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DETERMINATION OF COLOUR REMOVAL EFFICIENCY OF *Lemna minor* L. FROM INDUSTRIAL EFFLUENTS

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Abstract. Industrial and domestic wastewater treatment by using floating aquatic plants such as duckweed (*Lemna minor* L.) is a promising technology and, also an alternative method to traditional treatment methods in recent years. Some characteristics of this natural treatment technology such as its low capital and operation costs, easy to operate and low energy requirement make duckweed-based systems very attractive for wastewater treatment. Coloured industrial effluents that are very important for environment because of containing more pollutants are mostly resulted from dyeing processes. The most important parameter of these wastewaters is colour, and there are a lot of physical and chemical methods to remove it from wastewater. In this study, the potential of duckweed, *L. minor* grown in a laboratory scale pond system to remove colour from industrial effluent was investigated. According to the results, the percentage colour removal of system continuously decreased with time. The maximum removal efficiencies of the system having HRT (hydraulic retention time) of 3 days were found to be 85.9% for COD (chemical oxygen demand), 79.4% for MLSS (mixed liquor suspended solids), 66.1% for Pt–Co (platinum–cobalt colour unit), and 68.2% for DFZ (European norm for colour at three wavelengths)-436 nm, 68.4% for DFZ-525 nm, and 83.8% for DFZ-620 nm.

Keywords: *Lemna minor*, duckweed, colour, wastewater treatment, phytoremediation.

AIMS AND BACKGROUND

Phytoremediation is an alternative method to traditional expensive treatment methods in order to remove different pollutants such as heavy metals and xenobiotics from wastewater. In this study, it was aimed to find the efficiency of *Lemna minor* to remove colour from coloured industrial effluents as an alternative phytoremediation technology.

One of the negative consequences of industrialised societies is the spread of several xenobiotic compounds contaminating our environment. These compounds are causing a lot of public and ecological health problems. These potential health risks are mostly associated with increasing of wastewater discharges spreading the entire of aquatic environment containing toxic chemicals such as heavy metals, phenolic compounds and dyes. Such extensive use of dyes and pigments in the

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industries such as textile, leather, paper, cosmetic, plastic and food often poses problems in the form of coloured wastewater that require pre and/or advance treatment prior to discharge the receiving water bodies. Unless they are properly treated, these dyes may significantly affect photosynthetic activity in aquatic life due to reduced light penetration, and may also be toxic to some aquatic life due to the presence of metals[1].

The chemical composition of the colour contaminating effluents has rapidly changed due to a shift in the consumer preferences; the most significant reason of these contaminations is greater usage of synthetic reactive dyes and azo dyes[2]. Wastewater is a mixture of colourants (dyes and pigments) and various organic compounds used as cleaning solvents, and have more chemicals. It also contains high concentrations of heavy metals and total dissolved solids[3]. Some organic and inorganic compounds at toxic levels have been detected in industrial discharges resulting in plant upsets and discharge permit violations[4]. Conventional wastewater treatment plants have low removal efficiency for reactive and other anion soluble dyes due to their stability towards light, wash, heat and oxidising agents. The operational costs of these physicochemical treatment processes also are very high. Coloured industrial wastewaters are also less treatable through biological aerobic processes. Although biodegradation is a potential possibility of removal of soluble dyes, the reactive dyes are an exception[5].

In recent years, phytoremediation has emerged as an alternative technology that uses living organisms for recovery or cleanup of contaminated sites (soil, sediment, air and water)[6]. A wide variety of organisms are able to degrade textile dyes, including bacteria[7], fungi[8–10], yeast[11], and algae[12–14]. It has been found that plants also can be used as a remediation tool[15–17]. The integration of plants with the intensive aquaculture offers the advantage to have clean effluents and also possible a second crop production[18]. The higher aquatic plants have an important role in the biological purification of water sources. Structural investigations on these plant species offer scientific basis for their rational utilisation during water purification[19]. Plants are highly sensitive to many environmental pollutants but few cases have been reported that the plants have the potential capacity to degrade textile dyes[20]. Among different plant species, *Lemna minor* L. posses physiological properties such as small size, high multiplication rates and vegetative propagation. These characteristics make *L. minor* as an ideal test system for water quality studies to monitor heavy metal and other aquatic pollutants. The main objective of the present study is to find the colour removal efficiency of *L. minor* from an industrial textile wastewater using a laboratory scale pilot system by phytoremediation technology.

EXPERIMENTAL

In this study, the potential of duckweed, *L. minor*, grown in a laboratory scale pond system to remove colour from industrial effluent was investigated. Prior the experiments, plants were obtained from the logical quiescent water bodies and acclimated to laboratory conditions for a month. Then they were set into pilot system consisted of three Plexiglas ponds with a capacity of 14 l filled with Synthetic Duckweed Nutrient Solution (DNS)[21]. In the acclimation period, plants were fed only with DNS. Then, real coloured industrial wastewater was fed to pilot system. DNS was prepared by using the following salts in stock solution: $NaNO_3$, 25.50 g/l; $NaHCO_3$, 15.0 g/l; K_2HPO_4, 1.04 g/l; $CaCl_2.2H_2O$, 4.41 g/l; $MgCl_2$, 5.70 g/l; $FeCl_3$, 0.096 g/l; $MnCl_2$, 0.26 g/l; $MgSO_4.7H_2O$, 14.7 g/l; H_3BO_3, 0.186 g/l; $Na_2MoO_4.2H_2O$, 7.26 mg/l; $ZnCl_2$, 3.27 mg/l; $CoCl_2$, 0.78 mg/l; and $CuCl_2$, 0.009 mg/l. This stock solution was diluted with deionized water. The real untreated raw wastewater from a textile industry taken from the Bursa city was used in this study, and its characteristics were determined in the initial of experiments (Table 1). All experiments were carried out in two ponds. One pond was used as control pond without any addition of wastewater, and only fed with synthetic duckweed nutrient solution (DNS). All ponds were fed using a peristaltic pump continuously (Fig. 1).

Table 1. Wastewater characteristics of the system

Parameter	Value
pH	8.22±0.5
COD (mg/l)	300±100
DFZ – 436 nm	21±9.6
DFZ – 525 nm	15.2±6.2
DFZ – 620 nm	8.8±3.2
Pt-Co	2200±800
MLSS (mg/l)	165±80

Temperature was maintained at 23–25°C, and 16 h of photoperiod was applied to plants. Each pond was segmented into twelve 10×10 cm sections connected to each other, and hydraulic retention times (HRT) for the ponds were changed as 3–10 days. Water samples were collected from the effluent of the system, and analysed for suspended solids (MLSS), chemical oxygen demand (COD) and colour (in Pt–Co and RES units), periodically. COD, colour and MLSS values were measured following standard methods (APHA)[21]. All reagents used were of analytical grade. Standards of European Norm EN ISO 7887 were considered as basis and DFZ parameter was also chosen for colour measurements with a HACH Lange DR-5000 spectrophotometer. The measured absorbance values at three wavelengths of 436, 525 and 620 nm were converted to 'Indices of Transparency' (DFZ = Durchsichts-Farb-Zahl). DFZ limits, determined according to the European norm, are 7 m^{-1} for 436 nm, 5 m^{-1} for 525 nm and 3 m^{-1} for 620 nm (Europa Norm, 1994). DFZ calculation was made as follows:

$$\mathrm{DFZ} = 100\ (E_\lambda/d) \tag{1}$$

where E_λ is extinction (at a known wavelength) and d – the thickness of cell in cm.

All the samples were filtered (0.45 μm) before colour measurement test. In order to determine growth rates of plants grown in these mediums, frond numbers were counted daily to calculate growth rate constants according to the standard methods. At the beginning of each run, 40 fronds were set in each flask of 250 ml capacity containing 200 ml DNS and wastewater. Plants which are healthy, light green and in same size were chosen. Typical plant size was two to four fronds on each plant. Plant growth was explained depending on the increase of the frond number of *Lemna*. The growth rate is expressed as the difference of the logarithms of the final frond number (F_{t_2}) and the initial number (F_{t_1}) divided by the number of days of growth. The growth rate is then represented by the following formula[22]:

$$\mathrm{RGR} = \mathrm{d}F/\mathrm{d}t = kF;\ k = (\ln F_{t_2} - \ln F_{t_1})/(t_2 - t_1), \tag{2}$$

where RGR is the relative growth rate (frond number/day); k – the growth rate constant (1/d); F_{t_1} – the frond number at t_1, and F_{t_2} – the frond number at t_2.

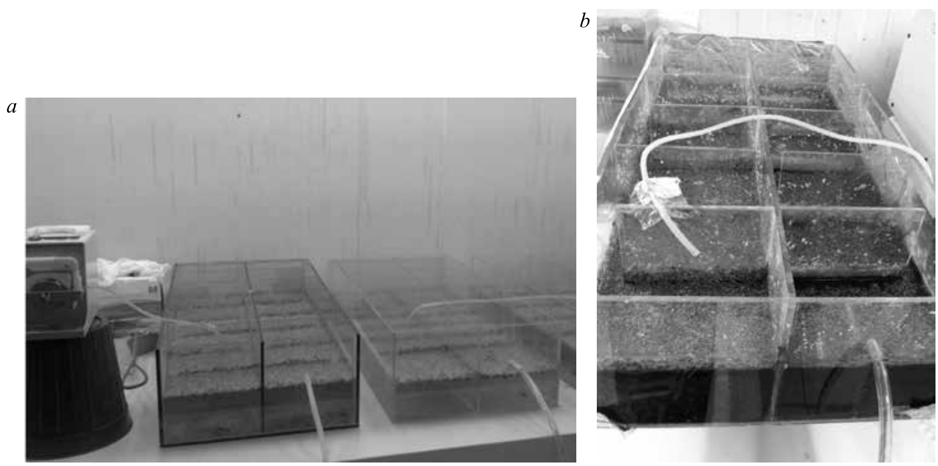

Fig. 1. Pilot system with *L. minor* (*a* – photo of the system in the initial of the experiments; *b* – photo of the system after adding of coloured wastewater)

RESULTS AND DISCUSSION

Plants that grow in contaminated environments due to industrial discharge of dye-containing wastewater are exposed constantly to these xenobiotic compounds. Therefore, survival plants and their ability for continuous bioremoval have been considered as an important criterion in the selection of plants with high potential for phytoremediation purpose[23]. In order to investigate this ability, experiments were performed by *Lemna* pilot system with different HRT values. In order to determine colour removal efficiency of *Lemna* in different HRTs, pilot system was operated in different HRT values of 3, 7 and 10 days. System was fed continuously and treatment performance of wastewater was determined by measuring colour, COD and MLSS parameters. The initial solution pH value of wastewater is a factor affecting pollutant removal performance of plants due to its effect on the growth rate of them. In this study, pH value of wastewater entering the system was not changed because it was in the range of plants optimum pH range of 6.5–8.0, normally. Water samples were taken from the effluents of the system in the first HRT for all studied different HRT values, and sampling was continued with time intervals. Calculated percentage removal values for the system having HRT of 10 days are shown in Fig 2. According to the results, the maximum removal efficiencies of treatment parameters for pond system having HRT of 10 days were found to be 75.0% for COD, 94.5% for MLSS, 62.4% for Pt–Co, and 59.5% for DFZ-436 nm, 62.9% for DFZ-525 nm, and 65.7 % for DFZ-620 nm.

The percentage removal of colour continuously decreased with time. Colour removal efficiency of the system for HRT of 10 days was in the range of 62.4–3.3% for Pt-Co; 59.5–3.2% for DFZ-436 nm; 62.9–4.6% for DFZ-525 nm and 65.7–8.2% for DFZ-620 nm, respectively. However, COD and MLSS removal efficiencies of this run were in the range of 75.0–52.0% for COD and 94.5–55.4% for MLSS. While colour removal efficiency of the system decreased with time, MLSS removal increased, but COD removal did not change after second HRT of 20 days. According to Fig. 2, treatment performances of the system get worse after second HRT value. The decreasing removal rate by aquatic plants with increasing contact time is indicative of a fast attainment of saturation state in the plants[24]. Thus, it is possible to say *Lemna* reached to saturation for colour and other parameters in 20 days.

Calculated percentage removal values for HRT of 7 days are shown in Fig. 3. According to the results, the percentage removal of colour continuously decreased with time. Colour removal efficiency of system for HRT of 7 days was in the range of 33.9–12.3% for Pt–Co; 34.2–5.8% for DFZ-436 nm; 34.9–4.1% for DFZ-525 nm; 34.0–23.3% for DFZ-620 nm, respectively. However, COD and MLSS removal efficiencies were in the range of 57.7–43.1% and 91.7–80.4%, respectively. Colour removal of the system decreased with time while MLSS and COD removal did not changed significantly. According to this system for HRT of 7 days, the optimum operating time was found as 15 days (namely second HRT).

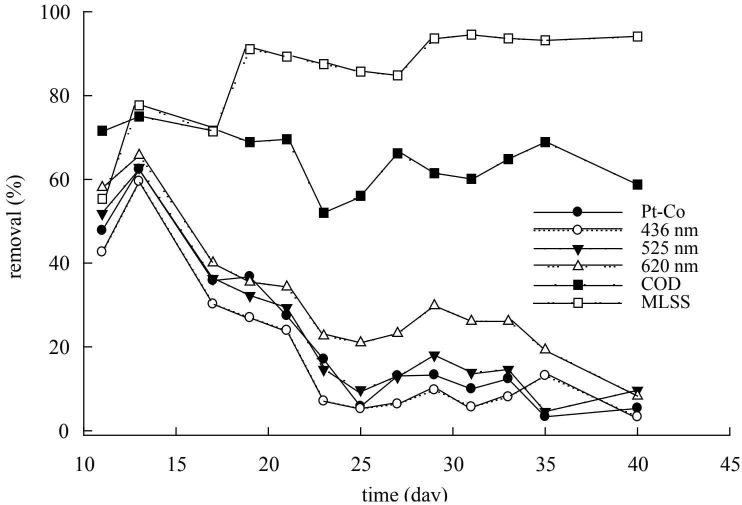

Fig. 2. Removal efficiency of the system in HRT of 10 days

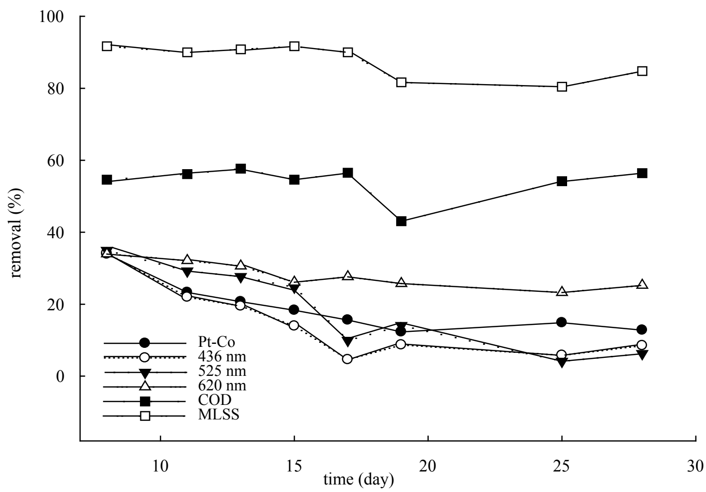

Fig. 3. Removal efficiency of the system in HRT of 7 days

Percentage removal values for the system having HRT of 3 days are shown in Fig. 4. According to the results, the percentage removal of colour continuously decreased in the first two HRT of 6 days, and did not change significantly after that with time. Colour removal efficiency was in the range of 66.1–55.5% for Pt–Co; 68.2–58.5% for DFZ-436 nm; 68.4–58.9% for DFZ-525 nm; 83.8–76.4% for DFZ-620 nm, for HRT of 3 days, respectively. COD and MLSS removal efficiencies were in the range of 85.9–75.4% and 79.4–35.2%, respectively. However, their removal efficiencies increased with time up to 15 days. Thus, it can be said that

Lemna system have good treatment performance for coloured textile wastewater for HRT of 15 days.

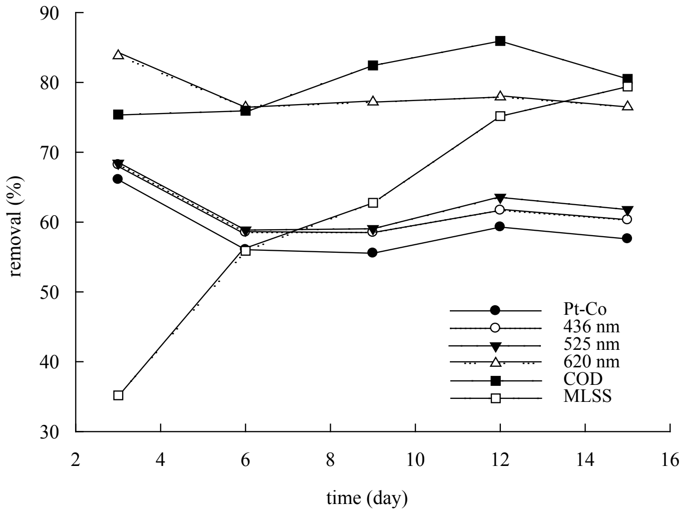

Fig. 4. Removal efficiency of the system in HRT of 3 days

In order to determine growth rate changes of plants when they were exposed to coloured real wastewater, plants were grown in flasks containing 200 ml wastewater and DNS. These flasks were incubated under the same medium conditions. The frond numbers of plants were counted daily to calculate the growth rate constants, and compared to that of control to determine the percentage inhibition. Growth rate values of *L. minor* were studied by batch technique and shown in Table 2. Results showed that plants growth was faster in the DNS solution than those plants grown in the wastewater. Results also showed that complex structure of untreated textile wastewater effected the plants growth negatively with time.

Table 2. Relative growth rate values of plants grown in the DNS and wastewater

Time (days)	RGR (d^{-1})		Percentage of control (%)
	DNS	wastewater	
3	0.037	0.024	64.8
6	0.032	0.023	71.8
9	0.031	0.024	77.4
12	0.036	0.016	44.4
15	0.036	0.015	41.7
18	0.038	0.013	34.2

CONCLUSIONS

The present study revealed that a pond system with continuous flow containing duckweed, *L. minor* as an aquatic plant is able to remove colour from untreated textile wastewater effectively. In addition to this, variation in HRT values was an important factor in the colour removal by phytoremediation systems like this. The results obtained from the experiments showed that high colour, COD and MLSS removal efficiencies can be obtained by these plants when they are exposed to coloured wastewater continuously. The best treatment performance of the system was obtained at HRT of 3 days, and it increased with increasing HRT up to 15 days. The results presented demonstrate that a laboratory-scale *Lemna* system has a potential to treat coloured real textile wastewater. It has been observed that *Lemna minor* has a high capacity of adaptation, and duckweeds play a substantial role in the pollutant removal from industrial wastewater.

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