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1 **Removal of micro-pollutants from urban wastewater by constructed wetlands with *Phragmites***

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2 *australis* and *Salix matsudana*

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28 **Abstract**

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This study assessed the ability to remove micro-pollutants from wastewater using herbaceous species (*Phragmites australis* L.) and trees (*Salix matsudana* Koidz.) in constructed wetland (CW) systems. The targets of the study were: i) pharmaceuticals like diclofenac, ketoprofen, atenolol; ii) 4-*n*-NP (4-*n*-nonylphenol) and the ethoxylated derivatives monoethoxylated nonylphenol (NP<sub>1</sub>EO) and diethoxylated nonylphenol (NP<sub>2</sub>EO); iii) triclosan, a bactericide used in personal-care products. The twelve CW systems, filled with clay and gravel, were irrigated with wastewater from municipal area of Pagnana (Tuscany, Italy) and influent and effluent water samples analyzed periodically by gas chromatography–mass spectrometry (GC-MS/MS). The removal efficiency of CWs planted with willow and common reed ranged from 8.4% up to 100%, with the higher removal efficiency for triclosan. On the contrary, the removal efficiency of NPs and NPEOs appears lower than pharmaceuticals. Data demonstrated that *P. australis* efficiently removed NP, diclofenac and atenolol, while *S. matsudana* preferentially removed NP<sub>1</sub>EO, NP<sub>2</sub>EO, ketoprofene and triclosan. A specific selection of plants used in CWs could be exploited for the removal of specific xenobiotics from wastewater.

**Keywords** Atenolol • Common reed • Diclofenac • Ketoprofen • Nonylphenols • Removal efficiency • Triclosan • Willow •

## 47 Introduction

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248 In the recent years, the occurrence of an emerging class of contaminants named micro-pollutants in  
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549 the aquatic environments has become a global issue of increasing environmental concern. Micro-  
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750 pollutants, include a massive range of anthropogenic substances such as pharmaceuticals, surfactants,  
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51 steroids, hormones, personal care products, industrial chemicals, pesticides, etc. All the above  
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152 mentioned organic substances are not often completely and consistently removed during conventional  
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53 wastewater treatment processes, and thus they are frequently detectable in reclaimed surface water at  
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154 concentrations ranging from ng/L to mg/L (Hollender et al. 2008).

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55 Polyethoxylated nonylphenols (NPnEOs) are non-ionic surfactants that are used in the tannery  
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2256 industry (Langford and Lester, 2002), but also as domestic surfactants in dispersants, emulsifiers,  
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2457 detergents, dyes, pesticides, cosmetics, etc. (Soares et al. 2008). NPnEOs are only partially degraded  
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58 in conventional wastewater treatment plants (WWTPs), and the main problem related to these  
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2959 surfactants is the evidence that the conventional bacterial metabolic activity of the activated sludges  
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60 is capable to depolymerize the ethoxylated group producing compounds more toxic and resistant to  
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3461 biological degradation with reference to NPnEOs, such as nonylphenols (NPs), monoethoxylated  
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62 nonylphenol (NP<sub>1</sub>EO) and diethoxylated nonylphenol (NP<sub>2</sub>EO) (Koh et al. 2005). Their persistence  
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3963 in river sediments and several environmental compartments have been investigated (Soares et al.  
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4164 2008) and the demonstrated negative effects of NPnEOs and of NP/NP<sub>1</sub>EO/NP<sub>2</sub>EO on animals and  
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4465 plants induced the Authority to ban in Europe this class of compounds. In fact, starting from 2000,  
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66 NPnEOs but also NP/NP<sub>1</sub>EO/NP<sub>2</sub>EO have been classified as priority hazardous substances (Directive  
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67 2000/60/EC). After few years, other marketing restrictions for NPnEOs were introduced under the  
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5168 Directive 2003/53/EC, while the Directive 2008/105/EC limited (below 0.3 µg/L) the average annual  
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69 concentration of NPnEOs and of NP/NP<sub>1</sub>EO/NP<sub>2</sub>EO in surface waters. Recently, the presence of  
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60 NPnEOs used in textiles, have been limited to 0.01% of weight of textile good produced (Commission  
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6172 regulation EU–2016/26). Despite the legislation in force, NPnEOs, NPs/NPEOs are still frequently  
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60 recorded in the environment.  
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73 Together with NPnEOs and derivatives, pharmaceuticals and personal care products (PPCPs) are  
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274 considered active organic pollutants. The global PPCPs use, coupled with the fast introduction of new  
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575 pharmaceuticals to the market, contributing significantly to the presence of these substances and their  
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776 active metabolites in the aquatic environment (Ebele et al. 2017).

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1077 The most common PPCPs include several pharmaceutical drugs such as analgesics, anti-  
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1278 inflammatory, anti-bacterial, anti-epileptics,  $\beta$ -blockers, etc. (Miege et al. 2009). Non-steroidal anti-  
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1579 inflammatory drugs comprise diclofenac and ketoprofen, which are used to treat rheumatic diseases  
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1780 and suppress inflammation (Cuklev et al. 2012). Other classes of pharmaceuticals considered active  
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2081 organic pollutants detected in wastewaters, include  $\beta$ -blockers like atenolol used to treat human  
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2282 hypertension (Kasprzyk-Hordern et al. 2009). The legislation does not limit the human use of PPCPs  
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2483 and these compounds increase in concentration in the environment with the increase of the population.  
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2784 Since conventional decontamination methods are not able to efficiently remove all these classes of  
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2985 micro-pollutants from wastewaters, new methods and technologies have been tested in the recent  
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3286 years. In this context, the use of herbaceous plants could be considered a good tool for a  
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3487 phytoremediation approach of these micro-pollutants in the aquatic environment. The efficiency of  
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3688 *Phragmites australis* on removing ibuprofen (Kotzya et al. 2010) or herbicides (Schröder et al. 2005)  
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3989 under hydroponic conditions have been described. *Scirpus validus* was also defined able to uptake  
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4190 diclofenac and caffeine from contaminated water (Zhang et al. 2012, 2013).

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4491 Recently, it has been demonstrated that poplar plants are able to take up and metabolize caffeine  
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4692 (Pierattini et al. 2016b), erythromycin, diclofenac and sodium dodecyl sulphate (Pierattini et al.  
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4993 2016a, 2018a,b). The capability of Salicaceae family (i.e. willow) to uptake and degrade organic  
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5194 contaminants from soils and waters has been reviewed by Marmiroli et al. (2011) and *Salix fragilis*  
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5395 was considered useful for phytoremediation of soil spiked with 10 and 200 mg kg<sup>-1</sup> of sulfadiazine  
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5696 (Michelini et al. 2012).

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5897 The aim of this research was: *i*) test the ability of *P. australis* and *S. matsudana* to remove some  
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6198 common micro-pollutants such as NPs, NP<sub>1</sub>EO and NP<sub>2</sub>EO and pharmaceuticals (atenolol,

99 diclofenac, ketoprofen, triclosan) from wastewaters in vegetated CWs; ii) evaluate the removal  
100 efficiency of each specific CW systems in function of arboreal or herbaceous plants used.

## 102 **Materials and methods**

103 The experimental plan was set up within the municipal WWTPs in municipal area of Pagnana  
104 (Tuscany, Italy). A total of 12 experimental replicates – CWs (115\*76\*69 cm) were placed under an  
105 open greenhouse system covered with green net in order to avoid ambient precipitation  
106 (Supplementary Fig. 1). CWs - were filled with 15 cm of gravel (Ø 20-30 mm, porosity of 50%) 45  
107 cm of expanded Agrileca clay (Ø 8-20 mm) and again 5 cm of gravel (Ø 20-30 mm) (Laterlite, Milano  
108 Italy) for a final volume of 115\*76\*55 cm.

109 In spring, the CW systems were planted using two-year-old uniform rooted cuttings of similar height  
110 and weight woody cuttings of *Salix matsudana* Levante (six for CW), provided by Istituto  
111 Sperimentale per la Pioppicoltura (Casale Monferrato, Italy) and *Phragmites australis*. Four CW  
112 systems were used as control without plant inside, four planted with *S. matsudana* and four with *P.*  
113 *australis*. A total of 9 plants were used for CW. The pilot study began once the system was stable. In  
114 order to determine the ability of plants to remove the contaminants, the CWs were programmed in  
115 batch mode during the experiment. After each treatment trial, the pilot was continuously powered  
116 (flow loading 30 mm day<sup>-1</sup>) until the next input wastewater when the CWs were again used in batch  
117 mode.

118 Wastewater was analyzed three times during the experiment (July the 2<sup>nd</sup> and the 30<sup>th</sup>, September the  
119 24<sup>th</sup>), before the treatment in CWs and the parameters pH, conductivity, chemical oxygen demand  
120 (COD), suspended solids (SS), and ammonium nitrogen (NH<sub>4</sub>-N) were determined using standard  
121 methods.

122 In order to test the yields of the final process of water treatment according to seasonal variations (from  
123 July to September), CWs were provided with a leachate collection system and the effluents of each  
124 CW systems (50 ml) were analyzed at 2 or 3 days' interval. During July (from July 2<sup>nd</sup> to 9<sup>th</sup>) and

125 August (from July 30 to August 6), the CWs were set up in batch mode and the wastewater was  
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126 retained in each CWs for 7 days, while in September (from 24 to 28 September) for 4 days.  
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127 At the end of the third trial, leaves of *S. matsudana* and *P. australis* were collected and three biological  
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128 replicates for each set of condition were separately analyzed for phenolic and pharmaceutical contents  
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129 at each sampling time. The 4-*n*-NP (4-*n*-nonylphenol) selected as representative of NPs, NP<sub>1</sub>EO,  
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130 NP<sub>2</sub>EO, atenolol (belonging to the group of “beta blocker”), diclofenac (non-steroidal ant-  
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131 inflammatory drug NSAID), ketoprofen (non-steroidal ant-inflammatory drug NSAID) and triclosan  
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132 (antibacterial and antifungal agent) were quantified in the water used as input in CWs, in water  
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133 collected as CWs output and also in leaves of both plant species studied.  
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134 The wastewater samples were spiked with 25 ng of [3,5-<sup>2</sup>H<sub>2</sub>]-NP<sub>1</sub>-EO, [3,5-<sup>2</sup>H<sub>2</sub>]-NP<sub>2</sub>-EO and <sup>13</sup>C<sub>6</sub>-  
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135 Diclofenac as internal standards to account for process losses acidified to pH 2.8-3.0 and extracted  
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136 three times with an equal volume of dichloromethane (DCM). The DCM extracts were reduced to  
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137 lower volumes with a rotary evaporator and dried under a gentle flow of dry nitrogen for  
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138 derivatization (Yang et al. 2011). All chemicals used for analyses set up, were purchased from Sigma-  
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139 Aldrich, Germany.  
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140 Vegetative tissues (1 g) were added with 50 ng of [3,5-<sup>2</sup>H<sub>2</sub>]-NP<sub>1</sub>-EO, [3,5-<sup>2</sup>H<sub>2</sub>]-NP<sub>2</sub>-EO and <sup>13</sup>C<sub>6</sub>-  
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141 Diclofenac as internal standards to account for process losses, homogenized under liquid nitrogen  
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142 and extracted with 10 ml in water with stirrer. Samples were centrifuged at 12,000 rpm and 4 °C for  
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143 15 min, acidified to pH 2.8-3.0 and the water phase was partitioned with DCM (1:1 v/v). The DCM  
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144 extracts were reduced to lower volumes with a rotary evaporator and dried under a gentle flow.  
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145 Derivatization was performed with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) containing  
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146 1% trimethylchlorosilane for 20 minutes at 70 °C following the protocol of Samaras et al. (2011).  
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147 Quantification was accomplished by GC–MS/MS analysis by a Saturn 2200 quadrupole ion trap mass  
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148 spectrometer coupled to a CP-3800 gas chromatograph (Varian Analytical Instruments, Walnut  
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149 Creek, CA, USA) equipped with a MEGA 1 MS capillary column (30 m; 0.25 mm i.d., 0.25 µm film  
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150 thickness, MEGA s.n.c., Milan, Italia). The carrier gas was helium, which was dried and air free, with  
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151 a linear speed of 60 cm s<sup>-1</sup>. The oven temperature was maintained at 80 °C for 1 min, increased to  
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152 210 °C at a rate of 15 °C/min, further increased to 235 °C at a rate of 5 °C/min and further increased  
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153 to 300 °C at a rate of 20 °C/min. Full-scan mass spectra were obtained in EI<sup>+</sup> mode with an emission  
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154 current of 10 µA and an axial modulation of 4 V. Data acquisition was from 150 to 450 Da at a speed  
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155 of 1.4 scan s<sup>-1</sup>.

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156 The acquisition of the mass spectra was obtained by singular ions system (SIS). **In the case of**  
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157 **uncertainty on the identification of the analyte the re-fragmentation of a characteristic ion (MS/MS)**  
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158 **was adopted.** The quantification of the molecules was carried out by comparing the peak area of one  
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159 or more ions characteristic of the internal standard with that obtained for the corresponding analyte.  
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160 For compounds that do not have the internal standard, e.g: atenol, ketoprofene and triclosan, the  
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161 quantification was performed using the calibration curve with these compounds. **The minimum level**  
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162 **of quantification (LOQ) and the minimum level of detection (LOD) were monitored daily with**  
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163 **standard and with the signal/noise ratio respectively.**

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164 Removal efficiency of micro-pollutants was evaluated by means of Eq. (1): Removal efficiency (%)  
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165 =  $(M_{inf} - M_{eff}) / M_{inf} \times 100$ , where  $M_{inf}$  is the load of micro-pollutant in CW influent and  $M_{eff}$  is the  
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166 load of micro-pollutant in CW systems effluent.

## 168 **Statistical analysis**

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169 At each sampling time, data (n=3) of 4-n-NP, NP<sub>1</sub>EO, NP<sub>2</sub>EO, atenolol, diclofenac, ketoprofen and  
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170 triclosan, **in each CWs**, were subjected to a one-way analysis of variance (ANOVA). Separation of  
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171 means was done using a Bonferroni's multiple comparison test at P=0.05. Unpaired *t*-test analysis at  
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172 0.05 probability level was performed on data about concentrations of pharmaceuticals and  
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173 nonylphenols in leaves of *Salix matsudana* Levante and *Phragmites australis* and on removal  
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174 efficiency of CW. Statistical analysis was performed using NCSS 2000 Statistical Analysis System  
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175 Software.

177 **Results and Discussion**

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278 During the study period, water samples from the CWs influent were collected and analyzed in July  
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179 the 2<sup>nd</sup> and the 30<sup>th</sup> and September the 24<sup>th</sup>. The average wastewater quality parameters of the influent  
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180 in the three sampling times were: 7.4±0.30 pH, 2233±105.9 µS/cm conductivity, 31.6±2.55 mg/L  
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181 COD, 9±2 mg/L SS and 1±0.98 mg/L NH<sub>4</sub><sup>+</sup>-N (Table 1). As far as the concentration of the selected  
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182 micro-pollutants analyzed (Supplementary Table 1), it has changed significantly during the  
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183 experimental period following a specific trend month by month, but always in the range of ng ml<sup>-1</sup>.  
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184 The presence of PPCPs (ketoprofen, diclofenac, atenolol and triclosan) and NPs in the influent  
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185 wastewater, confirmed their widespread use and high frequency of detection reported in previous  
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186 studies (Matamoros and Bayona, 2006; Matamoros et al. 2007; Soares et al. 2008).  
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187 The main physical-chemical and biological mechanisms contributing to the elimination of micro-  
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188 pollutants in CWs are: *i*) photolytic degradation, *ii*) sorption, *iii*) plant uptake, *iv*) microbial  
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189 degradation. Moreover, some CW design parameters could have an effect on micro-pollutants  
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190 removal such as: configuration of CWs, presence and types of vegetation, operational mode, soil  
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191 matrix and hydraulic retention time (Zang et al. 2014).  
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192 In this applicative research, we focused the attention on plant uptake ability (presence and type of  
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193 vegetation used), since the role carried out by plants within a CW wastewater treatment system,  
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194 results to be crucial (Lee and Scholz, 2007, Dordio et al., 2009; Zhang et al., 2012) and to better  
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195 understand the influence of plants, we assessed the comparative removal efficiency of planted CWs  
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196 with herbaceous on one hand and arboreal plant species on the other hand (*P. australis* -CWs and *S.*  
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197 *matsudana* -CWs) with reference to unplanted CWs (control).  
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198 The growth substrate matrix is considered very important in CWs, not only because it supports the  
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199 growth of plants and microorganisms, but also because it can adsorb the micro-pollutants. Therefore,  
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200 the solid matrix can play an important role in contaminant retention (Dordio et al. 2011). Clay and  
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201 gravel are commonly used materials in CWs approaches in order to assure homogenized water flow.  
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202 Some authors found that among different substrates (vesuvianite, gravel, and zeolite), gravel appeared



203 to be the most efficient filter material (Xiaoyan et al. 2015). The sorption capacity of light expanded  
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204 clay aggregates was proved evaluating the ability to remove carbamazepine, clofibric acid, and  
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205 ibuprofen (Dordio et al. 2009). In July, in CWs with only clay and gravel, the removal capability for  
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206 diclofenac, ketoprofen, and atenolol was lower compared to CWs vegetated with willow and common  
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207 reed, indicating the importance to introduce also plants for improving the removal activity of micro-  
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208 pollutants (Fig. 1, 2).  
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209 Only for the pharmaceutical triclosan the concentration removed by each CWs (in the first sampling  
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210 time) was independent to the setup used. In fact, when triclosan concentration was lower into  
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211 wastewater input, the three different CW systems tested were able to remove totally the compound  
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212 (Fig. 2B). Triclosan was not detectable until some days after the first application. On the 2<sup>nd</sup> August,  
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213 on the contrary, the concentration of triclosan in effluents of non-planted CWs was higher than in the  
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214 influent. This result could due to a delay in the transport/diffusion through the gravel and clay layer,  
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215 since soil matrix has a direct effect on the sorption as it has been demonstrated in previous works  
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216 (Zang et al. 2014). On the other hand, it is worth mentioning that the bacterial biofilms eventually  
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217 colonizing the gravel and clay, substrate of plant growth, might be responsible for the transformation  
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218 and eventually degradation of contaminants. In fact, in our experimentation the depletion of the  
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219 contaminants in the unplanted CWs can be associated to both the adsorption and transformation  
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220 capacity of the bacterial community colonizing the substrate. On the other hand, the depletion of the  
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221 planted CWs can be associated to the combination of the adsorption and degradation of the bacterial  
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222 community colonizing the gravel and clay and the uptake capacity of the plant. However, in this  
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223 context, the role of bacteria colonizing the plant growth substrate was not studied, because of the  
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224 reasonable consideration that the plant capacity to uptake the micro-pollutants was predominant on  
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225 the bacterial capacity to transform and eventually degrade the adsorbed one. As a matter of the fact,  
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226 the bacterial load (*E. coli*), routinely measured before the discharge in superficial water, at the end of  
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227 the pipeline of the WWTP of Pagnana, was null as shown in Table 1. Assuming that the total bacterial  
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228 load entering in the CWs was low and not necessarily specialized in the degradation of the  
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229 contaminants of interest, since deriving from the activated sludge not capable to transform the micro-  
1 pollutants during the time of residence in the WWTP and considering that the residence time of the  
230 wastewater in the CWs was even shorter, in this phase of the study, we considered the contribution  
231 of the microbial community in micro-pollutants removal, negligible, if compared to the possible  
232 uptake capacity of the different plant species. This assumption was adopted for all the micro-  
233 pollutants of interest.

234 Atenolol was the pharmaceutical detected at higher concentrations in influent wastewater. In July the  
235 2<sup>nd</sup>, Atenolol reached the values of 13.6 ng mL<sup>-1</sup> in the wastewater influent, then declined during the  
236 experimental period with values below 0.5 ng mL<sup>-1</sup> in CW effluents (Fig. 1A).

237 It appeared evident that the amount of wastewater produced plays a role on the concentration of  
238 compounds investigated. The lower input of all pharmaceuticals analyzed was observed in the month  
239 of August and could be related to the paucity of the people in the city area studied and consequently  
240 in the quality of wastewater produced in this period.

241 In August, CWs with willow and common reed, showed similar ability to uptake contaminants (Figs.  
242 1, 2) while, in September, *P. australis*-CWs exhibited the higher capability to remove the  
243 pharmaceutical atenolol, ketoprofen, and triclosan from wastewater (Fig. 1A, 2).

244 In relation to the concentration on NP and ethoxylates a clear decline of these compounds in  
245 wastewater input was observed during the experiment (Fig. 3). It is interesting to note that the amount  
246 of 4-n NP and NP<sub>2</sub>EO measured in July in effluents of empty CWs increased with the time. The  
247 NP<sub>2</sub>EO was even higher than in influent. This increase of concentration in the effluent compared to  
248 influent in the month of July, can derive from a previous accumulation of NP<sub>2</sub>EO during the  
249 continuous flow CWs setup before the experiment started. A subsequently delay in the diffusion  
250 through the gravel and clay could be the cause of the observed phenomena.

251 As for pharmaceuticals the empty CWs with only clay and gravel showed a lower capability to remove  
252 phenols compared to those containing *P. australis* or *S. matsudana*, while similar capability to remove  
253 NPs from wastewater was observed between willow and common reed (Fig. 3).

255 Multiple studies using different pharmaceuticals and different macrophyte species have been carried  
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256 out indicating that specific macrophytes could influence the removal efficiencies of pharmaceuticals  
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257 (Hijosa-Valsero et al. 2010; Zarate et al. 2012). Hijosa-Valsero et al. (2010) reported that *P. australis*  
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258 was more efficient than *Typha angustifolia* for the removal of ibuprofen, diclofenac, caffeine, and  
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259 methyl dihydrojasmonate. Stevens et al. (2009) also provided the evidence that specific differences  
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1260 existed for accumulation of triclosan in CWs for *Bidens frondosa* and *Sesbania herbacea* plants. To  
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261 date, there is very little information about the use of macrophyte selection for both pharmaceuticals  
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1262 and NPs removal in CWs. Most of the studies concerning plant uptake are based on herbaceous  
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263 species such as *Scirpus* (Zhang et al. 2012; 2013), *Phragmites* (Di Gregorio et al. 2015), and *Vicia*  
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264 *faba* (Sjöström et al. 2008), while very few studies investigated the removal efficiency of these  
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265 compounds by tree species (Iori et al. 2013; Pierattini et al. 2016a,b; 2018). The selection of the plant  
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266 species is recognized as an important element for contributing to the efficiency of CW for the removal  
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267 of pollutants as well as the selection of the appropriate phytoremediation strategies. The research on  
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268 the plant species with specific ability to take up organic contaminants is very important and need to  
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269 be further explored.

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270 Under our experimental conditions, the removal efficiency of plant system ranged from 8.4% up to  
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271 100%, with higher value for triclosan in both CW systems studied (Fig. 4). On the contrary, the  
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272 removal efficiency of NPs appeared lower than pharmaceuticals, with some cases of inefficiency for  
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273 NP<sub>1</sub>EO and NP<sub>2</sub>EO removal from CW with *P. australis* or *S. matsudana* (Fig. 5).

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274 Throughout the exposure period, no plant mortality was recorded on *S. matsudana* (Levante) or *P.*  
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275 *australis*, suggesting that the tested micro-pollutants and the concentrations applied did not  
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276 compromise the survival of the plants. These results agree with the literature showing that compounds  
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277 such as diclofenac, ketoprofen, and atenolol do not exhibit acute toxicity or lethal effects on  
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278 organisms at the concentration levels of 1.0 mg L<sup>-1</sup> (Prášková et al. 2013).

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279 Anyway, a different pattern of pharmaceuticals accumulation in the harvestable part of *P. australis*  
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280 and *S. matsudana* was observed (Fig. 6). Considering the single compound, atenolol accumulation in

281 leaves of common reed was above  $80 \mu\text{g g}^{-1}$  FW, eight times higher than willow ( $10 \mu\text{g g}^{-1}$  FW). The  
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282 amount of atenolol accumulated by plants was one order of magnitude more than the other compounds  
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283 (Fig. 6). The high amount of atenolol in plant tissues could be related to the higher amount of this  
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284 compound detected in the influent but also to its physical-chemical properties such as water solubility.  
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285 The water soluble organic compounds, such as atenolol, could be more easily taken up by the plant  
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1286 roots and then translocated to shoot tissues. The ability of vegetables to accumulate atenolol was  
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1287 demonstrated in tomato and potato grown in soils fertilized with commercial biosolids (Sabourin et  
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1288 al. 2012). Moreover, the greenhouse experiment of Wu et al. (2012) demonstrated, for the first time,  
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1289 the ability of plant to take up atenolol and other compounds in tomato, carrot, broccoli, bell pepper,  
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2290 and spinach. In both cases the concentration of atenolol found was in the range of  $\text{ng g}^{-1}$  DW, one  
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2291 order of magnitude less than *P. australis* and *S. matsudana* plants.  
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2292 Photolytic degradation in CWs depends on light intensity and light attenuation by water depth (Zhang  
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2293 et al. 2014). During the batch mode setup, diclofenac, ketoprofen, and triclosan, can undergo a  
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2294 photolytic degradation and this process may play a major role in elimination of these compounds as  
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2295 also observed in free water-surface-CWs or hydroponic systems (Zhang et al. 2014).  
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2296 The capability of plants to take up ketoprofen is poorly documented and in several studies this  
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2297 molecule is not determined (Goldstein et al. 2014). Also for triclosan there are few published papers  
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2298 reporting its uptake, but recently it was demonstrated that *Eichhornia crassipe* and *Pistia stratiotes*  
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2299 are able to remove 30.4% and 67.1% respectively of triclosan added in a nutrient solution (Victor et  
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2300 al. 2016). Willow plants take up ketoprofen and triclosan at higher concentrations than common reed  
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2301 ( $125 \text{ ng g}^{-1}$  FW vs  $75 \text{ ng g}^{-1}$  FW and  $90 \text{ ng g}^{-1}$  FW vs  $50 \text{ ng g}^{-1}$  FW, respectively), while diclofenac  
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2302 concentration in leaves of *P. australis* (Fig. 6) was double compared to leaves of *S. matsudana* ( $15$   
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2303  $\text{ng g}^{-1}$  FW and  $7.5 \text{ ng g}^{-1}$  FW, respectively). Herbaceous species such as *Scirpus* and *Typha* have been  
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2304 studied in mesocosms setup under diclofenac (Zhang et al. 2012; Bartha et al. 2014), and in particular  
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2305 *Scirpus validus* has been found to be tolerant up to  $2 \text{ mg L}^{-1}$  of diclofenac (Zhang et al. 2012). The  
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2306 low amount detected in leaves of *P. australis* and *S. matsudana* (not more than  $20 \text{ ng g}^{-1}$  FW)  
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307 compared to other pharmaceuticals studied, could be associated to its sensibility to photodegradation  
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308 under UV radiations (Zhang et al. 2012). Pierattini et al. (2018a) observed a rapid photolysis of  
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309 diclofenac that resulted in only 30% of the diclofenac initial concentration remaining in water within  
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310 3 days of exposure to sunlight. Moreover, this chemical has been classified as poorly degradable as  
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311 confirmed by Garcia-Rodríguez et al. (2015) using *Lemna* plants. Since photo-degradation process  
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314 In the same way to pharmaceuticals, the accumulation in the harvestable part of NPs/NPsEO was  
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Plant organic micro-pollutants uptake and translocation to the aerial parts is recognized to be related  
to the octanol-water partition coefficient ( $\log K_{ow}$ ) of the molecule (Miller et al. 2016), but contrasting  
results derived from correlation between pharmaceuticals uptake in plant and different  $\log K_{ow}$   
values. Although Briggs et al. (1982) demonstrated that the uptake of many *O*-  
methylcarbamoyloximes chemical and substituted phenylureas into plant tissues of barley is directly  
proportional to  $\log K_{ow}$ , Zhang et al. (2013) reported that there was no significant correlation between  
 $\log K_{ow}$  of 5 pharmaceuticals and the uptake concentration in *S. validus*.

*P. australis* and *S. matsudana* demonstrated different trend of correlation between micro-pollutants  
uptake in plant and  $\log K_{ow}$  (Supplementary material Fig. 2), in fact, *P. australis* plants are able to  
translocate in the aerial part higher amounts of the micro-pollutants with lower  $\log K_{ow}$ . These data  
suggest that different macrophyte could be associated in the phytoremediation approach in order to  
obtain maximum results in terms of contaminants uptake.

## Conclusion

333 The removal of micro-pollutants from wastewaters in CW system is a result of different complex  
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334 processes. Regarding the ability of different macrophyte species to bioaccumulate pharmaceuticals  
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335 and NPs/NPsEO, this research demonstrates that *P. australis* and *S. matsudana* are able to remove  
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336 these contaminants from wastewater. In fact, *P. australis* uptakes efficiently 4-*n*-NP, diclofenac and  
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337 atenolol, while *S. matsudana* can translocate in the aerial part preferentially NP<sub>1</sub>EO, NP<sub>2</sub>EO,  
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338 ketoprofene, and triclosan. The different plant uptake of micro-pollutants suggests a very interesting  
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339 use of the consociation of different species to remove, in a more effective way, xenobiotic compounds  
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340 from wastewater. CWs with clay and gravel as substrates and willow/common reed as plant species  
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341 can be considered additional systems to conventional wastewater treatments for improving the micro-  
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342 pollutants removal.  
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32  
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36 University of Pisa and Acque Spa  
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471 Table 1. Average wastewater quality parameters (means  $\pm$  standard deviation) of the influent in the  
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 472 tree sampling times (July the 2<sup>nd</sup> and the 30<sup>th</sup> and September the 24<sup>th</sup>). nd= not detected.  
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<b>Parameters</b>	
pH	7.4 $\pm$ 0.3
Conductivity ( $\mu$ S/cm)	2233 $\pm$ 106.0
Total Solid sediments (mL/L)	9.0 $\pm$ 2.0
BOD (mg/L O <sub>2</sub> )	nd
COD (mg/L O <sub>2</sub> )	31.7 $\pm$ 2.5
N (mg/L)	4.1 $\pm$ 1.7
NH <sub>4</sub> (mg/L)	1.0 $\pm$ 0.9
N-NO <sub>2</sub> (mg/L)	0.2 $\pm$ 0.2
NO <sub>3</sub> (mg/L)	4.6 $\pm$ 2.5
N- <i>tot</i> (mg/L)	8.2 $\pm$ 4.4
P- <i>tot</i> (mg/L)	0.2 $\pm$ 0.0
Cl <sup>-</sup> (mg/L)	414 $\pm$ 34.6
SO <sub>4</sub> (mg/L)	115 $\pm$ 9.5
<i>E. coli</i> UCF (100 mL)	nd

486 Figure legends

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**Fig. 1.** Temporal variability of contaminants concentration atenolol (A) and diclofenac (B), in wastewater input, and in effluent from control constructed wetland (CW) systems with clay and sand, CW with *Phragmites australis* or *Salix matsudana*. Data represent means  $\pm$  standard deviation of three replicates. For each sampling time mean were analyzed with one-way ANOVA. **At each sampling time statistically significant differences among CWs system have been calculated with Bonferroni post-test ( $P < 0.05$ ) and indicated with different letters.** ns= not significant.

**Fig. 2.** Temporal variability of contaminants ketoprofen (A) and triclosan (B), in wastewater input, and in effluent from control constructed wetland systems (with clay and sand), with *Phragmites australis* or with *Salix matsudana*. Data represent means  $\pm$  standard deviation of three replicates. For each sampling time mean were analyzed with one-way ANOVA. **At each sampling time statistically significant differences among CWs system have been calculated with Bonferroni post-test ( $P < 0.05$ ) and indicated with different letters.** ns= not significant.

**Fig. 3.** Temporal variability of 4-nNP (nonylphenol, A), NP1EO (nonylphenol-monoethoxylate, B), NP2EO (nonylphenol-diethoxylate, C), in wastewater input, and in effluent from control constructed wetland systems (with clay and sand), with *Phragmites australis* or with *Salix matsudana*. Data represent means  $\pm$  standard deviation of three replicates. For each sampling time mean were analyzed with one-way ANOVA. **At each sampling time statistically significant differences among CWs system have been calculated with Bonferroni post-test ( $P < 0.05$ ) and indicated with different letters.** ns= not significant.

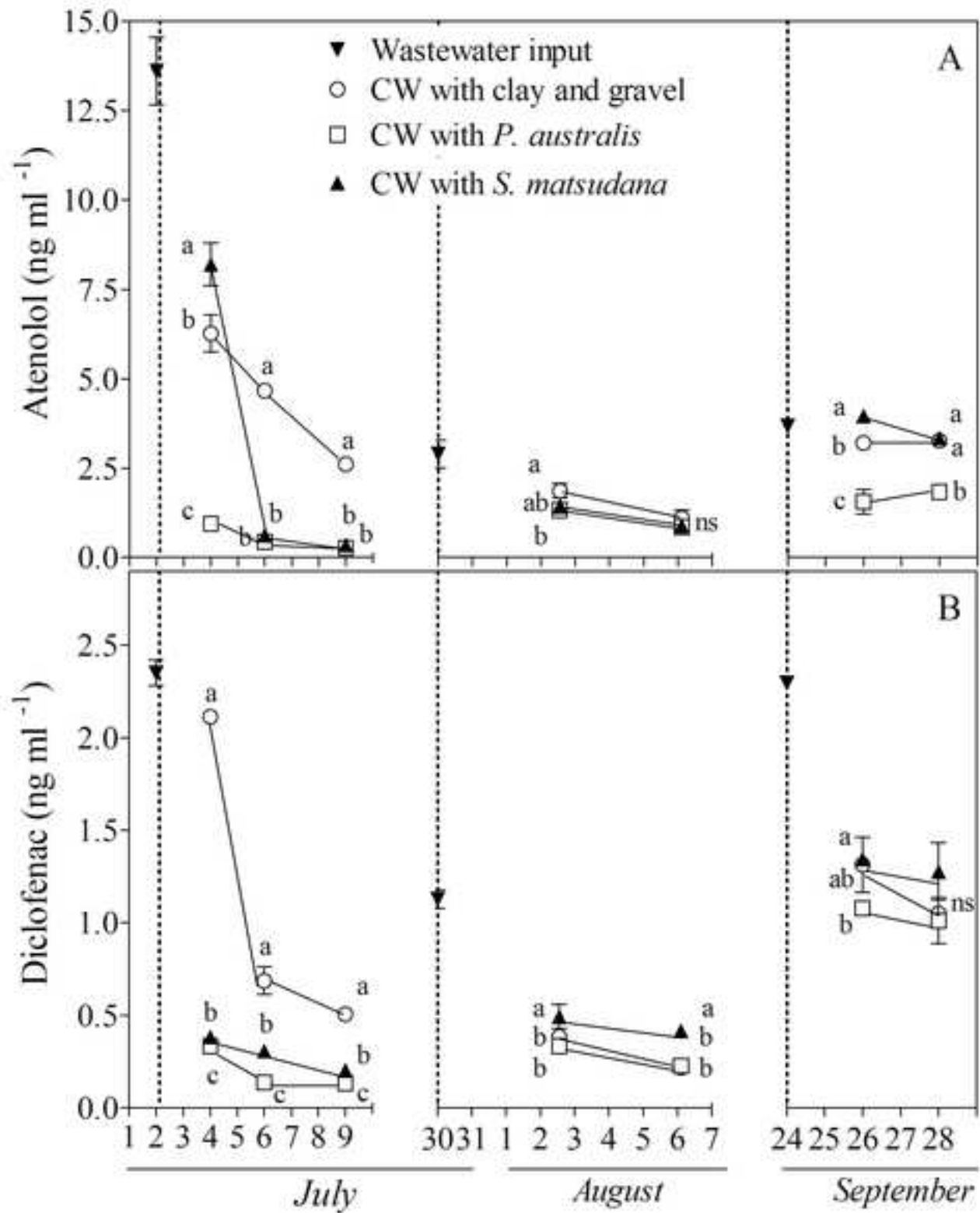
**Fig. 4.** Removal efficiency (%) of pharmaceutical atenolol, diclofenac, triclosan and ketoprofen from contaminated water in constructed wetland systems with *Phragmites australis* or with *Salix*

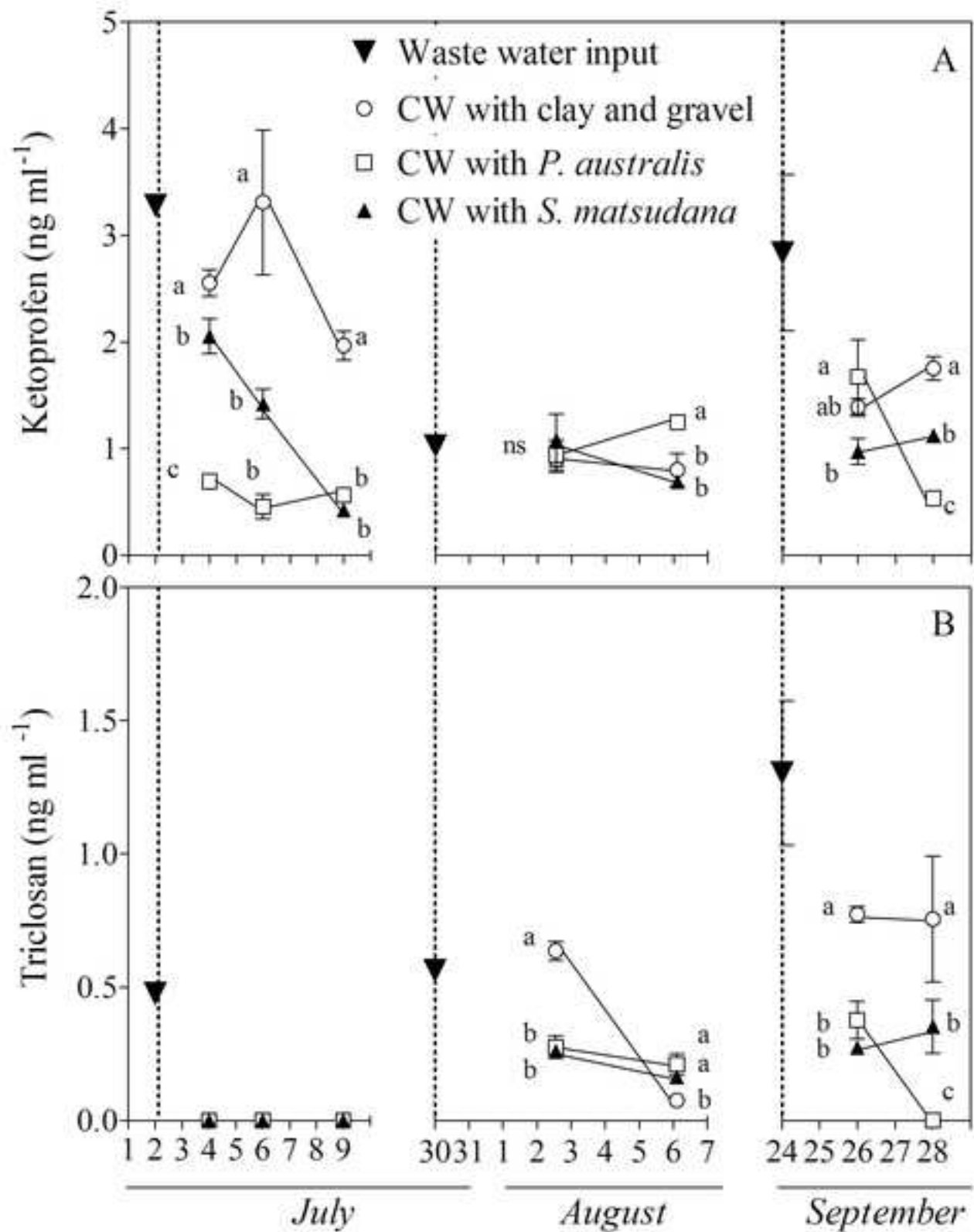
512 *matsudana*. Data represent means of three replicates. *t*-test analysis was performed at each sampling  
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513 time: ns, not significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

514  
515 **Fig. 5.** Removal efficiency (%) of 4-*n*-NP (nonylphenol), NP<sub>1</sub>EO (nonylphenol-monoethoxylate),  
516 NP<sub>2</sub>EO (nonylphenol-diethoxylate), from contaminated water in constructed wetland systems with  
517 *Phragmites australis* or with *Salix matsudana*. Data represent means of three replicates. *t*-test analysis  
518 was performed at each sampling time: ns, not significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

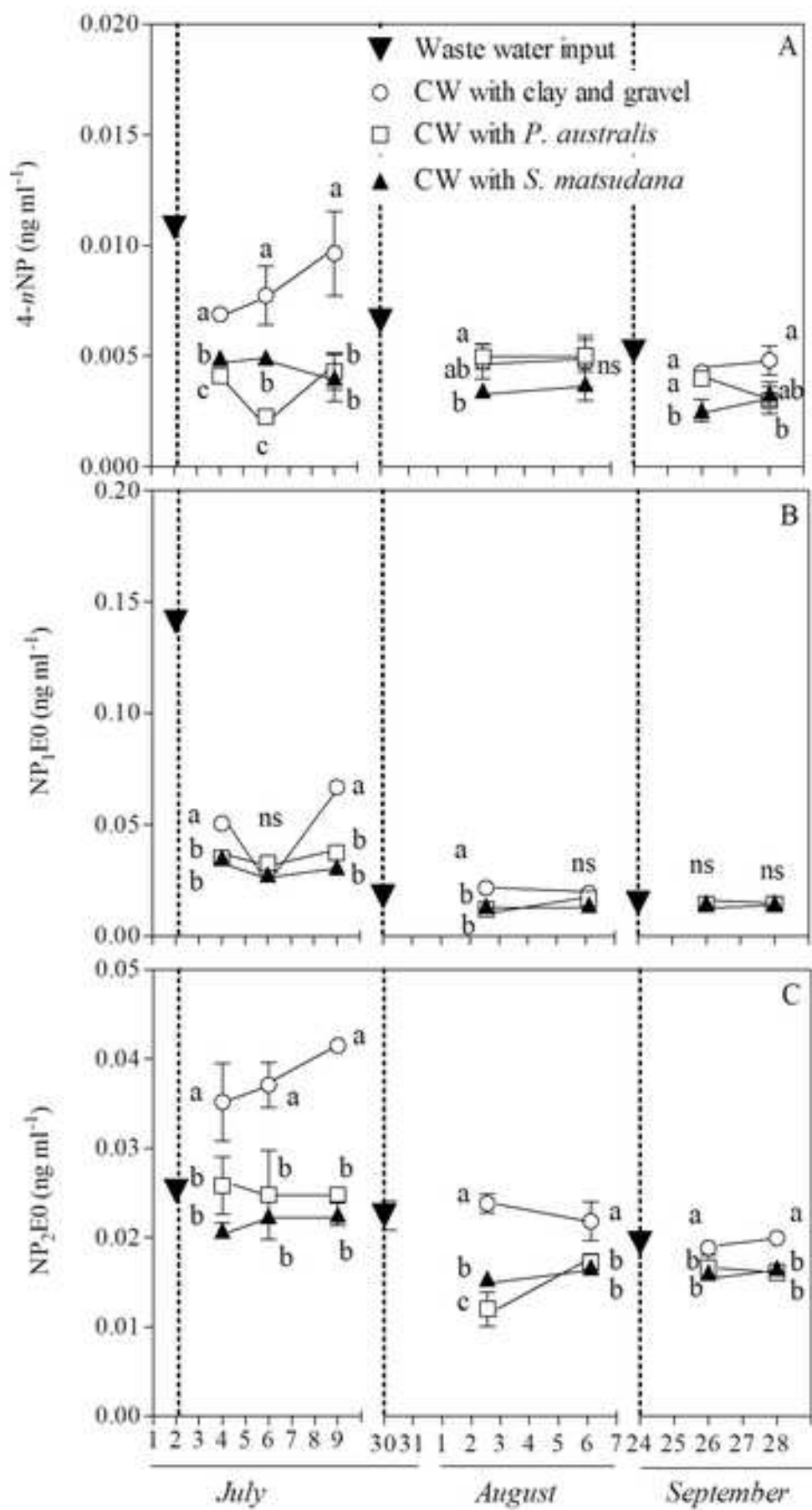
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520 **Fig. 6.** Concentrations of diclofenac, ketoprofen, triclosan (ng g<sup>-1</sup> FW) and atenolol (μg g<sup>-1</sup> FW) in  
521 *Phragmites australis* or *Salix matsudana* plants after 90 days of growth into constructed wetland  
522 systems. Data represent means + standard deviation of three biological replicates. *t*-test analysis was  
523 performed at each sampling time: ns, not significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

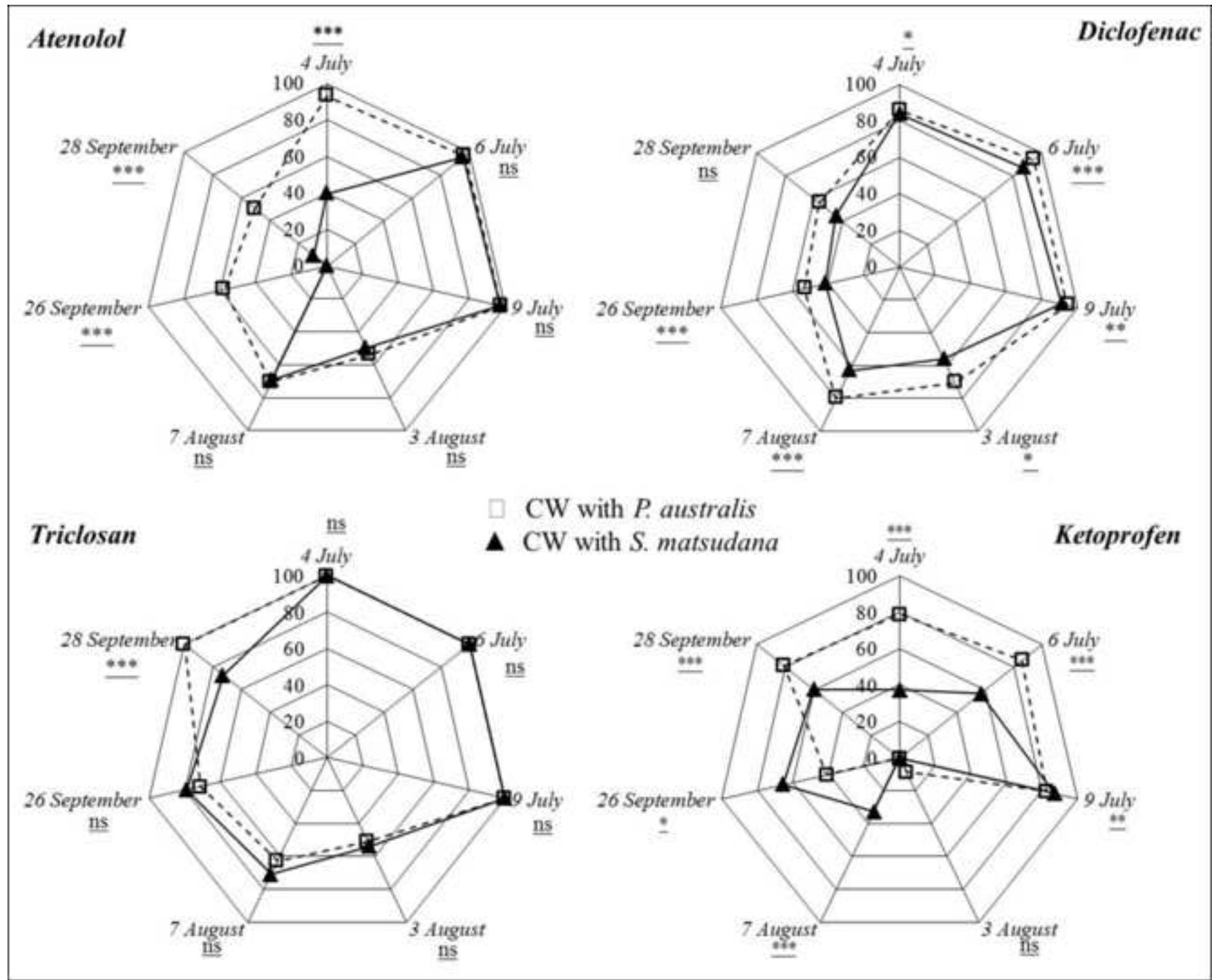
524  
525 **Fig. 7.** Concentrations of 4-*n*NP (nonylphenol), NP<sub>1</sub>EO (nonylphenol-monoethoxylate), NP<sub>2</sub>EO  
526 (nonylphenol-diethoxylate) (ng g<sup>-1</sup> FW) in *Phragmites australis* or *Salix matsudana* plants after 90  
527 days of growth into constructed wetland systems. Data represent means + standard deviation of three  
528 biological replicates. *t*-test analysis was performed at each sampling time: ns, not significant; \*  
529 P<0.05; \*\*P<0.01; \*\*\*P<0.001.

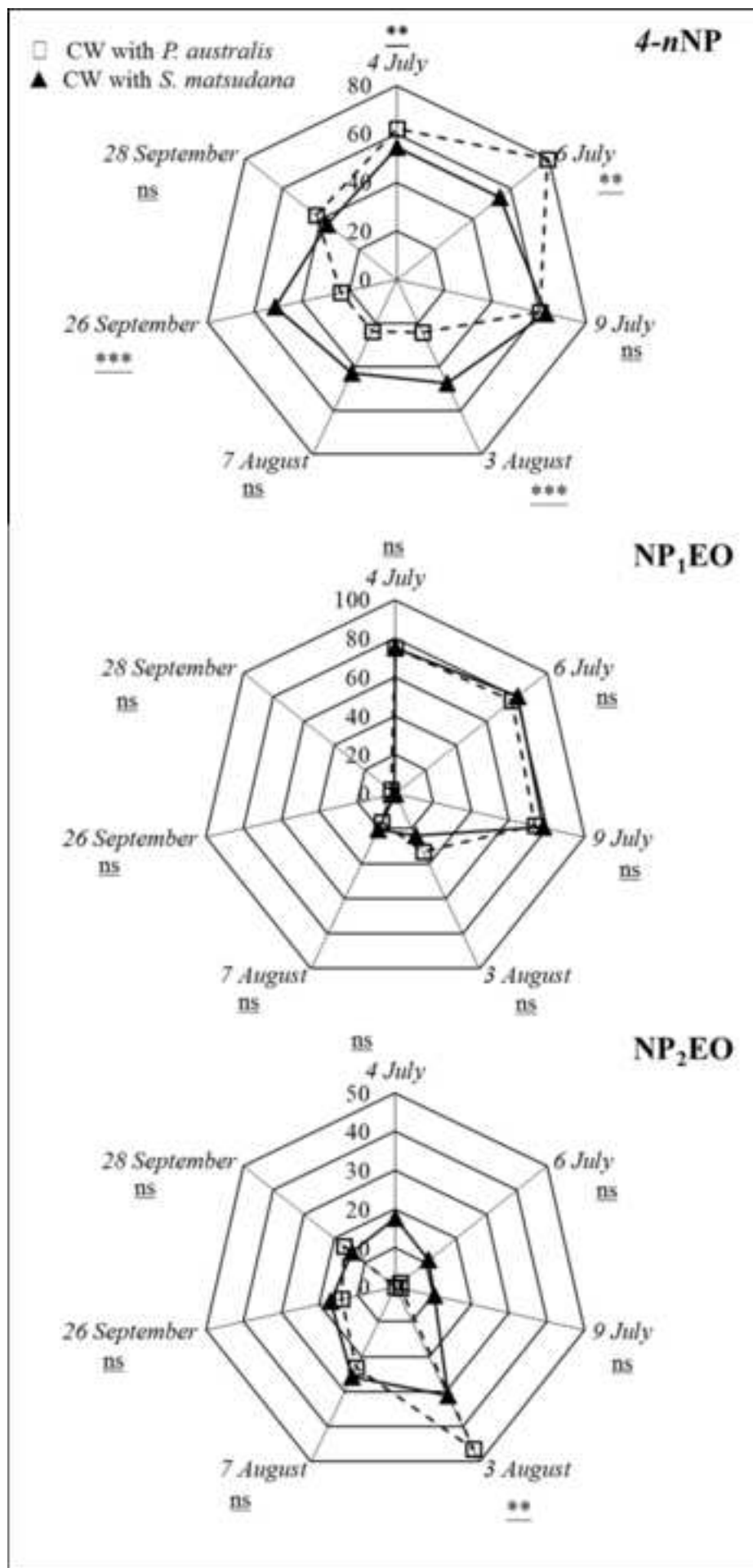


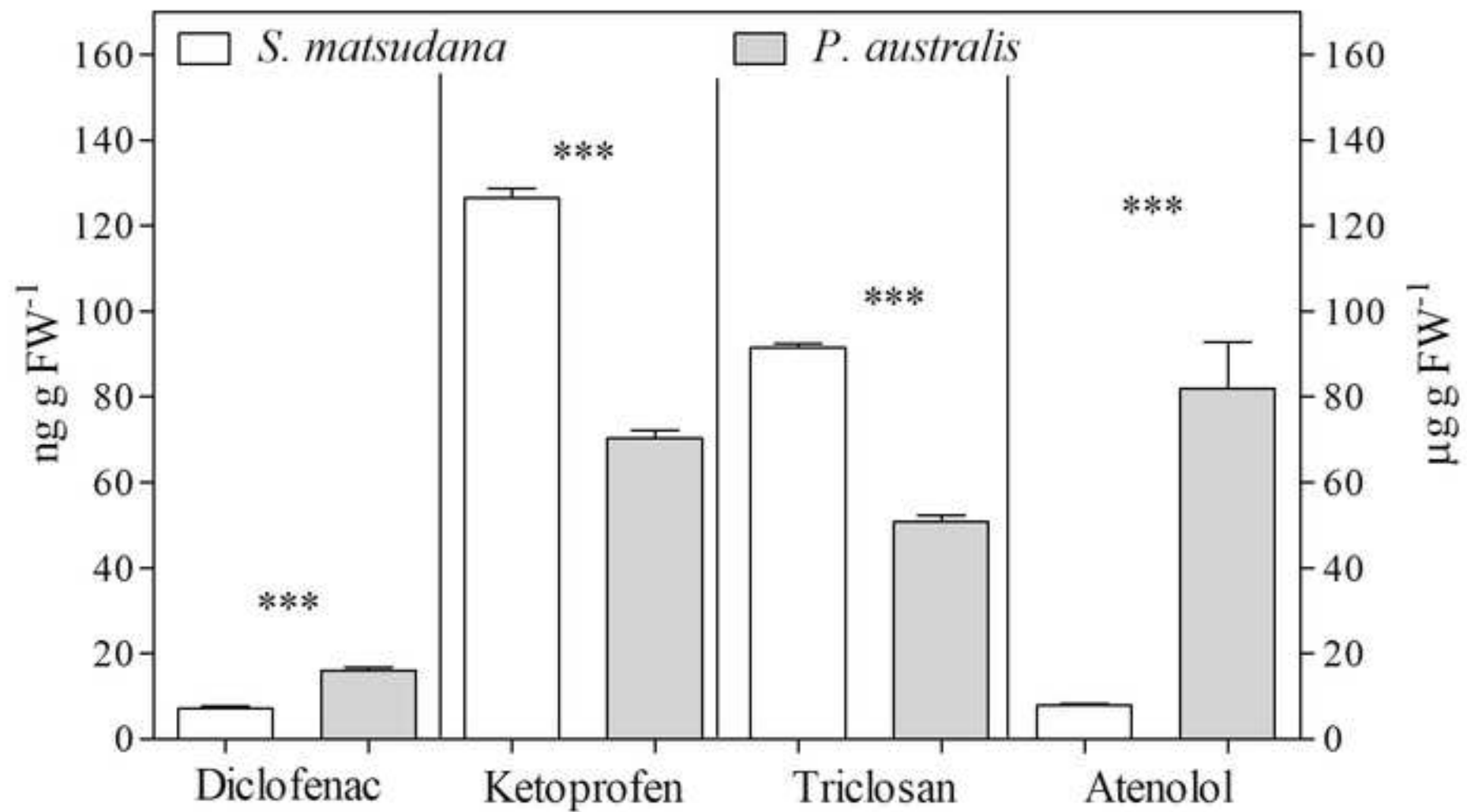












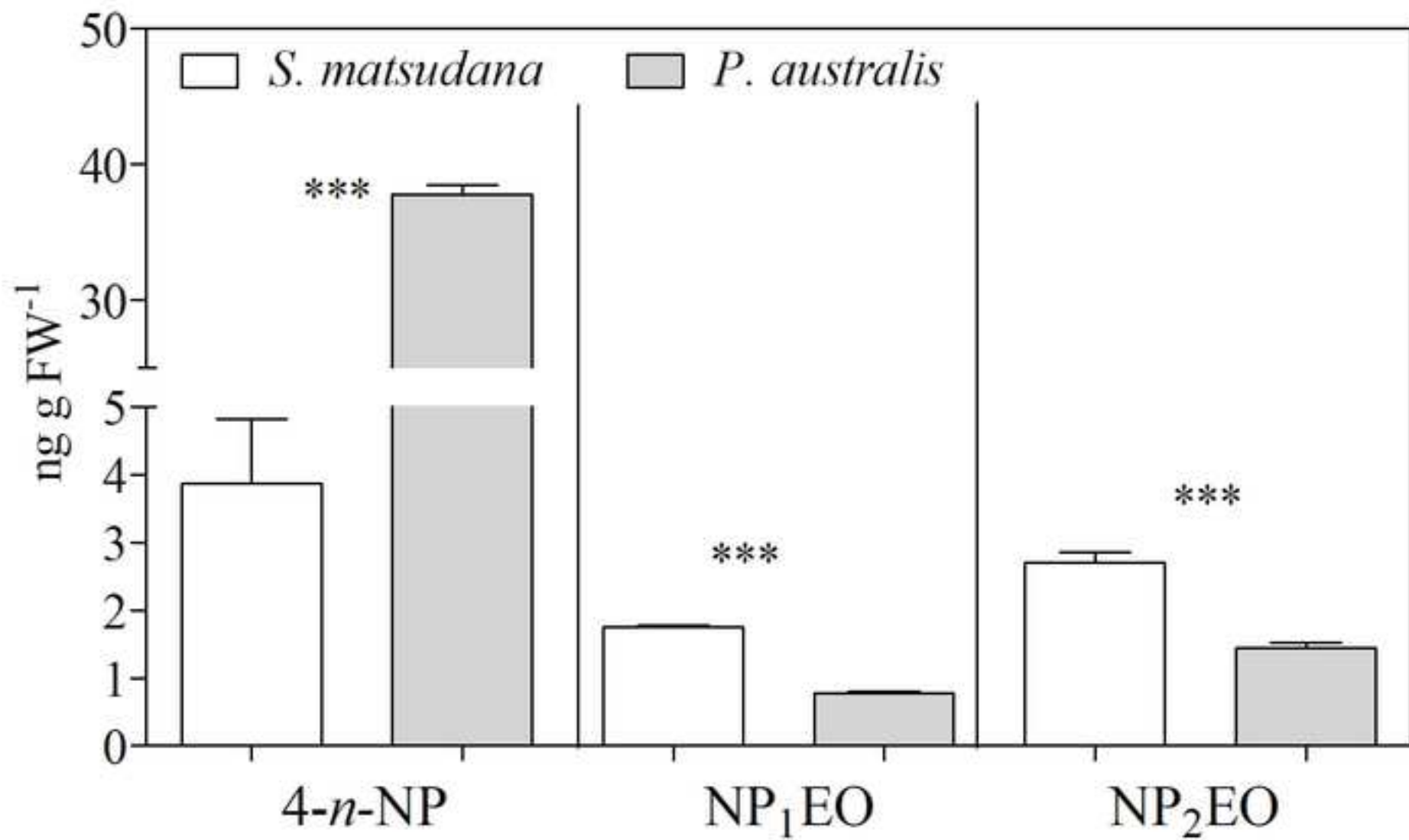


Table 1. Average wastewater quality parameters (means  $\pm$  standard deviation) of the influent in the tree sampling times (July the 2<sup>nd</sup> and the 30<sup>th</sup> and September the 24<sup>th</sup>). nd= not detected.

<b>Parameters</b>	
pH	7.4 $\pm$ 0.3
Conductivity (mS/cm)	2233 $\pm$ 106.0
Total Solid sediments (mL/L)	9.0 $\pm$ 2.0
BOD (mg/L O <sub>2</sub> )	nd
COD (mg/L O <sub>2</sub> )	31.7 $\pm$ 2.5
N (mg/L)	4.1 $\pm$ 1.7
NH <sub>4</sub> (mg/L)	1.0 $\pm$ 0.9
N-NO <sub>2</sub> (mg/L)	0.2 $\pm$ 0.2
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SO <sub>4</sub> (mg/L)	115 $\pm$ 9.5
<i>E. coli</i> UCF (100 mL)	nd



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**Supplementary Material**  
SM\_Fig1,2 and Table1 (1).docx

