Removal of micro-pollutants from urban wastewater by constructed wetlands with *Phragmites australis* and *Salix matsudana*

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Abstract

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_1EO) and diethoxylated nonylphenol (NP_2EO); 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 red 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_1EO, NP_2EO, 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 •

Introduction

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

Polyethoxylated nonylphenols (NPnEOs) are non-ionic surfactants that are used in the tannery industry (Langford and Lester, 2002), but also as domestic surfactants in dispersants, emulsifiers, detergents, dyes, pesticides, cosmetics, etc. (Soares et al. 2008). NPnEOs are only partially degraded in conventional wastewater treatment plants (WWTPs), and the main problem related to these surfactants is the evidence that the conventional bacterial metabolic activity of the activated sludges is capable to depolymerize the ethoxylated group producing compounds more toxic and resistant to biological degradation with reference to NPnEOs, such as nonylphenols (NPs), monoethoxylated nonylphenol (NP_1EO) and diethoxylated nonylphenol (NP_2EO) (Koh et al. 2005). Their persistence in river sediments and several environmental compartments have been investigated (Soares et al. 2008) and the demonstrated negative effects of NPnEOs and of NP/NP_1EO/NP_2EO on animals and plants induced the Authority to ban in Europe this class of compounds. In fact, starting from 2000, NPnEOs but also NP/NP_1EO/NP_2EO have been classified as priority hazardous substances (Directive 2000/60/EC). After few years, other marketing restrictions for NPnEOs were introduced under the Directive 2003/53/EC, while the Directive 2008/105/EC limited (below 0.3 µg/L) the average annual concentration of NPnEOs and of NP/NP1EO/NP2EO in surface waters. Recently, the presence of NPnEOs used in textiles, have been limited to 0.01% of weight of textile good produced (Commission regulation EU–2016/26). Despite the legislation in force, NPnEOs, NPs/NPEOs are still frequently recorded in the environment.

Together with NPnEOs and derivatives, pharmaceuticals and personal care products (PPCPs) are considered active organic pollutants. The global PPCPs use, coupled with the fast introduction of new pharmaceuticals to the market, contributing significantly to the presence of these substances and their active metabolites in the aquatic environment (Ebele et al. 2017).

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

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

The aim of this research was: *i*) test the ability of *P. australis* and *S. matsudana* to remove some common micro-pollutants such as NPs, NP_1EO and NP_2EO and pharmaceuticals (atenolol,

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

Materials and methods

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

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

Wastewater was analyzed three times during the experiment (July the 2nd and the 30th, September the 24th), before the treatment in CWs and the parameters pH, conductivity, chemical oxygen demand (COD), suspended solids (SS), and ammonium nitrogen (NH_4-N) were determined using standard methods.

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

August (from July 30 to August 6), the CWs were set up in batch mode and the wastewater was retained in each CWs for 7 days, while in September (from 24 to 28 September) for 4 days.

At the end of the third trial, leaves of *S. matsudana* and *P. australis* were collected and three biological replicates for each set of condition were separately analyzed for phenolic and pharmaceutical contents at each sampling time. The 4-*n*-NP (4-*n*-nonylphenol) selected as representative of NPs, NP_1EO, NP_2EO, atenolol (belonging to the group of "beta blocker"), diclofenac (non-steroidal ant-inflammatory drug NSAID), ketoprofen (non-steroidal ant-inflammatory drug NSAID) and triclosan (antibacterial and antifungal agent) were quantified in the water used as input in CWs, in water collected as CWs output and also in leaves of both plant species studied.

The wastewater samples were spiked with 25 ng of [3,5-$^2H_2$]-NP_1-EO, [3,5-$^2H_2$]-NP_2-EO and $^{13}C_6$-Diclofenac as internal standards to account for process losses acidified to pH 2.8-3.0 and extracted three times with an equal volume of dichloromethane (DCM). The DCM extracts were reduced to lower volumes with a rotary evaporator and dried under a gentle flow of dry nitrogen for derivatization (Yang et al. 2011). All chemicals used for analyses set up, were purchased from Sigma-Aldrich, Germany.

Vegetative tissues (1 g) were added with 50 ng of [3,5-$^2H_2$]-NP_1-EO, [3,5-$^2H_2$]-NP_2-EO and $^{13}C_6$-Diclofenac as internal standards to account for process losses, homogenized under liquid nitrogen and extracted with 10 ml in water with stirrer. Samples were centrifuged at 12,000 rpm and 4 °C for 15 min, acidified to pH 2.8-3.0 and the water phase was partionated with DCM (1:1 v/v). The DCM extracts were reduced to lower volumes with a rotary evaporator and dried under a gentle flow.

Derivatization was performed with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane for 20 minutes at 70 °C following the protocol of Samaras et al. (2011).

Quantification was accomplished by GC–MS/MS analysis by a Saturn 2200 quadrupole ion trap mass spectrometer coupled to a CP-3800 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA, USA) equipped with a MEGA 1 MS capillary column (30 m; 0.25 mm i.d., 0.25 µm film thickness, MEGA s.n.c., Milan, Italia). The carrier gas was helium, which was dried and air free, with

a linear speed of 60 cm s^{-1}. The oven temperature was maintained at 80 °C for 1 min, increased to 210 °C at a rate of 15 °C/min, further increased to 235 °C at a rate of 5 °C/min and further increased to 300 °C at a rate of 20 °C/min. Full-scan mass spectra were obtained in EI^+ mode with an emission current of 10 μA and an axial modulation of 4 V. Data acquisition was from 150 to 450 Da at a speed of 1.4 scan s^{-1}.

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

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

Statistical analysis

At each sampling time, data (n=3) of 4-n-NP, NP_1EO, NP_2EO, atenolol, diclofenac, ketoprofen and triclosan, in each CWs, were subjected to a one-way analysis of variance (ANOVA). Separation of means was done using a Bonferroni's multiple comparison test at P=0.05. Unpaired *t*-test analysis at 0.05 probability level was performed on data about concentrations of pharmaceuticals and nonylphenols in leaves of *Salix matsudana* Levante and *Phragmites australis* and on removal efficiency of CW. Statistical analysis was performed using NCSS 2000 Statistical Analysis System Software.

Results and Discussion

During the study period, water samples from the CWs influent were collected and analyzed in July the 2nd and the 30th and September the 24th. The average wastewater quality parameters of the influent in the tree sampling times were: 7.4±0.30 pH, 2233±105.9 µS/cm conductivity, 31.6±2.55 mg/L COD, 9±2 mg/L SS and 1±0.98 mg/L NH_4^+-N (Table 1). As far as the concentration of the selected micro-pollutants analyzed (Supplementary Table 1), it has changed significantly during the experimental period following a specific trend month by month, but always in the range of ng ml^{-1}. The presence of PPCPs (ketoprofen, diclofenac, atenolol and triclosan) and NPs in the influent wastewater, confirmed their widespread use and high frequency of detection reported in previous studies (Matamoros and Bayona, 2006; Matamoros et al. 2007; Soares et al. 2008).

The main physical-chemical and biological mechanisms contributing to the elimination of micro-pollutants in CWs are: *i*) photolytic degradation, *ii*) sorption, *iii*) plant uptake, *iv*) microbial degradation. Moreover, some CW design parameters could have an effect on micro-pollutants removal such as: configuration of CWs, presence and types of vegetation, operational mode, soil matrix and hydraulic retention time (Zang et al. 2014).

In this applicative research, we focused the attention on plant uptake ability (presence and type of vegetation used), since the role carried out by plants within a CW wastewater treatment system, results to be crucial (Lee and Scholz, 2007, Dordio et al., 2009; Zhang et al., 2012) and to better understand the influence of plants, we assessed the comparative removal efficiency of planted CWs with herbaceous on one hand and arboreal plant species on the other hand (*P. australis* –CWs and *S. matsudana* -CWs) with reference to unplanted CWs (control).

The growth substrate matrix is considered very important in CWs, not only because it supports the growth of plants and microorganisms, but also because it can adsorb the micro-pollutants. Therefore, the solid matrix can play an important role in contaminant retention (Dordio et al. 2011). Clay and gravel are commonly used materials in CWs approaches in order to assure homogenized water flow. Some authors found that among different substrates (vesuvianite, gravel, and zeolite), gravel appeared

to be the most efficient filter material (Xiaoyan et al. 2015). The sorption capacity of light expanded clay aggregates was proved evaluating the ability to remove carbamazepine, clofibric acid, and ibuprofen (Dordio et al. 2009). In July, in CWs with only clay and gravel, the removal capability for diclofenac, ketoprofen, and atenolol was lower compared to CWs vegetated with willow and common reed, indicating the importance to introduce also plants for improving the removal activity of micro-pollutants (Fig. 1, 2).

Only for the pharmaceutical triclosan the concentration removed by each CWs (in the first sampling time) was independent to the setup used. In fact, when triclosan concentration was lower into wastewater input, the three different CW systems tested were able to remove totally the compound (Fig. 2B). Triclosan was not detectable until some days after the first application. On the 2nd August, on the contrary, the concentration of triclosan in effluents of non-planted CWs was higher than in the influent. This result could due to a delay in the transport/diffusion through the gravel and clay layer, since soil matrix has a direct effect on the sorption as it has been demonstrated in previous works (Zang et al. 2014). On the other hand, it is worth mentioning that the bacterial biofilms eventually colonizing the gravel and clay, substrate of plant growth, might be responsible for the transformation and eventually degradation of contaminants. In fact, in our experimentation the depletion of the contaminants in the unplanted CWs can be associated to both the adsorption and transformation capacity of the bacterial community colonizing the substrate. On the other hand, the depletion of the planted CWs can be associated to the combination of the adsorption and degradation of the bacterial community colonizing the gravel and clay and the uptake capacity of the plant. However, in this context, the role of bacteria colonizing the plant growth substrate was not studied, because of the reasonable consideration that the plant capacity to uptake the micro-pollutants was predominant on the bacterial capacity to transform and eventually degrade the adsorbed one. As a matter of the fact, the bacterial load (*E. coli*), routinely measured before the discharge in superficial water, at the end of the pipeline of the WWTP of Pagnana, was null as shown in Table 1. Assuming that the total bacterial load entering in the CWs was low and not necessarily specialized in the degradation of the

contaminants of interest, since deriving from the activated sludge not capable to transform the micro-pollutants during the time of residence in the WWTP and considering that the residence time of the wastewater in the CWs was even shorter, in this phase of the study, we considered the contribution of the microbial community in micro-pollutants removal, negligible, if compared to the possible uptake capacity of the different plant species. This assumption was adopted for all the micro-pollutants of interest.

Atenolol was the pharmaceutical detected at higher concentrations in influent wastewater. In July the 2nd, Atenolol reached the values of 13.6 ng mL^{-1} in the wastewater influent, then declined during the experimental period with values below 0.5 ng mL^{-1} in CW effluents (Fig. 1A).

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

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

In relation to the concentration on NP and ethoxylates a clear decline of these compounds in wastewater input was observed during the experiment (Fig. 3). It is interesting to note that the amount of 4-n NP and NP_2EO measured in July in effluents of empty CWs increased with the time. The NP_2EO was even higher than in influent. This increase of concentration in the effluent compared to influent in the month of July, can derive from a previous accumulation of NP_2EO during the continuous flow CWs setup before the experiment started. A subsequently delay in the diffusion through the gravel and clay could be the cause of the observed phenomena.

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

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

Under our experimental conditions, the removal efficiency of plant system ranged from 8.4% up to 100%, with higher value for triclosan in both CW systems studied (Fig. 4). On the contrary, the removal efficiency of NPs appeared lower than pharmaceuticals, with some cases of inefficiency for NP_1EO and NP_2EO removal from CW with *P. australis* or *S. matsudana* (Fig. 5).

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

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

leaves of common reed was above 80 µg g^{-1} FW, eight times higher than willow (10 µg g^{-1} FW). The amount of atenolol accumulated by plants was one order of magnitude more than the other compounds (Fig. 6). The high amount of atenolol in plant tissues could be related to the higher amount of this compound detected in the influent but also to its physical-chemical properties such as water solubility. The water soluble organic compounds, such as atenolol, could be more easily taken up by the plant roots and then translocated to shoot tissues. The ability of vegetables to accumulate atenolol was demonstrated in tomato and potato grown in soils fertilized with commercial biosolids (Sabourin et al. 2012). Moreover, the greenhouse experiment of Wu et al. (2012) demonstrated, for the first time, the ability of plant to take up atenolol and other compounds in tomato, carrot, broccoli, bell pepper, and spinach. In both cases the concentration of atenolol found was in the range of ng g^{-1} DW, one order of magnitude less than *P. australis* and *S. matsudana* plants.

Photolytic degradation in CWs depends on light intensity and light attenuation by water depth (Zhang et al. 2014). During the batch mode setup, diclofenac, ketoprofen, and triclosan, can undergo a photolytic degradation and this process may play a major role in elimination of these compounds as also observed in free water-surface-CWs or hydroponic systems (Zhang et al. 2014).

The capability of plants to take up ketoprofen is poorly documented and in several studies this molecule is not determined (Goldstein et al. 2014). Also for triclosan there are few published papers reporting its uptake, but recently it was demonstrated that *Eichhornia crassipe* and *Pistia stratiotes* are able to remove 30.4% and 67.1% respectively of triclosan added in a nutrient solution (Victor et al. 2016). Willow plants take up ketoprofen and triclosan at higher concentrations than common reed (125 ng g^{-1} FW *vs* 75 ng g^{-1} FW and 90 ng g^{-1} FW *vs* 50 ng g^{-1} FW, respectively), while diclofenac concentration in leaves of *P. australis* (Fig. 6) was double compared to leaves of *S. matsudana* (15 ng g^{-1} FW and 7.5 ng g^{-1} FW, respectively). Herbaceous species such as Scirpus and Typha have been studied in mesocosms setup under diclofenac (Zhang et al. 2012; Bartha et al. 2014), and in particular *Scirpus validus* has been found to be tolerant up to 2 mg L^{-1} of diclofenac (Zhang et al. 2012). The low amount detected in leaves of *P. australis* and *S. matsudana* (not more than 20 ng g^{-1} FW)

compared to other pharmaceuticals studied, could be associated to its sensibility to photodegradation under UV radiations (Zhang et al. 2012). Pierattini et al. (2018a) observed a rapid photolysis of diclofenac that resulted in only 30% of the diclofenac initial concentration remaining in water within 3 days of exposure to sunlight. Moreover, this chemical has been classified as poorly degradable as confirmed by Garcia-Rodrìguez et al. (2015) using *Lemna* plants. Since photo-degradation process of diclofenac could be considered similar in all CWs tested, the different plant physiology (in terms of transpiration and metabolism) could make the differences observed between plant species.

In the same way to pharmaceuticals, the accumulation in the harvestable part of NPs/NPsEO was different between willow and common reed. In particular, the NP_1E0 and NP_2E0 were absorbed in higher amounts in leaves of willow, showing concentrations of 1.75 ng g^{-1} FW and 2.70 ng g^{-1} FW, respectively (Fig. 7). The *P. australis*, instead, absorbed higher amount of 4-*n*-NP with an average concentration of 38.8 ng g^{-1} FW, while *S. matsudana* only ten times less (3.86 ng g^{-1} FW) (Fig. 7).

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 *0*-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

The removal of micro-pollutants from wastewaters in CW system is a result of different complex processes. Regarding the ability of different macrophyte species to bioaccumulate pharmaceuticals and NPs/NPsEO, this research demonstrates that *P. australis* and *S. matsudana* are able to remove these contaminants from wastewater. In fact, *P. australis* uptakes efficiently 4-*n*-NP, diclofenac and atenolol, while *S. matsudana* can translocate in the aerial part preferentially NP_1EO, NP_2EO, ketoprofene, and triclosan. The different plant uptake of micro-pollutants suggests a very interesting use of the consociation of different species to remove, in a more effective way, xenobiotic compounds from wastewater. CWs with clay and gravel as substrates and willow/common reed as plant species can be considered additional systems to conventional wastewater treatments for improving the micro-pollutants removal.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1. Average wastewater quality parameters (means ± standard deviation) of the influent in the tree sampling times (July the 2nd and the 30th and September the 24th). nd= not detected.

Parameters	
pH	7.4±0.3
Conductivity (µS/cm)	2233±106.0
Total Solid sediments (mL/L)	9.0±2.0
BOD (mg/L O_2)	nd
COD (mg/L O_2)	31.7±2.5
N (mg/L)	4.1±1.7
NH_4 (mg/L)	1.0±0.9
N-NO_2 (mg/L)	0.2±0.2
NO_3 (mg/L)	4.6±2.5
N-*tot* (mg/L)	8.2±4.4
P-*tot* (mg/L)	0.2±0.0
Cl^- (mg/L)	414±34.6
SO_4 (mg/L)	115±9.5
E. coli UCF (100 mL)	nd

Figure legends

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 ± 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 ± 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 ± 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*

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

Fig. 5. Removal efficiency (%) of 4-n-NP (nonylphenol), NP1EO (nonylphenol-monoethoxylate), NP2EO (nonylphenol-diethoxylate), from contaminated water in constructed wetland systems with *Phragmites australis* or with *Salix matsudana*. Data represent means of three replicates. *t*-test analysis was performed at each sampling time: ns, not significant; *P<0.05; **P<0.01; ***P<0.001.

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

Fig. 7. Concentrations of 4-*n*NP (nonylphenol), NP_1EO (nonylphenol-monoethoxylate), NP_2EO (nonylphenol-diethoxylate) (ng g^{-1} FW) in *Phragmites australis* or *Salix matsudana* plants after 90 days of growth into constructed wetland systems. Data represent means + standard deviation of three biological replicates. *t*-test analysis was performed at each sampling time: ns, not significant; *P<0.05; **P<0.01; ***P<0.001.

Figure 1

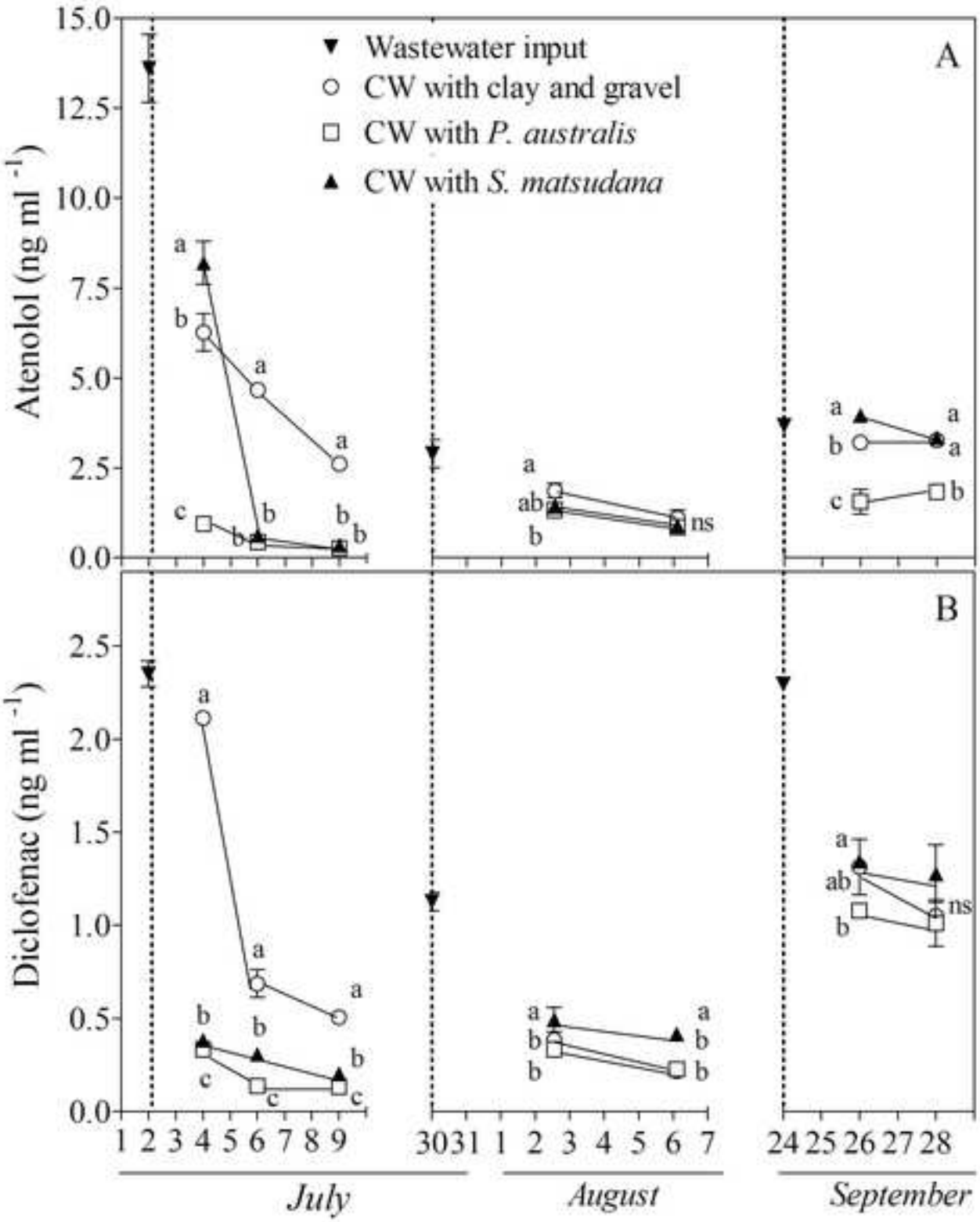

Figure 2

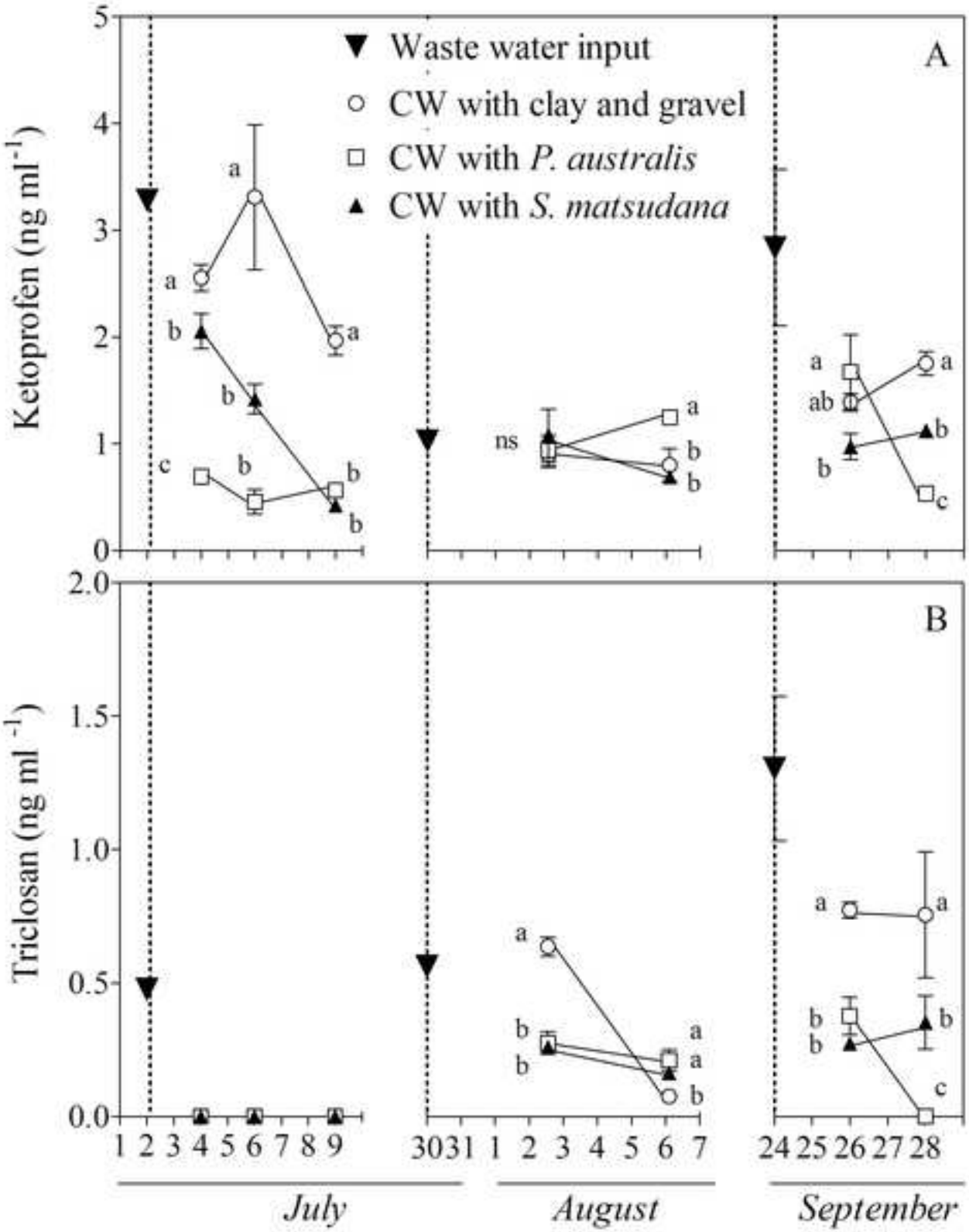

Figure 3

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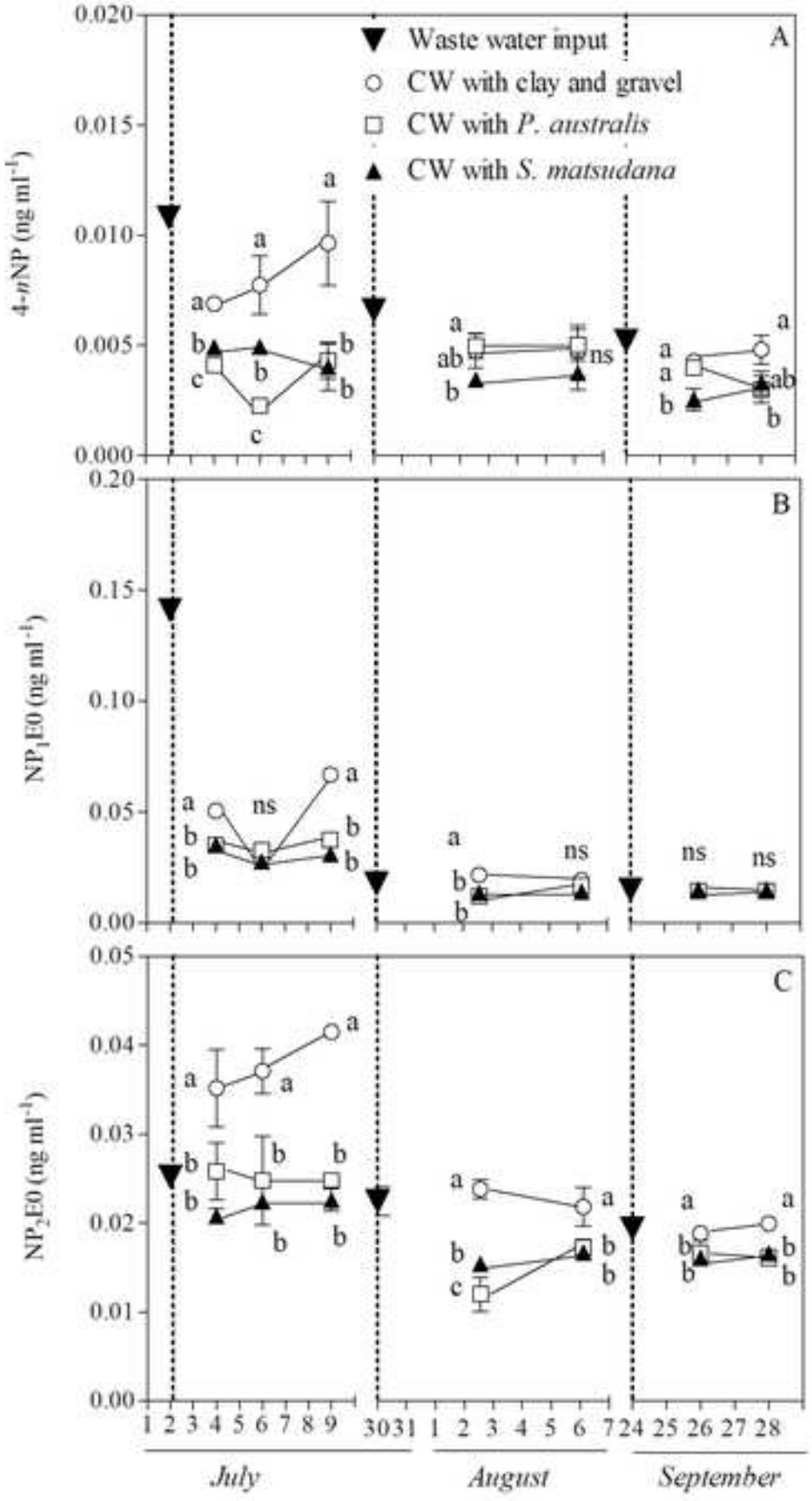

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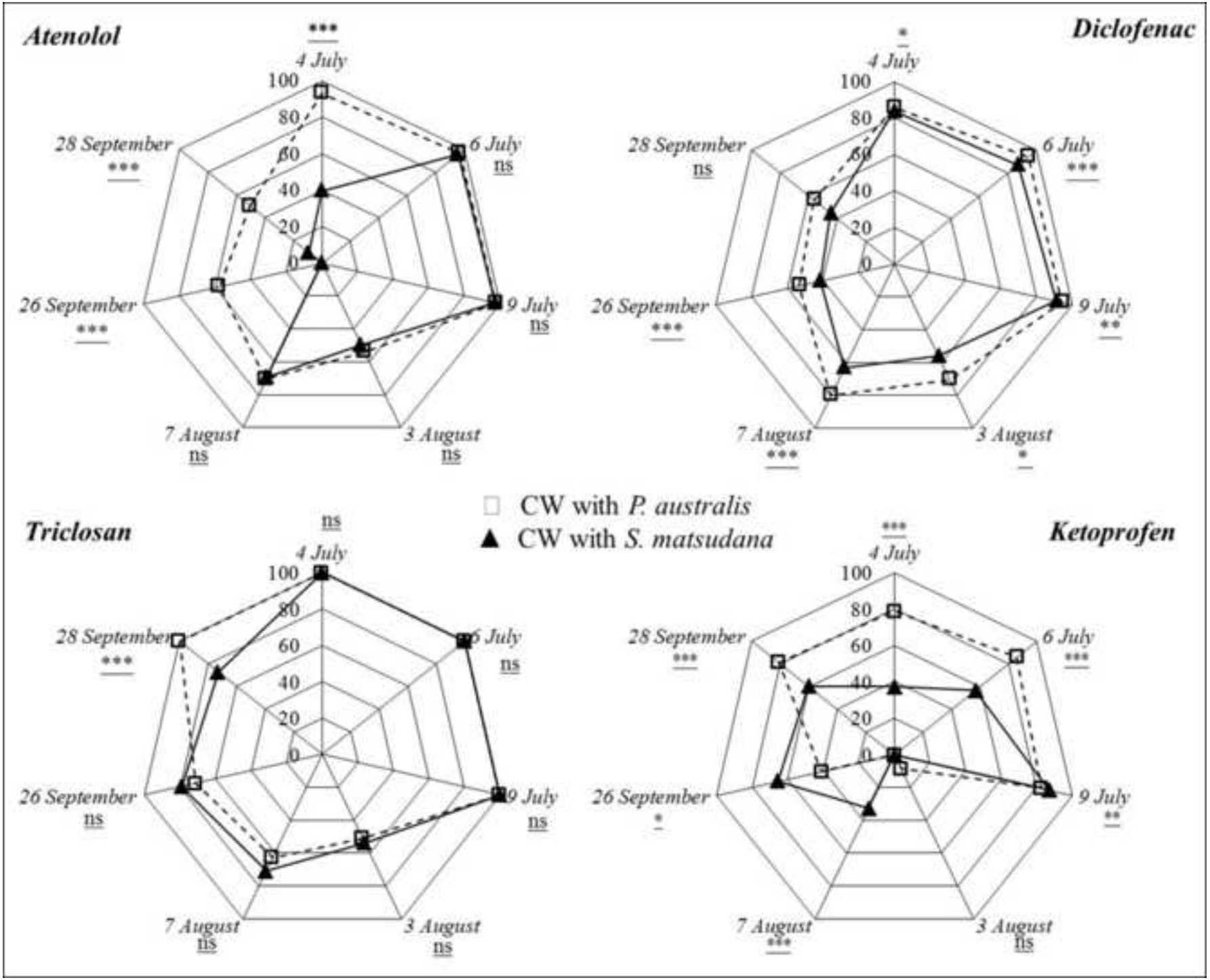

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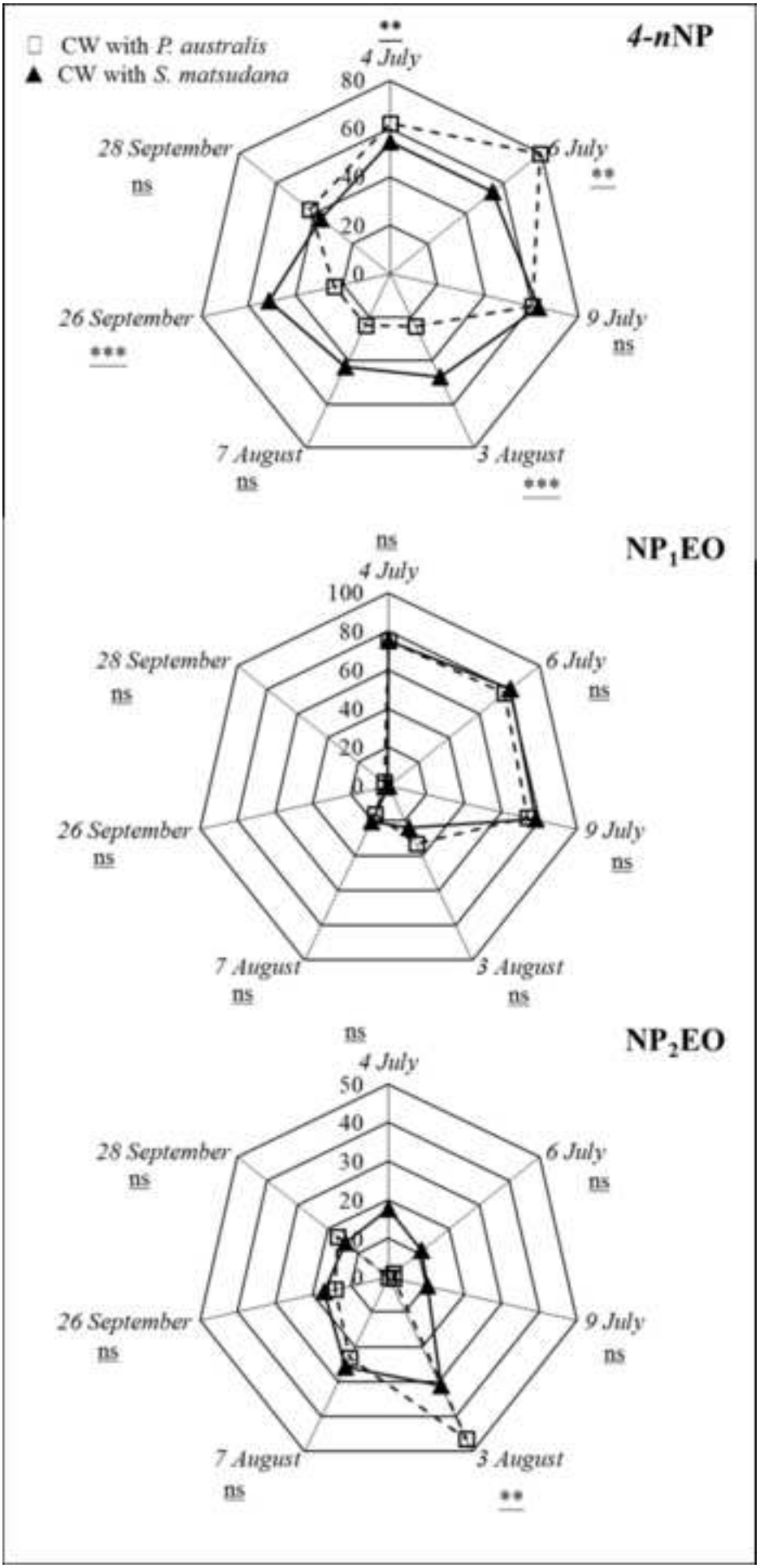

Figure 6

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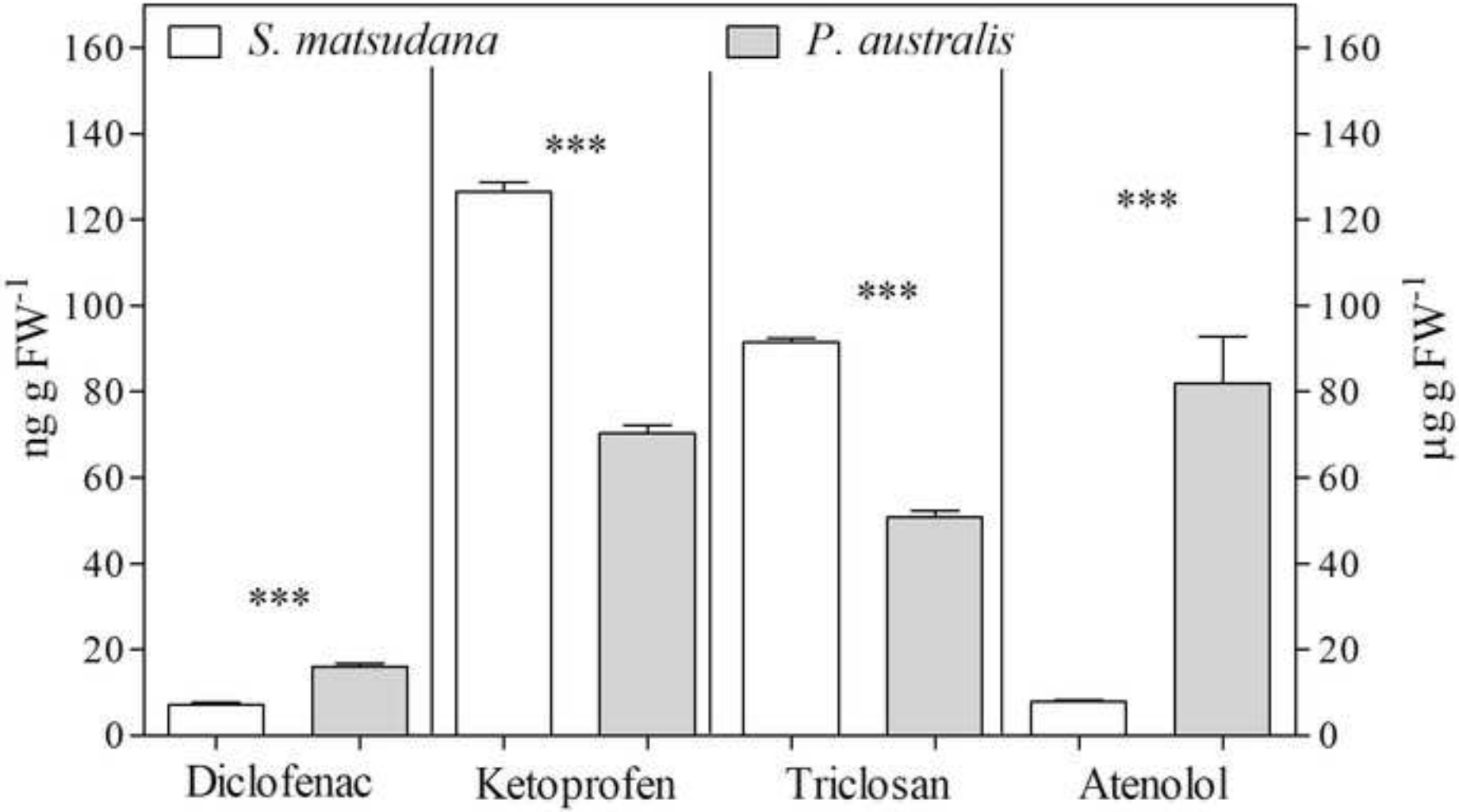

Figure 7

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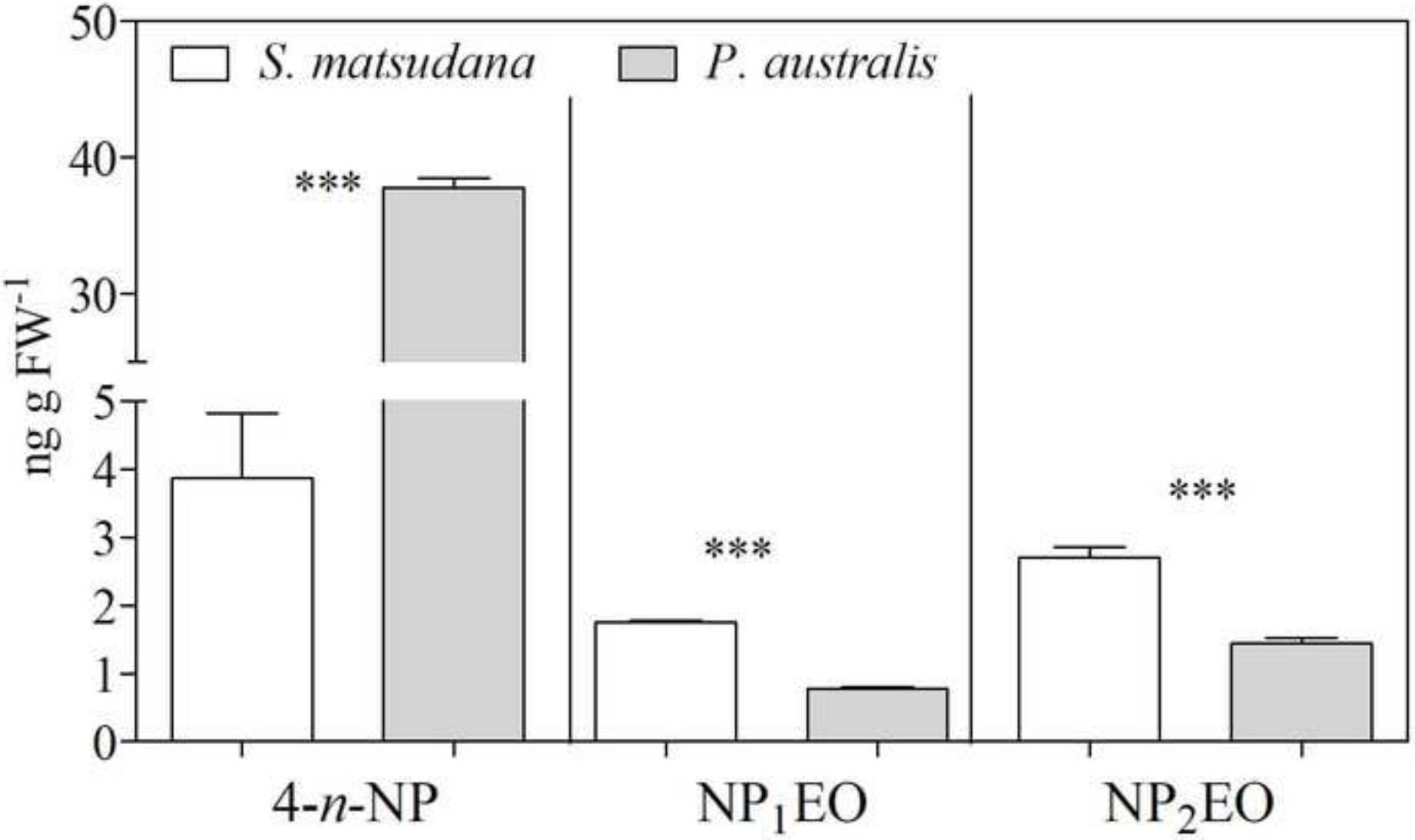

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