Chemosphere

Use of constructed wetlands to prevent overloading of wastewater treatment plants --Manuscript Draft--

Manuscript Number:		
Article Type:	Research paper	
Section/Category:	Treatment and Remediation	
Keywords:	Contaminants of emerging concern; potentially toxic elements; Arundo donax; vertical constructed wetlands	
Corresponding Author:	Andreina Traversa, Ph.D. Universita degli Studi di Bari Aldo Moro Dipartimento di Scienze del Suolo della Pianta e degli Alimenti Bari, ITALY	
First Author:	Claudio Cocozza	
Order of Authors:	Claudio Cocozza	
	Claudio Di Iaconi	
	Sapia Murgolo	
	Andreina Traversa, Ph.D.	
	Francesco De Mastro	
	Marco De Sanctis	
	Valerio Guido Altieri	
	Claudio Cacace	
	Gennaro Brunetti	
	Giuseppe Mascolo	
Abstract:	 Cluseppe Mascolo The fluctuation in the number of people in tourist areas affects the wastewater quality and quantity. Constructed wetlands (CWs) aim to simulate physical, chemical, and biological processes occurring in natural environments for wastewater treatment and are considered a sustainable system. The current study aimed at evaluating the effectiveness of containerized CWs for supporting the wastewaters treatment plants i periods of overloading. Such approach can be quickly implementable, economic, and the CWs can be fast regenerated in the framework of sustainable good practices. Three CWs were prepared in as many containers layering 10 cm of gravel, 60 cm of sand and 10 cm of gravel, and placing pieces of giant reed rhizomes in the upper layers. The bottom of each CW had a tap, and CWs were irrigated with a real municip sewage three times a week. Before each new irrigation, the tap was opened, and the effluent collected for determining gross parameters, elemental composition and contaminants of emerging concern (CECs). CWs significantly reduced almost all gross parameters considered and half the CECs except for a couple of metabolites of corresponding parental compounds. With regard to the potentially toxic elements, all reduced their concentration from the influents to the effluents. The results of this study were promising and highlighted good efficiency of constructed wetlands as pre-treatment of real municipal sewage to reduce the overloading of the wastewater treatment plant. 	
Suggested Reviewers:	Athanasios S Stasinakis University of the Aegean astas@env.aegean.gr Paolo Roccaro University of Catania proccaro@dica.unict.it Karam Farrag	

	National Water Research Center karam_farrag@cleqm.org.eg
	Sarit L Kaserzon The University of Queensland Queensland Alliance for Environmental Health Sciences k.sarit@uq.edu.au
	Cesar Plaza de Carlos Spanish Scientific Research Council c.plaza@ica.csic.es
	Hamada Abdelrahman Cairo University hamada@cu.edu.eg
Opposed Reviewers:	

1	Use of constructed wetlands to prevent overloading of wastewater treatment plants
2	
3	Claudio Cocozza ¹ , Claudio Di Iaconi ² , Sapia Murgolo ² , Andreina Traversa ^{1*} , Francesco De
4	Mastro ¹ , Marco De Sanctis ² , Valerio Guido Altieri ² , Claudio Cacace ¹ , Gennaro Brunetti ¹ ,
5	Giuseppe Mascolo ²
6	
7	¹ Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, University of Bari, Via Amendola
8	165/A, 70126 Bari, Italy
9	² CNR, Istituto di Ricerca Sulle Acque, Via F. De Blasio 5, Bari, 70132, Italy
10	
11	
12	* Corresponding Author
13	Postal address: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, University of Bari,
14	Via Amendola 165/A, 70126 Bari, Italy
15	Tel. (+39) 080 544 2282
16	e-mail: andreina.traversa@uniba.it
17	
18	

19 Abstract

The fluctuation in the number of people in tourist areas affects the wastewater quality and quantity. 20 Constructed wetlands (CWs) aim to simulate physical, chemical, and biological processes occurring 21 in natural environments for wastewater treatment and are considered a sustainable system. The 22 current study aimed at evaluating the effectiveness of containerized CWs for supporting the 23 wastewaters treatment plants in periods of overloading. Such approach can be quickly implementable, 24 25 economic, and the CWs can be fast regenerated in the framework of sustainable good practices. Three CWs were prepared in as many containers layering 10 cm of gravel, 60 cm of sand and 10 cm of 26 gravel, and placing pieces of giant reed rhizomes in the upper layers. The bottom of each CW had a 27 tap, and CWs were irrigated with a real municipal sewage three times a week. Before each new 28 irrigation, the tap was opened, and the effluent collected for determining gross parameters, elemental 29 30 composition and contaminants of emerging concern (CECs).

CWs significantly reduced almost all gross parameters considered and half the CECs, except for a couple of metabolites of corresponding parental compounds. With regards to the potentially toxic elements, all reduced their concentration from the influents to the effluents.

The results of this study were promising and highlighted good efficiency of constructed wetlands as pre-treatment of real municipal sewage to reduce the overloading of the wastewater treatment plant.

Keywords: Contaminants of emerging concern; potentially toxic elements; *Arundo donax*; vertical
constructed wetlands

40 **1. Introduction**

Tourist areas are commonly characterized by a considerable increase of people during the holiday 41 42 season, which leads to an intense seasonal water demand (Salgot and Tapias, 2004). The fluctuation 43 in the number of people affects not only water demand but also wastewater quality and quantity. In fact, in comparison with a typical municipal sewage, the sewages generated in tourist areas are usually 44 more concentrated in terms of typical pollutant parameters, more variable in terms of both flow and 45 46 contaminant variation, and less disintegrated in terms of particulate matter because of the shorter sewerage system (Odegaard, 1989). Wastewater treatment plants operated in tourist areas often suffer 47 48 from a series of problems, including shock loads and and flow fluctuations, which may affect effluent 49 quality (Christoulas and Andreadakis, 1989). Therefore, a more reliable and robust wastewater treatment train is needed in tourist areas for coping with the organic load variations typical of these 50 51 areas.

Constructed wetlands (CWs) aim to simulate physical, chemical and biological processes 52 occurring in natural environments for wastewater treating (Bakhshoodeh et al., 2017), and, due to 53 54 their significant reduction in energy demand, economic costs, less operational and maintenance requirements and environmental pollution, they can be considered a sustainable system capable to 55 enhance the treatment of municipal sewage in tourist areas (Nuamah et al., 2020). With respect to 56 57 conventional treatment systems, CWs require renewable sources, emit very low amount of greenhouse gas, do not produce by-products, and have small problems of odours and insects 58 (Stefanakis et al., 2014). 59

The physical and chemical processes that occur in CWs are adsorption, oxidation, chemical degradation, photodegradation, volatilization and hydrolysis of contaminants, while biodegradation and plant absorption are the main biological processes (Hijosa-Valsero et al., 2016). These processes can occur simultaneously and lead to several advantages, such as plant nutrients cycling, adsorption of potentially toxic elements (PTE) and organic pollutants.

The components of CWs that contribute to the partial or total removal of pollutants are the 65 66 substrate, the plant, the quality of the influent and the microbial population associated to the CWs (Kosolapov et al., 2004). The substrate plays the most important role in removing contaminants 67 through filtration and adsorption of contaminants, electron donor process, support for 68 microorganisms and wetland plants. Gravel is the most common substrate used in CWs (Li et al., 69 2014) because provides sufficient hydraulic conductivity, supports the vegetation, retains suspended 70 71 solids and affects the percolation and infiltration of contaminants (Nuamah et al., 2020; Vymazal et 72 al., 2021) and is largely used also in combination with sand for their affordability and large availability. In addition, sand provides an ideal texture for the establishment of plants, even if it 73 74 requires frequent addition of wastewater because dries out quickly.

Plants play an important role both alive and dead; when alive they deflect flows and provide 75 attachment sites for microorganisms, while when dead they release organic carbon usable by 76 77 microorganisms for their metabolism (Davis, 1995). In this regard, giant reed (Arundo donax) is a perennial herbaceous plant widely spread all over the world that requires high amount of water, and 78 79 is highly tolerant to salinity, with a fast growth rate and a large amount of biomass produced that 80 make it suitable for use in CWs (Allinson, 2017). Giant reed is also capable to absorb PTE and to improve saline soils (Herrera-Alamillo and Robert, 2012; Quinn et al., 2015) and, due to its great 81 82 biomass production, can be suitable for the anaerobic digestion for production of energy and digestate (Shilpi et al., 2019; Zhang et al., 2021). 83

The microbial diversity depends on the configuration and design of the CWs (Rajan et al., 2019), and is a function of the concentration of different forms of nitrogen and carbonaceous materials, amount of plant litter, amount of oxygen and available nutrients, and the type of plant used (Liang et al., 2003; Ligi et al., 2014).

The level of contamination of influents affects the efficiency of CWs in the removal of contaminants influencing the composition of the microbial community. In fact, several studies have reported the presence of certain microorganisms in presence of specific compounds (Guo et al.,

2020; Li et al., 2020; Liu et al., 2020). In addition, CWs can also allow a partial removal of 91 compounds of emerging concern (CECs), such as pharmaceuticals and personal care products, 92 among others. Indeed, this is a side benefit that can be also obtained in the soli due to some 93 94 components present in it having catalytic behaviour (Pizzigallo et al., 1998; Herrera et al., 2001). CECs, in fact, are poorly removed by the activated sludge process in conventional wastewater 95 treatment plants (WWTPs) and the long-term exposure to them could cause reproductive and 96 hormonal disorders as potential health problems and the increase of bacterial antibiotic resistance 97 (Gonsioroski et al., 2020). Therefore, CECs removal usually requires an additional process (tertiary 98 treatment) after the biological step that can be effectively carried out employing one of the advanced 99 100 oxidation processes (Murgolo et al., 2021; Maniakova et al., 2020).

The current study aims at evaluating the effectiveness of containerized CWs for supporting the wastewaters treatment plants in periods of overloading. Such approach can be quickly implementable, economic, and the CWs can be fast regenerated in the framework of sustainable good practices.

104

105 2. Materials and Methods

106 *2.1. Experimental site and constructed wetlands*

The study was carried out with three verticals containerized CWs that were built at the IRSA-107 CNR located in Bari, Southern Italy (5-meter a.s.l., 41°07'31"N, 16°52'00"E). The CWs have been 108 set up according to Hoffmann et al. (2011). In details, from the bottom to the top of each CW, there 109 were 10 cm of gravel, 60 cm of sand and 10 cm of gravel, and the volume of each CW was 0.8-1 m³. 110 The bottom of each CW had a tap and pieces of giant reed rhizomes have been placed in the upper 111 112 layer. Each CW was irrigated with a real municipal sewage, collected from a local municipal wastewater treatment plant in Bari (a city of Southern Italy), three times a week keeping the tap closed 113 until a 15-20 cm layer of wastewater was obtained on the surface (about 400-500 L for each irrigation 114 per CW). Before each new irrigation, the tap was opened, and the effluent collected for further 115 analyses. 116

According to the Köppen–Geiger climate classification (Kottek et al., 2006), the location is characterized by a warm temperate Mediterranean climate with dry and hot summers. With reference to time series 1921–2019, annual average rainfall is approximately 543 mm, mainly distributed between September and March. July is the driest month with 16.9 mm of rain, while November is the month with the greatest rainfall, having an average of 69.8 mm. With reference to time series 1926– 2019, the annual average temperature was 17.0 °C, with average maximum temperature of 25.4 °C in July and average minimum temperature of 9.4 °C in January.

124

125 2.2. Gross parameters

126 Composite samples of influent and the effluent of the biological treatment of wastewater were collected three times a week and subjected to gross parameter analysis: electrical conductivity (EC), 127 chemical oxygen demand (COD), soluble COD (sCOD), total suspended solids (TSS), volatile 128 129 suspended solids (VSS), total nitrogen (TN), ammonia (NH₄⁺), total phosphorus (TP), phosphates, chlorides, and sulphates. COD and TN were measured using DR Lange test kits. sCOD concentration 130 131 was determined after sample filtration through a 0.45 µm nylon filter. TSS and VSS were analysed 132 according to the procedure proposed by APHA (2005). For NH4⁺ determination, an online water/liquid L800 ammonia analyser was utilized (Hemera Analyzers, Meylan, France), while anion 133 species such as PO₄³⁻, Cl⁻ and SO₄²⁻ were measured by ion chromatography (Thermo Scientific 134 Dionex Aquion; column IonPac[™] AS23; carbonate/bicarbonate as eluent). 135

136

137 2.3. Elemental analysis

For the determination of the total content of PTE, plant micronutrients and the exchangeable bases, 3 mL of HNO₃ were added to 5 mL of influents or effluents and microwave digested (Multiwave Perkin Elmer 3000). Finally, all samples have been filtered through Whatman 42-filter paper and analysed by using an inductively coupled plasma optical emission spectrometer (ICAP 6300 Thermo Electron).

144

2.4. Contaminants of emerging concern (CECs)

The occurrence of CECs in both influent and effluent wastewater was performed according to an 145 experimental procedure described elsewhere (Montagna et al., 2020). Briefly, influent and effluent 146 samples where diluted 1:4 and 1:2, respectively. They were filtered (0.2 µm nylon filter) and aliquots 147 of 2 mL were analyzed by on-line solid phase extraction (SPE) by a Hypersil GOLDaQ column (20 148 x 2.1 mm, 5 µm) that was used operating at a flow rate of 0.250 mL min⁻¹ of 100 % water. The 149 analytes retained on the SPE column were then separated and detected by ultra-high pressure liquid 150 chromatograph (Ultimate 3000 System, Thermo Fisher Scientific) interfaced with a high-resolution 151 tandem mass spectrometer (TripleTOF® 5600+ System, AB Sciex) by means of a duo-spray ion 152 source operated in electrospray (ESI) positive mode was employed. All MS analyses were acquired 153 by an acquisition method based on double experiments TOFMS/IDA (Information dependent 154 155 Acquisition) in the mass scan range 50-1000 Da. In order to make the analytical method more robust, each analysed sample was added with the internal standard carbamazepine D10, prior to the analytical 156 157 determination. In this way, it was possible to take into consideration the relative reduction of the signal intensity due to the matrix effect of each sample. 158

All the raw data obtained by the high-resolution mass spectrometry analysis were processed using 159 160 a database of emerging pollutants from the NORMAN network and Sciex OS software (AB Sciex). Briefly, the data processing was performed as follows: each sample was screened, principally on the 161 basis of the mass exact and isotopic pattern, using CECs list of the SusDat database (20 suspect lists 162 comprising ~58000 compounds) compiled by the Norman network (Alygizakis et al., 2019). A 163 confident identification of detected compounds in investigated samples was performed by comparing 164 retention time and MS/MS fragmentation pattern of the suspect identified compounds with those of 165 relative standard compounds, where available, analyzed employing the same analytical method. The 166 list of identified compounds was then re-processed in Sciex OS software for confirmation of detected 167 CECs. The screening allowed the confident detection of 20 compounds listed in Table 1. 168

170 2.5 Statistical analysis

Experimental data were tested against the normal distribution of variables (Shapiro—Wilk test) and the homogeneity of variance (Bartlett test) using R studio software. The variables normally distributed with homogeneity of variances verified were subjected to an ANOVA and HSD test. Data not normally distributed were subjected to the Levene test and a nonparametric ANOVA analysis (Kruskal-Wallis test) and Dunn test.

176

177 **3. Results and discussion**

178 **3.1.** Gross parameters

The main gross parameters of influents and effluents of the three vertical CWs are reported in Fig. 1. Inspecting Fig. 1A, it is possible to observe that a significant increase of EC was obtained after the percolation of wastewater through the CWs. This should be ascribed to the evapotranspiration process that increased the solute concentration in the effluents. This was also confirmed by chloride (Fig. 1B) which shows a similar trend, also because it is a soluble anion, a plant micronutrient and apparently the excess was not uptaken by *Arundo donax*.

The COD content was significantly and drastically reduced by CWs thanks to the action of sand 185 used as adsorbent material. In fact, removal efficiencies in the range of 78.7%-85.7 % were observed 186 with residual effluent concentrations always lower than 200 mg L⁻¹ and sometimes even below 100 187 mg L⁻¹. Similar results have been obtained by Healy et al. (2007) who adopted a stratified sand filter 188 for removal of COD from wastewaters. Other recent studies on CWs have shown a significant 189 reduction in COD, even using different absorbent materials and different technologies (Tuttolomondo 190 et al., 2020; Ruan et al., 2021). The soluble COD shows a trend similar to the COD but less 191 pronounced. 192

193 TSS and VSS have practically zeroed out after crossing the wetlands due to the substrate's 194 filtration and sedimentation (Batool and Saleh, 2020) with removal efficiencies comprise in the range 89-94.9% and 87.7-93.3%, respectively. Regarding the role of the plant, Toscano et al. (2015) found
that *Arundo donax* showed the highest removal rate of TSS with respect to *Vetiveria, Miscanthus* and *Phragmites*.

As far as nitrogen is concerned, a significant removal of influent TN and ammonia content was 198 also recorded. In fact, looking at Fig. 1B, it is possible to observe that TN and ammonia removal 199 efficiencies from 86.1 to 93.2% and from 77.4 to 98.1%, respectively, were recorded. Considering 200 201 the absence of an external aeration system, the atmospheric oxygen diffusion through the upper layer into the bed is normally low and therefore nitrification process will not occur. Hence, ammonia 202 removed, included ammonia from ammonification process of organic nitrogen, should be ascribed 203 204 mainly to the giant reed uptake and, secondarily, to ion exchange with cations in substrates (Ji et al., 2022; Jóźwiakowski et al., 2017). 205

Finally, phosphorous removal was almost completed with removal efficiencies higher than 95% 206 207 and residual effluent concentration practically nil (Fig. 1B). A part of P was subjected to biotic uptake by plants and microorganisms, and another one was possibly retained by the substrate through abiotic 208 209 processes such as co-precipitation, sedimentation, adsorption, and burial (Reddy et al., 1999). The 210 abiotic P removal is mainly governed by the nature and the properties of substrates, by their content of Ca, Fe, and Al (Vohla et al., 2011; Park et al., 2017). Wang et al. (2008) tested the potential of 211 212 seven plants in removal COD, total nitrogen and total phosphorus from sewage and found that giant reed was the most efficient. 213

214

215 3.2. Potentially toxic elements, plant micronutrients and exchangeable bases

This study highlighted different results related to the destiny of PTE, plants micronutrients and exchangeable bases in effluents (Figs. 2-3). Generally speaking, all PTE considered showed a large variability of concentration in the influents, while their content in the effluents was lower and less variable (Fig. 2). In particular, Cu and Zn content was reduced in effluents reaching almost zero (Fig. 2A), according to various Authors that considered *Arundo donax* promising for the removal of

different metals from the environment (Miao et al., 2012; Delplace et al., 2020; Danelli et al., 2021). 221 222 Birch et al. (2004) observed a significant reduction of Cr, Cu, Pb and Zn, and a variable removal of Ni from stormwater by CWs in accord with our study in which the removal of Ni did not present any 223 trend (Fig. 2B). Another study supported the reduction of Cu, Pb and Zn content thanks to the CW 224 (Walker and Hurl, 2002), and the mechanism proposed by Authors was the sedimentation. Kadlec et 225 226 al. (2000) reported different processes involved in removal of PTE in CWs depending on the type of plants adopted, the substrate used and the type of water to restore. According to Ji et al. (2022), the 227 substrate plays the most important role in the removal of PTE and the greater the contact surface, the 228 greater the absorption (Yadav et al., 2010). 229

230 Apparently, B passed through the CWs without any interaction with the substrate (Fig. 3A), while Türker et al. (2014) found the removal of B from wastewater by adsorption onto the substrate, 231 especially in the presence of organic matter. Therefore, the initial absence of organic matter in the 232 233 inorganic substrate could explain the present result. The behaviour of Mn was different because it significantly increased in effluents with respect to influents (Fig. 3A), and a similar result has been 234 235 found by Birch et al. (2004). These Authors hypothesized a release of Mn from the coarse-grained 236 Mn-oxides coated grains during the periods of addition of the influent and more abundant flow. In addition, Mays and Edwards (2001) have shown a poor potential of plants in the removal of Fe and 237 238 Mn because found in plants only 1% and 2% of the annual Fe and Mn loading in the wetlands. In fact, those elements are micronutrients, required in concentration of mg kg⁻¹ in the plant tissues. 239

Figure 3B shows the content of exchangeable bases: Mg and Na content increased significantly in the effluents possibly because the plant uptake was lower than the sum of input and enrichment deriving from the cation exchange between the bases from the CW substrate and the PTE in wastewaters, as reported by Yu et al. (2021). In contrast, K and Ca concentration did not change significantly between influents and effluents due to their higher plant uptake with respect to Mg and Na. Anyway, the exchangeable bases are not of concern.

247 *3.3. CECs removal*

The screening for CECs in the influent wastewater revealed the presence of 20 main compounds that were detected in all samples, being both influent and effluent. They include pharmaceuticals, cosmetics and personal care products, metabolites of both pharmaceuticals and drugs of abuse, food additives and chemicals employed in various preparations. In Fig. 4 are depicted the box and whiskers plots of abundance of detected CECs in influent and effluents during the whole experimental campaign. Half the CECs showed a statistically significant reduction of abundance when flowing through the CWs.

The processes responsible for reducing the presence of organic pollutants in effluents are 255 photodegradation, volatilization, adsorption/sorption, plant uptake and accumulation, and 256 biodegradation (aerobic and anaerobic) (Zhang et al. 2014; Gorito et al. 2017), even if the 257 volatilization and the photodegradation can be insignificant in the case of pollutants with relatively 258 259 greater molecular weight and in cloudy wastewater, respectively (Wu et al., 2020). A crucial role in the removal of organic pollutants in vertical CWs is generally played by aerobic biodegradation (Ilyas 260 and van Hullebusch, 2020) and plants influence the potential of CWs by creating suitable conditions 261 262 for the pollutant's removal processes (Ji et al., 2022). Processes of adsorption and desorption of organic pollutants can also occur in the surface of substrates (Karimi et al., 2019). 263

264 Two CECs, namely Atenolol acid (Fig. 4A) and Deet-carboxylic acid (Fig. 4B), showed a significantly and numerically increase of abundance in the effluents, respectively. This is consistent 265 with the concomitant statistically significant reduction of Atenolol and Deet that are the respective 266 parent compounds of the aforementioned CECs. This finding supports the evidence that 267 biodegradation processes are occurring in the CWs resulting in the transformation of some CECs. In 268 any case, such biodegradation processes are not expecting to allow the complete mineralization of 269 270 CECs due to the residence time in the CWs not being sufficiently long. The CECs biodegradation is also evident on some CECs metabolite, namely Benzoylecgonine that is a metabolite of cocaine (Fig. 271 4A), resulting in its numerically removal. 272

Overall, the CWs showed a reduction of detected CECs between 10 and 90 %, being the lowest removal percentage (10 %) for the 1,5-Dihydro-3-ethyl-4-methyl-2H-pyrrol-2-one and the highest removals for 2-(phenylmethylidene)octanal (83 %), Equol (89 %) and Deet (87 %) (Fig. 5). The two negative removal values are for the transformation of Deet and Atenolol (Fig. 5) into Deet-carboxylic acid and Atenolol acid, respectively, as reported before. It is worth noting that abundance of transformation products could not be directly compared to the respective parent compounds due to the different response in the mass spectrometer.

280

281 Conclusions

Containerized constructed wetlands can be useful as pre-treatment in case of overloading of the wastewater treatment plants. In fact, CWs significantly reduce the main gross parameters and PTE content of wastewater to be sent to the main wastewater treatment facility. In addition, the evapotranspiration of the CWs reduces slightly the volume of wastewater. With regards to the CECs, CWs contribute to lowering some organic contaminants significantly. The number of CWs can be adjusted to seasonal needs, they do not release anything into the environment, and can be set aside during periods of low load and regenerated for subsequent high load seasons.

Further studies are required to test their efficiency in longer periods of time, and to improve theirfeatures for obtaining safe effluents for irrigation.

291

292 **References**

- Allinson, G., 2017. Effect of increasing salinity on development of Giant Reed (Arundo donax) from
- rhizome and culms. Bull. Environ. Contam. Toxicol. 99, 743–747. 10.1007/s00128-017-2197-0
- APHA, A. &W. E. F, 2005. Standards methods for the examination of water and wastewater. 21st ed.
- 2005. American Public Health Association, Washington, DC. https://www.standardmethods.org/.
- 297 Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T.,
- Oswaldova, M., Slobodnik, J., 2019. NORMAN digital sample freezing platform: A European

- virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and
 screen suspects in "digitally frozen" environmental samples. TrAC, Trends Anal. Chem. 115, 129137.
- Bakhshoodeh, R., Alavi, N., Majlesi, M., Paydary, P., 2017. Compost leachate treatment by a pilotscale subsurface horizontal flow constructed wetland. Ecol. Eng. 105, 7–14.
 https://doi.org/10.1016/j.ecoleng.2017.04.058
- Batool, A., Saleh, T.A., 2020. Removal of toxic metals from wastewater in constructed wetlands as
 a green technology; catalyst role of substrates and chelators. Ecotoxicol. Environ. Saf. 189,
 109924. 10.1016/j.ecoenv.2019.109924
- Birch, G.F., Matthai, C., Fazeli, M.S., Suh, J.Y., 2004. Efficiency of a constructed wetland in
 removing contaminants from stormwater. Wetlands 24, 459–466. https://doi.org/10.1672/02775212(2004)024[0459:EOACWI]2.0.CO;2
- Christoulas, D.G., Andreadakis, A.D., 1989. A system for on site treatment and disposal of
 wastewaters from tourist resorts. Water Science and Technology, 21, Issue 1, Pages 37–45
- 313 Danelli, T., Sepulcri, A., Masetti, G., Colombo, F., Sangiorgio, S., Cassani, E., Anelli, S., Adani, F.,
- Pilu, R., 2021. *Arundo donax* L. biomass production in a polluted area: Effects of two harvest
 timings on heavy metals uptake. Appl. Sci. 11, 1147. 10.3390/app11031147
- Davis, L., 1995. A Handbook of Constructed Wetlands: General considerations. U.S. Government
 Printing Office, pp. 54.
- 318 Delplace, G., Schreck, E., Pokrovsky, O. S., Zouiten, C., Blondet, I., Darrozes, J., Viers, J., 2020.
- Accumulation of heavy metals in phytoliths from reeds growing on mining environments in
- 320 Southern Europe. Sci. Total Environ. 712, 135595. 10.1016/j.scitotenv.2019.135595
- 321 Gorito, A.M., Ribeiro, A.R., Almeida, C.M.R., Silva, A.M.T., 2017. A review on the application of
- 322 constructed wetlands for the removal of priority substances and contaminants of emerging concern
- 323 listed in recently launched EU legislation. Environ Pollut 227, 428–443.
- 324 https://doi.org/10.1016/j.envpol.2017.04.060

- Gonsioroski, A.; Mourikes, V.E.; Flaws, J.A. Endocrine Disruptors in Water and Their Effects on the
 Reproductive System. *International Journal of Molecular Sciences* 2020, *21*, 1929,
 doi:10.3390/ijms21061929.
- Guo, F., Zhang, J., Yang, X., He, Q., Ao, L., Chen, Y., 2020. Impact of biochar on greenhouse gas
 emissions from constructed wetlands under various influent chemical oxygen demand to nitrogen
- 330 ratios. Bioresour. Technol. 303, 122908. https://doi.org/10.1016/j.biortech.2020.122908
- Healy, M.G., Rodgers, M., Mulqueen, J., 2007. Performance of a stratified sand filter in removal of
 chemical oxygen demand, total suspended solids and ammonia nitrogen from high-strength
- 333 wastewaters. J. Environm. Manage. 83, 409–415. https://doi.org/10.1016/j.jenvman.2006.03.005
- Herrera F., Lopez A., Mascolo G., Albers P., Kiwi J.
 Catalytic decomposition of the reactive dye Uniblue A on hematite. Modelling of the reactive
 surface. Water Research, 2001, 35(3), 750-760.
- Herrera-Alamillo, M.Á., Robert, M.L., 2012. Liquid in vitro culture for the propagation of *Arundo donax*. Methods Mol. Biol. 877, 153–160. 10.1007/978-1-61779-818-4_12
- Hijosa-Valsero, M., Reyes-Contreras, C., Dominguez, C., Becares, E., Bayona, J.M., 2016.
- 340 Behaviour of pharmaceuticals and personal care products in constructed wetland compartments:
- influent, effluent, pore water, substrate and plant roots. Chemosphere 145, 508–517.
 https://doi.org/10.1016/j.chemosphere.2015.11.090
- Hoffmann, H., Platzer, C., Winker, M., von Muench, E., 2011. GIZ (Editor) (2011): Technology
 review of constructed wetlands. Subsurface flow constructed wetlands for greywater and domestic
 wastewater treatment. Eschborn: Deutsche Gesellschaft fuer Internationale Zusammenarbeit (GIZ)
 GmbH, Germany.
- Ji, Z., Tang, W., Pei, Y., 2022. Constructed wetland substrates: A review on development, function
 mechanisms, and application in contaminants removal. Chemosphere 286, 131564.
 https://doi.org/10.1016/j.chemosphere.2021.131564

- Jóźwiakowski, K., Bugajski, P., Mucha, Z., W'ojcik, W., Jucherski, A., Nastawny, M., Siwiec, T.,
- 351 Mazur, A., Obro´slak, R., Gajewska, M., 2017. Reliability and efficiency of pollution removal
- during long-term operation of a one-stage constructed wetland system with horizontal flow. Separ.
- 353 Purif. Technol. 187, 60–66. https://doi.org/10.1016/j.seppur.2017.06.043
- 354 Kadlec, R.H.; Knight, R.L.; Vymazal, J.; Brix, H.; Cooper, P.; Haberl, R. Constructed Wetlands for
- Pollution Control: Processes, Performance, Design and Operation, 1st ed.; IWA Publishing:
 London, UK, 2000; pp. 17–90.
- Karimi, S., Tavakkoli Yaraki, M., Karri, R.R., 2019. A comprehensive review of the adsorption
 mechanisms and factors influencing the adsorption process from the perspective of bioethanol
 dehydration. Renew. Sustain. Energy Rev. 107, 535–553.
 https://doi.org/10.1016/j.rser.2019.03.025.
- Kosolapov, D.B., Kuschk, P., Vainshtein, M.B., Vatosourina, A.V., Weibner, A., Kastner, M.,
 Muller, R.A., 2004. Microbial processes of heavy metal removal from carbon_deficient effluents
 in constructed wetlands. Eng. Life Sci. 4, 403–411. https://doi.org/10.1002/elsc.200420048.
- 364 Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F., 2006. World map of the Köppen-Geiger
- 365 climate classification updated. Meteorol. Z. 15, 259–263. 10.1127/0941-2948/2006/0130
- Ilyas, H., van Hullebusch, E.D., 2020. Performance comparison of different types of constructed
 wetlands for the removal of pharmaceuticals and their transformation products: a review. Environ.
- 368 Sci. Pollut. Res. 27, 14342–14364. https://doi.org/ 10.1007/s11356-020-08165-w.
- Li, Y., Zhu, G., Ng, W. J., Tan, S. K., 2014. A review on removing pharmaceutical contaminants
 from wastewater by constructed wetlands: Design, performance and mechanism. Sci. Total
 Environ. 468, 908–932. 10.1016/j.scitotenv.2013.09.018
- Li, T., Fan, Y., Cun, D., Song, X., Dai, Y., Wang, F., Wu, C., Liang, W., 2020. Treatment
 performance and microbial response to dibutyl phthalate contaminated wastewater in vertical flow
 constructed wetland. Chemosphere 246, 125635. https://doi.org/
 10.1016/j.chemosphee.2019.125635

- Liang, W., Wu, Z.B., Cheng, S.P., Zhou, Q.H., Hu, H.Y., 2003. Roles of substrate microorganisms
- and urease activities in wastewater purification in a constructed wetland system. Ecol. Eng. 21,
 191–195. 10.1016/j.ecoleng.2003.11.002
- Ligi, T., Oopkaup, K., Truu, M., Preem, J.K., Nõlvak, H., Mitsch, W.J., Mander, U., Truu, J., 2014.
- 380 Characterization of bacterial communities in soil and sediment of a created riverine wetland
- complex using high-through put 16S rRNA amplicon sequencing. Ecol. Eng. 72, 56–66.
 https://doi.org/10.1016/j.ecoleng.2013.09.007
- Liu, T., Lu, S., Wang, R., Xu, S., Qin, P., Gao, Y., 2020. Behavior of selected organophosphate flame
- retardants (OPFRs) and their influence on rhizospheric microorganisms after short-term exposure
- in integrated vertical-flow constructed wetlands (IVCWs). Sci. Total Environ. 710, 136403.
- 386 https://doi.org/10.1016/j. scitotenv.2019.136403
- 387 G. Maniakova, K. Kowalska, S. Murgolo, G. Mascolo, G. Libralato, G. Lofrano, O. Sacco, M. Guida,
- L. Rizzo, Comparison between heterogeneous and homogeneous solar driven advanced oxidation
 processes for urban wastewater treatment: Pharmaceuticals removal and toxicity, Sep. Purif.
 Technol. 236 (2020). https://doi.org/10.1016/j.seppur.2019.116249.
- Mays, P.A., Edwards, G.S., 2001. Comparison of heavy metal accumulation in a natural wetland and
 constructed wetlands receiving acid mine drainage. Ecol. Eng. 16, 487–500.
 https://doi.org/10.1016/S0925-8574(00)00112-9
- Miao, Y., Xiao, X.Y., Miao, X.F., Guo, Z.H., Wang, F.Y., 2012. Effect of amendments on growth
 and metal uptake of Giant Reed (*Arundo donax* L.) grown on soil contaminated by arsenic,
 cadmium and lead. Trans. Nonferrous Met. Soc. China 22, 1462–1469. 10.1016/S10036326(11)61342-3
- 398 M.T. Montagna, O. De Giglio, C. Calia, C. Pousis, F. Triggiano, S. Murgolo, C. De Ceglie, F.
- Bagordo, F. Apollonio, G. Diella, M. Narracci, M.I. Acquaviva, G. Bonanno Ferraro, P. Mancini,
- 400 C. Veneri, S. Brigida, T. Grassi, A. De Donno, C. Di Iaconi, M.C. Caputo, R.A. Cavallo, G. La
- 401 Rosa, G. Mascolo, 2020. Microbiological and Chemical Assessment of Wastewater Discharged

- by Infiltration Trenches in Fractured and Karstified Limestone (SCA.Re.S. Project 2019–2020). 402 Pathogens, 9(12),1010, 1-20. 403
- Murgolo, S., De Ceglie, C., Di Iaconi, C., Mascolo, G., 2021. Novel TiO2-Based Catalysts Employed 404
- in Photocatalysis and Photoelectrocatalysis for Effective Degradation of Pharmaceuticals (PhACs) 405
- in Water: A Short Review. Current Opinion in Green and Sustainable Chemistry, 30, 100473, 406
- doi:10.1016/j.cogsc.2021.100473. 407

- Nuamah, L.A., Li, Y., Pu, Y., Nwankwegu, A.S., Haikuo, Z., Norgbey, E., Banahene, P., Bofah-408
- Buoh, R., 2020. Constructed wetlands, status, progress, and challenges. The need for critical cleaner productive 410 operational reassessment for а ecosystem. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2020.122340 411
- Odegaard, H., 1989. Appropriate technology for wastewater treatment in coastal tourist areas. Water 412 Science and Technology, 21, Issue 1, 1–17. 10.2166/wst.1989.0002 413
- 414 Park, J.-H., Wang, J.J., Kim, S.-H., Cho, J.-S., Kang, S.-W., Delaune, R.D., Seo, D.-C., 2017.
- Phosphate removal in constructed wetland with rapid cooled basic oxygen furnace slag. Chem. 415
- 416 Eng. J. 327, 713-724. https://doi.org/10.1016/j.cej.2017.06.155
- Pizzigallo, M.D.R.; Ruggiero, P.; Crecchio, C.; Mascolo, G. 1998. Oxidation of Chloroanilines at 417 Metal Oxide Surfaces. J. Agric. Food Chem. 46, 2049–2054, doi:10.1021/jf9707905. 418
- Quinn, L.D., Straker, K.C., Guo, J., Kim, S., Thapa, S., Kling, G., Lee, D.K., Voigt, T.B., 2015. 419
- Stress-tolerant feedstocks for sustainable bioenergy production on marginal land. Bioenerg. Res. 420
- 8, 1081–1100. 10.1007/s12155-014-9557-y 421
- Rajan, R.J., Sudarsan, J.S., Nithiyanantham, S., 2019. Microbial population dynamics in constructed 422
- wetlands: Review of recent advancements for wastewater treatment. Environ. Eng. Res. 24, 181-423
- 190. https://doi.org/10.4491/eer.2018.127 424
- Reddy, K.R., Kadlec, R.H., Flaig, E., Gale, P.M., 1999. Phosphorus retention in streams and wetlands 425
- А Rev. Environ. Sci. Technol. 29. 83-146. review. Crit. 426 https://doi.org/10.1080/10643389991259182 427

- 428 Ruan, W., Cai, H., Xu, X., Man, Y., Wang, R., Tai, Y., Chen, Z., Vymazal, J., Chen, J., Yang, Y.,
- 429 Zhang, X., 2021. Efficiency and plant indication of nitrogen and phosphorus removal in
- 430 constructed wetlands: A field-scale study in a frost-free area. Sci. Tot. Environm. 799, 149301.
- 431 https://doi.org/10.1016/j.scitotenv.2021.149301
- 432 Salgot, M., Tapias, J.C., 2004. Non-conventional water resources in coastal areas: A review on the
 433 use of reclaimed water. Geologica Acta, 2, Issue 2, 121–133.
- Shilpi, S., Lamb, D., Bolan, N., Seshadri, B., Choppala, G., and Naidu, R., 2019. Waste to watt:
 Anaerobic digestion of wastewater irrigated biomass for energy and fertiliser production. J.
 Environ. Manag. 239, 73–83. 10.1016/j.jenvman.2019.02.122
- 437 Stefanakis, A.I., Akratos, C.S., Tsihrintzis, V.A., 2014. Vertical flow constructed wetlands:
 438 Ecoengineering systems for wastewater and sludge Treatment (1st ed.). Amsterdam, The
 439 Netherlands: Elsevier Publishing.
- 440 Toscano, A., Marzo, A., Milani, M., Cirelli, G. L., Barbagallo, S., 2015. Comparison of removal
- 441 efficiencies in Mediterranean pilot constructed wetlands vegetated with different plant species.
- 442 Ecol. Eng. 75, 155–160. 10.1016/j.ecoleng.2014.12.005
- Türker, O.C., Vymazal, J., Türe, C., 2014. Constructed wetlands for boron removal: a review. Ecol.
 Eng. 64, 350–359. 10.1016/j.ecoleng.2014.01.007
- 445 Tuttolomondo, T., Virga, G., Licata, M., Leto, C., La Bella, S., 2020. Constructed wetlands as
- sustainable technology for the treatment and reuse of the first-flush stormwater in agriculture—A
- 447 case study in Sicily (Italy). Water 12, 2542. doi:10.3390/w12092542
- Vymazal, J., Zhao, Y., Mander, U., 2021. Recent research challenges in constructed wetlands for
 wastewater treatment: A review. Ecol. Eng. 169, 106318.
 https://doi.org/10.1016/j.ecoleng.2021.106318
- 451 Vohla, C., K^oiv, M., Bavor, H.J., Chazarenc, F., Mander, Ü., 2011. Filter materials for phosphorus
- 452 removal from wastewater in treatment wetlands—a review. Ecol. Eng. 37, 70–89.
- 453 https://doi.org/10.1016/j.ecoleng.2009.08.003

- Walker, T. A., Hurl., S., 2002. The reduction of heavy metals in a stormwater wetland. Ecol. Eng. 18,
 407–414. https://doi.org/10.1016/S0925-8574(01)00101-X
- Wang, R., Li, H., Sun, H., 2019. Bismuth: Environmental Pollution and Health Effects. Encyclopedia
 Environ. Health 1, 415–423. 10.1016/b978-0-12-409548-9.11870-6
- 458 Wu, G., Wang, J., Geng, J., 2020. Chemical HRPs in wastewater. In: High-risk pollutants in
- 459 wastewater (Ren, H., Zhang, X., Eds.). Elsevier, pp. 5-39. https://doi.org/10.1016/C2018-0-00194-
- 460 2
- Yadav, A.K., Kumar, N., Sreekrishnan, T.R., Satya, S., Bishnoi, N.R., 2010. Removal of chromium
 and nickel from aqueous solution in constructed wetland: mass balance, adsorption–desorption
- 463 and FTIR study. Chem. Eng. J. 160, 122–128. 10.1016/j.cej.2010.03.019
- Yu, G., Li, P., Wang, G., Wang, J., Zhang, Y., Wang, S., Yang, K., Du, C., Chen, H., 2021. A review 464 on the removal of heavy metals and metalloids by constructed wetlands: bibliometric, removal 465 466 pathways, and key factors. World J. Microbiol. Biotechnol. 37, 157. https://doi.org/10.1007/s11274-021-03123-1 467
- Zhang, D., Gersberg, R.M., Ng, W.J., Tan, S.K., 2014. Removal of pharmaceuticals and personal care
 products in aquatic plant-based systems: a review. Environ Pollut 184:620–639.
 https://doi.org/10.1016/j.envpol.2013.09.009
- Zhang, D., Jiang, Q.W., Liang, D.Y., Huang, S., Liao, J., 2021. The potential application of Giant
 Reed (*Arundo donax*) in ecological remediation. Front. Environ. Sci.
 |https://doi.org/10.3389/fenvs.2021.652367

475	Figure	captions

Figure 1. Gross parameters values in influents and effluents during the whole trial. Error bars
correspond to the standard deviations and columns with the same letter are not statistically
different.

- Figure 2. Concentrations of potentially toxic elements in influents and effluents during the whole
 trial. Error bars correspond to the standard deviations and columns with the same letter are not
 statistically different.
- 483 Figure 3. Concentrations of micronutrients (A) and exchangeable bases (B) in influents and effluents
- 484 during the whole trial. Error bars correspond to the standard deviations and columns with the same
- 485 letter are not statistically different.
- Figure 4. Box and whiskers plots of abundance of detected CECs in influents and effluents during
 the whole trial. Error bars correspond to the standard deviations and columns with the same letter
 are not statistically different.
- **Figure 5.** Average percent removal of detected CECs as a result of percolation through the CWs.

- Containerized constructed wetlands were used to pre-treat real municipal sewage
- Almost all gross parameters significantly reduced in effluents
- Almost all potentially toxic elements significantly reduced in effluents
- Ten contaminants of emerging concern out of 20 significantly reduced in effluents
- Two metabolites increased their concentration in effluents



Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti -Di.S.S.P.A.

Bari, 5th of August, 2022

Dear Prof.
Yeomin Yoon, PhD
Editor
Chemosphere

Dear Editor,

On behalf of all Authors, I like to submit the manuscript entitled "Use of constructed wetlands to prevent overloading of wastewater treatment plants" by Claudio Cocozza, Claudio Di Iaconi, Sapia Murgolo, Andreina Traversa, Francesco De Mastro, Marco De Sanctis, Valerio Guido Altieri, Claudio Cacace, Gennaro Brunetti, Giuseppe Mascolo.

All the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal.

I look forward to hearing from you about this matter at your nearest convenience.

With my best regards

Sincerely yours

Andreina Traversa, PhD Corresponding Author Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti Università degli Studi di Bari "Aldo Moro" Via Amendola, 165/A - 70126 Bari (Italy)

Declaration of interests

⊠The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:









A

Figure 2





В

A







(b)

acid INT

(b)

Nide IN

ate OUT

(a)

acid OLY

D

(a)

lide OUT -

(a)

(a)

the M

(a)

dine IN -

(a)

- Uno

Wizne Intrance





Table 1. CECs detected in the influent wastewater employing the database of Norman network and

 then refined by Sciex OS software.

Organic pollutant	Molecular formula	Molecular configuration	Use
1,5 dihydro - 3 - ethyl - 4- methyl - 2H - pyrrol- 2 one	C7H11NO	N HO	Chemical used for the preparation of Glimepiride, and its intermediates, used as antidiabetic agents.
N-(1,1,3,3 tetramethyl butyl) acrylamide	C11H21NO	O H H	Cosmetic (protects against UV rays)
7 hydroxyheptane 1 nitrile	C7H13NO	но	Industrial and manufacturing fields
Atenolol acid	C14H21NO4		"Cardioselective" <u>beta-</u> adrenoceptor antagonist
Triethyl phosphate	C6H15O4P		Plasticizer
Atenolol	C14H22N2O3		Beta blocker pharmaceutical
Fenofibric acid	C17H15ClO4	CI CI	Food diets
2- (phenylmethylidene)oc tanal	C15H20O		Natural product found in <i>Plectranthus glabratus</i>
Benzoylecgonine	C16H19NO4		Metabolite of cocaine

3 etoxy 4 hydroxybenzaldehyde	C9H10O3	O OH	Food additive
Deet	C12H17NO	O N	Mosquito repellent
Menadiol	C11H10O2	OH OH OH	Food supplements
DEET-Carboxylic Acid	C12H17NO	ООН	Mosquito repellent
Tranexamic acid	C8H15NO2	H ₂ N	Used in medicine against fibrinolysis
Equol	C15H14O3	НОСООС	Food supplements
2 - (2-vinyloxyethoxy) ethyl acrylate	C9H14O4		Chemical used to make special coatings, dehydrating agents, adhesives
Dibenzylamine	C14H15N	H	Chemical harmful to aquatic life
Tofogliflozin	C22H26O6	HO OH	Drug for the treatment of diabetes mellitus
Formylpiperidine	C6H11NO	O N H	Solvent
N-(2-methyl-1phenyl- 2propanyl) acetamide	C12H17NO	O N H	Derives from phentermine that is similar to amphetamines and acts at

	the level of the nervous
	system

Conceptualization	Claudio Cocozza, Gennaro Brunetti, Claudio Di Iaconi, Giuseppe Mascolo		
Methodology	Claudio Cocozza, Gennaro Brunetti, Claudio Di Iaconi, Giuseppe Mascolo, Andreina Traversa		
Formal analysis	Sapia Murgolo, Andreina Traversa, Francesco De Mastro, Marco De Sanctis, Valerio Guido Altieri, Claudio Cacace		
Investigation	Claudio Cocozza, Claudio Di Iaconi, Sapia Murgolo, Andreina Traversa, Francesco De Mastro, Marco De Sanctis, Valerio Guido Altieri, Claudio Cacace, Gennaro Brunetti, Giuseppe Mascolo		
Resources	Claudio Cocozza, Gennaro Brunetti, Claudio Di Iaconi, Giuseppe Mascolo		
Data Curation	Claudio Cocozza, Claudio Di Iaconi, Sapia Murgolo, Andreina Traversa, Francesco Mastro, Marco De Sanctis, Valerio Guido Altieri, Claudio Cacace, Gennaro Brunetti Giuseppe Mascolo		
Writing - Original Draft	Claudio Cocozza, Claudio Di Iaconi, Andreina Traversa, Giuseppe Mascolo		
Writing - Review & Editing	Claudio Cocozza, Claudio Di Iaconi, Andreina Traversa, Giuseppe Mascolo		
Supervision	Claudio Cocozza, Gennaro Brunetti, Claudio Di Iaconi, Giuseppe Mascolo, Andreina Traversa		
Project administration	Claudio Cocozza, Gennaro Brunetti, Claudio Di Iaconi, Giuseppe Mascolo		
Funding acquisition	Claudio Cocozza, Gennaro Brunetti, Claudio Di Iaconi, Giuseppe Mascolo		