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TEXTILE DYE REMOVAL USING EXPERIMENTAL WETLAND PONDS PLANTED WITH COMMON DUCKWEED UNDER SEMI-NATURAL CONDITIONS

The study assesses the performance of experimental wetland pond systems vegetated by *Lemna minor* L. (common duckweed or lesser duckweed) for textile azo dye removal. The objectives are to assess the influence of *L. minor* on water quality parameters, compare the dye and chemical oxygen demand (COD) removal of four dyes (Acid Blue 113, Reactive Blue 198, Direct Orange 46 and Basic Red 46) with each other, and monitor the effect of dye accumulation as a function of the relative growth rate (RGR) of *L. minor*. Findings indicate that the simulated shallow pond systems remove BR46 (low concentration) significantly ($p < 0.05$) higher than other dyes, and the ponds containing *L. minor* outperformed control ponds by around 51% in mean removal efficiency with a potential of *L. minor* for phytoremediation of approximately 13% efficiency.

1. INTRODUCTION

Textile effluents containing synthetic dyes are one of the main causes of watercourse pollution particularly in developing countries [1–3]. Most dyes cannot bind with textiles entirely, which leads to the residuals being released into watercourses as waste [4]. The dye wastewater effluents are high in color, pH, suspended solids, COD [5], biochemical oxygen demand, metals [6] and temperature [2]. Typically, textile industry processing effluents contain dyes in the range between 10 and 200 mg/dm^3 [7].

Various methods have been used for textile dye removal including chemical and physical processes such as oxidation, coagulation and flocculation, adsorption by activated carbon and membrane filtration [2, 3, 8, 9]. Biological treatment alternatives such as constructed wetland ponds are likely to be sustainable and cost-effective [10, 11].

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However, apart from Yaseen and Scholz [4], only short-term studies treating wastewater contaminated with textile dyes in shallow pond and wetland systems have been conducted in Turkey and India [12].

Lemna minor is a very small free-floating macrophyte growing rapidly and adapting easily to various environmental conditions in stagnant and slow-flowing watercourses [1, 13]. Lemna accumulates and assimilates contaminants in aquatic systems [14] and can be utilised for metal removal [6]. This plant is often used as a fodder due to elevated protein concentrations and a lack of fibre [14, 15].

The aim of this paper was to assess the performance of simulated shallow wetland pond systems vegetated by *L. minor* for the treatment of artificial textile dye wastewater under semi-natural environment conditions. The objectives are to assess the influence of *L. minor* on the water quality of the dye solutions, compare the dye and COD removals of four dyes with each other, and assess the effect of dye accumulation as a function of the growth rate of *L. minor*.

2. EXPERIMENTAL

Dyes and nutrients. Four commercially available dyes were used in this study (Table 1): Acid Blue 113 (AB113), Reactive Blue 198 (RB198), Basic Red 46 (BR46) and Direct Orange 46 (DO46) which were supplied by Dystar UK Limited (Colne Side Business Park, Huddersfield, UK) except for AB113, which was obtained from Sigma-Aldrich Company UK Limited (The Old Brickyard, New Road, Gillingham, UK).

Table 1

Characteristics of dyes used in the study

Dye	Molecular composition	Chemical class	Molecular weight [g/mol]	λ_{max} [nm]
Acid Blue 113	$C_{32}H_{21}N_5Na_2O_6S_2$	diazo	681.6	566
Reactive Blue 198	$C_{41}H_{30}Cl_4N_{14}Na_4O_{14}S_4$	diazo/oxazine	1304.8	625
Basic Red 46	$C_{18}H_{21}N_6$	monoazo	321.4	530
Direct Orange 46	$C_{12}H_{10}N_3NaO_3S$	monoazo	299.2	421

λ_{max} is the wavelength at the maximum absorption of the dye.

TNC Complete, which is an aquatic plant nutrient supplied by TNC Limited (Spotland Bridge Mill, Mellor Street, Rochdale, UK), was used. The corresponding ingredients were as follows: nitrogen (1.5 wt. %), phosphorus (0.2 wt. %), potassium (5 wt. %), magnesium (0.8 wt. %), iron (0.08 wt. %), manganese (0.018 wt. %), copper (0.002 wt. %), zinc (0.01 wt. %), boron (0.01 wt. %) and molybdenum (0.001 wt. %). Ethylenediaminetetraacetic acid, which was a part of the fertiliser, contained copper, iron, manganese and zinc. About 1 cm^3 of fertiliser was added to 10 dm^3 of dechlorinated tap water.

Experimental set-up. The experiment was carried out at the University of Salford using simple plastic washing-up containers (33 cm long, 25.5 cm wide, and 14 cm deep) located outside. Six containers were allocated for each dye. An additional 5 containers without dyes (controls) were also monitored. From 10 July 2014 (Table 2), each container was filled with tap water to the desired level of 6.9 cm, which was equivalent to 5 dm^3. Subsequently, 200 healthy *L. minor* plants (2.600±0.0292 g), which had a maximum number of four fronds each, were added to each container, and the system was fed weekly with water and fertiliser (see above for volume). The plants were collected from a small pond (Cowpe Lodge, Cowpe Reservoir, Rossendale, UK). Dyes at a concentration of 5 mg/dm^3 were added to undertake initial tests to examine plant survival.

Table 2

Experimental phases

Range of dates	Notes
10/07/2014 –11/08/2014	Set-up period: plants were added to the containers and fed with fertiliser weekly.
11/08/2014 –15/12/2014	Monitoring period: the plants acclimatised. Dyes were added to the system and plants were monitored (data not shown).
15/12/2014 –2/2/2016	Main experiment: systems were operated under semi-natural conditions and data recorded.

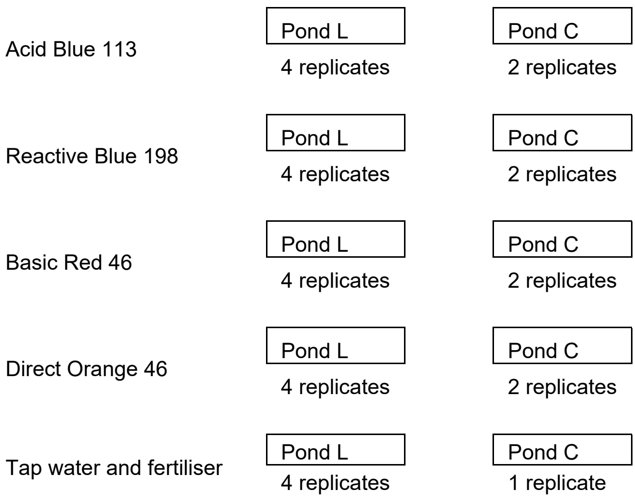

Fig. 1. Scheme of the shallow pond systems (L – *Lemna minor* L., C – control)

The main study started on 15 December 2014, and ended on 2 February 2016. Figure 1 indicates the diagram for the experimental set-up. Twenty-nine containers were used: six for each dye (four dyes in total) and five without dye. The set-up consisted of two treatment groups. The first group comprised *L. minor* (four replicates) for the ponds with and without dye (L ponds). The second group represented controls without plants for the ponds (two replicates) containing dye and ponds (one replicate) without dye (C ponds).

The experiment was conducted in a batch mode. Dye wastewater of a constant concentration (5 mg/dm^3) was added to the system once per week on the same day to achieve a contact time of seven days. However, the quantity of the dose added was different, depending on evaporation and precipitation. In the case of high precipitation, water was removed from the ponds to keep the corresponding volume at 4 dm^3, and 1 dm^3 of raw or initial water (inflow) was added. The solution was topped-up weekly to the same desired level as required to compensate for water loss due to evaporation and transpiration.

Water quality analysis. Routine water quality sampling (50 cm^3) was carried out according to APHA [16] to monitor the system performance. The spectrophotometer DR 2800 Hach Lange (Hach Lange, Willstätterstrsse, Düsseldorf, Germany) was used for standard water quality analysis for variables including COD, absorbance, apparent colour and suspended solids. Apparent colour (unit: Pt-Co) was measured at 455 nm when dissolved and suspended matter was present. This variable should not be confused with any specific dye colour. For more details, readers may refer to method 8025 stated in the manual of the DR 2800 Hach Lange equipment.

The turbidity was determined with a turbicheck turbidity meter (Tintometer GmbH, Lovibond Water Testing, Dortmund, Germany). The redox potential (redox) and pH were measured with a WTW Vario meter (Cole-Parmer Instrument Co., Ltd., Unit 3 River Brent Business Park, Trumpers Way Hanwell London, UK). Dissolved oxygen (DO) was measured with a Hach HQ30d flexi meter (Hach, 5 Pacific Way, Salford, UK). The electrical conductivity (EC) was determined using the meter METTLER TOLEDO Five Go^{TM} (Keison Products, Chelmsford, UK).

The analysis was performed for 12-cm^3 samples, which were filtered through a 0.45 μm pore diameter Whatman filter paper (Scientific Laboratory Suppliers, Ltd. Wilford Industrial Estate, Ruddington Lane, Wilford, Nottingham, UK). The filtered water sample was then analysed with a UV-Vis spectrophotometer at the maximum absorption wavelengths for each dye which was determined for aqueous solutions using a scanning UV-Vis spectrophotometer WPA Bio Wave II (Biochrom, Ltd., Building 1020, Cambourne Business Park, Cambourne, Cambridge, UK). The corresponding wavelengths were 566, 625, 530 and 421 nm for AB113, RB198, BR46 and DO46, respectively.

Environmental and plant growth monitoring. The outside temperatures were between –3 °C and 29 °C (mean of 11.4±6.5 °C) at about 10:45. Light measurement readings were performed by applying the lux meter ATP-DT-1300 (TIMSTAR, Road Three, Winsford Industrial Estate, Winsford, UK) for the range 200–50 000 lux. Readings were between 1079 lux and 49 150 lux (mean of 12 718 lux) above and close to the plants.

The RGR was calculated indirectly by measuring the fresh weight of the harvested *L. minor* plants. To assess the impact of dye accumulation on *L. minor*, the plants were monitored and harvested to avoid overcrowding, which inhibits 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 h. The RGR based on fresh weight was calculated as an indirect indicator of possible dye toxicity [13].

Statistical analysis. IBM SPSS Statistics Version 20 was used to compute the non--parametric Mann–Whitney U ($p < 0.05$) and the parametric *t*-tests. One-way and univariate ANOVA tests using Tukey post hoc test multiple comparison were also conducted. The correlation coefficients between variables were calculated using the Spearman's test.

3. RESULTS AND DISSCUSSION

3.1. RAW WATER QUALITY PARAMETERS

Table 3 summarises the raw water quality. The values of pH and apparent color were within the ranges of 6 to 10, and 50 to 2500 Pt-Co, respectively [17]. The concentration of the dye was only 5 mg/dm^3 but it compared well with concentrations used for Basic Red 46 [13] and Acid Blue 92 [1]. The reason for using low raw water concentrations is because duckweed ponds are often used as a polishing step (i.e., last step in multi-stage treatment) to remove dyes, organic matter [12] and heavy metals [6].

Table 3

Raw wastewater quality for each system between 15 December 2014 and 2 February 2016

Parameter	Mean	Standard deviation	Minimum	Maximum	Sample number
Acid Blue 113					
Dye concentration, mg/dm^3	5.0	0.40	4.5	5.6	58
pH	7.3	0.12	7.1	7.5	58
Redox potential, mV	–24.4	8.40	–37.0	–12.0	58
Dissolved oxygen, mg O_2/dm^3	9.6	0.28	9.2	10.5	58
Electrical conductivity, μS/cm	113.9	6.06	106.0	133.0	58
Suspended solids, mg/dm^3	3.7	1.33	2.0	6.0	58
Turbidity, NTU	1.6	0.64	0.7	3.0	58
Colour, Pt Co	445.6	25.14	416.0	482.0	14
COD, mg O_2/dm^3	24.7	0.96	23.0	26.0	13

Table 3

Raw wastewater quality for each system between 15 December 2014 and 2 February 2016

Parameter	Mean	Standard deviation	Minimum	Maximum	Sample number
Reactive Blue 198					
Dye concentration, mg/dm^3	5.1	0.39	4.4	5.6	58
pH	7.3	0.10	7.2	7.5	58
Redox potential, mV	–31.6	5.24	–38.0	–23.0	58
Dissolved oxygen, mg O_2/dm^3	9.6	0.20	9.3	10.5	58
Electrical conductivity, µS/cm	123.7	6.63	112.0	135.0	58
Suspended solids, mg/dm^3	4.9	0.82	3.0	7.0	58
Turbidity, NTU	2.0	0.36	1.3	2.7	58
Colour, Pt Co	167.4	38.14	123.0	201.0	14
COD, mg O_2/dm^3	9.4	0.61	8.5	10.1	13
Basic Red 64					
Dye concentration, mg/dm^3	5.1	0.30	4.4	5.5	58
pH	7.3	0.05	7.3	7.4	58
Redox potential, mV	–31.7	2.52	–37.0	–28.0	58
Dissolved oxygen, mg O_2/dm^3	9.6	0.24	9.2	10.3	58
Electrical conductivity, µS/cm	114.1	4.99	103.0	121.0	58
Suspended solids, mg/dm^3	3.9	0.95	2.0	5.0	58
Turbidity, NTU	3.1	0.83	1.9	6.0	58
Colour, Pt Co	410.1	12.25	389.0	424.0	14
COD, mg O_2/dm^3	13.3	0.88	12.0	14.5	13
Direct Orange 46					
Dye concentration, mg/dm^3	5.0	0.35	4.4	5.6	58
pH	7.4	0.06	7.3	7.4	58
Redox potential, mV	–32.2	3.04	–37.0	–29.0	58
Dissolved oxygen, mg O_2/dm^3	9.5	0.06	9.5	9.6	58
Electrical conductivity, µS/cm	118.9	10.32	105.4	133.0	58
Suspended solids, mg/dm^3	3.2	0.98	2.0	5.0	58
Turbidity, NTU	2.0	0.65	0.9	3.5	58
Colour, Pt Co	674.4	38.13	620.0	721.0	14
COD, mg O_2/dm^3	14.5	0.97	12.5	16.0	13
Tap water and fertiliser without dye addition					
pH	7.3	0.07	7.2	7.3	13
Redox potential, mV	–27.2	5.27	–33.0	–19.0	13
Dissolved oxygen, mg O_2/dm^3	9.4	0.35	8.9	9.9	13
Electrical conductivity, µS/cm	85.7	0.88	84.0	87.0	13
Suspended solids, mg/dm^3	2.0	1.47	0.0	4.0	13
Turbidity, NTU	3.2	0.76	2.0	4.1	13
Colour, Pt Co	3.0	0.78	2.0	4.0	13
COD, mg O_2/dm^3	4.1	0.30	3.6	4.4	12

3.2. TREATED WATER QUALITY

Both contact time and the presence of plants are key parameters influencing the final water quality. The experiment was conducted for seven days of contact time, which matched values commonly reported in the literature regarding *L. minor* [12, 13] for the treatment of textile dyes. In addition, Reema et al. [9] mentioned that the ability of *L. minor* for uptake of dye molecules escalates with an increase in contact time for initial dye concentrations.

Table 4

Treated wastewater (outflow) water quality for each system between 15 December 2014 and 2 February 2016

Parameter	Mean	Standard division	Minimum	Maximum	Sample number
Acid Blue 113 with tap water and fertiliser within *Lemna minor* L. ponds					
Dye concentration, mg/dm^3	1.1	0.46	0.1	2.2	59
pH	7.9	0.52	7.2	9.1	58
Redox potential, mV	–50.1	12.30	–71.3	–18.3	58
Dissolved oxygen, mg O_2/dm^3	10.4	1.17	8.2	13.9	57
Electrical conductivity, µS/cm	72.4	20.04	35.5	120.0	58
Suspended solids, mg/dm^3	24.1	23.60	3.3	94.5	58
Turbidity, NTU	7.2	5.66	2.2	27.4	58
Colour, Pt Co	194.0	91.97	75.8	355.3	18
COD, mg O_2/dm^3	38.4	24.37	4.2	76.0	14
Acid Blue 113 with tap water and fertiliser within control ponds					
Dye concentration, mg/dm^3	1.3	0.55	0.3	2.5	59
pH	7.8	0.49	7.1	9.7	58
Redox potential, mV	–50.8	10.98	–79.6	–16.0	58
Dissolved oxygen, mg O_2/dm^3	10.4	1.10	8.0	13.4	57
Electrical conductivity, µS/cm	88.2	22.76	49.8	134.3	58
Suspended solids, mg/dm^3	21.2	17.96	2.0	82.0	58
Turbidity, NTU	5.8	3.64	1.5	20.6	58
Colour, Pt Co	189.1	98.96	41.0	381.5	18
COD, mg O_2/dm^3	36.0	24.01	3.7	77.1	14
Reactive Blue 198 with tap water and fertiliser within L. *minor* ponds					
Dye concentration, mg/dm^3	1.6	0.56	0.7	3.1	59
pH	7.7	0.43	7.0	9.3	58
Redox potential, mV	–47.5	9.76	–71.5	–18.0	58
Dissolved oxygen, mg O_2/dm^3	10.4	1.18	8.3	13.6	57
Electrical conductivity, µS/cm	71.8	20.81	34.5	139.4	58
Suspended solids, mg/dm^3	13.2	9.90	1.8	42.0	58
Turbidity, NTU	5.0	2.45	2.0	11.4	58
Colour, Pt Co	61.9	38.72	10.3	135.0	18
COD, mg O_2/dm^3	26.6	18.49	2.5	68.1	14

Table 4

Treated wastewater (outflow) water quality for each system
between 15 December 2014 and 2 February 2016

Parameter	Mean	Standard division	Minimum	Maximum	Sample number
Reactive Blue 198 with tap water and fertiliser within control ponds					
Dye concentration, mg/dm^3	2.1	0.79	0.8	4.0	59
pH	7.7	0.57	7.1	9.7	58
Redox potential, mV	–46.6	11.19	–74.5	–20.0	58
Dissolved oxygen, mg O_2/dm^3	10.4	1.29	8.01	13.6	57
Electrical conductivity, µS/cm	95.0	21.71	64.5	155.5	58
Suspended solids, mg/dm^3	11.1	8.60	2.0	36.0	58
Turbidity, NTU	4.7	3.03	1.0	13.5	58
Colour, Pt Co	91.7	67.50	6.5	221.5	18
COD, mg O_2/dm^3	29.8	21.03	2.6	72.2	14
Basic Red 46 with tap water and fertiliser within L. *minor* ponds					
Dye concentration, mg/dm^3	0.7	0.41	0.1	1.7	59
pH	7.7	0.57	7.0	10.0	58
Redox potential, mV	–46.8	12.92	–77.3	–16.3	58
Dissolved oxygen, mg O_2/dm^3	10.5	1.19	8.2	13.9	57
Electrical conductivity, µS/cm	77.5	23.54	32.4	131.6	58
Suspended solids, mg/dm^3	31.7	31.36	2.3	100.3	58
Turbidity, NTU	8.9	7.54	1.8	35.6	58
Colour, Pt Co	51.7	35.65	14.3	126.5	18
COD, mg O_2/dm^3	30.0	17.75	2.1	61.1	14
Basic Red 46 with tap water and fertiliser within control ponds					
Dye concentration, mg/dm^3	1.0	0.59	0.2	2.5	59
pH	7.7	0.46	7.0	9.9	58
Redox potential, mV	–45.9	11.76	–76.2	–15.5	58
Dissolved oxygen, mg O_2/dm^3	10.3	1.12	8.1	13.4	57
Electrical conductivity, µS/cm	84.7	30.02	34.1	154.8	58
Suspended solids, mg/dm^3	24.6	32.10	1.0	111.0	58
Turbidity, NTU	7.4	7.33	1.0	31.3	58
Colour, Pt Co	63.6	35.94	19.0	148.0	18
COD, mg O_2/dm^3	35.8	27.82	2.5	88.5	14
Direct Orange 46 with tap water and fertiliser within L. *minor* ponds					
Dye concentration, mg/dm^3	2.6	1.43	1.0	7.2	59
pH	7.8	0.57	7.1	9.9	58
Redox potential, mV	–48.6	12.76	–79.0	–25.3	58
Dissolved oxygen, mg O_2/dm^3	10.4	1.14	8.2	13.4	57
Electrical conductivity, µS/cm	72.6	19.80	36.0	131.9	58
Suspended solids, mg/dm^3	12.8	13.28	2.0	54.3	58
Turbidity, NTU	4.7	2.95	1.7	18.3	58
Colour, Pt Co	177.8	60.61	85.8	303.3	18
COD, mg O_2/dm^3	32.5	25.10	2.7	79.0	14

Table 4

Treated wastewater (outflow) water quality for each system
between 15 December 2014 and 2 February 2016

Parameter	Mean	Standard division	Minimum	Maximum	Sample number
Direct Orange 46 with tap water and fertiliser within control ponds					
Dye concentration, mg/dm^3	3.5	1.72	1.3	8.0	59
pH	7.8	0.56	7.1	10.1	58
Redox potential, mV	–48.7	12.36	–82.0	–25.5	58
Dissolved oxygen, mg O_2/dm^3	10.4	1.10	8.1	13.5	57
Electrical conductivity, µS/cm	88.0	24.94	45.9	144.0	58
Suspended solids, mg/dm^3	11.5	8.33	1.0	34.0	58
Turbidity, NTU	4.1	1.85	1.3	10.3	58
Colour, Pt Co	230.6	100.30	72.5	398.5	18
COD, mg O_2/dm^3	33.4	26.81	2.8	88.8	14
Tap water and fertiliser within L. *minor* ponds					
pH	7.7	0.39	6.9	8.6	58
Redox potential, mV	–46.2	11.22	–64.3	–9.5	58
Dissolved oxygen, mg O_2/dm^3	10.5	1.16	7.8	13.8	57
Electrical conductivity, µS/cm	77.1	19.83	34.8	115.8	58
Suspended solids, mg/dm^3	8.0	5.56	2.3	37.8	58
Turbidity, NTU	4.6	1.93	1.2	10.4	57
Colour, Pt Co	36.0	20.99	8.5	69.8	18
COD, mg O_2/dm^3	28.4	20.59	3.3	71.0	14
Tap water and fertiliser within control ponds					
pH	8.0	0.49	7.1	8.9	53
Redox potential, mV	–53.7	12.28	–72.0	–21.0	55
Dissolved oxygen, mg O_2/dm^3	10.4	1.24	7.8	13.8	57
Electrical conductivity, µS/cm	84.3	18.92	44.0	123.0	58
Suspended solids, mg/dm^3	44.1	22.80	2.0	82.0	58
Turbidity, NTU	8.9	3.49	1.0	16.1	57
Colour, Pt Co	63.3	11.91	41.0	85.0	18
COD, mg O_2/dm^3	39.6	18.58	10.0	86.1	13

The mean values of the treated or final (outflow) dye concentrations for all dyes were higher in the control ponds than in the planted ponds (Table 4). Table 5 summarises the statistical analysis of treated water quality parameters and the removal efficiencies. Lower treated water values were noticed for planted and control ponds during periods of relatively high rainfall (Fig. 2). Except for AB113, the mean treated water dye concentrations were significantly higher within the control ponds than within the *L. minor* ponds (Table 5).

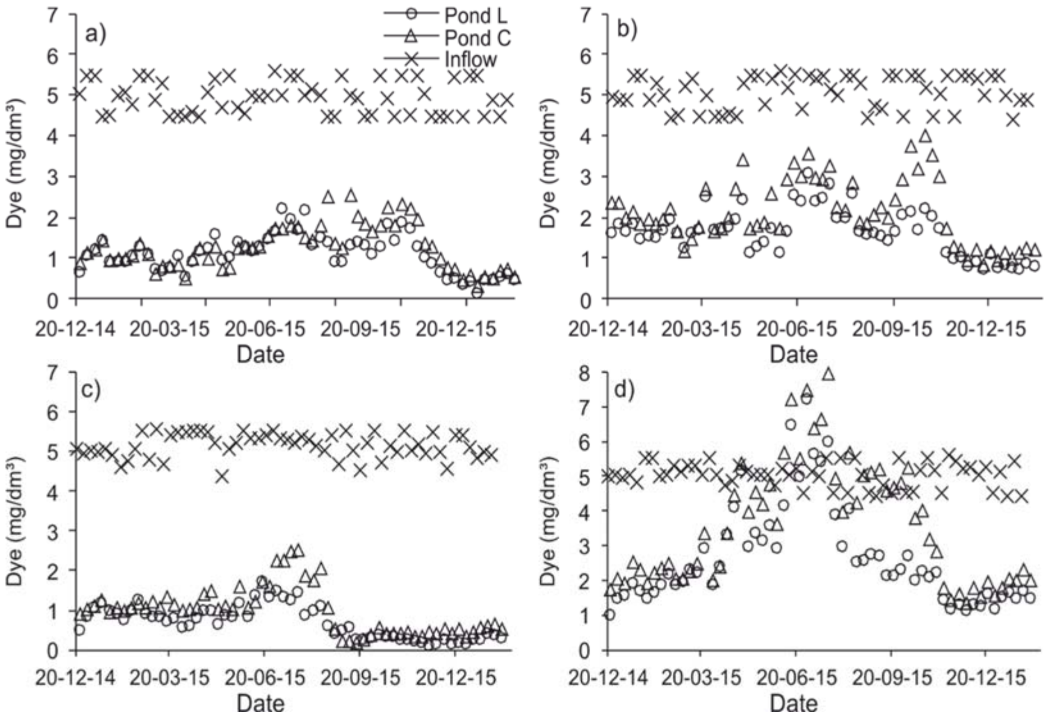

Fig. 2. Raw water (inflow) and mean treated water (outflow) dye concentrations (L – *Lemna minor* L., C – control) for a) Acid Blue 113, b) Reactive Blue 198, c) Basic Red 46, and d), Direct Orange 46

Low standard deviations are associated with the treated water AB113, RB198 and BR46 concentrations (Fig. 2a–c). In contrast, the highest water quality standard deviations were linked to DO46 (Table 4). The final water concentrations were lower than the raw water concentrations for all dyes except for DO46 during the period between 15/4/2015 and 15/10/2015.

Table 5

Overview of the statistical analysis for treated wastewater (outflow) quality parameters and corresponding removal efficiencies (where appropriate)

Type of the dye	Shapiro –Wilk test (*p*-value[1])	Statistical test	*p*-values[2] between L&C	Type of the dye	Shapiro –Wilk test (*p*-value[1])	Statistical test	*p*-values[2] between L&C
Dye [mg/dm^3]				Dye removal [%]			
AB113	0.034	M–W	0.195	AB113	0.030	M–W	0.201
RB198	0.002	M–W	<0.001	RB198	0.247	*t*-test	<0.001
BR46	<0.001	M–W	0.004	BR46	0.013	M–W	<0.001
DO46	<0.001	M–W	0.003	DO46	<0.001	M–W	<0.001

Table 5

Overview of the statistical analysis for treated wastewater (outflow) quality parameters and corresponding removal efficiencies (where appropriate)

Type of the dye	Shapiro–Wilk test (*p*-value[1])	Statistical test	*p*-values[2] between L&C	Type of the dye	Shapiro–Wilk test (*p*-value[1])	Statistical test	*p*-values[2] between L&C
Chemical oxygen demand [mg/dm^3]				Chemical oxygen demand removal [%]			
AB113	0.095	*t*-test	0.826	AB113	<0.001	M–W	0.627
RB198	0.275	*t*-test	0.777	RB198	<0.001	M–W	0.233
BR46	0.094	*t*-test	0.531	BR46	0.121	*t*-test	0.596
DO46	0.094	*t*-test	0.942	DO46	0.009	M–W	0.691
TW + F	0.354	*t*-test	0.167	TW + F	<0.001	M–W	0.043
Colour [Pt Co]				Dissolved oxygen [mg/dm^3]			
AB113	0.046	M–W	0.950	AB113	0.002	M–W	0.625
RB198	0.008	M–W	0.327	RB198	<0.001	M–W	0.838
BR46	0.003	M–W	0.261	BR46	<0.001	M–W	0.993
DO46	0.318	*t*-test	0.103	DO46	<0.001	M–W	0.910
TW + F	0.019	M–W	<0.001	TW + F	<0.001	M–W	0.598
Suspended solids [mg/dm^3]				pH			
AB113	<0.001	M–W	0.897	AB113	<0.001	M–W	0.633
RB198	<0.001	M–W	0.309	RB198	<0.001	M–W	0.262
BR46	<0.001	M–W	0.005	BR46	<0.001	M–W	0.855
DO46	<0.001	M–W	0.332	DO46	<0.001	M–W	0.989
TW + F	<0.001	M–W	<0.001	TW + F	0.075	*t*-test	0.001
Turbidity [NTU]				Redox potential [mV]			
AB113	<0.001	M–W	0.473	AB113	0.020	M–W	0.879
RB198	<0.001	M–W	0.196	RB198	<0.001	M–W	0.282
BR46	<0.001	M–W	0.069	BR46	0.059	*t*-test	0.693
DO46	<0.001	M–W	0.886	DO46	0.008	M–W	0.884
TW + F	<0.001	M–W	<0.001	TW + F	0.001	M–W	<0.001
Electrical conductivity [µS/cm]							
AB113	0.141	*t*-test	<0.001				
RB198	0.008	M–W	<0.001				
BR46	<0.001	M–W	0.143				
DO46	0.001	M–W	0.001				
TW + F	0.048	M–W	0.081				

[1]Test of normality (if $p > 0.05$, data are normally distributed, if $p < 0.05$, data are not normally distributed).

[2]*p*-value, probability of the statistic test (if $p > 0.05$, the variables are not statistically significantly different, if $p < 0.05$, the variables are statistically significantly different).

Note: L – *Lemna minor* L. pond, C – control pond, AB113 – Acid Blue 113, RB198 – Reactive Blue 198, BR46 – Basic Red 46, DO46 – Direct Orange 46, TW + F – tap water and fertiliser, M–W, Mann –Witney U test.

Color mean treated water values (Table 4) were higher for control ponds regarding all types of wastewater except for ponds contained AB113. Overall, the treated water color values were lower than the raw inflow water for all ponds containing dyes.

The threshold value for dye effluent in China (country with the highest dye consumption) is 80 Pt-Co [18]. In total, samples were 17 and 15, 6 and 9, 4 and 4, 18 and 15, and 2 times non-compliant for planted and control ponds, respectively, which contained AB113, RB198, BR46 and DO46, as well as control ponds (only containing tap water and fertilizer) in this order.

The European and international standards set typical lower and upper pH thresholds of 6.5 and 8.5, respectively [3]. In total, samples were 9 and 3, 4 and 6, 4 and 1, 6 and 7, and 2 and 8 times non-compliant for ponds contained AB113, RB198, BR46, DO46, and tap water and fertiliser for planted ponds and control ponds, respectively. However, in Thailand, where a lot of coloured textiles are produced, the thresholds values are 5.5–9.0 [19], and samples were 1 and 2, 2 and 4, 3 and 1, and 3 and 2 times non-compliant for systems containing AB113, Rb198, BR46 and DO46 for planted ponds and control ponds in that order. According to [18], the lower and upper threshold values for pH in China are 6 and 9, respectively. In comparison, the non-compliant values were similar to the ones set for Thailand.

pH influenced the capacity of the dye uptake and plant growth. The optimum pH for a high removal efficiency depends on the type of dye itself, e.g., 7 for Methylene Blue, 8 for Basic Blue 9 [9], 6–7.5 for BR46 [13] and 6.5 for Acid Blue 92 [1]. Saratale et al. [8] indicated that the optimum pH for high colour removal should be within the range of 6–10. The removal efficiency considerably declines at strong acidic or strong alkaline conditions in biological treatment by bacteria. In comparison, the allowable range of pH for growth of *L. minor* is between 4.5 and 8.3 [9].

Dissolved oxygen and redox potential are indicators for aerobic and anaerobic conditions [10]. Overall, the values of treated water DO ranged between 7.8 and 13.9 mg O_2/dm^3. However, the raw water values varied between 8.9 and 10.5 mg O_2/dm^3. In addition, the mean treated water values (Table 4) in terms of DO for AB113, RB198 and DO46 were similar for *L. minor* ponds, and control ponds. However, the mean treated water values of DO in *L. minor* ponds were slightly higher than those in the unplanted ponds for systems containing BR46, and tap water and fertiliser.

The biodegradation of organic contaminants in the wetland ponds was boosted by the presence of a high concentration of DO, which facilitates the growth of aerobic microorganisms eliminating organic substances. However, high DO inhibits the dye removal, because of electrons released by microbial cells during the oxidation process utilising oxygen instead of azo dyes during the degradation processes [4].

In terms of redox potential, the mean treated water values (Table 4) were higher in *L. minor* ponds than those in control ponds for systems containing AB113, DO46, and tap water and fertiliser. In contrast, the mean treated waters in terms of redox potential for wastewaters contained RB198 and BR46 were higher for control ponds than for

L. minor ponds. Ponds containing only tap water and fertiliser as well as *L. minor* ponds had significantly higher redox potentials than the control ponds containing tap water and fertiliser (Table 3). As shown in Table 4, the minimum and maximum redox potentials indicated anoxic conditions. An aerobic environment is linked to redox potentials higher than 100 mV, while anaerobic conditions are associated with a redox potential lower than –100 mV [10].

The highest concentrations of treated water suspended solids (SS) were noted for control ponds containing tap water and fertiliser followed by *L. minor* ponds comprising BR46. In comparison, the lowest values were observed for planted ponds fed by tap water and fertiliser followed by control ponds containing RB198, and control ponds fed by DO46 (Table 4). The European and many international standards for SS are 35 mg/dm^3 in the case of effluents discharged directly to receiving freshwater bodies. The results indicated that the *L. minor* ponds and control ponds containing AB113, *L. minor* ponds and control ponds containing RB198, *L. minor* ponds and control ponds containing BR46, *L. minor* ponds and control ponds containing tap water and fertiliser, and *L. minor* comprising DO46 were 13 and 13, 2 and 1, 20 and 17, 1 and 36, and 5 times non-complaint, respectively. However, in the case of discharge to urban wastewater sewerage networks, many regulations set a threshold of 350 mg dm^3. The SS concentrations for all treated water ponds were within the standard [3]. However, the Chinese threshold is only 150 mg dm^3 [18], and all samples were also less than this value.

The mean final water values for SS in the control ponds comprising tap water and fertiliser were significantly higher than those for *L. minor* ponds, because of the addition of fertiliser. In contrast, the mean treated water values of SS for ponds containing BR46 were significantly higher in *L. minor* ponds than those in control ponds (Table 5).

Based on the mean turbidity treated water values (Table 4), *L. minor* pond final waters were characterized by higher values than those for control ponds for all dyes. However, the mean treated water turbidity values for *L. minor* ponds were lower than those for the control ponds for systems fed only by tap water and fertiliser. The mean treated water turbidity values in control ponds comprising tap water and fertiliser were significantly higher than *L. minor* ponds (Table 5).

The highest and lowest turbidity values mirrored those for SS. A correlation analysis showed that SS was significantly ($r = 0.917$, $p = 0.000$) positively correlated with turbidity and significantly ($r = –0.471$, $p = 0.000$) negatively correlated with DO. Therefore, high values of DO in the systems may suggest low microbial activities for organic matter degradation, which consequently reduces SS [11] and COD removal [20]. A correlation analysis highlighted that the COD removal was significantly ($p < 0.01$) negatively correlated with DO ($r = –0.456$, $p = 0.000$).

The EC is an important indicator to assess indirectly the salinity of a system. An increase in EC can inhibit the growth of *L. minor* [21]. All mean final water EC values were less than the raw water ones (Table 4), and the highest EC mean values were observed for control ponds, whereas the lowest values were found in *L. minor* ponds for all types of wastewater. This indicates that the presence of plants in the ponds is responsible for the reduction of the EC as explained by Nilratnnisakorn et al. [19] suggesting that the dye molecules are caught in barriers within the vascular plant system. In addition, the plants are able to remove small amounts of salts by passing them through their semi-permeable membrane [22].

The mean final water EC values for the control ponds were significantly higher than those for *L. minor* ponds with AB113, Rb198 and DO46. However, no significant differences were found between *L. minor*, and control ponds for BR46, and tap water and fertiliser (Table 5).

The values of total dissolved solids (TDS) were equal to the half of the EC values (data not shown). The final total dissolved solids (TDS) values for all treatment systems were compliant with the standard threshold of 3000 mg TDS/dm^3 set in Thailand [19]. Moreover, the values even complied with the European standard (class I – natural non-polluted state) threshold of 500 mg/dm^3 [3].

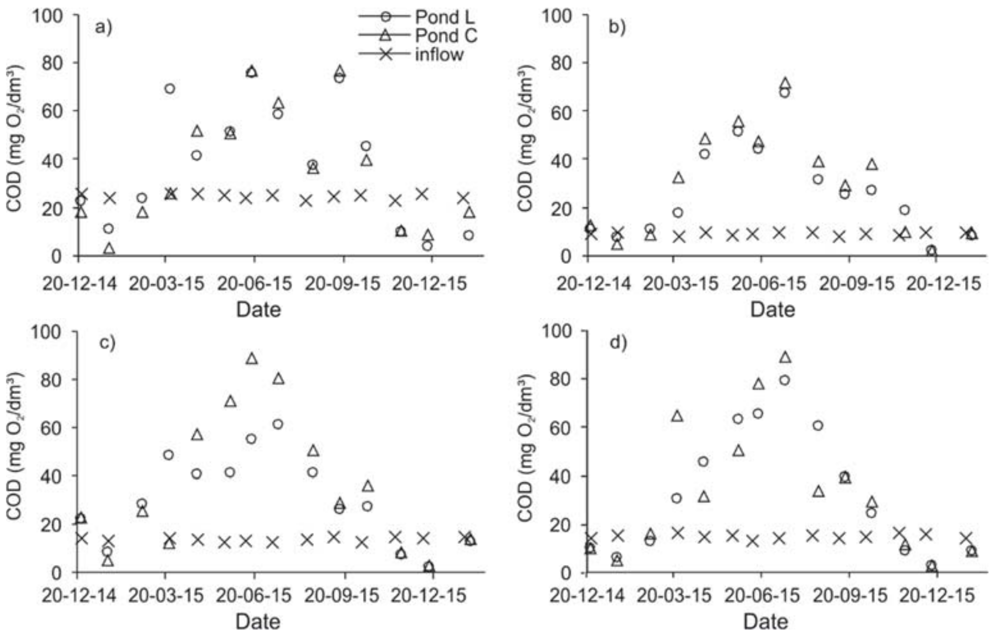

Fig. 3. Monthly raw water (inflow) and treated water (outflow) concentrations of COD for different treatments (L – *Lemna minor* L., C – control): a) Acid Blue 113, b) Reactive Blue 198, c) Basic Red 46, d) Direct Orange 46, and (e) water and fertiliser (control)

Figure 3 shows the COD final water concentration profiles. In general, the COD concentrations fluctuated over time, and were higher than the raw water ones during the period from 20/3/2015 to 10/11/2015 for all types of wastewater. This indicates low microbial activity for degradation of the organic matter associated with high levels of DO. However, during high rainfall periods, particularly in winter at the beginning and the end of the experiment, the COD concentrations were lower or similar to the raw water values for all dyes (Fig. 3a–d), and higher than the raw water values for ponds fed by tap water and fertilizer (Fig. 3e) in both planted and control ponds. The COD mean treated water values were higher in control ponds than in *L. minor* ponds for all types of wastewater except for ponds fed by AB113 (Table 4). The presence of plants did not affect significantly ($p < 0.05$) on the COD values. The European and most international [3] as well as the Chinese [18] standards set threshold values for COD of 125 mg O_2/dm^3 and 200 mg O_2/dm^3, respectively. These values are valid for the discharge of effluent directly into waterbodies. The results show that all COD values are within the standard thresholds.

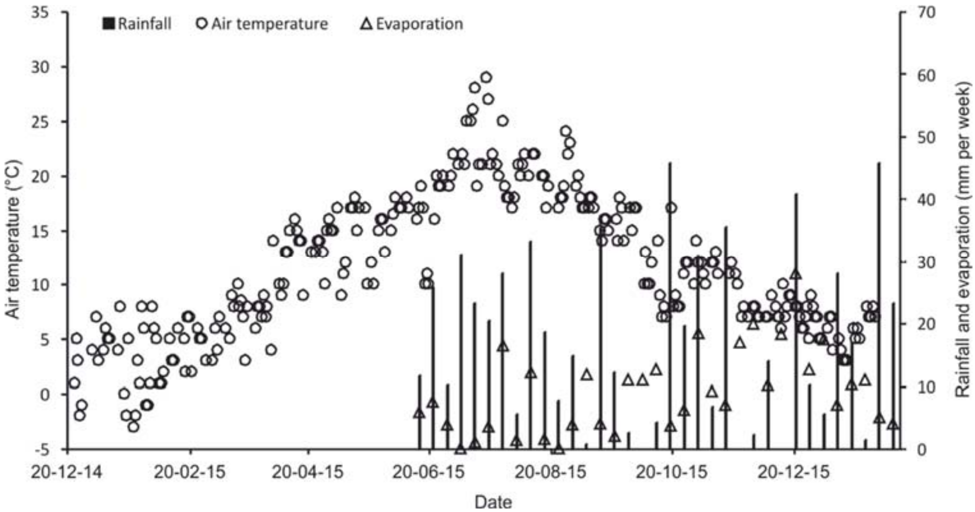

Fig. 4. Environmental conditions in Salford (UK) during the experiment period between 15 December 2014 and 2 February 2016

The long-term optimum temperature for *L. minor* growth is 26 °C [14]. The minimum and maximum temperatures are 4 and 33 °C, respectively. Out of 274 temperature recordings (Fig. 4), 27 were lower than the minimum recommended ranges. Movafeghi et al. [13] found that elevated temperature increases the removal efficiency. Dye biosorption is an endothermic process when using *L. minor*.

3.3. DYE REMOVAL

Table 6 shows the average values of dye removal. The mean removal values were higher within *L. minor* ponds than those in control ponds. The mean removals, which are equal to 32% for *L. minor* ponds, and 28% for control ponds, were impacted by the dilution effect considering that rainfall added more water to the ponds than evapotranspiration removed water from the ponds. However, significant differences were found between *L. minor* ponds, and control ponds regarding mean BR46 and RB198 removals (Table 5).

Table 6

Dye and COD removals

Pond	Load in [mg]			Load out [mg]			Removal [%]	*n*
	Minimum	Maximum	Mean	Minimum	Maximum	Mean		
Acid Blue 113 – dye								
L	6.0	14.7	9.4±2.02	1.3	12.3	6.4±2.25	32	58
C	6.5	15.2	10.1±2.31	3.0	13.5	7.3±2.84	28	
Acid Blue 113 – COD								
L	68.1	548.6	261.5±147.64	36.4	494.5	236.8±143.00	9	9
C	100.6	552.6	265.8±133.73	80.0	513.0	244.4±129.98	8	
Reactive blue 198 – dye								
L	7.4	18.6	11.8±2.34	4.9	15.3	9.5±2.25	19	58
C	8.4	21.1	13.7±3.29	6.7	21.8	12.2±3.34	11	
Reactive blue 198 – COD								
L	50.0	365.2	200.5±88.83	20.4	322.8	175.3±90.77	13	9
C	51.2	365.6	211.6±109.50	21.5	332.1	192.7±109.69	9	
Basic red 46 – dye								
L	5.2	13.7	8±1.96	0.8	8.2	3.9±2.01	51	58
C	5.5	16.4	9.1±2.59	1.1	13.7	5.6±2.88	38	
Basic red 46 – COD								
L	50.0	360.4	211.2±91.44	18.1	278.6	145.1±75.50	31	9
C	50.4	444.0	267.4±127.23	21.5	382.2	200.6±115.72	25	
Direct orang 46 – dye								
L	9.0	31.8	15.6±5.57	8.0	34.6	15.0±5.48	4	58
C	10.5	36.5	19.1±6.67	11.1	38.9	19.9±7.28	-4	58
Direct orange 46 – COD								
L	52.0	659.0	291.4±198.76	23.3	548.2	274.3±194.78	6	9
C	46.8	488.5	262.1±158.23	23.9	580.3	260.1±179.21	1	9
Water and fertiliser – COD								
L	64.4	444.3	252.9±128.28	28.4	345.2	173.7±110.42	31	9
C	208.4	528.3	351.8±97.62	174.4	499.4	307.4±92.82	13	8

L – *Lemna minor* L., C – control, *n* – sample number.

The removal efficiencies for BR46 and RB198 due to the impact of the pond system (microbial and plant removal as well as container wall adsorption effects) were approximately 51% and 19%, respectively. However, the corresponding removal efficiencies by plants due to biosorption and biochemical degradation processes were only around 13% and 8%, because the control ponds removed these dyes by around to 38% and 11% for BR46 and RB198. respectively. These results resemble outcomes for treatment of Basic Blue 41 using submerged plants [23].

For the remaining dyes, there was little difference between planted and unplanted ponds, which indicates that the plants were unable to remove them. Regarding the treatment performance of ponds containing DO46, although a significant difference in mean removal between design variables was found (Table 3), the mean removal values were only 4% and –4% (i.e. the system became a source rather than a sink) for planted and unplanted ponds, which indicates that these pond systems are unable to break down DO46 molecules. Note that slight negative removals are predominantly linked to small loads (mg) and measurement inaccuracies.

The longitudinal profile of the dye removal is shown in Fig. 5. Most of the removal efficiencies in planted ponds were higher than the control ponds for all dyes. In addition, the same longitudinal trend was noted for all dyes throughout the year (Fig. 5a–c), except for some fluctuating within the DO46 profile (Fig. 5d). The dye removal fluctuated with the variation of rainfall. A considerable increase was noted at the end of experiment due to high amount of rainfall in winter. The maximum and minimum removals were 82% and 7% for *L. minor* ponds, and 63% and –1% for control ponds for the treatment of AB11, as well as 43% and 7%, and 34% and –13% for *L. minor* and control ponds, respectively, for the treatment of RB198 (Fig. 5b). Negative removal efficiencies can be explained by phenomena such as water surface evaporation and pond edge effects. The DO46 removal ranged between 18% and –25%, and between 15% and –41% for *L. minor*, and control ponds, respectively (Fig. 5d). High removal efficiencies were recorded for BR46 (Fig. 5c). The minimum and maximum removals were 18% and 86% for *L. minor* ponds, and 14% and 85% for control ponds, respectively.

Multiple comparisons of removal efficiencies among dyes showed a significant difference among all dyes. The corresponding dye values ranked as follow: BR46 > AB113 > RB198 > DO46. The *p*-values were <0.001 between the dye removal efficiencies using a univariate analysis of variance between the types of dyes for the following dye combinations: AB113 and RB198, AB113 and BR46, AB113 and DO46, RB198 and BR46, RB198 and DO46, and BR46 and DO46.

This indicates that BR46 is treated easily by shallow pond systems under natural conditions in Salford. This is due to the simple structure and small molecular weight of BR46 [22]. Moreover, the absence of sulpho groups in BR46 is linked to good degradation during biological treatment [4]. The removal by biosorption processes associated with the roots and leaves of *L. minor* is likely. Furthermore, pH values, which ranged from 7 to 10 were suitable for BR46 uptake. Movafeghi et al. [13] reported that pH between 5.3 and 9.3 do not affected noticeably the treatment of BR46. They considered that pH = 7 is optimal for decolourization of this dye.

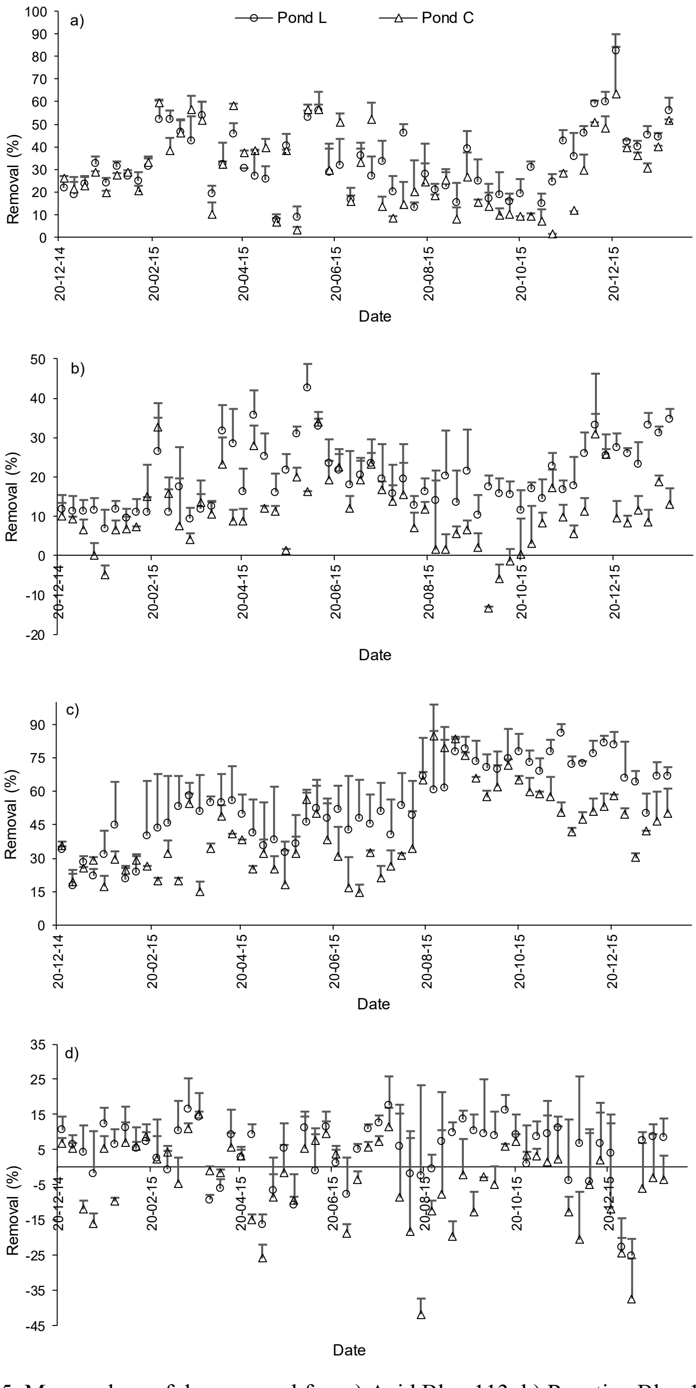

Fig. 5. Mean values of dye removal for: a) Acid Blue 113, b) Reactive Blue 198, c) Basic Red 46, and d) Direct Orange 46; L – *Lemna minor* L., C – control

3.4. CHEMICAL OXYGEN DEMAND REMOVAL

The COD removal efficiency (Table 6) was low for all ponds, which was evident from the inflow waters corresponding to the high treated water values as discussed in section 3.2 (Tables 3 and 4). In addition, mean COD removals were higher in *L. minor* ponds than those in control ponds for all ponds containing dyes, but the difference was not significant. However, for ponds comprising only tap water and fertiliser, outcomes showed that the mean values of COD removal were significantly higher in *L. minor* ponds than in the corresponding control ponds (Table 5). These results indicate that the presence of the dyes impact on the plant performance by reducing their ability to remove COD. Low removal in all ponds ranged between 1 and 31%, indicating a low level of dye mineralisation occurring in the ponds due to poor microbial degradation. The results of the correlation analysis indicate that the COD removal was significantly ($p < 0.01$) positively correlated with dye removal.

3.5. PLANT MONITIRING

The mean values of RGR, which are based on the fresh weight for plants harvested from each pond are shown in Fig. 6. This growth parameter has been used as an indirect indicator for the toxic effects of dyes on *L. minor* growth. The results clearly show that dyes negatively influence the RGR, which ranked as follows: AB113 > RB198 > DO46 > BR46. This outcome suggests that BR46, which is treated better than other dyes, has a more negative effect on the plant growth rate.

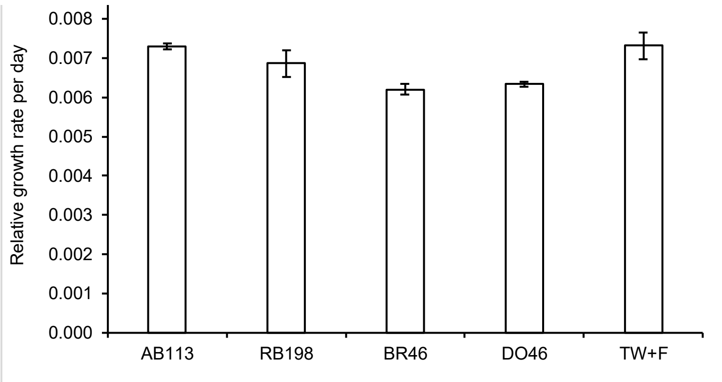

Fig. 6. Overview of the mean relative growth rate for *Lemna minor* L. in planted ponds: AB113 – Acid Blue 113, RB198 – Reactive Blue 198, BR46 – Basic Red 46, DO46 – Direct Orange 46, and TW + F – tap water and fertiliser) between 10 July 2014 and 2 February 2016. Note that standard deviations are presented by error bars based on four replicates

The same impact was observed for Brilliant Blue R special, which inhibited *L. minor* growth [24]. However, statistical analysis shows that there is no significant difference in RGR values among the ponds containing AB113, RB198, and tap water and fertiliser (Table 7). This indicates that the effect of AB113 and RB198 as growth inhibitors was very low. In addition, no significant differences in RGR between ponds comprising BR46 and DO46 were recorded.

Table 7

Overview of the statistical analysis for the relative growth rate of *Lemna minor* L. ponds between the wastewater types using the parametric one-way ANOVA

Variable combination	*p*-Value	Variable combination	*p*-Value
AB113 & RB198	0.155	RB198 & DO46	0.102
AB113 & BR46	<0.001	RB198 & TW + F	0.173
AB113 & DO46	<0.001	BR46 & DO46	0.947
AB113 & TW + F	1.000	BR46 & TW + F	<0.001
RB198 & BR46	0.027	DO46 & TW + F	0.001

Note: *p*-value – probability of the statistical test (if $p > 0.05$, the variables are not statistically significantly different, if $p < 0.05$, the variables are statistically significantly different). AB113 – Acid Blue 113, RB198 – Reactive Blue 198, BR46 – Basic Red 46, DO46 – Direct Orange 46, TW + F – tap water and fertiliser. Test of normality using Shapero–Wilk: 0.061. Significant value among the wastewater types is <0.001.

Moreover, the RGR for BR46 and DO46 were significantly lower than those for ponds containing only tap water and fertiliser (Table 7). The plants harvested in May and July originated only from ponds which were completely covered by *L. minor*. The last harvest took place in February at the end of the experiment (Table 8). Zhao et al. [15] highlighted that temperature and light intensity changes impact on the growth rate of duckweed, which increases during summer and reduces during winter.

Table 8

Mean values of the fresh and dry weights [g] of the plants harvested for each system between 10 July 2014 and 2 February 2016

<table>
<tr><th rowspan="2">Type of wastewater</th><th rowspan="2">Weight</th><th colspan="3">Date</th></tr>
<tr><th>12/05/15</th><th>06/07/15</th><th>02/02/16</th></tr>
<tr><td rowspan="2">Acid Blue 113</td><td>fresh</td><td>18.06</td><td>18.05</td><td>31.43</td></tr>
<tr><td>dry</td><td>0.910</td><td>0.903</td><td>1.570</td></tr>
<tr><td rowspan="2">Reactive Blue 198</td><td>fresh</td><td rowspan="7">not applicable</td><td>22.33</td><td>31.30</td></tr>
<tr><td>dry</td><td>1.121</td><td>1.558</td></tr>
<tr><td rowspan="2">Basic Red 46</td><td>fresh</td><td>N/A</td><td>37.33</td></tr>
<tr><td>dry</td><td>N/A</td><td>1.900</td></tr>
<tr><td rowspan="2">Direct Orange 46</td><td>fresh</td><td>N/A</td><td>40.03</td></tr>
<tr><td>dry</td><td>N/A</td><td>2.002</td></tr>
<tr><td>Tap water and fertiliser</td><td>fresh</td><td>14.78</td><td>53.50</td></tr>
</table>

4. CONCLUSIONS AND RECOMMENDATIONS

The shallow ponds removed the Basic Red 46 (BR46) at low concentration of 5 mg/dm^3 better than the other dyes (Acid Blue 113 (AB113, Reactive Blue 198 (RB198) and Direct Orange 46 (DO46)) under semi-natural conditions in Salford. The mean removal efficiency of BR46 for *L. minor* ponds was around 51% with a considerable phytoremediation potential of around 13%. Biosorption processes may be enhanced due to the effect of a simple chemical structure and small molecular weight associated with the absence of sulpho-groups in BR46. The treated wastewater values of total dissolved solids and chemical oxygen demands were within the allowable ranges for discharge to the watercourses. The growth of *L. minor* was inhibited significantly ($p < 0.05$) by BR46 and DO46 compared to ponds fed by tap water and fertiliser.

Further experiments should be conducted under controlled conditions using *L. minor* ponds for treatment of artificial textile wastewater using the same dyes after changing the operation parameter, which may affect the dye removal efficiencies such as concentration, contact time and pH value. At the same time, plant uptake, microbial degradation and particle sedimentation processes could be assessed in more detail. Further research on the treatment of other dyes using pond systems planted by *L. minor* or other macrophytes should be undertaken.

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