Phenomenex, USA). The mobile phase consisted of 0.1% formic acid in ultra-pure water as solvent A and acetonitrile as solvent B. The gradient used was as follows: initial conditions 10% B; 0-10 min: 10-100% B; 10-15 min maintain 100% B; 15-18 min return to initial conditions; 18-25 min equilibration of the column. The flow rate was settle at 1.0 mL min-1. The injection volume was 20 µL and the column was kept at 35ºC. Fluorescence detection was carried out at an excitation/emission wavelength pair of 274/610 nm. Labsolution software version 5.82 was used for control and data processing. Under the describer experimental conditions, venlafaxine retention time was 7.78 min.

Quantification of venlafaxine was performed using an external calibration curve generated using linear regression analysis. Linearity was achieved over the concentration range of 50 to 5000 µg L-1 with a good fit (r2 > 0.999). Limit of detection (LOD) and limit of quantification (LOQ) of venlafaxine were 9.15 and 30.5 µg L-1, respectively.

Results and discussions

Since classical remediation methods have failed to purify contaminated water with pharmaceuticals and POPs, various studies have been carried out to identify alternative methods.

In 2012, Chen et al. (CHEN & al. [16]) have carried out cypermethrin degradation experiments using pure and mixed cultures of *Bacillus cereus* and *Streptomyces aureus*. The results obtained showed that in pure cultures the half-life for cypermethrin ranged between 32.6h and 43 h and in mixed crops of 13 h.

Mack'ak et al., in 2015 (MACKUL'AK & al. [17]) tested the bioremediation capacity of *Cabomba carolinian*, *Limnophila sessiliflora*, *Egeria najas* and *Iris pseudacorus* for a range of pharmaceutical products including venlafaxine. The results obtained showed that these plants had a venlafaxine removal capacity between 29% and 62%, with the required time of 96h. *Iris pseudacorus* has the best removal capacity.

Cruz-Morato et al., in 2014, studied the effectiveness of removing pharmaceutical products from fungal contaminated waters (CRUZ-MORATO & al. [18]). Of the 52 products used in the experiment, 48 were partially or completely removed. However, the remediation process has a very long duration, the water remediation being done only after 8 days.

In 2015, Catala et al. performed experiments to remediate contaminated water with pharmaceutical products using photo-Fenton treatment (CATALA & al. [19]). Following experiments, product concentrations have fallen below the detection limit, however, water toxicity has not been achieved. Moreover, in some cases it has increased due to the reaction products used, especially peroxides.

Also in 2015, Calisto et al. conducted experiments to remove pharmaceuticals from water using two forms of carbon (CALISTO & al. [6]). They found that the method is effective only for a used carbon form and not for all analyzed products.

Since marine algae have been successfully used in the bioremediation of heavy metals and because they are cheap and easy to obtain, in our experiments we have chosen to use them in bioremediation experiments of contaminated water with pharmaceutical products. We chose the *Saccorhiza sp*. because it was easy to obtain, being picked from the Atlantic Ocean shoreline, in the area of Porto, Portugal. Of all the pharmaceutical products and POPs we have chosen venlafaxine because it has proved to be one of the hardest to remove from the water (RUA-GOMEZ & al [20]) and cypermethrin. The results obtained from our experiments have shown that *Saccorhiza sp*. has a high adsorption capacity for venlafaxine and cypermethrin and the removal rates were high compared to the results obtained in the previous research.

Kinetics. Results obtained from GC-ECD analyzes for cypermethrin are shown in Table 1, where C_i is the initial concentration of cypermethrin, C_t isthe concentration of cypermethrin remaining in the aqueous solution at time t, V is the volume of cypermethrin solution, q_t is the amount of cypermethrin removed at time t and % is the percentage of cypermethrin removal at time t. The percentages of cypremethrin removed from the aqueous solution were calculated and these were between 14 and 55%.

Table 1. The concentrations of cypermethrin removed from aqueous solutions and its removal percentages

Ci (ppb)	Ct (ppb)	V (L)	mass of algae (mg)	qt (mg/g)	% removal conc.
209.1	209.1	0.3	400	0.000	0
	170.9			0.048	14.57
	161.0			0.060	19.52
	158.4			0.063	20.81
	153.8			0.069	23.10
	137.3			0.090	31.34
	149.9			0.074	25.03
	131.3			0.097	34.35
	133.9			0.094	33.03
	119.8			0.112	40.11
	123.5			0.107	38.23
	117.1			0.115	41.44
	95.0			0.143	52.51
	90.8			0.148	54.61
	89.4			0.150	55.29

Experimental data has been adjusted to kinetic models using the ORIGIN program, version 9.0. The equilibrium time for cypermethrin and alga is relatively quickly reached. The time needed to reach the equilibrium was 90 minutes.The fitting parameters are summarized in Table 2.

Results obtained from HPLC analyzes for venlafaxine are shown in Table 3, where Ci is the initial concentration of venlafaxine, Ct is the concentration of venlafaxine remaining in the aqueous solution at time t, V is the volume of venlafaxine solution, qt is the amount of venlafaxine removed at time t and % is the percentage of venlafaxine removal at time t. The percentages of venlafaxine removed from the aqueous solution were calculated and these were between 64 and 71%.

Experimental data has been adjusted to kinetic models using the ORIGIN program, version 9.0. The equilibrium time for venlafaxine and alga is relatively quickly reached. The time needed to reach the equilibrium was 30 minutes. The fitting parameters are summarized in Table 4.

Equilibrium. The adsorption capacity of *Saccorhiza sp*. for each initial concentration of cypermethrin was determined.

In Table 5 are presented the amounts of cypermethrin adsorbed on the algae for each initial concentration where C_i isthe initial concentration of cypermethrin, C_e is the concentration of cypermethrin at equilibrium, V is the volume of cypermethrin solution, q_e is the concentration of cypermethrin adsorbed at equilibrium. The adsorption capacity of Saccorhiza sp. for cypermethrin varied between 0.0447 mg g^{-1} and 1.196 mg g^{-1}.

Table 2. Fitting parameters of kitetic models for cypermethrin

Pseudofirst model	q(mg g^{-1})	635899.9011
	k_1 (min^{-1})	6.87E-06
	R^2	**0.73898**
Pseudosecond model	q_t (mg g^{-1})	364560.8609
	k_2 (min^{-1})	3.29E-11
	R^2	**0.73895**

Experimental data has been adjusted to Langmuir and Freundlich models using the ORIGIN program, version 9.0.

Experimental data are better described by Freundlich model. The fitting parameters are summarized in Table 6.

Experimental data has been adjusted to Langmuir and Freundlich models using the ORIGIN program, version 9.0.

Experimental data are well described by Langmuir model. *Saccorhiza sp.* has a high adsorption coefficient (0.39 mg/g). The fitting parameters are summarized in Table 8.

Table 3. The concentrations of venlafaxine removed from aqueous solutions and its removal percentages

Ci (ppb)	Ct (ppb)	V (L)	mass of algae (mg)	qt (mg/g)	% removal conc.
4000	1430.3275	0.5	500.18	2.568747751	64.24
	1410.2375			2.588830521	64.74
	1366.872			2.632180415	65.83
	1353.706			2.645341677	66.16
	1295.38			2.703646687	67.62
	1284.364			2.714658723	67.89
	1277.5755			2.72144478	68.06
	1197.051			2.801940301	70.07
	1198.0965			2.800895178	70.05
	1197.7555			2.801236055	70.06
	1174.6245			2.824358731	70.63
	1166.949			2.832031469	70.83
	1195.7915			2.803199348	70.11
	1211.2			2.787796393	69.72

Table 4. Fitting parameters of kitetic models for venlafaxine

Pseudofirst model	q(mg g^{-1})	0.40364
	k_1 (min^{-1})	1.40E-01
	R^2	0.88286
Pseudosecond model	q_t (mg g^{-1})	4.37E-01
	k_2 (min^{-1})	0.48771
	R^2	**0.93548**

Table 5. The amounts of cypermethrin adsorbed on the algae at equilibrium

Samples	Ci (ppb)	Ce (ppb)	V (L)	mass of algae (mg)	qe (mg/g)
1	78.811	7.195	0.03	400.0000	0.044760
2	141.467	11.480		400.0000	0.081242
3	299.465	96.239		400.0000	0.127016
4	1083.250	238.052		400.0000	0.528249
5	159.378	37.602		400.0000	0.076110
6	1482.012	336.045		400.0000	0.716230
7	178.484	16.761		400.0000	0.101077
8	454.543	102.352		400.0000	0.220119
9	493.730	133.554		400.0000	0.225110
10	899.775	193.589		400.0000	0.441367
11	3031.741	833.835		400.0000	1.373691
12	1337.442	329.535		400.0000	0.629942
13	3605.908	1317.795		400.0000	1.430070
14	3228.860	1054.196		400.0000	1.359165
15	4512.559	2199.599		400.0000	1.445600
16	4905.173	2991.330		400.0000	1.196152

Table 6. Fitting parameters of Langmuir and Freundlich equilibrium models for cypermethrin

Langmuir isotherm	Q_L (mg mg-1)	18546600.00
	b (L mg-1)	3.92E-06
	R^2	0.61286
Freundlich isotherm	K_F (µg mg-1)	1.89E-04
	n (L µg-1)	0.17125
	R^2	**0.8773**

Table 7. The amounts of venlafaxine adsorbed on the algae at equilibrium

Samples	Ci (ppb)	Ce (ppb)	V (L)	mass of algae (mg)	qe (mg/g)
1	2243.8820	912.2940	0.05	250.06	0.0419
2	2243.8820	954.3435		250.47	0.0335
3	2542.6915	1033.5935		250.01	0.0475
4	2542.6915	1075.7535		250.17	0.0391
5	2665.5345	1101.5520		250.01	0.0462
6	2665.5345	1080.6060		250.02	0.0504
7	3508.4245	1464.9495		250.47	0.0577
8	3508.4245	1441.6930		250.30	0.0624
9	3222.1495	1344.5860		250.03	0.0533
10	3222.1495	1340.1710		250.16	0.0541
11	7387.7580	3072.2915		250.27	0.1242
12	7387.7580	3065.9060		250.10	0.1255
13	4760.9340	1955.7730		250.38	0.0848
14	4760.9340	1917.5500		250.00	0.0926
15	10050.1020	4444.3440		250.80	0.1158
16	10050.1020	4375.6210		250.40	0.1297
17	8678.3110	3550.8780		250.28	0.1575
18	8678.3110	3561.3010		250.50	0.1553

Table 8. Fitting parameters of Langmuir and Freundlich equilibrium models for venlafaxine

Langmuir isotherm	Q_L (mg mg-1)	0.392
	b (L mg-1)	0.00014
	R^2	**0.8780**
Freundlich isotherm	K_F (µg mg-1)	0.00022
	n (L µg-1)	1.287
	R^2	0.859

Conclusions

Saccorhiza sp. is suitable for use in the bioremediation of contaminated water with pharmaceutical products, the antidepressant venlafaxine respectively, and for POPs, cypermethrin respectively because the equilibrium is quickly reached. The time required to reach the equilibrium state was 30 minutes for venlafaxine and 90 minutes for cypermethrin.

In terms of kinetics, experimental data are best described by the pseudo-order two kinetic model for venlafaxine and by both kinetic models for cypermethrin.

In terms of equilibrium, experimental data are best described by the Langmuir model for venlafaxive and by Freundlich model for cypermethrin.

Adsorption capacity of *Saccorhiza sp*. for venlafaxine was 0.39 mg g^{-1}, and venlafaxine removal rates in the aqueous solution were 64-71%. Adsorption capacity of *Saccorhiza sp*. for cypermethrin varied between 0.0447 mg g^{-1} and 1.196mg g^{-1}, and cypermethrin removal rates in the aqueous solution varied between 14 and 55%.

Because *Saccorhiza sp*. is a widely grown marine alga and is easy and inexpensive to obtain, it is a good candidate for use in the bioremediation of venlafaxine-contaminated water.

The data obtained in our research demonstrates that brown marine algae have a decontamination capacity of water contaminated with cypermethrin and venlafaxine higher than other studied organisms, and the time required to clean up contaminated water is much shorter.

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