Simultaneous Removal of Some Pesticides from Aqueous Solution by Using Submerged Aquatic Plant (*Nasturtium officinale)*

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Abstract
In this study, the potential biosorption characteristics of the submerged aquatic plant *Nasturtium officinale* was investigated for the removal of the chlorophenoxy acid derivates, namely, 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4-dichlorophenoxy propanoic acid (2,4-DP) and 2,4-dichlorophenoxy butyric acid (2,4-DB). The experiments were performed as simultaneous biosorption of the studied pesticides. Optimum biosorption conditions were determined as a function of pH, contact time and initial pesticidec concentrations. The concentrations of the pesticides in the remaining solutions after biosorption were simultaneously analyzed by high performance thin layer chromatography (HPTLC). The experimental adsorption data were fitted both the Langmuir and Freundlich adsorption models to describe the biosorption isotherm of the pesticides by aquatic plant *Nasturtium officinale* used as biomass. The highest pesticide uptake was calculated from Langmuir isotherm and found to be 11.24 mg g^{-1} for 2,4-DP.
Keywords: Pesticides, biosorption, HPTLC , *Nasturtium officinale*

Özet
Bu çalışmada, pestisit türevlerinden 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4-dichlorophenoxy propanoic acid (2,4-DP) ve 2,4-dichlorophenoxy butyric acid (2,4-DB)'nin giderimi sucul bitki *Nasturtium officinale* kullanılarak çalışılmıştır. Pestisitlerin sulu çözeltiden uzaklaştırılması, eş zamanlı olarak gereçekleştirilmiştir. Biyosorbsiyon sonucunda çözeltide kalan pestisit konsantrasyonu yüksek performanslı ince tabaka kromatografisi (HPTLC) kullanılarak belirlenmiştir. Optimum biyosorbsiyon koşullarının tespiti için temas süresi, pH ve pestisit başlangıç konsantrasyonu çalışılmıştır. Langmuir ve Freundlich adsorbdiyon modellerinin plotlarından elde edilmek suretiyle en yüksek pestisit alımı Langmuir izoterminden hesaplanmış ve 2,4-DP için 11.24 mg g^{-1} olarak bulunmuştur.

Anahtar kelimeler: Pestisit, biyosorbsiyon, HPTLC , *Nasturtium officinale*

1. Introduction

Pesticides have been generally used to protect the agricultural products from the harmfulls. Among these compounds, chlorophenoxy acid herbicides are used extensively as plant growth regulator. Chlorophenoxy acid herbicides are widely used in agriculture to control the weeds and other vegetation. It is well known that the excessive use of herbicides lead to different disorders on human and animals. Acidic herbicides are toxic for many living organisms even though none belongs to a group of very toxic pollutants. They may be mutagenic, tetragenic and carcinogenic while others have detrimental effects on mammals causing pyrexia, nausea, hyptonia, coma, metabolic

acidosis, convulsions, cytoskeletal perturbation and renal damage. Toxicity of phenoxy acids changes depending on the type of the compound: and its lethal dose (LD50) ranges from 300 to 3000 mg kg-1 body weight [1-3]. When applied, the herbicides are easily transferred to surface and groundwater, due to their polar nature and good solubility. Therefore, it may be a potential and serious risk to living organisms because the massive use of herbicides in the agriculture industry has led to measurable levels in the natural bodies of water, many of which supply the human population with fresh drinking water. Although their composition in the presence of oxygen is relatively fast, these herbicides are persistent under reductive conditions and their extended use can lead to pollution of surface and ground waters. European Community has established legal directives to restrict the use and to control their maximum residue level in several matrices [4]. However, many of these are being used in developing and undeveloped countries [3].

Currently, the activated carbon is the most widely used and effective physical sorbent to remove the pollutants. However, operation cost is expensive for its regeneration. Therefore, the development of analytical procedures for the simultaneous of chlorophenoxy acid herbicides and phenolic compounds is important. In recent years, biosorption has been considered as an alternative technique [5-7].

Biosorption can be defined as the removal of the organic and inorganic substances, via various physicochemical mechanisms including ion exchange, sorption, complexation, chelating by biological materials [8]. The use of aquatic plants in water quality assessment has been common for years as in-situ biomonitors and for in-situ remediation [9]. Aquatic plants have been used frequently to remove suspended solids, nutrients, heavy metals, toxic organic and bacteria from acid mine drainage, agricultural landfill and urban storm-water runoff [10]. Considerable research has been focused on determining the usefulness of macrophytes, as biomonitors of polluted environments and as bioremediative agents in waste water treatments [11,12]. Such studies done under defined experimental conditions provide results that can be extrapolated to natural environment. Several advantages of aquatic plants, have been reported for phytoremediation [10]. They are cost-effective universally available aquatic plants and with their ability to survive adverse conditions and high colonization rates. In addition, the submerged aquatic plants have very thin cuticle and therefore readily take up metals

or toxic organics from water through the entire surface. It is therefore of interest to assess the levels of organo metalic compounds in *N. officinale* due to its importance ecological processes.

To date, there is no report for the simultaneous removal of the 2,4-D, 2,4-DP and 2,4-DB by using submerged aquatic plant. Aquatic plants such as *Myriophyllum spicatum, Ceratophyllum demersum and Nasturtium officinale* were successfully used to remove the heavy metals from aqueous solutions [13-17]. Morever, *Emericella nidulans, Phanerochaete chrysosporium, Penicillium miczynskiia* and *Oscillatoria* sp. were also used to remove the 2,4-D [18-20]. The aim of this study is to use of submerged aquatic plant *N. officinale* as biosorbent simultaneous removal of 2,4-D, 2,4-DP and 2,4-DB from solution.

2. Material and Methods

2.1. Reagents and chemical

2,4-D, 2,4-DP and 2,4- DB were obtained as individual standards from Sigma Aldrich and used for preparing the stock standard solutions without purification. Chromatographic grade organic solvents were supplied from Merck and Riedel. The double deionized water was used for preparation of all solutions. All solutions were passed through from 0.45 μ m membrane filter (Sartorius Goettingen-Germany) before injection to the TLC plate. All solutions were stored at 4 ^{o}C in the dark when needed. High performance thin layer chromatography (HPTLC, Camag) was used for the analysis of the pesticides. *N. officinale* was harvested from a small-local lake in the vicinity of the Tigris River. Then, the plants were washed with diluted HCl solution (0.1 M) and distilled water before used.

2.2. Chromatographic separation

Chromatographic conditions for HPTLC were similar in literature [21,22]. The solutions of the 2,4-D, 2,4-DP and 2,4-DB in different concentrations were spotted on TLC Aluminum plate pre-coated with Silica Gel 60 F_{254} (layer thickness 0.2 mm) using Camag Linomat V and 100 µL syringe. The plates prewashed with methanol and activated 50ºC for 5 min prior to chromatography. The samples were streaked in the form narrow bands of length 6 mm, 15 mm from the bottom and 15 mm from the

margin using a nitrogen aspirator. Distances between the tracks were automatically selected. Camag twin trough chamber (10 cm ×10 cm) was saturated for 20 min. After the chamber saturation, the plates were developed to a distance of 9.5 cm with the development time being 20 min. A mixture of ethyl acetate-acetonitrile-methanol-ammonium-benzenexylene (2.1:1.3:1.0:0.2:1.0:0.3 v/v/v/v/v/v) was used as a mobile phase. Following to the development, the plates were dried in a current of air at the 25 ºC. Spectrodensitometric analyses were carried out using Scanner III in the absorbance mode. All the analysis was performed at the maximum wavelength of the pesticides as 232 nm. The colorless bands for each of the pesticides were scanned on TLC plate to obtain the UV-vis spectra. It was found that the selected wavelength was suitable for the analysis. The parameters used were: slit dimension 6.00×0.45 mm, scanning speed 1 mm s^{-1}, data resolution 100 μm s^{-1}. The source of radiation was D2&W lamps emitting continuous UV spectra. The chromatograms were integrated using winCATS evaluation software.

2.3. Biosorption experiments

The biosorption experiments were performed in 100 ml Erlenmeyer at 25ºC using an orbital shaker in a constant temperature. The initial pH values of the solutions were about 6 during the batch experiments and no pH adjustment was tested as a control. Hence, all the biosorption experiments were carried out at pH value of 6.0. Approximately, 2 g of *N. officinale* as a biomass was added to each flask and placed on the orbital shaker. The initial pesticide concentrations for the contact time experiments were 10 mg l^{-1} for each of the pesticides and the incubation times ranged from 2 to 120 min. The data used to derive the Langmuir constants were obtained using *N. officinale* biomass (about 2 g wet weight) and using different concentrations of 5, 10, 15, 25 and 50 mg l^{-1} of pesticides. The contact time was 120 min. After contacting, the contents of the flask were filtered to separate the biomass from the solution. The filtrates were then analyzed with an HPTLC for simultaneous determination of the pesticide concentrations in the samples by injecting the 20 μl of sample to the TLC plate. The control experiments were performed for each pesticide to measure any adsorption onto the glassware. The results of pesticides analysis were used to calculate specific adsorption

(mg pesticides adsorbed per g of biomass, dry weight). All the experiments were repeated three times and mean values were given.

3. Results and Discussion

3.1. Calibration graphs of the pesticides

Calibration graphs were found to be linear in the concentration range of 500-2500 ng spot^{-1} for 2,4-D,2,4-DB, 2,4-DP. The peak areas and concentrations were subjected to least square linear regression to calculate the calibration equations and correlation coefficients. The regression data as (not given) a good linear relationship over the low concentration range of 500-2500 ng spot^{-1}.

3.2. Effect of contact time on pesticide biosorption

Time of contact of adsorbate and adsorbent is great importance in adsorption, because it depends on the nature of the system used [23]. The effect of contact time on 2,4-D, 2,4-DB and 2,4-DP were studied between 2-120 minutes. As shown in Fig. 1 it was found that adsorptive quantity of pesticides on *N. officinale* increased when the contact time increased. The biosorption of pesticides by *N. officinale* was very rapid 20 min and the equilibrium was nearly reached after 60 min. Thus, the experimental period was determined as 60 min.

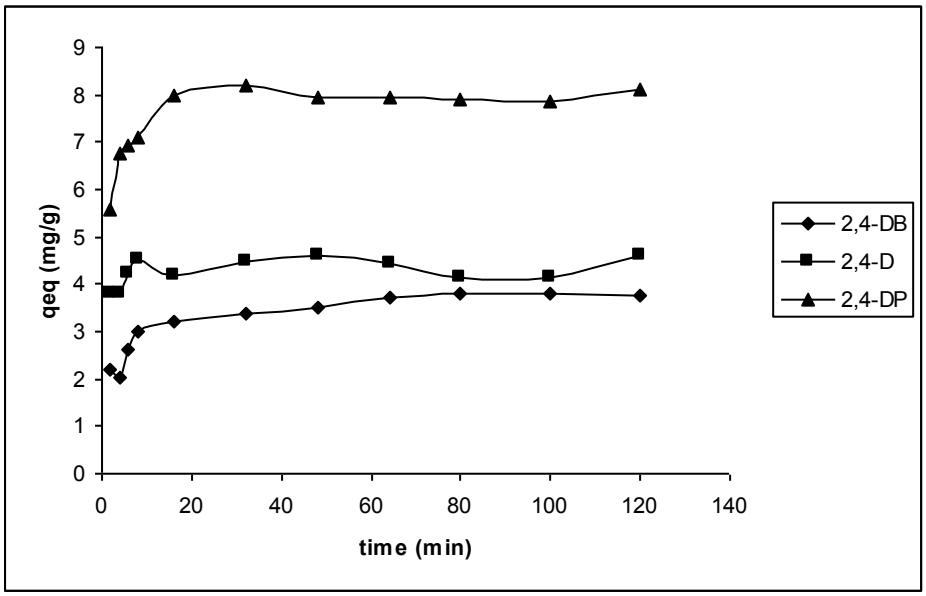

FIGURE 1 Effect of contact time on equilibrium bisorption capacity (initial pesticide concentration (C_o)= 10 mg/l, pH 4.0, temperature (T)= 25 ^{O}C, biosorbent amount (m)= 2 g)

3.3. Effect of pH on pesticide biosorption

It is well known that the pH of the initial solution has a critical role in the biosorption both metals and organic compounds [6-8]. Therefore, the pH of the initial solutions must adjust in this kind of experiments,. To remove the pesticides by using *N. officinale* as a biomass, the pH values ranging from 3.0 to 8.0 were studied in the experimental run. Then, the pesticide concentrations of the remaining solution after 60 minutes were determined by HPTLC. It was found that the initial pH of the solutions were similarly effect of the equilibrium pesticide concentrations. The chemical structures of the pesticides studied were not different from each other. It was found that the studied pesticides were maximum adsorbed on the biomass at pH 6.0 (Fig. 2.) because the highest biosorption efficiency was obtained in this pH value. At the pH values higher than 6.0, the pesticides precipitated and biosorption studies at these pH values could not be performed. As expected, the pHs of the initial solutions were the same for the pesticides studied.

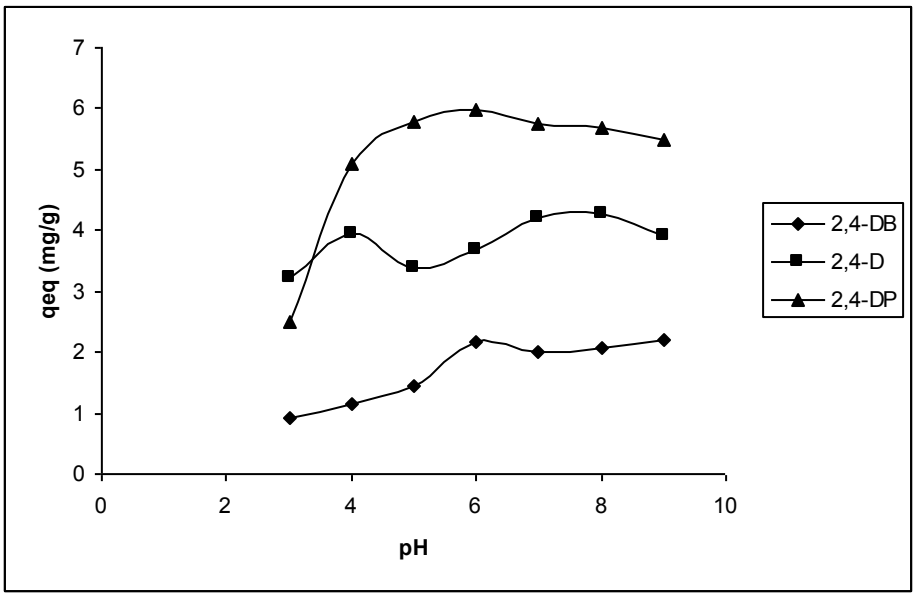

FIGURE 2 Effect of pH of the solution on equilibrium bisorption capacity (initial pesticide concentration (C_o)= 10 mg/l, contact time 60 min, temperature (T)= 25 ^{O}C, biosorbent amount (m)= 2 g)

3.4. *Effect of biomass concentration on pesticide biosorption*

Figure 3 shows that the pesticide adsorptions obtained at various concentrations (0.5- 2.5 g/l) of biomass of *N. officinale* were decreased with increasing wet mass

concentrations. It was observed that a 2.0 g/l concentration of biosorbent was enough for biosorption of the pesticides. Increasing biosorbent concentration can be attributed to increased adsorbent surface area and the availabilty of more adsorption sites [24]. Conversely, the quantity of biosorbed solute per unit weight of biosorbent decrease with increasing biosorbent concentration, which may be due to the complex interaction of several factors. An important factor at high sorbent dosages is that the available solute is insufficient to completely cover the available exchangeable sites on the biosorbent, usually resulting in low solute uptake [25].

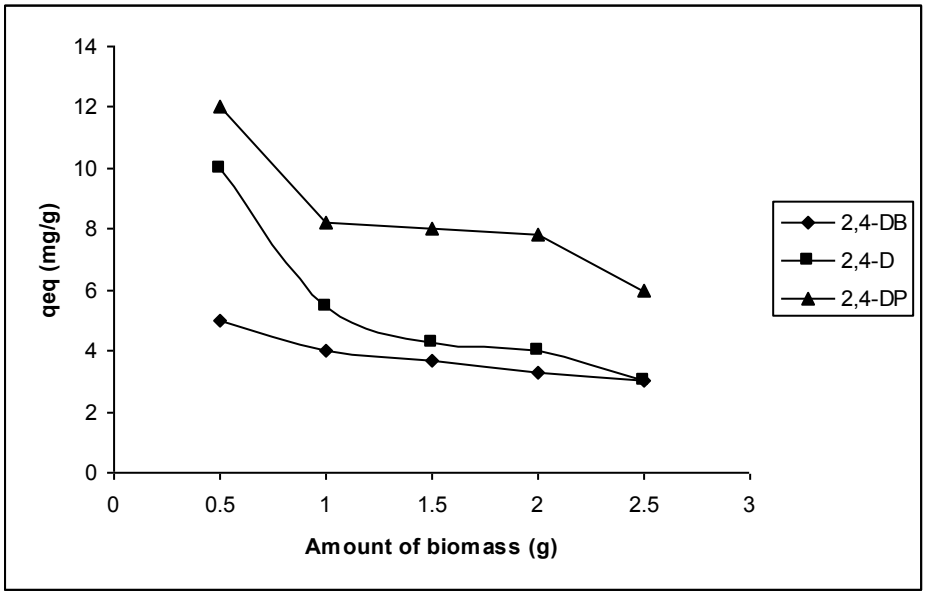

FIGURE 3 Effect of amount of biomass on equilibrium bisorption capacity (initial pesticide concentration (C_o) = 10 mg/l, pH 6.0, contact time 60 min, temperature (T)= 25 ^{O}C)

3.5. Effect of initial pesticides concentration on pesticide biosorption

The initial solute concentration seems to have impact on biosorption, with a higher concentration resulting in a high solute uptake. The effect of initial pesticide concentrations on the biosorption capacity of *N. officinale* biomass is shown in Fig. 4. It can be seen from Fig. 4 that, equilibrium uptake increased with the increasing of initial pesticides concentration at the range of experimental concentration. This is because at lower initial solute concentrations, the ratio of the initial moles of solute to the available surface area is low; subsequently, the fractional sorption becomes independent of the initial concentration. However, at higher concentrations, the sites

available for sorption become fewer compared to the moles of solute present and; hence, the removal of solute is strongly dependent upon the initial solute concentration [25]. So the values of q_e increase with the increase of initial pesticides concentration.

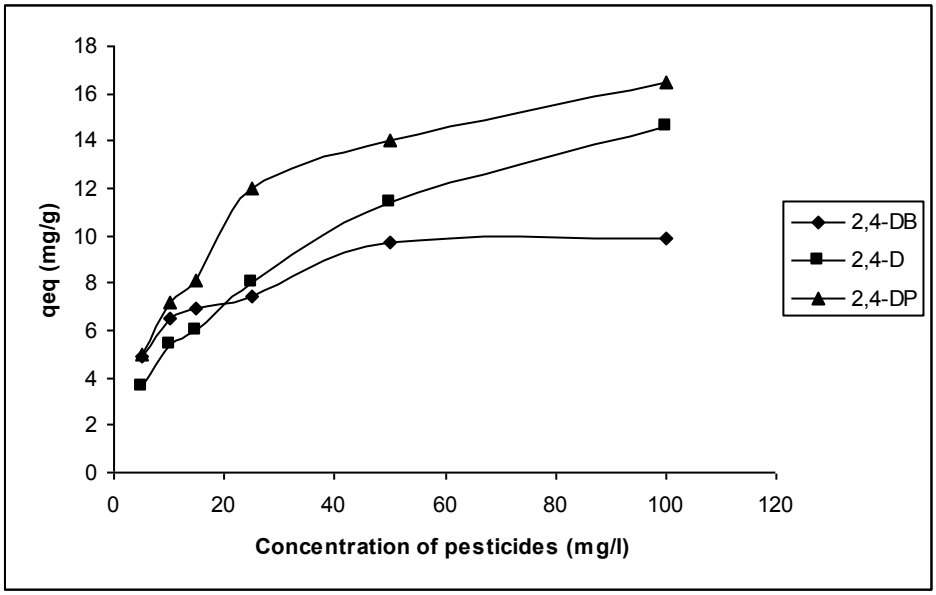

FIGURE 4 Effect of initial pesticides concentration on equilibrium bisorption capacity (pH 6.0, temperature (T)= 25 ^{O}C, biosorbent amount (m)= 2 g, contact time 60 min)

3.6. Adsorption isotherms

The biosorption capacity of the *N. officinale* as a biosorbent which is obtained from the mass balance on the sorbate in a system with solution volume, V is often used to acquire the experimental adsorption isotherms. Under the optimum conditions, the biosorption capacities of biosorbent for each of the pesticides at equilibrium were calculated from the following equations.

$$q_{eq} = \frac{(c_o - c_{eq})V}{X} \qquad (1)$$

where c_o is the initial concentrations of each of the pesticides in solutions, c_{eq} is the concentrations of the each of the pesticides in solutions at equilibrium, V is the volume of the

solution and X is the mass of biosorbent.

To understand the distribution of the solutes between the liquid and solid phases equilibrium conditions, the equilibrium concentration of the each of pesticides in the solution and the concentration sorbed onto the surface of the biomass were fitted to the most popular adsorption models, namely Langmuir (Eq. 2) and Freundlich (Eq. 3) sorption models. The Langmuir and Freundlich models are expressed as:

$$\frac{c_{eq}}{q_{eq}} = \frac{1}{K_b A_s} + \frac{c_{eq}}{A_s} \qquad (2)$$

$$\ln q_{eq} = \ln K_F + \frac{1}{n} \ln c_{eq} \qquad (3)$$

where q_{eq} and c_{eq} were described above. A_s, K_b, K_F and n are the adsorption isotherm parameters. A_s is the maximum amount of the pesticides per unit weight of biosorbents to form a complete monolayer on the surface bound at high c_{eq}, and K_b is a constant related to the affinity of the binding sites. A_s represents a practical limiting adsorption capacity when the surface is fully covered with pesticides and assists in the comparison of adsorption performance, particularly in cases where the biosorbent did not reach its full saturation in experiments. The Langmuir equation is valid for monolayer sorption onto a homogeneous surface with a finite number of identical sites. According to Langmuir equation, some assumptions are made as: the adsorption phenomenon is a reversible interaction, the properties of the adsorbed molecules do not change, there isn't lateral interaction between the adsorbed molecules and all of the adsorption sites have the same affinity for the sorbent [23]. The empirical Freundlich equation given above is based on a monolayer adsorption by the adsorbent with a heterogeneous energy distribution of active sites. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively. The Freundlich isotherm is also more widely used but provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model [16,17**,** 26-28].

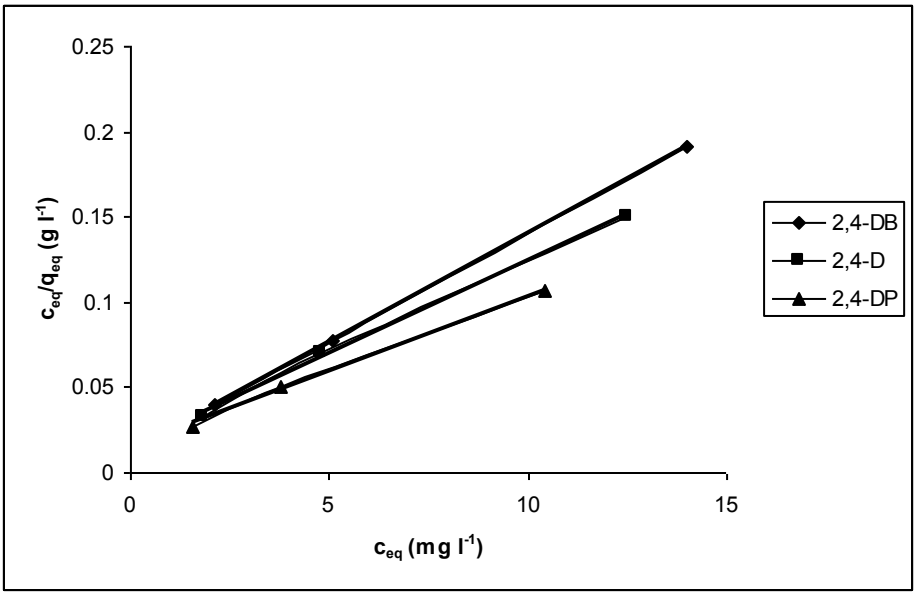

FIGURE 5 Langmuir adsorption isotherms of the pesticides adsorption on *N. officinale*

The Langmuir and Freundlich adsorption isotherms of pesticides obtained at 25 ºC were given in Figs. 5 and 6. The adsorption constants and correlation coefficients obtained from the Langmuir and Freundlich isotherms at 25 ºC were also given in Table 1. As seen from this table, high regression correlation coefficients (>0.97) were obtained and the results showed that the adsorption equilibrium data have fitted very well both adsorption models in the concentration ranges studied.

TABLE 1 The adsorption isotherm parameters for 2,4-D, 2,4-DB and 2,4-DP by using *N. officinale* as biosorbent

Compound	Langmuir isotherm			Freundlich isotherm		
	A_s (mg g^{-1})	K_b (l mg^{-1})	r^2	K_F	n	r^2
2,4-D	9.26	0.69	0.9979	4.81	0.46	0.9998
2,4-DP	11.24	0.58	0.9980	4.75	0.58	0.9416
2,4-DB	7.88	0.98	0.9999	4.75	0.58	0.9416

The uptake values obtained in this study are comparable with those values. The applicability of Langmuir isotherm to the pesticide biosorptions express that monolayer

adsorption on the surface of adsorbent conditions exist under the experimental conditions employed.

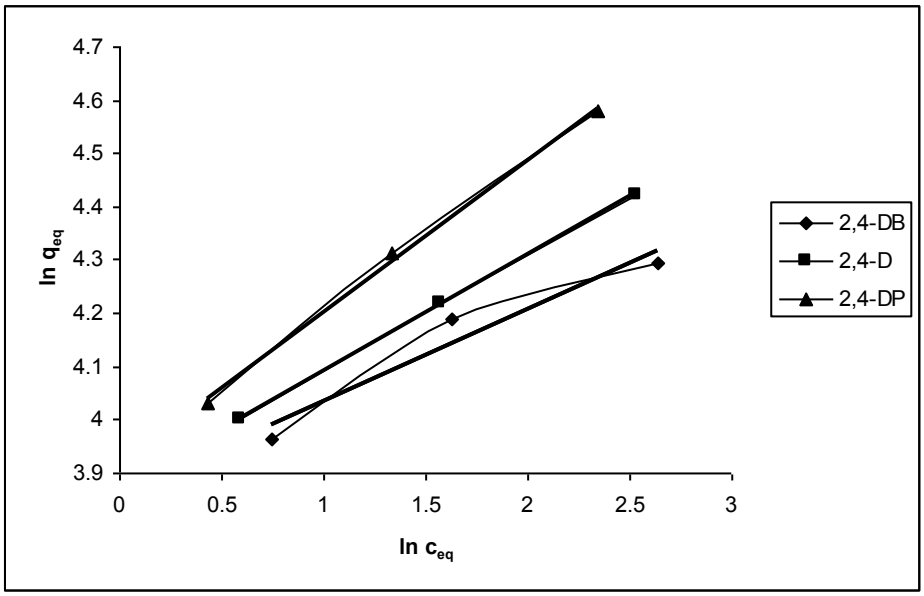

FIGURE 6 Freundlich adsorption isotherms of the pesticides adsorption on *N. officinale*

4. Conclusions

The biosorption of chlorophenoxy acid herbicides from aqueous solution by *N.officinale* was investigated by using HPTLC. As an alternative to the bacterial biosorbent, submerged aquatic plant *N. officinale* was used to remove the 2,4-D, 2,4-DP and 2,4-DB from aqueous solution. The parameters such as initial pesticides concentrations, contact time, the amount of biosorbent and pH which influence the biosorption were examined for each pesticides. The biosorption results obtained from experiments were applied to the Langmiur and Freundlich isotherm. The excellent applicability of Langmuir isotherm to the pesticides biosorption showed the occurrence of monolayer adsorption of active sites on the surface of biosorbent. The results obtained from the biosorption of the studied pesticides showed that *N. officinale* would adsorb 2,4-D, 2,4-DP and 2,4-DB based on the Langmuir coefficients, the maximum adsorption capacities of 9.26, 11.24 and 7.88 mg/g, respectively. The approach is suitable for routine monitoring as information on the acidic herbicides are necessary to understand the environmental rate of these compounds.

In view of the eco-friendly feature and generally easy procedures, the biosorption has being an alternative to the separation and preconcentration methods. This technology provides the using of the various types of biomass as a source to remove heavy metals from different matrices. From this point of view, the using of the *N. officinale* as biosorbent will facilitate the removel of pesticides by following points;

a) *N. officinale* can effectively remove the studied pesticides from aqueous solutions.

b) The proposed method required a small amount of biosorbent.

c) The method can be easily applied to river samples polluted with pesticides

d) *N. officinale* can be growth in short time. There are no special conditions for growthing.

e) It is not necessary to adjustment of pH. Because, the optimum pH of the biosorption was not so extreme. The pH's of the river water is close to optimum pH values.

f) The cost of the growthing for *N. officinale* is cheap. So, our method provides an economical approach.

When the effectiveness of the suggested biosorption method by using submerged aquatic plant *N. officinale* was compared with literature, it could be said that biosorbent used in our study was used for biosorption of pesticides. In addition, the maximum adsorption capacities calculated from Langmuir isotherm show that it has high adsorption capacity.

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