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Shallow pond systems planted with *Lemna minor* treating azo dyes

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A B S T R A C T

A higher demand on textile materials has resulted in an increase of the number of textile factories particularly in the developing world, which consequently negatively effects the environment due to their contaminated effluents. Textile effluents are highly coloured and mixed with different chemicals and pollutants. Shallow pond systems are a promising, cheap and effective technique for the treatment of contaminated wastewater. The aim of this study is to assess the performance of pond systems vegetated by *Lemna minor* L. (duckweed) for textile dye removal under controlled laboratory conditions. The key objectives of this study are to assess the influence of design variables on water quality parameters, the dye and chemical oxygen demand (COD) removal of dyes, and the effect of dye accumulation as a function of the relative growth rate of *L. minor*. Findings indicate that the simulated shallow pond system (as a polishing step) is able to remove only Basic Red 46 (BR46) in low concentrations, and ponds containing *L. minor* significantly ($p < 0.05$) outperformed algae-dominated ponds and control ponds. The simple chemical structure, absence of sulpho-group and small molecular weight associated with neutral pH values enhanced the capacity of the uptake of BR46 molecules. Furthermore, the total dissolved solid concentrations were within the threshold set for discharge to the aquatic environment.

Keywords:

Colour red

Biological treatment

Duckweed

Colorant removal

Textile wastewater

Water quality assessment

1. Introduction

1.1. Background

The industrial revolution and rapid population growth have increased the demand for textile materials, which has consequently increased the number of textile industries and their effluents (Khataee et al., 2012). Synthetic dyes are used to add colour to fibres (Sivakumar et al., 2013). However, in this process, a large volume of water is used, which as a result, discharges considerable amounts of dyeing effluent as waste into receiving waters.

In commercial terms, azo dyes are seen as the largest group of synthetic dyes (Pandey et al., 2007), and it is estimated that between 60% and 70% of the dyes applied in the textile industry are azo compounds. This group of dyes is distinguished by the presence of one or more double bonds between nitrogen atoms (Pandey et al., 2007;

Cumnan and Yimrattanabovorn, 2012). In textile dyebaths, dyes cannot bind with fabrics completely, resulting in some of the dyes being lost (Pearce et al., 2003). The dye wastewater effluents are high in colour, pH, suspended solids (SS), COD (Verma et al., 2012), biochemical oxygen demand and metals (Sekomo et al., 2012). Typically, textile industry-processing effluents contain dyes in the range between 10 and 200 mg/l (Pandey et al., 2007).

Most textile dyes with a rather low concentration of below 1 mg/l can be detected by the human eye (Pandey et al., 2007). Therefore, this aesthetic problem is one of the major challenges for receiving watercourses. In addition, a high concentration of these dyes in the receiving water body will prevent light penetration and negatively affect the ecosystem by reducing respiration and photosynthesis of aquatic organisms (Reema et al., 2011; Saratale et al., 2011). Moreover, these effluents pass through soil layers and may contaminate nearby surface and ground waters (Sivakumar, 2014). Furthermore, some textile dyes and their intermediate products are deleterious due to their toxicity, mutagenicity and carcinogenicity to life (Khataee et al., 2012).

Biological treatment alternatives using constructed wetlands are sustainable and cost-effective (Means and Hinchee, 2000). This technology is well-accepted to be environmentally friendly, cheap, simple-to-use and effective to treat diverse municipal sewage, storm water, agricultural runoff and industrial wastewaters worldwide (Scholz, 2010; Sani et al., 2013). Literature indicates promising results for textile dye removal using vertical and/or horizontal up-flow or down-flow wetland systems (Davies et al., 2005; Mbuligwe, 2005; Bulc and Ojstrsek, 2008; Ong et al., 2009; Cumnan and Yimrattanabovorn, 2012). The high removal efficiency in terms of dye, COD and other contaminants was due to the complex interactions between plants, water, soil and micro-organisms. Recently, there has been attention towards using shallow pond systems to treat textile wastewater contaminated with dyes using aquatic plants as a cheap, effective and environmentally friendly method. However, the literature in this area is still limited, and there are only short-term studies treating wastewater contaminated with textile dyes in shallow pond and wetland systems in Turkey and India (Muthunarayanan, 2011; Sivakumar et al., 2013; Sivakumar, 2014; Uysal et al. 2014).

1.2. Lemna minor

Wetland plants can play a major role in dye wastewater treatment (Mbuligwe, 2005). For example, *Lemna minor* is a rather small free-floating macrophyte, which grows rapidly and adapts easily to diverse aquatic conditions in stagnant ponds or slow-flowing streams (Movafeghi et al., 2013; Khataee et al., 2012). This plant has the ability to accumulate and assimilate pollutants from wastewater (Bekcan et al., 2009). It is also used for the removal of heavy metals (Sekomo et al., 2012) from industrial and textile wastewaters. In addition, it is a good source of fodder, because it has high concentrations of protein and low fibre content (Bekcan et al., 2009).

1.3. Aim and objectives

The overall aim is to assess the performance of simulated shallow pond wetland systems vegetated by *L. minor* for the treatment of artificial textile dye wastewater under controlled conditions. The corresponding objectives are to (a) evaluate and compare the water quality of different design variables such as the presence of *L. minor* and/or algae; (b) assess the influence of the design variable on water quality parameters; (c) assess and compare the dye and COD removal of four classes of dyes (Acid Blue 113, Reactive Blue 198, Direct Orange 46 and Basic Red 46) with each other; and (d) monitor the effect of dye accumulation as a function of the relative growth rate of *L. minor*.

2. Materials and methods

2.1. Dyes and nutrients

Four dyes were used in this study: Acid Blue 113 (AB113), Reactive Blue 198 (RB198), BR46 and Direct Orange 46 (DO46), which were supplied by Dystar UK Limited (Colne Side Business Park, Huddersfield, United Kingdom) except for AB113, which was obtained from Sigma-Aldrich Company UK Limited (The Old Brickyard, New Road, Gillingham, United Kingdom). The studied azo dyes are different in structure, molecular weight, mode of applications and number of azo bonds (Table 1).

The fertiliser TNC Complete, which is an aquatic plant nutrient supplied by TNC Limited (Spotland Bridge Mill, Mellor Street, Rochdale, United Kingdom), was used in this study. The corresponding ingredient composition was as follows: nitrogen (1.5%), phosphorus (0.2%), potassium (5%), magnesium (0.8%), iron (0.08%), manganese (0.018%), copper (0.002%), zinc (0.01%), boron (0.01%) and malybdenum (0.001%). Ethylenediaminetetraacetic acid, which is used as a source for copper, iron, manganese and zinc, was also provided by TNC Complete. One millilitre of fertiliser was added to 10 l of dechlorinated tap water.

Dye stock solutions were arranged for each dye by dissolving 5 g of a dye in one litre of distilled water and stored in the dark at 4ºC. The synthetic wastewater applied in this project was prepared by mixing the dye solution with dechlorinated tap water and fertiliser (TNC Complete), providing a 5-mg/l concentration for each dye.

2.2. Experimental set-up phases

The experiment was carried out at the university using plastic containers (length, 33 cm; width, 25.5 cm; depth, 14 cm) located outside. Twenty containers simulating shallow ponds were allocated for each dye. An additional 20 containers without dyes (controls) were also monitored. The containers were manually operated; e.g., contaminated inflow water was added by the operator. It follows that there were no physical inflow or outflow structures in the containers.

In the first phase between 10 July 2014 and 11 August 2014, each container was filled with tap water to the desired level of 6.9 cm depth, which is equivalent to 5 l. Subsequently, 200 healthy *L. minor* plants were added to each container, and the system was fed weekly with water and fertiliser (for composition; see section 2.1.). The plant was collected from a small pond close to Cowpe Reservoir (Cowpe, Rossendale, United Kingdom).

In the second phase between 11 August 2014 and 15 December 2014, the system was kept outside for acclimatisation and monitoring purposes. During this period, the plants grew very well, and some algae started to develop in most systems naturally, which were detected by using Leica DM750 LED Biological Microscope with ICC50W Camera Module-5.0 Mega Pixel (New York Microscope Co., Lauman Lane, Hicksville, New York, USA). Algal species were identified with the help of standard textbooks such as Nakada and Nozaki (2015). From 9 September 2014, dyes at a concentration of 5 mg/l were added to undertake initial tests to examine plant survival (data not shown).

On 15 December 2014, the third phase was started. The experiment was performed under controlled laboratory conditions by moving 69 containers (simulated pond environments) to the Maxwell Building (The University of Salford). The experiment comprised 14 ponds for each dye and 13 ponds without any dye. The set-up consisted of four treatment groups. The first group comprised *L. minor* and algae (LA Pond), the second one used only *L. minor* (L. pond), the third group used only algae (A Pond) and the fourth group represented the control without using *L. minor* and algae (C. pond). Four replicates for each group and two replicates for each control were used.

The group of artificial ponds containing only *L. minor* as well as the control group without any plants were kept free of algae. Considering that *L. minor* grows very rapidly, the surface areas of the ponds were frequently covered preventing sunlight from reaching any traces of algae in the *L. minor* ponds. However, any visual traces of algae were removed manually. This was only the case for *L. minor* ponds at the start of the experiment before *L. minor* got fully established. Furthermore, control ponds were free of any algae from the start.

The first dose of the dyes was applied to the pond systems after the transfer date on 23 December 2014. The concentrations of all doses were 5 mg/l and the contact time was seven days. The solution was topped-up weekly to the same desired level (equivalent of 5 l) as required to compensate for water loss due to evaporation and transpiration. Figure 1 presents the diagram for the experimental set-up. Table 2 shows a summary for the experimental phases.

2.3. Environmental boundary conditions

OSRAM HQL (MBF-U) High Pressure Mercury Lamp (400 W; Base E40) grow lights supplied by OSRAM (North Industrial Road, Foshan, Guangdong, China) and supported by a H4000 Gear Unit, which was provided by Philips (London Road, Croyden, United Kingdom), were used in the laboratory during the third phase of the experiment. Light was controlled by a timer, simulating the daylight conditions in Salford, with the help of the website Time and Date (2016).

The relative humidity values were between 67% and 48% (mean of 54%) and temperature readings were between 22°C and 29°C (mean of 27.3±2°C). They were measured using the Thermometer-Hygrometer-Station supplied by wetterladen24.de (JM Handelspunkt, Geschwend, Germany).

Light measurements were performed by using the lux meter ATP-DT-1300 for the range between 200 lux and 50000 lux (TIMSTAR, Road Three, Winsford Industrial

Estate, Winsford, United Kingdom). Readings were between 2023 lux and 2450 lux (mean of 2215 lux) directly above the plants. A summary of the environmental boundary conditions in the laboratory is shown in the Supplementary Material 1.

2.4. Water quality analysis

Water quality sampling (50 ml) was carried out according to APHA (2005). The spectrophotometer DR 2800 Hach Lange (Hach Lange, Willstätter Strasse, Düsseldorf, Germany) was used for standard water quality analysis for variables including (dye) absorbance, colour, SS and COD. The turbidity was determined with a Turbicheck Turbidity Meter (Tintometer, Lovibond Water Testing, Dortmund, Germany). The redox potential and pH were determined with a WTW Vario meter (Cole-Parmer Instrument Co., River Brent Business Park, Hanwell, London, United Kingdom). Dissolved oxygen (DO) was measured with a Hach HQ30d flexi meter (Hach, Pacific Way, Salford, United Kingdom). The electric conductivity was determined using the conductivity meter METTLER TOLEDO Five Go^{TM} (Keison Products, Chelmsford, United Kingdom).

The dye analysis was performed for 12-ml samples, which were filtered through a 0.45-μm pore diameter Whatman filter paper (Scientific Laboratory Suppliers, Wilford Industrial Estate, Nottingham, United Kingdom). The filtered water sample was then analysed with a UV-vis spectrophotometer (DR 2800 Hach Lange) at the maximum absorption wavelengths for each dye. The maximum absorption wavelength of each dye was determined for an aqueous solution using a scanning UV-vis spectrophotometer

WPA Bio Wave II (Biochrom, Cambourne Business Park, Cambourne, Cambridge, United Kingdom). The corresponding wavelengths were 566, 625, 530 and 421 nm for AB113, RB198, BR46 and DO46, respectively. The concentrations of dyes were determined depending on standard calibration curves.

2.5. Plant growth monitoring

In order to assess the impact of dye accumulation on *L. minor*, the plants were monitored and harvested to avoid over-crowding, which inhibit the optimum growth of *L. minor*. The fresh biomass weights were taken after putting the harvested plants on absorbent paper for five minutes. The dry weights were recorded after the plants were dried in an oven at 105°C for 24 hours. To measure the growth rate of *L. minor,* the frond numbers and coverage areas were monitored and recorded using the Aletheia Lemna Edition software (not in the public domain) developed by Christian Clausner (c.clausner@primaresearch.org). The relative growth rate based on the frond number was calculated, and the relative frond number of the plants was used as an indicator of possible toxicity (Horvat et al., 2007). The colour of the fronds were determined with the help of the Munsell colour chart (Munsell Color, 1977).

Regarding to the Aletheia Lemna Edition software application, the digital images were taken using OLYPUS VH 520, a compact digital camera with 14 megapixels on a full liquid crystal display screen. Natural light was used and the flash was switched off. To obtain a good resolution, the camera was in the automatic option mode (denoted "auto") and the distance was 60 cm above the water level. After taking pictures of the buckets containing *L. minor* from the top and of the relevant colours in the Munsell colour chart, the Aletheia Lemna Edition was applied.

2.6. Data analysis

Microsoft Excel (www.microsoft.com) was applied for the standard data analysis. IBM SPSS Statistics Version 20 (www.ibm.com) was used to compute the non-parametric Kruskal-Wallis ($p < 0.05$) statistic for comparing the medians among the non-normal-distributed dependent variables. The parametric one-way and univariate analysis of variance (ANOVA) was used for comparing the medians among the groups for the normality-dependent variables. The parametric *t*- test for comparisons between two groups was also applied. The Shapero-Wilko test was applied to check for normality. The Person and Spearman tests were used to calculate the correlation coefficients of different parametric and non-parametric variables, respectively.

3. Results and discussion

3.1. Inflow water quality parameters

Supplementary Material 2 shows the inflow water quality parameters of the prepared synthetic wastewaters, which include fertiliser and textile dye. These parameters compared well with the typical characteristics of textile wastewater

(Upadhye et al., 2012). Only the pH and the colour values were within the typical range of 6 to 10 and 50 to 2500 Pt Co, respectively. The dye inflow concentration was 5 mg/l lower than the typical range, but it compared well with concentrations used in the literature for the treatment of Brilliant Blue R (Kilic et al., 2010), Basic Red 46 (Movafeghi et al., 2013) and Acid Blue 92 (Khataee et al., 2012). In addition, the treated effluents in preliminary or secondary treatment stages are associated with concentrations lower than the textile factory outflow values.

3.2. Comparison of outflow water quality

This section covers the objectives (a) and (b). The contact time may influence the outflow water quality of pond treatment systems. A contact time of seven days is similar to those commonly published in the literature regarding *L. minor* (Kilic et al., 2010; Movafeghi et al., 2013; Sivakuma, 2014) used for the treatment of textile dyes. In addition, Reema et al. (2011) stated that the potential of *L. minor* for uptake of dyes accelerates with an increase in contact time for all initial dye concentrations.

Supplementary Material 3 shows the outflow water quality, and Table 3 summarises the statistical analysis of outflow water quality parameters and the removal efficiencies. The mean values of the outflow dye concentrations for AB113 showed no differences among the design variables. The ranges were between 6.3 mg/l for the control ponds and 7.6 mg/l for ponds containing *L. minor*. The outflow AB113 concentrations increased gradually over time during all experimental periods except for the control ponds, where they decreased in August and then sharply enhanced at the end of the experiment (Supplementary Material 4a). However, for the other dyes, the outflow dye concentrations were higher within the control ponds than the remaining ponds (Supplementary Material 4b,c,d). No difference was found between combined *L. minor* and algae ponds and ponds planted by *L. minor*, which indicates that the presence of algae does not affect the dye outflow concentrations. The outflow concentrations from algae ponds and control ponds were significantly higher than other design variables in ponds containing BR46 and DO46. In comparison, only algae ponds were significantly lower using RB198 (Table 3).

The lowest standard deviations were linked to BR46, because the outflow values were stable (Supplementary Material 4c), except for those of the control ponds, which fluctuated highly. Environmental policies in the UK require concentrations of the synthetic dye wastewaters to be discharged to the aquatic environment to be zero (Pearce et al., 2003). Colour mean outflow values were higher in control and algae ponds. However, the lowest mean values were in *L. minor* and, *L. minor* and algae ponds for all types of dye. The colour mean values in ponds containing tap water and fertiliser were ranked as follow: control > *L. minor* and algae > algae > *L. minor* ponds. Nevertheless, no differences among design variables for AB113, RB198 and DO46 were noted. In contrast, ponds with *L. minor* only and control ponds were significantly different than other ponds for BR46 (Table 3). Correlation analysis results indicated that the colour was significantly ($p < 0.01$) positively correlated with the dye ($r = 0.703$, $p = 0.000$) and COD ($r = 0.638$, $p = 0.000$) concentrations. This positive correlation can be explained by the deteriorations in dye and COD removal.

The mean values of pH obtained from the outflow (Supplementary Material 3) were slightly higher than the inflow values. Nonetheless, the pH values (6.6 to 9.0) for all pond outflows were within the pollution control standards (5.5 to 9) set, for example, in Thailand, where a lot of coloured textiles are produced (Nilratnisakorn et al., 2009). However, the maximum outflow values in the control ponds containing tap water and fertiliser are beyond European and international standard thresholds (6.5-8.5) according to Carmen and Daniela, (2012). In total, samples were 11 times non-compliant. The highest mean values for the pH outflow were observed in all control ponds, using tap water and fertiliser, followed by control ponds comprising dyes. In comparison, the lowest mean values were found in *L. minor* ponds containing BR46 followed by ponds containing *L. minor*, algae and the dye RB198 or BR46. The mean pH values for algae ponds were either similar or higher than the ponds comprising *L. minor* only, and *L. minor* combined with algae. The outflow pH values increased slightly compared to the inflow of all ponds. However, the small differences found between the ponds containing *L. minor* were lower compared to other ponds without *L. minor*. This may be because the plants try to gain an equilibrium between chemicals in the cells by proton and ion exchanges from the artificial wastewater (Noonpui and Thiravetyan, 2011).

The pH value made an important impact on the capacity of dye uptake and plant growth. The optimum pH to obtain a high removal efficiency depends on the type of dye itself; e.g., 7.0 for Methylene Blue, 8.0 for Basic Blue nine (Reema et al., 2011), 6.0-7.5 for BR46 (Movafeghi et al., 2013) and 6.5 for Acid Blue 92 (Khataee et al., 2012). Saratale et al. (2011) indicated that the optimum pH for high colour removal should be within the range 6-10. The removal efficiency considerably declines at strong acid or alkaline conditions for biological treatment systems. In comparison, the allowable range of pH for growth of *L. minor* is 4.5-8.3 (Reema et al., 2011).

Dissolved oxygen and redox potential are indicators for the aerobic and anaerobic conditions in the aquatic life (Ong et al., 2009). Overall, the values of outflow DO, which ranged between 7.9 and 10.1 mg/l, were lower than the inflow values, which varied between 8.6 and 10.8 mg/l. In addition, the mean outflow values (Supplementary Material 3) in terms of DO were relatively similar; no differences among the design variables for AB113, RB198, BR46 and DO46 were calculated. In contrast, significant differences were noted for the mean values of control ponds containing tap water and fertiliser (Table 3), where the DO was higher compared with ponds containing *L. minor* and/or algae. However, these findings do not match those published by Sekomo et al. (2012), which are related, however, to the treatment of heavy metals. In their study, higher values of DO were noted for algae ponds than duckweed ponds, because the photosynthetic process in algae ponds happen within the water body, whereas in duckweed ponds, the activities of oxygen production occur at the top layer, where consequently some of the oxygen is lost to the atmosphere and small amounts move to the water via the roots (Sekomo et al., 2012). This difference in results is expected, because the amount of algae in this research is limited and oxygen diffusion by the atmosphere affects the DO level rather than the impact of the system type.

Ong et al. (2011) reported that the biodegradation of organic contaminants in wetlands was boosted remarkably by the presence of a high amount of DO, which can facilitate the growth of aerobic microorganisms for eliminating organic substances. But high DO inhibited the dye removal mechanism because of the electrons released by microbial cells during the oxidation process utilising oxygen instead of azo dyes during degradation processes (Pearce et al., 2003).

Based on redox potential monitoring, higher mean outflow values (Supplementary Material 3) were observed in ponds comprising *L. minor* and ponds linked to both *L. minor* and algae in comparison to ponds with algae only and control ponds for all types of dyes. The mean outflows in terms of redox potential for wastewaters contained tap water and fertiliser ranked as follows: *L. minor* ponds > algae ponds > *L. minor* and algae ponds > control ponds. Statistically, no differences were observed for the mean values of the redox potential between algae and control ponds for all dyes. Furthermore, the mean redox potential values in *L. minor*, and *L. minor* and algae ponds were also not different, except for BR46 (Table 3). This is possibly because of the treated effluents from ponds containing AB113, RB198 and DO46 were coloured, which interferes with the photosynthesis of algae (Carmen and Daniela, 2012). However, the effluents from ponds comprising BR46 were colourless due to their high removal efficiencies. Regarding ponds containing tap water and fertiliser, only algae ponds, and *L. minor* and algae ponds were not different. The maximum and minimum values of redox potential indicating anoxic conditions, except for the control pond containing tap water and fertiliser, the maximum value was -116 mV, indicating anaerobic conditions (Supplementary Material 3).

The highest value of mean outflow SS concentration was noted for control ponds containing tap water and fertiliser followed by the control pond containing BR46, then by *L. minor* ponds, and *L. minor* and algae ponds comprising AB113. In contrast, the lowest value was observed in *L. minor* and algae ponds fed by tap water and fertiliser followed by *L. minor* ponds containing BR46 (Supplementary Material 3). The European and international standards for SS are 35 mg/l in case of discharge directly into receiving freshwater bodies. The results indicated that the *L. minor* and algae ponds, *L. minor* ponds, algae ponds and control ponds contain AB113, *L. minor* ponds comprise RB198, control ponds fed by BR46, tap water and fertiliser were 10, 9, 1, 1, 3, 22 and 29 times non-complaint, respectively. However, in case of discharge to urban wastewater sewerage networks, the regulations set a value of 350 mg/l. Only control ponds containing tap water and fertiliser were three times non-compliant (Carmen and Daniela, 2012).

Statistically, the mean outflow values of SS in the control ponds comprising BR46, tap water and fertiliser were significantly higher than other treatments (Table 3). However, for AB113 and RB 198, outflow values of SS were significantly higher for ponds containing *L. minor*, and *L. minor* and algae than algae only and control ponds (Table 3). Also, for AB113 and RB 198, no differences were found between the *L. minor*, and *L. minor* and algae ponds. No dissimilarity was also found between algae and control ponds. In terms of DO46, no difference was found among the design variables.

Based on the mean outflow values of turbidity (Supplementary Material 3), statistical analysis shows that *L. minor*, and *L. minor* and algae ponds were significantly higher than algae and control ponds containing AB113 when applying the Kruskal-Wallis test (Table 3). The control ponds comprising BR46, tap water and fertiliser were significantly higher than other design variables (Table 3). However, no dissimilarity was found for the mean values in terms of outflow turbidity among the design variables in ponds containing DO46. The highest and lowest turbidity values mirrored those for SS. A correlation analysis indicated that SS was significantly ($r = 0.773$, $p < 0.001$) positively correlated with turbidity and significantly ($r = -0.132$, $p < 0.001$) negatively correlated with DO. Therefore, high values of DO in the systems may reflect the low micro-organism activities for organic matter degradation, which consequently reduced the SS particles (Sani et al., 2013) and the COD removal (Scholz, 2010). A correlation analysis indicated that COD removal was significantly (p < 0.05) negatively correlated with DO (r = -0.249, p = 0.026).

An increase in the electronic conductivity (EC) values can inhibit the growth of *L. minor* as stated by Wendeou et al. (2013). The optimum growth rate of *L. minor* is associated with EC values ranging between 600 and 1400 μS/cm. All mean outflow EC values increased more than the inflow ones (Supplementary Material 3), and the highest EC mean values were observed for both control and algae ponds, whereas the lowest values were found in *L. minor*, and *L. minor* and algae ponds for all types of wastewater. This may indicate that the presence of plants in the ponds is responsible for the reduction of the EC. In addition, the conductivity mean values for planted ponds containing only water and fertiliser were higher than those for ponds comprising dyes. These findings match observations by Nilratnnisakorn et al. (2009) suggesting that large numbers of dye molecules might be caught in barriers within the vascular plant system. However, salts originate from synthetic wastewater. The plants are able to remove small molecules of these salts from solutions by passing them through their semi-permeable membrane (Noonpui and Thiravetyan, 2011).

Statistically, the mean outflow values of EC in algae ponds were significantly higher than those values of the other treatments (*L. minor*, *L. minor* and algae, and control ponds) for AB113 and DO46 (Table 3). However, for RB198, BR46, and tap water and fertiliser, the mean outflow values of EC in algae ponds and control ponds were significantly higher than those values of the other treatments (*L. minor*, and *L. minor* and algae) as shown in Table 3. In addition, no difference was found between *L.*

minor, and *L. minor* and algae ponds, and no dissimilarity was also noted between algae and control ponds for RB198, BR46, and tap water and fertiliser (Table 3).

The total dissolved solids (TDS) values for all treatment system outflow waters were compliant with the standard set in Thailand, which includes a threshold of 3000 mg/l (Nilratnnisakorn et al., 2009). Moreover, the values complied also with the European standard (class I -natural non polluted state) threshold of 500 mg/l (Carmen and Daniela, 2012).

Figure 2 shows the COD outflow concentration profile. In general, the COD concentrations increased gradually, and were higher than the inflow, which indicates low microbial activity for degradation of the organic matter associated with high levels of DO. The COD mean outflow values fluctuated and ranked for AB113 and RB198 as follow (Supplementary Material 3): *L. minor* and algae > *L. minor* > algae > control. The ranking was control > *L. minor* and algae > *L. minor* > algae for BR46, tap water and fertiliser. Finally, the ranking was *L. minor* and algae > algae > *L. minor* > control for DO46. Nevertheless, no differences were found among design variables in systems containing AB113, RB198, DO46 and BR46. However, in treatment systems comprising only tap water and fertiliser, the COD of the control pond was significantly higher than the COD for those ponds containing *L. minor* and/or algae (Table 3). This indicates that the absence of plants and dyes in the system increases the COD values. The European and international standards (Carmen and Daniela, 2012) set a threshold value for COD of 125 mg/l with respect to the discharge of effluent directly into water bodies. The results have shown that the COD values in *L. minor* and algae containing AB113, *L. minor* with AB113, algae with AB113 and control ponds containing AB113, *L. minor* and algae ponds comprising DO46, control ponds containing BR46, and control ponds with tap water and fertiliser were 6, 6, 2, 3, 1, 4 and 6 times non-compliant, respectively.

According to Bekcan et al. (2009), the long-term optimum temperature for *L. minor* growth is 26°C. The minimum and maximum temperatures are 4°C and 33°C, respectively. The temperature records (Supplementary Material 1) were within the recommended ranges. Movafeghi et al (2013) found that dye removal improved with an increase in temperature. They suggested that dye biosorption is an endothermic process linked to *L. minor*. Light intensity values were close to the optimum value of 2400 lux as documented by Kilic et al. (2010).

3.3. Dye Removal

Supplementary Material 5 shows the average values of dye removal. Table 4 provides an overview of the statistical analysis for dye removal efficiency. In general, very low dye removal efficiency was observed for AB113, RB198 and DO46. Statistically, no difference was found for the mean values of dye removal efficiency among the design variables for ponds containing A113, DO46 and RB198. This indicates that the biological treatment method of using shallow ponds with *L. minor* and/or algae, which is associated with high DO values and the presence of anoxic conditions, inhibits the removal of dye molecules. However, pond systems treating BR46 show mean removal efficiencies, which are highest for *L. minor* ponds (69%) followed by *L. minor* and algae ponds (67%), and then algae only ponds (53%). The lowest mean removal (31%) is linked to the control ponds. These results indicate that the impact of *L. minor* and algae on BR46 removal are 38% and 22%, respectively. These data resemble outcomes using submerged plants for handling Basic Blue 41 (Keskinkan and Goksu, 2007). Statistically, no difference in mean BR46 removal was found between ponds containing *L. minor*, and *L. minor* and algae, which indicates that the presence of a limited amount of algae does not affect the removal in *L. minor* and algae ponds. This is because *L. minor* covers the surface area, preventing light penetration and consequently inhibits the growth of algae biomass in the system (Sekomo et al., 2012). In addition, ponds with *L. minor* as well as ponds with *L. minor* and algae had significantly higher mean removal efficiencies than those without *L. minor* (algae and control ponds). Differences between control ponds were significantly ($p < 0.05$) lower than for the other ponds containing *L. minor* and/or algae (Table 3).

The longitudinal profile of the dye removal is shown in Fig. 3. The maximum and minimum removal were as follow: 26% and -4% for *L. minor* and algae ponds, respectively, and 26% and -6% for *L. minor* ponds in that order, 39% and -5% in algae ponds, respectively, and 29% and 11% in control ponds in that order for the treatment of AB113 However, these values were 21% and 5%, 28% and 3%, 29% and 1%, and 34% and -10% for *L. minor* and algae ponds, *L. minor* ponds, algae ponds and control ponds, respectively, for the treatment of RB198 (Fig. 3b). The DO46 removal ranged between 26% and -6%, 24% and -9%, 22% and -5 %, and 16% and -7% in *L. minor* and algae ponds, *L. minor* ponds, algae ponds and control ponds, respectively (Fig. 3d).

For BR46, the maximum and minimum removals were as follows: 85% and 26% in *L. minor* and algae ponds, 88% and 24% in *L. minor* ponds, 84% and 19% in algae ponds, and 60% and 3% in control ponds. Negative dye removal efficiencies can be explained by variations in influent concentrations and by the effect of plants harvested from the system, which consequently decreased the removal efficiency by reducing the surface area for sorption of dye molecules (Khataee et al., 2010) as indicated in Table 5.

Multiple comparisons of removal efficiencies among dyes showed no difference between AB113 and RB198. However, BR46 was significantly higher and DO46 was significantly lower than other dyes (Table 4), and the corresponding dye values ranked as follow: BR46 > RB198 > AB113 > DO46. This can be explained by the simple structure and small molecular weight of BR46. These findings match related ones by Noonpui and Thiravetyan (2011).

Furthermore, the neutral pH in the system was suitable for BR46 uptake. Similar results have been reported by Movafeghi et al. (2013) and Reema et al. (2011). There is electrostatic attraction between the sorbent surface, which is negatively charged, and the dye cation, that has a positive charge (Reema et al., 2011). Moreover, the absence of sulpho-groups in BR46 exhibits a good level of degradation during biological treatment methods (Pearce et al., 2003). AB113 contained two solphonation groups and was not treated by shallow pond systems, but Forgaces et al. (2004) reported that this dye adsorbs well in activated sludge treatment systems.

The control pond was significantly different from the other ponds (Table 4). The performance ranked as follows: *L. minor* ponds > *L. minor* and algae ponds > algae ponds > controlled ponds. Note that this sections and the subsequent section 3.4 cover objective (c).

3.4. Chemical oxygen demand removal

The average COD removal efficiency (Supplementary Material 5) was low for all ponds, which was clearly noticed from the inflow values corresponding to the high outflow values as discussed in section 3.2 (Supplementary Materials 2 and 3). Statistically, no significant difference was found in COD removal among the design variables for ponds containing AB113, RB198 and DO46 (Table 3). Concerning ponds comprising tap water and fertiliser, the control pond was significantly different than ponds containing *L. minor* and algae (Table 3). However, for ponds containing BR46, mean values of COD removal were higher in *L. minor* and algae, and *L. minor* ponds than algae and control ponds: *L. minor* > *L. minor* and algae > algae > control. Statistical analysis showed that the mean COD removal in the control ponds was significantly lower than the removals for *L. minor* and algae, and *L. minor* ponds. Also, *L. minor* ponds were significantly dissimilar than algae ponds. Low COD removal in all ponds ranged between 22% and -4%, indicating a low level of dye mineralisation occurring in the ponds due to the poor microbial degradation. Correlation analysis results indicate that the COD removal was significantly (r = 0.275, p = 0.014) positively correlated with dye removal.

3.5. Plant monitoring

This section covers objective (d). Findings show differences in the colour of *L. minor* leaves between ponds with and without dye (Supplementary Material 6). For ponds containing tap water and fertiliser (without dyes), the fronds colour analysis showed that a high percentage of area is covered by 7GY (mostly green colour), and a small percentage of the area is covered by 2.5GY (mostly yellow colour) according to Munsell Color (1977) compared with ponds containing dyes. However, it is important to note that the fronds of colour 2.5 GY cover around 1% in ponds without dyes compared with 2.8% minimum and 6.5% maximum in ponds with dyes. These small differences may not affect negatively the photosynthetic pigments as highlighted by Khataee et al. (2012). Acid Blue 92 at low concentrations of around 10 mg/l does not affect significantly the chlorophyll content.

Figure 4 provides an overview of the mean total coverage area including death and life fronds for all ponds during the experimental period. Table 6 provides an overview of the statistical analysis for the growth parameters between *L. minor* and algae, and *L. minor* ponds. The results indicate that the total coverage area is higher in ponds containing only tap water and fertiliser followed by ponds containing AB113, and then RB198. However, low coverage areas were found in ponds containing DO46 and BR46. Statistically, no difference was found between ponds containing *L. minor* and algae, and ponds comprising only *L. minor* in AB113, RB198, BR46, DO46 and tap water with fertiliser.

The overall mean values of relative growth rate are based on the fronds number and relative fronds number, which are shown in Figs. 5 and 6, respectively. These growth parameters have been used as indicators for the toxic effects of dyes on *L. minor* growth. Results clearly indicate that dyes negatively impact on relative fronds number and relative growth rates, which had the same trend and ranked as follows: Tap water and fertiliser > AB113 > RB198 > DO46 > BR46. This outcome suggests that BR46, which is successfully treated, had a negative effect on the plant growth rate. The same impact was observed for Brilliant Blue R special, which is a strong inhibitor regarding

L. minor growth (Khataee et al., 2012). For relative growth rate and relative fronds number calculations, no dissimilarities were found between ponds containing *L. minor* and algae, and ponds with *L. minor* only according to a *t*-test undertaken for all wastewaters (Table 6). The correlation analysis resulted in a significantly ($p < 0.01$) positive correlation between coverage area and relative frond number (r = 0.715, $p < 0.001$).

4. Conclusions

- Planted ponds performed better than control ponds (objective (a)).
- Total dissolved solid concentrations were within the threshold set for discharge to the environment (objective (a)).
- The design variables did not affect the mean outflow AB113 concentrations (objectives (a) and (b)).
- Control ponds had significantly higher concentrations than the other design variables for mean outflows of RB198, BR46 and DO46 (objectives (a) and (b)).
- For BR46, control ponds had significantly higher values than those for the other treatments, and *L. minor* ponds had significantly lower outflow colour values than those of the other design variables (objectives (a) and (b)).
- Outflow values of SS in control ponds comprising BR46, tap water and fertiliser were significantly higher than those for the other treatments (objectives (a) and (b)).

- Low concentrations of the dye BR46 can be successfully treated in laboratory-scale shallow ponds (objective (c)).
- No differences were found for COD removal among the ponds containing AB113, RB198 and DO46 (objective (c)).
- For BR46, the mean COD removal in the control ponds was significantly lower than those for *L. minor* and algae, and *L. minor* ponds (objective (c)).
- For BR46 dye removal, ponds containing *L. minor* outperformed other ponds (objective (c)).
- The simple chemical structure, absence of sulpho-group and small molecular weight associated with neutral pH values enhanced the capacity of the uptake of BR46 molecules (objective (c)).
- The presence of dyes inhibited the growth rate of plants (objective (d)).

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Supplementary Material

This article is supported by supplementary information that is linked to the article version published online. Supplementary Materials 1 to 6 provide information on environmental boundary conditions, inflow water quality, outflow water quality, inflow/outflow dye concentrations, dye/COD removal and plant colours, respectively.

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