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### Phytoremediation capabilities of Salvinia molesta, water hyacinth, water lettuce, and duckweed to reduce phosphorus in rice mill wastewater

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#### **ABSTRACT**

The objective of this study was to investigate the reduction of phosphorus from rice mill wastewater by using free floating aquatic plants. Four free floating aquatic plants were used for this study, namely water hyacinth, water lettuce, salvinia, and duckweed. The aquatic plants reduced the total phosphorus (TP) content up to 80% and chemical oxygen demand (COD) up to 75% within 15 days. The maximum efficiency of TP and COD reduction was observed with water lettuce followed by water hyacinth, duckweed, and salvinia. The study also aims to predict phosphorus removal by three modeling techniques, for example, linear regression (LR), artificial neural network (ANN), and M5P. Prediction has been done considering hydraulic retention time (HRT), hydraulic loading rate (HLR), and initial concentration of phosphorus ( $C_{\rm in}$ ) as input variables whereas the reduction rate of TP (R) has been considered as a predicted variable. ANN shows promising results as compared to M5P tree and LR modeling. The model accuracy is analyzed using three statistical evaluation parameters which are coefficient of determination  $(R^2)$ , root mean square error (RMSE), and means absolute error (MAE).

Abbreviations: %: percentage; °C: degree centigrade; A: surface area of tub; ANN: artificial neural network; APHA: American Public Health Association; BOD: biochemical oxygen demand; cm: centimeter; CaCO<sub>3</sub>: calcium carbonate; C<sub>in</sub>: initial concentration of total phosphorus; C<sub>out</sub>: final concentration of total phosphorus; CO2: carbon dioxide; c/p: carbon/phosphorus; COD: chemical oxygen demand; d. day; DIP: dissolved inorganic phosphorus; DNA: deoxyribonucleic acid; EC: electric conductivity; Eq.: equation; Fig.: figure; g: gram; g/l: gram per litter; HLR: hydraulic loading rate; HRT: hydraulic retention time; ln: natural log; L \* W \* D: length \* width \* depth; MAE: means absolute error; MLP: multilayer perceptron;  $m^2$ : square meter; max: maximum; mg/g: milligram per gram; mg/l: milligram per liter; min: minimum; min: minute; ml: milliliter; pH: potential of hydrogen; RRSE: root relative squared error; R: reduction rate;  $R^2$ : coefficient of determination; RAE: relative absolute error; ROL: radial oxygen loss; RMSE: root mean square error; St. dev.: standard deviation; T: experiment time; Temp.: temperature; TP: total phosphorus; V: volume of tub

#### **KEYWORDS**

Aquatic plants; eutrophication; artificial neural network; M5P tree; total phosphorus

#### Introduction

Phytoremediation is a technique in which plants are used to remove, transfer, stabilize, or degrade the contamination from soil and water. It is a low-cost and solar-energy-driven process and helpful in removing nutrients from water and wastewater. These nutrients, if unattended, ultimately cause eutrophication of water bodies. Eutrophication is when a waterbody becomes overly enriched with nutrients. Nitrogen and phosphorus are the main nutrients responsible for eutrophication. These nutrients support algal bloom, which depletes oxygen from water and results in the mortality of aquatic animals. Some algal blooms are toxic in nature and harmful for plants and animals. Improving the quality of a waterbody through physical and chemical methods could be expensive and strenuous. Phytoremediation is a suitable alternative for removing nutrients from water and wastewater, which requires availability of aquatic plants and some maintenance. The removal efficiency of various aquatic plants for toxic elements has been studied by several researchers and the results have been satisfactory. Aquatic plants can reduce various types of pollutants from wastewater, such as nitrogen, phosphorus, nitrate, potassium, calcium, metals, magnesium, sodium, heavy organic matter.

Daud et al. (2018) used duckweed for landfill leachate treatment, Rahman and Hasegawa (2011) used floating plants to remove arsenic from water, Singh et al. (2012) used duckweed to remove lead from wastewater, Favas and Pratas (2013) studied the potential of aquatic plants to remove uranium from water, Axtell et al. (2003) studied the removal of lead and nickel from wastewater by using aquatic plants, Abu Bakar et al. (2013) used phytoremediation to remove arsenic, zinc, and aluminum from gold mine wastewater, Tanhan et al. (2007) used phytoremediation for the removal of cadmium, zinc, and lead from wastewater,



Azeez and Sabbar (2012) used Lemna minor L. to remove pollutants from oil refinery wastewater, Saha et al. (2017) studied removal of chromium from mine wastewater by using water hyacinth. Reddy et al. (2015) studied pollutant removal from sugar industry wastewater by using floating aquatic plants. Mishra et al. (2013) studied heavy metal removal from paper mill wastewater by using aquatic plants. Ajayi and Ogunbayio (2012) studied pollutant removal from textile, metallurgical, and pharmaceutical wastewaters by using water hyacinth.

In this paper, phosphorus reduction efficiency of four free floating aquatic plants, namely water hyacinth, water lettuce, salvinia, and duckweed, is compared. Phosphorus is a macronutrient essential for floral life, and it is found in various forms in water: (a) dissolved inorganic phosphorus (DIP), (b) particulate inorganic phosphorus, (c) dissolved organic phosphorus, and (d) particulate organic phosphorus. DIP is the most bio-absorbable form of phosphorus. All the other forms must be first converted into DIP and then utilized by aquatic plants (Ready et al. 1999). In a plant body, phosphorus is accumulated as polyphosphate, which is acid soluble and used for plant metabolism and for the production of deoxyribonucleic acid (DNA) and protein. Insoluble phosphorus stored in plant biomass are used by plants during a scarcity of phosphorus in the environment. Excess phosphorus concentration in water leads to toxic algal bloom, which causes low oxygen levels, fish mortality, and loss of aquatic biodiversity. Phosphorus is eliminated through various mechanisms such as direct uptake by plants, assimilation by microbes, filtration by rhizosphere, adsorption on roots, and precipitation with the help of metal ions. Phosphorus removal depends on the growth rate and phosphorus content of plants. It is faster when the phosphorus concentration of water is lower than a specific limit. Water lettuce can tolerate up to 50 mg/l phosphate, and the accumulation rate was  $6.12 \pm 0.95$  mg/g dry weight of plant after 35 days of investigation under greenhouse condition (Ready et al. 1999). A summary of phosphorus removal by aquatic plants and their system details are given in Table 1. Although studies on contamination removal from wastewater are numerous, only a few studies have been performed on parboiled rice mill wastewater. Therefore, we performed a study on rice mill wastewater and investigated the performance of each aquatic plant compared with the control and with each other.

The objective of this study was to compare the phosphorus reduction capabilities of four aquatic free floating plants, namely water hyacinth, water lettuce, salvinia, and duckweed, in parboiled rice mill wastewater. This study also aimed to quantify the biomass growth of each plant. All plants' growth was inhibited in rice mill wastewater and limited growth was observed in 10%, 20%, and 30% dilution with tap water. Hence, a dilution of 1:1 of raw wastewater and tap water was chosen to perform the experiment. In this paper, the authors also try to predict the values of total phosphorus (TP) reduction from rice mill wastewater by using soft computing techniques like linear regression (LR), artificial neural network (ANN), and M5P. The ANN

models show the most promising results as compared to M5P tree and LR models. Soft computing techniques have been found very useful in the past studies related to environmental engineering, to predict, control, and monitor problems, such as meteorology, soil pollution, air pollution, environmental impact assessment, environmental hydraulics, environmental geology, etc. Karul et al. (2000) used a threelayer feed forward algorithm model to predict the eutrophic mechanism in three water bodies. The correlation coefficient was found to be between 0.60 and 0.75. Baxter et al. (2004) developed a model for turbidity removal from water. They obtained a high coefficient of determination  $R^2 = 0.96$  and a low value of mean absolute error, MAE = 0.08 NTU. Onkal-Engin et al. (2005) used back propagation based ANN model to determine the relationship between sewage odor and BOD. The correlation coefficients obtained from training and testing data sets were 0.98 and 0.91, respectively. The RMS errors were 0.04 for training data set and 0.07 for testing data set. Karaca and Özkaya (2006) developed a model to determine relationship between leachate flow rate and local meteorological data, based on back propagation algorithm. The coefficient of determination  $(R^2)$ and mean squared error (MSE) obtained from this model were 0.847 and 0.00168, respectively. Akratos et al. (2008) used ANN technique to estimate the values of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and found the coefficient of determination,  $R^2 = 0.52$ for BOD removal, and  $R^2 = 0.44$  for COD removal. Zhang et al. (2008) used self-organizing map (SOM) to predict soluble reactive phosphorus in integrated constructed wetlands (ICWs) by using dissolved oxygen (DO) and conductivity as input variable, obtained coefficient of determination,  $R^2 = 0.951$  and MAE: 0.048. El-Gendy (2008) investigated the removal of heavy metals by water hyacinth and predict the output with the help of exponential and LR equations. The model achieved high coefficient of determination of 0.996 with an average absolute deviation of 1.7%. Yetilmezsoy and Sapci-Zengin (2009) developed a ANN model with nine input variables and 12 hidden neurons to predict COD removal from an up-flow anaerobic sludge blanket (UASB) reactor for treating cotton textile wastewater. They found that the ANN model prediction was satisfactory and the coefficient of correlation obtained was 0.82. Akratos et al. (2009a, 2009b) used ANN model to predict orthophosphate, TP, and total nitrogen (TN) removal in constructed wetland and found low values of regression coefficient. They observed the coefficient of determination  $(R^2)$  as 0.43 and 0.53 for TP and TN removal, respectively. Bagheri et al. (2015) predict the removal of TP in sequencing batch reactor by using radial basis function (RBF) and multilayer perceptron (MLP) and got the coefficient of determination 0.95 and 0.99, respectively. Zhang and Pan (2014) used back propagation ANN-genetic algorithm to predict phosphate removal efficiency by hydrated ferric oxide-based nanocomposite and got the coefficient of determination as 0.9931. Ozengin et al. (2016) applied ANN computing technique for the estimation of TN and TP removal from water. The correlation coefficient between

References	Polluted water/waste water quality	System and study details	Observations
Debusk <i>et al.</i> (1995)	1:1 diluted primary lagoon dairy effluent Initial concentration: 7.3 mg/l	Batch study HRT: 7 days Tank volume: 70 l Plants: water hyacinth and duckweed	Phosphorus removal by water hyacinth and duckweed: 97% and 67%, respectively Maximum uptake by water hyacinth in winter and summer: 59 and 200 mg/m² d, respectively Maximum uptake by duckweed: 20 mg/m² d in both seasons.
Fonkou <i>et al.</i> (2002)	Domestic sewage Initial phosphorus concentration: 47.5 mg/l	HRT: 10 days Total area of 7 ponds: 702 m <sup>2</sup> Depth: 0.5–0.9 m Flow rate: 45 m <sup>3</sup> /d Plant: water lettuce	Phosphorus removal: 73.9%
Sooknah and Wilkie (2004)	Anaerobically digested flushed dairy manure wastewater diluted with tap water 1:1	HRT: 31 days  Tank dimensions L * B * H:  0.5 * 0.36 * 0.4 m  Working depth: 0.3 m  Plants used: water lettuce, pennywort and water hyacinth	Plants failed to grow in undiluted wastewater due to high salinity and high COD Maximum phosphorus removal by water hyacinth, pennywort and water lettuce were 98.5%, 71.3%, and 64.2%, respectively
Kutty et al. (2009)	STP effluent	HRT: 6 days Tank dimensions L * W * D: 90 * 40 * 25 cm Plant: water hyacinth	Total phosphorus removal: 72%
Mohedano et al. (2012)	Swine wastewater Mean initial concentration of Pond 1 and Pond 2: 92 and 10 mg/l, respectively	Pond 1 Pond dimensions: (L * W * D): 20.7 * 7.5 * 0.8 Pond volume: 102 m³ HRT: 102 days Pond 2 Dimensions L * W * D: 15 * 6 * 0.4 Pond volume: 34 m³ HRT: 34 days Plant used: duckweed	Pond 1 and Pond 2 removal rate were 89% and 47%, respectively.
Li et al. (2013)	Livestock wastewater	HRT: 40 days  Tank dimensions L * W * D: 120 * 60 * 60 cm  Working depth: 45 cm  Plants: Elodea nuttallii, Potamogeton pectinatus, and Duckweed	Total phosphorus removal by <i>Elodea nuttallii</i> , <i>Potamogeton pectinatus</i> , and Duckweed: 84.4%, 90.5%, and 95.95%, respectively
Shah et al. (2014)	Municipal wastewater Initial phosphorus concentration: 2.1 mg/l	HRT: 30 days  Tank dimensions L * W * D:  1.52 * 1.83 * 0.91 m  Plants used: water hyacinth, duckweed, and water lettuce	Average phosphorus removal by water hyacinth, duckweed, and water lettuce were 18.76%, 15.25%, and 10.69%, respectively
Mukherjee <i>et al.</i> (2015)	1:1 diluted rice mill effluent	Batch study HRT: 15 days Tank volume: 10 L Depth: 13 cm Diameter: 38 cm Temp: 26–37 °C Plant: water lettuce	Total phosphorus removal: 71–73%
Niveth <i>et al.</i> (2016)	Domestic sewage initial phosphorus concentration: 23.4 mg/l	HRT: 10 days Tank dimensions $L * B * H$ : 1 * 0.3 * 0.3 m Plant: water lettuce	Phosphorus removal: 81.3%
Rezania <i>et al.</i> (2014)	Domestic wastewater Initial phosphate concentration: 0.53Rezania mg/l	HRT: 21Rezania days Tank dimensions $L * B * H$ : $48 \times 90 \times 90$ cm Plant: water hyacinth	Optimum phosphate removal at 15 days: 70%
Victor <i>et al.</i> (2016)	Industrial wastewater initial phosphorus concentration: 39.23 mg/l	HRT: 20 days Tank dimensions: 1.6 m diameter and 0.5 m depth	Phosphorus removal: by water hyacinth and water lettuce were 65% and 50.04%, respectively
Indah <i>et al.</i> (2019)	Treated swine water Of initial total phosphorus concentration: 82.77 mg/l	Batch study Tank water volume: 10 l, surface area: 0.10 m², temp: 25–37 °C, HRT: 21 days Plant used: Eichhornia crassipes, Pistia stratiotes, Limnobium laevigatum, and Lemna sp.	Removal rate by Eichhornia crassipes, Pistia stratiotes, Limnobium laevigatum, and Lemna sp. were 19.05%, 32.34%, 31.13%, and 43.62%, respectively

predicted values and observed values were as high as 0.9463 for TN and 0.9161 for TP. Ergönül et al. (2020) predict the bioaccumulation efficiency of heavy metals by water lettuce. They used LR equation to predict output variable and obtained the coefficient of determination as 0.984. So based on the above discussed studies, it is evident that soft computing is a strong statistical tool in learning the problems related to water and wastewater in the field of



Figure 1. Pictorial view of the experimental setup.

environmental engineering. The use of soft computing in the area of phosphorus removal by free floating aquatic plants is still unexplored. To the best knowledge of authors, there is no such research found in which modeling is used for the prediction of phosphorus removal by free floating aquatic plants. However, some research found on constructed wetlands in which modeling is used to predict phosphorus removal. Hence an effort has been made to predict phosphorus removal by using LR, ANN, and M5P techniques.

#### Materials and methods

#### Study site

These experiments were performed in a rice mill in Kurukshetra district, Haryana, India. Kurukshetra is located at 160 km northward to the national capital, New Delhi. It is located at 29°58′10.2468″N latitude and 76°52′41.8116″E longitude coordinates, and its elevation is 258 m above the mean sea level.

#### Experimental setup and procedure

#### Experimental setup

A batch scale mesocosm study was performed in five identical polyvinyl chloride tubs, with dimensions of 44.25 cm in diameter, 13 cm in depth, 0.1541 m<sup>2</sup> in surface area, and 20 L in volume (Figure 1). The study was conducted from May 1, 2019 to May 15, 2019. During the study, the average high temperature was 38 °C and average low temperature was 22 °C. On May 1, the tanks were filled with rice mill wastewater and tap water in a dilution ratio of 1:1. Of the five tubs, one was used as control in which no aquatic plant was placed, and the other four were planted with salvinia, water lettuce, water hyacinth, and duckweed, respectively. On the same day, water samples were checked for TP, COD, pH, and conductivity (EC). Water samples were monitored daily. When the water level dropped due to evaporation and transpiration, tap water was used to top up the water level in the tubs.

#### Plant collection and sample analysis

Aquatic plants were collected from nearby wetlands and their roots washed with tap water to remove dirt. All the plants were placed in tap water for 5 days to remove soil and other materials deposited on roots. After 5 days, healthy plants were selected, and their dead leaves were chopped off. The selected plants were put in 20% diluted rice mill wastewater for 1 week to acclimatize them to the rice mill wastewater environment. Plants were selected from the 20% diluted water and finally placed in their designated tubs. Out of all plants, 15 plants of water lettuce with a total weight of 200 g, 10 plants of water hyacinth with a total weight of 300 g, 200 g of salvinia and, 50 g of duckweed selected for final experiments. Samples were collected from water tubs by using 50 mL sample bottles to obtain water from three sites; these samples were then mixed. All the parameters were analyzed in triplicate within 4h of the sample collection according to the American Public Health Association (APHA) methods (APHA 2005).

#### Physicochemical characterization of wastewater

Table 2 shows the physiochemical characterization of the effluent collected from the rice mill.

#### Overview of modeling techniques used in this study

#### Artificial neural network

ANN is the most common soft computing technique practicing almost in every stream. This technique is highly complex and nonlinear, based on the human nervous system. It is used to model the complicated relationship between input and output. There are many types of ANN applications, and the selection of the best depends on the nature of the work and the availability of the data. The most commonly used ANN in the field of environmental hydraulics is MLP (Govindaraju 2000). ANN with backpropagation algorithm, composed of three layers: input layer, hidden layer, and output layer and is used in this study. Logistic sigmoid function (Eq. (1)) is used as an activation function.

Table 2. Physicochemical characterization of wastewater.

vater Diluted water
4 6.8
51 1,945
3 34.5
50 1,280
96 –
4 16.2
-
5 –
֡

The activation function used in this study is as follows:

$$f(u_j) = \frac{1}{1 + e^{-u_j}} \tag{1}$$

#### M5P tree model

Model trees were first introduced by Quinlan (1992), then the concept was rebuilt and improved by Wang et al. (1997) into a program known as the M5P. M5P model is the modification and combination of conventional tree with LR at the terminal nodes. In this model, data are divided into subsets and all the subsets make a tree. Out of the many tree structures made with subsets, a tree structure with minimal errors is to be constructed. To eliminate the problem of overfitting, the tree must be pruned back by replacing a sub-tree with a leaf. Thus, the second stage in the design of a model tree involves pruning the overgrown tree and replacing the sub-trees with LR functions.

#### Performance evaluation criteria

To check the effectiveness of ANN and M5P tree modeling, three parameters are used to evaluate the model performance:

- Coefficient of determination  $(R^2)$ .
- Root mean square error (RMSE).
- Mean absolute error (MAE).

All the above parameters are calculated using training and testing data sets. The formula of the above parameters are as follows:

$$R^{2} = \frac{\left(n \sum a_{i} p_{i} - \sum a_{i} \sum p_{i}\right)^{2}}{\left[n \sum (a_{i})^{2} - (\sum a_{i})^{2}\right] \left[n \sum (p_{i})^{2} - (\sum p_{i})^{2}\right]}$$
(2)

RMSE = 
$$\sqrt{\frac{\sum_{i=1}^{n} (p_i - a_i)^2}{n}}$$
 (3)

$$MAE = \frac{\sum_{i=1}^{n} |p_i - a_i|}{n} \tag{4}$$

where a is actual value, p is predicted value, and n is the number of observations.

Table 3. Characteristics of water hyacinth dataset used in this study.

			Train data			Test data			
Parameter	Unit	Min	Max	Mean	St. dev.	Min	Max	Mean	St. dev.
R	-	0.028	0.761	0.377	0.217	0.031	0.772	0.425	0.216
HRT	days	1	13	5.342	3.465	1	14	5.313	3.355
HLR	$m^3/m^2.d$	0.007	0.097	0.033	0.029	0.007	0.097	0.031	0.028
$C_{\text{in}}$	mg/l	3.872	16.2	8.907	3.934	4.115	16.2	10.583	3.95

Table 4. Characteristics of water lettuce dataset used in this study.

			Train data			Test data			
Parameter	Unit	Min	Max	Mean	St. dev.	Min	Max	Mean	St. dev.
R	_	0.06	0.769	0.385	0.189	0.077	0.8	0.44	0.235
HRT	days	1	12	5.123	2.803	1	14	5.813	4.533
HLR	$m^3/m^2 d$	0.008	0.097	0.03	0.026	0.007	0.097	0.037	0.033
$C_{in}$	mg/l	3.502	16.2	8.668	3.799	4.312	16.2	11.104	3.63

Table 5. Correlation matrix among input and output variables (water hyacinth).

	HRT	HLR	$C_{in}$	R
HRT	1			
HLR	-0.77547	1		
$C_{\text{in}}$	0.470793	-0.3158	1	
R	0.89957	-0.77708	0.719158	1

Table 6. Correlation matrix among input and output variables (water lettuce).

	HRT	HLR	C <sub>in</sub>	R
HRT	1			
HLR	-0.77547	1		
$C_{\text{in}}$	0.466329	-0.3139	1	
R	0.946906	-0.81698	0.651836	1

#### Data set for static analysis

A detailed study on rice mill wastewater has been conducted to get credible data. There are about 106 number of data available each for water hyacinth and water lettuce on TP reduction. Both types of data sets are divided into two separate parts: training (70%) and testing (30%). Tables 3 and 4 show the features of training and testing data set of water hyacinth and water lettuce, respectively, in which hydraulic loading rate (HLR), hydraulic retention time (HRT), and initial concentration of total phosphorous (C<sub>in</sub>) are considered as input parameters whereas reduction rate of total phosphorous (R) is considered as output parameter. Tables 5 and 6 show the correlation matrix among input and output variables data for water hyacinth and water lettuce plant, respectively. The original data set is modified according to the requirement of the paper. The reduction rate was calculated as:

$$R = \frac{C_{\rm in} - C_{\rm out}}{C_{\rm in}} \tag{5}$$

 $C_{\rm in}$ , initial concentration of TP and  $C_{\rm out}$ , final concentration

HLR is calculated as:

$$HLR = \frac{V}{A*HRT}$$
 (6)

V, volume of tub (m<sup>3</sup>); A, surface area of tub (m<sup>2</sup>), and HRT is in days.

#### Various mechanisms of phosphorus removal

#### Direct uptake by plants

Phosphorus is a vital macronutrient, which is essential for plant growth. Approximately 1.1-2.2 mg/g (dry weight of plant) of phosphorus is required for normal plant growth (Ready et al. 1999). DIP is easily absorbed by plants, which is stored as polyphosphate in plant biomass. The accumulation of phosphorus is rapid during peak growing seasons. Phosphorus removal from water depends on the harvesting of plants, though most of the phosphorus is released back into the water column after the decomposition of plants. The rate of uptake by plants depends on the type of plant, root and soot ratio, c/p (carbon/phosphorus) ratio of detrital tissue, and physicochemical properties of water.

#### Assimilation by periphyton and microorganism

Periphyton is a biofilm float in an aquatic ecosystem. It consists of algae, cyanobacteria, autotrophic, and heterotrophic microbes, and detritus that are attached to plants. Periphyton uptake phosphorus from the water column for their growth. Periphyton is capable of assimilating both organic and inorganic phosphorus (Ready et al. 1999) and can induce changes in the pH and DO of water. Microorganisms convert organic phosphorus into inorganic phosphorus, which is ultimately uptaken by plants and periphyton.

#### **Adsorption**

Radial oxygen loss (ROL) and the presence of oxidizing bacteria cause iron or manganese plaques (metal hydroxides) formed on the roots surface (Emerson et al. 1999; Batty et al. 2002). Metals like copper and zinc, or phosphorus can be sorbed on these metal hydroxides (Ye et al. 2001).

#### Precipitation as phosphate

Photosynthesis and respiration initiate changes in the pH of water column, which can reach as high as 10. In a system where enough dissolved calcium is present in the water column, phosphorus precipitates as calcium phosphate (Ready et al. 1999). Moreover, periphyton is responsible for changes in the pH of water, which helps in the precipitation of phosphorus with CaCO<sub>3</sub> within the periphyton mat (Ready et al. 1999).

#### Factors affecting the performance of an aquatic treatment system

#### **Temperature**

Temperature plays a crucial role in the performance of the plant. Almost all the pollutants' removal rate is dependent on the temperature of water and air. For removing nutrients from wastewater, plant uptake and microbial activity play major roles. At temperatures below 10 °C, the growth rate of plants decreases and microbes do not function optimally, indicating low removal efficiency. Microorganisms responsible for nutrient removal work optimally at temperatures

more than 15 °C (Akratos and Tsihrintzis 2007). At temperatures below 15 °C, phosphate and TP removal were 50.7% and 41.8%, respectively, whereas at temperatures above 15 °C, the results improved to 79.2% and 70.1%, respectively (Akratos and Tsihrintzis 2007). Studies have shown that the removal rates of the TN and TP by water cress in eutrophic water of 22 °C were higher than that of 10 and 35 °C (Deng and Ni 2013).

#### Type of plant

Selection of proper plant is a key factor which affects water purification capacity of aquatic plant system. It has been shown that the removal rate of yellow flag for TN and TP are 2.82 and 5.31 times than removal rate of canna's, thaliadealbata's and willow herb's (Wu et al. 2012). The studies have shown that the order of TN purification capacities was A. Calamus > M. Korsakowil > L. Sagittifolial. For TP the order of removal was A. Calamus > L. Salicaria > M. Korsakowil > A. Orientale > S. Sagittifolia (Fu and He 2015).

#### Hydraulic retention time

HRT plays a major role in nutrient removal. At retention time of 10 h or less, the removal rate is proportional to the retention time, which was 10-70% for TN removal and 30-80% for TP removal. As the time increased to more than 10 h, the TN removal was 50% and TP removal was 60% (Sato et al. 2002). Akratos and Tsihrintzis (2007) showed that TP removal for HRT for 6 days was 33.6%, and as the HRT increases, the removal rate also increases to 56.3% for 8 days, 76.5% for 14 days, and 88.1% for 20 days. Thus, the removal rate is dependent on HRT.

#### Hydraulic loading rate

HLR plays a major role in pollutant removal. The relationship between loading rate and removal rate is inversely proportional (Sato et al. 2002). For the TN and TP, the loading rate proposed by Sato et al. (2002) for TN and TP are  $0.5-1 \text{ g/m}^2/\text{d}$  and  $0.05-0.15 \text{ g/m}^2/\text{d}$ , respectively. At a low loading rate, the removal rate is proportional to the loading rate, but when it exceeds  $1 \text{ g/m}^2/\text{d}$  for TN and  $0.15 \text{ g/m}^2/\text{d}$ for TP, the removal rate becomes constant.

#### Results and discussions

#### Visual observations

In undiluted wastewater, the growth of all aquatic plants was inhibited possibly because of low oxygen level and high organic matter concentration. The leaves of the plants started turning yellow and finally dried up. Sooknah and Wilkie (2004) and Mukherjee et al. (2015) showed similar results using water lettuce to treat dairy manure wastewater and parboiled rice mill wastewater, respectively. In the second phase, and the wastewater was diluted with tap water in a ratio of 1:1. From Day 2, water started turning black, which may be due to exhaustion of DO in water. The leaves of the water lettuce started turning yellow, possibly owing to

high concentration of COD and low level of DO in water. From Day 3, periphyton started growing in the salvinia tub; water hyacinth showed some progress in this period. From Day 6, periphyton started growing in all the tubs except the control; new leaves began to grow on water lettuce. After Day 9, the growth rate of all plants was fast, except salvinia. Salvinia started decomposing and finally settled down at the bottom, and periphyton was present in all the tubs. The plants covered the entire surface area with vegetation within 15 days. After Day 9, water turned brown and then cleared in all the tubs. This phenomenon might have occurred in the control tub owing to periphyton.

# Plant biomass and relative growth rate (RGR) determination

The production of all the aquatic plants was measured based on their growth rate. The overall process lasted for 15 days. Fresh biomass was measured on the first day, that is, before plantation, and on the last day of the experiment. The RGR was calculated to quantify the production. The RGR is defined as the change in size or mass of a plant with respect to its initial size or mass. In this study, RGRs of all four plants were calculated using the following formula

$$RGR = \frac{\ln W_2 - \ln W_2}{T} \tag{7}$$

 $W_1$ , weight of the biomass of the plant at the beginning of the experiment;  $W_2$ , weight of the biomass of the plant at the end of the experiment; ln, natural log; and T, experiment time.

This formula has been widely used to measure the growth rate of plants (Indah et al. 2019). The initial weight of all plants based on the fresh biomass was measured using a digital balance. After the completion of experiment, the final weight of all the plants based on fresh biomass was measured, and their relative growth was calculated according to the aforementioned formula. Plants predominantly exhibit a positive growth rate; however, in some cases plants may exhibit a negative growth rate. In this study, all the plants exhibited a positive growth rate, except Salvinia. The RGR of Salvinia was  $-0.1386 \,\mathrm{g/g}$  per day. This negative growth is attributed to various reasons such as high conductivity, the best grow conductivity for Salvinia is ranging from 240 to 500 μS/cm (Owens et al. 2014) which is much less than the conductivity of rice mill wastewater used in this experiment. There may be some others factors like high concentration of COD and phosphorus were responsible for this negative growth. Water hyacinth showed the highest RGR of 0.09877 g/g/day, followed by water lettuce with 0.0780 g/g/ day and duckweed with 0.0462 g/g/day growth rates.

#### **Phosphorus reduction**

Phosphorus removal from water column occurs through various mechanisms, which includes uptake by plants, assimilation by periphytons, adsorption, and precipitation. The effectiveness of four aquatic plants for removing

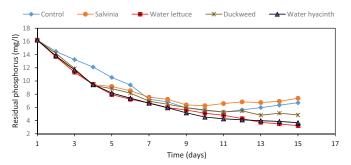


Figure 2. Reduction of total phosphorus by aquatic plants.

phosphorus was tested, and it is evident from the results that these plants are capable of removing phosphorus. Water lettuce showed the maximum efficiency among all the plants, followed by water hyacinth, salvinia, duckweed, and control. Water lettuce showed the maximum removal efficiency of 80.04% on Day 15, followed by water hyacinth (77.2%), salvinia (61.38% on Day 10), duckweed (70.21% on Day 13), and control (67.38% on Day 11). The growth rate of salvinia was negative; the phosphorus removal may have occurred owing to the periphyton present in the salvinia tub. After Day 15, phosphorus concentration in the water started increasing possibly owing to the decomposition of leaves and roots of these plants. Figure 2 shows the details of phosphorus removal by the four aquatic plants. Similar results were obtained by different researchers, Akinbile and Yusoff (2012) performed a study on aquaculture wastewater by using water hyacinth and water lettuce and found that water hyacinth reduces phosphorus by 85%, whereas water lettuce reduces phosphorus by approximately 70% in 3 weeks. Moreover, Mukherjee et al. (2015) performed a laboratory-scale experiment on parboiled rice mill wastewater by using water lettuce in a small container of 38 cm diameter and 10 L capacity and found that phosphorus removal efficiency was 73% within 2 weeks. Similarly, Kutty et al. (2009) used water hyacinth and found the TP removal was 72% in 6 days. Li et al. (2013) used livestock wastewater and found the TP removal by duckweed to be 96% in 40 days. Rezania et al. (2014) used domestic wastewater and found the results to be 70% on Day 15.

#### **COD** reduction

The reduction in COD concentration is presented in Figure 3. COD removal is higher in aquatic plant systems than in a control system. COD removal efficiency was the highest in the water lettuce tub (74.53%), followed by water hyacinth (71.87%), duckweed (62.19%), and salvinia (60%). The least removal was in the control system (50%).

Similar results were obtained by various researchers. Mukherjee *et al.* (2015) performed laboratory-scale experiments on rice mill wastewater by using water lettuce and found COD removal to be 76%. Sooknah and Wilkie (2004) performed an experiment using anaerobically digested flushed dairy manure wastewater diluted with tap water in a 1:1 ratio and floating aquatic plants and found COD reduction of 80% by water hyacinth and water lettuce

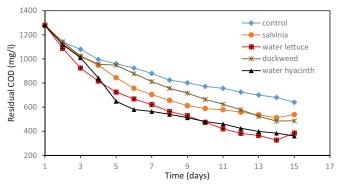


Figure 3. Reduction of COD by aquatic plants.

individually. Akinbile and Yusoff (2012) performed a study on aquaculture wastewater by using water hyacinth and water lettuce and found that COD removal was 59% by water hyacinth and 54% by water lettuce by the end of Week 4.

#### pH and EC variation

The results before and after treatment show an increase in pH value of all the systems. The initial pH of the aquatic plant systems and control system was 6.8. As the experiment progressed, the pH increased. The maximum changes were observed in the control system and the minimum changes in the water hyacinth tub. The pH values went up to 9 in all the tubs, except water hyacinth. Figure 4 shows the pH variation of all the tubs. The increase in pH was due to the photosynthesis of plants and periphyton present in water (National Oceanic and Atmospheric Administration 2019). They took  $\rm CO_2$  from water and expelled oxygen, and as the  $\rm CO_2$  dissolved in water, it becomes carbolic acid, which resulted in low pH. Thus,  $\rm CO_2$  removal increases the pH.

The conductivity remained close to the initial concentration of 1,945  $\mu$ s/cm. Due to high conductivity, the growth of salvinia was inhibited. Sooknah and Wilkie (2004) reported that the conductivity of 2,683  $\mu$ s/cm is toxic for water lettuce and the conductivity of 4,040  $\mu$ s/cm is toxic for water hyacinth. Figure 5 shows the conductivity variation of all tubs.

#### Static analysis

A comparative analysis of the data has been done by using LR, ANN, and M5P tree modeling techniques. The data obtained from two most efficient plants have been considered for this analysis. WEKA 3.8 software was used to derive regression or equation coefficients using training data-set.

#### Results of LR

In case of phosphorus removal by water hyacinth plant, the following equation is the outcome of training data set after applying LR (Eq. (8))

$$R = 0.0337 * HRT - 1.5442 * HLR + 0.0224 * C_{in} + 0.0473$$

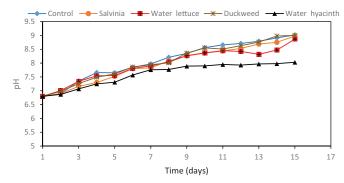


Figure 4. Variation of pH in the water tubs.

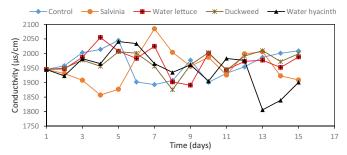


Figure 5. Variation of conductivity in the water tubs.

The parameters used for the performance evaluation are shown in Table 9. The values obtained are:  $R^2 = 0.978$ , RMSE = 0.0448, MAE = 0.0341, and  $R^2 = 0.957$ , RMSE = 0.0624, MAE = 0.0526 for training data set and testing data set, respectively.

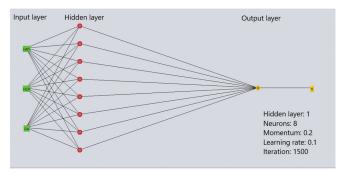
In case of water lettuce plant, the following equation is the outcome of training data set after applying LR (Eq. (9))

$$R = 0.0438 * HRT - 1.4022 * HLR + 0.0138 * Cin + 0.0833$$
(9)

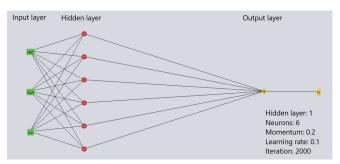
The parameters used for the performance evaluation are shown in Table 10. The values obtained are:  $R^2 = 0.9873$ , RMSE = 0.0298, MAE = 0.0253, and  $R^2 = 0.99$ , RMSE = 0.0428, MAE = 0.0337 for training data set and testing set, respectively. The performance of LR models for both the aquatic plants are shown in Figure 10 (water hyacinth) and Figure 12 (water lettuce).

#### Results of ANN model

ANN is a trial and error method which consist of three main components viz. input layer, hidden layer, and output layer. In this case, ANN is implemented to predict the reduction of TP by two aquatic plants, that is, water hyacinth and water lettuce. The model developed in this study is based on trial and error process for both the plants. All the parameters are checked by varying their values. The models with maximum coefficient of determination ( $R^2$ ) and least errors (MAE and RMSE) are selected. In case of water hyacinth, one hidden layer with eight neurons closely predicted the results to the actual values of reduction in TP (Figure 6). Other parameters used in this model: momentum = 0.2, learning rate = 0.1, and iteration = 1,500. In case of



**Figure 6.** Details of water hyacinth ANN model (input layer, hidden layer, and output layer).



**Figure 7.** Details of water lettuce ANN model (input layer, hidden layer, and output layer).

water lettuce, best results are obtained by using one hidden layer with six neurons having momentum = 0.2, learning rate = 0.1, and iteration = 2,000 (Figure 7). The performance of ANN models for both aquatic plants is shown in Figure 10 (water hyacinth) and Figure 12 (water lettuce). As shown in Tables 9 and 10, the values of  $R^2$ , RMSE, and MAE of ANN models are 0.9945, 0.0243, and 0.02 for water hyacinth, and 0.9962, 0.0201, and 0.014 for water lettuce, respectively for test data set. The results of ANN model show that the use of ANN model is suitable to predict the reduction of phosphorus by aquatic plants from wastewater.

Developing the ANN model is a trial and error process similar to developing GP, SVM, M5P, and RF models, based on the same dataset. Design of ANN includes the number of hidden layers, neurons in hidden layers, momentum, learning rate, and iterations.

#### Results of M5P tree model

M5P model is the modification and combination of conventional tree with LR at the terminal nodes. In this model, data are divided in subsets and all the subsets make a tree. Out of lot of trees made with subsets, the tree with minimum errors is to be constructed. The optimum results were obtained by setting the value of parameter M (instances) to 6.0. Tables 9 and 10 show the statistical evaluation parameter of the models for both water hyacinth and water lettuce, respectively. It is clear from Figures 10 and 12 that the prediction of phosphorus removal from water by both aquatic plants is close to the actual value. The performance evaluation parameters obtained from these models are  $R^2$  values (0.9758 and 0.9868), MAE values (0.0415 and 0.0368), and

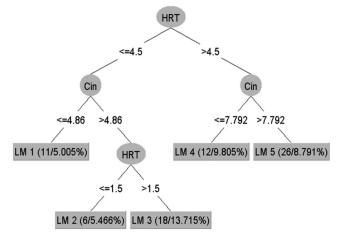
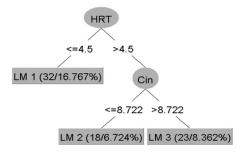


Figure 8. Diagram of M5P model with different conditions and rules (water hyacinth) (number of rules: 5).

**Table 7.** Pruned model tree equations for different conditions (water hyacinth).

Tree rule	Equation
LM num: 1	$R = 0.0522 * HRT + 0.0149 * C_{in} - 0.0609$
LM num: 2	$R = 0.0566 * HRT + 0.0164 * C_{in} - 0.05$
LM num: 3	$R = 0.0623 * HRT + 0.0189 * C_{in} - 0.0662$
LM num: 4	$R = 0.025 * HRT + 0.0467 * C_{in} - 0.0757$
LM num: 5	$R = 0.0331 * HRT + 0.0202 * C_{in} + 0.0678$



**Figure 9.** Diagram of M5P model with different conditions and rules (water lettuce) (number of rules: 3).

Table 8. Pruned model tree equations for different conditions (water lettuce).

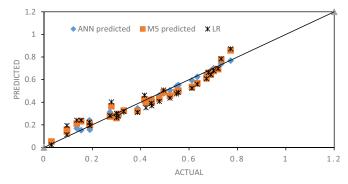
Tree rule	Equation
LM num: 1	$R = 0.0689 * HRT + 0.0113 * C_{in} - 0.0371$
LM num: 2	$R = 0.0488 * HRT + 0.0058 * C_{in} + 0.0832$
LM num: 3	$R = 0.0412 * HRT + 0.0149 * C_{in} + 0.0776$

RMSE values (0.0486 and 0.0455) in testing period for water hyacinth and water lettuce, respectively. When M5P model tree is pruned to get the results using smoothed linear models, there are five conditional linear equations (Figure 8 and Table 7) best fit for water hyacinth and three conditional equations best fit for water lettuce (Figure 9 and Table 8) developed, respectively.

#### **Comparison of models**

Figures 10 and 12 show a comparison of the modeling techniques used to predict phosphorous reduction from rice mill wastewater by water hyacinth and water lettuce plants, respectively. A LR relation is formed with the training data and the results are compared with the machine learning





**Figure 10.** Scattered diagram of actual and predicted phosphorus reduction by water hyacinth using LR, ANN, and M5P tree model in testing dataset.

Table 9. Performance of models with water hyacinth plant.

		Training data			Testing data			
Model	$R^2$	MAE	RMSE	$R^2$	MAE	RMSE		
ANN	0.995	0.0159	0.0214	0.994	0.0200	0.0243		
MP5	0.987	0.0256	0.0337	0.975	0.0415	0.0486		
Linear regression	0.978	0.0341	0.0448	0.957	0.0526	0.0624		

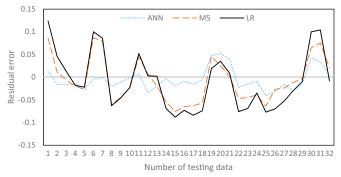
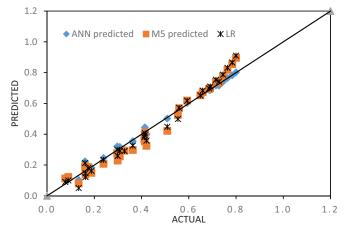


Figure 11. Residual error versus test data-set number using different regression approaches (water hyacinth).

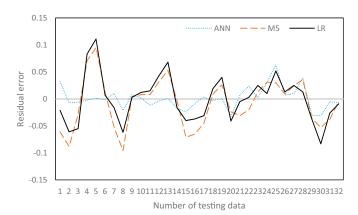
based ANN and M5P tree methods. The results of training and testing data with the LR model suggest that the models work fairly well in prediction of phosphorus reduction observed with water hyacinth and water lettuce plant. The RMSE values observed during testing with LR equations in estimating the phosphorous reduction using water hyacinth plant and water lettuce plant are 0.0624 ( $R^2 = 0.957$ ) and 0.0428 ( $R^2 = 0.99$ ), respectively, which is fairly acceptable. However interestingly, in case of the data observed with water hyacinth plant, the prediction of phosphorous reduction is relatively superior with machine learning based models (ANN and M5 tree) as compared to the LR model. The error values (RMSE and MAE) observed with ANN and M5 tree models are quite less in the training as well as in testing stage (Table 9). The estimated data points lie in the closer proximity of the perfect prediction line (x = y). The modeling results obtained from the inter-comparison of ANN and M5 tree models suggest that the prediction of phosphorous reduction (water hyacinth) with ANN is significantly better than that of the M5 tree model. The RMSE and MAE achieved with ANN are 0.0243 and 0.0200, respectively, while from M5P tree are 0.0486 and 0.0415, respectively. Elucidated from Figure 11, the residual errors from experimental and predicted values are minimal in the case of ANN as the error line is relatively closer to the zero error

Table 10. Performance of models with water lettuce plant.

	T	Training data			Testing data		
Model	$R^2$	MAE	RMSE	$R^2$	MAE	RMSE	
ANN	0.9946	0.0163	0.0204	0.9962	0.0140	0.0201	
M5	0.9906	0.0203	0.0257	0.9868	0.0368	0.0455	
Linear regression	0.9873	0.0253	0.0298	0.9900	0.0337	0.0428	



**Figure 12.** Scattered diagram of actual and predicted phosphorus reduction by water lettuce using LR, ANN, and M5P tree model in testing dataset.



**Figure 13.** Residual error versus test data-set number using different regression approaches (water lettuce).

line or base line as compared to M5 and LR. Thus, the overall modeling comparison shows that ANN is performing better, followed by the M5 and LR models, respectively. So this tool can be effectively employed in predicting the actual phosphorous reduction.

In the same way, all three modeling techniques are used to estimate the phosphorous reduction from water lettuce plant. The selection of input attributes is similar to water hyacinth plant. The LR equation developed using the training data is checked for accuracy on the testing data and the results are compared with ANN and M5P tree model. The performance of LR model is inferior in training as compared to M5P model but slightly improves during testing (Table 10). However, the comparison of both models (LR and M5P) with ANN specifies the strong performance of the ANN model in generalizing the data on phosphorous reduction. Figure 12 reveals that the predicted LR and M5P data points are much more scattered as compared to ANN data points. Based on the test results (Table 10), the error values are minimal with the ANN (RMSE = 0.0201, MAE =



Table 11. Summary of the modeling used in phosphorus and heavy metal removal.

Reference	System	Model	Input variables	Output variable	Performance
Thisstudy	Water hyacinth	Linear regression, ANN, M5P	Hydraulic retention time (HRT), hydraulic loading rate (HLR), and initial concentration of phosphorus (C <sub>in</sub> )	Phosphorus reduction (R)	RMS ERror LR: 0.0624 ANN: 0.0243 M5P: 0.0486
This study	Water lettuce	Linear regression, ANN, M5P	Hydraulic retention time (HRT), hydraulic loading rate (HLR), and initial concentration of phosphorus (C <sub>in</sub> )	Phosphorus reduction (R)	RMS error LR: 0.0428 ANN: 0.0201 M5P: 0.0455
Zhang et al. (2008)	Integrated constructed wetlands (ICWs)	Self-organizing map (SOM)	DO and conductivity	Soluble reactive phosphorus	R <sup>2</sup> : 0.951 MAE scaled: 0.048
El-Gendy (2008)	Water hyacinth	Exponential and linear regression	Initial concentration, time	Heavy metal removal	R <sup>2</sup> : 0.996 Average absolute deviation: 1.7%
Zhang and Pan (2014)	Column phosphate removal by hydrated ferric oxide-based nanocomposite	Back propagation ANN- genetic algorithm	Initial pH, sulfate concentration, operation temperature and adsorbent dosage	Phosphate removal efficiency	R <sup>2</sup> : 0.9931 MAE: 3.4
Bagheri <i>et al.</i> (2015)	Sequencing batch reactor	Multilayer perceptron (MLP)	Influent concentration (C <sub>in</sub> ), filling time (FT), reaction time, aeration intensity, sludge residence time (SRT), and MLVSS	Total phosphorus (mg/l)	R <sup>2</sup> : 0.99 RMSE: 0.15 mg/l
Bagheri <i>et al</i> . (2015)	Sequencing batch reactor	Radial basis function (RBF)	Influent concentration $(C_{\rm in})$ , filling time (FT), reaction time, aeration intensity, sludge residence time (SRT), and MLVSS	Total phosphorus (mg/l)	R <sup>2</sup> : 0.95 RMSE: 0.19 mg/l
Ozengin <i>et al.</i> (2016)	Subsurface horizontal flow constructed wetland planted with P. australis Phragmites australis	MLP feed forward and Levenberg–Marquardt back-propagation architecture	HRT, dissolved oxygen (DO), pH, temperature (T), NH4–N, NO3, NO2, ortho- phosphate (OP)	Total phosphorus	R <sup>2</sup> : 0.91 MAE: 0.14
Ergönül et al. (2020)	Water lettuce	Linear regression	Exposure duration	Bioaccumulation efficiency of heavy metals	R <sup>2</sup> : 0.984

0.0140). ANN model shows stronger approximations of the phosphorous reduction followed by the LR and the M5P tree, respectively. Elucidated from Figure 13, the residual errors from experimental and predicted values are minimal in the case of ANN as the error line is relatively closer to the zero error line or base line as compared to LR and M5P.

In reviewing the previous studies, few studies have been found that use machine learning to predict the removal of phosphorus from wastewater. Most of the studies are related to the constructed wetlands which utilized machine learning based methods. Although, some studies found on free floating aquatic plants pertaining to the removal of heavy metals but the application of machine learning is yet to be documented for the case of phosphorous removal from water by free floating plants. The authors, however, found some research papers on prediction of phosphorus reduction from other treatment processes, as well as the prediction of heavy metal removal by free floating plants using machine learning based regression models. The results of those existing studies are therefore incorporated into this work and summarized in a table along with the results of the current study (Table 11).

maximum efficiency as it reduced 80.04% of the phosphorus and 74.53% of COD from the water within 15 days. Water hyacinth showed the highest RGR of biomass (0.09877 g/g/ d), whereas salvinia showed a negative growth rate. Thus, aquatic plants have the potential to remove nutrients and organic matter from water. A statistical comparison between two modeling techniques with reference to LR has been done. ANN model predicted better results as compare to M5P tree and LR. In case of water hyacinth, the RMSE is 0.0243, 0.0486, and 0.0624 for ANN model, M5P, and LR model, respectively. In case of water lettuce, the RMSE is 0.0201, 0.0455, and 0.0428 for ANN model, M5P and LR model, respectively. So it can be concluding from the results that ANN model is better than M5P and LR. After a period of 15 days, the phosphorus content of the water started increasing due to the falling of leaves. Hence, it is advisable that plants must be harvested after15 days. Although this mesocosm study was successful but to verify the success and feasibility of the system, pilot-scale, and full-scale field studies are required.

#### **Conclusion**

The growth of all aquatic plants was found to be inhibited in undiluted raw rice mill wastewater. A dilution approach of mixing tap water with the wastewater in 1:1 ratio was adopted. Among the plants, water lettuce showed the

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#### **Disclosure statement**

The authors declare that there is no conflict of interests regarding the publication of this manuscript

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