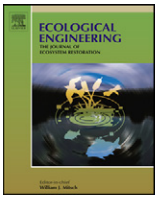
Ecological Engineering 44 (2012) 102–110

Contents lists available at SciVerse ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment

Christian B. Sekomo[a,b], Diederik P.L. Rousseau[a,c,*], Saleh A. Saleh[a], Piet N.L. Lens[a]

[a] UNESCO-IHE Institute for Water Education, Department of Environmental Resources, P.O. Box 3015, 2601DA Delft, The Netherlands
[b] National University of Rwanda, Department of Chemistry, P.O. Box 117, Butare, Rwanda
[c] Howest University College West-Flanders, Research Group Enbichem, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium

ARTICLE INFO

Article history:
Received 1 July 2011
Received in revised form 5 March 2012
Accepted 26 March 2012
Available online 2 May 2012

Keywords:
Algae
Duckweed
Textile wastewater
Heavy metals
Tracer test

ABSTRACT

Untreated textile wastewater is a typical source of heavy metal pollution in aquatic ecosystems. In this study, the use of algae and duckweed ponds as post-treatment for textile wastewater has been evaluated under the hypothesis that differing conditions such as pH, redox potential and dissolved oxygen in these ponds would lead to different heavy metal removal efficiencies. Two lab-scale systems each consisting of three ponds in series and seeded with algae (natural colonisation) and duckweed (*Lemna minor*), respectively, have been operated at a hydraulic retention time of 7 days and under two different metal loading rates and light regimes (16/8 h light/darkness and 24 h light). Cr removal rates were 94% for the duckweed ponds and 98% for the algal ponds, indifferently of the metal loading rate and light regime. No effect of pond type could be demonstrated for Zn removal. Under the 16/8 light regime, Zn removal proceeded well (~70%) at a low metal loading rate, but dropped to below 40% at the higher metal loading rate. The removal efficiency raised back to 80% at the higher metal loading rate but under 24 h light regime. Pb, Cd and Cu all showed relatively similar patterns with removal efficiencies of 36% and 33% for Pb, 33% and 21% for Cd and 27% and 29% for Cu in the duckweed and the algal ponds, respectively. This indicates that both treatment systems are not very suitable as a polishing step for removing these heavy metals. Despite the significant differences in terms of physico-chemical conditions, differences in metal removal efficiency between algal and duckweed ponds were rather small.

1. Introduction

Heavy metal contamination of the environment has increased sharply since 1900 (Nriagu, 1979) and actually the increasing contamination of freshwater systems with thousands of industrial and natural chemical compounds is one of the major environmental and human health problems worldwide (Ensley, 2000; Schwarzenbach et al., 2006). According to Johnson and Hallberg (2005) for example, it was estimated that in 1989 ca. 19,300 km of streams and rivers, and ca. 72,000 ha of lakes and reservoirs worldwide had been seriously damaged by mine effluents, although the true scales of the environmental pollution caused by mine water discharge is difficult to assess accurately. Other well-known anthropogenic sources of heavy metal pollutants are smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludge to land, and industrial manufacturing as the textile industry (Raskin et al., 1994; Cunningham et al., 1997; Blaylock and Huang, 2000).

Textile wastewater is a mixture of colorants (dyes and pigments) and various organic compounds used as cleaning solvents, and has a high chemical as well as biological oxygen demand. It also contains high concentrations of heavy metals and total dissolved solids (Sharma et al., 2007). For example, its discharge led to the complete disappearance of submerged and free floating hydrophytes, as well as also to the disappearance of certain marshy species in the pools in the area of Sanganer town, Jaipur (India).

Several technologies are available to remove heavy metals (HM) from wastewater such as chemical precipitation, flotation, coagulation-flocculation, ion exchange and membrane filtration (Kurniawan et al., 2006); all have their advantages and limitation in application, require high capital investment as well as tend to generate a sludge disposal problem (Cohen, 2006; Aziz et al., 2008). Thus for most developing countries, alternative technologies are needed that are within the economical and technological capability of these nations.

Wetland systems have been used as an alternative cost-effective technology to conventional wastewater treatment methods. It has

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[*] Corresponding author. Tel.: +3256241254.
E-mail address: diederik.rousseau@howest.be (D.P.L. Rousseau).

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http://dx.doi.org/10.1016/j.ecoleng.2012.03.003

been shown that they can efficiently remove heavy metals from both domestic and industrial wastewater (Rodgers and Dunn, 1992; Tang, 1993; Lakatos et al., 1997; Qian et al., 1999; LeDuc and Terry, 2005). Plants play important roles in these systems for the removal of pollutants (Brix, 1994; Oporto et al., 2006). Plants offer nutrition and protection against the physical environment and act as hosts for endophytic bacteria. Free-living, plant growth-promoting rhizobacteria, as well as symbiotic bacteria can improve plant nutrition and growth, and plant competitiveness, as well as responses to external stress factors such as contaminant (nutrients, heavy metals, ...) exposure through their mobilization (Doty, 2008; Trapp and Karlson, 2001; Jacob and Otte, 2004; Marchand et al., 2010). Microbial siderophores can interact with metals, reducing their toxicity or increasing labile metal pools and root uptake (Lemanceau et al., 2009; Mench et al., 2009).

Water hyacinth (*Eichhornia crassipes*) and duckweed (*Lemna* sp.) are commonly used in aquatic treatment systems. These plants and algae influence the redox and pH conditions of the aquatic systems as a result of photosynthesis and respiration processes (Shilton, 2005). Since photosynthesis requires solar radiation, oxygen is produced during daylight and consumed at night by algae and bacteria. Most algae require inorganic CO_2 as carbon source. During the photosynthesis CO_2 is consumed, consequently the pH may increase. At night, when CO_2 is produced from bacterial and algal respiration, the pH will decrease. Therefore, it is expected that they will also contribute to the metal removal processes. Given that pH and redox fluctuations are much higher in algal ponds than in duckweed ponds (Vymazal, 1995; Kadlec and Wallace, 2008), one may expect different metal removal efficiencies in these pond systems.

In wetlands, the pH affects the heavy metal removal efficiency. Ammonium conversion into nitrites during nitrification leads to proton production. These hydrogen ions are then neutralized by bicarbonate ions. Macrophytes, in releasing oxygen, promote the nitrification process. Protons produced due to nitrification may not all be neutralized by HCO_3^- ions, resulting in a pH decrease (Lee and Scholz, 2007). The overall mean surface charge of ferric (oxyhydr)oxides changes from a positive to a negative value as pH increases. Hence, to promote adsorption and removal of oxyanions of, for example, As, Sb, and Se, iron co-precipitation must occur under acidic conditions (Sheoran and Sheoran, 2006). Conversely, alkaline conditions are necessary to promote coprecipitation of cationic metals, such as Cu, Zn, Ni, and Cd. A high rate of nitrification can therefore reduce the efficiency of a constructed wetland in terms of cationic metal removal (Lee and Scholz, 2007). Furthermore, Galun et al. (1987) and Nyquist and Greger (2009) also reported that the pH affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions. This has in turn a direct effect on the adsorption, desorption, precipitation and co-precipitation process of metallic ions on the biosorbent.

Redox conditions affect the mobility of metals in two ways. Firstly, there are direct changes in the oxidation states of certain metals. Secondly, redox conditions may indirectly influence the mobility of metals that occur in only one oxidation state (e.g. cadmium and zinc). This is the case when these metals are bound to sulphides, organic matter or chelating agents (Stigliani, 1992). For heavy metals in wastewater, the effect mainly occurs to the heavy metals bound to reducible or oxidisable compounds.

In this study, we are presenting results of one step from a study that aspires to devise a sustainable low-cost heavy metals treatment technology for textile wastewater based on a two-step process: (1) heavy metals precipitation after sulphate reduction in anaerobic bioreactors and (2) a polishing step by means of aquatic treatment systems. The whole system constitutes an integrated system for heavy metals treatment (Sekomo et al., 2011b). The present study focuses on the second step, with the objective to study the effect of heavy metal loads on their removal efficiency and to find out how this is influenced by the light regime, pH and redox potential conditions in duckweed (DP) and algae (AP) ponds.

2. Materials and methods

2.1. Laboratory set-up

The set-up comprised two treatment lines, one with AP and one with DP, each consisting of three glass aquaria in series (L × W × D: 50 cm × 30 cm × 30 cm; maximum water depth 23.5 cm) as described by Shi et al. (2010). They were coded A1–A3 for the AP, and D1–D3 for the DP (Fig. 1).

The hydraulic behaviour of the ponds was studied using a tracer continuous loading technique using lithium chloride (LiCl) (Zimmo et al., 2000). Initial background of the tracer concentration in the pond water was measured in all sampling points immediately prior to the tracer addition. The flow pattern was characterised according the models described by Levenspiel (1972) and García et al. (2004).

2.2. Wastewater characteristics

Synthetic wastewater was pumped from a holding tank at equal rates to the AP and DP. This wastewater was based on the Hunter

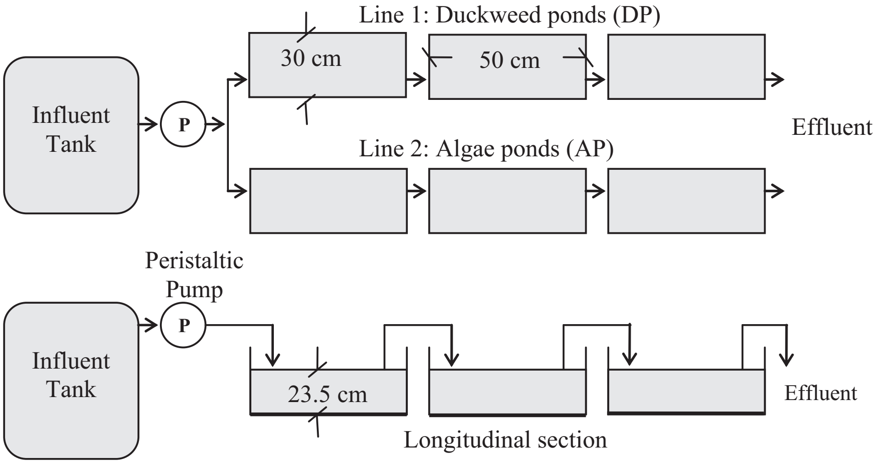

Fig. 1. Schematic representation of the AP and DP set-up.

nutrient solution (Leman, 2000), whose general characteristics are: 17.7 mg/L of TOC, 4.6 mg/L of NH_4^+-N, 4.8 mg/L NO_3^-, 7.3 mg/L of PO_4^{3-}, 3.6 mg/L of SO_4^{2-}, 6.3 mg/L of Mg^{2+}, 0.5 mg/L of Fe^{2+} and spiked with HM to a level equal to what can be expected of anaerobically pre-treated textile wastewater (see Section 2.3; based on Sekomo et al., 2011b). The hydraulic retention time (HRT) was set to seven days; evapotranspiration losses were compensated daily by adding demineralised water.

2.3. Operating conditions

The system was initially operated under a 16/8 h light/dark regime with a light intensity of 125 $\mu E\,m^{-2}\,s^{-1}$ on the water surface. The DP were inoculated with *Lemna minor* species at a density of 600 g fresh weight m^{-2} collected in Delft canal water. AP were inoculated with Delft canal water at start-up for algae (natural colonisation) development. After an initial start-up period of two weeks to allow plant and algae growth, three experiments were done: (1) Cd, Cr, Cu, Pb, and Zn at influent concentrations of 0.05, 1.5, 0.1, 0.25 and 1.25 mg/L, respectively, named Run 1; (2) double metal concentrations of Run 1, named Run 2; and (3) double metal concentrations of Run 1 and switch to a 24 h light regime, named Run 3. All experiments were done for 3 weeks (i.e. 3 times the HRT) to allow the system to reach steady state conditions. Duckweed biomass was harvested every five days to restore the duckweed density to 600 g fresh weight/m^2. This density was selected to prevent overcrowding and to maintain sufficient cover to minimize the development of algae in duckweed ponds (Zimmo, 2003). Floating algae formed over the water surface were collected regularly to improve oxygen levels in the system since a thick layer was obstructing light penetration.

2.4. Sampling, preservation and analytical procedures

Dissolved oxygen (DO) was measured using HACH HQ10-LDO (Luminescent Dissolved Oxygen). pH, temperature and ORP measurements were done with a WTW pH 340i. All equipment was properly calibrated following the manufacturer instructions. Readings were taken at 10 cm below the water surface every hour (one pond/24 h) during the experimental periods to produce physico-chemical profiles for each pond under the different conditions of each run. As the system was operated under constants conditions of flow and temperature, several samples ($N = 3$) were taken to determine the degree of variations in the system.

Sampling, preservation and analytical procedures followed the Standard Methods for Water and Wastewater Examination (APHO, 1995). Once a week, after good mixing, 250 mL of composite water samples were taken from tanks collecting all effluent during that week for mass balance purposes. Grab samples were collected from the influent and the effluents of each pond at 4 instances during the last two weeks of each run. During the first 10 days of each run, no grab samples were taken as it was assumed that the system was adapting to the newly applied influent conditions.

Biofilm accumulated on the walls of the ponds, floating algae and duckweed biomass were harvested and stored in a freezer until further HM analysis. Those samples were first dried at 105 °C, and then the biomass was digested with nitric acid using a microwave technique. HM analysis (Cd, Cr, Cu, Pb and Zn) was conducted for all water samples by using the flame and flameless atomic absorption spectrometer models Perkin-Elmer 3110 and Thermo elemental – SOLAR 95/Furnace.

2.5. Data treatment

Data were treated with Microsoft Excel™, Excel Stat Pro software. Data were tested for normality and then the *T*-test and ANOVA were conducted on them. Where the normality test was not successful, the Kruskal–Wallis non parametric test was conducted. For physico-chemical data, the *t*-test ($\alpha = 0.05$) and for heavy metal data, the ANOVA two-factor (concentration and light regime) with replication test ($\alpha = 0.01$) were applied to compare the algal and duckweed treatment ponds.

Knowing that in a continuous flow system there is always a variation in density and type of plants and microbes as the water passes through the system, the hydraulic model must account for these effects as reported by Kadlec and Wallace (2008). The tanks-in-series model (TIS) was applied and the removal profile for the contaminant in that system is given by:

$$\frac{(C - C^*)}{(C_i - C^*)} = \left(1 + \frac{k\tau}{Nh}\right)^{-N} \tag{1}$$

where C_i the initial concentration, C is the final concentration, C^* is the background concentration, k is the first-order rate constant, N is the number of tanks in series, h is the depth and τ is the hydraulic retention time.

The mass balance analysis was performed for algae and duckweed systems as follows:

(A) **Duckweed system**:

$$\text{Plant accumulation} + \text{Sedimentation} = \sum(Q_{in} \times C_{in}) - \sum(Q_{out} \times C_{out}) - R \tag{2}$$

where Q_{in}: influent flow rate (L/day); C_{in}: average influent concentration (mg/L); Q_{out}: effluent flow rate (L/day); C_{out}: average effluent concentration (mg/L); R: remaining load in water phase of the ponds at t_{final} (mg), hence

$$R = \text{total volume of the ponds} \times \text{average conc. of last grab samples} \tag{3}$$

(B) **Algal system**:

$$\text{Algal accumulation} + \text{Biofilm accumulation} = \sum(Q_{in} \times C_{in}) - \sum(Q_{out} \times C_{out}) - R \tag{4}$$

3. Results

3.1. Tracer test

Tracer recovery was consistently 97% or higher. Table 1 shows good agreement between theoretical and actual hydraulic retention times, indicating the absence of short-circuiting and/or dead zones. Dispersion numbers and *N* (number of tanks according to the tanks-in-series model) indicate well-mixed conditions.

3.2. Physico-chemical parameters

Water temperature ranged between 19 and 22 °C (data not shown), with only small daily variations. Looking at the longitudinal profile, no significant differences ($P > 0.05$) in temperature values were found for all the duckweed ponds when compared to the algal ponds. Nevertheless, different temperature profiles were recorded when comparing DP to AP. In the AP, maximum and minimum temperatures were recorded, respectively, at 1:30 AM and 9:30 PM during Run 1 and Run 2. In Run 3, the temperature profile showed a constant profile. In the DP, a constant temperature profile was recorded during all runs.

During all runs, higher dissolved oxygen concentrations and pH values (Fig. 3A, Table 2) were recorded in the algae ponds

Table 1
Hydraulic characteristics of the duckweed and the algal pond systems.

Parameters	Eff_DP1	Eff_DP2	Eff_DP3	Eff_AP1	Eff_AP2	Eff_AP3
HRT_{theo} (days)	2.31	4.62	6.93	2.31	4.62	6.93
HRT_{act} (days)	2.29	4.81	6.90	2.27	4.88	6.92
σ^2 (h^2)	4006	17,573	13,112	4039	15,624	13,160
R_n	0.75	0.76	2.09	0.73	0.88	2.10
Dispersion number d	0.66	0.65	0.24	0.68	0.57	0.24
Dead zone (%)	0.87	−4.17	0.43	0.87	−5.52	0.43
Recovery (%)	100.00	100.00	100.00	100.00	97.60	97.60
Dead time (h)	21.25	44.68	69.50	21.25	44.68	69.50

when compared to the duckweed ponds. However, the redox potential was high in the DP when compared to the AP (Table 2). Statistical analysis revealed significant differences (t-test, $P < 0.0001$) between AP and DP in dissolved oxygen, pH and redox potential.

3.3. Metal removal

Table 3 gives an overview of influent and effluent concentrations based on four grab samples taken once the system has adapted to the influent composition. In general, looking at the longitudinal profile all five heavy metals were characterised by a decreasing pattern in their removal efficiencies when comparing Run 1, Run 2 and Run 3. Overall Cr and Zn showed the highest removal rates, both in the duckweed and algae ponds. The system performance was very good, with maximum removal efficiencies varying between 91–99% for Cr and 51–82% for Zn both under low and high metal loading rates as well as under 24 h light regime. No significant differences were found between AP and DP in metal removal (ANOVA, $P = 0.66$) and after application of different light regimes (ANOVA, $P = 0.89$). However, the type of organisms did have a highly significant effect on the metal removal efficiency (ANOVA, $P < 0.001$) with algal ponds having a better performance.

3.4. Mass balance

The accumulation of heavy metals has been calculated for both duckweed and algal treatment ponds by the difference between inflows and outflows in the system including the quantity accumulated by the duckweed or algae, the quantity removed by sedimentation and the quantity remaining in the water phase of the ponds (Tables 4 and 5). The mass balance has been calculated for the entire experimental period, i.e. from the start up phase until the end of Run 3.

In Table 4, it should be noted that sedimentation is presenting the accumulation of heavy metals at the bottom of the pond. In Table 5, the final resident biofilm is representing the layer of algae that was covering the walls of the algal ponds. Error is a closing term representing the difference in concentration found after summation of all components considered.

4. Discussion

4.1. General performance

Heavy metal concentrations in the effluents supplied to the experimental set-up were high compared to the standards reported in the WHO (2006) guidelines for the safe use of wastewater, excreta, and grey water and EPA (2009) drinking water contaminant limits. The following orders were observed in metal removal under different conditions: Cr > Zn > Pb > Cd > Cu in Run 1 for the duckweed system, Cr > Zn > Cu > Cd > Pb in Run 1 for the algal system, Cr > Zn = Pb > Cd = Cu in Run 2 for duckweed system, Cr > Zn > Pb > Cu > Cd in Run 2 for algae system and Cr > Zn > Pb > Cu > Cd in Run 3 for both systems. The first-order model for 2 tanks-in-series was fitted to our data and the rate constant k was determined. In general, the rate constant was low and followed the order Cr > Zn > Pb > Cu > Cd (Table 6).

The system performance was good when compared with other reported natural systems. Maine et al. (2001) measured Cd uptake by *E. crassipes*, *Hydromistia stolonifera*, *Pistia stratiotes* and *Salvinia herzogii*. Little difference among species was observed and the approximate first order rate constant was 0.5 d^{-1}. Miretsky et al. (2004) reported on Cr removal by *S. herzogii* and *P. stratiotes*. They found that the Cr uptake increases with increasing Cr concentration in water up to 6200 mg/g at a water concentration of 6 mg/L. Removal of Pb in wetlands is highly variable with removal efficiencies between −220% and 98% for eight systems with a median removal of 25% (Kadlec and Wallace, 2008). Mitchell et al. (2002) reported a rate constant of 6 days^{-1} for Zn in wetlands treating highway runoff.

Mass balance analyses showed that the duckweed has a high binding capacity when compared to algae, specifically for Cr, Zn and Pb. However, high metal concentrations were encountered in the final resident biofilm in the AP. This gives an indication of the mechanism of metal retention within the system. As we did not investigate the metal partitioning in the plant, it is difficult to precise which part of the plant played a major role in metal retention or accumulation. However, biosorption (Dhabab, 2011) and bio-uptake (Oporto et al., 2006; Hou et al., 2007) are reported heavy metal removal mechanisms. In the AP, it is clear that removal was occurring mainly via precipitation due to the high pH combined

Table 2
Mean values of ORP, DO and pH for the AP and DP systems during all runs.

	ORP (mV)			DO (mg/L)			pH		
	Run_1	Run_2	Run_3	Run_1	Run_2	Run_3	Run_1	Run_2	Run_3
DP1	262.5 ± 46	332.5 ± 51.6	319 ± 7.1	0.7 ± 0.8	1.6 ± 0.5	2.1 ± 0.7	7.1 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
DP2	208 ± 9.9	228 ± 12.7	362 ± 28.3	3.3 ± 0.4	2.2 ± 1.0	1.3 ± 0.8	6.9 ± 0.1	6.6 ± 0.2	7.0 ± 0.1
DP3	285 ± 12.7	335 ± 14.1	361 ± 8.5	3.9 ± 0.9	2.7 ± 1.1	1.9 ± 0.4	7.0 ± 0.1	6.8 ± 0.0	6.9 ± 0.1
AP1	137 ± 19.8	276 ± 90.5	232 ± 15.6	16 ± 4.8	8.6 ± 2.4	14.3 ± 0.8	9.4 ± 0.4	8.4 ± 0.7	9.1 ± 0.1
AP2	153 ± 43.8	271 ± 42.4	196.5 ± 21.9	16.8 ± 3.7	11.6 ± 5.1	14.2 ± 4.9	9.5 ± 0.4	8.9 ± 0.5	9.5 ± 0.4
AP3	165 ± 22.6	251 ± 31.1	186.5 ± 9.2	11.8 ± 2.9	10 ± 2.9	16.3 ± 1.6	9.2 ± 0.3	8.9 ± 0.3	9.8 ± 0.1

1, 2 and 3 after AP and DP: stands for the number of the pond in series.

Table 3
Influent and effluent concentrations (average ± standard deviation; $n = 4$) for different runs and ponds system.

	Influent			Run_1					
	Run 1	Run 2	Run 3	DP1	DP2	DP3	AP1	AP2	AP3
Cd (mg/L)	0.06 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.053 ± 0.005	0.048 ± 0.005	0.040 ± 0.000	0.055 ± 0.006	0.053 ± 0.005	0.048 ± 0.005
Cr (mg/L)	1.35 ± 0.08	2.68 ± 0.15	2.68 ± 0.02	0.38 ± 0.04	0.18 ± 0.02	0.07 ± 0.01	0.19 ± 0.02	0.15 ± 0.01	0.05 ± 0.01
Cu (mg/L)	0.11 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.1 ± 0.00	0.10 ± 0.01	0.08 ± 0.01	0.1 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Pb (mg/L)	0.26 ± 0.01	0.61 ± 0.01	0.54 ± 0.01	0.23 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.21 ± 0.01
Zn (mg/L)	1.70 ± 0.05	3.14 ± 0.03	3.03 ± 0.02	1.25 ± 0.04	0.87 ± 0.05	0.48 ± 0.05	1.29 ± 0.03	0.94 ± 0.04	0.54 ± 0.09

	Run_2						Run_3					
	DP1	DP2	DP3	AP1	AP2	AP3	DP1	DP2	DP3	AP1	AP2	AP3
Cd (mg/L)	0.118 ± 0.005	0.108 ± 0.005	0.098 ± 0.005	0.118 ± 0.005	0.110 ± 0.000	0.108 ± 0.010	0.118 ± 0.01	0.113 ± 0.010	0.105 ± 0.010	0.118 ± 0.010	0.115 ± 0.010	0.110 ± 0.000
Cr (mg/L)	0.78 ± 0.15	0.25 ± 0.08	0.17 ± 0.02	0.53 ± 0.11	0.18 ± 0.10	0.06 ± 0.01	0.42 ± 0.25	0.21 ± 0.10	0.15 ± 0.05	0.37 ± 0.05	0.24 ± 0.51	0.07 ± 0.00
Cu (mg/L)	0.21 ± 0.03	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.20 ± 0.02	0.19 ± 0.02	0.2 ± 0.00	0.22 ± 0.01	0.19 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
Pb (mg/L)	0.52 ± 0.03	0.48 ± 0.03	0.41 ± 0.01	0.54 ± 0.03	0.48 ± 0.02	0.44 ± 0.02	0.49 ± 0.01	0.44 ± 0.04	0.38 ± 0.02	0.48 ± 0.05	0.43 ± 0.05	0.36 ± 0.04
Zn (mg/L)	2.82 ± 0.09	2.48 ± 0.16	2.25 ± 0.11	2.53 ± 0.18	2.25 ± 0.20	1.95 ± 0.32	2.59 ± 0.03	2.06 ± 0.07	0.65 ± 0.01	2.2 ± 0.07	1.49 ± 0.03	0.59 ± 0.05

1, 2 and 3 after AP and DP: stands for the number of the pond in series.

with biosorption and uptake. Our results are in good agreement with the findings of other researchers (Vymazal, 1995; Murray-Gulde et al., 2005; Vymazal and Krasa, 2005; Kadlec and Wallace, 2008; Sekomo et al., 2010a) who reported that algae, bulrushes, cattails, *Cyperus papyrus*, *Schoenoplectus californicus* play a lesser role in metal retention in a wetland. However, depending on the engineering process in the wetland, the bulk pollutant removal is always achieved by physico-chemical processes and then after plants can be used as polishing step.

4.2. Effect of metal load

The wastewater composition, in particular the dissolved oxygen concentration, the pH and the redox potential affect the speciation of heavy metals (Eggleton and Thomas, 2004; Simpson et al., 2004). In this study, physico-chemical parameters were affected as the metal load was varied (Table 2). Variation of pH between duckweed and algal ponds was related to the decreasing plant activities affected by doubling the heavy metal concentrations in Run 2. In Run 3, the increase in heavy metal removal observed was due to the application of a 24 h light regime to the system. An increasing trend in redox potential was observed for both pond types in Run 2. That increase is directly linked to the decreasing DO influenced by higher metal loads in the system. In Run 3, a further increase was observed for the duckweed ponds.

Many wetland plants have constitutive metal tolerance and mechanisms of metal resistance (Matthews et al., 2005; Deng et al., 2006, 2009; Kanoun-Boulé et al., 2009). These mechanisms enable most plant cells to continue normal activities upon Pb exposure while sacrificing a few cells that accumulate large amounts of Pb (Zhou et al., 2010). In this study, the decrease in pH between Run 1 and 2 (figures not shown); corresponds to the doubling of the metal concentration. This pH decrease can be explained by a plant reaction to the metal concentration becoming toxic. This may result in the excretion of organic materials by plants and therefore decreasing the pH of the solution. This is supported by Lemanceau et al. (2009) and Mench et al. (2009) reporting that excessive ethylene production promoted by stress can depress growth. Many bacteria facilitate plant growth by metabolizing 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene, through the synthesis of ACC deaminase (ACCD). Bacterial strains with ACCD activity can prevent reduction in root and shoot growth resulting from stressful conditions. Microbial siderophores can interact with metals, reducing their toxicity or increasing labile metal pools and uptake by roots. Furthermore, Hou et al. (2007) reported on the way heavy metals enter frond chloroplasts and may be over-accumulated, thus inducing oxidative stress which causes damages like peroxidation of chloroplast membranes.

In Run 1, Zn exhibits the second best removal efficiency of all metals tested, reaching similar levels of around 70% in both AP and DP (ANOVA, $P = 0.36$). Each of the three ponds in series seems to contribute to an almost equal way to the Zn removal, resulting in a linearly increasing removal efficiency. This linear trend could be explained by the same amount of pollutant being taken up by approximately equal amounts of duckweed in each pond. Important improvements can thus still be expected by enlarging the system; extrapolating the data shows that removal levels of 90% and more could be reached by just adding one more pond.

For Run 2 the situation is quite different, with much lower overall Zn removal efficiencies: 28% for DP and 38% for AP. High metal concentrations thus seem to have a negative effect on Zn removal. Statistical analysis revealed significant differences between AP and DP (ANOVA, $P < 0.001$). Zn like Cu is known to be an essential micronutrient for normal plant metabolism, playing an important role in a large number of metalloenzymes, photosynthesis

Table 4
Mass balance calculations for the duckweed system (load in mg/total operational time).

Metals load (mg/11 weeks)	Total influent load	Total effluent load	Harvested duckweed	*R*[a]	Sedimentation
Pb	447 (100%)	288 (64%)	101 (23%)	48 (11%)	10 (2%)
Cd	98 (100%)	75 (76%)	7 (7%)	12 (12%)	5 (5%)
Cr	2124 (100%)	100 (5%)	1130 (53%)	17 (1%)	878 (41%)
Zn	2851 (100%)	1173 (41%)	1123 (39%)	189 (7%)	366 (13%)
Cu	213 (100%)	166 (78%)	20 (9%)	23 (11%)	5 (2%)

[a] *R*: remaining load of water phase in the ponds.

Table 5
Mass balance calculations for the algal system (load in mg/total operational time).

Metals load (mg/11 weeks)	Total influent load	Total effluent load	Floating algal layer	Final resident biofilm	*R*[a]	Error
Pb	447 (100%)	280 (63%)	10 (2%)	108 (24%)	42 (9%)	7 (1.6%)
Cd	98 (100%)	78 (79%)	1 (1%)	6 (6%)	12 (12%)	2 (2%)
Cr	2124 (100%)	49 (2%)	134 (6%)	1924 (91%)	12 (1%)	6 (0.3%)
Zn	2851 (100%)	1090 (38%)	45 (2%)	1558 (55%)	152 (5%)	7 (0.2%)
Cu	213 (100%)	161 (75%)	2 (1%)	40 (19%)	21 (10%)	−10 (−4.7%)

[a] *R*: remaining load of water phase in the ponds.

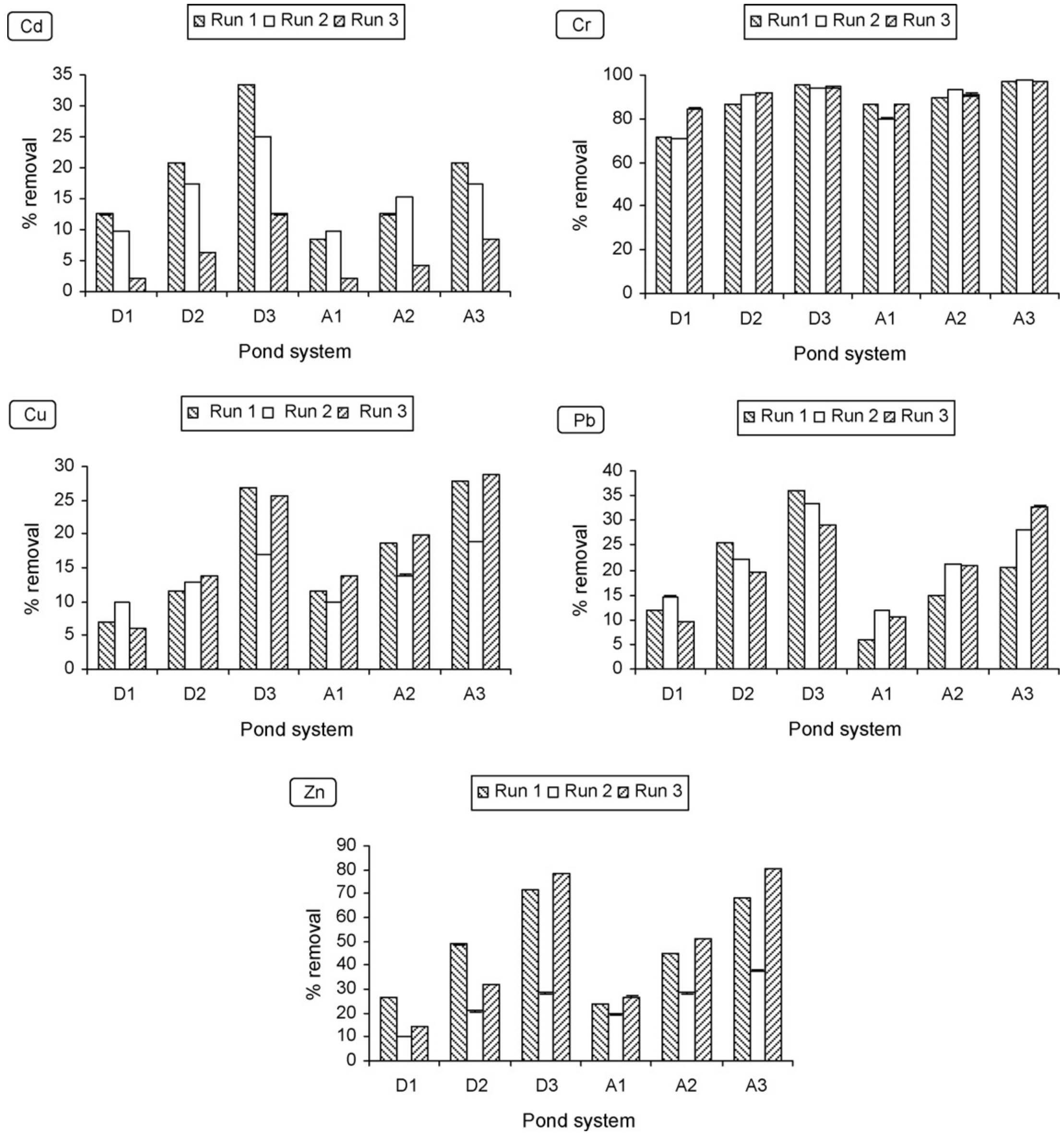

Fig. 2. Removal % of heavy metal in duckweed and algal ponds for Run 1 (low HM concentration, 16/8 h light regime), Run 2 (high concentration, 16/8 h light regime) and Run 3 (high concentration 24 h light regime), respectively.

Table 6
Rate constant (k) for the first order equation for the algal (AP) and the duckweed (DP) ponds.

	k (day^{-1})					
	Run 1		Run 2		Run 3	
	AP	DP	AP	DP	AP	DP
Cd	0.0151	0.0080	0.0103	0.0066	0.0047	0.0012
Cr	0.2310	0.2849	0.2023	0.3860	0.2198	0.3523
Cu	0.0120	0.0120	0.0065	0.0084	0.0100	0.1200
Pb	0.0160	0.0077	0.0150	0.0120	0.0130	0.0130
Zn	0.0600	0.0530	0.0085	0.0180	0.0790	0.0860

related plastocyanin and membrane structures (Megatelli et al., 2009; Williams et al., 2000). However, Cu and Zn are also known to toxic heavy metals (Li and Xiong, 2004; Drost et al., 2007). Megatelli et al. (2009) reported Zn toxicity on *Lemna* at concentrations of 6.5 mg/L. The removal efficiency is still linearly increasing (about 10% per pond). Extrapolating these data shows that one would have to triple the number of ponds to reach removal levels of 90% and more.

In Run 3, a good removal efficiency for Zn was achieved: 82% in AP and 79% in DP. The light regime, stimulating the photosynthetic activity of the plants in the system, seems to be beneficial to the system (ANOVA, $P < 0.001$; Kruskal–Wallis, $P = 0.235$). An exponential increase in the removal efficiency was observed from pond 1 to pond 3 (Fig. 2).

Cd, Cu and Pb all show relatively similar patterns with removal efficiencies varying between min. 17% (Run 2: Cd in AP and Cu in DP) and max. 36% (Run 1: Pb in DP) which indicates that both systems are not very suitable as a polishing step for removal of those heavy metals. ANOVA showed a statistically significant effect of the metal loading rate on Cu removal ($P < 0.05$) and of the pond type on Cd and Pb removal ($P < 0.01$ and $P < 0.001$, respectively). However, the Kruskal–Wallis test showed no statistically significant difference on the metal loading rate on Cu, Cd and Pb. The reason for such low removal efficiency could be explained by a toxicity effect affecting the plant performance. Megatelli et al. (2009) reported that Cd at 0.2 mg/L was the most toxic metal for *Lemna* followed by Cu at 0.6 mg/L and finally Zn at concentrations above 6.5 mg/L. Miretsky et al. (2004) also reported the non-survival of *Lemna* on a mixture of 4 mg/L of Zn, Cu, Fe, Mn, Cr and Pb each. Cd at concentrations above 1.12 mg/L and Cu at concentrations above 3.18 mg/L promote pigment degradation and photosynthesis inhibition in *Lemna trisulca* L. (Prasad et al., 2001). In this study, the low removal efficiency recorded in both systems could be also attributed to that toxic effect as the concentration used was close to the reported one.

Heavy metal toxicity to algae has been investigated for quite a long time. Many redox active and non redox reactive metals are known to cause oxidative stress, as indicated by lipid peroxidation and H_2O_2 accumulation in the cells (Schutzendubel et al., 2001). Copper is a redox-active transition metal, known to catalyse hydroxyl radical production. Furthermore, zinc and lead are redox inactive metals which inactivate the cellular antioxidant pool and disrupt the metabolic balance (Stohs and Bagchi, 1995; Briat, 2002). Shehata et al. (1999) reported on the toxic effect of multi metal mixtures which exist simultaneously in aquatic ecosystems on natural phytoplankton assemblages (green algae, cyanobacteria and diatoms). They found substantial changes in phytoplankton community structure and the most tolerant group were the blue-green algae, followed by green algae while diatoms were the most sensitive group. These studies investigated toxicity effects of metal concentrations ranging between 0.05 and 0.2 mg/L of heavy metals. In our study, we investigated 5–10 times higher concentrations.

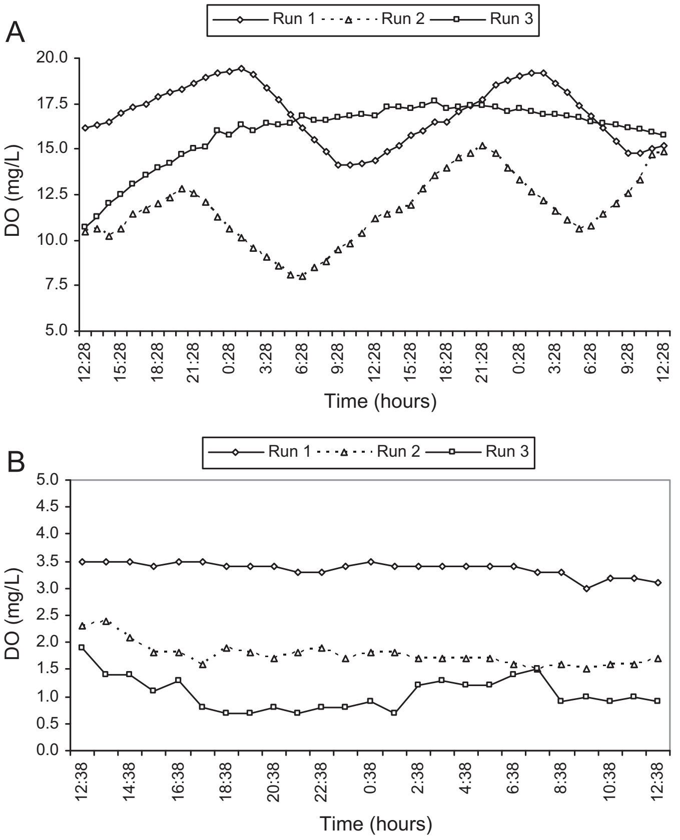

Fig. 3. Typical DO profiles for algae (A) and duckweed (B) ponds for day/night regime during Run 1, Run 2 (doubling the heavy metal concentration) and Run 3 (24 h light regime), respectively. Heavy metal concentrations 0.06, 1.35, 0.11, 0.26 and 1.7 mg/L in Run 1 and 0.12, 2.68, 0.25, 0.54 and 3.03 mg/L in Run 2 and 3 respectively for Cd, Cr, Cu, Pb and Zn.

4.3. Effect of light

Exposure of the system to a 24 h light regime has induced a change in physico-chemical parameters like DO, pH and redox potential. The sigmoid pattern recorded in Run 2 changed to a more or less constant pattern in Run 3 (Fig. 3). The reason for high oxygen levels in AP when compared to DP was due to algae photosynthetic activity within the water phase (Vymazal, 1995). In the DP, oxygen production occurred at the top surface of the plants (which is in the air phase) and oxygen tends to be lost to the atmosphere. Only a small portion is transported via the roots to the water phase (Bonomo et al., 1997). The duckweed mat at the top of the pond further prevents atmospheric re-aeration and light penetration hence limiting algae growth (Caicedo, 2005). In general, except Cd which showed high toxicity, the removal efficiencies of other metals have increased in Run 3 when compared to Run 2. This shows a positive effect of 24 h light application to the system.

5. Conclusions

This study showed that duckweed and algal ponds are suited as polishing step for heavy metal removal, especially Cr and Zn at lower concentration. However, for the treatment of high pollutant loads, a pre-treatment step is required. Heavy metal removal and abiotic conditions (pH, DO, ORP) and light regimes did not yield differences in removal efficiency. The difference expected in metal removal based on the pond type was not confirmed. In general, the overall performance of both ponds was similar.

Acknowledgments

The authors are grateful to the Dutch Government and the National University of Rwanda for the financial support provided for this research through the NPT/RW/051 Project. The authors would like also to thank the laboratory staff for UNESCO-IHE for their technical assistance during this research.

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