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Original Research

Phytoremediation and Biosorption Potential of *Lythrum salicaria* L. for Nickel Removal from Aqueous Solutions

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Abstract

Pollution of water sources with heavy metals is one of the most important global environmental issues. Even though there are many physical and chemical methods for removing heavy metals from contaminated water, in recent years cost-effective and environmentally friendly techniques such as phytoremediation and biosorption are used to remove heavy metals from water. In this study, phytoremediation and biosorption potential of *Lythrum salicaria* L. for nickel removal from aqueous solutions were investigated. Phytoremediation experiments were conducted at 10% Hoagland solution with 0, 10, 15, 20, 25, 50, and 100 mg/L nickel, and pH levels of 5, 6, and 7 to determine the accumulation of nickel in vegetative parts of *L. salicaria*. Phytoremediation results indicated that maximum Ni (II) accumulation by *L. salicaria* was at pH 7 with 10 mg Ni/L and distribution of Ni (II) was in the root (3,737.8 mg/kg DW) > shoot (697 mg/kg DW) > leaf (418.4 mg/kg DW) of *L. salicaria*. On the other hand, the effect of pH, biomass dosage, contact time, and initial Ni (II) concentration on the biosorption potential of *L. salicaria* roots was investigated in a batch system at room temperature. Optimum conditions were achieved at pH 7 with the biomass dosage of 6 g/L at an equilibrium contact time of 40 min. Equilibrium data was adapted to Langmuir and Freundlich isotherm models to find the best-fitting model. The Langmuir isotherm model described the biosorption process best with a maximum monolayer sorption capacity of 9.1580 mg/g for Ni (II) ions.

Keywords: hydroponic culture, *Lythrum salicaria*, nickel pollution, phytoremediation

Introduction

Heavy metal pollution of freshwater is a serious issue worldwide, particularly in developing countries such as Turkey [1-4]. Natural and anthropogenic activities such as urban sewage, tanneries, and the textile industry have contaminated fresh water with various metals, including Zn, As, Pb, Ni, Cr, and Cd [5-6].

Heavy metals are non-biodegradable and therefore it is very important to remove them from the environment in terms of the health of living organisms. Some technologies – including ion exchange, reverse osmosis, electro dialysis, coagulation-precipitation, electrochemical operation, and filtration – have been used for their removal from aquatic systems [7-9]. Because most of these techniques mentioned above are costly, economical, and

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environmentally friendly, techniques such as biosorption and phytoremediation have been used to remove heavy metals from aquatic environments in recent years [10-12]. Biosorption is known as a metabolically passive process. There is no danger of toxicity of sorbent by sorbate solution. Heavy metal removal mechanism by this process depends on the adsorption of metals onto the surface of dead cells, and the rate of the process is very quick and reversible. On the other hand, phytoremediation technique remediates contaminated water by using hydroponically cultivated plants. Both of the methods are originated on biological materials; while the biomass in biosorption is not alive, living cells are used in phytoremediation [13-14]. Wetland plants that showed high biomass are mostly used to remove heavy metals from aquatic systems such as water hyacinths, water lettuce, and cattail [15].

Under the present investigation, *Lythrum salicaria* L. was used to determine its potential in removing nickel from aquatic systems. The geographic origin of *L. salicaria* (Lythraceae) is Eurasia. It is the most common aquatic plant in Turkey and shows a distribution along rivers, lakes, and canals. *L. salicaria* is a herbaceous perennial plant that grows up to 20-180 cm tall along the wetlands between 0-1,400 m altitude. The color of flowers is red to purple with 6-12 petals and 12 stamens and they appear from July to September. Fruit is capsule. A single mature plant can produce 2-3 million seeds per year [16-17]. The Lythrum generic name comes from Greek Word "luthron," or blood, referring to its ability to stop bleeding. The main compounds of the plant are tannins, flovans, and anthocyanins. *L. salicaria* is used medicinally to treat diarrhea, varicose veins, hemorrhoids, bleeding gums, and eczema [18-20].

One of the heavy metals that has reached high levels dangerous for human beings and aquatic organisms is nickel (Ni). Ni (II), one of its toxic elements, is widely distributed in aquatic systems. The origins of Ni (II) contamination in the environment are metal mining, glass industry, fossil fuel burning, industrial wastes, fertilizers, and vehicle emissions [21-22]. Human exposure to nickel over 0.04 mg/L in consumption water causes allergies in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases, and lung and nasal cancers [23-24]. In this study we tested the biosorption and phytoremediation potential of *L. salicaria* for nickel removal from aquatic systems.

Material and Methods

Phytoremediation Experiment

Seeds of *L. salicaria* were randomly collected during September to October 2015 from populations along the Porsuk River, Kütahya (39°25′N and 29°58′E), where the plant showed optimum distribution (Fig. 1). The seeds were sewn into pots filled with soil and placed in pools filled with water in the greenhouse. The plants were grown in this condition until we had about 10 cm-high seedlings.

Fig. 1. *Lytrum salicaria* population along the Porsuk River (Kütahya).

For acclimatization, seedlings were trans-ferred to hydroponic culture containing 10% Hoagland for 15 days.

Experiments were performed in pots (2.5 L capacity) of hydroponic culture system. The study had three steps. After adaptation, in the first step *L. salicaria* seedlings were treated with 10% Hoagland solution with eight different Ni (II) concentrations, prepared by using $NiCl_2.6H_2O$ (0, 5, 10, 15, 20, 25, 50, and 100 mg Ni/L) for seven days to determine the maximum Ni (II) concentration that plants accumulate. In the second step, *L. salicaria* seedlings were treated to 10% Hoagland solution containing 10 mg/L Ni (II) at three different pH levels (5, 6, and 7). In the last step, *L. salicaria* seedlings were treated to 10% Hoagland solution containing 10 mg/L Ni (II) to determine the accumulation of Ni (II) in vegetative parts of the plant (root, shoot, and leaf). In the first and second steps of the experiment, the roots of seedlings were washed with 1% Na-EDTA and ultra-pure water. Then the whole seedlings were dried at 70ºC for 48 hours. In the last step, the same processing was followed as mentioned above, but instead of using whole seedlings, the roots, shoots, and leaves of the *L. salicaria* were separated, dried, and ground to prepare samples for digestion. 0.1 g plant sample was digested by wet digestion method based on nitric acid and hydrogen peroxide [25].

Biosorption Experiment

Dried roots of *L. salicaria* were used as biomass for the biosorption of Ni (II) ions. Firstly, dried roots were washed with deionized water and then dried at 80ºC. Then the dried biomass was ground to a particle size of 300 µm and stored in a desiccator for further use. A stock solution of Ni (II) was prepared using $NiCl_2.6H_2O$. The working solutions were prepared by the appropriate dilutions from the stock solution. All the experiments were performed at room temperature. pH of the solutions were adjusted by HCl or NaOH. Biosorption experiments were carried out at different pH values. 0.1 g of dried biomass was immersed in 50 mL of 100 mg/L Ni (II) solution and pH of the solutions were adjusted from 1 to 7 and agitated at

a constant speed for an hour. The effect of biomass dosage was controlled by ranging the amount of samples from 0.5 to 10 g/L at the determined pH. Then the effect of contact time was investigated at various time intervals between 5 and 120 minutes at the constant pH and biomass dosage. And lastly the effect of initial concentration was studied. The concentrations of Ni (II) solutions varied between the ranges of 2.5-100 mg/L while the other parameters were stable at the determined conditions. After the batch experiments were completed, the solutions were centrifuged.

Ni (II) concentration of plant samples from biosorption and phytoremediation experiments were analyzed using an atomic absorption spectrometer (Analytikjena ContrAA 300) at Dumlupinar University, Advanced Technologies in Design, Research and Development and Application Centre.

Data Analysis

Data were analyzed using the JMP 6 SAS [26] program. F-test was used to determine the differences between the applications at $p<0.05$ level. TUKEY-HSD multiple comparison test was used on applications that were statistically different according to the F-test.

Results and Discussion

Phytoremediation Experiment

Ni (II) can cause toxic effects in plants over certain concentrations (11.74 ppm), such as chlorosis, necrosis, and retardation of germination, inhibition of growth, and reduction of yield [21]. Also, Singh and Pandey [27] reported that Ni induced visible toxicity symptoms in water lettuce subjected to exposure of higher concentrations (1.0 and 10.0 ppm), and maximum reduction in chlorophyll and protein content was observed at 10 ppm Ni. On the other hand, Chen et al. [21] and Chami et al. [28] indicated that 15, 20, 25, 50, and 100 mg Ni/L solutions caused death of the above- and below-ground parts of all seedlings. In this study, results of the first step of the experiment showed that maximum Ni (II) accumulation by *L. salicaria* was obtained in solutions containing 5 and 10 mg Ni/L, and solutions containing 15, 20, 25, 50, and 100 mg Ni/L caused death of the above- and below-ground parts of all *L. salicaria* seedlings. Ni (II) accumulation at concentrations of 5 and 10 mg Ni/L in *L. salicaria* were 2,582 and 5,523.2 mg/kg DW (dry weight), respectively (Fig. 2a). Thus, Ni accumulation in the whole plant increased with increasing nickel concentrations up to 10 mg Ni/L.

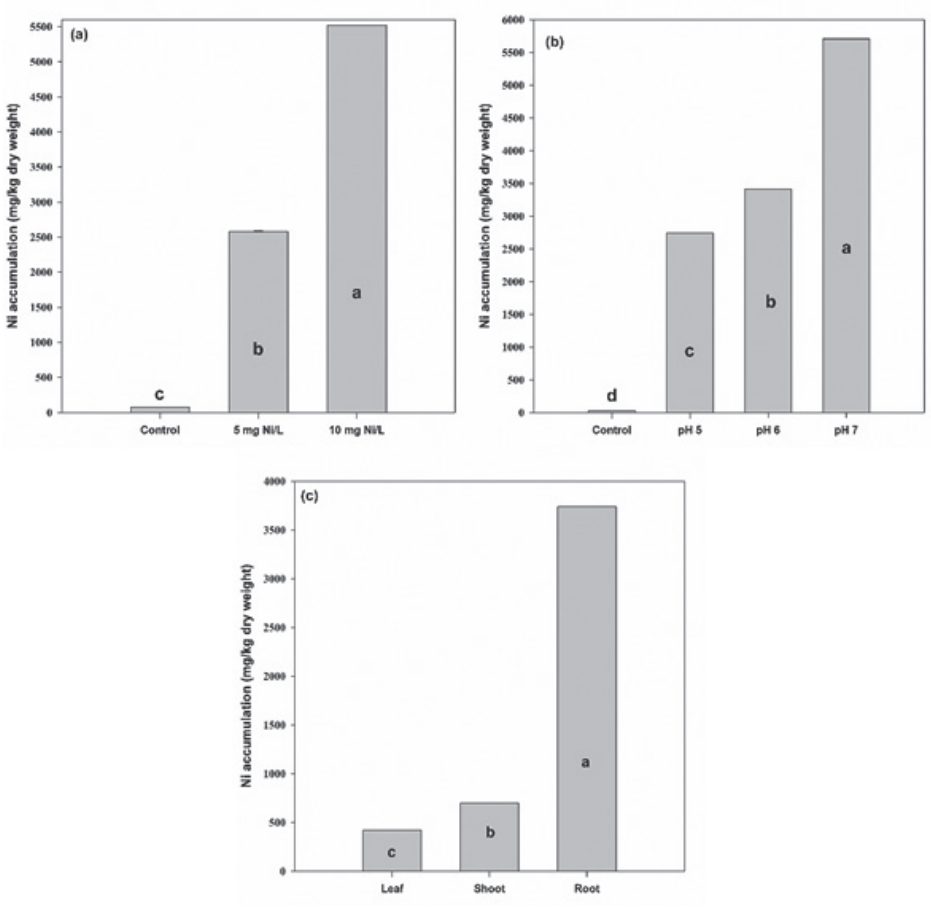

Fig. 2. The accumulation of Ni (II) (mg/kg dry weight) in *L. salicaria*; a) different concentrations of Ni (II) (Control, 5 and 10 mg Ni/L) ($F = 106,442.5$; $p<0.05$), b) different pH levels ($F = 714,909.5$; $p<0.05$) and c) Ni (II) accumulation in different organs of *L. salicaria* at pH 7 with 10 mg Ni/L ($F = 166,367.5$; $p<0.05$).

Changes in the physiochemical conditions of the aquatic environment such as pH, redox potential, organic ligands and temperature have impacts on proportion of the metal ions that plants can accumulate [15]. The pH of the solution is the most important variable affecting accumulation capacity in nickel-contaminated water. According to Kaur et al. [29], the highest Ni accumulation by *Lemna minor* was determined at pH 6 and pH 7. Kukier et al. [30] also stated that the accumulation of nickel by Alyssum increased with increasing pH in soil. Results of the second step of the experiment showed that Ni (II) accumulation at 10 mg Ni/L by *L. salicaria* was significantly increased at higher pH values. While the lowest Ni (II) accumulation was obtained at pH 5 (2739.5 mg/kg DW) and pH 6 (3413.3 mg/kg), the highest Ni (II) accumulation was obtained at pH 7 (5708.3 mg/kg DW) in *L. salicaria* seedlings (Fig. 2b).

Analyses of Ni (II) in the roots, shoots, and leaves of *L. salicaria*, grown in 10 mg Ni/L concentration, showed statistically significant differences (Fig. 2c). Results indicated that maximum Ni (II) accumulation was at pH 7 with 10 mg Ni/L, and the distribution of Ni (II) was in the root (3737.8 mg/kg DW) > shoot (697 mg/kg DW) > leaf (418.4 mg/kg DW) of *L. salicaria*, which showed that *L. salicaria* had great potential for phytoremediation of nickel in its root. According to our results, 77% of Ni (II) accumulation by *L. salicaria* was retained in the root of the plant. Recent studies indicated that aquatic fern *Salvinia minima* Baker also accumulated Ni more in roots than in leaves [31]. Yusuf et al. [32] and Vajpayee et al. [33] also reported that Ni accumulation was more pronounced in roots rather than the shoot in barley and maize, and aquatic plant *Vallisneria spiralis* L., respectively. Chen et al. [21] stated that over 50% of Ni (II) absorbed by the plants was retained in the roots of xylem parenchyma cell walls and vacuoles. When comparing its accumulation capacity and tolerance to Ni, *L. salicaria* is a suitable plant to use in phytoremediation at the aquatic environments contaminated with Ni.

Biosorption Experiments

Effect of pH

In the biosorption process pH is known as the most significant parameter that depends on the competition between the metal ions and protons for the binding sites [13, 34]. pH effect on the biosorption of Ni (II) ions onto *L. salicaria* biomass between the pH range of 1-7 was studied.

Fig. 3 clearly indicates that as pH of the solutions increases, uptake capacity of biomass increases. pH 7 is the optimum pH for the biosorption of Ni (II) ions onto biomass. At lower pH values the binding sites on the surface of the biomass were about completely protonated and positively charged Ni (II) ions that were repulsed by protons. By increasing pH, the surface of the biosorbent was more negatively charged and this resulted with the electrostatic interactions with Ni (II) ions [8, 35-36].

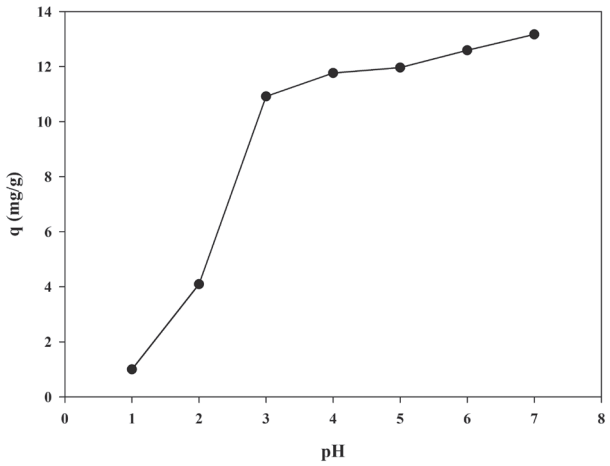

Fig. 3. Effect of pH on Ni (II) ions biosorption onto *L. salicaria* roots (Ni (II) concentration:100 mg/L, adsorbent dosage: 2 g/L).

Similar results have been reported by other researchers. Akar et al. [37] indicated that a precipitate occurs in the form of $Ni(OH)_2$ at higher pH values greater than 8.3 in dilute solutions of Ni (II) ions and preferred to study at pH 6.5 (the original pH of Ni (II) solution). Hanif et al. [38] and Kaur et al. [29] also mentioned that at pH values lower than 6, Ni (II) ion removal was partly inhibited due to the competition between hydrogen and Ni (II) ions onto the sorption sites.

Effect of Biomass Dosage

As can be seen from Fig. 4, varying the biomass dosage from 0.5 to 10 g/L at pH 7 and contact time of 60 min. increases the percentage of Ni (II) removal from 18 to 65%. The biosorption process reaches an equilibrium at a dosage of 6 g/L. A further increase doesn't change the uptake ratio. Therefore, 6 g/L biomass dosage was selected as the optimum value for biosorption experiments. This result can be attributed to the saturation of binding sites

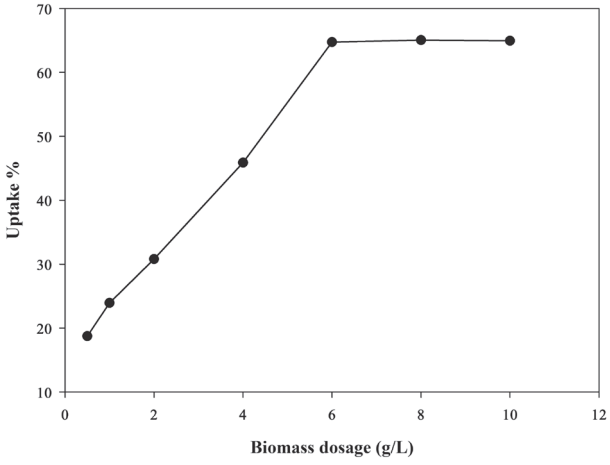

Fig. 4. Effect of biomass dosage on Ni (II) ions biosorption onto *L. salicaria* roots (Ni (II) concentration:100 mg/L, pH: 7).

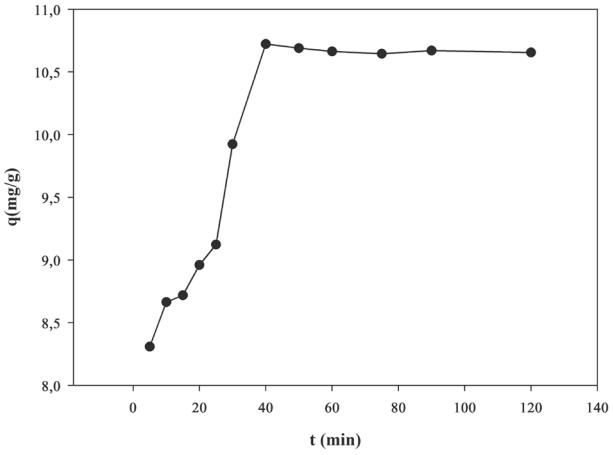

Fig. 5. The effect of contact time for Ni (II) ions biosorption onto *L. salicaria* roots (Ni (II) concentration:100 mg/L, pH:7, biomass dosage: 6 g/L).

with the Ni (II) ions [39]. Also, the partial aggregation that occurs at higher dosages decreases the active sites on the biomass. Experimental results of Sarı et al. [8], Reddy et al. [40], and Fawzy et al. [41] are compatible with the literature. In these studies biosorption capacity increases to an equilibrium dosage and then tends to be almost the same.

Effect of Contact Time

In the biosorption of Ni (II) ions by *L. salicaria* roots, the effect of contact time was shown in Fig. 5. It can be seen that biosorption capacity increases with contact time up to 40 min. After that point, extending the time does not have a positive influence on adsorption capacity. Therefore, further experiments were carried out at 40 min. At the beginning of the biosorption process, there are many vacant active binding sites on the biomass surface. However, in the process of time, metal uptake of biomass decreases due to the gradual occupancy of the active binding sites until they reach saturation [41]. In a similar study by Torab-Mostaedi (2013), biosorption of Ni (II) reached equilibrium within the 60 minutes for the initial Ni (II) concentration of 50 mg/L at pH 5 and adsorbent dosage of 4 g/L. After that optimum contact time, Ni (II) uptake capacity of the biomass was insignificantly effected by the extending time [42].

Biosorption Isotherms

Langmuir and Freundlich isotherm models are the most-used linear models that explain the mechanism of biosorption between the metal ions and biomass. In the study the obtained data were also applied to these two models. Equations of both of the models are:

$$\frac{C_e}{q_e} = \frac{1}{(q_{\max} K_L)} + \frac{C_e}{q_{\max}} \quad (1)$$

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (2)$$

…where q_{max} is the maximum biosorption capacity of biomass (mg/g), q_e is the amount of Ni (II) ions biosorbed by the per gram of biomass at the equilibrium (mg/g), C_e is the equilibrium concentration of Ni (II) solution (mg/L), K_L is the Langmuir biosorption constant (L/g) related to the free energy of biosorption, and K_F (L/g) and n (dimensionless) are the Freundlich isotherm constants that are related to the biosorption capacity and biosorption intensity [43].

Figs 6 and 7 show the linear form of Langmuir and Freundlich isotherms obtained by plotting C_e/q_e versus C_e and $\ln q_e$ versus $\ln C_e$ for the biosorption of Ni (II) ions onto

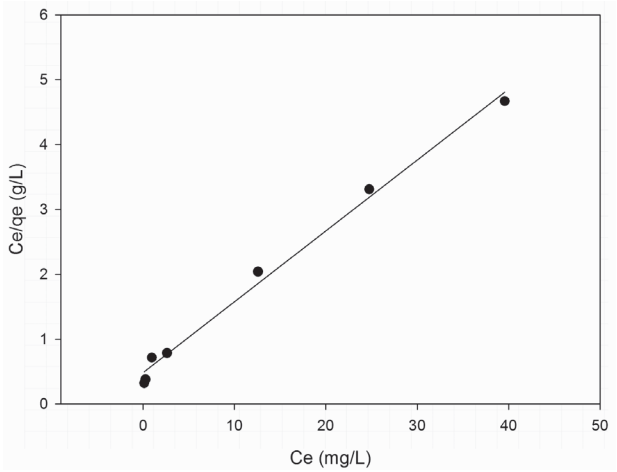

Fig. 6. Langmuir plots for Ni (II) ions biosorption onto *L. salicaria* roots (pH:7, biomass dosage: 6 g/L, contact time: 40 min.).

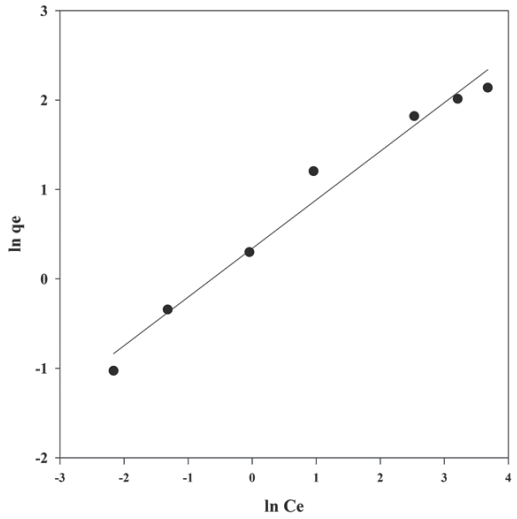

Fig. 7. Freundlich plots for Ni (II) ions biosorption onto *L. salicaria* roots (pH:7, biomass dosage: 6 g/L, contact time: 40 min.).

Table 1. Isotherm constants for the biosorption of Ni (II) ions onto *L. salicaria* roots.

Langmuir			Freundlich		
q_{max} (mg g^{-1})	K_L (L mg^{-1})	r^2	n	K_F (Lg^{-1})	r^2
9.1580	0.2264	0.9923	1.8400	1.4015	0.9772

L. salicaria roots at room temperature, respectively, and isotherm model parameters are listed in Table 1.

The Langmuir isotherm model has one more constant defined as separation factor, R_L, that is used to determine whether the process is favorable or not. It can be calculated by the following equation (Eq. 3):

$$R_L = \frac{1}{(1 + K_L C_0)} \quad (3)$$

…where C_0 (mg/L) is the initial Ni (II) concentration in the solution. The calculated result from Eq.3 for the biosorption of Ni (II) at room temperature is found to be 0.0423. The R_L constant being in the interval of 0 and 1 indicates that the biosorption of Ni (II) onto roots of *L. salicaria* is favorable [29].

All the parameters for the Lanqmuir and Freundlich isotherms are given in Table 1. When the r² values are compared in Table 1, it is clearly seen that the Ni (II) adsorption data of biomass as a function of equilibrium concentrations at room temperature were fitted to the Langmiur isotherm equation very well. From the Langmuir model, the monolayer biosorption capacity of *L. salicaria* biomass was found to be 9.1580 mg/g for Ni (II) ions.

Conclusions

Soil and water contamination with Ni has become a worldwide problem [21]. Nickel is one of the most important heavy metals that is widely distributed in rivers and lakes of Kütahya. Thus, Ni has been released into the environment from wastewater of ceramic, porcelain, and tile industries in Kütahya [1].

This study investigated phytoremediation and biosorption of Ni (II) by *L. salicaria*. The data showed that *L. salicaria* was an efficient biomaterial for removal of Ni (II) from aquatic systems in phytoremediation and biosorption techniques. While 3.7378 mg/g Ni (II) was removed by *L. salicaria* roots at 10 mg/L Ni (II) and pH 7 in a phytoremediation study, 9.1580 mg/g Ni (II) was removed at pH 7 at a dosage of 6 g/L and contact time of 40 min. in a biosorption study. In conclusion, *L. salicaria* roots are the most effective biomaterial for biosorption and phytoremediation studies for the removal of Ni (II) from aquatic systems.

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