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Low-Quality Irrigation Water Treated Using Waste Biofilters

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Abstract: Although in water-deficient regions, agricultural runoff, drainage water or surplus irrigation water is often used, there are constraints related to its quality to be considered (salinity, nutrients and pollutants). Thus, it is necessary to treat surplus irrigation water considering the low-energy supply systems available to farmers. This work focuses on a nature-based water treatment system consisting of two prototypes of anaerobic bioreactors with horizontal or vertical flow. To enhance the circular economy strategy, two different wastes (coarse sand and almond pruning) were used as bioreactor components. The aim of the research was to monitor the quality of the water (pH, electrical conductivity, suspended solids, chemical oxygen demand, alkalinity and bicarbonate, carbonate and nitrogen contents) before and after the treatment. All the parameters studied (except chemical oxygen demand) were reduced by the treatments, but with large variations. Furthermore, there was 100% nitrogen reduction in the horizontal water flow treatment with the filter bed formed by coarse sand and almond pruning. It was observed that the variation in the concentration of some parameters was associated with the type of filter bed (i.e., the C/N ratio of the residue) and with the design for water circulation flow. Although the findings are promising, further research is needed to achieve reductions in all studied parameters.

Keywords: eutrophication; non-conventional water resources; nature-based solution; zero waste



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1. Introduction

Worldwide water resources are increasingly coming under pressure, leading to water scarcity and a deterioration in water quality. The expected growth of the human population [1,2] entails an increase in global demand for resources such as food or water, 60% and 55%, respectively, by 2050 [3–5]. Future projections consider that a lack of water will affect 60% of the world's population by 2025 [6,7]. However, global water scarcity is caused not only by the physical scarcity of the resource, but also by the progressive deterioration of water quality; so, this reduces the quantity of water that is safe to use [8]. In 2015, 60% of surface waters in the European Union (EU) had a poor ecological status, mainly due to point pollution (e.g., wastewater) or non-point pollution (e.g., agriculture) [9,10]. Agriculture is the largest water user worldwide, and it accounts for 70 to 95 percent of total freshwater withdrawals, depending on the degree of the country's development [3,8].

Currently, water scarcity affects more than 40% of the global population [11], and in the EU, 29% of its territory was affected by water scarcity in 2019 [12]. In this context, non-conventional water resources are becoming more prominent [6]. To improve the worldwide water supply and sanitation infrastructure, it is estimated that USD 6.7 trillion are needed [4].

As a result of the increasing reuse and recirculation of water, water quality tends to deteriorate, and this restricts its future uses [13]. The reuse of wastewater for irrigation is widespread to improve the circular economy of water in urban settlements [14]. By 2023, it is expected that global water reuse will achieve 1.66% of total water use, with 32% of reclaimed

water used for irrigating [15]. In 2006, EU countries reused 964 million m³ year⁻¹, and Spain had the best share, 347 million m³ year⁻¹ [16].

Although agricultural runoff, drainage water or surplus irrigation water are often used in water-deficient regions, there are some constraints to be considered, such as salts, pathogens, emerging contaminants and nutrients because of fertilizer use [7–9,14]. Nitrogen (N) is an essential nutrient for crop yields and food production, but its excessive presence in aquatic ecosystems can trigger eutrophication processes. In Europe, for the period 2016–2019, water categorized as eutrophic included 81% of marina waters, 31% of coastal waters, 36% of rivers and 32% of lakes [17]. This poses problems for crop yield, ecosystems sustainability and human health [18–20]. Therefore, its repeated use should be carried out when an adequate quality is ensured. If not, agricultural drainage water (marginal water) must be treated, which implies addressing the difficulty of installing treatment plants in rural settings covering large or scattered agricultural areas.

To overcome this issue, and in relation to the European Green Deal [21], the EU Action Plan: “Towards a Zero Pollution for Air, Water and Soil” aims to reduce soil, water and air pollution, improving soil quality by reducing nutrient losses and chemical pesticides use by 50%. Additionally, in March 2020, the European Commission announced the adoption of the circular economy action plan (CEAP) [22,23] and prioritized the reduction, reuse, recycling and alternative management of waste materials. The CEAP represents a new economic and production paradigm that requires a shift in mindset, recognizing waste as a potential resource rather than a burden to be managed and discarded in landfills, as in the previous linear economy [24]. In addition, the Water Framework Directive [25] aims to ensure the sustainable use of water resources and its quality by 2027. Materials in suspension, substances that contribute to eutrophication and substances which have an unfavorable influence on the oxygen balance, among others, are a main concern. Moreover, the Nitrates Directive is an important instrument to achieve and proposes the use of eco-agricultural practices and nature-based solutions for water treatment and soil remediation [17].

In such a way, green treatment technology (constructed wetlands, waste stabilization ponds and infiltration land) is being used to model nature works mainly for wastewater remediation [26–30]. Nature-based solutions have more benefits compared to those of traditional wastewater treatments, such as a low maintenance requirement, cost effectivity, removal efficiency [29,30] and extensive design possibilities based on the element to be removed (water level and flow movement, phytoremediation, phycoremediation, substrate, aerobic or anaerobic conditions, whether it is energetically self-sufficient or not and nutrient recovery, among others). Bioreactors are one of the most used treatments since pollution removal is conducted due to retention on adsorbent material (biofilter) and microorganisms that accumulate on the adsorbent [31]. The surface of the biofilter is key for determining the biomass growth rate and biomass retention capacity [7,32,33]. Accordingly, the selection of adsorbent will determine the efficiency of the adsorption process [7]. A wide range of adsorbent materials, both inorganic and organic ones (agricultural waste, among others), have been studied for wastewater treatments, confirming its effectiveness for removing pollutants [32–34]. The use of waste can enhance the circular economy and avoid the costs associated with management [33]. Moreover, it can be a helpful practice as the increase in food production will lead to an increase in food waste. Agricultural waste, such as pruning residues, due to its porous and multi-hierarchical lignocellulosic composition, have intrinsic mesoporous structure, exceptional optical and mechanical characteristics and a high capacity for water transportation, which offers them interesting opportunities for water treatment [7].

Several authors consider that technosols can be designed to provide ecosystem services like a natural soil does and to recover a degraded ecosystem, including aquatic ones [35–42]. Technosols, have been successfully used to improve the surface runoff water quality in mining areas, urban stormwater and wastewater [7,32,33,43–47]. However, their ability to treat irrigation water has not been studied as much, especially when macrophytes are not involved [27].

Based on the previous ideas, the aim of this research was to study a nature-based treatment free of emergent vegetation by using residues as the adsorbent and the design of pilot biofilter systems to improve the quality of agricultural water. The physical and chemical parameters (pH, electrical conductivity, suspended solids, chemical oxygen demand, alkalinity and bicarbonate, carbonate and total nitrogen contents) of low-quality irrigation water before and after the treatments were determined to check the effectiveness of the treatments designed.

2. Materials and Methods

2.1. Irrigation Water Source

The irrigation water has its origin in the Main Irrigation Channel of Elche's reservoir (Alicante, Spain). Elche's reservoir is in the north of the city and receives water from Vinalopó river. This river is fed by natural waters and treated water from wastewater treatment plants situated along its basin. The irrigation channel of Elche's reservoir begins at the dam reservoir and runs in the same directions as Vinalopó river does, crossing the city of Elche from the north to the south.

The experiment was conducted over twenty weeks. Water was collected weekly (Figure 1) (UTM geographical coordinates X: 701,170.5 m; Y: 4,239,112.38 m), and fed into the biofilters systems. Irrigation water samples were analyzed immediately.



Figure 1. Sampling location map (National Geographical Institute of Spain).

2.2. Bioreactor Designs

Water pilot treatment plants were inspired by the performance of nature-based solutions using wastes as the adsorbent material. They were located inside the greenhouse of the University Miguel Hernández of Elche (Alicante, Spain) and were kept under controlled conditions. Two types of anaerobic bioreactors were designed, one with subsurface water and horizontal flow, and the other with subsurface water and vertical flow (Figure 2).

Both biofilters were made of fiberglass-reinforced polyester (Figure 2, part c). The horizontal bioreactor size was 120 cm × 15 cm × 35 cm (L × W × H), and the vertical bioreactor of 15 cm × 15 cm × 60.5 cm (L × W × H), and they had three sections. The first and last one (10 cm × 15 cm × 27 cm) were the water inlet zone and the water outlet zone, which were full of volcanic gravel (diameter approximately between 3–5 cm) and worked as pre-treatment and homogenization areas prior to the introduction of water to the anaerobic treatment. The middle section (length 100 cm) held the natural adsorbent, and both horizontal treatments had two layers. The bottom one contained wastes (22 cm) and the top one contained coarse sand (4 cm) to control and reduce the evapotranspiration of subsurface flow. The inlet point was 24 cm high, and the outlet point 20 cm high from the bottom of the bioreactor.



Figure 2. Bioreactors diagrams. At the top: anaerobic bioreactor with subsurface water and horizontal flow. At the bottom: anaerobic bioreactor with subsurface water and vertical flow. (a) Irrigation water in polyethylene deposits; (b) peristaltic pump; (c) biofilter; (d) effluent recovered in polyethylene deposits.

The vertical bioreactor had one section with two layers. The bottom one contained the wastes (48 cm high), and the top one contained sand (high 4 cm). The inlet point was situated at the top of the bioreactor, and the outlet point was 45 cm high from the bottom. Both types of bioreactors maintained the anaerobic conditions, and water (inlet and outlet) was disposed in polyethylene deposits.

The wastes used were selected for treatments based on their availability in the area (considering circular economy and zero waste strategy) and their adsorption potentiality. Inorganic residue was collected from the extractive activities of limestone deposits and fine gravel/coarse sand (2–3 mm) (G). This was composed mainly of calcium carbonate (over 99%), and to a lesser extent, magnesium carbonate, and the bed had a porosity of 41.8%. Further, an organic residue of almond tree pruning (A) was collected from agricultural areas close to Elche (Alicante, Spain). Almond tree pruning was subjected to conditioning processes consisting of air drying at room temperature and chopping (5 cm size). The porosity was 69.6%, and its characterization is provided in Table 1, and methods of analysis were previously published [48,49].

Table 1. Almond tree pruning characterization: organic matter content (OM), pH, electrical conductivity (EC) and bulk density (ρ_b), mean value (M) and standard deviation (SD) [48,49].

Residue	OM (%)		pH (units)		EC ($\mu\text{S cm}^{-1}$)		ρ_b (g cm^{-3})	
	M	SD	M	SD	M	SD	M	SD
G	0	0	9.90	0.03	107.85	17.62	1.55	0.05
A	93.2	0.6	4.66	0.007	665	0.80	0.36	0.006

Therefore, by combining the wastes and bioreactors design, four treatments were studied:

- Horizontal water flow with filter of G (HG).
- Horizontal water flow with filter of G and A (HA).
- Vertical water flow with filter of G (VG).
- Vertical water flow with filter of G and A (VA).

The constant supply of irrigation water to the bioreactors was achieved using peristaltic pumps (inlet point) from polyethylene deposits, keeping the flow rate in all the treatments (2.3 L day^{-1}) and the hydraulic retention time (4 days) the same. Bioreactors were covered with a black mesh of 1 mm situated over them (5 cm) to reduce evapotranspiration (0.5 mm m^{-2}) and protect from insect access and seed germination. Influent water in the deposits was replaced weekly to avoid water degradation. The effluent, as well, was taken weekly and directly from the source point as it arrived for an hour to ensure that we had enough water to analyze. Therefore, the bioreactors were used for substrate adsorption and microbial degradation as removal mechanisms.

2.3. Water Characterization Methods

Influent (I) and effluent water (E) -EHG, EHA, EVG and EVA- from each treatment was analyzed weekly: pH, electrical conductivity (EC), total suspended solids (SS), chemical oxygen demand (COD), total alkalinity and bicarbonate, carbonate and total nitrogen contents (N). Analysis of water samples was based on the APHA standard methods [50]. The pH was measured (method 4500-H+ and 2580) by using a CRISON GLP 21 pH-meter, and electrical conductivity (EC) was measured with a CRISON GLP 31 conductivity meter (method 2510). SS values were obtained after filtering the samples with 47 mm glass microfiber filters and heating them in an oven (J.P SELECTA CONTEM) at $105 \text{ }^\circ\text{C}$ (method 2540 D). The COD was tested using a digestion vials reagents kit, a thermoreactor (HI 839800-02) at $150 \text{ }^\circ\text{C}$ and a multiparameter photometer (HI 83300) (all from HANNA INSTRUMENTS (method 5220)). Alkalinity, bicarbonates and carbonates contents were measured according to the methods, 4500-CO₂ and 2320 D. The N content was measured using the HANNA kit (HI94767). The persulfate method was used to determine the total nitrogen content via the oxidation of all nitrogenous compounds to nitrate with the HANNA reactor (HI839800) at $105 \text{ }^\circ\text{C}$ and HANNA multiparameter photometer (HI83399).

Weekly changes in irrigation water characteristics were calculated as the percentage of variation according to Equation (1) [51]:

$$\text{Variation (\%)} = (1 - (C_e/C_i)) \times 100 \quad (1)$$

C_e : the value of the analyzed parameter in the bioreactor outlet water (effluent); C_i : the value of the analyzed parameter in the bioreactor inlet water (influent). When the result of variation is positive, there is a reduction of the analyzed parameter; on the contrary, when it is negative, there is an increment.

2.4. Statistical Analysis

Descriptive statistics were used to calculate the mean and standard deviation for each individual water test (five repetitions per each treatment). Analysis of variance (ANOVA) and Tukey's multiple comparisons test were conducted using SPSS Statistics (v.26).

3. Results and Discussion

3.1. Irrigation Water Characterization

Table 2 provides the mean value of the parameters analyzed in the influent (I) in the horizontal bioreactors (HG and HA) and in the vertical bioreactors (VG and VA).

Table 2. Irrigation water (influent) characteristics used for each type of bioreactor (horizontal and vertical), mean value (M) and standard deviation (SD).

Parameter	Units	Horizontal		Vertical	
		M	SD	M	SD
pH	(units)	8.25	0.09	8.27	0.11
EC	(mS cm ⁻¹)	17.45	1.55	18.26	0.78
SS	(mg L ⁻¹)	41.38	8.50	40.37	8.60
COD	(mg L ⁻¹ O ₂)	96.84	61.99	100.29	60.79
Alkalinity	(mg CaCO ₃ L ⁻¹)	250.69	18.16	260.12	14.85
Bicarbonates	(mg HCO ₃ ⁻ L ⁻¹)	150.15	10.76	155.60	8.78
Carbonates	(mg CO ₃ ⁻² L ⁻¹)	2.66	0.50	2.96	0.88
Nitrogen	(mg N L ⁻¹)	15.40	5.22	20.15	9.22

As it was expected, the inlet water characteristics were similar in both treatments; although, the water derived from the deposits used to fill the horizontal bioreactors and vertical bioreactors was obtained from the same source (time needed to prepare the systems and refill the deposits). So, there are slightly variations in the composition of the inlet water.

3.2. Effluent Characterization

pH, EC, SS, COD, alkalinity, bicarbonates, carbonates and N data obtained weekly are provided in a graphic format (Figures 3 and 4) and in detail in Appendix A (Tables 1–8).

All of the treatments showed a pH in the effluent (Figure 3a,b) lower than the pH of the influents (Table 2). The maximum pH value (8.48) was reached in the EHA in the fifth week, and the minimum (5.06) one was obtained in the EHA in the first week (Table 1). The contribution of almond pruning residue leads to greater fluctuations in the pH of the effluent (Figure 3a,b). Acidification in the first week of the EHA are due to the contribution of the highly soluble compounds from the almond pruning that can acidify water, e.g., (dissolved organic matter). According to Rodríguez-Espinosa et al. [49], the pH of the aqueous extract of almond pruning shows a value of 4.66 (Table 1). However, in the EHA, as the weeks passed, the pH values increased, obtaining the same as that in the EHG in week 20 (Table 1). However, the changes in the pH in the VA treatment, after an initial reduction, increased; although, at week 20, the lowest pH value of all effluents was observed (8.01). This may be associated with the type of bioreactor. The mean pH of EHG and EVG, both only with an inorganic bed, were similar and quite stable over time.

All the effluents showed a mostly higher EC than the incoming water did (Table 2). However, some differences were observed between the types of bioreactor (Figure 3c,d). Both horizontal effluents achieved lower EC during weeks 2 and 3, and only the EVA among vertical effluents maintained reached a lower EC than the inlet water did in weeks 1, 4 and 20. EC may be influenced by the type of bioreactor and, in general, an increment in the salinity was noticed in all the effluents. This means that these treatments have low efficiency, reducing the salinity of low-quality water.



Figure 3. pH, EC, SS and COD results of horizontal and vertical water flow bioreactors. (a) Weekly pH (units) of horizontal water flow bioreactors. (b) Weekly pH (units) of vertical water flow bioreactors. (c) Weekly EC (mS cm^{-1}) of horizontal water flow bioreactors. (d) Weekly EC (mS cm^{-1}) of vertical water flow bioreactors. (e) Weekly SS concentration (mg L^{-1}) of horizontal water flow bioreactors. (f) Weekly SS concentration (mg L^{-1}) of vertical water flow bioreactors. (g) Weekly COD concentration (mg L^{-1}) of horizontal water flow bioreactors. (h) Weekly COD concentration (mg L^{-1}) of vertical water flow bioreactors.

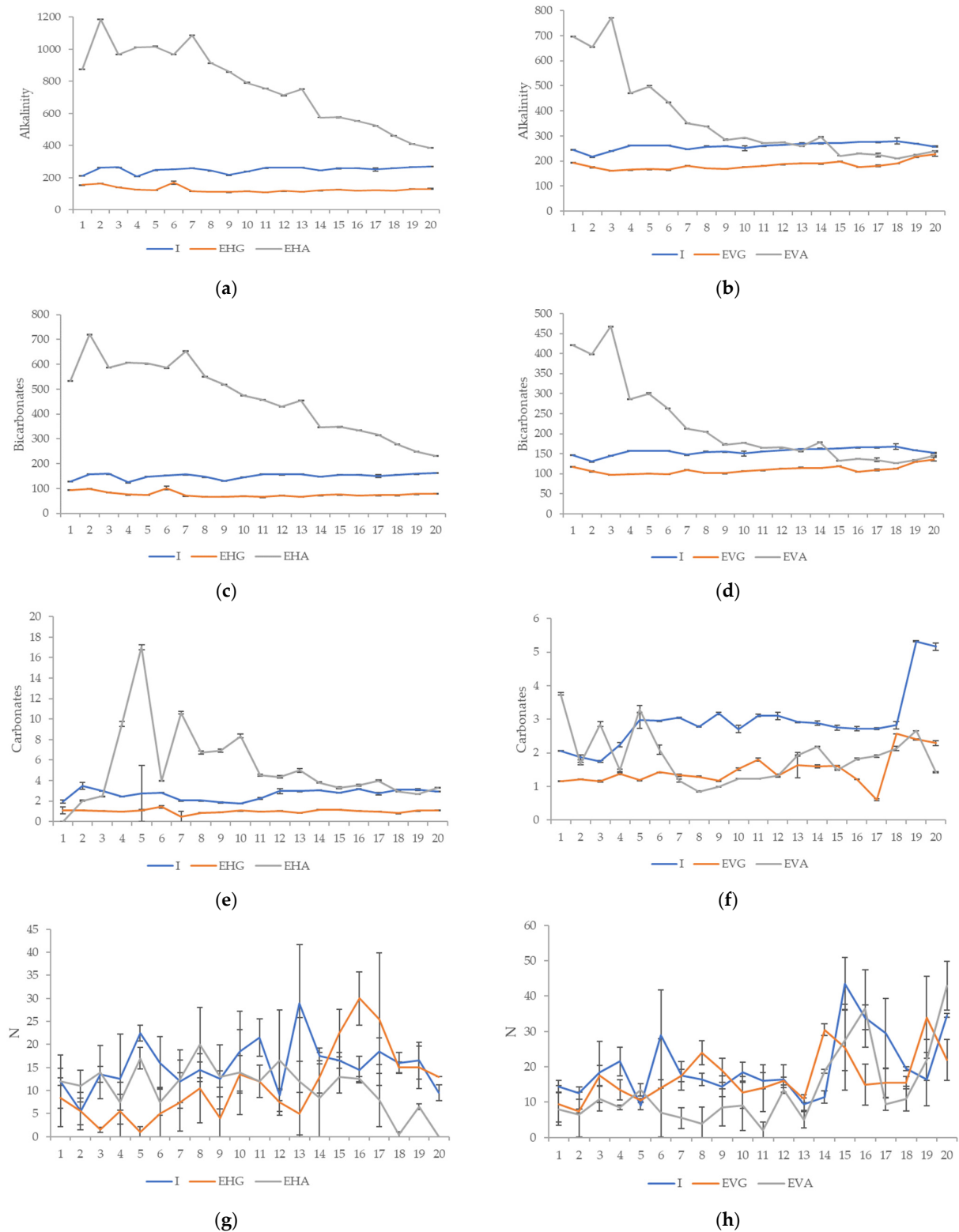


Figure 4. Alkalinity, bicarbonates, carbonates and N results of horizontal and vertical water flow bioreactors. (a) Weekly alkalinity concentration (mg L⁻¹) of horizontal water flow bioreactors. (b) Weekly alkalinity concentration (mg L⁻¹) of vertical water flow bioreactors. (c) Weekly bicarbonates concentration (mg L⁻¹) of horizontal water flow bioreactors. (d) Weekly bicarbonates concentration (mg L⁻¹) of vertical water flow bioreactors. (e) Weekly carbonates concentration (mg L⁻¹) of horizontal water flow bioreactors. (f) Weekly carbonates concentration (mg L⁻¹) of vertical water flow bioreactors. (g) Weekly N concentration (mg L⁻¹) of horizontal water flow bioreactors. (h) Weekly N concentration (mg L⁻¹) of vertical water flow bioreactors.

The values obtained for SS in the outlet waters in the EHA and the EVA were generally higher than those in the inlet water (Figure 3e,f). The use of organic waste in these cases favored the increment of the SS. The SS in the EHA was very high throughout the experiment, except for the last week (43.58 mg L^{-1}), when it was close to the inlet value (41.38 mg L^{-1}), as it is showed in the Appendix A (Table 3). In the VA treatment, there was an initial contribution to the SS that was stabilized from week 6, even reaching a lower concentration than the inlet water had until the last week (Figure 3e,f). The SS in the outlet water was always under the value of the inlet water in the EHG (except in week 4). The SS in the EVG was below the inlet water during all 20 weeks. Although, the SS concentration in the EVA reached the lowest value (22.07 mg L^{-1}) in week 16 (Table 3). Therefore, the SS was better controlled by the vertical bioreactors to facilitate precipitation and sedimentation processes and favoring the diminution of the SS in the outlet water.

None of the four bioreactors achieved a weekly lower COD than that of the inlet water (Figure 3g,h). A contribution of oxidizable organic matter released from the organic waste (A) can be observed in both type of bioreactors (Table 4). However, the concentration of the COD in the EVA was better, and even in week 20, the COD concentration was lower (346.75 mg L^{-1}) than the achieved in the EHG (396.25 mg L^{-1}). The inorganic bioreactors reached lower COD values comparing with the values of those containing almond pruning (Figure 3g,h). During experimentation, the COD reached similar values in the four treatments. In fact, this parameter is related to the biological activity of bioreactors and also dead matter coming from the biomass formed in the bioreactors.

Figure 4a,b shows the weekly alkalinity concentrations of the effluents. The weekly alkalinity concentration was always lower than the initial one (inlet waters) in the EHG and EVG, and they were the most stable systems to control this parameter. Although, the alkalinity concentration in the EVA fluctuated, from week 14, the results were below those of the influent water (Table 2). Inorganic bioreactors obtained the best values (109.36 mg L^{-1} in EHG and 162.19 mg L^{-1} in EVG), although they are composed of fine gravel/coarse sand composed mainly by calcium carbonate (Table 5).

The trend in the bicarbonate content of the effluents (Figure 4c,d) is like that shown for alkalinity (Figure 4a,b). Inorganic bioreactors achieved weekly concentrations lower (Table 6) than the initial ones (Table 2). Despite the high initial contribution of bicarbonates from the EHA and EVA effluents, due to the organic waste and the acidity of this residue, the VA system stabilized it, and from week 15, it showed a concentration lower than that of the influent (Table 6).

Figure 4e,f shows the concentration of carbonates determined in all the treatments over the 20 weeks in each effluent. Inorganic bioreactors showed lower carbonate concentrations than the incoming water did (Table 2). In the organic bioreactors, an initial contribution of carbonates was observed, which was greatly exacerbated in the case of EHA (Table 7). However, in the organic vertical system (VA), from week 6, the carbonate concentration was lower than the concentration presented in the low-quality irrigation water, and it reached the lowest value among all treatments in the first week (0.01 mg L^{-1}).

Regarding the most important parameters of water quality, N concentration is one of the most relevant due to the possible eutrophication that can be caused by inorganic N in water (lakes and coastal areas). The results in the effluents are shown in Figure 4g,h and in Table 8. All the treatments reached lower N concentrations than the inlet water did for several weeks (Table 2), but fluctuations in N reduction are seen every 2–3 weeks. This variability is associated with changes in the microbial activity and the removal capacity associated with the increment of biomass and the needs of N for this increase (Table 8). The HA treatments showed fewer fluctuations in the N concentration. In fact, from week 17, this treatment reached a substantial reduction of N, reaching an almost total reduction in the last week. At this point, the microbial activity was very consolidated, and in the last weeks, the inlet water shows a lower N concentration, so that the need for N by the microbial population (sized for a higher N input) may not be met; so, there is a higher N demand. Probably, this means that this treatment would be the best to control N.

Table 3 provides the weekly variation, in percentages, for each parameter analyzed. In all treatments, a pH variation was observed, reducing the pH of the effluents (0.8%, 0.8%, 3.6% and 6.5% in EHG, EHA, EVG and EVA, respectively) at the end of the 20 weeks. In the systems with organic wastes, although there were fluctuations (increase and reduction), the pH reduction was predominant, which may be due to the action of anaerobic microorganisms' metabolisms [52]. The inorganic vertical system achieved higher percentages of pH reduction, reaching its maximum at week 17 with 6.8%. The highest percentages of pH reduction were obtained in the EHA (38.2% in week 1) and EVA (8.4% in week 8) effluents, mainly due to the initial contribution of the most soluble organic acids from the organic waste. VA achieved the greatest reduction.

The trend of the EC was associated with the type of flow: water circulation, horizontal or vertical (Table 3). In the horizontal systems, there was a very high contribution of EC during the first week (−24.8% in EHG and −39.6% in EHA), but both systems reached positive variations in the second and third weeks. However, from the third week, the percentages of reduction, although fluctuating, remained negative. For the vertical systems, though they also obtained negative percentages (except for the first week), the EVA one obtained an EC variation percentage of 0.1 in the last week, which was compared to −0.2% for the EVG one. In general, salinity was affected negatively, with slight increments in the effluents.

Table 3 shows how the variation in the SS in the effluents depends to a greater extent on the type of absorbent (inorganic or a combined organic+inorganic bed). Thus, EHG and EVG showed positive SS variation over the 20 weeks, except in weeks 5 and 6 (EHG) and in weeks 16 and 20 (EVG). EHG and EVG reached maximum SS variation percentages of 54.8% and 58.2%, respectively. The bioreactors with organic waste showed greater difficulties in reducing the SS, especially with horizontal water flow. EHA had a high initial SS input (up to −1650.8%), so that its variation percentages up to week 17 showed very high negative values. EVA managed to reach positive percentages of variation from week 7, ending with the best percentage of variation (15.8%) in the last week. Particulate matter from the bed of the bioreactors was responsible for this increment, mainly in the bioreactors with the presence of almond waste.

None of the systems achieved a positive weekly variation in the COD percentage (Table 3). These results agree, in some way, with the results obtained for the SS presented in the effluents. The biological activity after the first few weeks can help to maintain a higher COD in the effluents regarding the values of influents.

The inorganic systems showed positive variations in alkalinity (reducing the alkalinity) during all the weeks (Table 3). In fact, EHG reached its maximum positive variation in week 11 (58.2%), and EVG reached its maximum positive variation in week 6 (36.8%). EHG maintained high percentages of variation until week 20 (51.4%); however, EVG at week 20 obtained a 12% variation. High initial alkalinity was observed in the organic treatments with the presence of almond pruning; although, EVA continued to have a positive variation from week 13 (except for week 14), and at week 20, this was 7%. The same trend of variation was observed for bicarbonates (Table 3).

High percentages of variation were obtained with carbonates (Table 3). The systems with only inorganic waste showed positive variations in all the weeks, obtaining the highest percentages of variation in week 7 (78.2%) for EHG and in week 17 (77.5%) for EVG. Regarding the bioreactors with almond waste, EVA started with negative variations, but from week 5, the values were positive, ending in week 20 with the maximum value of reduction (72.5%). However, EHA started with positive reduction percentages (99.7% at week 1), but from week 4 (except for weeks 18 and 19), the percentages were negative.

Table 3. Variation in the parameters analyzed (%) in horizontal and vertical bioreactors from weeks 1 to 20.

pH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	−0.6	3	2.6	2.8	0.7	1.4	0	−0.5	0.3	−1.6	−0.8	1.2	2.3	1.3	0.6	1.7	1.7	2.4	1.6	0.8
EHA	38.2	9.7	8.4	1.4	−2.8	4.3	−1.3	0.3	−0.4	−2.6	1.4	2.9	3.2	3.3	3.5	3.6	1.5	2.7	2.7	0.8
EVG	1.1	1.4	−0.6	−0.2	2.5	1.9	2.7	1.8	2.4	1.1	0.3	2.1	0	0.7	1.3	1.9	6.8	−1.7	2.6	3.6
EVA	2.2	6.5	2	5	2.5	4.1	6.8	8.4	6.1	4.9	6.4	4.8	1.7	1.7	2.3	1.9	0.7	0	2.3	6.5
EC	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	−24.8	10.4	0.7	−3.2	−16	−14.3	−14.9	−14.5	−14.9	−13.4	−11.7	−15	−8.8	−13.6	−5.1	−11.6	−9.8	−8.6	−5.3	−13.0
EHA	−39.6	8.2	0.4	−11.5	−14.8	−6.8	−8.4	−5.3	−11.1	−15.8	−9.4	−10.5	−7.3	−13.5	−2.8	−8.2	−6.7	−8.9	−5.5	−11.8
EVG	1.0	−2	−1.1	−1.6	−2.6	−1.1	−2.8	−3.7	−2.9	−1.8	−2.4	−0.1	−8.2	−6.8	−4.8	−5.5	−1.5	−2.7	−1.7	−0.2
EVA	0.3	−0.7	−3.3	0.7	−2	0	−1.6	0.9	−3.0	−1.6	−2.7	−0.1	−6.6	−0.7	−5.1	−1.3	−0.4	−2.5	−2.4	0.1
SS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	54.8	31.6	22	0.2	−80	−2.8	30.6	19.1	22.0	25.3	39.6	15.0	26.4	31	47.2	23	7.7	36.1	49.4	7.7
EHA	−81.1	−1650.8	−1080.3	−994.1	−607.2	−499.2	−416.3	−651.1	−354.4	−93.6	−114.4	−148.0	−49.4	−134.2	−162.8	−104.3	−93.6	−14.8	1.7	−22.2
EVG	25.8	37.8	43.5	47.9	29.6	44.7	37.9	42.5	39.9	36.8	58.2	55.5	21.9	27.0	44.1	−13.3	8.3	23.8	23.1	−6.6
EVA	−452.6	−354.4	−273.9	−108.2	−10.4	−50.2	18.9	36.2	17.5	36.7	41.1	48.6	24.1	12.3	31.7	24.9	−9.7	21.9	0.3	15.8
COD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	−695.3	−281.3	−401.9	−378	−508.6	−376.3	−390.3	−434.1	−344.4	−420.3	−315.9	−2.1	−192.3	−390.2	−303.1	−401.7	−268.4	−395.7	−344.9	−323.8
EHA	−34,561.8	−7253.6	−4156.6	−3088.8	−2122.1	−1048.8	−951.0	−846.4	−689.4	−711.6	−545	−52.3	−314.2	−501.1	−424.2	−508	−371.3	−445.7	−344.1	−364.4
EVG	−454.7	−233.2	−310.5	−246.2	−1.8	−141.8	−322.1	−233	−273.5	−183.1	−350.6	−292.9	−303.5	−204.0	−242.7	−351.3	−444.2	−275.5	−415.6	−316
EVA	−1896.4	−1490.1	−1217.2	−410.6	−34.4	−137.7	−376.1	−259.8	−315.7	−247.1	−350.9	−265.1	−279.1	−258.5	−222.8	−281.7	−396.5	−298.6	−361.5	−391.8
Alkal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	26.8	37.9	47.2	39.5	50.2	33.2	55.6	54.4	48.5	51.7	58.2	55	57.1	50.8	51.2	53.8	51.6	53.7	51.2	51.4
EHA	−310.3	−348.3	−264.4	−385.3	−309.5	−281.6	−316.8	−274	−296.4	−229.5	−189.1	−171.4	−187.4	−133.3	−123.3	−112.9	−109.1	−77.0	−54.8	−42.4
EVG	20.6	19.2	32.4	36.7	36.2	36.8	26.3	34.1	35	29.7	30.2	29.5	29.3	29.8	27.3	36.5	34.5	32.2	19.2	12
EVA	−184.8	−201.8	−221.6	−80.1	−89.5	−65.7	−42	−30.9	−9.8	−16.7	−4.2	−3.4	4	−9.9	19.1	17	18.9	24.5	16.2	7
Bicarb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	26.5	37.1	46.8	39.1	50	33	55.3	54.4	48.5	51.9	58.2	54.8	56.8	50.6	51.1	53.5	51.4	53.3	50.9	51.2
EHA	−316.8	−357.8	−270	−387.5	−305.6	−286.2	−315.6	−274.7	−296.8	−227.7	190.5	−173.9	−189.8	−135.7	−125.4	−115.1	−110.2	−78.8	−56.2	−43
EVG	20.3	18.9	32.4	36.7	35.8	36.6	25.7	33.7	34.4	29.4	29.9	28.9	29.1	29.5	27.1	36.2	33.7	32.6	17.9	10.5
EVA	−186.3	−204.9	−223.6	−81.8	−91.1	−67.6	−44.2	−32.8	−11.4	−18	−5.9	−4.6	3.4	−10.5	18.6	16.8	18.7	24.6	15	4.7
Carbo.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	43.5	67.9	65.3	59.8	59.5	48.5	78.2	57.9	51.4	38.7	56.2	64.6	71.1	62.9	57.9	67.1	64.4	73.4	66	63
EHA	99.7	41.9	17.2	−287.3	−519.7	−41.8	−417.2	−226.5	−270.3	−379.2	−100.9	−46.8	−68.8	−25.1	−18.3	−11.6	−47.2	6.2	14.2	−12.3
EVG	43.6	34.9	33.2	38.8	60	51.9	56.2	53.2	63.1	43.9	42.4	57.2	43.9	44.7	41.4	55.3	77.5	9.1	54.9	55.7
EVA	−82.8	10	−64.1	34	−11	29.2	61.6	69.6	68.6	54.6	60.5	57.4	33.9	24.4	45.6	33.1	30	24.5	50	72.5
N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
EHG	29.2	0	88.9	56	95.6	68.8	37.5	27.6	68	27	44.2	16.7	82.8	25.7	−36.4	−106.9	−37.8	6.3	9.1	−36.8
EHA	0	−100	−3.7	40	24.4	53.1	−4.2	−37.9	−4	24.3	44.2	−83.3	58.6	51.4	21.2	13.8	56.8	96.9	60.6	100
EVG	34.5	40	5.4	37.2	−16.7	51.7	0	−45.5	−31	31.1	12.5	3	−15.8	−165.2	41.4	55.9	47.5	20.5	−106.1	36.2
EVA	44.8	48	40.5	60.5	−50	75.9	68.6	75.8	41.4	51.4	87.5	18.2	47.4	−63	36.8	−7.4	67.8	43.6	−33.3	−24.6

Note(s): Alkal.: alkalinity; Bicarb.: bicarbonates; Carbo.: carbonates.

Biological nitrogen removal is based on the process of the oxidation of ammonium to nitrate (nitrification) and the denitrification of nitrate to nitrogen gas and the efficiency of these processes. Increased dissolved oxygen contents can negatively affect nitrogen removal [53]. So, maintaining anaerobic conditions would facilitate N removal. Although the reactors are anaerobic, the best anaerobic conditions prevail in the deeper layers [20]. A priori, by checking the great N results (reduction of 100%) of the EHA reactors at week 20 (Table 3), which were better than the others, we came to think that the absence of oxygen contributed to N removal [54,55]. However, EHG and EVA reached high values of N reduction at weeks 5 (95.6%) and 11 (87.5%), respectively.

The results of previous studies indicate that the pH can influence N removal processes. Although Wu et al. [56] concluded that alkalinity enhances a higher denitrification rate, Feng et al. [57] showed that the N removal was higher when reactors use acid-treated carriers. As mentioned before, the pH of the aqueous extract of almond pruning shows a value of 4.66 [49]. In these pilot bioreactors, the best nitrogen reduction values were obtained in the presence of almond residue. Moreover, this waste facilitates the microbial biomass growth due to its porous structure.

The C/N ratio is also a determinant for denitrification processes; so, at a low C/N ratio, denitrification is reduced [56], and the opposite is also true. According to the results obtained by Rodríguez-Espinosa et al. [58], almond pruning residues have a high C/N ratio (C/N = 89), which could facilitate nitrogen removal (denitrification). As a consequence, microorganisms need an extra N supply (coming, in this case, from inlet water) to process N from almond tree pruning. Therefore, this result is in line with the conclusions obtained by the authors of the above-mentioned reference.

4. Conclusions

Water quality assurance is starting to be of interest mainly in water-deficient regions. Technologies based on nature-based solutions are a valid option to improve the quality of such water resources, as well as to promote the circular economy when using waste as adsorbent materials. However, the changes in water quality parameters are not the same for all of them, and the design and construction of pilot plants to improve water quality should be considered for each case.

For most of the studied parameters in this work (pH, SS, COD, alkalinity, bicarbonates, carbonates and N), the type of waste used in the bioreactors has a large influence. However, the design and flow of water (horizontal or vertical circulation) is important. In general, the vertical flow regime was favorable for reducing the parameters analyzed. The exception may be salinity, which was not strictly affected by the treatments, and this is an issue for the future study of treatment systems, and the same is true for the COD, which was increased.

The most important result was that the N content was reduced and reached almost a total diminution in water in the treatment EHA. In general, the C/N ratio, in this case of the almond residue, is the key for N reduction.

Therefore, bioreactors can be helpful to improve the characteristics of irrigation water. In view of the many design possibilities, future studies should be carried out to achieve reductions in all the studied parameters, and a combination of several systems can favor the treatment of the low-quality water by using nature-based solutions.

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Appendix A

Table 1. Mean value (M) and standard deviation (SD) of pH (units of pH) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	8.18	0.012	8.44	0.012	8.35	0.006	8.36	0.006	8.25	0.006	8.29	0.017	8.13 a	0.012	8.12 a	0.010	8.15	0.015	8.10	0.006
EHG	8.23	0.012	8.19	0.013	8.13	0.008	8.13	0.006	8.19	0.008	8.17	0.006	8.13 a	0.013	8.16	0.019	8.13	0.006	8.23	0.0010
EHA	5.06	0.006	7.62	0.008	7.64	0.008	8.24	0.006	8.48	0.006	7.93	0.006	8.24	0.006	8.09 a	0.013	8.19	0.017	8.31	0.013
F	1 × 10 ⁶ ***		5906 ***		9400 ***		1588 ***		2057 ***		1100 ***		133 ***		19.3 ***		19.4 ***		2820 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
I	8.15	0.021	8.25	0.010	8.29	0.010	8.30	0.021	8.26	0.012	8.31	0.017	8.27	0.006	8.28	0.013	8.29	0.0010	8.25	0.013
EHG	8.21	0.019	8.15	0.006	8.11	0.006	8.19	0.005	8.21	0.005	8.17	0.006	8.12 a	0.021	8.08	0.006	8.16	0.006	8.18 a	0.005
EHA	8.04	0.006	8.01	0.008	8.03	0.017	8.03	0.005	7.98	0.006	8.01	0.008	8.14 a	0.008	8.05	0.008	8.06	0.008	8.18 a	0.008
F	119 ***		888 ***		532 ***		466 ***		1440 ***		653 ***		138 ***		723 ***		817 ***		63.0 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	8.12	0.010	8.15	0.015	8.10	0.006	8.15 a	0.021	8.25	0.010	8.29	0.010	8.30	0.021	8.26	0.012	8.31	0.017	8.27	0.006
EVG	8.03	0.008	8.04	0.029	8.14	0.013	8.17 a	0.005	8.05 a	0.006	8.14	0.013	8.08	0.006	8.11	0.005	8.11	0.006	8.17	0.010
EVA	7.94	0.006	7.63	0.013	7.94	0.013	7.74	0.036	8.04 a	0.017	7.96	0.017	7.74	0.021	7.57	0.017	7.80	0.008	7.86	0.008
F	522 ***		759 ***		396 ***		407 ***		399 ***		613 ***		1080 ***		3531 ***		1940 ***		2820 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
I	8.28 a	0.013	8.29	0.010	8.25 a	0.013	8.26	0.006	8.27	0.012	8.26	0.006	8.23	0.006	8.26 a	0.010	8.53	0.006	8.57	0.012
EVG	8.26 a	0.006	8.12	0.005	8.25 a	0.006	8.20	0.006	8.16	0.005	8.10 a	0.008	7.67	0.006	8.40	0.006	8.31	0.006	8.27	0.006
EVA	7.75	0.017	7.89	0.012	8.11	0.006	8.12	0.006	8.08	0.005	8.10 a	0.008	8.17	0.013	8.26 a	0.008	8.33	0.008	8.01	0.010
F	2230 ***		1909 ***		336 ***		592 ***		609 ***		577 ***		4862 ***		264 ***		1309 ***		3620 ***	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001$, 0.01 and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 2. Mean value (M) and standard deviation (SD) of EC (mS cm⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	11.27	0.059	17.65	0.008	17.64	0.099	18.32	0.046	17.50	0.039	17.54	0.072	17.42	0.101	17.48	0.064	16.77	0.061	17.4	0.102
EHG	14.07	0.993	15.82	0.045	17.53	0.021	18.91	0.078	20.29	0.085	20.04	0.008	20.02	0.055	20.01	0.025	19.27	0.148	19.73	0.041
EHA	15.73	0.047	16.20	0.084	17.58	0.015	20.44	0.048	20.08	0.029	18.73	0.051	18.88	0.050	18.42	0.070	18.63	0.057	20.15	0.058
F	5455 ***		1219 ***		3.80 ns		1357 ***		3018 ***		2397 ***		1303 ***		1518 ***		702 ***		1703 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
I	17.76	0.078	17.42	0.095	17.64	0.024	16.99	0.070	18.07	0.043	18.05	0.034	18.37	0.086	18.62	0.056	18.71	0.053	18.49	0.176
EHG	19.84	0.028	20.03	0.019	19.20	0.177	19.30 a	0.061	18.99	0.081	20.14	0.140	20.16	0.158	20.23 a	0.096	19.70 a	0.141	20.90 a	0.141
EHA	19.43	0.083	19.26	0.049	18.93	0.119	19.28 a	0.161	18.57	0.010	19.53	0.140	19.60	0.148	20.28 a	0.050	19.73 a	0.054	20.68 a	0.150
F	1059 ***		1280 ***		180 ***		620 ***		300 ***		345 ***		206 ***		725 ***		161 ***		289 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	17.65	0.008	16.77 a	0.061	17.40	0.102	17.76 a	0.078	17.42	0.095	17.64 a	0.024	16.99	0.070	18.07 a	0.043	18.05	0.034	18.37	0.086
EVG	17.47	0.108	17.11	0.107	17.60	0.021	18.04	0.067	17.87 a	0.041	17.84	0.070	17.46	0.031	18.73	0.154	18.56 a	0.015	18.71 a	0.069
EVA	17.60	0.166	16.89 a	0.033	17.98	0.067	17.64 a	0.109	17.77 a	0.010	17.65 a	0.057	17.27	0.013	17.90 a	0.087	18.59 a	0.054	18.67 a	0.139
F	1.45 ns		21.2 ***		68.0 ***		22.4 ***		61.7 ***		16.8 ***		112 ***		69.5 ***		259 ***		13.1 **	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
I	18.62	0.056	18.71	0.053	18.49	0.176	18.69	0.062	18.94	0.017	19.14 a	0.065	19.21	0.026	18.95	0.070	19.03	0.083	19.41	0.039
EVG	19.06 a	0.080	18.73	0.125	20.00	0.164	19.96	0.008	19.85 a	0.071	20.19 a	0.257	19.50	0.025	19.47 a	0.048	19.34 a	0.031	19.45	0.062
EVA	19.12 a	0.042	18.73	0.053	19.72	0.021	18.82	0.050	19.90 a	0.019	19.40	0.031	19.29	0.026	19.42 a	0.026	19.48 a	0.124	19.39	0.057
F	79.6 ***		0.16 ns		133 ***		923 ***		611 ***		50.7 ***		134 ***		124 ***		27.4 ***		1.36 ns	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001$, 0.01 and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 3. Mean value (M) and standard deviation (SD) of SS (mg L⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	51.96	3.18	35.64	1.14	31.31 a	8.29	29.49 a	2.69	35.13	4.47	30.78 a	2.55	41.08	0.88	34.98	0.84	36.7	0.07	44.55	0.58
EHG	23.50	6.18	24.39	3.22	24.43 a	2.36	29.43 a	0.05	63.24	5.17	31.66 a	0.40	28.52	1.92	28.31	2.18	28.63	2.74	33.26	4.44
EHA	94.08	3.57	624.00	2.31	369.56	15.90	322.65	18.07	248.39	14.47	184.44	10.90	212.05	1.88	262.69	4.60	166.78	4.63	86.24	8.68
F	248 ***		82,946 ***		1431 ***		1030 ***		629 ***		748 ***		15,774 ***		8098 ***		2491 ***		98.0 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
I	53.12	6.22	35.60 a	0.79	41.64	0.73	42.39	1.13	47.99	2.93	42.42 a	1.05	42.55 a	1.01	57.98	2.55	56.73 a	1.03	35.67 a	0.55
EHG	32.09	1.07	30.25 a	0.84	30.65	6.28	29.27	0.11	25.35	1.59	32.68 a	0.18	39.29 a	0.34	37.05	2.80	28.70	2.44	32.91 a	0.29
EHA	113.90	2.62	88.28	14.10	62.22	6.80	99.30	6.81	126.11	10.89	86.68	9.87	82.39	27.97	66.58	2.12	55.75 a	0.58	43.58	4.95
F	464 ***		61.7 ***		35.8 ***		349 ***		258 ***		101 ***		8.82 **		147 ***		412 ***		14.8 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	34.98	0.69	36.70 a	0.07	44.55	0.58	53.12	6.22	35.60 a	0.79	41.64	0.73	42.39	1.13	47.99	2.93	42.42	1.05	42.55	1.01
EVG	25.97	1.69	22.82 a	1.06	25.18	1.88	27.67	1.30	25.06	2.66	23.04	2.27	26.34	0.67	27.60 a	0.58	25.51	1.07	26.90 a	2.67
EVA	193.26	4.25	205.36	13.72	166.57	5.44	110.58	2.95	39.31 a	6.50	62.54	3.45	34.39	0.84	30.62 a	0.50	35.01	4.82	26.93 a	1.99
F	4967 ***		655 ***		2109 ***		441 ***		13.1 **		266 ***		318 ***		158 ***		33.9 ***		80.7 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
I	57.98	2.55	56.73	1.03	35.67	0.55	37.93	0.20	43.64	3.34	29.37	1.18	30.02 a	1.42	32.43	0.28	31.92 a	7.47	29.89 a	4.43
EVG	24.25	1.63	25.22	0.51	27.86 a	1.32	27.68	2.11	24.41	3.08	33.29	2.36	27.53 a	6.24	24.72 a	5.45	24.54 a	4.28	31.88 a	1.92
EVA	34.15	1.47	29.19	0.94	27.08 a	0.87	33.25	1.44	29.82	1.29	22.07	0.91	32.95 a	1.61	25.34 a	3.64	31.83 a	6.36	25.18 a	5.98
F	318 ***		1598 ***		96.9 ***		48.1 ***		52.8 ***		50.1 ***		2.03 ns		5.11 *		1.88 ns		2.4 ns	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001$, 0.01 and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 4. Mean value (M) and standard deviation (SD) of COD (mg L⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	43	5.20	80	3.77	67 a	10.11	72	6.35	70	2.31	80	0.00	75	1.73	69	0.01	81	1.73	89	10.97
EHG	338	0.02	306	0.01	335 a	0.82	342	15.84	426	22.00	381	15.01	365	10.81	369	36.37	358	24.54	461	30.60
EHA	14,731	94.37	5901	16.52	2841	515.40	2280	208.01	1556	35.22	919	121.25	783	0.01	653	12.73	636	4.04	718	2.50
F	94,705 ***		4.5 × 10 ⁶ ***		106 ***		400 ***		4173 ***		145 ***		12,693 ***		689 ***		1487 ***		1132 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	85	2.31	351 a	7.53	120	8.66	87	10.39	97	0.02	88	13.00	104	0.58	87	5.20	103	8.66	94	1.73
EHG	354	26.56	359 a	8.10	349	10.11	427	17.90	391	3.56	440	11.84	381	92.68	429	33.20	456 a	25.40	396	26.29
EHA	548	10.53	535	22.52	495	9.24	523	42.15	509	8.66	534	9.81	488	25.12	472	0.82	445 a	10.98	434	2.63
F	790 ***		206 ***		1638 ***		285 ***		6150 ***		1636 ***		51.2 ***		474 ***		576 ***		597 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	69	0.01	81	1.73	89	10.97	85	2.31	351 a	7.53	120	8.66	87	10.39	97	0.02	88	13.00	104	0.58
EVG	383	27.43	268	9.54	363	10.69	294	75.93	357 a	0.50	289 a	2.31	367	0.50	323 a	32.33	328 a	31.48	293	11.55
EVA	1378	37.53	1280	20.80	1166	36.69	434	35.22	472	0.96	284 a	19.63	414	15.88	349 a	6.93	365 a	24.45	359	22.81
F	2592 ***		9486 ***		2378 ***		52.1 ***		959 ***		240 ***		1042 ***		211 ***		154 ***		323 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	87	5.20	103	8.66	94	1.73	106	1.15	97	1.73	78	1.41	65	1.29	74	0.58	66	5.20	71	7.51
EVG	390 a	11.30	403	6.40	377 a	28.58	322	3.20	331	7.23	352	4.08	351	15.64	276 a	28.87	338 a	36.11	293	17.63
EVA	390 a	16.79	374	1.5	355 a	8.66	380	1.15	312	15.02	298	25.12	320	8.54	293 a	4.62	302 a	13.57	347	6.08
F	843 ***		2789 ***		333 ***		19,382 ***		722 ***		389 ***		930 ***		209 ***		174 ***		637 ***	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001$, 0.01 and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 5. Mean value (M) and standard deviation (SD) of alkalinity (mg L⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	213.11	1.17	264.47	0.17	265.40	0.90	208.32	0.15	248.33	0.15	253.55	1.21	261.01	0.10	244.50	0.58	217.00	2.66	240.00	1.13
EHG	156.10	0.64	164.27	0.80	140.22	0.34	125.99	0.30	123.66	2.37	169.26	11.18	115.97	0.59	111.40	1.62	111.67	0.77	115.99	1.86
EHA	874.33	1.95	1185.71	0.58	967.23	0.60	1011.05	1.21	1016.84	1.82	967.56	0.62	1087.99	0.65	914.37	1.24	860.11	0.62	790.81	2.48
F	3.4×10^5 ***		3.9×10^6 ***		1.9×10^6 ***		1.8×10^6 ***		3.1×10^5 ***		18,195 ***		4.2×10^6 ***		5×10^5 ***		2.4×10^5 ***		1.4×10^5 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	261.43	0.65	262.68	0.32	262.03	1.68	246.50	1.73	257.91	2.21	259.70	0.34	251.17	9.51	260.85	0.32	265.47	1.13	270.33	1.54
EHG	109.36	0.41	118.25	0.81	112.50	0.06	121.26	0.86	125.93	0.82	120.00	1.15	121.47	0.00	120.74	0.29	129.65	0.40	131.27	2.26
EHA	755.85	0.63	712.91	0.62	753.20	1.24	575.08	0.00	576.11	3.57	552.95	1.73	525.10	0.60	461.77	2.32	411.00	1.15	384.98	1.13
F	1.4×10^6 ***		1×10^6 ***		3.1×10^5 ***		1.8×10^5 ***		35,122 ***		1.3 $\times 10^5$ ***		5616 ***		63,407 ***		85,661 ***		22,125 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	244.50	0.58	217.00	2.66	240.00	1.13	261.43	0.65	262.68	0.32	262.03	1.68	246.50	1.73	257.91	2.21	259.70	0.34	251.17	9.51
EVG	194.05	0.20	175.39	2.31	162.19	0.22	165.47	0.40	167.48	0.60	165.50	0.58	181.61	0.45	170.00	0.00	168.74	0.29	176.70	0.80
EVA	696.33	1.17	654.82	1.68	771.88	0.00	470.96	1.17	497.76	3.43	434.28	0.34	349.95	1.12	337.73	0.55	285.04	2.29	293.00	0.10
F	5.3×10^5 ***		55,542 **		9.9×10^5 ***		1.5×10^5 ***		28,388 ***		67,609 ***		19,423 ***		16,304 ***		8262 ***		458 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	260.85	0.32	265.47	1.13	270.33	1.54	270.72	2.22	272.64	0.00	276.00	0.00	276.24	2.26	280.16	11.31	269.00	1.15	258.00	2.31
EVG	182.10	0.12	187.16	1.06	191.00	1.15	190.06	0.02	198.13	0.00	175.35	1.13	181.00	3.46	190.00	0.00	217.47	2.26	227.00	8.08
EVA	271.92	0.25	274.42	0.16	259.59	1.13	297.39	1.20	220.60	0.00	229.00	1.15	224.00	6.93	211.40	1.18	225.39	0.00	240.00	0.00
F	1.6×10^5 ***		11,356 ***		4472 ***		5885 ***		4.3×10^3 ***		11,649 ***		419 ***		206 ***		1432 ***		41.1 ***	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001$, 0.01 and 0.05. F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 6. Mean value (M) and standard deviation (SD) of bicarbonates (mg L⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	127.97	0.57	157.73	0.22	158.78	0.54	124.50	0.09	148.63	0.31	151.76	0.80	157.08	0.06	147.01	0.35	130.43	1.55	144.59	0.66
EHG	94.06	0.76	99.04	0.50	84.44	0.19	75.81	0.15	74.25	1.39	101.74	6.70	70.26	0.19	67.03	0.99	67.15	0.45	69.62	1.15
EHA	533.34	1.19	721.22	0.30	587.47	0.36	607.00	0.98	602.86	0.86	586.15	0.32	652.83	0.53	550.90	0.93	517.59	0.56	473.85	1.71
F	3.1 × 10 ⁵ ***		3.6 × 10 ⁶ ***		1.9 × 10 ⁶ ***		1.0 × 10 ⁶ ***		3.6 × 10 ⁵ ***		18,642 ***		3.7 × 10 ⁶ ***		4.1 × 10 ⁵ ***		2.4 × 10 ⁵ ***		1.2 × 10 ⁵ ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	157.14	0.33	157.15	0.05	156.77	1.01	147.20	1.03	154.44	1.32	155.12	0.25	150.40	5.69	155.88	0.23	158.72	0.80	161.87	0.92
EHG	65.66	0.22	71.02	0.50	67.71	0.05	72.77	0.50	75.57	0.48	72.09	0.68	73.07	0.01	72.78	0.18	77.97	0.23	78.93	1.39
EHA	456.44	0.50	430.40	0.49	454.34	0.95	346.89	0.05	348.05	2.25	333.66	0.95	316.21	0.30	278.67	1.36	247.97	0.73	231.46	0.72
F	1.2 × 10 ⁶ ***		8.5 × 10 ⁵ ***		2.6 × 10 ⁵ ***		1.8 × 10 ⁵ ***		33,592 ***		1.5 × 10 ⁵ ***		5694 ***		66,589 ***		70,420 ***		21,167 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	147.01	0.35	130.43	1.55	144.59	0.66	157.14	0.33	157.15	0.05	156.77	1.01	147.20	1.03	154.44	1.32	155.12	0.25	150.40	5.69
EVG	117.16	0.12	105.71	1.41	97.71	0.17	99.49	0.22	100.91	0.35	99.46	0.35	109.39	0.23	102.35	0.02	101.71	0.16	106.19	0.52
EVA	420.91	0.76	397.72	1.07	467.92	0.08	285.76	0.75	300.24	2.20	262.75	0.35	212.26	0.73	205.14	0.36	172.84	1.40	177.46	0.08
F	4.8 × 10 ⁵ ***		56,969 ***		1.0 × 10 ⁶ ***		1.5 × 10 ⁵ ***		24,450 ***		65,361 ***		19,651 ***		16,878 ***		834 ***		475 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	155.88	0.23	158.72	0.80	161.87	0.92	162.14	1.29	163.46	0.07	165.54	0.07	165.69	1.36	167.97	6.78	158.57	0.68	152.02	1.29
EVG	109.20	0.12	112.78	0.62	114.80	1.09	114.26	0.06	119.18	0.02	105.69	0.70	109.77	2.08	113.22	0.10	130.15	1.35	136.09	4.85
EVA	165.00	0.13	166.03	0.04	156.35	0.71	179.15	0.73	133.01	0.02	137.80	0.67	134.77	4.19	126.73	0.65	134.71	0.07	144.92	0.02
F	1.3 × 10 ⁵ ***		9676 ***		3126 ***		6195 ***		9.5 × 10 ⁵ ***		11,418 ***		396 ***		210 ***		1213 ***		30.4 ***	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001, 0.01$ and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 7. Mean value (M) and standard deviation (SD) of carbonates (mg L⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	1.94	0.1377	3.47	0.3173	3.00	0.0102	2.46	0.0018	2.75	2.7481	2.80	0.0596	2.05	0.0008	2.06	0.0049	1.87	0.0718	1.74	0.0311
EHG	1.10	0.3475	1.11	0.0092	1.04	0.0162	0.99	0.0283	1.11	0.0505	1.44	0.1141	0.45	0.5160	0.87	0.0012	0.91	0.0182	1.07	0.0108
EHA	0.01	0.0001	2.01	0.0544	2.48	0.0015	9.53	0.2380	17.03	0.2505	3.97	0.0550	10.61	0.1324	6.72	0.1672	6.92	0.1765	8.35	0.1918
F	81.2 ***		163 ***		33,501 ***		4358 ***		8307 ***		983 ***		1262 ***		4098 ***		3416 ***		5128 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	2.25	0.0646	2.97	0.2359	2.96	0.0190	3.05	0.0214	2.78	0.0239	3.17	0.0371	2.71	0.1027	3.12	0.0368	3.10	0.1080	2.92	0.0166
EHG	0.98	0.0295	1.05	0.0065	0.85	0.0107	1.13	0.0228	1.17	0.0230	1.04	0.0237	0.97	0.0127	0.83	0.0020	1.05	0.0171	1.08	0.0097
EHA	4.52	0.1152	4.36	0.1110	5.00	0.1887	3.81	0.0501	3.29	0.0663	3.54	0.1042	3.99	0.0569	2.92	0.0531	2.66	0.0275	3.28	0.0333
F	2105 ***		488 ***		1426 ***		6567 ***		2676 ***		1705 ***		1985 ***		4625 ***		1097 ***		11,286 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	2.06	0.0050	1.87	0.0718	1.74	0.0311	2.25	0.0646	2.97	0.2359	2.96	0.0190	3.05	0.0214	2.78	0.0239	3.17	0.0371	2.71	0.1027
EVG	1.16	0.0010	1.22	0.0001	1.16	0.0290	1.38	0.0214	1.19	0.0199	1.42	0.0050	1.33	0.0383	1.29	0.0169	1.17	0.0174	1.52	0.0329
EVA	3.76	0.0432	1.68	0.0402	2.86	0.0755	1.49	0.0356	3.30	0.1074	2.10	0.1363	1.17	0.0426	0.85	0.0211	1.00	0.0052	1.23	0.0158
F	11,045 ***		200 ***		1186 ***		462 ***		230 ***		375 ***		3466 ***		9507 ***		10,292 ***		622 ***	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	3.12	0.0368	3.10	0.1088	2.92	0.0166	2.89	0.0614	2.75	0.0719	2.72	0.0712	2.72	0.0223	2.83	0.1141	5.32	0.0229	5.16	0.1125
EVG	1.80	0.0458	1.33 a	0.0250	1.64 a	0.3716	1.60	0.0417	1.61	0.0211	1.22	0.0081	0.61	0.0279	2.57	0.0000	2.40	0.0250	2.29	0.0816
EVA	1.23	0.0158	1.32 a	0.0531	1.93 a	0.0169	2.18	0.0088	1.50	0.0197	1.82	0.0331	1.91	0.0340	2.13	0.0676	2.66	0.0000	1.42	0.0187
F	3040 ***		836 ***		39.0 ***		897 ***		963 ***		1108 ***		5597 ***		83.7 ***		27,339 ***		2342 ***	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001, 0.01$ and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

Table 8. Mean value (M) and standard deviation (SD) of total nitrogen (mg L⁻¹) in horizontal and vertical flow bioreactors.

Horizontal	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	12	0.82	5.5	2.89	13.5 a	1.73	12.5	9.81	22.5 a	1.73	16 a	5.77	12	4.62	14.5	1.73	12.5	1.73	18.5	8.66
EHG	8.5	6.35	5.5	4.04	1.5	0.58	5.5	6.35	1 b	1.15	5 b	5.77	7.5	6.35	10.5	7.51	4	4.62	13.5	4.04
EHA	12	5.77	11	3.46	14 a	5.77	7.5	1.73	17 c	2.31	7.5 ab	2.89	12.5	6.35	20	8.08	13	6.93	14	9.24
F	0.66 ns		3.3 ns		16.4 ***		1.12 ns		155 ***		5.32 *		0.89 ns		2.19 ns		4.24 ns		3.59 ns	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	21.5	4.04	9	1.15	29 a	12.70	17.5	1.73	16.5 ab	1.73	14.5 a	2.89	18.5	2.89	16 a	2.31	16.5 a	4.04	9.5 a	1.73
EHG	12 a	3.46	7.5	2.89	5 b	4.62	13	3.46	22.5 a	5.20	30	5.77	25.5	14.43	15 a	1.15	15 a	4.62	13 b	0.00
EHA	12 a	3.46	16.5	10.97	12 ab	13.86	8.5	9.81	13 b	3.46	12.5 a	0.58	8	5.77	0.5	0.58	6.5	0.58	0.00 c	0.00
F	8.95 **		2.15 ns		4.89 *		2.18 ns		6.60 *		26.2 ***		3.72 ns		129 ***		9.18 **		181 ***	
Vertical	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	14.5	1.73	12.5	1.73	18.5	8.66	21.5	4.04	9 a	1.15	29	12.70	17.5 a	1.73	16.5 a	1.73	14.5 ab	2.89	18.5	2.89
EVG	9.5	5.20	7.5	0.58	17.5	2.89	13.5 a	2.89	10.5 a	0.58	14	13.86	17.5 a	4.04	24 b	3.46	19 a	3.37	12.75	4.50
EVA	8	4.62	6.5	6.35	11	3.46	8.5 a	0.58	13.5	1.73	7	6.93	5.5	2.89	4 c	4.62	8.5 b	5.20	9	6.93
F	2.71 ns		2.84 ns		2.09 ns		20.6 ***		13.5 ***		3.78 ns		20.8 ***		33.7 ***		7.14 *		3.59 ns	
	Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
I	16 a	2.31	16.5	4.04	9.5 a	1.73	11.5 a	1.73	43.5	7.51	34 a	3.46	29.5	9.81	19.5 a	0.58	16.5 a	7.51	34.5 a	0.58
EVG	14 a	6.63	16	1.15	11 a	1.15	30.5 b	1.73	25.5	12.12	15	5.77	15.5 a	4.04	15.5 a	1.73	34 b	11.55	22	5.77
EVA	2	2.31	13.5	0.58	5	2.31	18.75 c	0.50	27.5	8.66	36.5 a	10.97	9.5 a	1.73	11	3.46	22 ab	5.77	43 a	6.93
F	12.6 ***		1.72 ns		12.1 **		177 ***		4.19 ns		10.0 **		10.9 **		14.1 **		4.31 ns		16.4 ***	

Note(s): F values followed by ***, ** and * indicate significant differences at $p = 0.001$, 0.01 and 0.05 . F values followed by ns indicates no significant differences. In the columns, mean values followed by a letter in common are statistically equal to $p = 0.05$.

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