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Biosorption of Tetracycline and Cephalexin onto Surfactant-Modified Waste Biomass Using Response Surface Methodology and Ecotoxicological Assessment: Phytotoxicity and Biotoxicity Studies

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Abstract Waste hazelnut shell was modified using hexadecyltrimethylammonium (HDTMA) to remove tetracycline and cephalexin from water and minimize the residual antimicrobial activity of tetracycline and cephalexin. Response surface methodology (RSM) was used to determine the effect of solution pH (3.0–6.0–9.0), initial pollutant concentration (5–52.5–100 mg/L), contact time (5–92.5–180 min), and temperature (20–35–50°C) on the removal efficiency of tetracycline and cephalexin. Comparison between model results and experimental data gave a high coefficient of determination (R^2_{TC} 0.94, R^2_{CPX} 0.99). The predicted removal efficiency of tetracycline and cephalexin by the RSM design was 37.34% and 83.07%, respectively. Langmuir, Freundlich, D-R, and Temkin isotherm were applied to equilibrium data. The Q^o values for tetracycline and cephalexin were 6.97 and 47.77, respectively. Acute tests were performed before and after biosorption using *Lepidium sativum* and *Daphnia magna*. IC_{50}, $LC_{50,}$ and toxic unit were determined. IC_{50} values for root and shoot for tetracycline and cephalexin were 50 mg/L, 50 mg/L, 140 mg/L, and 270 mg/L, respectively. LC_{50} values of tetracycline and cephalexin were 58 mg/L and 37 mg/L for 48 h, respectively. There was a large decrease in mortality (%) after biosorption. This biosorbent was effective in the biosorption of tetracycline and cephalexin and in reducing toxicity.

Keywords Biosorption · Cephalexin · HDTMA · Ecotoxicity · RSM · Tetracycline

1 Introduction

Antibiotics are widely used worldwide to treat human, animal, and plant infections (Aslan & Şirazi 2020). A large part of antibiotics are not used in metabolism and they are excreted directly into the receiving environment via urine and feces (Bangari & Sinha 2019; Xu et al. 2019; Grenni et al. 2018). These substances, even in trace amounts (ng/L or µg/L), have high acute toxicity and cause environmental degradation (Gil et al., 2019). Thus, it is of great significance to remove antibiotic residues in wastewater from sources such as residences, hospitals, and pharmaceutical factories before discharging them to the environment (Bangari & Sinha 2019). Methods such as adsorption, filtration, sedimentation, flotation, and flocculation are traditionally used to remove pharmaceuticals from water (Priyan et al., 2021). Among these methods, adsorption is preferred because of its simple design, ease of manufacture, high efficiency, low cost, and

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no high toxic by-products (Bangari & Sinha 2019; Zhang et al. 2017; Xiang et al. 2019). The key factor for the adsorption process is adsorbents. The usability of waste biomass as a biosorbent was studied for the removal of many chemicals from wastewater (Al-Yousef et al., 2021; Paunovic et al., 2019; Karri et al., 2020; Chen et al., 2021). Furthermore, the reuse of waste represents both one of the foundations of the Circular Economy philosophy and an example of a green process and solid waste utilization (Vilardi et al., 2020).

One of the main forms of agricultural waste in Turkey is hazelnut shells (Sürmen, 2003). Various studies have shown that unprocessed almond, hazelnut, and walnut shells have a biosorption capacity against both metal cations and metal anions (Vilardi et al., 2020; Cataldo et al., 2018; Cimino et al., 2000; Mehrasbi et al., 2009). In the removal of antibiotics from wastewater by the adsorption process, the adsorbent-antibiotic complex is excreted into the environment (Mosa et al., 2020; Lv et al., 2019). According to previous studies, desorption process can follow the adsorption process, especially in the soil environment under the appropriate conditions (Mosaleheh & Sarvi 2020; Riaz et al., 2019; Zhang et al., 2017a). This desorption process can be a major drawback of the adsorption process. Generally, the antimicrobial activity of adsorbent-antibiotic complexes has been ignored in studies (Mosaleheh & Sarvi 2020). A study by Mosaleheh and Sarvi (2020) was evaluated the residual antimicrobial activity of adsorbent-antibiotic complex using an agar disk (Mosaleheh & Sarvi 2020).

This study aimed to develop an adsorbent with minimized residual antimicrobial activity after adsorption. To this end, the waste hazelnut shell was organically modified with hexadecyltrimethylammonium (HDTMA). Thus, the study results were intended to give a new perspective on the safe biosorption of tetracycline (TC) and cephalexin (CPX) with minimal residual antimicrobial activity. The reason for using TC and CPX in the study is that they are one of the most commonly prescribed antibiotics and are frequently detected in the groundwater and surface water (Lin et al., 2009; Watkinson et al., 2009; Charuaud et al., 2019). In addition, TCs are used as a growth promoter in livestock (Ian & Marilyn, 2001). Most TC antibiotics are excreted in the feces as a parent compound and reach the food chain through biological enrichment and concentration (biomagnification) (Rodrigues et al., 2019).

Daphnia magna has been widely used for many years in toxicity studies (Wollenberger et al., 2000; Tongur & Yıldırım, 2015). The *D. magna* test is cost-effective and meets the criteria of the 3R rule (Tkaczyk et al., 2021; Bownik, 2019; Krewski et al., 2020).

In recent years, response surface methodology (RSM) is used for modeling and optimizing various processes (Zhang et al., 2017b; Gangil & Pradhan, 2017). In this study, RSM was used to obtain the best system performance using a limited number of experimental experiments and to interpret the effects of the variables.

In light of all the information explained above, this study has many aims. (i) Firstly, it aims to develop a biosorbent with minimized residual antimicrobial activity after adsorption. To this end, the waste hazelnut shell was modified with HDTMA. After then, it was used for the biosorption of TC and CPX. (ii) Important experimental parameters affecting biosorption, such as initial pollutant concentration, solution pH, contact time, and temperature, were investigated using the RSM approach. Analysis of variance (ANOVA) was conducted to investigate the relative importance of independent variables. Isotherm studies were carried out to examine the behavior of the biosorption process. (iii) Phytotoxicity studies were performed on TC and CPX exposure with *Lepidium sativum* seed, and growth inhibition (%), inhibition concentration (IC_{50}), and toxic unit (TU) values were obtained. (iv) Acute biotoxicity studies were performed on tetracycline and cephalexin exposure on *Daphnia magna*, and lethal concentration (LC_{50}) and toxic unit (TU) values were obtained. (v) The change in phytotoxicity and biotoxicity after biosorption was evaluated.

To summarize, this study aims is to ensure efficient removal of tetracycline and cephalexin from wastewater with waste biosorbent with minimized residual antimicrobial activity, to determine the IC_{50}, LC_{50}, and TU values of tetracycline and cephalexin with acute toxicity studies, to perform their toxicological classifications, and to determine the change in acute toxicity after biosorption.

2 Material and Methods

2.1 Materials and Reagents

Tetracycline (TC) and cephalexin (CPX) were obtained from Sigma-Aldrich. All chemical reagents

were of analytical grade and purchased from Merck and Sigma-Aldrich companies. NaOH and HCl were used to adjust the pH of the mixture. The preparation and modification of biosorbent (waste biomass; (WB)) and schematic diagram of the cationic exchange reaction between Na and HDTMA are given in section S1 and Fig. S1 (in Supplementary Material).

2.2 Biosorption Testing

The solution volume of 100 ml was added in an Erlenmeyer, and shaken at 180 rpm for different contact times and subsequently centrifuged for 15 min. TC (λ 357 nm) and CPX (λ 230 nm) concentrations were determined using a spectrophotometer (Spectroquant Pharo 300, Merck). In the study, initial pollutant concentration (5–52.5–100 mg/L), solution pH (3.0–6, 0–9.0), contact time (5–92.5–180 min), and temperature (20–35–50°C) were varied. The amount of biosorbent was kept constant at 5 g/L. The experiments were designed with various combinations to study synergistic effects on the removal efficiency. The biosorbed amount of biosorbate at equilibrium time (qe; mg/g) and (%) removal efficiency were calculated according to Eqs. 1 and 2, respectively.

$$qe = \frac{(Co - Ce)}{m} \times \mathrm{V} \tag{1}$$

$$\text{Removal}\ (\%) = \frac{(Co - Ce)}{Co} \times 100 \tag{2}$$

where Co and Ce are the initial and equilibrium biosorbate concentrations (mg/L), V is the volume of solution (L), and m is the mass of biosorbent.

2.3 Statistical Experimental Design

In this study, a subset of RSM was applied to study the adsorption parameters of TC and CPX (pH of solution-X_1, initial concentrations-X_2, temperature-X_3, and contact time-X_4). The Design Expert 7.0.0 (trial version, Stat Ease Inc., Minneapolis, USA) statistical software was used for the model fitting and significance for the adsorption efficiency and the adsorbed quantity of TC and CPX. The analysis of ANOVA was used to ensure a satisfactory model for biosorption work. Removal efficiency (%) was used as the response variable. The removal efficiencies (%) of TC and CPX are shown as Y1 and Y2, respectively. Initial pollutant concentration (5–52.5–100 mg/L), solution pH (3.0–6.0–9.0), contact time (5–92.5–180 min), and temperature (20–35–50°C) were chosen as the independent process variables. The amount of biosorbent was kept constant at 5 g/L. As indicated in Table 1, four independent process variables, initial pollutant concentration, solution pH, contact time, and temperature were segregated into four levels with a coded value. Then, the RSM with a Box–Behnken design was applied.

2.4 Isotherm Studies and Batch Data Modeling

Isotherm studies were performed based on the optimum removal conditions obtained from RSM experiments at different initial concentrations (2.5–5–10–20–30–60–90–120 mg/L) for TC and CPX. Biosorption systems were investigated using Langmuir, Freundlich, Dubinin-Radushkevich (D-R), and Temkin isotherm models.

2.4.1 Langmuir Isotherm

The Langmuir equation is represented by the following expression (Tomul et al., 2019):

$$\mathrm{qe} = \frac{\mathrm{Q^o K_L Ce}}{1 + \mathrm{K_L Ce}} \tag{3}$$

where Q^o denotes the amount of biosorbed monolayer capacity (mg/g); K_L is the Langmuir isotherm constant (L/mg).

2.4.2 Freundlich Isotherm

Freundlich isotherm describes the exponential distribution and energies of active centers by the biosorption process occurring on a multilayered and

Table 1 Levels of factors and independent variables used for Box–Behnken experimental design

Variables	Symbols		Coded levels		
	Uncoded	Coded	−1	0	1
pH	X_1	x_1	3.0	6.0	9.0
C_0 (mg/L)	X_2	x_2	5	52.5	100
T (°C)	X_3	x_3	20	35	50
time (min)	X_4	x_4	5	92.5	180

heterogeneous surface (Khandaker et al., 2020). Freundlich isotherm is represented as follows:

$$qe = K_F Ce^{1/n} \quad (4)$$

where K_F denotes the Freundlich isotherm constant (L/g) and n represents the exponent of the Freundlich constant. When ($1/n>1$) the biosorption is unfavorable, when ($1/n=1$) the biosorption is homogeneous, and when ($0<1/n<1$) the biosorption is favorable (Bougdah et al., 2020).

2.4.3 D-R Isotherm

D-R isotherm model is used to predict the physical or chemical mechanism of biosorption process (Mate & Mishra, 2020). The equation for D-R isotherm is given as:

$$qe = q_m \exp^{-\beta\varepsilon^2} \quad (5)$$

where q_m is the maximum biosorption capacity (mg/g); β corresponds to the activity coefficient (mol^2/kJ^2), and ε represents the Polanyi potential (kJ/mol), (RTln(1+(1/Ce)). The parameter β could be used to estimate the mean free energy $\left(E = 1/\sqrt{2\beta}\right)$ which could distinguish the type of biosorption process. When the magnitude E was $<8 kJmol^{-1}$, the biosorption process was physical adsorption, and when E was between 8 and 16 $kJmol^{-1}$, the process was chemical adsorption (Chabani et al., 2006).

2.4.4 Temkin Isotherm

The equation for Temkin isotherm represented as (Abutaleb et al., 2020):

$$qe = \frac{RT}{b_T} \ln\left(K_T Ce\right) \quad (6)$$

where b_T is Temkin isotherm constant (g kJ/mg mol); K_T is Temkin isotherm equilibrium binding constant (L/mg); T is temperature (K), and R is universal gas constant (8.314 J/molK).

2.5 Toxicity Experiments

Phytotoxicity experiments with *Lepidium sativum* and biotoxicity experiments with *Daphnia magna* are given in sections S2 and S3 (S2 and S3 in Supplementary Material).

2.6 Toxicity Classification

The toxicities of TC and CPX were checked following the guidelines of the Commission of the European Communities (96/67/EEC) (EC, 1996). Toxic Unit (TU) values were calculated based on Eq. 7. The toxicity classification of the substance based on TU value is given in Table S1 (Table S1 in Supplementary Material).

$$TU = \frac{1}{LC50\ \left(IC_{50}\right)} \times 100 \quad (7)$$

where;

LC_{50} or IC_{50} is the concentration of water samples when half of the aquatic organisms are dead or showed growth inhibition

3 Results and Discussion

3.1 Optimization and Statistical Analysis Using RSM for TC and CPX Biosorption

A total of 29 runs proposed by the RSM design were prepared with different combinations of solution pH-X_1, initial pollution concentration-X_2, temperature-X_3, and contact time-X_4. Five experiments were repeated for the center points. Experimental design as coded equations for TC and CPX is given in Table S2 and Table S3, respectively (Table S2 and S3 in Supplementary Material). The results for TC and CPX could be expressed by a quadratic equation (Açıkel et al., 2010). Linear equations related to the parameters affecting biosorption of TC and CPX by HDTMA-WB are presented in Eqs. (8) and (9), respectively:

$$\begin{aligned} Y_1 = {} & +31.4467 - 4.93469\,X_1 - 0.44298\,X_2 + 0.40141 \\ & X_3 - 0.16568\,X_4 - 0.059855\,X_1 X_2 - 0.027724\,X_1 X_3 \\ & + 1.14738E^{-3}\,X_1 X_4 - 2.30614E^{-3} \\ & X_2 X_3 + 1.72427E^{-3}\,X_2 X_4 - 1.77619E^{-3}\,X_3 \\ & X_4 + 0.96252\,X_1{}^2 + 4.27828E^{-3} \\ & X_2{}^2 + 1.39109E^{-3}\,X_3{}^2 + 3.61499E^{-4}\,X_4{}^2 \end{aligned} \quad (8)$$

$$Y_2 = -4.62782 + 6.59986\,X_1 - 0.027110\,X_2 + 1.24682\,T - 0.012598\,X_3 + 1.75439E^{-5}\,X_1\,X_2 - 0.037667\,X_1\,X_3 + 9.52381\mathrm{E}^{-6}\,X_1\,X_4 - 7.71930\,E^{-5}\,X_1\,X_3 + 3.72932E^{-4}\,X_1\,X_4 - 6.47619E^{-5}\,X_3\,X_4 \quad (9)$$

Analysis of variance (ANOVA) was used to assess the adequacy of the model and all associated data reported in Table S4 (Table S4 in Supplementary Material). The statistical accuracy of the model was checked with the *F*-test, and the *F*-values were found to be 16.79 and 236.72 for biosorption of TC and CPX onto HDTMA-WB, respectively. Since the probability value is smaller than the *F*-value, it is understood that the model is compatible. (*P*)>*F* less than 0.05 indicates that the model terms are significant (Mohammadi et al., 2022). In cases when the probability value is greater than 0.1000, the design becomes insignificant. The *p*-values for TC and CPX were found to be <0.01, and the validity of the model was proved. The coefficient of determination R^2 value of the model was found to be 94.38 and 99.25 for the removal of TC and CPX, respectively. The closer the R^2 value is to 1, the better correlation between experimental values and model values (Erşan & Açıkel 2014). Only 5.62% and 0.75% of the model for the removal of TC and CPX cannot be expressed with the equation. R^2_{Adj} (0.89) and R^2_{Pred} (0.67) values for TC removal are close and compatible. R^2_{Adj} (0.99) and R^2_{Pred} (0.98) values for CPX removal are close and compatible. The values of lack of fit (0.49) and fit (0.8542) showed that the equation is sufficient to provide for the removal of TC and CPX. Experimental data, "Adeq Precision," measures the signal-to-noise ratio. The values predicted by the model should be a ratio greater than 4 (Safari et al., 2018). A ratio of 16.198 and 64.353 for TC and CPX indicates an adequate signal, respectively.

The three-dimensional response surfaces obtained for the removal of TC and CPX using the design expert are shown in Fig. 1a, b, and c; Fig. 2a, b, and c; Fig. 3a, b, and c; and Fig. 4a , b, and c, respectively.

According to Fig. 1a, it was observed that the initial TC concentration and pH increase together affected the TC biosorption and the increased pH values increased the removal efficiency. When the effects of temperature and pH are examined together, it is understood from the linear effects that the temperature is not very effective on TC removal efficiency at high pH values (Fig. 1b). According to Fig. 1c, it is observed that TC removal efficiency decreases with the increased contact time at a high pH value. It is thought that the increased contact time may cause desorption (Fig. 1c). When Fig. 2a is examined, it is understood that the temperature at a high initial TC concentration affects the removal efficiency. At the increased initial TC concentration, the second-order effects of time on TC removal were dominant (Fig. 2b). The increase in contact time and temperature together had a linear effect on TC removal (Fig. 2c). The changes observed in the graphs are confirmed by Eq. 8.

According to Fig. 3a, the initial CPX concentration and pH increase had a linear effect on CPX biosorption. It was observed that the increased pH values increased the removal efficiency. When the effects of temperature and pH were examined together, CPX removal efficiency at the maximum level was obtained at high pH and temperature values (Fig. 3b). At increased pH values, CPX removal is quite high even at low contact times (Fig. 3c). When Fig. 4 a is examined, it is observed that the initial concentration is not very effective on removal efficiency at high temperature values. The collective and second-order effects of contact time on CPX removal at increased initial CPX concentration values were dominant (Fig. 4b). It was observed that the effect of increasing contact time and temperature together on CPX removal was more effective than TC (Fig. 4c). The changes observed in the all graphs are confirmed by equation 8 and 9.

3.2 Evaluation of Optimum Conditions and Adsorption Mechanism

The optimal conditions for the removal of TC using HDTMA-WB with RSM experimental design were determined as the initial TC concentration of 9.40 mg/L, solution pH 8.66, a temperature 25.96 °C, and contact time 146.47 min. The optimal conditions obtained from CPX removal studies using HDTMA-WB with RSM experimental design were determined as the initial CPX concentration of 29.26 mg/L, solution pH 8.31, a temperature 36.53 °C, and contact time 100.71 min. Under optimal conditions, the removal efficiencies of TC and CPX by HDTMA-WB were found to be 37.34% and 83.07 %, respectively.

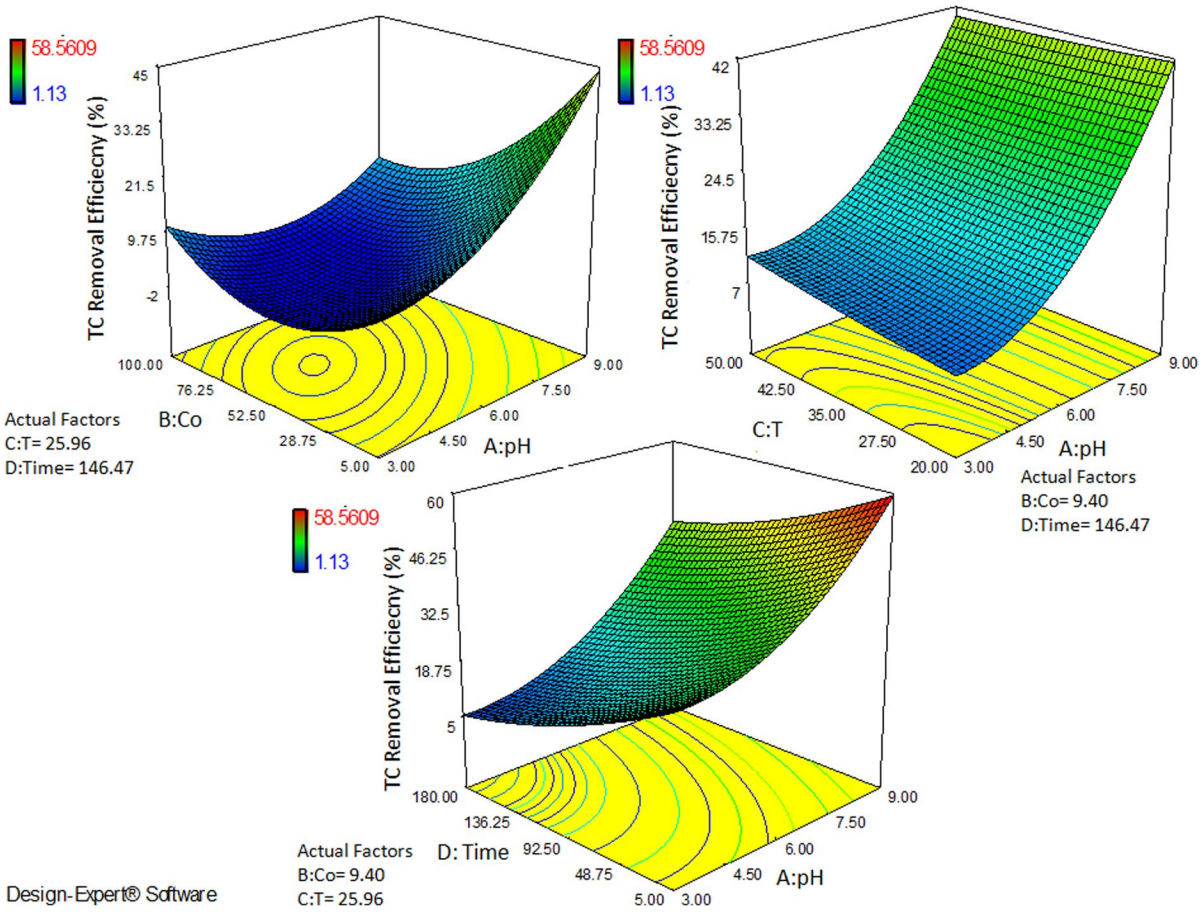

Fig. 1 The 3D graph showing the change in TC biosorption by HDTMA-WB with RSM with **a** pH-initial TC concentration, **b** pH-temperature, **c** pH-contact time

The removal efficiency of pharmaceutical compounds is directly affected by the pH of the solution (Hiew et al., 2019). The molecular structure of TC includes different functional groups such as amino, carboxyl, ketone, and phenols. TC has three pKa values (pka_1 3.30, pka_2 7.70, pka_3 9.70). There are four types of TC ions, including TCH_3^+ (cationic) (pH <3.30), TCH_2^0 (zwitterionic) (3.30 <pH <7.70), TCH^- (anionic) (7.70 <pH <9.70), and TC^{2-} (anionic) (pH ˃ 9.70), under different pH conditions (Wu et al., 2016). TC removal efficiency increased with the increase in pH and reached the optimum condition (37.34%) at pH 8.66. At this pH, TC is in anionic form. Therefore, TC in the anionic form and HDTMA-WB in the cationic form can easily interact with the surface complexation reaction.

The pka_1 and pka_2 values of CPX are 2.56 and 6.88, respectively (Legnoverde et al., 2014). This indicates that CPX molecules exist as cation, zwitterion, and anion forms at pH <2.56, 2.56–6.88, >6.88, respectively (Zhao et al., 2020). Although the CPX removal efficiency was high at all pH values studied, it increased with the increase in pH and reached the optimum condition (83.07%) at pH 8.31. The increase in solution pH gradually turns CPX molecules into a negatively charged form and causes an increase in electrostatic attraction between organic clusters (polarity/positive charge derived from quaternary ammonium group, etc.) of HDTMA-WB and organic group –COOH, $–NH_2$) of CPX (Zhao et al., 2020; Karnjanakom & Maneechakr, 2019). Similar results in different studies reported the effect of pH in increasing the removal of TC and CPX (Bangari

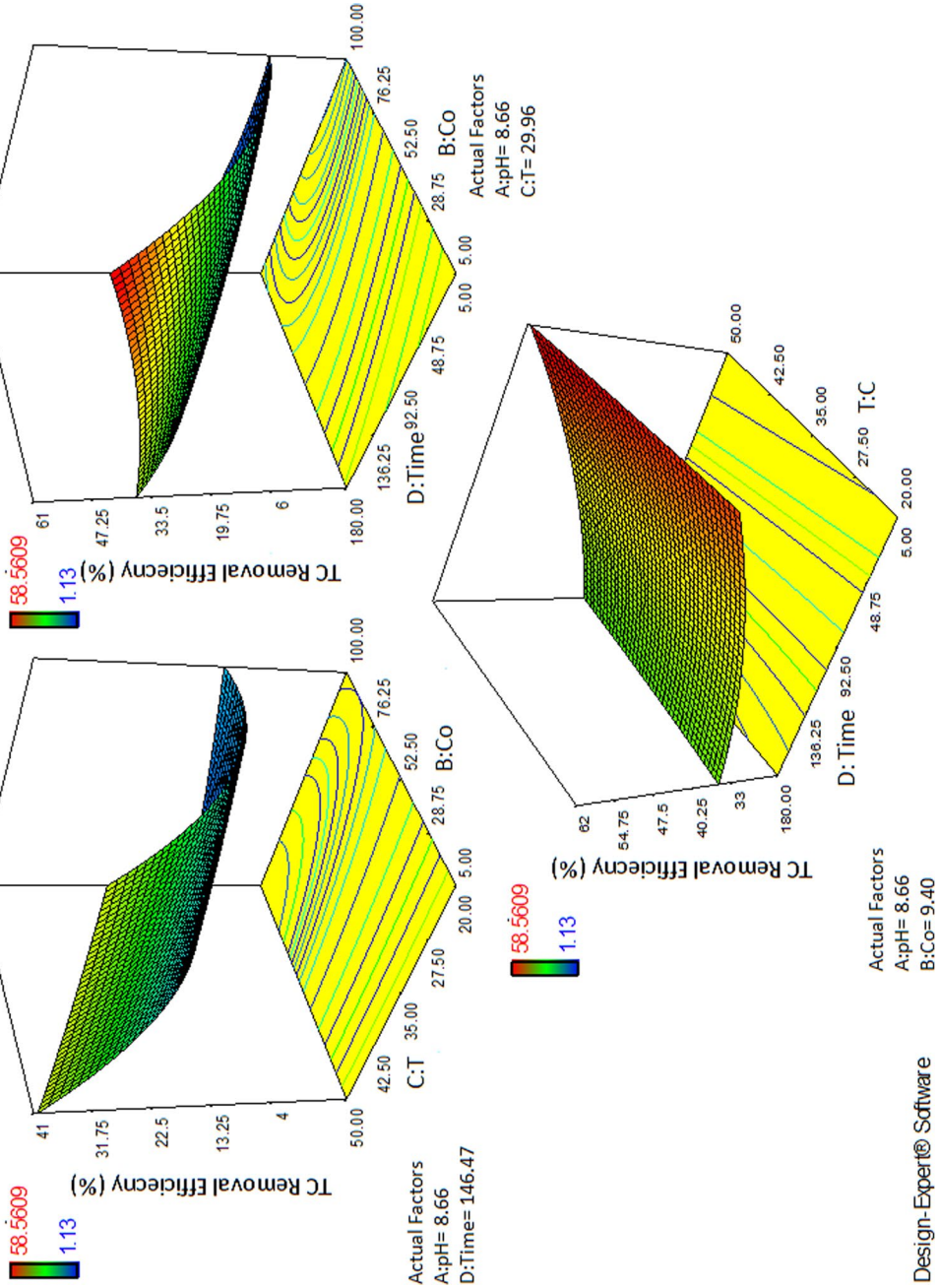

Fig. 2 The 3D graph showing the change in TC biosorption by HDTMA-WB with RSM with **a** initial TC concentration-temperature, **b** initial TC concentration-contact time, **c** temperature-contact time

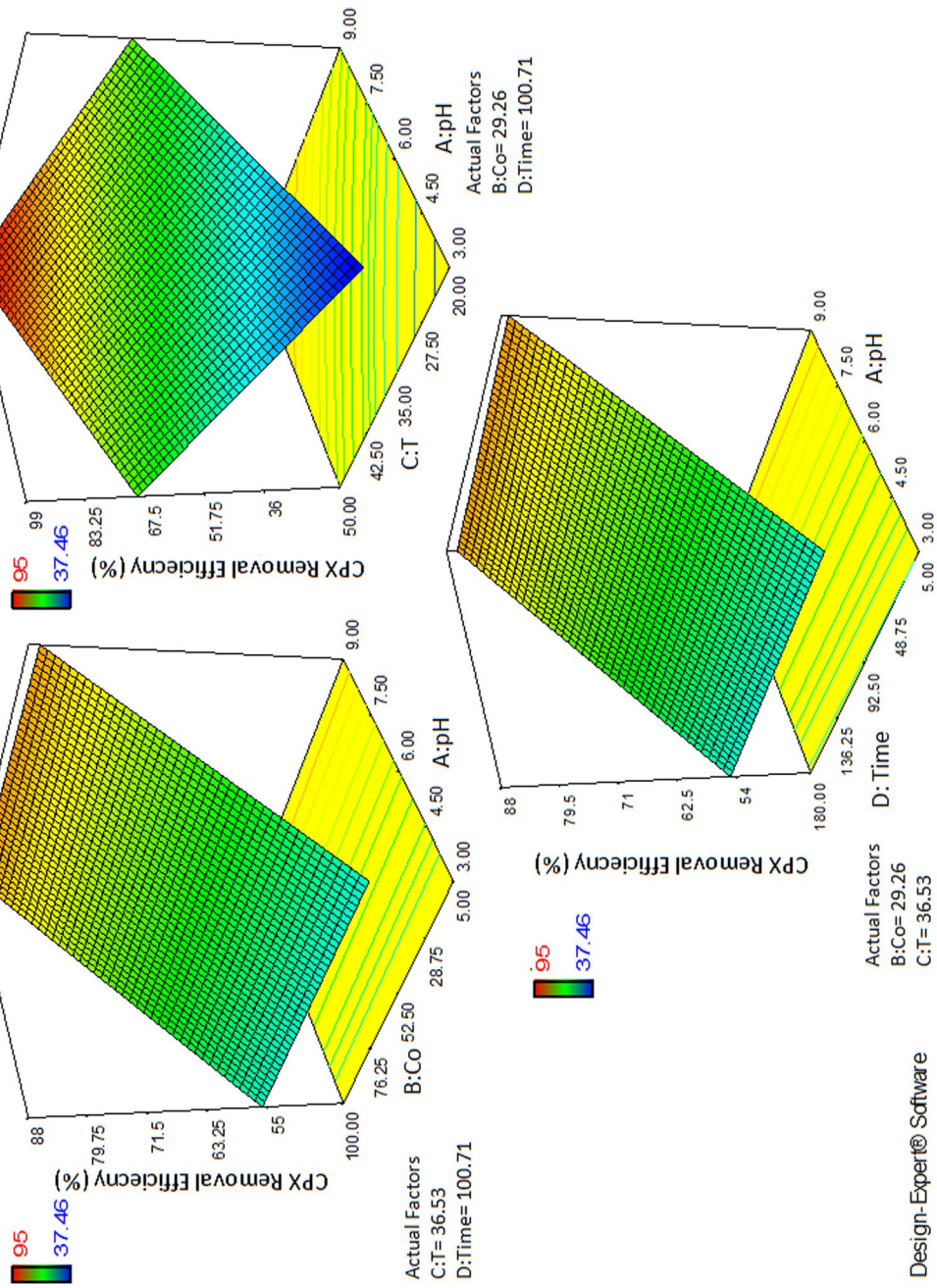

Fig. 3 The 3D graph showing the change in CPX biosorption by HDTMA-WB with RSM with **a** pH-initial TC concentration, **b** pH-temperature, **c** pH-contact time

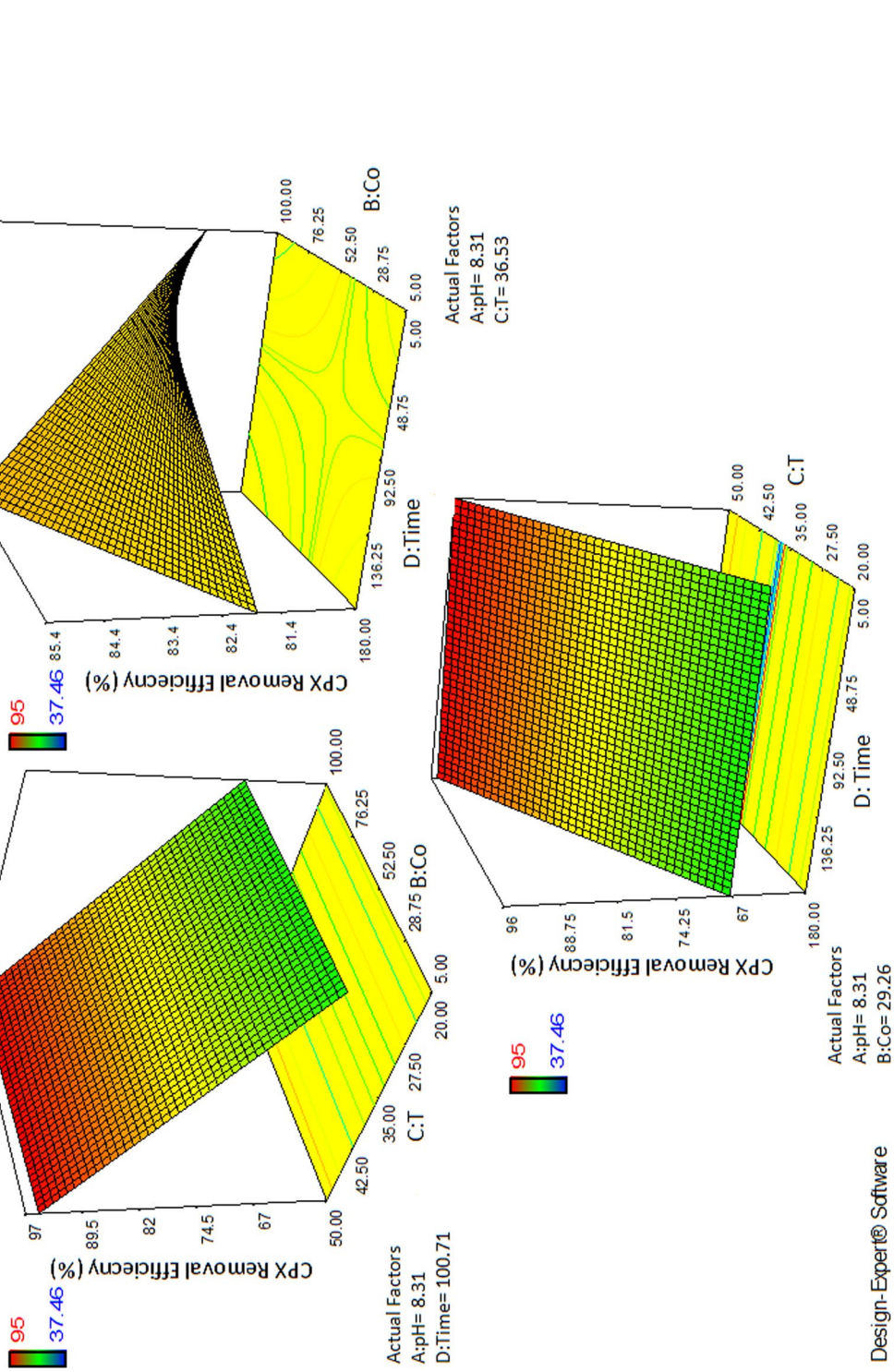

Fig. 4 The 3D graph showing the change in CPX biosorption by HDTMA-WB with RSM with **a** initial CPX concentration-temperature, **b** initial CPX concentration-contact time, **c** temperature-contact time

& Sinha, 2019, Zhao et al., 2020; Topal & Arslan Topal, 2020). Furthermore, at high pH values, more OH^- ions in the solution activate the amino group in TC and CPX. In this case, the adsorption mechanism between antibiotics and HDTMA-WB is related to the amino affinity (Bangari & Sinha, 2019).

Initial pollutant concentrations are an important factor affecting removal efficiency. Initial concentrations of TC and CPX under optimal conditions were found to be 9.40 mg/L and 29.26 mg/L, respectively. The removal efficiency decreased with increasing initial TC concentration. This situation was explained by Topal and Arslan Topal (2020) with the decrease of adsorbent activity. When the initial CPX concentration increased, removal efficiency increased. Increasing initial CPX concentrations provided higher collision between CPX and HDTMA-WB surface and increased uptake of CPX (Taoufik et al., 2021).

The optimal temperature for the removal of TC and CPX was found to be 25.96 °C and 36.53 °C, respectively. The decrease in removal efficiency with increasing temperature indicates an exothermic sorption process (Shams et al., 2021). The increase in CPX removal efficiency with the increased temperature may be related to the expansion of the active sites of the adsorbent, and they are becoming more attractive to the adsorbate (Pouretedal & Sadegh, 2014).

3.3 Biosorption Isotherms

The biosorption process of TC and CPX onto HDTMA-WB was investigated using adsorption isotherms such as Langmuir, Freundlich, D-R, and Temkin isotherm models. Plots and parameters of isotherms are shown in Fig. S2 and Table 2 (Fig. S2 in Supplementary Material).

The value of Q^o (mg/g) for TC and CPX was found to be 6.97 and 47.77, respectively. From the plot, n values for TC and CPX were found to be 2.00 and 1.16, respectively. The value of R^2 was found to be more significant, showing that the biosorption of TC and CPX follows Langmuir isotherm indicating to monolayer adsorption of TC and CPX on the adsorbent surface via electrostatic force. According to the above result, CPX biosorption presented a higher qmax value than that of TC biosorption. Q^o values of various biosorbents in the literature and the present study were compared in Table S5 (Table S5 in Supplementary Material). The apparent energy for biosorption of TC and CPX was found to be 7.70 and 9.18 which shows that the biosorption processes of TC and CPX are physical and chemical, respectively. The values for b_T for TC and CPX are 1725.18 and 286.54, respectively.

Table 2 Isotherm parameters for biosorption of TC and CPX onto HDTMA-WB

Isotherm model	TC	CPX
Langmuir isotherm		
R^2	0.9597	0.8257
Q^o	6.97	47.77
K_L	0.0467	0.0428
Freundlich isotherm		
R^2	0.9404	0.8072
n	2.00	1.16
k_F	0.64	1.89
D-R isotherm		
R^2	0.9705	0.7612
q_m	1.176×10^{-4}	0.028
β	8.462×10^{-9}	5.932×10^{-9}
E	7.70	9.18
Temkin isotherm		
R^2	0.9615	0.8739
b_T	1715.18	286.54
K_T	0.541	0.563

3.4 Phytotoxic Effects

The growth inhibition (%), IC_{50}, and toxic Unit (TU) values for TC and CPX determined by the inhibition of root and shoot growth of *Lepidium sativum* are given in Fig. 5a and b, and Table S6, respectively (Table S6 in Supplementary Material). All *Lepidium sativum* test cultures showed higher toxicity for TC than for CPX, but a difference in toxicity trend was observed depending on the pollutant concentration. The length of the root and shoot decreased and growth inhibition (%) increased as the concentrations of TC and CPX increased.

The IC_{50} values for root and shoot for TC and CPX were 50 mg/L, 50 mg/L, 140 mg/L, and 270 mg/L, respectively. According to the toxicity classification, TC showed acute toxicity (III) to root and shoot of the *Lepidium sativum*. CPX showed slight acute toxicity (II) to the root of *Lepidium sativum*, no acute toxicity (I) to the shoot of a plant. TC has been found to have stronger detrimental effects on

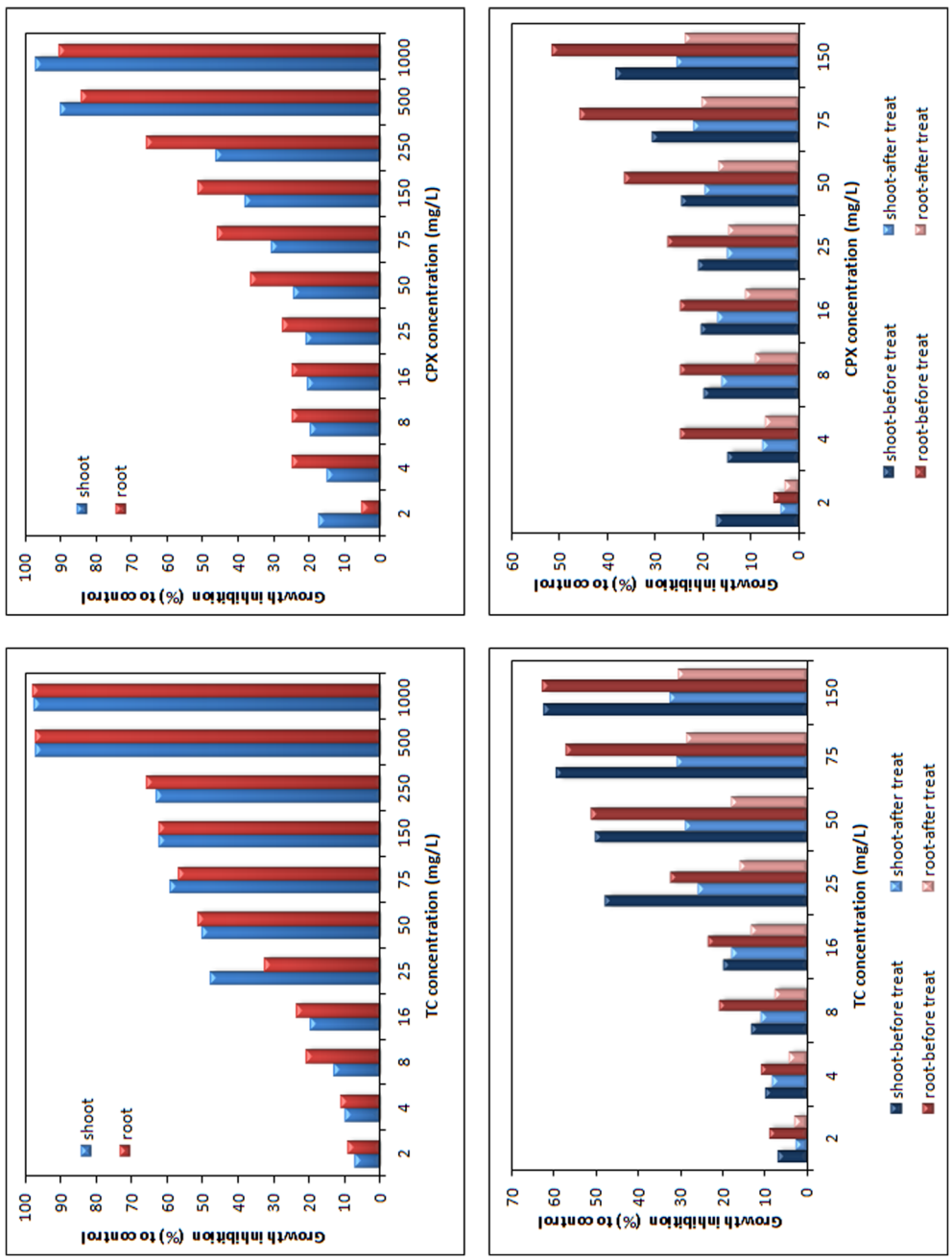

Fig. 5 Inhibition of growth (%) of *Lepidium sativum* seeds at various concentrations of TC (**a**) and CPX (**b**), ecotoxicological response of *Lepidium sativum* seeds to pollution of TC (**a1**) and CPX (**b1**) before and after biosorption

Lepidium sativum than on CPX. In this process, acute TC concentrations are thought to affect the photosynthetic process of *Lepidium sativum* chloroplasts by inhibiting oxygen production and the growth of plants (H´ajkov´a et al., 2019). According to Boonsaner and Hawker (2012), the uptake and accumulation of TC found as zwitterion under typical environmental conditions passes through passive diffusion like neutral organics. Organic and cation forms of TC are transported through aqueous diffusion or active transport mechanisms. The uptake and translocation of different TC forms can vary within plants (Boosaner & Hawker 2012). Boosaner and Hawker (2012) reported that in the rice root exposed to aqueous zwitterionic TC concentrations of 50 mg L for 15 days, no TC bioconcentration and translocation to shoots or harmful effects on plants were observed (Boosaner & Hawker 2012). Tongur and Yıldırım (2015) found the EC_{50} values of TC towards *Lepidium sativum* as 4.75 mg/L and 5.90 mg/L for root and hypocotyls (Tongur & Yıldırım, 2015). EC_{50} values of TC towards *L. minor* and *L. Gibba* were found to be 1.06 mg/L and 0.723 mg/L respectively Brain et al. (2008). In the literature review, no acute toxicity studies of CPX were found.

The phytotoxic effects of TC and CPX before and after biosorption were determined by the inhibition of root and shoot growth of *Lepidium sativum* (Fig. 5a1, b1).

After the biosorption process, we see a large increase in root and shoot length of all seeds and a large decrease in growth inhibition (%). Before biosorption, at 150 mg/L initial TC and CPX solutions, the highest growth inhibition (%) for shoot and root was calculated as 62%, 62.3%, 37.8%, and 51.3%, respectively. After biosorption, growth inhibition (%) for shoot and root decreased to 32.2%, 30.2%, 25.2%, and 23.3%. The decrease in growth inhibition (%) is due to the removal of toxic micropollutants TC and CPX from water by HDTMA-WB.

3.5 Biotoxic Effects

The mortality (%), LC_{50}, and toxic unit (TU) values for TC and CPX determined by the number of immobilized *D. magna* are given in Fig. 6a and b, and Table S7, respectively (Table S7 in Supplementary Material).

After 48-h exposure, LC_{50} values were 58 mg/L for TC, and 37 mg/L for CPX. TC and CPX showed acute toxicity (III) for 48 h towards the *D. magna*. CPX has been found to have a more lethal effect on *D. magna* than TC. *D. magna* acute biotoxicity was highly dependent on the exposure time of the test organisms. As the incubation time increased, the toxicity of the substances tested increased. Wollenberger et al. (2000) studied the acute and chronic toxicity of nine antibiotics commonly used in intensive fisheries on freshwater crustaceans and suggested that the no observed effect concentration (NOEC) of TC on *D. magna* was 340 mg/L (Wollenberger et al., 2000). Wang et al. (2008) studied the acute toxicity of TC on D. *magna,* and the 48-h LC_{50} value for *D. magna* was reported as 617.2 mg/L. Considering these studies, the lowest LC_{50} values and the highest toxicity values were found in our study. In this case, we determined that, unlike other studies, TC has an acute toxic effect on the aquatic organism (Wang et al., 2008).

The biotoxic effects of TC and CPX before and after biosorption were evaluated on *D. magna* (Fig. 6a1, b1).

After the biosorption process, there was a large decrease in the mortality rate (%). Before biosorption, at 150 mg/L initial TC and CPX solutions, mortality (%) was calculated as 60%, 100%, 40%, and 60%, respectively. After biosorption, mortality (%) was decreased to 20%, 40%, 20%, and 40%. This shows that the effective removal of TC and CPX from aqueous solution by HDTMA-WB. Similar results to the results obtained before and after removal of TC have also been confirmed by other authors Huo et al. (2022) and Zhang et al. (2022).

4 Conclusion

Waste hazelnut shell (WB) was modified with HDTMA to reduce its antimicrobial activity and used for biosorption of TC and CPX. The RSM was used to evaluate the effects of solution pH, contact time, initial pollutant concentration, and temperature parameters on removal efficiency. The RSM results showed that these parameters have a direct effect on biosorption efficiency. In the predicted optimal conditions to RSM, the removal efficiency of TC (37.34%) was optimized at pH 8.66, 9.40 mg/L initial TC concentration, 146.47 min contact time, and

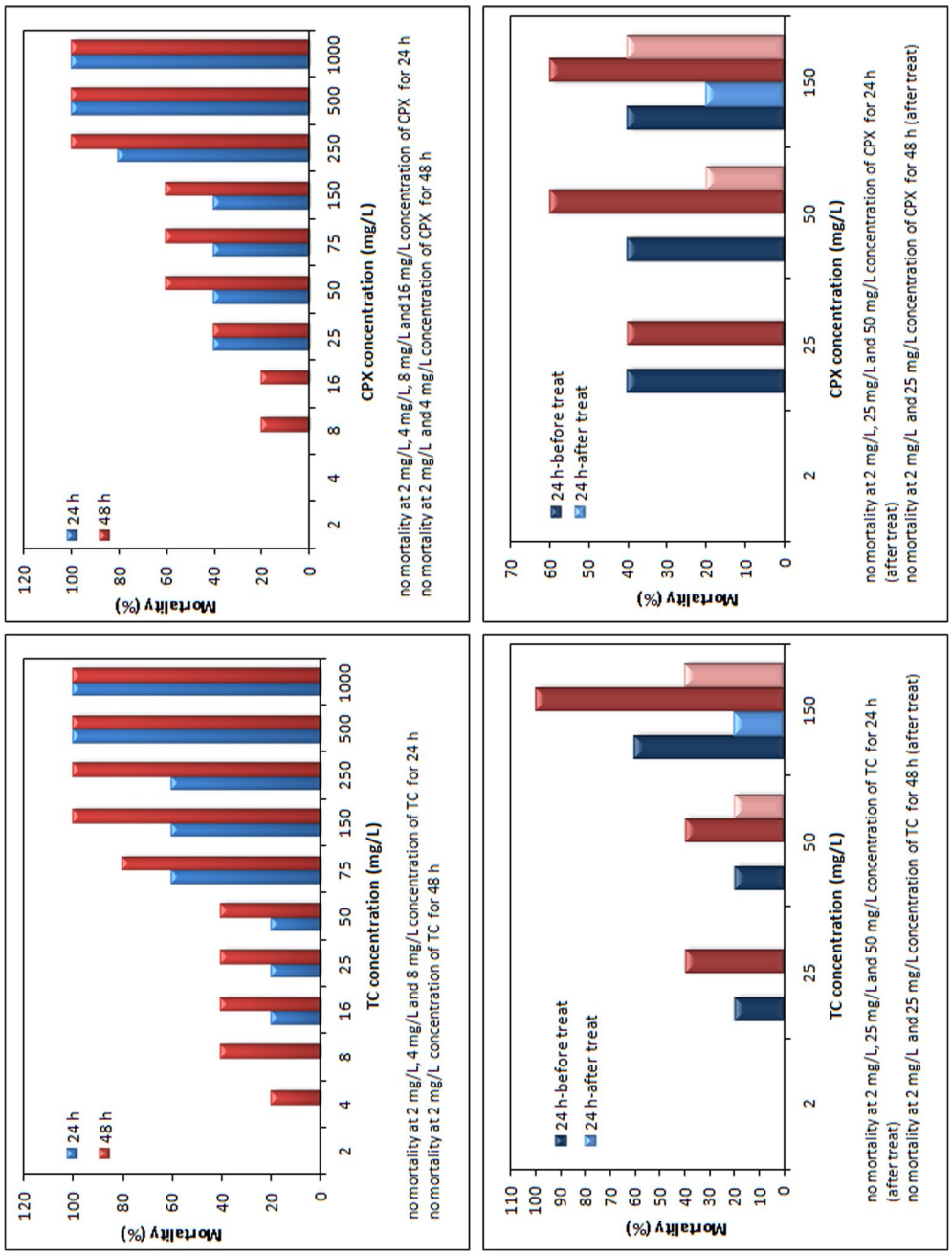

Fig. 6 Mortality (%) of *D. magna* at various concentrations of TC (**a**) and CPX (**b**), ecotoxicological response of *D. magna* to pollution of TC (**a1**) and CPX (**b1**) before and after biosorption

25.96 °C temperature. The removal efficiency of CPX (83.07 %) was optimized at pH 8.31, 29.26 mg/L initial CPX concentration, 100.71 min contact time, and 36.53 °C temperature. The biosorption process was investigated using Langmuir, Freundlich, D-R, and Temkin isotherm models. The value of Q^o (mg/g) for TC and CPX was found to be 6.97 and 47.77, respectively. The ecotoxicological assessment was determined using *Lepidium sativum* and *D. magna*. According to the toxicity classification, TC showed acute toxicity (III) to root and shoot of the *Lepidium sativum*. CPX showed slight acute toxicity (II) to the root of *Lepidium sativum*, no acute toxicity (I) to the shoot of a plant. After 48 -h exposure, TC and CPX showed acute toxicity (III) towards *D. magna*. After the biosorption process, a large decrease in inhibition rate (%) and mortality rate (%) occurred. This biosorbent showed good efficiency for biosorption of TC and CPX and reduction of toxicity. The results can be used for the risk assessment of the two antibiotics in the environment and their removal from wastewater.

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Data Availability All data generated or analysed during this study are included in this published article (and its supplementary information files).

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing İnterests The authors declare no competing interests.

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