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Ecological design of constructed wetlands in cold mountainous region: from literature to experience --Manuscript Draft--

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Corresponding Author:	Alessio Cislighi Universita degli Studi di Milano Facolta di Scienze Agrarie e Alimentari ITALY	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Universita degli Studi di Milano Facolta di Scienze Agrarie e Alimentari	
Corresponding Author's Secondary Institution:		
First Author:	Rachele Stentella	
First Author Secondary Information:		
Order of Authors:	Rachele Stentella Alessio Cislighi Lorenzo M. W. Rossi Luca Giupponi Enzo Bona Alberto Zambonardi Luigi Rizzo Francesco Esposto Gian Battista Bischetti	
Order of Authors Secondary Information:		
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Abstract:	<p>Constructed wetlands (CWs) are effective and sustainable engineered systems for domestic and/or municipal wastewater treatments. In the last half-century, CWs have become common solutions for small communities in remote mountainous areas. However, cold climate in mountain environment often causes a significant reduction of the pollutants removal performance compared to warmer environments. Therefore, in the present study, we aim to help build consensus on the best design practices for CWs in mountain environments. A meta-analysis of the scientific literature focusing on the CWs in cold climate was conducted. Meanwhile, several monitoring activities (chemical analysis on wastewater and treated water and analysis of changes in the plant communities) were carried out on 6 CWs observing the different conditions between immediately after the construction and 3-7 years later. The results showed an encouraging agreement between scientific studies and observations from the monitored case studies. Lack of plan space, low temperatures and supply meteoric inflows prompted the engineers, in the case studies, to design systems composed of septic tank and horizontal subsurface bed. Such a solution showed a good treatment efficiency (removal rates were more than 60% for most pollutants) in a harsh mountainous environment. A significant difference was evident in the choice of plants. Although the common reeds (<i>Phragmites australis</i>) remain an excellent solution, in plain and hilly areas, planting of tufted hairgrass (<i>Deschampsia cespitosa</i>) ensures</p>	

	<p>similar average performance but is more suitable for the mountain ecosystems. In conclusion, the present study proposes technical and engineering recommendations and a sort of ecological design to increase wastewater treatment efficiency and adapt the systems to a natural and cold environment.</p>
<p>Suggested Reviewers:</p>	<p>Claudia Ferrario Mario Negri Institute for Pharmacological Research Branch of Milan: Istituto di Ricerche Farmacologiche Mario Negri claudia.ferrario@marionegri.it Expert in wetland plants</p> <hr/> <p>Stefano Puricelli Politecnico di Milano stefano.puricelli@polimi.it Expert in wastewater treatment</p> <hr/> <p>Azade Deljouei University of Teheran a.deljouei@ut.ac.ir Expert in vegetation conservation</p> <hr/> <p>Cosimo Peruzzi Politecnico di Torino cosimo.peruzzi@polito.it Expert in wastewater treatment</p>

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1 [Title](#)

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3 experience

4 [Authors](#)

5 Rachele Stentella¹, Alessio Cislaghi^{1,2}, Lorenzo M.W. Rossi¹, Luca Giupponi^{1,2}, Enzo Bona³, Alberto
6 Zambonardi⁴, Luigi Rizzo⁴, Francesco Esposito⁴, Gian Battista Bischetti^{1,2}

7 [Affiliation](#)

8 ¹ Department of Agricultural and Environmental Sciences (DiSAA), University of Milan, Via Celoria
9 2, 20133 Milan, Italy

10 ² Centre of Applied Studies for the Sustainable Management and Protection of Mountain Areas
11 (Ge.S.Di.Mont), University of Milan, Via Morino 8, Edolo, 25048 Brescia, Italy

12 ³ Adamello Regional Park, Piazza Tassara 3, 25043, Breno (Brescia), Italy

13 ⁴ Acque Bresciane S.r.l., Via Cefalonia, 70 – 25124 Brescia, Italy

14 * Corresponding author, Department of Agricultural and Environmental Sciences (DiSAA),
15 Università degli Studi di Milano, via Celoria 2, 20133, Milan, Italy. Phone: +39 02 503 16903; Fax:
16 +39 02 503 16911. E-mail address: alessio.cislaghi@unimi.it (Alessio Cislaghi)

17

18 Abstract

19 Constructed wetlands (CWs) are effective and sustainable engineered systems for domestic and/or
20 municipal wastewater treatments. In the last half-century, CWs have become common solutions
21 for small communities in remote mountainous areas. However, cold climate in mountain
22 environment often causes a significant reduction of the pollutants removal performance
23 compared to warmer environments. Therefore, in the present study, we aim to help build
24 consensus on the best design practices for CWs in mountain environments. A meta-analysis of the
25 scientific literature focusing on the CWs in cold climate was conducted. Meanwhile, several
26 monitoring activities (chemical analysis on wastewater and treated water and analysis of changes
27 in the plant communities) were carried out on 6 CWs observing the different conditions between
28 immediately after the construction and 3-7 years later. The results showed an encouraging
29 agreement between scientific studies and observations from the monitored case studies. Lack of
30 plan space, low temperatures and supply meteoric inflows prompted the engineers, in the case
31 studies, to design systems composed of septic tank and horizontal subsurface bed. Such a solution
32 showed a good treatment efficiency (removal rates were more than 60% for most pollutants) in a
33 harsh mountainous environment. A significant difference was evident in the choice of plants.
34 Although the common reeds (*Phragmites australis*) remain an excellent solution, in plain and hilly
35 areas, planting of tufted hairgrass (*Deschampsia cespitosa*) ensures similar average performance
36 but is more suitable for the mountain ecosystems. In conclusion, the present study proposes
37 technical and engineering recommendations and a sort of *ecological design* to increase
38 wastewater treatment efficiency and adapt the systems to a natural and cold environment.

39 Keywords

40 Wastewater treatment; common reed; tufted hairgrass; wetlands plants; pollutant removal.

41 Introduction

42 Wastewater treatment is an extensively explored practice that constantly requests methodological
43 and technological developments for enhancing the efficiency in removing contaminants, reducing
44 the costs, and for improving environmental sustainability. Constructed Wetlands (CW) are a
45 suitable technique, widely adopted for their cost-effective, energy-efficiency, and environmental-
46 friendly impact on treating various types of wastewaters (municipal-domestic, industrial,
47 agricultural runoff, landfill leachate, polluted rivers, etc.) (Stefanakis and Tsihrintzis 2012; Flores et
48 al. 2019). CWs are an integrated ecosystem that remove efficiently several pollutants such as

49 biochemical oxygen demand (BOD), chemical oxygen demand (COD), total phosphorus (TP), and
50 total nitrogen (TN), by a synergic process, involving plant uptake, microbe assimilation, and
51 substrate adsorption (Wu et al. 2015; Jafarinejad and Jiang 2019). In addition, more than 25 years
52 of experience showed that CWs assure lower cost, fewer operation and maintenance
53 requirements than the traditional ones (Cooper 1999). Although these solutions bring evident
54 advantages, the research is still ongoing, especially in remote mountainous areas (Schwitzguébel
55 et al. 2011; Wang et al. 2017). Significant problems are related to i) the construction work where
56 large and flat surface space and unrestricted accessibility are often missing, and ii) to the cold
57 temperatures that reduce the pollution removal efficiency of the system (Varma et al. 2021).
58 Many studies agree on how pollutants removal efficiencies are higher in summer than in winter
59 (Kadlec et al. 2000; Tunçsiper 2009) and how the best nutrient removal rate occurs at a
60 temperature of 30°C (Akpor et al. 2013). Low temperatures negatively affect phytodegradation,
61 nitrification, denitrification, microbial degradation, and adsorption (Ji et al. 2020; Varma et al.
62 2021). Although some authors proposed solutions to overcome the impact of low temperature on
63 pollutants removal, the limits of CWs in cold environment are evident and design and plant species
64 selection remain a challenge (Yan and Xu 2014; Wang et al. 2017).

65 The designers could act over many parameters: hydrological and hydraulic characteristics of the
66 system (subsurface or open water surface flow), flow path (horizontal or vertical), medium (grain
67 size and additional filters), and type of plants (emergent, submerged, or free-floating) (Zhang et al.
68 2017). Three main types of phytoremediation beds can be found in literature: horizontal
69 subsurface flow (HSF), vertical subsurface flow (VSF), and free-water surface (FWS-CW). In HSF and
70 FWS-CW the bed is horizontal, dug in the ground at 0.5-1m-depth, while in the first a wastewater
71 flow that always is embedded, while in the second a free dispersion of water on the surface. In
72 VSF, instead, vertical tanks are filled with the medium and vegetated, and the wastewater is
73 inputted on the surface and moves vertically to the outlet.

74 In remote and cold mountainous areas, the selection of hydrological and hydraulic features and
75 direction of flow is often subject to external conditions, such as topography and accessibility, thus
76 the plant species begin a crucial design parameter (Mustafa et al. 2009; Brisson and Chazarenc
77 2009; Vymazal 2011a; Jain et al. 2020). In particular, the roots of plants play an indispensable role
78 in the subsoil producing organic matter that is the energy source for heterotrophic activity by
79 microorganisms and fungi, in their turn, responsible for transforming and decomposing the

80 contaminants (Brix et al. 2007; Kadlec and Wallace 2009). Meanwhile, the plants cover the surface
81 offering insulation and keeping substrate-free in colder periods (Vymazal 2011b). In addition, the
82 right choice of plants is key to maintaining the integrity of the landscape and enhance the
83 ecological and environmental value of a wetland ecosystem (Celewicz-Gołdyn and Kuczyńska-
84 Kippen 2017). Stems, roots, and leavers serve as a substrate for microbes and bacteria, and a
85 shelter for invertebrates and vertebrates (Rejmankova 2011).

86 In this context, despite the scientific communities making efforts to investigate the impact of
87 different plant species on pollutants removal, few are the studies focusing on the selection of
88 plant species that allow to balance the treatment performance and the ecological aspects in
89 remote and cold climate areas (Põldvere et al. 2009; Cicero Fernandez et al. 2019). Exploring the
90 experiences in high mountains is a necessity to address the design of new efficient systems.

91 In the present study, the main purpose is to provide some recommendations derived from a
92 detailed literature review and a mid-term monitoring of some case studies in mountainous areas
93 of the Alps, at low temperature. For this purposed, we organised the work into three main
94 chapters and objectives:

- 95 • A meta-analysis of the wide literature on CW applications in cold climates to investigate which
96 typology, medium, plant species and precautions can improve the performance in wastewater
97 treatment.
- 98 • A field monitoring that focuses on the removal rate over time of 6 CWs built with different
99 designs and plant species.
- 100 • The plant community evolution– the only design parameter that evolves over time – within the
101 6 studied CWs through the means of ecological criteria.

102 Furthermore, the present study provides an experience for an *ecological design* of new CW
103 systems that, as well as being efficient, have to be properly adapted to a natural and precious
104 mountainous landscape as in protected areas.

105 [Case studies](#)

106 The study sites are six CW systems situated in Camonica Valley (Lombardy, Northern Italy) in the
107 municipalities of Cedegolo, Cevo, Edolo, Lozio and Sonico (Figure 1 and Figure 2) and five of them
108 within the borders of Adamello Regional Park. These systems are situated at an altitude varying

109 from 600 m to 2450 m a.s.l. and are in operation for at least 2 years (Table 1). Two of them treat
110 municipal wastewater (50-1000 PE), while the others the domestic wastewater of four shelters
111 (30-50 PE). The CWs are constructed with a similar scheme composed of a septic tank as primary
112 treatment, connected to a horizontal subsurface flow system as secondary treatment, and in one
113 case with a free water surface system. The horizontal beds were built with an average depth of
114 0.6-0.7 m, filled with medium-size gravel and zeolite, and covered by plants.

115 *Table 1. Description of study sites: location, construction year, elevation, PE, system set-up,*
116 *medium and planted plant species.*

117 *Figure 1. Location of study sites.*

118 *Figure 2. Photographs of case studies: (Table 1): [a] Cevo, [b] Tonolini, [c] Aviolo, [d] Gnutti, [e]*
119 *Grevo, and [f] Lozio.*

120 [Material and methods](#)

121 [Meta-analysis](#)

122 The study provides a meta-analysis of the recent scientific publication focused on the use of CWs
123 for wastewater treatment in mountainous areas in cold climate. The selection includes all the
124 studies conducted on sites with an average annual temperature of less than 10°C (and an average
125 temperature in winter less than -5°C) and covers a wide range of developments in this field. In
126 particular, the meta-analysis focuses on several factors such as the CW typologies (horizontal
127 subsurface flow HSF, vertical subsurface flow VSF and free-water surface FWS-CW), the substrates
128 used as a medium, the plant species, and the capacity to treat different pollutants.

129 [Monitoring CWs](#)

130 [1.1.1 Water quality analysis](#)

131 Inlet and outlet samples of domestic wastewaters were collected from inspection wells after the
132 primary and secondary treatments. The water quality analyses were conducted for 3 out of the 6
133 case studies (Aviolo, Cevo, and Tonolini). Two replicates sampling were conducted in Tonilini and
134 Aviolo shelter, and three in the Chevo phytoremediation system. The replicates sampling was
135 conducted during the summer when the activity of the shelter is more intense. Samples were
136 transported to laboratory and analysed immediately for BOD, COD, nitrate (NO_3^-), ammonium
137 (NH_4^+), and phosphates (PO_4^{3-}) using conventional methods (American Public Health Association
138 2005).

139 1.1.2 Vegetation analysis

140 Floristic-vegetational data were collected in the 6 areas occupied by the CWs, immediately after
141 the construction (t_0) and after several years of operation (t_x , from 2 to 7 years later). Some
142 sampling plots of 2-16 m² were defined on the CW beds of each study area (Aviolo: 3; Tonolini: 3;
143 Gnutti: 3; Cevo: 2; Lozio: 3; Grevo: 3) where the plants were planted during the construction. The
144 plant species (tracheophytes) of each sampling plot were collected and identified in the months of
145 July for some consecutive years (2019-2022) using “Flora d’Italia” dichotomous keys (Pignatti,
146 1982; Pignatti et al., 2017) and the percentage of coverage was estimated for each of them.

147 1.1.3 Ecological indicators

148 Six floristic-ecological indicators proposed by Landolt et al. (2010) were used to evaluate the
149 environmental requirements of the plant species found in scientific literature and detected in the
150 sampling areas: soil moisture (F), humus content (H), light needs (L), nutrients in the soil (N), soil
151 reaction (R), and temperature (T). The values of these indexes range from 1 to 5 (Table 2). From
152 the floristic-vegetational data collected, the mean values of each indicator were calculated by
153 averaging the species values weighted on their abundance (percentage of coverage). Such indexes
154 were used to compare the ecological differences and the evolution trajectory between the plant
155 communities stabilized immediately after the CW construction and approximately 5-years later.

156 *Table 2. Range of values for the Landolt’s indicators.*

157 Results

158 Meta-analysis

159 The selected studies are 77 that investigated the removal performance of CWs in cold climates
160 located in 27 different countries (Supplementary Materials – Table A). The meta-analysis focused
161 on 4 different CW types: HSF, VSF and two hybrid systems, HSF+VSF and VSF+HSF. In fact,
162 subsurface systems are more suitable for treating wastewater at low temperature because the
163 heat loss is lower under the ground (Liang et al. 2020), whereas the free water surface systems are
164 heavily affected by adverse conditions such as water icing and plant withering (Kadlec and Wallace
165 2009).

166 1.1.4 CWs typologies

167 CWs typology significantly influences the performance of pollutants removal (TSS, BOD, COD, TN,
168 TP and NH₄⁺). Among the scientific studies, the majority focused on HSF systems (55%), whereas

169 hybrid systems (25%) and VSF systems (20%) were less investigated in mountainous areas. The
170 efficiencies of each CWs typology showed wide variability in the function of pollutants (Figure 3).
171 In general, the combination of VSF and HSF systems shows the best performance in reducing
172 contaminants in wastewater, with more than 78% for TSS, BOD, COD and TP and more than 67%
173 for nitrates and ammonia. The combination of HSF and VSF systems recorded the worst
174 performance with five out of six pollutants removal rates less than 75% on average. HSF systems
175 showed good performance over 77% of removal efficiencies for TSS, BOD, COD and NH_4^+ , whereas
176 moderate values for TN and TP, 52% and 66% removal rate respectively. Similar results were
177 obtained for VSF systems that guaranteed the best performance in removing TSS concentration (-
178 91% TSS concentration), good performance of over 80% of removal for BOD and COD, and a
179 moderate performance for the other pollutants (<80% removal rate). Table 3 summarizes the
180 efficiency of CWs typology in reducing some wastewater pollutants.

181 *Figure 3. Distribution of removal efficiencies of CW systems in cold climate collected in the*
182 *literature in function of CW's typology and pollutants.*

183 *Table 3. Average and standard deviation of removal efficiencies of CW systems in cold climate*
184 *collected in the literature in function of CW's typology and pollutants*

185 1.1.5 Medium

186 The choice of medium in CWs depends on hydraulic permeability and on the capacity in absorbing
187 pollutants. In the scientific literature, the CWs substrates, frequently used and investigated, are
188 numerous, grouped by natural material (sand, gravel, clay, calcite, marble, zeolite, bauxite,
189 bentonite, etc.), industrial by-product (slag, fly ash, mineralized refuse, hollow brick crumbs, etc.)
190 and artificial media (activated carbon, light weight aggregates, compost, biochar, calcium silicate
191 hydrate, etc.). By restricting the selection of the widespread natural media, sand enhanced the
192 best performance in terms of removal rates with all the pollutant removal rates higher than 70%
193 on average (Figure 4). Conversely, despite the good performance in removal TSS, BOD and COD,
194 gravel medium showed poorer results in reducing the concentrations of TN, TP and NH_4^+ . Clay
195 medium revealed the worst performance in reducing TSS and COD, but the best performance in
196 reducing TP. Table 4 summarizes the efficiency of CWs in function of the medium.

197 *Figure 4. Distribution of removal efficiencies of CW systems in cold climate collected in the*
198 *literature in function of CW's typology and medium.*

199 *Table 4. Average and standard deviation of removal efficiencies of CW systems in cold climate*
200 *collected in the literature in function of CW's typology and medium*

201 1.1.6 Plant species

202 Approximately 60% of the analysed studies investigated CWs where the common reed
203 (*Phragmites australis*) was exclusively planted without distinction for the typology (Figure 5).
204 Approximately 13% of the CWs planted common reed in combination with other species. The
205 second plant more used in CWs was the cattail (*Typha* spp.) for 6% of case studies. In addition,
206 20% of studies reported a wide spectrum of plant species (approximately 50), especially those of
207 the genera *Carex* (sedges), *Iris*, *Schoenoplectus* or *Scirpus* or *Bolboschoenus* (bulrush) and *Salix*
208 (willows). Among these genera, it was possible to identify 23 species of the Alps (Table 6Table 5).
209 According to the Landolt's indicators associated with each species, all these 23 species were not
210 tolerant to the alpine climate preferring the hilly climate ($T=3.67\pm 0.65$) and were more suitable
211 with a mid presence of humus content ($H=3.52\pm 1.08$), of nutrients ($N=3.52\pm 0.67$), and with an
212 extremely wet soil ($F=4.37\pm 0.80$). Moreover, such species prefer neutral soils ($R=3.30\pm 0.82$).

213 The Landolt's indicators showed how the commonly used species such as *P. australis* and *Typha*
214 spp. did not perfectly fit the cold climate features. In fact, *P. australis* grows well where the soil is
215 rich in nutrients ($N=4$), and high level of humidity ($F=4.5$), suitable for HSF system. Conversely, this
216 species requests mid-high temperature ($T=4$), light exposure ($L=3$) and moderate presence of
217 humus in the soil ($H=3$). Landolt's indicators for *Typha* spp. (*T. angustifolia* and *T. latifolia*) are
218 higher than for *P. australis*. Such species request alkaline soils ($R=4$), submerged water conditions
219 ($F=5$) and high light need ($L=4$).

220 *Figure 5. Distribution of plant species in CWs found in literature.*

221 *Table 5. List of the 23 plant species found in literature and commonly widespread over the Alps,*
222 *with Landolt's indicators values.*

223 Monitoring CWs

224 1.1.7 Chemical analysis

225 Chemical analyses were carried out for the first 4-5 years after the construction of CW. In Cevo
226 shelter, the pollution removal rates were 86.74% for BOD, 89.18% for COD, 59.45% for NO_3^- ,
227 78.37% for NH_4^+ , and 74.28% for PO_4^{3-} (Figure 6). This performance in removing pollution was the
228 best in the observed case studies. In Tonolini shelter, the pollution removal rates were 68.64% for

229 BOD, 60.66% for COD, 46.89% for NO_3^- , 75.59% for NH_4^+ , and 60.09% for PO_4^{3-} (Figure 6). In Aviolo
230 shelter, the pollution removal rates were 66.76% for BOD, 61.98% for COD, 24.40% for NO_3^- ,
231 70.06% for NH_4^+ , and 51.70% for PO_4^{3-} (Figure 6). Here, the performances were lower than the
232 others but satisfactory over 60% for BOD and COD, and over 70% for NH_4^+ . Scarce removal rates
233 were observed for NO_3^- and PO_4^{3-} . All 3 monitored CWs did not show a trend of removal pollution
234 rates over time, showing good performance already from the first year after the construction. The
235 complete results of the chemical analyses were reported in Supplementary Materials - Tables B, C
236 and D.

237 *Figure 6. Removal pollution rates over the time for the case studies of Cevo, Tonolini and Aviolo.*

238 1.1.8 Vegetation analysis

239 Once put into operation the CW, the plant species were few: in Tonolini, Gnutti and Aviolo
240 shelters (>1900 m asl), the number of species varied from 2 to 6, whereas in the other CWs only *P.*
241 *australis* was planted. In the first three case studies, tufted hair grass (*Deschampsia cespitosa*) was
242 always present, whereas *Trichophorum cespitosum* and *Festuca rubra* were two on three times.
243 The number of species sharply increased over the time as expected, while the plant coverage was
244 approximately always higher than 90%, except for Grevo where it was around 60%. Where *P.*
245 *australis* was planted, the number of species colonised the empty spaces among each other,
246 benefiting from a wet and rich nutrient shallower soil layer (especially in the first part of the CW
247 bed). In fact, 94 plant species were identified (Supplementary Materials – Table E). The maximum
248 number of species was found in Aviolo (n=25), whereas the minimum one was in Cevo (n=5).

249 Considering the floristic-physiognomic changes of plant communities (Figure 7), in Aviolo, Tonolini
250 and Gnutti, *D. cespitosa* was the dominant species increasing its coverage on the CW beds,
251 whereas deergrass (*T. cespitosum*) and red fescue (*F. rubra*) were gradually replaced by several
252 other species. In the other cases, *P. australis* remained the dominant plant of the community with
253 a slight increase of other species (<20% of the coverage) where the position of the CW bed
254 guaranteed a good brightness. Conversely, in Grevo, a poor brightness and a high humus content
255 on the CW bed because of the coverage by chestnut foliage did not favour the growth of the *P.*
256 *australis*. Among the 10 identified species, the common nettle (*Urtica dioica*) began the dominant
257 species. Finally, in Lozio and Cevo, *P. australis* remained the dominant species approximately 80%
258 of the plant community.

259 *Figure 7. Plant community distribution over the time: t_0 is the beginning of the operation (after the*
260 *construction) whereas t_x represents the time after 2-7 years after the construction; n.b. “other*
261 *species” represents the species with a plant community distribution less than 10%.*

262 1.1.9 Ecological indicators

263 Ecological indicators showed the plant evolution trajectories since the operations has been
264 starting. The evolution of plant communities after 2-7 years is very similar in all the sites where
265 only *P. australis* was planted (Table 6). In detail, the colonizing plant suits well towards an
266 environment with low variations of soil moisture and temperature, and with an increment in light
267 needs, nutrients, and humus concentration. Similar behaviour was observed in Gnutti, despite the
268 elevation difference, except for humus content and light needs. In this case, the colonizing plant
269 community seems to appreciate less humus content and light brightness. The remaining site
270 showed a different trend in terms of ecological indicators. In Aviolo, all the indicators tend to
271 increase especially in a wet and humus-rich environment. Conversely, in Tonolini, the results
272 showed the highest discrepancies: a significant decrease in humus content, light and nutrient
273 needs and a significant increase of temperature, soil moisture and soil reaction needed than the
274 plant community planted during the CW construction.

275 *Table 6. Variations of Landolt’s indicators immediately after the construction (t_0) and 2-7 years*
276 *later (t_x).*

277 Discussion

278 Design key parameters from literature to experience

279 The criteria for CW design and operation include the selection of site, substrate, wastewater type,
280 plant species, hydraulic loading rate, hydraulic retention time, water depth, operation mood and
281 maintenance procedures (Kadlec and Wallace 2009; Akrotos et al. 2009). In cold mountainous
282 regions, the choice of the design key parameters should be done considering both the effects of
283 the low temperature on the wastewater treatment performance (on phytodegradation,
284 nitrification, denitrification, microbial degradation, and adsorption of the pollutants) and the
285 impacts of the planted plants on the ecological landscape (including their ability to survive in a
286 specific environment) (Varma et al. 2021).

287 1.1.10 Typology

288 This study conducted a detailed review that identifies the VSF+HSF systems as the best solution in
289 terms of the reduction of water pollutants. However, this typology of CW requests wide and plain
290 spaces that are rarely available. For these reasons, the most adopted solution was the
291 combination between a Imhoff tank (or septic tank) and HSF. The first element settles the solids
292 on the bottom, while the wastewater treatment is conducted through the horizontal subsurface
293 flow. The subsurface flow is more suitable in cold climates as their wastewater treatment process
294 is under the ground where heat loss is less (Liang et al. 2020). Conversely, all those systems
295 including surface flow are strongly affected by wastewater disruption due to water icing and plant
296 withering (Kadlec and Wallace 2009). For these reasons, the typology composed of septic tank and
297 HSF is the most adaptable in cold mountain areas, as shown by the performance of our case
298 studies. Both septic tank and HSF bed have to be designed in function of PE: Many authors
299 recommend following standard designing criteria: 3-5 m² PE⁻¹, gravel-based with a mean grain size
300 <10 mm, with a slope of beds of 1-2 % and mean depth of beds of 0.7 m (Masi et al. 2000). These
301 designing parameters guarantee the best results in the removal of organic load, suspended solids,
302 hygienic load, irrespective of the variations in hydraulic load, the characteristics of water treated
303 and of seasonal temperature changes.

304 1.1.11 Medium

305 A design key parameter is certainly the medium that usually is selected in function of its hydraulic
306 permeability and the capacity of absorbing different pollutants. Poor conductivity causes an
307 increase of risk of clogging, whereas poor absorption by substrate means low long-term removal
308 performance (Wang et al. 2010). In this context, this study underlined the good performance in
309 removing pollutants provided by sand and fine gravel. However, these media increased the risk of
310 clogging the first part of the CW bed where the wastewater flow is concentrated. For this reason, a
311 reliable expedient is to fill the initial and outlet zones of the CW bed with coarse gravel to favour
312 the wastewater flow. Moreover, it is evident how sand and gravel are poor candidates for long-
313 term phosphorus and nitrogen storage; thus, the designers prefer to add other media
314 supplements. For example, at low temperatures, the performance could be improved mixing sand
315 and/or gravel with other materials such as zeolite, lightweight aggregates and carbonate that
316 reduce all the main pollutants. Another interesting media supplement was the biochar: recent
317 studies showed good performance in removing nitrates (Boehm et al. 2020) and was considered
318 an effective alternative technology for its low cost. However, under low-temperature conditions,

319 some authors noticed a decrease of microbial activity and as consequence nitrogen removal
320 efficiency (Zhang et al. 2021). In all our systems, the choice fell on a 50-100 mm gravel with zeolite
321 supplement; at an elevation higher than 1900 m asl the designers placed an additional layer of
322 natural soil above the substrate.

323 1.1.12 Plant species

324 A challenge in designing CWs is to select plant species or better plant communities that can
325 improve the pollutants removal rate. The most common plant used in CWs for wastewater
326 treatment were *P. australis*, *Typha* spp., and *Scirpus* spp. (Reed and Brown 1992). Undoubtedly, *P.*
327 *australis* is largely recognized as the “best plant” for treating wastewater and groundwater
328 contaminated by organic pollutants (Fester 2012), even in a low-temperature environment. *P.*
329 *australis* has a positive effect in removing organics, ammonia, phosphorus, and fecal coliforms,
330 and in reducing heavy metal and per-polyfluoroalkyl substances concentrations (Schierup and
331 Larsen 1981; Vymazal and Březinová 2016; Ferrario et al. 2022). This choice is confirmed also in
332 our cast studies (Figure 2). In Cevo and Lozio (800-1100 m als), 5 years after the construction, *P.*
333 *australis* has benefited from a good brightness and wet condition, remaining the dominant species
334 with a plant cover of around 80%. Nevertheless, *P. australis*, used in a cold climate, is probably
335 near close range to species ascension limit (Varma et al. 2021). In fact, *P. australis* (and *Typha*
336 spp.) often turns yellow when the temperature fell below 5°C and completely withers when the
337 temperature reached the freezing point (Yan and Xu 2014). In addition, *P. australis* generally
338 suffered a shaded environment (L=4) and an excessive presence of humus on the ground (H=3).
339 This fact was confirmed in Grevo, where the CW bed was degraded over time. The reasons were:
340 (i) dense foliage of three chestnut trees that covered the CW bed; (ii) a clogging of the inlet zone
341 due to the dense root network of the *P. australis* and the concentrated inlet wastewater flow; and
342 (iii) the presence of wild animals such as boars (*Sus scrofa*). *P. australis* cannot be the optimum
343 solution for high-altitude locations and/or for protected natural areas. As a potential alternative,
344 the first systems were covered by a shallower soil layer with meadow species, collected in
345 proximity. The monitoring conducted in the present study underlined how *D. cespitosa* was the
346 dominant species inside the conditions of the HSF beds. *D. cespitosa* is a common species of the
347 Alps, widespread in high-altitude meadows, marshes, and moorland (Davy 1980). Such species
348 grows colonizes on soils with impeded drainage, low oxygen concentration, a wide range of pH
349 (3.7-8.3) and poor nutrients (and also heavy metals) (Cox and Hutchinson 1979). Moreover, it has
350 little sensitivity to atmospheric humidity, wind-exposure and a longer period of cold temperature

351 (even producing more seed) (Davy 1982). As a tufted hairgrass plant, it was able to grow in a wide
352 range of light intensities, also acclimatising to shade. In terms of water pollutants treatment, *D.*
353 *cespitosa* tolerates high level of nitrogen and phosphorus especially ammonia and provides high
354 capacity in nutrient fixation.

355 [Problems, maintenance and possible solutions](#)

356 The literature overview and the monitoring of some case studies showed some
357 problems/solutions for the design of CWs in cold climate regions. This evidence can be resumed in
358 the following points.

- 359 • *Improving the wastewater treatment performance.* Some recommendations are: (i) control the
360 runoff flow and stormwater towards the system (directly or diluting the wastewater supply)
361 that strongly reduces the hydraulic retention time; (ii) if there is empty space, designing a VSF
362 upstream to HSF to promote nitrification and aerobic processes (Merlin et al. 2002; Mantovi et
363 al. 2003; Jenssen et al. 2005; Sayadi et al. 2012); (iii) sizing the CW beds at least $6 \text{ m}^2 \text{ PE}^{-1}$ to
364 reduce the impacts of external factors (Buchberger and Shaw 1995; Werker et al. 2002; Brix
365 and Arias 2005; Tunçsiper et al. 2015).
- 366 • *Sizing properly the CW bed.* Some recommendations are: (i) the wastewater has to be pre-
367 treated into a sedimentation tank (minimum volume 2 m^3 for a single household with up to 5
368 PE) (Brix and Arias 2005); (ii) the minimum length of the root-zone system is 10 m (Brix and
369 Arias 2005; Vymazal 2007); (iii) the bottom slope should be around 1% from inlet to outlet; (iv)
370 guarantee a minimum bed depth of 0.60 m at the inlet side of the bed and deeper towards the
371 outlet; (v) install a tight membrane (minimum 0.5 mm thickness) of polyvinyl chloride or
372 polyester reinforced with glass fibers to avoid the infiltration below; (vi) protect the membrane
373 with sand or a geotextile layer; and (vii) the substrate should be uniform sand and fine gravel
374 0.5-8 mm.
- 375 • *Selecting the most adapt plant species.* This study emphasized the importance of an *ecological*
376 *design* showing the potentialities of *P. australis* (as expected) and *D. cespitosa* in function of
377 the environment where the CW has to be constructed. *P. australis* guarantees good
378 performance especially at an altitude less than 1300 m a.l.s., making some few precautions: (i)
379 after planting, a periodic eradication of invasive species is recommended for the first 3 years
380 until complete colonization of the bed with a medium-high plant density (or plant cover)
381 (Budelsky and Galatowitsch 2000); (ii) maintain a water level of 5-10 cm under the ground to

382 favor the plant growth; (iii) avoid the presence of excessive level of humus content in the
383 shallower soil layer; and (iv) install a fence against wild animals (e.g. boars). Alternatively, for
384 locations at higher elevations and cold climates, *D. cespitosa* is a good solution. Some
385 recommendations are: (i) plant at the beginning of the growing season (end of the dormant
386 period); (ii) maintain the water level of 5-10 cm under the ground to favor the plant growth;
387 (iii) prevent stagnation in filtration bed that can degrade the plants, and (iv) ensure a good
388 brightness removing or thinning the trees canopy, if necessary.

389 • *Preventing clogging.* Some recommendations: (i) install an oil separator (degreaser) to prevent
390 flocculation, viscous layer and bulking and decrease solid waste floating (Perle et al. 1995;
391 Vidal et al. 2000; Cammarota and Freire 2006); (ii) spread the wastewater over the entire
392 width of the filtration bed; (iii) design inspection wells, in the proximity of the drainage pipes,
393 accessible to air pumps/metal stick/brushes to allow de-clogging actions. If the system allows
394 it (present a difference in ground level or it is raised compared to ground level) leave
395 horizontal access to the pipe. If this is not possible, provide the drainage pipe with inspection
396 manholes to insert an air pressure pump; (iv) design inlet and outlet zones as a transverse
397 trench filled with stones ensuring that no wastewater is exposed to the atmosphere; and (v)
398 use coarse and lightweight material (such as plastic media) for the inlet part of filtration bed to
399 get easier the cleaning and replacing if necessary (Tatoulis et al. 2017).

400 Conclusions

401 In the last decades, CWs have become increasingly common solutions for wastewater treatment
402 of small communities in remote mountainous areas. Although they enhanced good performance
403 in pollutants removal rate and had low impact on landscape. However, in cold climates, low
404 temperatures do not promote plant growth and reduced the removal performances of many
405 pollutants. The literature review showed that the combination VSF+HSF filling the beds with sand
406 and fine gravel guarantee a good performance also in cold climates. The plant that was largely
407 used is *P. australis* in the previous studies. The monitoring results partially reinforced the outcome
408 of the meta-analysis, observing a good treatment efficiency in the monitored sites also in harsh
409 mountainous environments (average concentration reductions were BOD=61%, COD=42%,
410 NH_4^+ =76%, PO_4^{3-} =61%). The lack of available space forced the engineers to design systems
411 composed of septic tank and horizontal subsurface beds. The substrate that balances the
412 requested dimensioning size of the bed and the performance is the fine gravel with a moderate

413 percentage of zeolite that favours pollutant abatement. Moreover, a significant result was
414 provided by the floristic-vegetational monitoring and the ecological indicators that showed as *P.*
415 *australis* remains an excellent solution for most warm environments, but where the CW has to be
416 integrated into natural and, sometimes, protected areas, planting *D. cespitosa* assures similar
417 removal performance and provides more eco-compatibility. Thus, *D. cespitosa* is a suitable
418 phytoremediation species for the mountain environments of the Alps. In addition, the present
419 study reinforced the importance of an ecological assessment of the plant communities, already
420 present in the areas and able to colonise the empty space, that has to be inserted in the design
421 process as a sort of *ecological design* of the CW. Finally, combining literature and experience is
422 possible to provide technical, engineering, and botanical measures to improve the design and
423 maintenance of the CWs, to increase the wastewater treatment efficiency and exploit the multi-
424 functionality of these areas.

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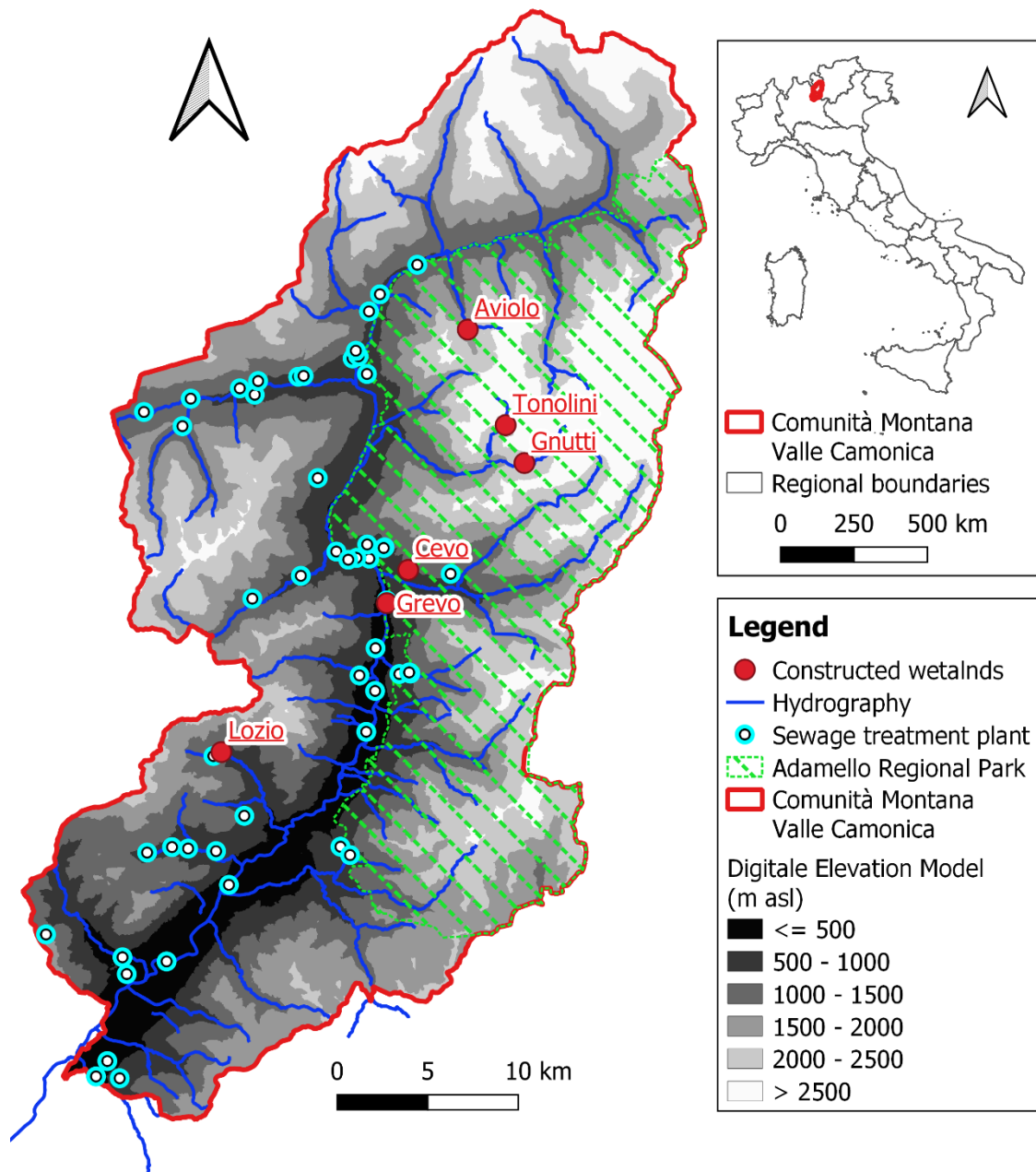
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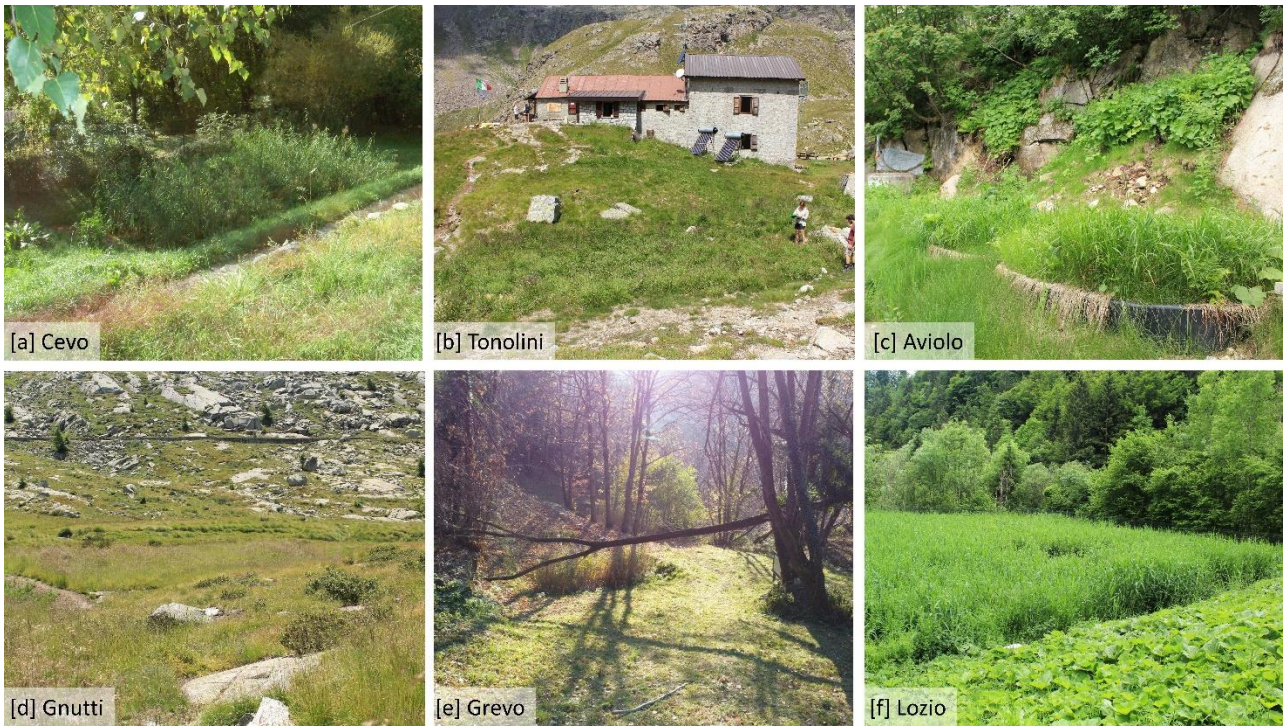
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1 Figures



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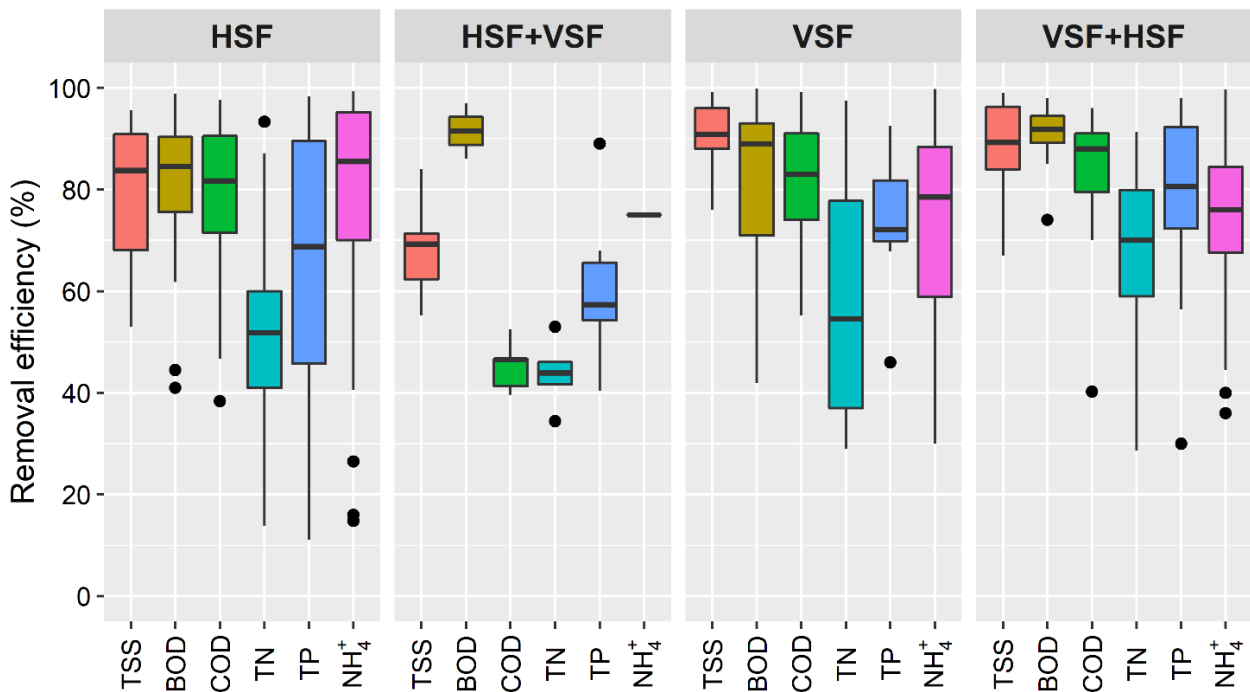
3 Figure 1. Location of study sites.



4

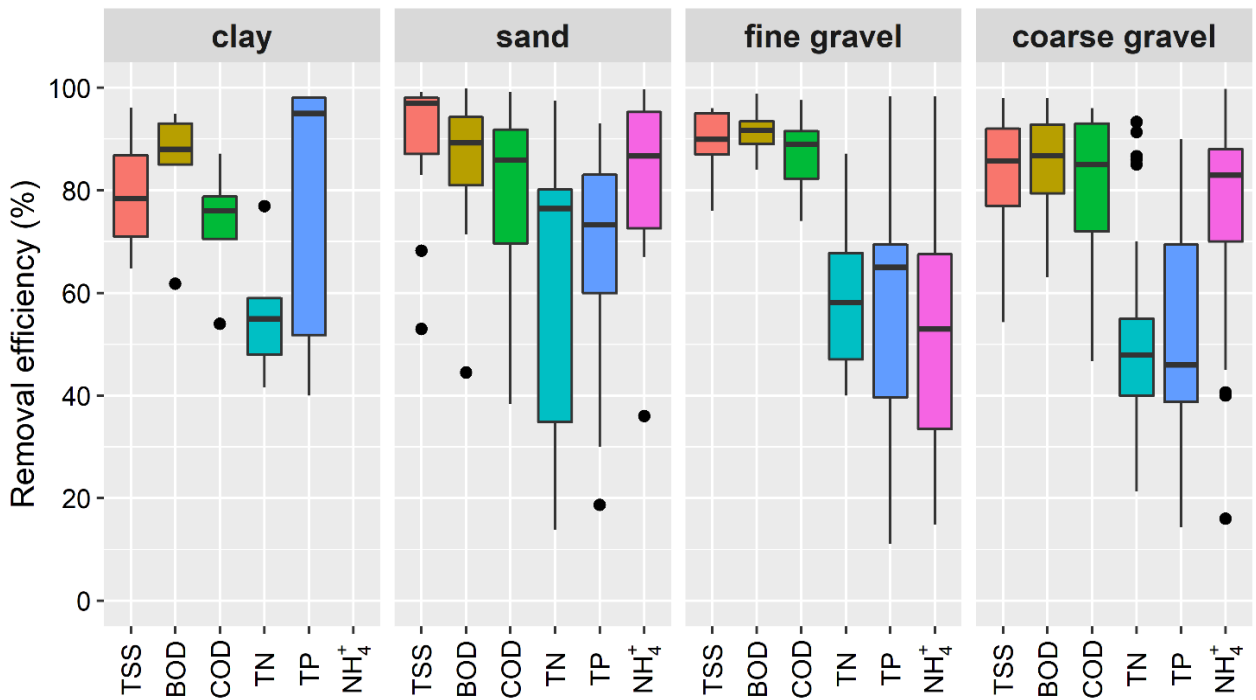
5 *Figure 2. Photographs of case studies: (Error! Reference source not found.): [a] Cevo, [b] Tonolini,*
 6 *[c] Aviolo, [d] Gnutti, [e] Grevo, and [f] Lozio.*

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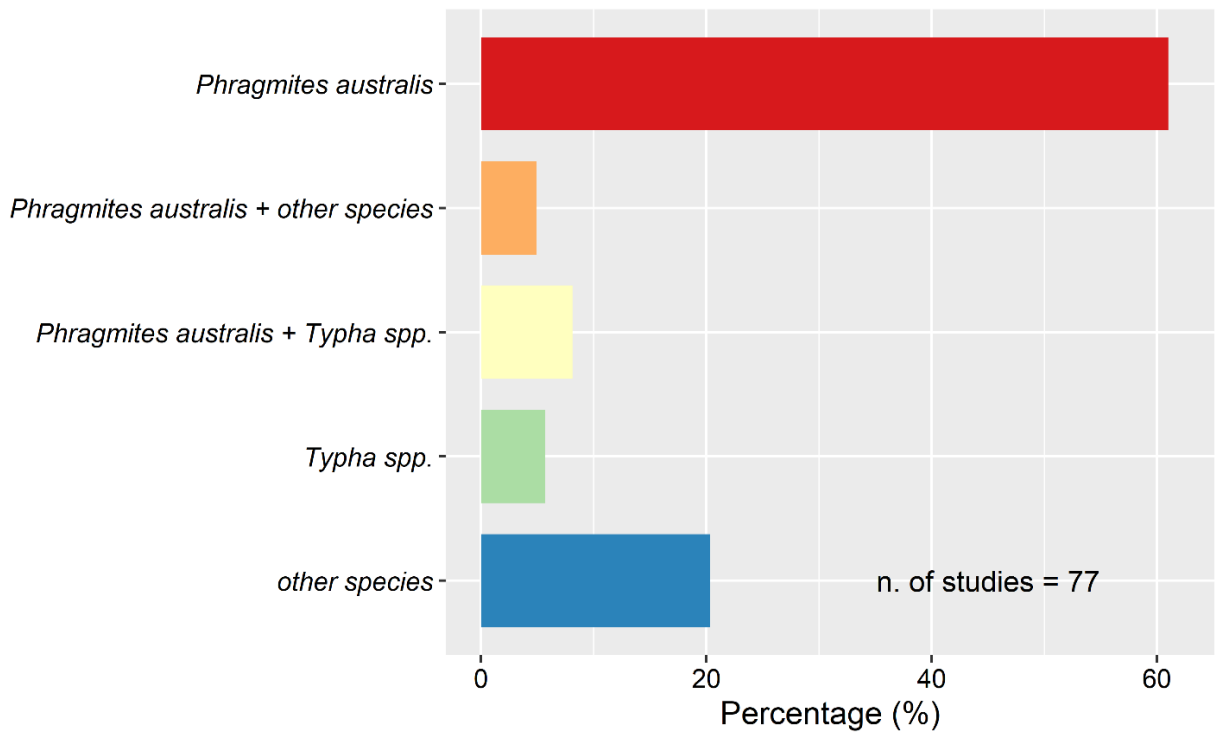
9 *Figure 3. Distribution of removal efficiencies of CW systems in cold climate collected in the*
 10 *literature in function of CW's typology and pollutants.*



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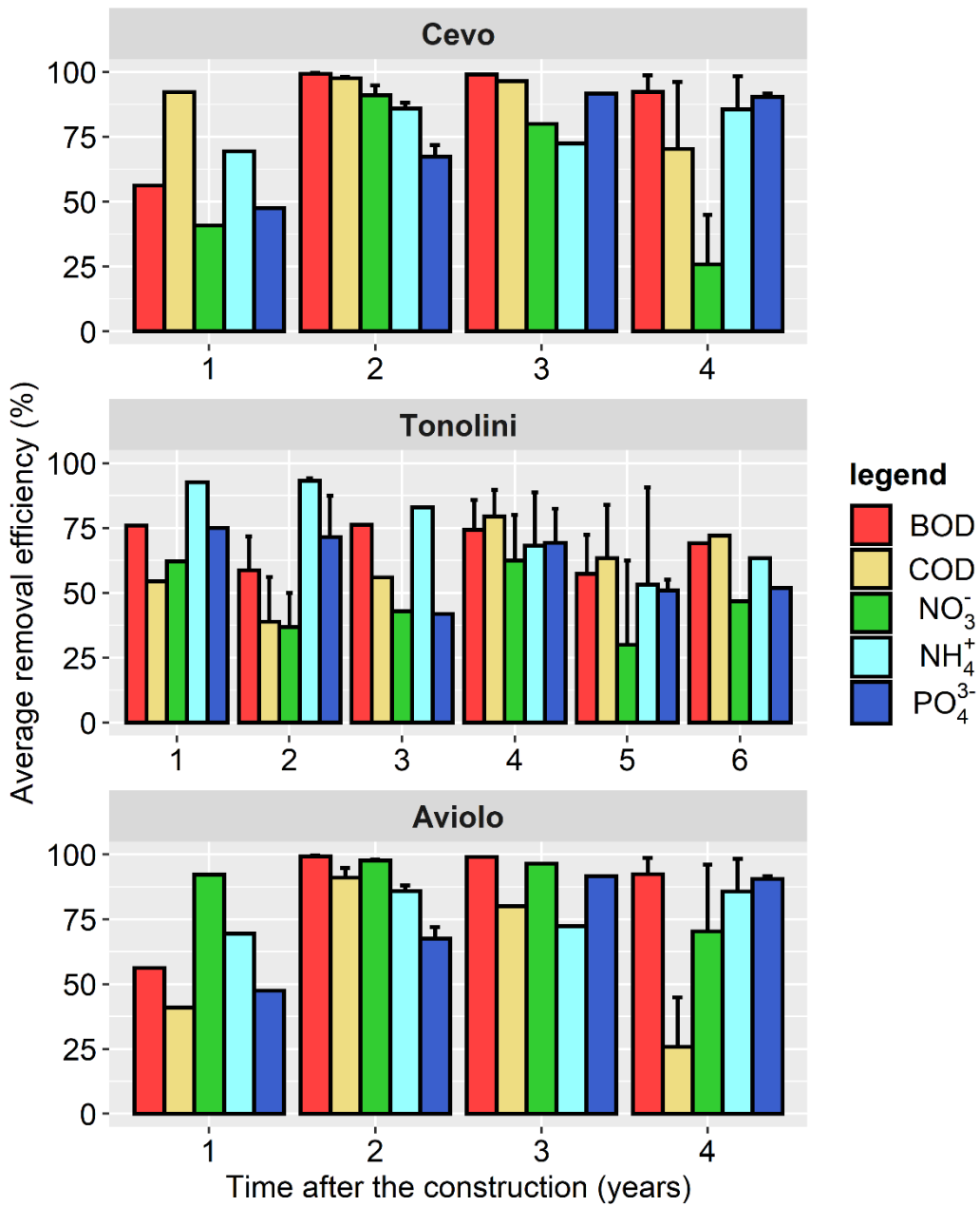
12 Figure 4. Distribution of removal efficiencies of CW systems in cold climate collected in the
 13 literature in function of CW's typology and medium.

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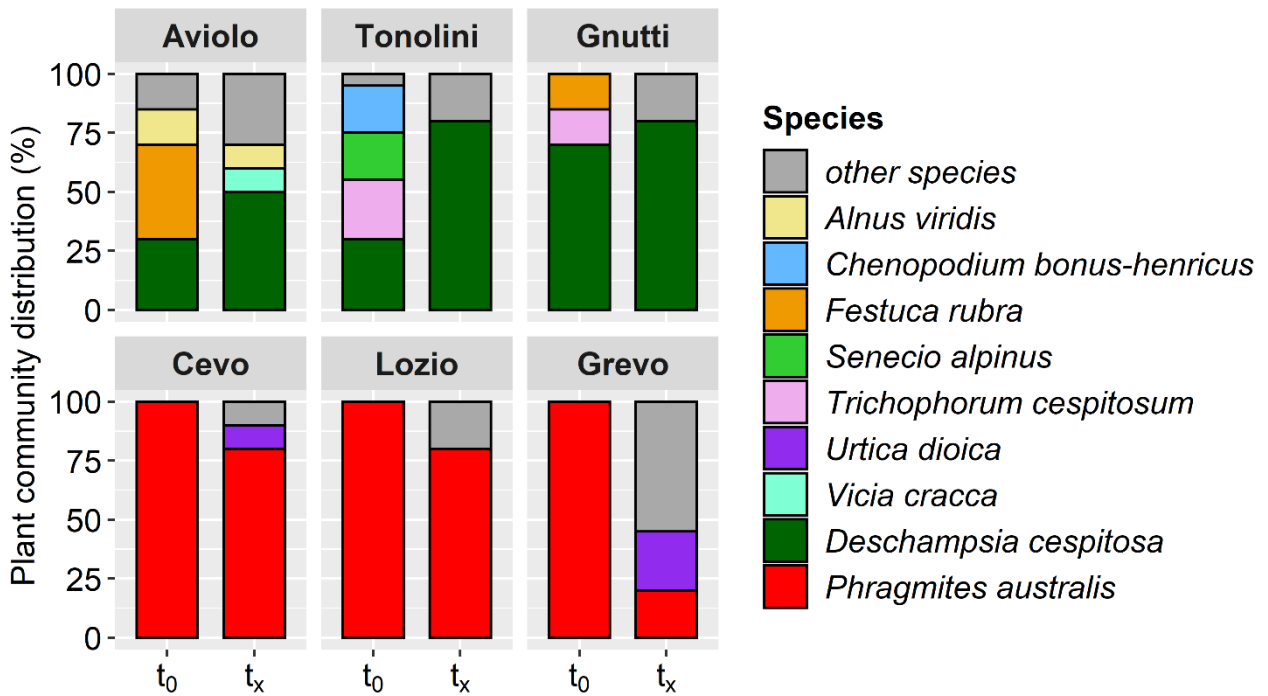
16 Figure 5. Distribution of plant species in CWs found in literature.



17

18 *Figure 6. Removal pollution rates over the time for the case studies of Cevo, Tonolini and Aviolo.*

19



20

21 Figure 7. Plant community distribution over the time: t_0 is the beginning of the operation (after the
 22 construction) whereas t_x represents the time after 2-7 years after the construction; n.b. "other
 23 species" represents the species with a plant community distribution less than 10%.

1 Tables

2 Table 1. Description of study sites: location, construction year, elevation, PE, system set-up, medium and planted plant species.

Study site (Abbreviation)	Location	Construction year	Elevation m a.s.l.	PE	CW description	Medium	Plant species
“Casa del Parco” shelter (Cevo)	Cevo (BS)	2014	1100	50	Septic tank + 2 horizontal subsurface beds (4.7m x 15.3m)	70 cm: zeolite + 50-200mm gravel	<ul style="list-style-type: none"> • <i>Phragmites australis</i>
Franco Tonolini Shelter (Tonolini)	Sonico (BS)	2013	2450	20	Degreaser + Septic tank + horizontal subsurface wetland (6m x 10m)	60 cm: zeolite + 50-100mm gravel	<ul style="list-style-type: none"> • <i>Senecio alpinus</i> • <i>Leucanthemopsis alpina</i> • <i>Chenopodium bonus-henricus</i> • <i>Deschampsia cespitosa</i> • <i>Trichophorum cespitosum</i>
Occhi Sandro all'Aviolo Shelter (Aviolo)	Edolo (BS)	2015	1935	30	Degreaser + Septic tank + 2 parallel series of 5 horizontal subsurface wetlands (2m x 2.5m)	60 cm: zeolite + 50-100mm gravel	<ul style="list-style-type: none"> • <i>Festuca rubra</i> • <i>Deschampsia cespitosa</i> • <i>Senecio nemorensis</i>

						10 cm: natural soil	<ul style="list-style-type: none"> • <i>Alnus viridis</i> • <i>Vaccinium myrtillus</i> • <i>Rubus idaeus</i>
Serafino Gnutti (Gnutti)	Sonico (BS)	2019	2166	40	Degreaser + Septic tank + horizontal subsurface wetland (9m x 15m)	60 cm: zeolite + 50-100mm gravel	<ul style="list-style-type: none"> • <i>Deschampsia cespitosa</i> • <i>Trichophorum cespitosum</i>
Grevo-Cedegolo CW (Grevo)	Cedegolo (BS)	2013	510	50	Septic tank + horizontal subsurface wetland + open water surface wetland	70 cm: 50-100 mm gravel	<ul style="list-style-type: none"> • <i>Phragmites australis</i> • <i>Nymphaea alba</i> • <i>Iris pseudacorus</i>
Villa-Lozio CW (Lozio)	Lozio (BS)	2014	884	1000	5 Septic tanks + 2 horizontal subsurface wetlands	50 cm: natural soil 15 cm: 10-20 mm gravel 20 cm: 40-70 mm gravel	<ul style="list-style-type: none"> • <i>Phragmites australis</i>

3 *Table 2. Range of values for the Landolt's indicators.*

Environmental factor	Indicator values [1-5]
Soil moisture (F)	Gradient from extreme dry soils (1) to plants growing underwater (5)
Humus content (H)	Gradient from humus-poor soils (1) to humus-rich soils (5)
Light needs (L)	Gradient from deep shade (1) to full light (5)
Nutrients (N)	Gradient from nutrient-poor soils (1) to nutrient-rich soils (5) mainly nitrogen
Soil reaction (R)	Gradient from acidic soils (1) to carbonate-containing alkaline soils (5)
Temperature needs (T)	Gradient from cold sites (alpine or nival belt) (1) to warm places (plain belt) (5) of the Alps

4

5

6 *Table 3. Average and standard deviation of removal efficiencies of CW systems in cold climate*
 7 *collected in the literature in function of CW's typology and pollutants*

Typology	TSS	BOD	COD	TN	TP	NH₄⁺
HSF	80.16±13.60	81.59±13.06	78.37±15.72	52.64±17.29	66.31±24.51	77.06±22.97
VSF	91.38±5.87	81.94±17.76	80.79±13.83	58.19±24.32	73.99±12.86	74.85±20.56
HSF+VSF	68.29±10.04	91.50±7.78	45.31±5.10	43.83±6.18	60.92±16.40	75.00±0.00
VSF+HSF	88.44±9.58	90.67±5.90	84.11±13.66	67.54±18.44	78.63±18.27	72.52±18.90

8

9

10 *Table 4. Average and standard deviation of removal efficiencies of CW systems in cold climate*
 11 *collected in the literature in function of CW's typology and medium*

medium	TSS	BOD	COD	TN	TP	NH₄
clay	79.4±13.54	84.54±13.31	73.29±13.89	56.1±13.4	76.56±28.32	±
sand	90.03±13.78	84.67±15.97	79.03±19.06	62.08±27.67	67.75±20.68	81.9±17.31
fine gravel	89.8±5.94	91.43±4.39	87.14±6.74	59.79±16.28	56.7±32.91	53.63±26.22
coarse gravel	82.66±12.24	86.13±9.15	81.46±14.06	51.56±19.85	52.48±21.48	77.28±20.01

12

13 Table 5. List of the 23 plant species found in literature and commonly widespread over the Alps,
 14 with Landolt's indicators values.

Plant species	F	H	L	N	R	T
<i>Acorus calamus</i>	5.00	5.00	4.00	4.00	4.00	4.00
<i>Bolboschoenus planiculmis</i>	4.00	3.00	4.00	3.00	4.00	4.50
<i>Caltha palustris</i>	5.00	5.00	3.00	3.00	3.00	3.00
<i>Cardamine amara</i>	5.00	3.00	4.00	3.00	3.00	3.50
<i>Carex paniculata</i>	5.00	5.00	4.00	4.00	4.00	3.00
<i>Carex riparia</i>	5.00	5.00	4.00	4.00	4.00	4.00
<i>Carex rostrata</i>	5.00	5.00	4.00	2.00	2.00	3.00
<i>Deschampsia cespitosa</i>	4.00	3.00	3.00	3.00	1.00	3.00
<i>Glyceria maxima</i>	4.00	3.00	4.00	4.00	4.00	4.00
<i>Hippuris vulgaris</i>	5.00	5.00	4.00	3.00	4.00	3.00
<i>Iris foetidissima</i>	2.00	3.00	4.00	3.00	3.00	5.00
<i>Iris pseudacorus</i>	4.50	5.00	3.00	4.00	3.00	4.00
<i>Juncus effusus</i>	4.00	3.00	3.00	4.00	2.00	3.50
<i>Lolium perenne</i>	3.00	3.00	4.00	4.00	3.00	3.50
<i>Phalaris arundinacea</i>	5.00	3.00	4.00	4.00	3.00	4.00
<i>Phragmites australis</i>	4.50	3.00	3.00	4.00	3.00	4.00
<i>Rumex alpinus</i>	3.50	3.00	4.00	5.00	3.00	2.00
<i>Salix babylonica</i>	3.50	3.00	4.00	3.00	3.00	4.50
<i>Salix viminalis</i>	4.00	1.00	4.00	3.00	4.00	3.50
<i>Schoenoplectus lacustris</i>	5.00	3.00	4.00	3.00	4.00	3.50
<i>Schoenoplectus tabernaemontani</i>	4.50	3.00	4.00	3.00	4.00	4.00
<i>Typha angustifolia</i>	5.00	3.00	4.00	4.00	4.00	4.00
<i>Typha latifolia</i>	5.00	3.00	4.00	4.00	4.00	4.00
<u>Average value</u>	4.37	3.52	3.78	3.52	3.30	3.67
<u>Standard deviation</u>	0.80	1.08	0.42	0.67	0.82	0.65

15

16 *Table 6. Variations of Landolt's indicators immediately after the construction (t_0) and 2-7 years*
 17 *later (t_x).*

Site	Time	T	F	H	L	N	R
Aviolo	t_0	2.83	3.45	3.20	2.95	3.20	2.75
	t_x	2.83±0.32	3.72±0.022	3.37±0.18	3.05±0.44	3.15±0.10	2.82±0.48
Tonolini	t_0	2.58	3.68	3.50	3.55	3.25	2.65
	t_x	2.79±0.01	3.78±0.00	3.00±0.00	3.20±0.01	2.78±0.01	2.91±0.04
Gnutti	t_0	2.93	3.93	3.30	3.15	2.70	2.70
	t_x	2.86	3.77	3.06	3.13	2.93	2.91
Cevo	t_0	4.00	4.50	3.00	3.00	4.00	3.00
	t_x	3.85±0.04	4.26±0.02	3.00±0.00	3.08±0.02	4.10±0.06	3.06±0.07
Lozio	t_0	4.00	4.50	3.00	3.00	4.00	3.00
	t_x	3.84±0.48	4.28±0.47	3.00±0.01	3.07±0.17	4.08±0.04	3.07±0.42
Grevo	t_0	4.00	4.50	3.00	3.00	4.00	3.00
	t_x	3.64±0.17	3.65±0.43	3.00±0.11	3.18±0.11	4.01±0.03	3.20±0.09

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