Eco-physiological characteristics of *Pistia stratiotes* and its removal of pollutants from livestock wastewater

Jinfa Chen, Qishan Nie, Yun Zhang, Jinzhao Hu and Lin Qing

ABSTRACT

The effectiveness of water lettuce in removing pollutants including organic pollutants, nitrogen (NH_3-N) and total phosphorus (TP) from livestock wastewater along with the physiological effects and their correlations, was studied for the first time. The results showed that the water lettuce had higher removal efficiency with low concentrations of livestock wastewater. The removal efficiency of water lettuce for different initial concentrations of livestock wastewater within 8 d was as follows: chemical oxygen demand (CODcr) (68–82%) > NH_3-N (57–69%) >TP (27–45%). The speed of purification of water lettuce for CODcr, NH_3-N and TP conformed to first order kinetics equations. The water quality indices CODcr, NH_3-N and TP had a higher linear correlation with peroxidase (POD) activity ($R^2 > 0.9^2$) than with superoxide dismutase (SOD) and catalase (CAT) activity, which indicates that the main reacting enzyme of water lettuce under high CODcr stress is peroxidase.

Key words | kinetic equation, livestock wastewater, POD, water lettuce

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INTRODUCTION

Livestock wastewater falls into the category of wastewater with a high concentration of organic matter, nitrogen and phosphorus. The severity of pollution caused by livestock wastewater is a matter of concern in many countries (Ren *et al.* 2010). In China, wastewater generated by intensive farms is commonly stored in anaerobic lagoons and partially treated, and is then sprayed onto the nearby crop fields. Excessive manure loading can cause environmental problems such as odor, greenhouse gas emissions and pollutant accumulation in the soil (He *et al.* 2006; Shen *et al.* 2008).

During the past decade, wetlands have been increasingly constructed worldwide as a low-cost environmentally friendly means of removing pollutants from domestic and sewage effluents (Lee *et al.* 2010). Several studies have explored their role in treating wastewater discharged from poultry or livestock farms (Hunt & Poach 2001; Lu *et al.* 2008). Aquatic plants play an important role in the treatment process. The ideal plant species to remediate a heavily contaminated site would be a rapidly growing, high biomass crop with an extensive root system that can both tolerate and accumulate the contaminants. Floating macrophytes such as water hyacinths (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes* L.) and water fern (*Salvinia sp.*) have been widely studied due to their ability to adsorb contaminants in water, easy manual manipulation and rapid growth, and their consequent use in wetlands constructed for wastewater treatment. *Pistia stratiotes* L., commonly known as water cabbage as well as water lettuce, is a perennial free-floating invasive weed, originally from Florida and Texas and now widespread in ponds in the warmer parts of the world (Sooknah & Wilkie 2004; Zimmels *et al.* 2006). It has been widely reported as a good bio-indicator of pollution and a potential candidate plant for bioremediation purposes (Akapo *et al.* 2011).

Water lettuce (*Pistia stratiotes* L.) has been shown to have great potential as a biological filter for adsorbing pollutants from wastewater. Odjegba & Fasidi (2004) reported that *Pistia stratiotes* is a potential candidate for the removal of Zn, Cr, Cu, Cd, Pb, Ag and Hg. The plant could be used to treat water containing low concentrations of these elements (Odjegba & Fasidi 2004). Further work on *Pistia stratiotes* as a means for adsorbing metals showed it to have good resistance to high lead-ion concentration during a long cultivation experiment and good removal capacity for the chosen types of metal (Espinoza-Quiñones *et al.* 2009). It also has considerable ability to accumulate Cd (Li *et al.* 2013). *Pistia stratiotes* also proved to have great potential for adsorbing non-metal

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pollutants. It has been proven that water lettuce has great potential for removing N and P from eutrophic stormwaters and improving other water quality properties (Lu *et al.* 2010). Both the leaves and the roots of *Pistia stratiotes* have a high capacity for removing and adsorbing crude oil from saline solutions (Sánchez-Galván *et al.* 2013).

In this experiment, three main objectives were pursued: (1) to evaluate the effectiveness of *Pistia stratiotes* in removing organic pollutants, N and P from different concentrations of livestock wastewater under simulated natural conditions; (2) to understand the cleaning process by analyzing the removal half-life and decay rate coefficient; (3) to study the mechanisms by which *Pistia stratiotes* cleans up livestock water pollutants (organic pollutants, N and P) by using correlation analysis between water quality indices and physiological properties of the plant.

MATERIALS AND METHODS

Chemicals and water samples

All chemicals used were of analytical grade. Livestock wastewater was collected from dairies located in Xichang city and was diluted to required concentrations with distilled water. The concentration of chemical oxygen demand (CODcr), NH_3-N, and total phosphorus (TP) in raw livestock wastewater was 4,988–8,560 mg/L, 312.78–509.74 mg/L and 117.39–188.40 mg/L respectively.

Plants and experimental design

Pistia stratiotes plants of similar sizes were obtained from outdoor ponds in the suburbs of Xichang City in mid-December. The plants were thoroughly washed with tap and double distilled water. They were then acclimatized in plastic basins with tap water indoors. After 7 days, healthy *Pistia stratiotes* specimens with abundant roots were selected and planted into eight plastic containers with 600–700 g biomass per container. Plants in treated groups were treated with diluted livestock wastewater with concentrations of about 500, 1,000, 1,500 and 2,000 mg/L, and were marked A, B, C and D respectively, corresponding to low to high CODcr concentrations. The concentrations of NH_3-N and TP in the four treated groups were determined by the dilute ratio by which the CODcr in the raw wastewater was diluted to the desired concentrations. The indoor air temperature was between 282 and 284 K; the water temperature in the plastic containers was between 289.5 and 292 K. Water quality indices CODcr, NH_3-N and TP, and physiological properties of the plants-superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity - were measured at the end of each time interval (2 days). The testing period was 8 days.

Chemical analyses

SOD activity was assayed according to the method described by Beauchamp and Fridovich (Beauchamp & Fridovich 1971). The activity was determined by calculating the inhibition effect of nitro-blue tetrazolium (NBT) photochemical reduction. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, 2 μM riboflavin and 60 μL enzyme extract. Riboflavin was prepared with deionized water. The rest of the reagents above were prepared with phosphate buffer (0.05 M, pH 7.8). The absorbance was then measured at 560 nm by a spectrophotometer. An active SOD unit was defined as the quantity of SOD required to result in a 50% inhibition effect of the reduction of NBT.

CAT activity was measured according to the method described by Aebi (Aebi 1974). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 30 mM H_2O_2 and 30 μL enzyme extract. The decrease in absorbance at 240 nm was measured. An active CAT unit was defined as the amount of enzyme required to decompose 1 nmol H_2O_2 per min. at a kinetic removal rate of 2.4.

POD activity was measured according to Kraus and Fletcher (Kraus & Fletcher 1994). The reaction mixture was 2.5 mL 50 mM potassium phosphate buffer (pH 6.1), 0.1 mL 3% H_2O_2, 0.2 mL 1% guaiacol and 30 μL enzyme extract. A unit of POD was defined as the amount of enzyme required to decompose 1 μmol guaiacol per min.

CODcr, NH_3-N and TP concentrations were determined according to the protocols in *Standard Methods for the Examination of Water and Wastewater* (National Environmental Protection Agency 2002).

Decay rate coefficient and half-life period equation

The first-order kinetics equation is:

$$C = C_0 \exp(-kt) \tag{1}$$

The relation between reaction rate and reactant concentration is linear in the first-order kinetics equation. Many studies indicate that the equation can be used for COD, NH_3-N and TP removal in constructed wetlands (Chen &

Cao 2007; Suwasa et al. 2009). The decay rate coefficients k (d^{-1}) for CODcr, NH_3-N and TP were calculated based on observed mass removal rates in the tanks using the following expression:

$$k = t(\ln C_0 - \ln C) \quad (2)$$

The half-life period equation is:

$$T_{1/2} = \frac{\ln 2}{k} \quad (3)$$

$T_{1/2}$ is defined as the time to reduce half of the nutrient concentration in aqueous solution, which reflects the reduction rate for nutrient material. It is a turning point for nutrients reduced in aqueous solution.

C_0 is the initial concentration, mg/L; C is the concentration at time t, mg/L; t is the reaction time, d; k is the decay rate coefficient, d^{-1}; $T_{1/2}$ is the half-life period.

Statistical analysis

Mean and standard deviation values of POD, SOD and CAT triplicates were calculated. The results for the different initial concentrations were regression calculated using Origin 7.5 and were generated to a linear equation.

RESULTS AND DISCUSSION

Removal efficiency of *Pistia stratiotes* for CODcr in wastewater

The CODcr removal efficiency data for different initial concentrations are shown in Figure 1.

Pistia stratiotes showed a high purifying capacity for organic pollutants in livestock wastewater, especially for treated groups A, B and C where the lowest removal efficiency was above 70%. For treated group D, however, the removal rate was about 44%. The CODcr removal rate in control groups was stable at 30%.

The decay rate coefficient and the half-life period equation results for CODcr removal are shown in Table 1. All data from treated groups showed high correlativity through first-order kinetic equation calculation, with the correlation coefficient R^2 higher than 0.92.

There was a big gap of about 10 days between groups A and a, B and b and C and c, which may indicate that the

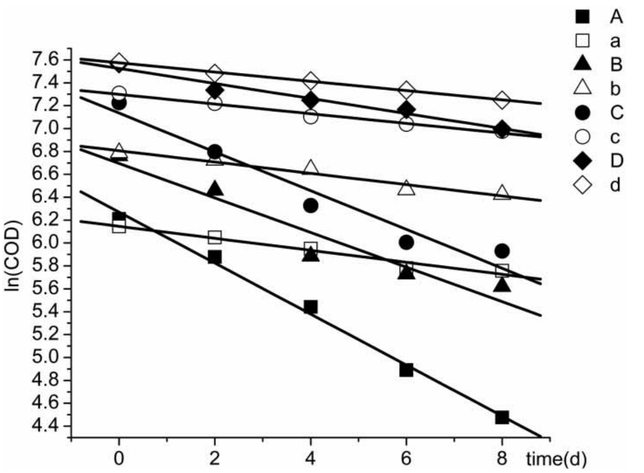

Figure 1 | Variation of CODcr with time in 30 L different initial livestock wastewater concentrations over 8 days (A, B, C, D treatment with 600–700 g *Pistia stratiotes* biomass; a, b, c, d with no plants).

Table 1 | CODcr reduction in different initial livestock wastewater concentrations over 8 days: reduction rate constant and half-life time

Group	Decay rate coefficient (d^{-1}) k	Half-life period (d) $T_{1/2}$	Correlation coefficient R^2	C_0 (mg/L)
A	0.2227	3.1	0.9938	498
a	0.0524	13.2	0.9667	467
B	0.1507	4.5	0.9262	864
b	0.0494	14.0	0.9602	888
C	0.1693	4.1	0.9501	1,376
c	0.0422	16.4	0.9840	1,493
D	0.0656	10.5	0.9638	1,933
d	0.0404	17.2	0.9967	1,960

consumption effect of the plants plays an important role in organic pollutant removal. But when the initial concentration was increased to a higher concentration, the half-life period increased sharply from about 4 to 10.5 d and the gap between D and d groups was reduced to 6.7 d. The decay rate coefficient k decreased greatly when C_0 increased from 1,376 to 1,933 mg/L. This variation may be attributed to the fact that the organic pollutant adsorbent effect of the plants was inhibited greatly at C_0 higher than 1,376 mg/L.

Removal efficiency of *Pistia stratiotes* for NH_3-N in wastewater

The purifying effect of *Pistia stratiotes* on NH_3-N in different initial concentrations of livestock wastewater is shown in

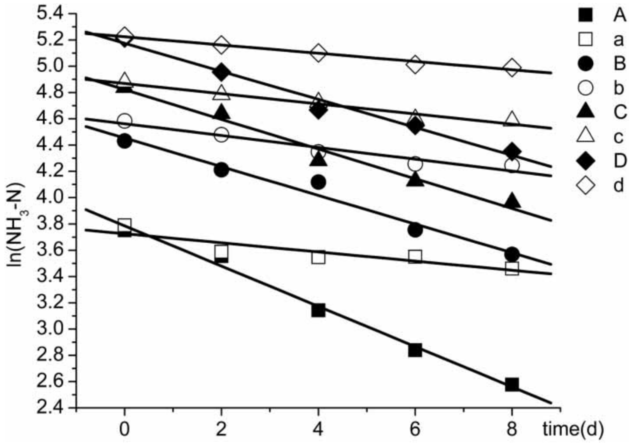

Figure 2 | Variation of NH_3-N with time in 30 L different initial livestock wastewater concentrations over 8 days (A, B, C, D treatment with 600–700 g *Pistia stratiotes* biomass; a, b, c, d with no plants).

Figure 2. The NH_3-N removal rates in treated groups were between 57 and 69%. The highest removal rate reached 69%, which was for the lowest initial concentration of NH_3-N. In treated groups B, C and D, the removal rates were stable around 58%.

The results of regression analysis demonstrated that all NH_3-N removal data in treated groups showed high correlativity in the first-order kinetic equation, with the correlation coefficient R^2 higher than 0.95 in treated groups. The decay rate coefficient of treated group A was higher than B, C and D which remained at around 0.11. The half-life period of treated group A was 4.5 d, lower than B, C and D which remained stable at between 6.1 and 6.6 d. For the control groups, the half-life periods were more than 15 d, distinctly higher than those for treated groups. Ammonia can be removed by nitrification, denitrification and volatilization at pH values of 9.0 and above, and plant consumption (Jing & Lin 2004; Lee *et al*. 2013). The consumption effect of *Pistia stratiotes* plays an important role in ammonia removal and accounts for the great difference between treated groups and control groups, especially in high concentration wastewater (Table 2).

The reduction rate of treated group A was higher than other treated groups, but remained constant when C_0 was increased from 84.09 to 184.1 mg/L. This phenomenon is normally related to the fact that increased COD levels may decrease nitrification rates in wetlands due to competition for available dissolved oxygen, and that with the increase of CODcr and NH_3-N concentrations in treated groups A to B the available dissolved oxygen was already in short supply.

Table 2 | NH_3-N reduction in different initial livestock wastewater concentration over 8 days: reduction rate constant and half-life time

Group	Decay rate coefficient (d^{-1}) k	Half-life period (d) $T_{1/2}$	Correlation coefficient R^2	C_0 (mg/L)
A	0.15322	4.5	0.9905	42.74
a	0.03475	19.9	0.8064	44.22
B	0.10915	6.3	0.9727	84.09
b	0.04509	15.4	0.9435	97.98
C	0.11299	6.1	0.9762	126.19
c	0.03870	17.9	0.9611	131.45
D	0.10663	6.5	0.9801	184.1
d	0.03135	22.1	0.9791	186.69

Removal efficiency of *Pistia stratiotes* for TP in wastewater

The removal rate of total P in different livestock wastewater concentrations was lower than that for COD and NH_3-N, ranging from 18 to 46% (see Figure 3). The removal gap between treated groups and control groups was also smaller than that for COD and ammonia. This can be explained by the fact that substrates for phosphorus adsorption and deposition capacity are more effective than the absorption and transportation effect of phosphate by plants (Zheng *et al*. 2013). The removal rate of TP in treated groups was strongly related through first-order kinetic equation calculation ($R^2 > 0.93$) (see Table 3).

The decay rate coefficient k of treated groups was higher than that of control groups especially at low initial TP concentrations. This demonstrates that plant consumption is an

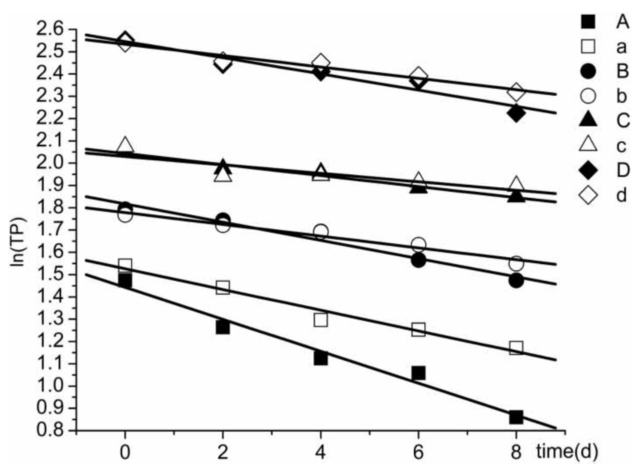

Figure 3 | Variation of TP with time in 30 L different initial livestock wastewater concentrations over 8 days (A, B, C, D treatment with 600–700 g *Pistia stratiotes* biomass; a, b, c, d with no plants).

Table 3 | TP reduction in different initial livestock wastewater concentration over 8 days: reduction rate constant and half-life time

Group	Decay rate coefficient (d^{-1}) k	Half-life period (d) $T_{1/2}$	Correlation coefficient R^2	C_0 (mg/L)
A	0.07166	9.7	0.9743	4.37
a	0.04647	14.9	0.9720	4.67
B	0.04081	17.0	0.9637	6.01
b	0.02636	26.3	0.9661	5.86
C	0.02473	28.0	0.9743	7.79
c	0.01896	36.6	0.7436	7.96
D	0.03641	19.0	0.9358	12.83
d	0.02550	27.2	0.9528	12.69

important mechanism of nutrient removal from wastewater. The half-life periods of treated groups and control groups increased with initial concentrations of C_0 ranging from 4.37 to 7.79 mg/L, but decreased at higher initial concentrations of C_0 ranging from 7.79 to 12.83 mg/L. The variation may be attributed to the precipitation function strengthening at a high initial concentration (Mufarrege *et al.* 2010).

Although the NH_3-N and TP removal rates for *Pistia stratiotes* in high-concentration wastewater were not as high as expected, they were substantially significant in low-concentration wastewater.

Variation in the activity of SOD, CAT and POD in leaves of *Pistia stratiotes*

Antioxidant enzyme systems in plants such as SOD, CAT and POD can be effective in removing excessive activated oxygen induced by adverse stress. They can help to maintain the activated oxygen metabolism balance and membrane structure (Dazy *et al.* 2009; Hasan *et al.* 2009; Sinha *et al.* 2009). However this protection is limited, and when the stress is increased or is endured for a long time, the defense system of plants will weaken as the stress goes beyond the plant's tolerance limit. The outcome is inhibited growth, destruction of the structure of the cell membranes and the enzyme system, accumulation of toxic materials and even death (Li *et al.* 2005; Tewari *et al.* 2006). Variations in the activity of different enzymes in the leaves of *Pistia stratiotes* are shown in Figures 4–6.

The main path to clear high concentrations of H_2O_2 from the plant body is CAT, which is maintained at a low level. In this experiment CAT fluctuated irregularly with time, but the variation range was small (from 0.1459–0.2195 U/g). CAT in all plants of the treated groups decreased after 8 d, which

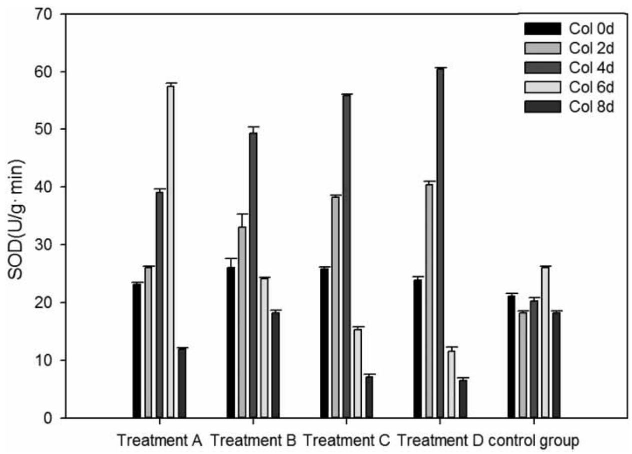

Figure 4 | Effect of different concentration of livestock waster on the activities of SOD in *Pistia stratiotes* leaves over 8 days (plants in control groups were cultivated in tap water).

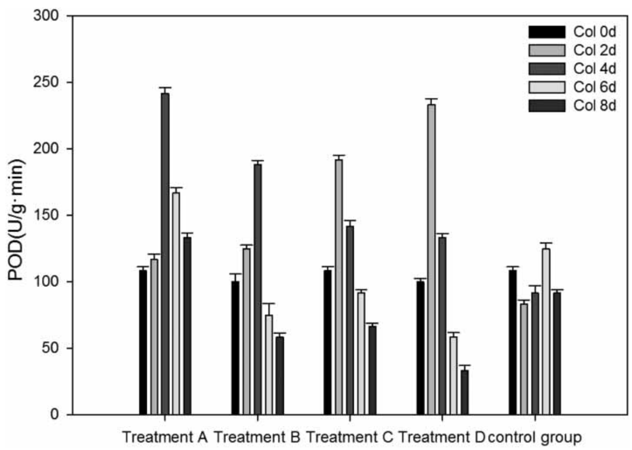

Figure 5 | Effect of different concentration of livestock waster on the activities of POD in *Pistia stratiotes* leaves over 8 days (plants in control groups were cultivated in tap water).

demonstrated that the tolerance of CAT in *Pistia stratiotes* to livestock wastewater was low; the decreased levels of enzyme activity could not maintain the system balance. SOD is the main antioxidant enzyme for clearing oxygen radicals such as superoxide radical O^{2-} and produce H_2O_2; POD can clear low concentrations of H_2O_2 from the plant body, protecting the metabolic balance of the plant. All SOD and POD in *Pistia stratiotes* leaves increased at first, but then decreased by stages under the stress of different initial concentrations of livestock wastewater. POD and SOD reached their lowest levels after 8 d, at 65.55, 100, 39.08 and 35.66% respectively for POD and 130.00, 70.00, 80.01 and 40.01% respectively for POD in each of the control groups. SOD and POD in *Pistia stratiotes* leaves showed a strong oxidative

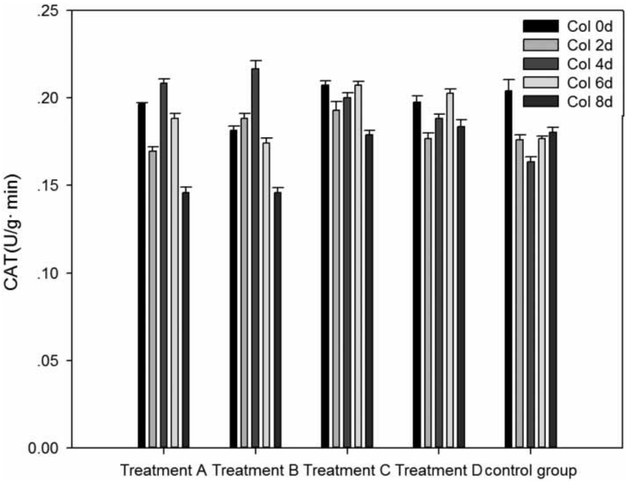

Figure 6 | Effect of different concentration of livestock waster on the activities of CAT in *Pistia stratiotes* leaves over 8 days (plants in control groups were cultivated in tap water).

stress reaction in livestock wastewater. The change trends correspond to the typical behavior of plants under stress: when stress begins they increase their resistance to adapt to the adverse environment, but when the stress exceeds their limits of endurance their defensive ability weakens accordingly until death ensues (Xu *et al.* 2011).

Correlation analysis between water quality indices and physiological indices of the plant

The correlations between the three water quality indices COD, NH_3-N TP and the physiological indices of plant POD, SOD and CAT were analyzed (see Table 4). The results revealed a highly significant difference ($P < 0.01$) in treated group D, a significant difference ($0.01 < P < 0.05$) in the COD of treated groups A, B and C, and a highly significant difference ($P < 0.01$) in the NH_3-N and TP of treated groups A, B and C. Concentrations of the three water quality indices were highly correlated linearly with POD ($R^2 > 0.9$), which demonstrated that the main reaction enzyme in *Pistia stratiotes* leaves in livestock wastewater was peroxidase. The correlation coefficient R^2 between COD, NH_3-N and TP and SOD showed an increase at first followed by a decrease, which reached its maximum in treated group B ($R^2 > 0.9$). This demonstrates that SOD is more effective at lower concentrations with increased pollutant concentration. SOD enzyme activity can help to maintain the balance of activated oxygen in the metabolism of *Pistia stratiotes* at low pollutant concentrations, but higher concentrations will destroy the relativities. The correlations of COD and CAT were low for all treated groups, which indicate that the organic pollutants did not promote the accumulation of H_2O_2. The increase of NH_3-N and TP at low concentrations promoted the accumulation of H_2O_2 in *Pistia stratiotes*, since the R^2 between NH_3-N and TP and CAT increased sharply in treated groups A to B. However, higher concentrations of NH_3-N and TP destroyed the correlations sharply.

Comparison with the effect of other wetland plants in removing pollutants from livestock wastewater

Some other wetland plants have been used in the removal of pollutants from livestock wastewater. A comparison between the results of this study and other relevant research is shown in Table 5.

Table 4 | Significant and correlation coefficients between water quality indices and physiological indices of the plant

		Treated group A	Treated group B	Treated group C	Treated group D
Significant coefficient P		0.02526	0.01256	0.01825	0.00049
Correlation coefficient R^2	COD/POD	0.99607	0.91409	0.98895	0.99724
	COD/SOD	0.13666	0.94539	0.54481	0.61686
	COD/CAT	0.28018	0.3155	0.2193	−1.15685E-13
Significant coefficient P		0.00906	0.00262	0.00371	0.00311
Correlation coefficient R^2	NH_3-N/POD	0.9944	0.91502	0.99705	0.98359
	NH_3-N/SOD	0.13666	0.94678	0.88103	0.57071
	NH_3-N/CAT	0.3142	0.83374	0.21924	−1.15907E-13
Significant coefficient P		0.00066	0.00006	0.00001	4.00000E-05
Correlation coefficient R^2	TP/POD	0.97242	0.94827	0.96813	0.99998
	TP/SOD	0.13665	0.97132	0.85105	0.66018
	TP/CAT	0.4236	0.9292	0.35589	−1.16E-13

Notes: $0.01 < p < 0.05$ significant difference, $p < 0.01$ highly significant difference, 0–0.19 very low correlation, 0.2–0.39 low correlation, 0.4–0.69 medium correlation, 0.7–0.89 high correlation, 0.9–1.0 very high correlation.

Table 5 | Comparison of the effectiveness of different wetland plants in removing pollutants from livestock wastewater

		Initial concentration (mg/L)			Removal rate (%)			
Plant system	**Treatment time**	**CODcr**	**NH_3-N**	**TP**	**CODcr**	**NH_3-N**	**TP**	**Reference**
Ligularia sibirica	9 d	238.4	31.94	15	82.78	87.75	92.73	Zhang *et al.* (2011)
Bidens frondosa and *Rumex dentatus*	4 d	520	110	10	60	67	70	Zhang *et al.* (2013)
Eichhornia crassipes	25 d	560 1,080		17 45.91	87.6 74.9		58.2 60.6	Sun *et al.* (2011)
Polygonum hydropiper	15			10.55 21.81			17.24 46.20	Zheng *et al.* (2013)
Pistia stratiotes	8 d	498 864	42.74 84.09	4.37 6.01	82.33 68.06	69.21 57.81	45.88 27.30	This study

It can be seen from Table 5 that compared with other plants *Pistia stratiotes* removes CODcr, NH_3-N and TP efficiently and shows good degradation capacity. The removal rate of *Pistia stratiotes* for CODcr of low initial concentrations was higher than other plants in fewer treatment days. For the removal rate of NH_3-N and TP, *Pistia stratiotes* was lower than other plants, which may be interpreted as that low initial CODcr concentrations did not promote the accumulation of H_2O_2, but the increase of NH_3-N and TP in a low initial concentration range did (Li *et al.* 2012).

CONCLUSION

In the removal of pollutants by *Pistia stratiotes,* the rate for CODcr was the most striking, with figures of 68–82%; NH_3-N came next at 57–69%, and TP was lower at 27–45%, all after 8 days of treatment. Compared with the control groups the presence of *Pistia stratiotes* brought an evident improvement in the removal rate of pollutants in livestock wastewater. The removal rates for CODcr, NH_3-N and TP alike were described well by the first-order kinetic equation, with a correlation coefficient R^2 greater than 0.92 in all cases. Compared with the control groups the half-life periods of the pollutants in livestock wastewater decreased significantly. This shows that *Pistia stratiotes* can play an important role in cleaning livestock wastewater. Correlation analysis between water quality indices in livestock wastewater and three endogenous enzymes of the protective system of *Pistia stratiotes* (SOD, POD and CAT) shows that POD plays an important role in the stress-resistance system ($R^2 > 0.9$, $p < 0.05$). POD provides a defense mechanism against oxidative damage induced by livestock wastewater.

The advantages of *Pistia stratiotes* include high biomass, easy cultivation, extensive competitive capability, good adaptability and wide geographic distribution. In light of these preponderant features, *Pistia stratiotes* is a promising species with great value for phytoremediation of wastewater. As water lettuce is an invasive species and will release pollutants when it decomposes, it is important that the plant should be strictly confined in the remediation system and that it should be salvaged before going rotten. In this way its nutrient scavenging ability can be exploited to the full without bringing unnecessary damage to the ecosystem (Lu *et al.* 2010; Li *et al.* 2013).

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REFERENCES

Aebi, H. 1974 *Methods of Enzymatic Analysis*. Academic Press, NewYork, 2, 673–680.

Akapo, A. A. R., Omidiji, S. O. & Otitoloju, A. A. 2011 Morphological and anatomical effects of crude oil on *Pistia stratiotes*. *The Environmentalist* **31** (3), 288–298.

Beauchamp, C. & Fridovich, I. 1971 Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44**, 276–287.

Chen, B. & Cao, W. H. 2007 Purification effect of water hyacinth on middle-high concentration wastewater from livestock. *ACTA Agriculture Jiangxi* **19** (2), 95–97.

Dazy, M., Masfaraud, J. F. & Férard, J. F. 2009 Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. *Chemosphere* **75** (3), 297–302.

Espinoza-Quiñones, F. R., Módenes, A. N., Costa, I. L., Palácio, S. M., Szymanski, N., Trigueros, D. E. G., Kroumov, A. D. & Silva, E. A. 2009 Kinetics of lead bioaccumulation from a hydroponic medium by aquatic macrophytes Pistia stratiotes. *Water Air and Soil Pollution* **203** (1), 29–37.

Hasan, S. A., Fariduddin, Q., Ali, B., Hayat, S. & Ahmad, A. 2009 Cadmium: toxicity and tolerance in plants. *Environmental Biology* **30** (2), 165–174.

He, L. S., Liu, H. L., Xi, B. D. & Zhu, Y. B. 2006 Enhancing treatment efficiency of swine wastewater by effluent recirculation in vertical-flow constructed wetland. *Journal of Environmental Science* **18** (2), 221–226.

Hunt, P. G. & Poach, M. E. 2001 State of the art for animal wastewater treatment in constructed wetlands. *Water Science and Technology* **44** (11), 9–25.

Jing, S. R. & Lin, Y. F. 2004 Seasonal effect on ammonia nitrogen removal by constructed wetlands treating polluted river water in southern Taiwan. *Environmental Pollution* **127** (2), 291–301.

Kraus, T. & Fletcher, R. 1994 Paclobutrazol protects wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? *Plant and Cell Physiology* **35**, 45–52.

Lee, S., Maniquiz, M. C. & Kim, L. H. 2010 Characteristics of contaminants in water and sediment of a constructed wetland treating piggery wastewater effluent. *Journal of Environmental Sciences* **22** (6), 940–945.

Lee, S. Y., Maniquiz, M. C., Choi, J. Y., Jeong, S. M. & Kim, L. H. 2013 Seasonal nutrient uptake of plant biomass in a constructed wetland treating piggery wastewater effluent. *Water Science and Technology* **67** (6), 1317–1323.

Li, F. Z., Jin, S. H. & Hu, G. H. 2005 Isolation and physiological characteristics of a premature senescence mutant in rice (*Oryza sativa* L). *Journal of Zhejiang University. Science* **6B** (8), 803–811.

Li, M., Ma, X. Z. & Wang, W. 2012 Effect of *Pistia stratiotes* L. on removal rate for nitrogen and phosphorus in polluted water body. *Resources and Environmental in the Yangtza Basin* **21** (9), 1137–1142.

Li, Y., Zhang, S., Jiang, W. & Liu, D. 2013 Cadmium accumulation, activities of antioxidant enzymes and malondialdehyde (MDA) content in *Pistia stratiotes* L. *Environmental Science Pollution Research International* **20**, 1117–1123.

Lu, J. B., Fu, Z. H. & Yin, Z. Z. 2008 Performance of a water hyacinth (*Eichhornia crassipes*) system in the treatment of wastewater from a duck farm and the effects of using water hyacinth as duck feed. *Journal of Environmental Science* **20** (5), 513–519.

Lu, Q., He, Z. L., Graetz, D. A., Stoffella, P. J. & Yang, X. E. 2010 Phytoremediation to remove nutrients and improve eutrophic storm-waters using water lettuce (*Pistia stratiotes* L.). *Environmental Science Pollution Research* **17** (1), 84–96.

Mufarrege, M. M., Hadad, H. R. & Maine, M. A. 2010 Response of *Pistia stratiotes* to heavy metals (Cr, Ni, and Zn) and phosphorous. *Archives Environmental Contamination Toxicology* **58** (1), 53–61.

Odjegba, V. J. & Fasidi, I. O. 2004 Accumulation of trace elements by *Pistia stratiotes*: implications for phytoremediation. *Ecotoxicology* **13**, 637–646.

Ren, L. J., Wu, Y., Ren, N. Q., Zhang, K. & Xing, D. F. 2010 Microbial community structure in an integrated AO reactor treating diluted livestock wastewater during startup period. *Journal of Environmental Science* **22** (5), 656–662.

Sánchez-Galván, G., Mercado, F. J. & Olguín, E. J. 2013 Leaves and roots of *Pistia stratiotes* as sorbent materials for the removal of crude oil from saline solutions. *Water, Air, and Soil Pollution* **224** (2), 1421–1433.

Shen, Y., Yuan, W., Pei, Z. & Mao, E. 2008 Culture of microalga *Botryococcus* in livestock wastewater. *Transactions of the ASABE* **51** (4), 1395–1400.

Sinha, S., Basant, A., Malik, A. & Singh, K. P. 2009 Multivariate modeling of chromium-induced oxidative stress and biochemical changes in plants of *Pistia stratiotes* L. *Ecotoxicology* **18**, 555–566.

Sooknah, R. D. & Wilkie, A. C. 2004 Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. *Ecological Engineering* **22** (1), 27–42.

Standard Methods for Water and Wastewater Monitoring and Analysis 2002 4th edn, State Environmental Protection Administration of China. Beijing, China.

Sun, J., Hao, Z. H., Yang, G. F. & Wang, J. Y. 2011 Purification effects of various combined hydrophytes on the waste water from livestock and poultry products. *Chinese Agricultural Science Bulletin* **27** (32), 270–274.

Suwasa, K., Supreeya, K. & Hans, B. 2009 Treatment of high-strength wastewater in tropical vertical flow constructed wetlands planted with *Typha angustifolia* and *Cyperus involucratus*. *Ecological Engineering* **35**, 238–247.

Tewari, R. K., Praveen, K. & Sharma, P. N. 2006 Magnesium deficiency induced oxidative stress and antioxidant responses in mulberry plants. *Scientia Horticulturae* **108** (1), 7–14.

Xu, J. T., Li, C., Yang, F., Dong, Z., Zhang, J., Zhao, Y. C., Qi, P. Y. & Hu, Z. 2011 *Typha angustifolia* stress tolerance to wastewater with different levels of chemical oxygen demand. *Desalination* **280** (1–3), 58–62.

Zhang, J. P., Wang, Y. J. & Deng, S. H. 2011 Purification effect of *Ligularia sibirica* on livestock wastewater. *Journal of Sichuan Agricultural University* **29** (3), 397–401.

Zhang, C. Y., Wang, Y. & Wang, Y. Y. 2013 Purification effect of subsurface flow constructed wetland on livestock wastewater. *Transactions of the Chinese Society of Agricultural Engineering* **29** (17), 160–168.

Zheng, Z. C., Li, T. X., Zeng, F. F., Zhang, X. Z., Yu, H. Y., Wang, Y. D. & Liu, T. 2013 Accumulation characteristics of and removal of nitrogen and phosphorus from livestock wastewater by Polygonum hydropiper. *Agricultural Water Management* **117**, 19–25.

Zimmels, Y., Kirzhner, F. & Malkovskaja, A. 2006 Application of Eichhornia crassipes and Pistia stratiotes for treatment of urban sewage in Israel. *Journal of Environmental Management* **81** (4), 420–428.

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