

## Article

# Investigation of the Factors Affecting the Treatment Performance of a Stormwater Horizontal Subsurface Flow Constructed Wetland Treating Road and Parking Lot Runoff

Hyeseon Choi, Franz Kevin F. Geronimo, Minsu Jeon and Lee-Hyung Kim \*

Department of Civil and Environmental Engineering, Kongju National University, Cheonan City 31080, Korea; hyeseon27@kongju.ac.kr (H.C.); fkgeronimo@kongju.ac.kr (F.K.F.G.); minsu91@kongju.ac.kr (M.J.)

\* Correspondence: leehyung@kongju.ac.kr

**Abstract:** This study assessed the factors affecting the growth and survival of microorganisms in a small horizontal subsurface flow constructed wetland (HSSF CW) treating stormwater runoff from highly impervious road and parking lot through long-term monitoring from 2010 until present. The HSSF CW facility consisted of sedimentation or pre-treatment zone, vegetation zone, and effluent zone, and employed filter media including bio-ceramics, sand, gravel, and wood chips. Results showed that flow reduction in the wetland through filtration and sedimentation played an important part in the overall performance of the HSSF CW. In addition, vegetation growth was found to be affected by pollutant and stormwater inflow in the HSSF CW. Vegetation near the outflow port exhibited greater growth rates by about 6.5% to 64.2% compared to the vegetation near the inflow port due to the less stormwater pollutant concentrations via filtration mechanism in the plant or media zone of the HSSF CW. The pollutant inflow from road and parking lot played an important role in providing good environment for microbial growth especially for the dominant microbial phyla including *Proteobacteria*, *Actinobacteria* and *Acidobacteria* in the HSSF CW. The findings of this research are useful in understanding treatment mechanisms and identifying appropriate design considerations for HSSF CW.

**Keywords:** horizontal subsurface flow constructed wetland; low impact development; microorganisms; stormwater



**Citation:** Choi, H.; Geronimo, F.K.F.; Jeon, M.; Kim, L.-H. Investigation of the Factors Affecting the Treatment Performance of a Stormwater Horizontal Subsurface Flow Constructed Wetland Treating Road and Parking Lot Runoff. *Water* **2021**, *13*, 1242. <https://doi.org/10.3390/w13091242>

Academic Editor: Miklas Scholz

Received: 8 February 2021

Accepted: 27 April 2021

Published: 29 April 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Constructed wetlands (CWs) are artificial systems designed to mimic the functions of natural wetlands [1]. CWs were mainly utilized for water treatment from different sources including agricultural, livestock, and urban areas. Apart from the intrinsic ability of CWs to improve water quality, CWs can also be used to manage flooding, serve as ecological habitats, secure water resources, provide recreational and educational benefits, and mitigate climate change [2]. The primary components of CWs include water, soil, plants, microorganisms, and animals. Additionally, the CWs are capable of maintaining mass and energy flow. Mass circulation pertains to the transformation and movement of water, organic matter, and nutrients [3,4]. Mechanisms including retention, detention, evapotranspiration, filtration, infiltration, phytoremediation and bioremediation serve as essential factors affecting the water treatment and flood management potential of CWs [5].

CWs are designed to optimize ecosystem services and highlight the benefits of natural processes. Depending on hydrological and flow characteristics, CWs can be classified as free water surface wetlands (FWS), horizontal sub-surface flow wetlands (HSSF), vertical flow wetlands (VF), and hybrid wetlands [6]. CWs can be designed as single-type wetlands or a series of connected systems with varying classifications. The types and configuration of CWs can vary greatly depending on the purpose, nature of influent, and availability of space [7]. In recent years, floating aquatic plant systems (FAPS) were widely utilized as compared with submerged plant systems, since these facilities can be easier to maintain [8].

The removal of nutrients (i.e., nitrogen and phosphorus) in CWs can be attained through phytoremediation and bioremediation mechanisms [9]. Nitrogen is generally removed through plant uptake mechanism and nitrification–denitrification processes [7]. However, in the case of environments with low dissolved oxygen (DO) concentration, the removal of nitrogen can be significantly decreased [10]. Phosphorus can be removed through particle adsorption, plant uptake, and microorganism respiration [7]. Phosphorus removal in CWs can be generally low, but increasing plant coverage can improve the rate at which phosphorus is removed in the system [11]. In a study conducted by Kadlec and Wallace (2008), plants accounted for approximately 20% of the total phosphorus removed by the CWs [12].

The inflow water in CWs may also contain considerable amounts of pathogens which may be removed through media filtration, precipitation, and adsorption mechanisms in the CWs [13–15]. Researches on CWs applied for various purposes are continuously being conducted; however, current studies mainly focused on the pollutant removal performance of the systems without a concrete explanation of the internal processes affecting the efficiency of the CWs. In addition, studies about the effects of microorganisms to the overall performance of CWs are still lacking. As such, this study investigated the physicochemical and biological mechanisms in a horizontal subsurface flow (HSSF) CW through long-term monitoring. Specifically, the factors affecting overall performance of HSSF CW were assessed.

## 2. Materials and Methods

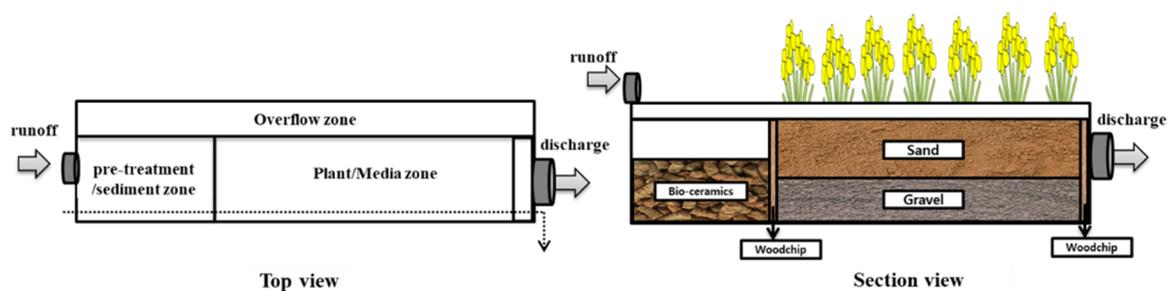
### 2.1. Site Description and Facility Characteristics

The catchment area characteristics and the properties of the small HSSF CW located at Kongju National University in Cheonan City, South Korea (36°51'1.11" N; 127°9'0.23" E) utilized in the study were summarized in Table 1. The HSSF CW, which was constructed in 2010, was installed to treat stormwater runoff from a 424 m<sup>2</sup> impermeable road and parking lot. The facility can be divided into four sections: (1) sedimentation or pre-treatment zone, (2) plant or the filter zone, (3) effluent zone, and the overflow zone as shown in Figure 1. The sedimentation or pre-treatment zone was underlaid with bio-ceramic media packs. On the other hand, a vertical woodchip layer was installed at the initial part of the plant or filter zone while the remaining parts of the plant or filter zone was filled with sand and gravel layers, respectively. Sand, gravel, woodchip, and bio-ceramics sizes were ranging from 2 to 5 mm, 20 to 30 mm, 10 to 20 mm, and 2 to 4.7 mm, respectively. The bio-ceramic layer in the sedimentation zone was utilized to enhance the reduction of particulate matter. The filter zone was designed to have sufficient infiltration function to retain sufficient water for the growth of vegetation [16]. *Iris ensata* var. *spontanea*, a native plant species in Korea, was selected based on a number of factors such as low maintenance requirements, fast growth, pollutant uptake performance and high tolerance towards toxicities [13]. The HSSF CW received stormwater runoff from a 424 m<sup>2</sup> combined road and parking lot catchment and has a facility storage volume of 1.56 m<sup>3</sup>. HSSF CW storage volume to total volume (SV/TV) ratio is 30.6% while pre-treatment volume to storage volume (PV/SV) ratio is 26.2%. Table 1 exhibited the other properties of the HSSF CW.

**Table 1.** Site characteristics and HSSF CW specifications.

Characteristics	Unit	HSSF CW
Year constructed		2010
Infiltration capability		No
Vegetation		<i>Iris ensata</i> var. <i>spontanea</i>
Overflow channel		Yes
Media		Sand, gravel, bio-ceramic and woodchips
Runoff source		Road and Parking Lot
Dimension (L × W × H)	m × m × m	7 × 1 × 0.7
Pre-treatment		Yes
Pre-treatment volume,	m <sup>3</sup>	0.67
Storage volume	m <sup>3</sup>	1.56
Catchment area	m <sup>2</sup>	424
SA/CA	%	1.4
SV/TV	%	30.6

SA/CA: ratio of facility surface area to catchment area; SV/TV: ratio of storage volume to total volume.

**Figure 1.** Schematic diagram of the HSSF CW.

## 2.2. Storm Event Monitoring, Water Quality Analyses and Calculation

A total of 27 rainfall events were monitored from 2010 up to 2019. Monitoring during rainfall events was carried out by considering the first flush phenomenon that is usually observed in highly urbanized land uses [17]. Manual grab sampling was used to collect water samples. The first sample was collected as soon as the inflow or outflow was observed. The succeeding samples for the first hour of the event were collected after five, 10, 15, 30, and 60 min, followed by an hourly interval until the end of the rainfall event. To effectively calculate the pollutant loads, flowrates were measured every five minutes [18]. The collected water samples were analyzed for different water quality parameters based on the Standard Methods for the Examination of Water and Wastewater including total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) [19]. Total heavy metal concentrations, including Cr, Fe, Ni, Cu, Zn Cd, and Pb, were also measured using inductively coupled plasma spectrometry [16]. Hydrologic records (i.e., rainfall depth, duration, and antecedent dry days) were obtained from the Korea Meteorological Administration. The event mean concentrations (EMCs) of various pollutants were calculated using Equation (1) [20]. The pollutant removal efficiency of the system was calculated by getting the difference between the inflow and outflow pollutant load divided by the inflow load multiplied to 100. Results were statistically analyzed using SYSTAT 12 and Excel software, including normality test and analysis of variance. Pearson correlation coefficient (R) was used to determine the dependence between each water quality parameter. Significant differences between parameters were accepted at 95% confidence level, signifying that the probability ( $p$ ) value was less than 0.05.

$$EMC = \frac{M}{V} = \frac{\sum_i c_i q_i}{\sum_i q_i} \quad (1)$$

### 2.3. Plant and Microorganism Monitoring and Sampling

P1 to P4 illustrated in Figure 2 were the soil sampling points in the plant or filter zone of the HSSF CW. The height and chlorophyll content of *Iris ensata* var. *spontanea* planted in the HSSF CW were measured monthly to quantify the difference every month except the winter season. Similarly, a total of six soil sampling were conducted distributed in all seasons except the winter season. For physico-chemical and microbial analysis. Soil quality parameters including pH, conductivity, nutrient content (TN and TP), and heavy metals (Cd, Cr, Cu, Pb, Zn, and As) were tested based on the soil sampling and methods analysis by Carter and Gregorich 2007 [21]. Soil microbial analyses through Soil samples were also subjected to microbial analysis through 16S rRNA gene sequence obtained by Roche 454 pyrosequencing technology [22]. This method is based on the idea that all bacteria host 16S rRNA and by using universal primers in a single PCR reaction, all bacteria in a target environment may be identified by the end of the sequence.

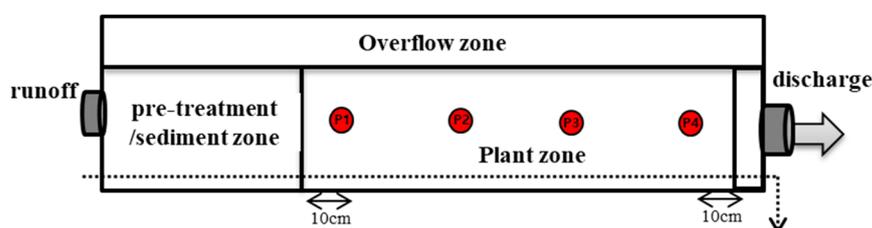


Figure 2. HSSF CW sampling points.

## 3. Results and Discussion

### 3.1. Analysis of Influent Water Quality and Pollutant Removal Efficiency

The 27 monitored storm events had an average  $\pm$  standard deviation rainfall depth and duration of  $8.4 \pm 8.6$  mm  $3.2 \pm 2.3$  h, respectively. Most of the storm events were monitored during summer season comprising 54.5% of the total sampling followed by spring and autumn seasons amounting to 31.8% and 13.7%. Parking lots and roads represent major sources of water pollution in urban areas with typically associated pollutants including TSS, metals, anthropogenic organic compounds, nutrients, and microbial contaminants [23,24]. Based on Figure 3, the mean  $\pm$  standard deviation inflow EMC or EMCin of TSS, COD, TN, and TP were  $143.3 \pm 88.2$  mg/L,  $155.2 \pm 106.9$  mg/L,  $8.5 \pm 4.9$  mg/L, and  $0.56 \pm 0.31$  mg/L, respectively. In addition, the EMCin for heavy metals were  $0.16 \pm 0.09$   $\mu\text{g/L}$ ,  $5.78 \pm 4.93$   $\mu\text{g/L}$ ,  $0.15 \pm 0.09$   $\mu\text{g/L}$ ,  $0.53 \pm 0.51$   $\mu\text{g/L}$ ,  $0.10 \pm 0.08$   $\mu\text{g/L}$ , and  $0.18 \pm 0.12$   $\mu\text{g/L}$  for Cr, Fe, Cu, Zn, Cd, and Pb, respectively. The effluent EMC (EMCout) of TSS, COD, TN, and TP amounting to  $47.8 \pm 31.9$  mg/L,  $80.2 \pm 52.2$  mg/L,  $6.4 \pm 3.7$  mg/L, and  $0.31 \pm 0.16$  mg/L, respectively, were found to be significantly less than the EMCin with probability ( $p$ ) value less than 0.001. TSS were reduced in the system through filtration and sedimentation in the CWs [25,26]. In a study conducted by Geronimo et al., 2014, it was found that the removal of other pollutants in a similar stormwater treatment system was attributed to TSS removal [27]. This finding highlighted the importance of sedimentation and filtration mechanism in the overall performance of CWs. The nitrogen removal mechanisms in CWs involve ammonification, nitrification–denitrification, plant uptake, and physicochemical methods such as sedimentation, ammonia stripping, breakpoint chlorination, and ion exchange [28]. On the other hand, the major phosphorus removal processes in CWs include plant uptake, formation of organic deposits through the immobilization of microorganisms, and sedimentation of particles containing adsorbed phosphorus [12]. The EMCout of Cr ( $0.15 \pm 0.10$   $\mu\text{g/L}$ ), Fe ( $2.31 \pm 2.10$   $\mu\text{g/L}$ ), Cu ( $0.15 \pm 0.03$   $\mu\text{g/L}$ ), Zn ( $0.30 \pm 0.28$   $\mu\text{g/L}$ ), Cd ( $0.10 \pm 0.09$   $\mu\text{g/L}$ ), and Pb ( $0.18 \pm 0.21$   $\mu\text{g/L}$ ) also exhibited lower values as compared with the EMCin ( $p < 0.05$ ). Heavy metal removal mechanisms in CWs include phytoremediation, adsorption, and ion exchange [29,30]. In the study conducted by [31], it was found that the stormwater runoff from a 75% impervious road contained 140 mg/L, 129 mg/L, 19.4  $\mu\text{g/L}$ , 13.2  $\mu\text{g/L}$ ,

and 81.1 µg/L of TSS, COD, Cu, Pb, and Zn, respectively. Similarly, Li et al., 2012, found Mn and Fe concentration in stormwater from 48.6% impervious road were 20.0 µg/L 42.5 µg/L, respectively [32]. Lower pollutant concentration in stormwater runoff from the other studies were attributed to lower imperviousness compared to the parking lot and road in this study which has 100% imperviousness thereby causing less infiltration of surface runoff.

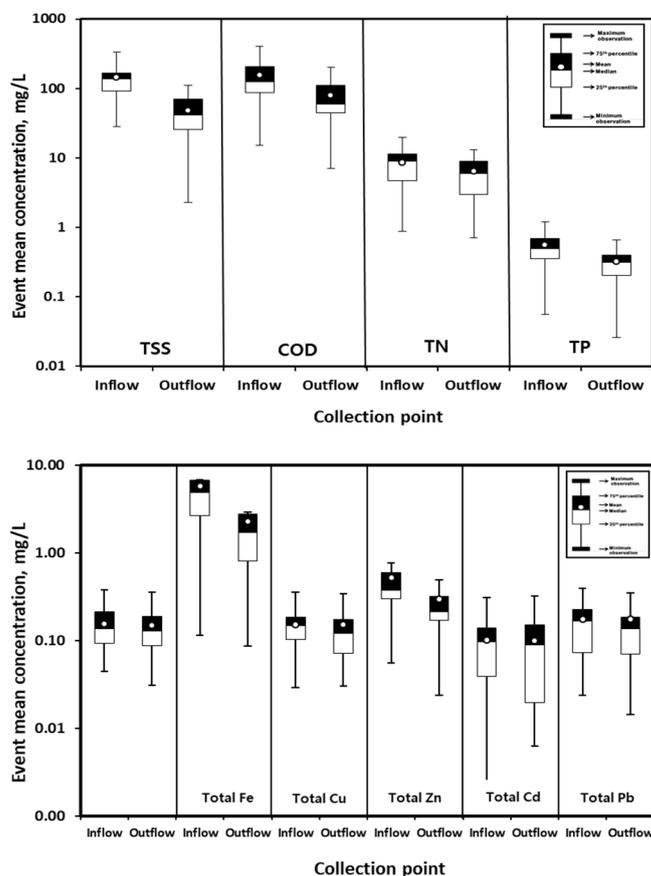
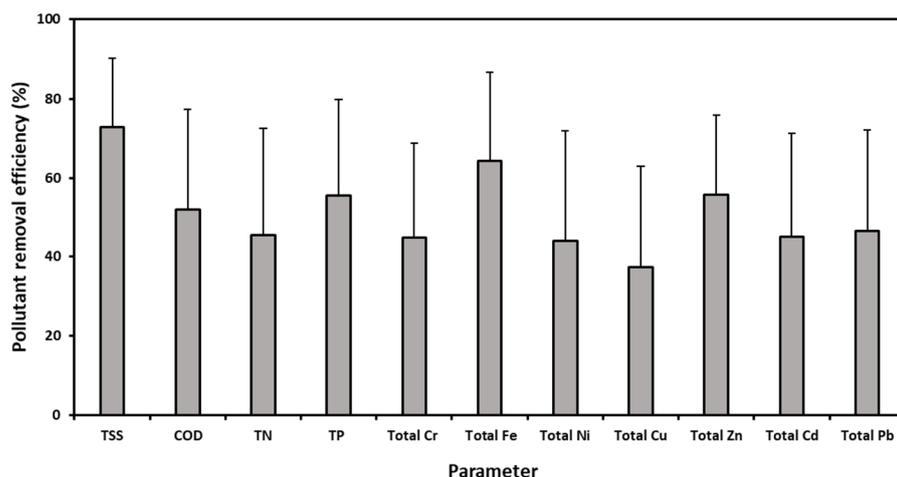


Figure 3. Inflow and outflow characteristics of HSSF CW.

The summary of the pollutant removal performance of the HSSF CW was illustrated in Figure 4. Among the water quality parameters analyzed, the facility exhibited the highest removal for TSS with a mean  $\pm$  standard deviation of  $71.5 \pm 16.8\%$  followed by organics, nutrients and heavy metals with mean  $\pm$  standard deviation of  $57.2 \pm 20.5\%$ ,  $50.5 \pm 22.6\%$ , and  $46.8 \pm 23.1\%$ , respectively. High removal of TSS, TP and heavy metals in the HSSF CW was attributed to the pre-treatment zone or sedimentation basin where these pollutants were deposited. The efficient removal of TSS, TP, and heavy metals in the stormwater runoff can be attributed to the effective settlement of sediment-bound pollutants and particulates in the facility's sedimentation basin. Previous studies also reported that CWs equipped with pre-treatment zones or sedimentation basins were capable of reducing incoming pollutant loads in the system, thus promoting a suitable environment for plant and microbial growth [33]. Apart from the effective sedimentation mechanisms in the facility, high TSS and heavy metal removal efficiencies can also be attributed to the bio-ceramic filter used in the facility [34,35]. These results implied that flow reduction in the wetland through infiltration and sedimentation played an important role in the overall performance of the HSSF CW.



**Figure 4.** Pollutant removal efficiency of the HSSF CW.

### 3.2. Implication of Plant Growth and Chlorophyll Changes in the HSSF CW

Plants in CWs can reduce NPS pollutant concentrations through phytoremediation [9,36]. Moreover, plants can also improve the water storage and infiltration functions of CWs through plant roots. The removal efficiency of a nature-based stormwater treatment facility varies depending on the influent water quality, filter media characteristics, microbiological activities, and the type of vegetation utilized in the system [34,36]. Iris, commonly planted in CWs, is effective in removing nitrogen in water [37,38]. Iris is capable of simultaneously absorbing nitrous oxide and deoxidizing nitrogen in its roots [12]. As illustrated in Figure 5, the height of Iris planted in the HSSF CW continued to increase from March to July, whereas the observed plant heights started to decrease from August. Iris growth rate near the inflow and outflow ports between March and April were almost similar, especially during the months of April and May when the plants experienced extended periods of sunlight exposure. Despite the lower sunlight duration due to rainy days between July to August, the growth of Iris was found to be steadily increasing at a range of 0.2 to 5.2%. The growth rate of Iris in the inflow part ranged from 27 to 103% was found to be significantly less than the growth rate of Iris near the outflow amounting to 28% to 139% ( $p < 0.001$ ). Higher Iris growth rates were observed near the outflow port due to the soluble pollutants resulting from filtration and sedimentation mechanisms in the sedimentation zone and initial part of the media zone. From May to June, the growth rate of Iris in the outflow was about 15% greater than the inflow due to the influx of large amounts of nutrients when rain frequently occurred. Iris growth in this study amounting from 32 to 80 cm was found to be almost in the same range as the Iris studied by Lee et al., 2018 over four months was about 10 cm to 60 cm and 10 cm to 45 cm considering 60 mg/kg and 23.9 mg/kg of nitrogen concentration, respectively.

Photosynthesis is the basic process in which plants reduce carbon dioxide, produce oxygen, and increase its own biomass. Plants play an important role in the removal metals in water and soil [39,40]. Plants employ physico-chemical processes such as filtration, adsorption, and cation exchange to sequester pollutants in the planting media or overlying water. The changes in chlorophyll concentration among the Irises were measured to evaluate the plants' activities and photosynthetic potential. As exhibited in Figure 6, the chlorophyll measurements were relatively higher in spring as compared to the measurements observed in other seasons. This trend is similar to the plants' growth rate, wherein the highest measurements were recorded during the months of March to April. The activity and growth rate of plants in the HSSF CW were expected to affect the species and quantity of microorganisms and the pollutant removal capability of the system. Chlorophyll levels in the inflow and outflow zones were similar during spring and summer seasons. Chlorophyll concentration in the inflow and outflow ranges from  $52.4 \pm 15.9 \text{ ug/cm}^2$  to  $52.4 \pm 24.3 \text{ ug/cm}^2$ , and  $49.8 \pm 14.4 \text{ ug/cm}^2$  to  $53.4 \pm 25.6 \text{ ug/cm}^2$ , respectively, during

summer and spring seasons. The difference in chlorophyll concentrations between the plants located in the influent and effluent zones during the fall season can be attributed to the pattern of nutrient deposition. Chlorophyll is the core of photosynthetic activity in vegetation, and it can provide a measure of plant growth conditions from another perspective [41,42]. In addition, chlorophyll represents the amount of nutrients that a plant contained through the leaf color [43,44]. During fall season, the amount of nutrients deposited in CW can be reduced due to the lower rainfall depth and frequency. Additionally, lower temperatures can also affect plant productivity and slow-down growth patterns. It was also found that the plants near inflow contained higher chlorophyll compared to the outflow due to the pollutant inflow during the rainfall affect both the growth and activity of the plant.

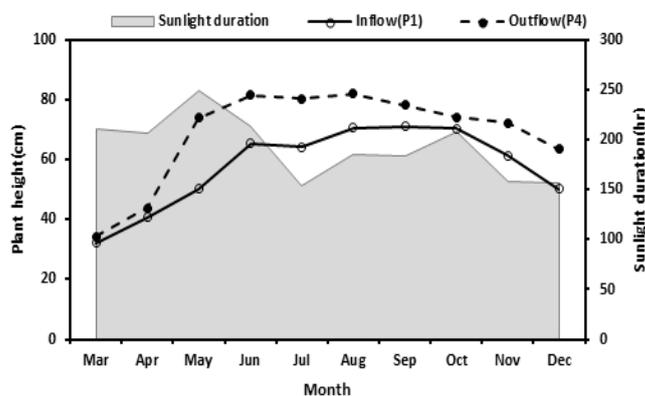


Figure 5. Relationship between sunlight duration and plant height.

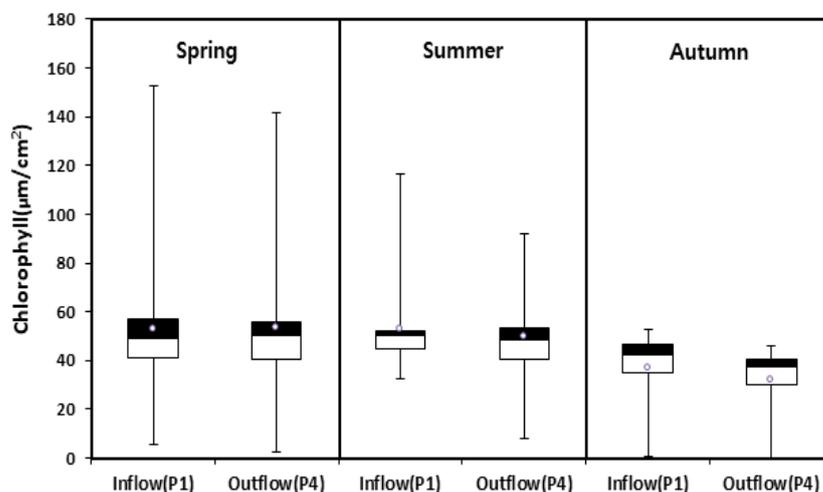


Figure 6. Seasonal comparison of chlorophyll concentration in Iris.

### 3.3. Soil Characteristics along the Plant Zone of the HSSF CW

CW soil is an important environment in which vegetation and microorganisms survive and perform pollutant decomposition and material circulation. Physico-chemical characteristics of soil from different sampling points were listed in Table 2. Based on the analyses, pH of P1 and P2 close to the inflow was 6.5 while relatively higher pH was observed for P3 and P4 amounting to 6.8 and 6.7, respectively, which are both near the outflow. Highest hydraulic conductivity was measured in P1 with similar concentrations at the succeeding points in the CW plant zone. On the other hand, water content and ignition loss were found to have the same trend. High water content in P1 was due to the continuous supply of water from the sedimentation or pre-treatment zone. Since the HSSF CW was designed to

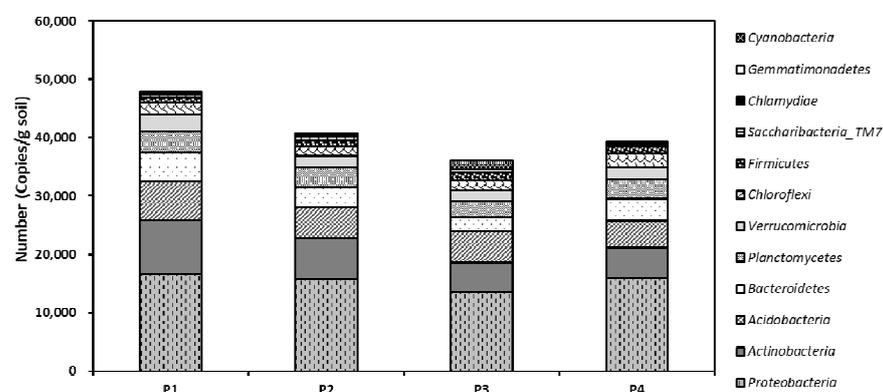
have 1% slope, lowest water content was observed in P2. TN and TP were found to be 2.3 to 3.5 times and 1.5 to 2.0 times, respectively, higher in P1 compared to other points along the plant zone. Among the heavy metals, Zn was found to have the highest concentration which was about 0.2 to 10.7 times higher compared to other heavy metals. It was analyzed that Zn is an essential element for plant growth and is easily absorbed by roots as such the concentration decreases along the plant zone to the outflow port [45]. TP and heavy metals decreased along the plant zone to the outflow port. This finding is attributed to the deposition of heavy metals and TP attached to sediments in the pre-treatment basin and were mostly absorbed in the top of the vegetation thereby reducing its concentration along the media in the plant zone.

**Table 2.** Soil characteristics per collection point along the HSSF CW.

Parameter	Unit	Point			
		P1	P2	P3	P4
pH		6.5	6.8	6.7	6.5
Conductivity		47.7	19.1	17.1	18.8
Water content	%	18.0	16.4	16.5	17.5
Ignition loss	%	28.8	26.3	27.5	27.4
TN		364.3	104.1	102.8	153.5
TP		213.7	114.0	141.3	105.6
Cd		5.4	1.5	0.8	1.1
Cr		0.8	0.3	0.3	0.3
Cu	mg/kg	2.3	1.0	0.4	0.7
Pb		0.7	3.2	1.6	1.8
Zn		25.6	9.9	2.4	3.0
As		1.8	0.9	0.7	0.7

### 3.4. Microbial Distribution along the Plant Zone of the HSSF CW

Figure 7 showed the count of microorganisms in the soil collected in the plant zone of the HSSF CW. Highest number of microorganisms was found in P1 amounting to 47,836 copies/g of soil which is located near the inflow of the HSSF CW. Microbial count in P2, P3 and P4 were almost in the same range and was found to be less than P1 amounting to 40,834 copies/g of soil, 36,110 copies/g of soil, and 39,376 copies/g of soil, respectively. It was also found that various stormwater pollutants accumulated pre-treatment zone or sedimentation zone provided good environment for microorganism growth [46]. The results of the microbial analysis by distance showed that Proteobacteria accounted for the highest proportion of about 37.8%, followed by Actinobacteria and Acidobacteria comprising 15.9% and 13.4%, respectively.



**Figure 7.** Microbial count per collection point in the plant zone of HSSF CW.

Proteobacteria survives through symbiosis with bacteria and other plant roots, and the high proportion of Proteobacteria have significant effect on nitrogen reduction and plant growth in the facility [47–50]. Actinobacteria is a microorganism that parasites on plant roots to create root holes, perform nitrogen fixation and reduce heavy metals [51,52]. High concentration of heavy metals in roads and parking lots, which accumulated in the HSSF CW, resulted to microorganism growth and survival related to heavy metals. Pollutants present in stormwater runoff entering the HSSF CW were found to have provided good environment for Proteobacteria, Actinobacteria and Acidobacteria to be the dominant species. Proteobacteria, Actinobacteria and Acidobacteria are almost always the most dominant microbial phyla in the environment, even in wastewaters [53].

Table 3 exhibited the Pearson correlation analysis between the CW soil microorganisms and the soil quality parameters. Most of the microorganisms were found to have high positive correlation to different soil quality parameters except for pH and Pb. Among the microorganisms, Gemmatimonadetes was found to have positive correlation with  $r$  value of 0.899 to pH while negative correlation ( $r$  value:  $-0.686$  to  $-0.931$ ) to other parameters including conductivity, water content and TN. This finding might have resulted from the characteristics of Gemmatimonadetes being rod-shaped aerobic bacteria that use photosynthesis based on chlorophyll [54,55]. On the other hand, pH was found to be negatively correlated ( $r$  value:  $-0.357$  to  $-0.667$ ) with most of the microorganisms indicating that higher pH will entail lower microorganisms count or less microorganism survival. This finding implied that soil pH is an important condition for microorganism growth. Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Planctomycetes and Verrucomicrobia were found to have high correlation with concentrations of heavy metals except Pb, which are considered to have an effect on pollutant reduction through bioremediation, sedimentation, and ion exchange. High correlation between heavy metals and microbial species was found to have influenced the production of characteristic enzymes, contributing to the improvement of removal efficiency of heavy metals.

**Table 3.** Pearson correlation matrix of microorganisms and soil quality parameters.

	pH	Conductivity	Water Content	LOI	TN	TP	Cu	Cr	Cd	Pb	Zn	As
Proteobacteria	−0.613	1.000	0.804	0.836	0.986	0.937	0.972	0.999	0.995	−0.720	0.954	0.982
Actinobacteria	−0.567	0.999	0.769	0.813	0.974	0.943	0.977	0.999	0.997	−0.693	0.966	0.989
Acidobacteria	−0.573	0.997	0.772	0.842	0.976	0.957	0.964	0.996	0.992	−0.729	0.951	0.980
Bacteroidetes	−0.635	0.999	0.820	0.833	0.990	0.925	0.974	0.999	0.995	−0.716	0.953	0.981
Planctomycetes	−0.599	1.000	0.793	0.832	0.983	0.941	0.973	0.999	0.995	−0.717	0.956	0.984
Verrucomicrobia	−0.615	1.000	0.804	0.841	0.986	0.939	0.970	0.999	0.994	−0.727	0.951	0.981
Chloroflexi	−0.667	0.995	0.842	0.870	0.995	0.930	0.956	0.993	0.984	−0.764	0.928	0.965
Firmicutes	−0.602	0.971	0.780	0.923	0.967	0.974	0.900	0.966	0.952	−0.840	0.879	0.927
Saccharibacteria_TM7	−0.357	0.985	0.640	0.802	0.934	0.993	1.000	0.988	0.995	−0.722	0.993	1.000
Gemmatimonadetes	0.899	−0.686	−0.929	−0.052	−0.931	0.911	−0.326	−0.485	−0.241	0.006	0.053	−0.0021

#### 4. Conclusions

CWs provide different benefits such as pollutant removal, water circulation and material circulation through different physical, chemical, and biological processes via different components such as soil, filter media, plants, and microorganisms. Analysis on the influent characteristics of stormwater entering the HSSF CW revealed that imperviousness rate greatly affected the generation of NPS pollutants. The pre-treatment zone or sedimentation zone of the CW provided several benefits not only in reducing stormwater pollutant load but also providing good growing environment for plant and microorganisms. Both plant growth rate and microorganism dominance were affected by the stormwater runoff quality entering the HSSF CW. Higher microorganism count was observed near the outflow of the CW compared to inflow since some of the pollutant present in stormwater runoff were detrimental to microorganism growth and survival. On the other hand, higher pollutant concentration in the inflow resulted to better growth of plants compared to

outflow part. Proteobacteria, Actinobacteria and Acidobacteria were found to be the most dominant microbial phylum in the HSSF CW. Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Planctomycetes and Verrucomicrobia were considered to have affected the pollutant removal mechanisms of through bioremediation, sedimentation, and ion exchange. These findings may be used in designing appropriate stormwater CWs considering different environmental conditions and CW components.

**Author Contributions:** H.C.: conceptualization; methodology; software; validation; formal analysis; investigation; resources; data curation; writing—original draft preparation; visualization. F.K.F.G.: methodology, investigation, writing—review and editing, visualization and supervision. M.J.: methodology; investigation; L.-H.K. conceptualization; writing—review and editing; supervision; project administration; funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Wu, S.; Lyu, T.; Zhao, Y.; Vymazal, J.; Arias, C.A.; Brix, H. Rethinking intensification of constructed wetlands as a green eco-technology for wastewater treatment. *Environ. Sci. Technol.* **2018**, *52*, 1693–1694. [[CrossRef](#)]
2. Park, J.B.; Craggs, R.J.; Tanner, C.C. Eco-friendly and low-cost Enhanced Pond and Wetland (EPW) system for the treatment of secondary wastewater effluent. *Ecol. Eng.* **2018**, *120*, 170–179. [[CrossRef](#)]
3. Li, D.; Zheng, B.; Liu, Y.; Chu, Z.; He, Y.; Huang, M. Use of multiple water surface flow constructed wetlands for non-point source water pollution control. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 5355–5368. [[CrossRef](#)]
4. Tanner, C.C.; Adams, D.D.; Downes, M.T. Methane emissions from constructed wetlands treating agricultural wastewaters. *J. Environ. Qual.* **1997**, *26*, 1056–1062. [[CrossRef](#)]
5. Zhuang, L.L.; Yang, T.; Zhang, J.; Li, X. The configuration, purification effect and mechanism of intensified constructed wetland for wastewater treatment from the aspect of nitrogen removal: A review. *Bioresour. Technol.* **2019**, *293*, 122086. [[CrossRef](#)]
6. Vymazal, J. Types of constructed wetland for wastewater treatment: Their potential for nutrient removal. In *Transformation of Nutrients in Natural and Constructed Wetlands*; Bachhuys: Leiden, The Netherlands, 2001.
7. Kadlec, R.; Knight, R.; Vymazal, J.; Brix, H.; Cooper, P.; Haberl, R. *Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation*; IWA Publishing: London, UK, 2000.
8. Kivaisi, A.K. The potential for constructed wetlands for wastewater treatment and reuse in developing countries: A review. *Ecol. Eng.* **2001**, *16*, 545–560. [[CrossRef](#)]
9. Horne, A.J. Phytoremediation by constructed wetlands. In *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*; John Wiley: New York, NY, USA, 2000.
10. Stenstrom, M.K.; Poduska, R.A. The effect of dissolved oxygen concentration on nitrification. *Water Res.* **1980**, *14*, 643–649. [[CrossRef](#)]
11. Mann, R.A.; Bavor, H.J. Phosphorus removal in constructed wetlands using gravel and industrial waste substrata. *Water Sci. Technol.* **1993**, *27*, 107–113. [[CrossRef](#)]
12. Kadlec, R.H.; Wallace, S. *Treatment Wetlands*; CRC Press: Boca Raton, FL, USA, 2008.
13. Fang, H.; Zhang, Q.; Nie, X.; Chen, B.; Xiao, Y.; Zhou, Q.; Liang, X. Occurrence and elimination of antibiotic resistance genes in a long-term operation integrated surface flow constructed wetland. *Chemosphere* **2017**, *173*, 99–106. [[CrossRef](#)] [[PubMed](#)]
14. Zhang, S.; Lu, Y.X.; Zhang, J.J.; Liu, S.; Song, H.L.; Yang, X.L. Constructed wetland revealed efficient sulfamethoxazole removal but enhanced the spread of antibiotic resistance genes. *Molecules* **2020**, *25*, 834. [[CrossRef](#)] [[PubMed](#)]
15. Lamori, J.G.; Xue, J.; Rachmadi, A.T.; Lopez, G.U.; Kitajima, M.; Gerba, C.P.; Sherchan, S. Removal of fecal indicator bacteria and antibiotic resistant genes in constructed wetlands. *Environ. Sci. Pollut. Res.* **2019**, *26*, 10188–10197. [[CrossRef](#)] [[PubMed](#)]
16. Choi, J.Y.; Maniquiz-Redillas, M.C.; Hong, J.S.; Lee, S.Y.; Kim, L.H. Comparison of the treatment performance of hybrid constructed wetlands treating stormwater runoff. *Water Sci. Technol.* **2015**, *72*, 2243–2250. [[CrossRef](#)] [[PubMed](#)]
17. Kim, L.-H.; Kang, H.-M.; Bae, W. Treatment of particulates and metals from highway stormwater runoff using zeolite filtration. *Desalination Water Treat.* **2010**, *19*, 97–104. [[CrossRef](#)]
18. Choi, H.; Geronimo, F.K.F.; Hong, J.; Kim, L.-H. Assessment of the influence of urban stormwater runoff on the growth of *Spiraea prunifolia* var. *simpliciflora*. *Desalination Water Treat.* **2019**, *158*, 225–232. [[CrossRef](#)]

19. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 1915.
20. Kim, L.H.; Lee, S. Characteristics of washed-off pollutants and dynamic EMCs in a parking lot and a bridge during storms. *J. Korean Soc. Water Environ.* **2005**, *21*, 248–255. [[CrossRef](#)]
21. Carter, M.R.; Gregorich, E.G. *Soil Sampling and Methods of Analysis*; CRC Press: Boca Raton, FL, USA, 2007.
22. D'Argenio, V.; Salvatore, F. The role of the gut microbiome in the healthy adult status. *Clin. Chim. Acta* **2015**, *451*, 97–102. [[CrossRef](#)]
23. Göbel, P.; Dierkes, C.; Coldewey, W.G. Storm water runoff concentration matrix for urban areas. *J. Contam. Hydrol.* **2007**, *91*, 26–42. [[CrossRef](#)]
24. Revitt, D.M.; Lundy, L.; Coulon, F.; Fairley, M. The sources, impact and management of car park runoff pollution: A review. *J. Environ. Manag.* **2014**, *146*, 552–567. [[CrossRef](#)]
25. Lee, S.; Maniquiz-Redillas, M.C.; Kim, L.H. Settling basin design in a constructed wetland using TSS removal efficiency and hydraulic retention time. *J. Environ. Sci.* **2014**, *26*, 1791–1796. [[CrossRef](#)]
26. Akinbile, C.O.; Yusoff, M.S.; Zuki, A.A. Landfill leachate treatment using sub-surface flow constructed wetland by *Cyperus haspan*. *Waste Manag.* **2012**, *32*, 1387–1393. [[CrossRef](#)]
27. Geronimo, F.K.F.; Maniquiz-Redillas, M.C.; Tobio, J.A.S.; Kim, L.H. Treatment of suspended solids and heavy metals from urban stormwater runoff by a tree box filter. *Water Sci. Technol.* **2014**, *69*, 2460–2467. [[CrossRef](#)] [[PubMed](#)]
28. Lee, C.G.; Fletcher, T.D.; Sun, G. Nitrogen removal in constructed wetland systems. *Eng. Life Sci.* **2009**, *9*, 11–22. [[CrossRef](#)]
29. Gill, L.W.; Ring, P.; Higgins, N.M.; Johnston, P.M. Accumulation of heavy metals in a constructed wetland treating road runoff. *Ecol. Eng.* **2014**, *70*, 133–139. [[CrossRef](#)]
30. Ben Salem, Z.; Laffray, X.; Ashoour, A.; Ayadi, H.; Aleya, L. Metal accumulation and distribution in the organs of Reeds and Cattails in a constructed treatment wetland (Etueffont, France). *Ecol. Eng.* **2014**, *64*, 1–17. [[CrossRef](#)]
31. Gnecco, I.; Berretta, C.; Lanza, L.G.; La Barbera, P. Storm water pollution in the urban environment of Genoa, Italy. *Atmos. Res.* **2005**, *77*, 60–73. [[CrossRef](#)]
32. Li, F.; Chen, J.; Engel, B.A.; Liu, Y.; Wang, S.; Sun, H. Assessing the effectiveness and cost efficiency of green infrastructure practices on surface runoff reduction at an urban watershed in China. *Water* **2021**, *13*, 24. [[CrossRef](#)]
33. Schulz, C.; Gelbrecht, J.; Rennert, B. Treatment of rainbow trout farm effluents in constructed wetland with emergent plants and subsurface horizontal water flow. *Aquaculture* **2003**, *217*, 207–221. [[CrossRef](#)]
34. Zhao, J.; Zhao, Y.; Xu, Z.; Doherty, L.; Liu, R. Highway runoff treatment by hybrid adsorptive media-baffled subsurface flow constructed wetland. *Ecol. Eng.* **2016**, *91*, 231–239. [[CrossRef](#)]
35. Babatunde, A.O.; Zhao, Y.Q.; Burke, A.M.; Morris, M.A.; Hanrahan, J.P. Characterization of aluminium-based water treatment residual for potential phosphorus removal in engineered wetlands. *Environ. Pollut.* **2009**, *157*, 2830–2836. [[CrossRef](#)]
36. Herath, I.; Vithanage, M. Phytoremediation in constructed wetlands. In *Phytoremediation*; Springer: Cham, Switzerland, 2015; pp. 243–263. [[CrossRef](#)]
37. Alihan, J.C.; Maniquiz-Redillas, M.; Choi, J.; Flores, P.E.; Kim, L.H. Characteristics and fate of stormwater runoff pollutants in constructed wetlands. *J. Wetl. Res.* **2017**, *19*, 37–44. [[CrossRef](#)]
38. Sundaravadivel, M.; Vigneswaran, S. Constructed wetlands for wastewater treatment. *Crit. Rev. Environ. Sci. Technol.* **2001**, *31*, 351–409. [[CrossRef](#)]
39. Gao, J.; Wang, W.; Guo, X.; Zhu, S.; Chen, S.; Zhang, R. Nutrient removal capability and growth characteristics of *Iris sibirica* in subsurface vertical flow constructed wetlands in winter. *Ecol. Eng.* **2014**, *70*, 351–361. [[CrossRef](#)]
40. Vymazal, J. The use of hybrid constructed wetlands for wastewater treatment with special attention to nitrogen removal: A review of a recent development. *Water Res.* **2013**, *47*, 4795–4811. [[CrossRef](#)]
41. Chandra, R.; Kumar, V. Mechanism of wetland plant rhizosphere bacteria for bioremediation of pollutants in an aquatic ecosystem. In *Advances in Biodegradation and Bioremediation of Industrial Waste*; CRC Press: Boca Raton, FL, USA, 2015; Volume 329.
42. Greenway, M. The role of macrophytes in nutrient removal using constructed wetlands. In *Environmental Bioremediation Technologies*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 331–351. [[CrossRef](#)]
43. Zhang, X.; Hu, Z.; Ngo, H.H.; Zhang, J.; Guo, W.; Liang, S.; Xie, H. Simultaneous improvement of waste gas purification and nitrogen removal using a novel aerated vertical flow constructed wetland. *Water Res.* **2018**, *130*, 79–87. [[CrossRef](#)] [[PubMed](#)]
44. Chang, S.X.; Robison, D.J. Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. *For. Ecol. Manag.* **2003**, *181*, 331–338. [[CrossRef](#)]
45. Liu, J.; Dong, Y.; Xu, H.; Wang, D.; Xu, J. Accumulation of Cd, Pb and Zn by 19 wetland plant species in constructed wetland. *J. Hazard. Mater.* **2007**, *147*, 947–953. [[CrossRef](#)]
46. Elis, J.B.; Shutes, R.B.E.; Revitt, D.M. *Guidance Manual for Constructed Wetlands*; Environment Agency: Bristol, UK, 2003.
47. Ahn, J.-H.; Choi, M.-Y.; Lee, H.-W.; Kim, B.-Y.; Song, K.; Kim, M.-S.; Weon, H.-Y. Analysis of community structure of metabolically active bacteria in a rice field subjected to long-term fertilization practices. *Korean J. Soil Sci. Fertil.* **2013**, *46*, 585–592. [[CrossRef](#)]
48. Podosokorskaya, O.A.; Kadnikov, V.V.; Gavrilov, S.N.; Mardanov, A.V.; Merkel, A.Y.; Karnachuk, O.V.; Ravin, N.V.; Bonch-Osmolovskaya, E.A.; Kublanov, I.V. Characterization of *M. elioribacter roseus* gen. nov.; sp. nov.; a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class I gnavibacteria, and a proposal of a novel bacterial phylum I gnavibacteriae. *Environ. Microbiol.* **2013**, *15*, 1759–1771. [[CrossRef](#)]

49. Thomas, F.; Barbeyron, T.; Michel, G. Evaluation of reference genes for real-time quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. *J. Microbiol. Methods* **2011**, *84*, 61–66. [[CrossRef](#)]
50. Zhang, H.; Gao, Z.; Shi, M.; Fang, S.; Xu, H.; Cui, Y.; Liu, J. Study of the effects of land use on hydrochemistry and soil microbial diversity. *Water* **2019**, *11*, 466. [[CrossRef](#)]
51. Sathya, A.; Vijayabharathi, R.; Gopalakrishnan, S. Plant growth-promoting actinobacteria: A new strategy for enhancing sustainable production and protection of grain legumes. *3 Biotech* **2017**, *7*, 1–10. [[CrossRef](#)] [[PubMed](#)]
52. Franco-Correa, M.; Chavarro-Anzola, V. Actinobacteria as plant growth promoting rhizobacteria. *Actinobacteria Basics Biotechnol. Appl.* **2016**, 249–270. [[CrossRef](#)]
53. Xue, J.; Schmitz, B.W.; Caton, K.; Zhang, B.; Zabaleta, J.; Garai, J.; Sherchan, S.P. Assessing the spatial and temporal variability of bacterial communities in two Bardenpho wastewater treatment systems via Illumina MiSeq sequencing. *Sci. Total Environ.* **2019**, *657*, 1543–1552. [[CrossRef](#)] [[PubMed](#)]
54. Raymond, J. Coloring in the tree of life. *Trends Microbiol.* **2008**, *16*, 41–43. [[CrossRef](#)] [[PubMed](#)]
55. Zeng, Y.; Feng, F.; Medova, H.; Dean, J.; Koblizek, M. Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7795–7800. [[CrossRef](#)]