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Influence of paper mill wastewater on reed chlorophyll content and biomass

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ABSTRACT

Studies on the influence of paper mill wastewater, which affects photosynthesis, on reed chlorophyll and biomass can provide a theoretical basis for the ecological restoration of wetland plants. The influence of different concentrations of wastewater (chemical oxygen demands (COD) of 300, 175 and 50 mg•L^{-1}) and different irrigation times (germination, blade-expansion, rapid-growth, heading and maturity stages) on the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and chlorophyll *a*/b in reeds were tested in experimental pools that simulated the wetland ecosystem of the Liaoning Shuangtai estuary. The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and chlorophyll *a*/b all increased significantly with increasing concentrations of wastewater, and their contents all differed significantly from each other. The contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll were maximal when irrigated at the beginning of all growth stages with COD of 300 mg•L^{-1}(C_1O) at 2.253, 0.458 and 2.711 mg•g^{-1} fresh weight (FW), respectively, with irrigation at the rapid-growth stage and were minimal in the control plants at 0.142, 0.068 and 0.210 mg•g^{-1} FW, respectively, with irrigation at maturity. Chlorophyll *a*/b was maximal at C_1O at 5.753 with irrigation at germination stage and was minimal in the control plants at 2.113 with irrigation at maturity. Reed biomass was maximal at C_1O at 3.26 kg, which was 2.76 times higher than the control. Reed biomass was positively correlated with the content of chlorophyll and with COD in the wastewater. Paper mill wastewater generally increased the content of chlorophyll and the net photosynthesis rate and enhanced the growth of reeds.

1. Introduction

Estuary wetland is a sensitive area of interaction between sea and land. Increased industrialization and global climate warming, though, are currently reducing the area of wetland, and the salinization of soil is seriously increasing. Many paper mills in China discharge large quantities of wastewater into wetland, mostly distributed along rivers. With proper treatment, wastewater from paper mills can be used for irrigation, alleviating the shortage of irrigation water from wetland, can promote plant growth and can improve soils (Kiziloglu et al., 2008; Pervaiz, 2003). With a certain concentration of wastewater irrigation, wetland can effectively remove the contaminants in the wastewater, and soil and water quality will not be imperiled (Xia et al., 2011). The characteristics of groundwater and the microbial and nutritional elements of soil in different areas and vegetational types have been studied under the current use of wastewater for irrigation (Singh, 2007; Patterson et al., 2008), mostly as analyses of the physical and chemical properties of wetland soil and of the status of reed growth (Ding et al., 2005; Li et al., 2008). The influence on chlorophyll in reeds, however, has rarely been reported (Chung et al., 2008). Chlorophyll is an important physiological index of photosynthesis and the growth of plants.

The Liaoning Shuangtai estuarine wetland is the final barrier of the Liao River's exit to the sea, and its degradation has become increasingly serious. In this study, we monitored chlorophyll levels and reed biomass by irrigating experimental pools, simulating the Liaoning Shuangtai ecosystem, with different concentrations of paper mill wastewater at different stages of reed development. Our findings may serve to provide reference points for the ecological restoration of salinized wetland.

2. Materials and methods

2.1. Materials

Selected rhizomes for transplanting were cut from nongerminating reeds from the Liaoning Shuangtai estuarine wetland in China. Soil samples from the same wetland were also collected. The soil type was meadow soil with a pH of 8.5, organic-matter content of 1.046% and bulk density of 1.03 g•cm^{-3}.

Fourteen 50-L barrels of wastewater were collected from the

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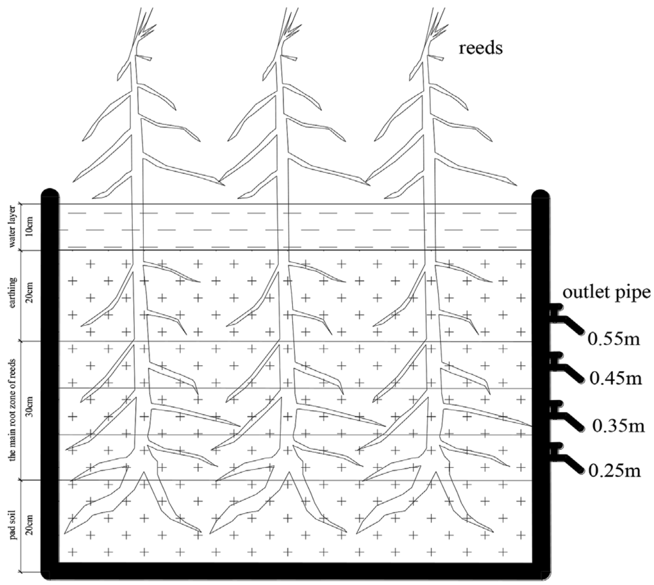

Fig. 1. The simulation device of reed wetland.

effluent of a paper mill in Liaoning province five times from May to September, 2012. The wastewater had a pH of 8.3, chemical oxygen demand (COD) of 300 mg•L^{-1}, total nitrogen content of 29.5 mg•L^{-1} and total phosphorus content of 3.28 mg•L^{-1} and was stored in the shade until used.

The experiments began in April, 2012. A total of 19 concrete test pools were constructed to simulate natural wetland. Each pool was 2 × 1 × 0.8 m in length × width × internal depth on a foundation embedded 0.8 m in the ground. The planting density of reed was 55 per square meter. The reed rhizomes were transplanted into the soil collected from the estuary and were irrigated with wastewater as shown in Fig. 1.

2.2. Methods

The growth of the reeds was classified into germination, blade-expansion, rapid-growth, heading and maturity stages, following the national specifications for irrigation tests. The reeds received six wastewater treatments and three concentrations of wastewater, as measured by CODs of 300, 175 and 50 mg•L^{-1}.

The 19 test pools received the following treatments. Fifteen pools were divided into five groups of three, numbered I, II, III, IV and V, representing the germination, blade-expansion, rapid-growth, heading and maturity stages, respectively. These groups of three pools were irrigated with the three concentrations of wastewater (see below), but only at the beginning of each respective growth stage; tap water was added at all other times. Three pools, designated by "O", were irrigated with the three concentrations of wastewater at the beginnings of all growth stages. One control pool (CK) was irrigated only with tap water.

The three concentrations of wastewater were designated as C_1, C_2 and C_3. C_1 was the undiluted wastewater with a COD of 300 mg•L^{-1}. C_2 and C_3 were obtained by dilution with tap water to CODs of 175 and 50 mg•L^{-1}, respectively. The solution was irrigated in the five growth stages of reed which were divided into germination period, leaf-expansion period, rapid growth period, heading period and maturity period which is respectively from late April to mid-May, from mid-May to late June, from early July to mid-august, from mid-august to mid-September, from mid-September to mid-October according to "the national standards for the division and observation of major crop growth". For example, C_1I represents undiluted wastewater (COD of 300 mg•L^{-1}) irrigating at the beginning of the germination stage, and C_3O represents wastewater diluted to a COD of 50 mg•L^{-1} irrigating at the beginnings of all growth stages. The volume of water in each experimental pool was 0.2 m^3. The depth of the water was maintained at 10 cm with tap water to ensure the normal growth of the reeds. Water surface is regularly observed and maintained by timely irrigation. Preliminary research showed that there is scarce nitrogen, phosphorus and heavy metals which could affect the results.

2.3. Sample collection and measurement

The acetone/ethanol method was adopted for measuring chlorophyll content (Hou, 2004). Fresh reed leaves (0.1 g) were cut and placed in test tubes containing 10 mL of extraction liquid. The tubes were sealed and placed in the dark at room temperature (25 °C), and the chlorophyll was extracted. When the leaves were completely white, the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll were determined by colorimetric analysis, using the Lambert-Beer law, with an ultraviolet spectrophotometer at wavelengths of 663 nm and 645 nm.

Aboveground parts of mature reeds were harvested and dried at 105 °C to determine the reed biomass of each experimental pool.

2.4. Data processing and analysis

The influence of wastewater concentration and irrigation time on the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and chlorophyll *a*/b and on biomass levels were analyzed by two-way ANOVAs with SPSS version 17.0 (statistical significance was set at $P < 0.05$; a significance level of 0.01 was deemed to indicate very high significance). Correlations among biomass, chlorophyll and the COD of the wastewater were also analyzed with liner regression.

3. Results

3.1. Effects of paper mill wastewater on chlorophyll a

Treatment with paper mill wastewater generally increased the content of chlorophyll *a* of the reeds significantly ($P < 0.05$) (Table 1, Fig. 2). Different wastewater concentrations and irrigation times had different influences on the content of chlorophyll *a*. Chlorophyll *a* content was lowest at 0.142 mg•g^{-1} fresh weight (FW) at maturity when irrigated with tap water and reached a maximum of 2.253 mg•g^{-1} FW at the rapid-growth stage when irrigated with wastewater at each growth stage of the reeds. The content of chlorophyll *a* showed the trend of $C_1O > C_1 > C_2O > C_2 > C_3O > C_3 >$ CK at each irrigation time, i.e. it was maximal at C_1O.At C_1O, the content of chlorophyll *a* at the germination, blade-expansion, rapid-growth, heading and maturity stages were 50.86%, 33.78%, 58.11%, 42.51% and 100.62% ($P < 0.01$) higher, respectively, than the content at CK. These results indicate that paper mill wastewater promoted the synthesis of chlorophyll *a* in the plants.

The content of chlorophyll *a* in reed leaves varied depending on the stage of growth and development, with the highest content at the rapid-

Table 1
Relationships between the chlorophyll contents of reeds and paper mill wastewater concentrations and irrigation times. * indicates a significance level of 0.05, ** indicates a significance level of 0.01. The numbers in the table are F values and the numbers between parentheses are P values.

Chlorophyll	Wastewater concentration	Irrigation time	Wastewater concentration × Irrigation time
Chlorophyll *a*	7.791**b	162.777**b	0.795 (0.730)
Chlorophyll *b*	4.235**b	96.436**b	0.854 (0.658)
Total chlorophyll	7.563**b	161.208**b	0.777 (0.751)
Chlorophyll *a*/b	2.459*a	48.405**b	1.080 (0.388)

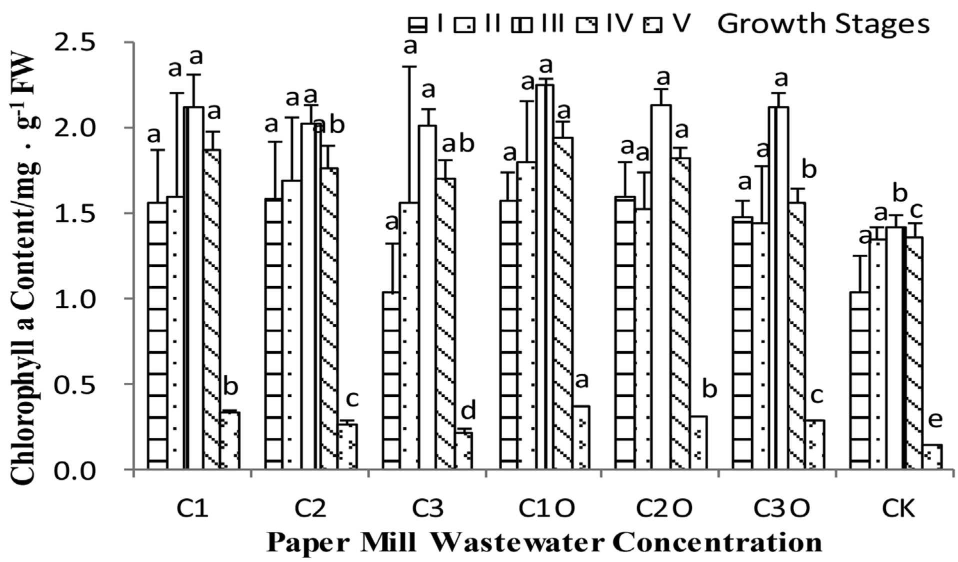

Fig. 2. The influence of paper mill wastewater on the content of chlorophyll *a*. Treatments without common letters in the figure indicate statistical significance at the 0.05 level. Error bars represent standard errors.

growth stage, followed by the heading, blade-expansion, germination and maturity stages. The contents of chlorophyll *a* at C_1, C_2, C_3, C_1O, C_2O, C_3O and CK were 5.42, 6.72, 8.12, 5.06, 5.81, 6.52 and 9.04 times higher, respectively, at the rapid-growth stage than at maturity. The contents of chlorophyll *a* at the various concentrations and irrigation times were significantly different from each other ($P < 0.05$), but concentration and irrigation time were not significantly correlated ($P > 0.05$). The contents of chlorophyll *a* at the germination and blade-expansion stages were not significantly different from each other ($P > 0.05$). At the blade-expansion, content was not significantly different between various concentrations but was significantly higher than the content of the control ($P < 0.05$). At the heading stage, the contents of chlorophyll *a* at C_1O, C_1 and C_2O were similar and were significantly higher than those of the other treatments ($P < 0.05$). The contents of chlorophyll *a* at C_2, C_3 and C_3O were not significantly different from each other but were significantly different from the control ($P < 0.05$). At maturity, the content of chlorophyll *a* was maximal at 0.372 mg•g^{-1} FW at C_1O, which was significantly different from the contents in all treatments except C_1, C_2O, C_2 and C_3O.

3.2. Effects of paper mill wastewater on chlorophyll b

The content of chlorophyll *b* in reed leaves differed very significantly at different wastewater concentrations and irrigation times (Table 1). At the same concentration, the content of chlorophyll *b* was highest at the rapid-growth stage, followed by the heading, blade-expansion, germination and maturity stages. These differences were statistically significant. At the same irrigation time, the content of chlorophyll *b* increased with increasing concentrations (Tables 1 and 2). At C_1O, the contents of chlorophyll *b* at the germination, blade-expansion, rapid-growth, heading and maturity stages were 7.94%, 31.52%, 40.06%, 34.81% and 75.00% higher, respectively, than the content of CK. At the rapid-growth stage, the contents of chlorophyll *b* at C_1, C_2, C_3, C_1O, C_2O, C_3O and CK were 2.57, 2.90, 2.54, 2.84, 2.85, 2.49 and 3.81 times ($P < 0.01$) higher, respectively, than the content at maturity, reaching a maximum of 0.458 mg•g^{-1} FW at C_1O. The content of chlorophyll *b* reached a minimum of 0.068 mg•g^{-1} FW at CK when irrigated at maturity.

3.3. Effects of paper mill wastewater on total chlorophyll

The content of total chlorophyll differed very significantly at different wastewater concentrations and irrigation times ($P < 0.01$) (Table 1, Fig. 3). The contents of total chlorophyll at C_1, C_2, C_1O, C_2O and C_3O were not significantly different at the germination stage but were significantly higher than those at C_3 and CK. The wastewater thus increased the content of total chlorophyll when irrigated at the germination stage. At the blade-expansion stage, the contents of total chlorophyll at the various concentrations were not significantly different from each other, perhaps because the differences in the contents of chlorophyll *b* at the various concentrations were not significant. At the rapid-growth and heading stages, the contents of total chlorophyll were not significantly different at all concentrations but were significantly higher than the content at CK. At maturity, the contents of total chlorophyll were significantly different at all concentrations except C_1 and C_2O ($P < 0.05$). The content reached a maximum of 2.711 mg•g^{-1} FW at C_1O when irrigated at the rapid-growth stage and reached a minimum of 0.210 mg•g^{-1} FW at CK when irrigated at maturity.

Table 2
The influence of paper mill wastewater on the content of chlorophyll *a*. Treatments without common letters in the same column indicate significance at the 0.05 level. $\overline{X}$ indicates the average and s indicates standard errors. The units are mg•g^{-1} FW.

Wastewater concentration	Germination stage ($\overline{X}$ ± s)	Blade-expansion stage ($\overline{X}$ ± s)	Rapid-growth stage ($\overline{X}$ ± s)	Heading stage ($\overline{X}$ ± s)	Maturity stage ($\overline{X}$ ± s)
C_1	0.359 ± 0.082a	0.366 ± 0.146a	0.382 ± 0.007c	0.375 ± 0.006b	0.107 ± 0.012a
C_2	0.363 ± 0.064a	0.373 ± 0.140a	0.437 ± 0.009b	0.386 ± 0.007b	0.112 ± 0.009a
C_3	0.227 ± 0.047a	0.355 ± 0.046a	0.372 ± 0.008c	0.367 ± 0.007b	0.105 ± 0.08a
C_1O	0.288 ± 0.080a	0.411 ± 0.098a	0.458 ± 0.006a	0.426 ± 0.009a	0.119 ± 0.010a
C_2O	0.336 ± 0.057a	0.319 ± 0.149a	0.447 ± 0.010ab	0.418 ± 0.012a	0.116 ± 0.003a
C_3O	0.338 ± 0.041a	0.320 ± 0.032a	0.381 ± 0.012c	0.367 ± 0.007b	0.109 ± 0.007a
CK	0.267 ± 0.078a	0.313 ± 0.002a	0.327 ± 0.008d	0.316 ± 0.008c	0.068 ± 0.006b

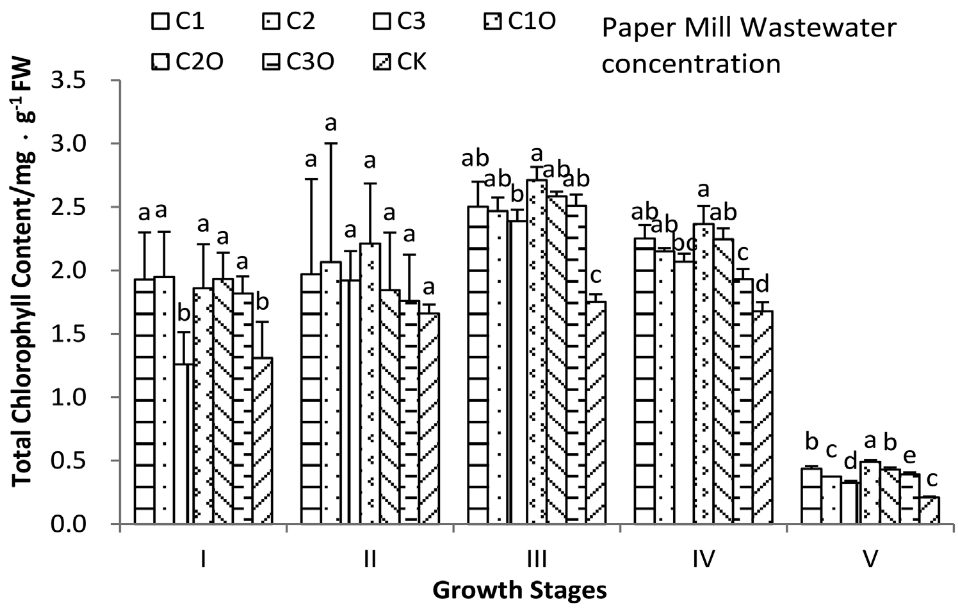

Fig. 3. The influence of paper mill wastewater on the content of total chlorophyll. Treatments without common letters in the figure indicate statistical significance at the 0.05 level. Error bars represent standard errors.

3.4. Effects of paper mill wastewater on chlorophyll a/b

The contents of chlorophyll *a*/b differed very significantly at the different irrigation times, varied significantly at the various concentrations and were higher than that of the control (Table 1). At the same concentration, chlorophyll *a*/b content was highest at the rapid-growth stage, decreased at the heading stage and was lowest at maturity. Chlorophyll *a*/b contents at the rapid-growth and heading stages were both significantly higher than the content at maturity. At the same irrigation time, the differences in chlorophyll *a*/b contents at different concentrations were not significant but were significantly higher than the content at CK (Table 3). Chlorophyll *a*/b content was maximal at C_1O at 5.753 when irrigated at the germination stage, which was higher than the chlorophyll *a* content at this stage. Chlorophyll *a*/b content was minimal at CK at 2.113 when irrigated at maturity. The levels of chlorophyll *a* decreased more quickly than those of chlorophyll *b* during the senescence of reed leaves at maturity.

3.5. Regression analysis of the concentration of paper mill wastewater and the photosynthesis index of reed

The results of the relationship between the concentration of wastewater and the photosynthesis index of reed investigated by regression analysis are shown in Table 4. It is known from Table 4 that the correlation of chlorophyll *a* and total chlorophyll to the concentration of wastewater was very significant in the stages of germination, blade-expansion, rapid-growth, heading and maturity ($P < 0.05$). And in the different irrigation period, the linear correlation between the net photosynthetic rate and the concentration of paper mill wastewater was the most significant, and the correlation coefficient reached over 0.90.

3.6. Effects of paper mill wastewater on reed biomass

The effects of paper mill wastewater on reed biomass are shown in Fig. 4. The reed biomass was maximal at C_1O at 3.26 kg, which was 2.76 times higher than in the control. The reed biomasses in the treated pools were higher than the control biomass. The paper mill wastewater thus promoted reed growth.

The correlations among biomass, total chlorophyll content and the COD of the wastewater are given in Table 5.

Reed biomass was positively correlated with chlorophyll content and the COD of the wastewater, and chlorophyll content was also correlated with the COD of the wastewater. The regression equation was [biomass] = 1.591 [chlorophyll] + 0.004 [COD] −0.526 ($R^2 = 0.674$, $P < 0.05$). These results indicated that the COD of the wastewater influenced biomass more than chlorophyll content.

4. Discussion

4.1. Chlorophyll content of reeds under wastewater treatment

With the increase of wastewater concentration, the content of chlorophyll *a*, chlorophyll *b*, total chlorophyll and chlorophyll *a*/b of Reed increased significantly. The chlorophyll content of reed was the most in the rapid-growth stage.

Research showed that the appropriate level of nitrogen in agricultural wastewater could reduced the toxicity of salinity alkalinity to reed PSII donor and recipient side, thereby promoting the growth and

Table 3
The influence of paper mill wastewater on chlorophyll *a*/b. Treatments without common letters in the same column indicate significance at the 0.05 level.

Wastewater concentration	Germination stage	Blade-expansion stage	Rapid-growth stage	Heading stage	Maturity stage
C_1	4.430 ± 0.624a	4.410 ± 0.092a	5.545 ± 0.393a	5.001 ± 0.198a	3.108 ± 0.361a
C_2	4.365 ± 0.190a	4.431 ± 0.588a	4.646 ± 0.156b	4.574 ± 0.375a	2.351 ± 0.094b
C_3	4.570 ± 0.091a	4.449 ± 0.681a	5.424 ± 0.368a	4.634 ± 0.072a	2.108 ± 0.072b
C_1O	5.753 ± 2.205a	4.416 ± 0.267a	4.918 ± 0.156b	4.554 ± 0.255a	3.147 ± 0.417a
C_2O	4.816 ± 0.733a	5.282 ± 2.169a	4.782 ± 0.142b	4.380 ± 0.363a	2.706 ± 0.076ab
C_3O	4.401 ± 0.261a	4.478 ± 0.736a	5.588 ± 0.033a	4.264 ± 0.267a	2.603 ± 0.179ab
CK	3.999 ± 0.518a	4.312 ± 0.262a	4.363 ± 0.315b	4.316 ± 0.354a	2.113 ± 0.415b

Table 4
Regression analysis between photosynthesis indicators of reed and wastewater concentration.* indicates a significance level of 0.05,** indicates a significance level of 0.01.

Wastewater irrigation period	Photosynthesis index	Line equations	Correlation coefficient r
Germination	Chlorophyll *a*	y = 1.075 + 0.002x	0.721**
	Chlorophyll *b*	y = 0.267 + 0.0004x	0.593*
	Chlorophyll *a*/b	y = 4.065 + 0.001x	0.404
	Total chlorophyll	y = 1.341 + 0.002x	0.706*
	Net photosynthetic rate	y = 8.811 + 0.022x	0.991**
Blade-expansion	Chlorophyll *a*	y = 1.454 + 0.001x	0.199
	Chlorophyll *b*	y = 0.332 + 0.002x	0.199
	Chlorophyll *a*/b	y = 4.375 + 0.0002x	0.059
	Total chlorophyll	y = 1.785 + 0.001x	0.202
	Net photosynthetic rate	y = 17.721 + 0.016x	0.983**
Rapid-growth	Chlorophyll *a*	y = 1.666 + 0.002x	0.700*
	Chlorophyll *b*	y = 0.354 + 0.0002x	0.565
	Chlorophyll *a*/b	y = 4.690 + 0.002x	0.476
	Total chlorophyll	y = 2.2020 + 0.002x	0.706*
	Net photosynthetic rate	y = 21.658 + 0.023x	0.993**
Heading	Chlorophyll *a*	y = 1.496 + 0.001x	0.802*
	Chlorophyll *b*	y = 0.340 + 0.0002x	0.694*
	Chlorophyll *a*/b	y = 4.387 + 0.002x	0.649*
	Total chlorophyll	y = 1.829 + 0.002x	0.802**
	Net photosynthetic rate	y = 0.691 + 0.003x	0.969**
Maturity	Chlorophyll *a*	y = 0.165 + 0.001x	0.949*
	Chlorophyll *b*	y = 0.085 + 0.0001x	0.624*
	Chlorophyll *a*/b	y = 1.987 + 0.003x	0.818*
	Total chlorophyll	y = 0.250 + 0.001x	0.917**
	Net photosynthetic rate	y = 0.122 + 0.0004x	0.901**

chlorophyll synthesis of the plants (Deng et al., 2011), which confirmed the results of this study although the experiment material is agricultural wastewater. Many beneficial elements were contained in papermaking wastewater which could become a new way to improve prodect level by motivating chlorophyll and promoting reed growth. Bouchama, Khaled found that after reed was treated by wastewater, low dose of cadmium in the wastewater could induce the total content of chlorophyll (a + b) in reed increased significantly when irrigated cadmium solution (Bouchama et al., 2016). While the results were opposite that content of chlorophyll (a + b) increased with the COD concentration of wastewater in this research. The reason probably because the effects are different between one metal element like Bouchama's research and the interaction of various chemical elements in paper mill wastewater like this research. Puteh and Adam found that when the concentration of wastewater was less than 25%, the content of chlorophyll *a* and *b* has a higher promotion effect and when the concentration of wastewater was 50%–100%, it was harmful to the content of chlorophyll *a* and *b* after treating rice seeds with different concentrations of municipal wastewater (Puteh et al., 2015). This research drew the similar thends while the concentration range is not very consistent. The differences might be due to different sensitivity to papermaking wastewater between rice and reed plant as mentioned in these two studies. Also the irrigating solution were different from urban sewage and papermaking wastewater, which brought different chemical elements and nutrients and made different effects on the plant. Moradi pointed out that the content of chlorophyll in the leaves of the oats under different irrigation concentration and irrigation interval was the highest in the whole plant period, and drought could reduce the chlorophyll content of leaves (Moradi et al., 2016). The same conclusion was drawn in this research. The oats had different optimal irrigation periods from reeds for their different physiological characteristics. This conclusions were verified by the Ahsan and Muhammad's research which showed that under the same wastewater treatment condition, the physiological characteristics of different Fragrant Rosa Species were different, R. bourboniana showed the highest photosynthetic rate, while R. gruss anteplitz showed the largest chlorophyll content (Ahsan et al., 2017). Researches also showed that seedlings was significantly related to the concentration of wastewater of textile mill (Ozel, 2014), which also confirmed that wastewater can promote plant growth as the conclusion of this research.

4.2. Reed biomass under treatment of paper mill wastewater

Paper mill wastewater irrigation can increase the biomass of reed, which was verified in Yu's studies that wastewater irrigation promoted the growth of reed via biomass distribution, morphology and growth characteristics under irrigated two effluent of paper mill wastewater (Yan et al., 2009). The same conclusion was also drawn in 2 years irrigation experiments from Zema, who irrigated three kinds of energy crops (Typha latifolia, Arundo donax and Phragmites australis) with city wastewater within 2 years, and the results show that wastewater irrigation can increase the yield of reed biomass (Zema et al., 2012). This research also provided a reference for the feasibility of using waste water to irrigate reeds for a long period of time.

4.3. Relationship of chlorophyll content and biomass of reed

The chlorophyll content of the reed was positively correlated with the biomass, and the biomass increased with the increase of chlorophyll content. Similar conclusions can be found in other studies: Ston-Egiert fond that there is a correlation between chlorophyll *a* and biomass through analyzing the distribution and concentration of phytoplankton biological factors (Ston-Egiert et al., 2010). Popoviciu found that the density and biomass of sediment bacteria were positively correlated with water temperature and chlorophyll concentration by studying intertidal sediments of bacterial density, biomass and diversity of shape and structure of the seasonal of three beach surface in Constanta (Popoviciu and Ardelean, 2012). The content of chlorophyll is also influenced by the total carbon biomass of phytoplankton (Eker-Develi et al., 2012). Although some differences between variety of plant species same result remained that some correlation existed between chlorophyll content and biomass as mentioned in this research.

5. Conclusions

This study concluded that paper mill wastewater increased reed biomass and promoted reed growth by increasing chlorophyll content and net photosynthetic rate of reeds. When the concentration of paper mill wastewater is 0–300 mg/L, the optimum irrigation concentration of papermaking wastewater is 300 mg/L, and the best period of irrigation is rapid growth period.

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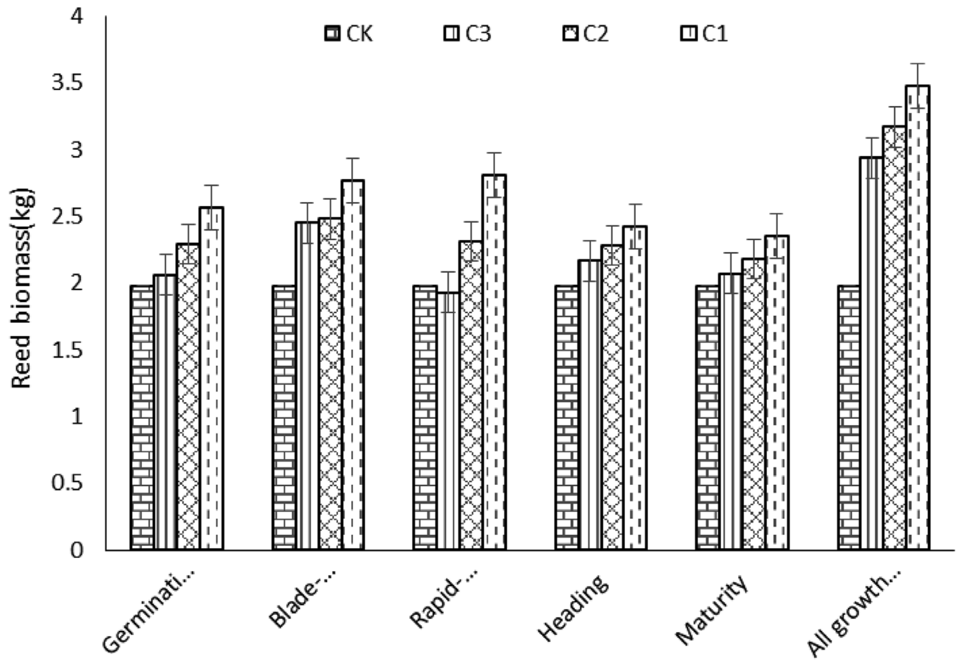

Fig. 4. The influence of paper mill wastewater on reed biomass.

Table 5
Correlations among reed biomass, chlorophyll contents and the COD of the wastewater.** indicates a significant correlation at the 0.01 level. The numbers in the table are R^2 values.

	Biomass	Chlorophyll	COD of wastewater
Biomass	1	0.749**	0.725**
Chlorophyll		1	0.614**
COD of wastewater			1

References

Ahsan, Muhammad, Riaz, Atif, Jaskani, Muhammad Jafar, 2017. Physiological and anatomical response of fragrant Rosa species with treated and untreated wastewater. Int. J. Agric. Biol. 19 (1), 13–22.

Bouchama, Khaled, Rouabhi, Rachid, Djebar, Mohammed Reda, 2016. Behavior of Phragmites australis (CAV.) Trin. Ex Steud used in phytoremediation of wastewater contaminated by cadmium. Desalination and water treatment 57 (12), 5325–5330.

Chung, A.K.C., Wu, Y., Tam, N.F.Y., Wong, M.H., 2008. Nitrogen and phosphate mass balance in a sub-surface flow constructed wetland for treating municipal wastewater. Ecol. Eng. 32 (1), 81–89.

Deng, Ch, Zhang, G., Pan, X., 2011. Photosynthetic responses in reed (Phragmites australis (CAV.) TRIN. Ex Steud.) seedlings induced by different salinity-alkalinity and nitrogen levels. J. Agric. Sci. Technol. 13 (5), 687–699.

Ding, C., Wang, S.H., Yang, C.S., 2005. Influence on the soil of seashore wetland and the growth of pulp wastewater irrigated reeds. Ecol. Environ. 14 (1), 21–25 (in Chinese).

Eker-Develi, Elif, Berthon, Jean-Francois, Canuti, Elisabetta, 2012. Phytoplankton taxonomy based on CHEMTAX and microscopy in the northwestern Black Sea. J. Mar. Syst. 94, 18–32.

Hou, F.L., 2004. Plant Physiology Experimentation. Science Press (in Chinese).

Kiziloglu, F.M., Turan, M., Sahin, U., Kuslu, Y., Dursun, A., 2008. Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower(*Brassica olerecea L.*var.botrytis)and red cabbage(*Brassica olerecea L.*var.rubra)grown on calcareous soil in Turkey Agriculture. Water Manag. 95 (6), 716–724.

Li, J.L., Lu, Z.H., Tian, J.Y., Wang, L., Li, P.H., Xiao, Z.F., 2008. Bioremediation of coastal saline-alkali marshy soil by irrigation with pulp and paper waste water. J. China Inst. Min. Technol. 37 (2), 281–286 (in Chinese).

Moradi, S., Heidari, H., Saeidi, M., Nosratti, I., 2016. Effect of sewage-contaminated water on seed production, heavy metals accumulation and seedling emergence in oat. Global Nest J. 18 (2), 329–338.

Ozel, Halil Baris, Ozel, Handan Ucun, 2014. Effects of industrial wastewater on germination and some physiological properties of Dormast oak (quercus petraea liebl.) seeds. Fresen. Environ. Bull. 23 (10A), 2615–2619.

Patterson, S.J., Chanasyk, D.S., Mapufumo, E., Naeth, M.A., 2008. Effects of diluted kraft pulp mill effluent on hybrid poplar and soil chemical properties. Irrigat. Sci. 26, 247–560.

Pervaiz, Z., 2003. Response of selected wheat cultivars to calcium in saline condition. Int. J. Agric. Biol. 5 (3), 265–269.

Popoviciu, Razvan, Ardelean, Ioan I., 2012. Seasonal variation of bacterial abundance, biomass and morpho-structural diversity in littoral sandy sediments on the Romanian black sea coast. In: 12h International Multidisciplinary Scientific Geoconference (SGEM). Albena, Bulgaria, Jun 17–23.

Puteh, Adam Bin, Gassama, Umaru Mohamed, Abd-Halim, Mohamed Ridzwan, 2015. Influence of municipal wastewater on rice seed germination, seedling performance, nutrient uptake, and chlorophyll content. J. Crop Sci. Biotechnol. 18 (1), 9–19.

Singh, S.K., 2007. Effect of irrigation with paper mill effluent on the nutrient status of soil. Int. J. Soil Sci. 2 (1), 74–77.

Ston-Egiert, Joanna, Lotocka, Maria, Ostrowska, Miroslawa, 2010. The influence of biotic factors on phytoplankton pigment composition and resources in Baltic ecosystems: new analytical results. Oceanologia 52 (1), 101–125.

Xia, J.B., Xie, W.J., Sun, J.K., Liu, J.T., Liu, Q., Lu, Z.H., 2011. Effects of papermaking wastewater irrigation on reed growth and its soil improvement. J. Soil Water Conserv. 25 (1), 110–118 (in Chinese).

Yan, Yu, Penghui, Li, Zhaohua, Lu, 2009. Effect of papermaking wastewater irrigation on growth of reed in saline-alkaline wetland. In: 3rd International Conference on Bioinformatics and Biomedical Engineering. Beijing, Peoples R China, Jun 11–16.

Zema, Demetrio Antonio, Bombino, Giuseppe, Andiloro, Serafina, 2012. Irrigation of energy crops with urban wastewater: effects on biomass yields, soils and heating values. Agric. Water Manag. 115, 55–65.