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Fungal Consortia Mediated Bio-Treatment of Organic Matter and Metals Uptake from Sewage Water: Maize Agro-Physiological Assessment

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Abstract: The present investigation aims to improve the efficiency of fungal mono- and mixed cultures in removing organic pollutants and metals from sewage water (SW) for further maize plant response assessments. The reduction in the organic load from the SW was harnessed using a co-culture consortium consisting of *Aspergillus niger* (KB5), *Sordariomycetes* sp. (D10), and *Coniochaetaceae* sp. (LB3). The testing results had evinced removal of up to 88% of the organic matter and more than 96%, 91%, 80%, and 47.6%, of removal percentages for Copper (Cu), Nickel (Ni), Cadmium (Cd), and Lead (Pb), respectively, with the developed fungal consortium [KB5 + D10 + LB3]. After treatment and lab experiments, a reuse of treated and untreated SW for plant irrigation was evaluated towards improving maize plant growth. Irrigation was conducted in pot experiments with three types of water: clean water (Control), untreated (USW), and treated SW by fungal consortia (TSW) and by station treatment plant STP (TSWP) using the randomized complete block (RCB) experimental design. Results of the pots trial revealed that the morphological parameters of SW-irrigated plants are slightly improved compared to water-irrigated plants. Data regarding assimilating area attributes indicated that the most significant enlargement of the assimilation area was observed with TSW-D (1/4) irrigation by 1051 cm^2, followed by TSWP-D (0) by 953.96 cm^2, then USW-D (1/4) by 716.54 cm^2, as compared to plants irrigated with clean water (506.91 cm^2). On average, the assimilation areas were larger by 51.76%, 46.86%, and 29.25% in TSW, USW, and TSWP-irrigated plants, respectively. Thus, SW irrigation supports the required qualities and quantities of microelements and water for plant growth. Oxidative stress assessment showed that irrigations with treated SW caused a significant decrease in both enzymatic and non-enzymatic antioxidants, depicting that the treatment lowered the stress of sewage water.

Keywords: sewage water; treatments; fungal consortia; growth performance; antioxidant enzymes; photosynthetic pigments

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1. Introduction

Maize or corn (*Zea mays* L., Family *Poaceae*) is a major cereal crop in the world, ranking third after wheat and rice [1]. Global cereal production [2] is expected to rise by 13% by 2027, mainly due to an increased supply of cereal crops for food, livestock feed, and energy sources. The arid and semi-arid areas of the world, especially the Gulf region, face challenges in meeting the growing freshwater demands for irrigated agricultural production. Crops and other socio-economic activities, accounting for 69% of all water withdrawals [2], raise concerns regarding food security. On the other hand, progress in industrial activities and growth in the human population place worldwide freshwater withdrawals under increasing stress, as well as shortages of water resources available on regional and global scales [3,4]. Nowadays, global water withdrawal has increased six-fold over the past 100 years and is still growing steadily at a rate of around 1% per year, posing a threat to the effective enjoyment of human rights to water and sanitation for billions of potential people [5]. Therefore, alternative water resources and the development of sustainable strategies for water resource management are currently some of the leading global issues in regard to meeting water supply and food demands. Various crops have gained importance worldwide due to the constraints in freshwater availability as an alternative to recycling and reusing wastewater for irrigation. Frequently, in arid or semi-arid regions, wastewater irrigation for crop production is a common agricultural practice that provides an additional water supply and limits the supplementing of chemical fertilizers in the land [6,7]. Meanwhile, depending on the origin and the level of treatment, wastewater might contain toxic elements that can accumulate in the soil and crops, posing a threat to human health [8].

Sewage water (SW) is the most important contributor to water resources' organic and inorganic pollution. Treated SW (TSW) is widely used worldwide for agricultural irrigation. SW contains a lot of macronutrients (N, P, K) and micronutrients that serve as valued resources for plant nutrients, water reuse, and energy [7]. Additionally, using sewage water may contribute to preserving the environment as an alternative to polluted water disposal with an agronomical and potential economic effect. Meanwhile, depending on wastewater sources, untreated or poorly treated wastewater may contain undesirable substances that adversely affect the wastewater-irrigated plants, soil properties, and food chain if their amount exceeds the permissible limit [9,10]. Experiments conducted on wastewater, especially sewage water irrigation, indicate wastewater could solve water scarcity; however, its impact varies in regard to crop production yield in the scientific literature, making assessing its role in food security difficult. For instance, the study of Singh et al. [11] reported an increase in Rabi crop yields and an enhancement of the organic carbon content (N, P, K) and (inorganic) micronutrients of the soil resulting from sewage effluent irrigation. In the same way, several studies recorded that sewage water irrigation improved soil fertility [12,13]. Elsewhere, Saxena et al. [14] reported, via quantitative contamination profiling, the persistence of such emerging pollutants in SW (collected from three STPs in India) like drug-resistant bacteria and pharmaceuticals and personal care products (PPCPs) that may have adverse effects on ecological compartments and human health. Therefore, SW requires specific treatment and qualitative and quantitative parameters for unrestricted use. Several conventional approaches have been adapted for wastewater treatment, although most are not economical and have proven costly [15].

Advanced new green technical approaches, such as biological treatments using microbes, have gained popularity in reducing the organic load and other toxic substances in wastewater. The use of microorganisms to transform or mineralize a wide range of contaminants has been reported in many studies as a safe and low-cost method compared to physicochemical techniques [16]. In this regard, fungi are actively potent in remediating various organo-pollutants due to their hyphal surface network, which generates mechanical pressure, allowing the mycelial thallus to uptake nutritional substances and pollutants in the contaminated areas. Further, through intercellular translocation, fungi can seek out and metabolize the adhered organic compounds on the cell wall for their growth. Moreover, fungi are well-recognized for secreting ligninolytic enzymes extracellularly, such as laccases, peroxidases, and ligninase, which are the most implicated in the biodegradation processes. The literature has reported that fungal mycelia inherit great features and surfaces for distinguished tolerance towards heavy metal stress [17]. Thus, fungi are effective in the remediation and elimination of various persistent organic structures and metals. This knowledge can be used to design biological remediation systems for nutrient recovery and to remove metals from wastewater creating an opportunity for recycling of natural resources.

Typically, effluents from STPs may contain several constituents and dissolved organic matter that can serve as sustainable nutrient sources for microbial growth [18]. Treated wastewater is one of the most essential sources of recycled water, as countries located in arid and semi-arid regions reuse treated wastewater due to the water shortages they face. Reusing treated wastewater for agricultural irrigation can protect the environment and public health while reducing the extraction of significant amounts of water [19,20]. Systems for intercropping and irrigation with treated wastewater enhance soil health and boost crop growth, yield, and grain nutrient content while lowering hazardous heavy metals beneath the intercropping system [21]. Treated sewage water irrigation can increase crop physiological parameters [22]. In the intercropping technique, treated wastewater could ease the strain on crop nutrient uptake from the soil and improve plant physiological characteristics [23]. The intercropping technology boosts crop productivity and growth while lowering the chance of soil heavy metal accumulation during irrigation with treated wastewater [21].

The present research work was planned to identify and evaluate the potential application of a fungal consortia treatment methodology for simultanous organic matters' biodegradation during microbal growth and the bio-adsorption of metal ions from sewage water before further recycling in regard to plant irrigation. On the other side, our research work evaluates the impact of treated sewage water by a selected fungal consortia and untreated sewage water, in comparison with STP irrigation water, on morphological parameters, photosynthetic pigments, and some activities of stress-responsible enzymes (Peroxidase (POD) and Catalase (CAT) activities) of *Zea mays* L. plants.

2. Results and Discussion

2.1. Sewage Water Analysis

The physicochemical characteristics of untreated SW, clean water, and the treated SW by STP were appraised and compared with the Ministry of Water and Electricity: Riyadh, Saudi Arabia, 2006 (2006-MWE) standards allowable in restricted and unrestricted irrigation waters [24] (Table 1).

Table 1. Physicochemical characterization of the irrigation water used in the study.

Property	Unit	Untreated SW (USW)	Treated SW by Fungal Biomass (TSW)	SW Treated by Station Treatment Plant STP	Freshwater	2006-MWE Standards for Unrestricted Irrigation
		Physical parameters				
Temperature	°C	20 ± 0.5	20 ± 0.2	21 ± 0.1	21 ± 0.1	40
pH	-	7.8 ± 0.2	6.5 ± 0.1	7 ± 0.1	8.14	6.0–8.4
TSS	mg/L	43 ± 0.5	38 ± 1.4	2 ± 0.05	ND	40
EC	uS/cm	2650 ± 10.4	2103	1150 ± 11.6	350 ± 0.01	<700
		Organic chemicals parameters				
COD	mg/L	2315 ± 4.2	276 ± 1.06	33 ± 2.15	ND	ND
BOD_5	mg/L	1065 ± 8.8	108 ± 2.5	12 ± 1.9	ND	40
TKN	mg/L	39 ± 1.3	18.5	2 ± 0.3	ND	5–40
		Inorganic chemicals parameters				
		Heavy metals				
Arsenic (As)	mg/L	0.088	ND		ND	0.1
Cobalt (Co)	mg/L	0.007 ± 0.01	ND	0.005	ND	0.05
Cadmium (Cd)	mg/L	0.0035 ± 0.02	0.0007 ± 0.025	0.002 ± 0.0052	ND	0.01

Table 1. *Cont.*

Property	Unit	Untreated SW (USW)	Treated SW by Fungal Biomass (TSW)	SW Treated by Station Treatment Plant STP	Freshwater	2006-MWE Standards for Unrestricted Irrigation
Nickel (Ni)	mg/L	0.012 ± 0.29	0.001 ± 0.05	0.02 ± 0.001	ND	0.2
Lead (Pb)	mg/L	0.21 ± 0.01	0.11 ± 0.01	0.1 ± 0.06	ND	0.1
Copper (Cu)	mg/L	0.5 ± 0.04	0.02 ± 0.02	0.03 ± 0.1	ND	0.4
Zinc (Zn)	mg/L	0.05 ± 0.8	0.015 ± 0.08	2 ± 0.8	ND	4.0
Iron (Fe)	mg/L	<4.988 ± 0.5	ND	<0.4 ± 0.3	0.05 ± 0.01	5.0
Calcium (Ca)	mg/L	93 ± 1.94	21.57 ± 2.75	66.99 ± 4.5	0.98 ± 0.03	230
Magnesium (Mg)	mg/L	93 ± 1.51	7.64 ± 1.8	32 ± 2.32	1.34 ± 0.06	100
Sodium (Na)	mg/L	472 ± 5.54	175 ± 8.026	128 ± 4.15	0.85 ± 0.02	230
Potassium (K)	mg/L	147 ± 2.06	19 ± 0.036	19.36 ± 1.3	0.14 ± 0.02	ND
Phosphorus (P)	mg/L	15 ± 1.5	7 ± 0.025	3.95 ± 0.25	ND	
* SAR			0.1 ± 0.05	2.51 ± 0.34	0.83 ± 0.03	
		Chemical compounds				
Total Dissolved Solids (TDS)	mg/L	1350 ± 10.5		129 ± 8.32	ND	2500
Chloride (Cl_2)	mg/L	215 ± 8.5		100	ND	100
Sulfate (SO_4)	mg/L	235 ± 8.24	165 ± 7.42	40 ± 2.28	0.81 ± 0.01	600
Nitrate (NO_3-N)	mg/L	25.5 ± 0.89	28.8 ± 0.76	9 ± 0.5	0	10
$PO_4{}^{3}$-P	mg/L	26.8 ± 1.4	20 ±1.02	4 ± 0.1	0	
		Biological parameters				
Fecal coliforms per 100 mL	/100 mL	$1.05 \times 10^5 \pm 465$	$1.58 \times 10^4 \pm 132$	2.1 ± 0.05	ND	2.2

(TSS): Total Suspended Solids ; (EC): Electronic Conductivity; (COD): Chemical Oxygen Demand; (TKN): Total Kjeldahl Nitrogen; (BOD_5): 5 days Biochemical Oxygen Demand; ND = Not Detected; ± = standard error of the mean, *n* = 3); N and P: Full ion record; (SAR): * Sodium Adsorption Ratio SAR= $\frac{[Na^+]}{\frac{\sqrt{Ca^{2+}+Mg^{2+}}}{\sqrt{2}}}$.

Table 1 illustrates the physicochemical characteristics of the sewage water (treated and untreated) used in maize irrigation. The pH values of the clean water (control) and the SW treated by the STP were neutral from 7.00 to 7.20, while the pH of the USW was 7.8. The SW BOD_5 and COD values were higher before the fungal treatment process than after. Typically, the biochemical oxygen demand (BOD_5) and the chemical oxygen demand (COD) were used to recognize wastewater composition. As presented in Table 1, SW is highly loaded in organic matter, as seen through the values of BOD_5 (1065 ± 8.8 mg/L), COD (2315 ± 4.2 mg/L), and TKN (39 ± 1.3 mg/L). This may be due to many oxidizable organic compounds and rapid consumption of the dissolved inorganic materials.

The conductivity (EC) and total dissolved solids (TDS) were higher in the raw sewage water, which describes the salinity level. Salt content is an essential parameter to be considered when using this water for irrigation. The high amounts of the total suspended solids (TSS) (43 ± 0.5 mg/L) and metal(loid)s, such as Cd (0.0035 ± 0.02 mg/L), Ni (0.012 ± 0.29 mg/L), Cu (0.5 ± 0.04 mg/L), and Pb (0.21 ± 0.01 mg/L), could be mainly due to the kind of waste coming from domestic or industrial areas. This high heavy metal content may cause metal(loid) accumulation in the soil, limiting plant growth and crop production [25]. SW has high values of cations like Na, K, Ca, and Mg, at (472 ± 5.54 mg/L), (147 ± 2.06 mg/L), (93 ± 1.94 mg/L), and (93 ± 1.51), respectively.

Data in Table 1 proves that raw SW is a rich source of organic and inorganic nutrients, making it suitable for irrigation reuse for plant growth. The SW quality reached the permissible limits for direct wastewater disposal, as prescribed by Kingdom of Saudi Arabia (KSA) norms [26]. The results agreed with the previous findings of Alawsy et al. [27] and Zidan et al. [28].

2.2. Screening of Single and Consortia Fungal Culture for COD Removal Efficiency

The removal of the organic and inorganic matter in the SW requires selective, efficient fungal strains, as well as favorable operative conditions. In this regard, pre-treatment by fungal pellets can be adapted, while the sewage water has a biodegradability capacity inferior to 3.0 (2 < COD/BOD5 = 2.17 < 3).

Earlier studies have demonstrated that, compared to other microorganisms, fungi have a high capacity to eliminate chemical oxygen demand (COD) from wastewater, which may be because fungi are heterotrophic organisms that utilize organic carbon as their single carbon source and primary form of energy [16]. The most exciting phenomenon of composites is that the properties that cannot be achieved with individual species can be attained by combining individual materials via a synergistic effect.

From a collection of fungal strains, recently isolated and identified by Daâssi and Almaghrabi [29], a screening of individual and consortia cultures was performed to reduce nutrients and some toxic pollutants from the SW. The tested fungal strains were characterized by diversity in terms of enzymatic metabolites (Lipase, Laccases, Cellulase, amylase...e.g.) [29].

The efficiency of the fungal treatment was evaluated through the COD parameter to predict the strength of the organic matter in the receiving water (Table 2).

Table 2. Comparison of sewage wastewater samples' COD values (mg/L) using single or mixed fungal strains.

Strain Numbers	GenBank Accession Number(s)	Identification	COD (mg/L)
Strain 1 (S1)	MZ817960.1	*Aspergillus niger* KB5	933 ± 7.35
Strain 2 (S2)	MZ817957.1	*Fusarium chlamydosporum* KB2	1054 ± 2.88
Strain 3 (S3)	OK668265.1	*Paecilomyces formosus* KW3	1512 ± 8.07
Strain 4 (S4)	MW699898.1	*Sordariomycetes* sp. D10	1206 ± 5.77
Strain 5 (S5)	MW699893.1	*Coniochaetaceae* sp. LB3	1795 ± 10.95
Consortium 1	S1 + S2	KB5 + KB2	650 ± 2.82
Consortium 2	S1 + S3	KB5 + KW3	517 ± 3.95
Consortium 3	S1 + S4	KB5 + D10	709 ± 2.14
Consortium 4	S1 + S5	KB5 + LB3	435 ± 1.55
Consortium 5	S2 + S3	KB2 +KW3	893 ± 1.07
Consortium 6	S2 + S4	KB2 +D10	1007 ± 2.33
Consortium 7	S2 + S5	KB2 + LB3	553 ± 3.11
Consortium 8	S3 + S4	KW3 + D10	965 ± 2.54
Consortium 9	S3 + S5	KW3 +LB3	352 ± 1.76
Consortium 10	S4 + S5	D10 + LB3	599 ± 1.88
Consortium 11	S1 + S2 + S3	KB5 + KB2 + KW3	375 ± 3.07
Consortium 12	S1 + S3 + S4	KB5 + KW3 + D10	288 ± 2.63
Consortium 13	S1 + S4 + S5	KB5 + D10 + LB3	276 ± 1.06
Consortium 14	S2 + S3 + S4	KB2 + KW3 + D10	375 ± 4.09
Consortium 15	S2 + S4 + S5	KB2 + D10 + LB3	396 ± 5.94
Consortium 16	S3 + S4 + S5	KW3 + D10 + LB3	498 ± 6.05

The results obtained in Table 2 refer to the COD values in the SW-amended medium during the fungal treatments using individual or mixed consortia strains.

A decline in the COD values of the treated SWs was recorded after 25 days by the tested strains cultivated in either mono or co-cultures. The COD values of treated sewage (TSW) with fungal monoculture (S1 to S5) amounted to 933 ± 7.35 to 1795 ± 10.95 mg/L, whereas COD values of the TSW with mixed cultures (consortium 11 to 16) oscillated from 276 ± 1.06 to 498 ± 6.05 mg/L. The fungal consortia, composed of two fungi (Consortium 1 to 10), exhibited lower values of COD than the fungal monoculture but was still limited compared to the consortia, consisting of three strains. As can be seen, the COD removal rate was influenced by the fungal strains; based on the combined potential for COD reduction, consortium 13, composed of KB5 + D10 + LB3, exhibited the lowest COD value with 276 ± 1.06 mg/L; therefore, those strains will further help the consortium-based sewage water stream treatment. In addition, the stability of the selected fungal consortium was tested on MEA plates. There was no antagonism among the fungal strains on the Petri plate. These findings demonstrated the synergetic action between the combined fungal species in using organic pollutants as a sole source of carbon or else degrading organic compounds in the presence of growth nutrients from cheap sources. Data from this study supports that the tested fungal strains were highlighted in several studies by their removal efficiency and potential for decomposing xenobiotic organic compounds from wastewater [25,27,30]. In some references, wood-decaying fungi are described as factories of lignin-modifying enzyme production, which is involved in fungal bioremediation processes, including laccases, manganese peroxidases, and lignin peroxidases [31,32].

In a related study on fungal strain application in treating organic pollutants, Daâssi and Almaghrabi [29] claimed the performance of *Fusarium chlamydosporum* [MZ817957] and *Coniochaeta* sp. [MW699893] aided in the removal of petroleum hydrocarbons from contaminated soil. Literature studies have demonstrated that biological treatment processes generally require collaborative enzymatic activities and the complex metabolic pathways of different species; however, single strains cannot treat high organic load in many cases, and their removal efficiency is still limited [33,34].

Similar studies demonstrated the enhancement of the biodegradation potential using microbial consortia to remove organic pollutants [35,36]; meanwhile, single strains still have limited bioremediation capacities, a high contamination risk, and required sterilization procedures [37]. In the same line with our results, Selim et al. [38] found a decrease in the COD with a removal percentage of 77.6% due to the bio-treatment of textile effluent using the fungal consortium *Aspergillus flavus* and *Fusarium oxysporium*.

2.3. Effect of Fungal Consortia Treatment on the Physicochemical Properties of SW

The effect of potential mixed culture of *Aspergillus niger KB5*, *Sordariomycetes* sp. D10 and *Coniochaetaceae* sp. LB3 was investigated for its efficiency in removing organic and inorganic matter in treated supernatants.

The observed results in Table 2 indicated that the fungi consortium (KB5 + D10 + LB3) cultivated in non-sterilized SW decreased the COD value, with a reduction rate of 88%, compared to USW. Moreover, the quantity of significant micronutrients and heavy metals in fungal-treated SW was below the treated wastewater standards for unrestricted irrigation in the Kingdom of Saudi Arabia (KSA) [26].

Data from this study supports that the fungal consortium utilized the soluble and insoluble organic substances in wastewater as nutrient and energy sources for their growth and accelerated the reduction process of COD and other indicator parameters of pollution.

Regarding data presented in Table 1, the fungal consortia enhance the removal of heavy metal ions from the SW. The reduction rates of metals were 47.6%, 80%, 91%, and 96% for Pb, Cd, Ni, and Cu, respectively. These findings demonstrated that the *Aspergillus niger*, *Sordariomycetes* sp., and *Coniochaetaceae* sp. species of the consortia have high metal ions uptake capacities in regard to sewage water. Similarly, many studies described the critical role of fungi as low-cost bio-adsorbant candidates for metal removal [27,28]. According to

Dusengemungu et al. [39], multiple genera of fungi, including *Aspergillus*, *Penicillium*, and *Trichoderma*, were recognized for their biosorption ability due to their capacity to bleach metal ions via cell-surface binding, cellular uptake, and compartmentalization. Other studies have reported the role of ligninolytic enzymes throughout oxidation, reduction, hydrolysis, and in degrading organic pollutants and xenobiotic compounds in sewage water [29,31].

2.4. Impact of Sewage Water on Maize Plant Growth

2.4.1. Morphological Plant Growth Traits

Compared to the control, sewage-irrigated plants' growth traits (Aerien part length, root length, and assimilating area) markedly increased (Table S1; Figure 1).

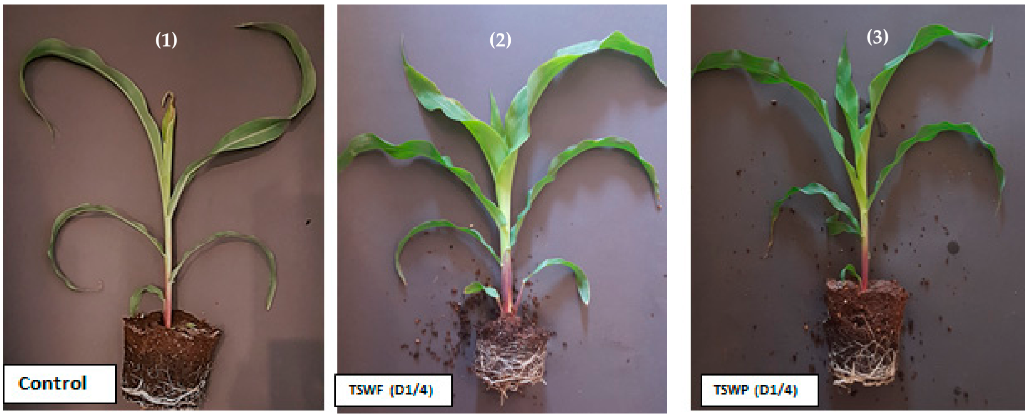

Figure 1. Aspect and sanitary maize (*Zea mays* L.) irrigated with water control (**1**); treated Sewage water by fungal consortia ($TSW_{D1/4}$) (**2**) in comparison with that irrigated with treated SW by STP ($TSWP_{D1/4}$) (**3**).

Regarding SW-irrigated maize, morphological traits, such as root elongation and assimilating area, were significantly stimulated during the irrigation with the fungal-treated SW (TSW) at dilution $D_{1/4}$, followed by the treated SW by STP at the dilution D_0, compared to control plants (irrigated with freshwater).

These morphological responses suggested the improvement of maize growth due to the potential role of SW irrigation to support the required qualities and quantities of microelements and water for plant growth. Similarly, morphological and physiological traits were claimed by Alawsy et al. [27], who recorded a significant increase in biomass, plant height, and dry weight of the treated maize with 50% of the SW. Applying 100, 75, and 50% concentrations of SW positively affected performance growth and nutrient accumulation in maize plants over the control.

The effect of SW on the assimilation area depended on water composition during maize growth (Figure 2). The greatest enlargement of the assimilation area was observed with TSW-$D_{(1/4)}$ irrigation by 1051 cm^2, followed by TSWP-D (0) by 953.96 cm^2, then USW-$D_{(1/4)}$ by 716.54 cm^2 as compared to plants irrigated with clean water (506.91 cm^2). On average, the assimilation areas were more significant by 51.76%, 46.86%, and 29.25% in TSW, USW, and TSWP-irrigated plants, respectively.

The effects of dilution on the SW-irrigation process of maize were also indicated in Figure 2. It showed that the dilutions $D_{(1/2)}$ and $D_{(1/4)}$ positively affected the assimilation areas of TSW-irrigated plants. However, negative impacts and no effects were observed on maize pots irrigated with TSWP and USW, respectively.

Data regarding assimilating area attributes indicated that the irrigation of maize with reclaimed water (TSW and TSWP) slightly improved plants' performance growth.

Likewise, USW irrigation enhanced the morphological traits of the plants (46.86%) compared to the control. Thus, SW contains beneficial elements that provide soil fertility and plant growth. Otherwise, the amount of pollutants in USW (Table 1) did not reach the toxic levels in maize plants for the agricultural experiments.

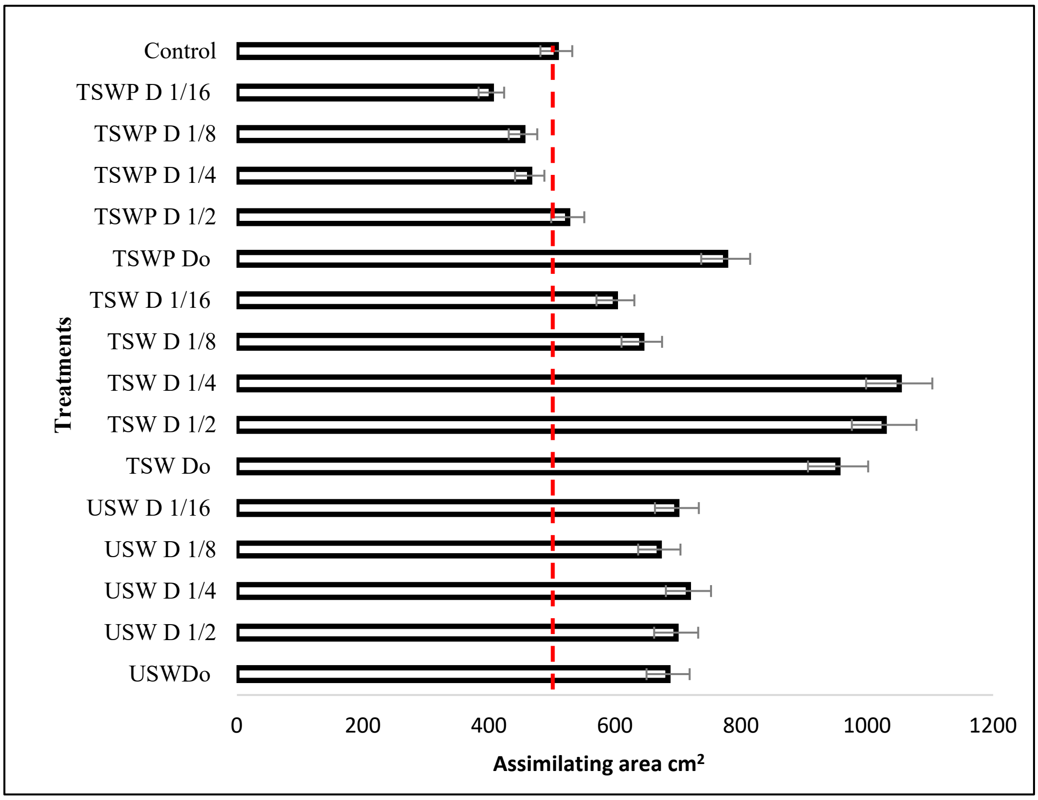

Figure 2. The effect of the treatment of sewage waters (SW), treated by fungal consortia (TSW), treated by SPT (TSWP), and untreated SW (USW) at different dilutions (D_0, $D_{1/2}$, $D_{1/4}$, $D_{1/8}$, $D_{1/16}$) on the assimilating area.

Our results demonstrated that irrigation with SW provides plants nutrients and water after being treated and converted into an available form by fungal consortium culture. Additionally, SW was rich in organic matter, nitrogen, and phosphorus, and those nutrients, as well as organic matter, could improve soil fertility. This result was consistent with Hawrot-Paw et al. [40], who reported that the amendment of maize pots with SW provides soil fertility.

Additionally, in the literature, many studies reported that dilutions might support the distribution and solubility of fertilizing elements and fulfill the water requirement of crops [41–43].

2.4.2. Physiological Traits

Applying SW to maize (*Zea mays* L.) plants, conducted in pot experiments, was based on a complete randomized experimental design with three blocks and three replicates.

A total of 61 runs with different combinations of treatments (A) and dilutions (B) were designed (Table 3). R1, R2, R3, R4, and R5 were the responses measured after 40 days of irrigation and corresponded to Total Phenol Content (TPC), Total chlorophyll Chll (a + b), Carotenoids, Peroxidase (POD) and catalase (CAT) activities, respectively (Tables S2 and 3).

Data presented in Table 3 showed that the adjusted sum of squares values were 96.98, 86.34, 85.74, 99.42, and 99.15% for R1, R2, R3, R4, and R5, respectively. At the same time, a relatively low value of the coefficient of variation (CV_{R1} = 12.37%; CV_{R2} = 12.37%; CV_{R3} = 13.7%; CV_{R4}= 3.68%; CV_{R5} = 3.65%) indicated a better accuracy and reliability of the experiments. The model's equations were presented in the Supplementary Materials (Table S3).

Table 3. Statistical analysis of the model (ANOVA).

Source	**Sum of Squares**					**Df * (υ)**	**Mean Square**					**F-Value**					***p*-Value**	**Sign ****
Responses	R1	R2	R3	R4	R5	R [a]	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R [a]	R [a]
Model	6.315×10^5	376.79	6.79	105.91	14.07	19	33,238.04	19.83	0.3576	5.57	0.7407	100.65	20.62	19.66	532.07	361.20	<0.0001	b
A-Treatment	1.118×10^5	318.14	3.34	102.23	9.67	3	37,267.55	106.05	1.11	34.08	3.22	112.85	110.2	61.31	3252.5	1571.02		
B-Dilution	2.078×10^5	21.65	1.41	1.62	1.16	4	51,947.84	5.41	0.3529	0.4046	0.2907	157.30	5.63	19.41	38.62	141.76		
AB	3.119×10^5	37.00	2.04	2.07	3.25	12	25,994.06	3.08	0.1698	0.1722	0.2705	78.71	3.21	9.34	16.44	131.90		
Pure Error	13,209.91	38.46	0.72	0.419	0.082	40	330.25	0.9616	0.0182	0.0105	0.0021							
Cor Total	6.447×10^5	415.26	7.52	106.33	14.16	59												

Fit Statistics

	R1	R2	R3	R4	R5		R1	R2	R3	R4	R5
Std. Dev.	0.9806	0.9806	0.1348	0.1024	0.1024	R^2	0.9795	0.9074	0.9033	0.9961	0.9942
Mean	7.92	7.92	0.9842	2.78	2.78	Adjusted R^2	0.9698	0.8634	0.8574	0.9942	0.9915
C.V. %	12.37	12.37	13.70	3.68	3.65	Predicted R^2	0.9539	0.7916	0.7824	0.9911	0.9870
						Adeq Precision	33.7729	14.2247	15.0090	61.5673	63.8280

[a] Responses R1, R2, R3, R4, R5: (R1 = Total Phenol (mg GA/g) /FW; R2 = Chll (a + b) (mg/g)/FW; R3 = Carotenoids (mg/g)/FW ; R4 = Peroxidase (Units mg^{-1}/FW); R5 = Catalase (Units mg^{-1} /FW).* df: Degrees of freedom. ** Significance.

3. Materials and Methods

3.1. Chemicals

Analytic compounds came from Sigma-Aldrich, St. Louis, MO, USA. Ampicillin/streptomycin solution was purchased from Gibco by Life Technologies (South San Francisco, CA, USA). Ultrapure de-ionized water was used throughout the experiments.

3.2. Fungal Strains

The fungal strains used in the present study were isolated from decaying wood in the Barzah and Rahat regions of Khulais, Jeddah City, Saudi Arabia, in March 2020. They were identified by Daâssi and Almaghrabi [29] as *Fusarium chlamydosporum* (KB2), *Aspergillus niger* (KB5), *Paecilomyces formosus* (KW3), and *Coniochaetaceae* sp. (LB3). Fungi were stored at 4 °C on Malt Extract Agar (MEA) plates supplemented with a 0.01% ampicillin/streptomycin solution (Gibco by Life Technologies) and subcultured monthly.

3.3. Irrigation Water Resources and Properties

The tap water in the faculty was used for irrigation as a freshwater source. Sewage water (SW) was obtained from a selected environmental service company from Jeddah City (Kingdom of Saudi Arabia), GPS 21°33′48.2″ N 39°11′33.4″ E. (average daily flow = 25,000 m^3/day).

The current study collected triplicate samples of raw sewage water (USW) from the influent at an STP directly downstream from a grit chamber. Then, treated sewage water samples from the plant (TSWP) were collected from the effluent at the STP. A composite sample represents the mixed samples from the influent or the effluent at the STP during the specific period (USW or TSWP). A dry, sterile Cap-Bottle Adaptor, 1 L bottle, and PFE Teflon were sampled in ice during transportation and then stored in the refrigerator (4 °C) until analysis. The quality of the sewage is shown in Table 1.

The electronic conductivity (EC) was measured with an advanced conductivity meter (Hanna instruments mod. HI 6321) and the pH with an advanced pH/ORP meter (Hanna instruments mod. HI6221, JEDDAH city, KSA), respectively.

The ion compositions Ca, K, Mg, Na, As, Co, Cd, Ni, Pb, and Fe of sewage wastewater were measured in a chemically digested sample by atomic absorption spectrometry. According to Standard Methods for Examining Water and Wastewater [44]. The COD, BOD_5, TDS, TSS, and TKN were determined [45,46].

Following the Swedish guidelines, all residues generated during experiments were sent for appropriate treatment, and the fungal biomass was sent to incineration.

3.4. Fungal Cultivation in Shake Flasks

As a first step, different fungal strains were cultivated in a SW-amended medium to test their potential to grow and remove the organic and inorganic matter from wastewater. SW was previously reported as containing the essential nutrients for microbial growth [17]; therefore, no nutritional amendment was used in any cultivation. A selection of flasks, both inoculated and non-inoculated with a Malt Extract broth medium, were used as controls.

Test flasks were inoculated with five agar plugs (Ø = 8 mm) from the 7-day-old Malt Extract Agar plates. The flasks were incubated at 30 °C for three days in a rotary shaker gyrating at 150 rpm. Afterward, half of the liquid medium was decanted, and the remaining fungal biomass was homogenized using an Ultra Turrax homogenizer (IKA T25, Jeddah, Saudi Arabia) for 3 min at 16,000 rpm. A volume of 3% of the homogenized fungal suspension was used to inoculate 500 mL baffled Erlenmeyer flasks containing 150 mL of SW previously filtrated and sterilized at 121 °C for 20 min. The SW-amended cultures were incubated in a rotary shaker at 150 rpm at 30 °C for 25 days.

3.5. Screening of Single and Consortia Fungal Culture for COD Removal Efficiency

Sixteen consortia were designed from five fungal strains. Out of 20 fungal consortia prepared for COD analysis, one fungal consortium, which exhibited the best values of COD in the SW-amended medium, was selected.

3.6. Pot Experiment Set-Up and Plant Growth

Maize (*Zea mays* L.) seeds were first submerged for 2 min in a sodium hypochlorite solution (1.5%) for disinfection (in accordance with the standard). Next, they were washed twice with MilliQ water and dried on sterile filter paper in a laminar airflow cabinet. Two maize seeds were sown in each hole filled with peat of seedling plates (54 × 28 × 4 cm, with a hole size of 5.5 × 5.5 × 4 cm) and regularly irrigated with freshwater.

OECD artificial soil was used in this study. The soil samples were air-dried, ground, and sieved through a 2 mm sieve.

After seedling growth reached 5–6 cm, one healthy plant was transplanted in pots (17 × 12 × 13 cm) containing 500 g of OECD soil. No chemical fertilizer was added to any experiment. Plant irrigations were arranged in a complete randomized design (RCBD) during March−May 2020. A total of 3 blocks (B1, B2, B3) were designed with three pots for each treatment (3 × 3 plots). The experiment was conducted from March to May 2020 under the following conditions: Temperature from 28 °C to 32 °C and humidity 60–75%. All pots were watered 3 days/week until they reached field capacity. Fresh and sewage water (USW, TSW, TSWP) were applied in different proportions 4 days/week (Table 4). The irrigation was applied using a plastic spray bottle. The raw and treated sewage water was manually shaken and diluted with ultrapure water every time before irrigation.

Table 4. Experimental randomized complete block design (RCBD).

Block	A	B	Run	Block	A	B	Run	Block	A	B
B1	USW	$D_{1/8}$	21	B2	USW	$D_{1/4}$	41	B3	TSWP	$D_{1/8}$
B1	Cont	$D_{1/4}$	22	B2	Cont	$D_{1/8}$	42	B3	TSW	$D_{1/8}$
B1	TSWP	$D_{1/4}$	23	B2	TSWP	$D_{1/4}$	43	B3	USW	$D_{1/4}$
B1	TSW	$D_{1/4}$	24	B2	TSW	$D_{1/4}$	44	B3	Cont	$D_{1/2}$
B1	Cont	$D_{1/8}$	25	B2	TSWP	$D_{1/8}$	45	B3	Cont	$D_{1/8}$
B1	Cont	$D_{1/2}$	26	B2	TSW	$D_{1/8}$	46	B3	TSWP	$D_{1/16}$
B1	TSWP	D_0	27	B2	Cont	$D_{1/16}$	47	B3	TSW	$D_{1/16}$
B1	TSW	D_0	28	B2	Cont	D_0	48	B3	TSWP	$D_{1/2}$
B1	TSWP	$D_{1/16}$	29	B2	USW	D_0	49	B3	TSW	$D_{1/2}$
B1	TSW	$D_{1/16}$	30	B2	USW	$D_{1/8}$	50	B3	Cont	$D_{1/16}$
B1	TSWP	$D_{1/2}$	31	B2	Cont	$D_{1/2}$	51	B3	USW	D_0
B1	TSW	$D_{1/2}$	32	B2	Cont	$D_{1/4}$	52	B3	USW	$D_{1/2}$
B1	USW	$D_{1/2}$	33	B2	TSWP	D_0	53	B3	USW	$D_{1/8}$
B1	Cont	D_0	34	B2	TSW	D_0	54	B3	Cont	D_0
B1	TSWP	$D_{1/8}$	35	B2	USW	$D_{1/2}$	55	B3	TSWP	D_0
B1	TSW	$D_{1/8}$	36	B2	TSWP	$D_{1/2}$	56	B3	TSW	D_0
B1	USW	D_0	37	B2	TSW	$D_{1/2}$	57	B3	USW	$D_{1/16}$
B1	Cont	$D_{1/16}$	38	B2	TSWP	$D_{1/16}$	58	B3	Cont	$D_{1/4}$
B1	USW	$D_{1/4}$	39	B2	TSW	$D_{1/16}$	59	B3	TSWP	$D_{1/4}$
B1	USW	$D_{1/16}$	40	B2	USW	D1/$_{16}$	60	B3	TSW	$D_{1/4}$

Cont: Control (Clean water), USW = Untreated Sewage Water, TSW = Treated Sewage Water by Fungal Consortia, TSWP = Treated Sewage water by the STP, (A: treatments; D: Dilution with Ultra-pure deionized water).

3.7. Plant Sample Collection and Preparation

After 56 days, *Zea mays* L. samples were collected in pre-cleaned plastic bags from the experimental design blocks, cultivated by freshwater (Control) and sewage water (treated or untreated) during March−May 2020. Three samples from each experiment were uprooted, gently washed, blotted, and divided into shoots (leaves and stem) and root parts.

The mean of separated parts was properly homogenized, labeled, and weighed for the fresh matter, then dried at 60 °C in an oven until constant weight for dry weight determination.

3.8. Growth Traits and Yield Parameters

3.8.1. Morphological Traits

Different morphological traits and metabolic enzymes determine plant growth, according to Dutta and Hyder [47].

After harvesting, all plant heights were measured with a simple rule from the ground level to the top of the plant without awns. The growth of maize was measured through several parameters, such as the plant height (cm) (PH), leaf height (cm) (LH), leaf breadth, leaf area (LA), the relative leaf height (RLH), length of root, and leaf number (Supplementary Materials Table S1).

The leaf area of the maize was estimated using Equation (1).

$$\text{LA} = \text{length} \times \text{breadth} \times 0.75 \quad (1)$$

The relative leaf height (RLH) was estimated by Equation (2).

$$\text{RLH} = \text{LH}/\text{PH} \quad (2)$$

The determination of the assimilation area (A.A) was performed according to Pace et al. [48] by Equation (3).

$$\text{Assimilating area} = \pi\,(22/7) \times \text{leaf radius} \times \text{leaf length} \quad (3)$$

3.8.2. Photosynthetic Pigments: Chlorophyll (a + b) and Carotenoids

Chlorophyll is dissolved in acetone when the sample is macerated. The optical density of the extract is measured with a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) at wavelengths of 663 nm and 645 nm, respectively, because these wavelengths are where chlorophyll 'a' and 'b' absorb the most. The amounts of chlorophyll 'a', 'b', and total chlorophyll are calculated using Ritchie formulas [49,50]. The sample extract produced for chlorophyll macerated with acetone can also be utilized for carotenoid quantification. In addition to measuring the extract's absorbance at 663 and 645 nm, spectrophotometric values at a 470 nm wavelength are taken. The formula used is according to Lichtenthaler and Buschmann [51].

3.8.3. Antioxidative Response to the SW-Irrigation

a. Estimation of total phenolic content (TPC)

Using the Folin–Ciocalteu technique, the TPC of shoot extracts was measured [52]. Gallic acid was used to perform calibration curves. Absorption was detected at a wavelength of 725 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The data were expressed as gallic acid mg/g dry weight (mg GAE/g) in milligram gallic acid equivalents per gram of fresh *Zea mays* L. material.

b. Antioxidant enzymes: Peroxidase and catalase activities

For antioxidant compound extraction, 0.5 g of fresh shoots were frozen in liquid nitrogen and then ground in a 4 mL solution containing a 50 mM phosphate buffer (pH 7.0), 1% (*w*/*v*) polyvinylpolypyrrolidone, and 0.2 mM ascorbic acid. The homogenate was centrifuged at 15,000× *g* for 30 min, and the supernatant was collected for enzyme assays.

Peroxidase activity (EC 1.11.1.7) was detected by spectrophotometer using the guaiacol oxidation technique, according to Hakiman and Maziah [53].

At 470 nm, the rise in absorbance owing to tetra-guaiacol production was detected. The change in absorbance per minute was used to measure peroxidase activity. A unit of peroxidase activity is defined as the specific activity expressed as enzyme units per milligram of protein with an extinction coefficient of 6.39 mM^{-1} cm^{-1}.

Catalase activity (EC 1.11.1.21) was evaluated by tracking the fall of peroxides using spectrophotometric measurement at 240 nm, as described by Hadwan [54]. One catalase unit is the quantity of enzyme that, at the specified conditions, decomposes one µmole of H_2O_2 per minute at pH 7 and 25 °C, as determined by Equation (4): at 25 °C and pH 7.0, one system degrades one micromole of H_2O_2 every minute.

$$\text{Units/mg} = (\Delta A/\text{min} \times 1000)/(43.6 \times \text{mg enzyme/mL reaction mixture}) \tag{4}$$

3.9. Statistical Analysis

After recording data on all tested parameters, the Design-Expert version 7.0 (STAT-EASE Inc., Minneapolis, MN, USA) software was used to analyze the randomized complete block design in a split-plot arrangement. The experimental results were analyzed by standard ANOVA.

3.9.1. Graphical Interpretation

The impact of irrigation using SW, at different dilutions and treatment methods, on maize plants for 40 days was assessed. The tested responses were R1, R2, R3, R4, and R5.

a. Response R1: Total Phenols Content (TPC)

Typically, the TPC content is an oxidative stress marker representing the non-enzymatic antioxidant contents in sewage-irrigated plants. The effect of SW irrigation on the TPC of maize plants grown at different concentrations is displayed in Figure 3.

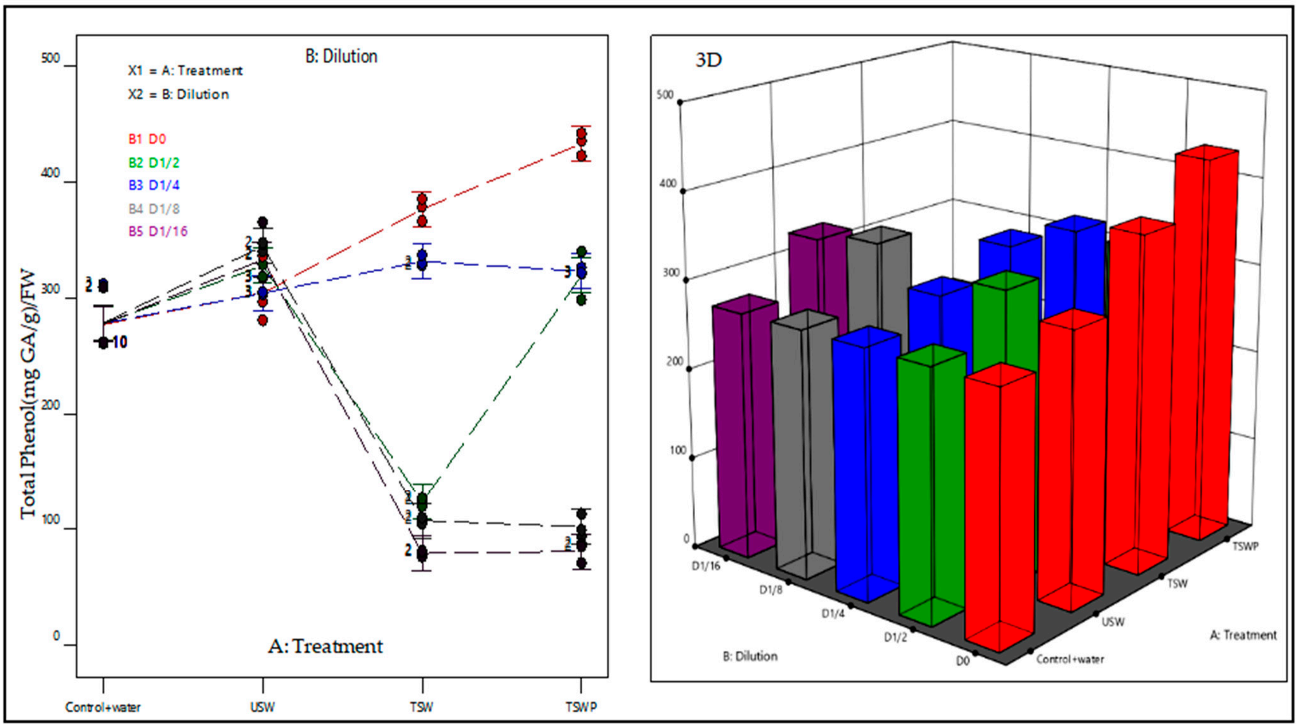

Figure 3. Effect of SW irrigation on Total phenols content (TPC) of maize plants grown in the function of SW treatment (factor A) at different dilutions (factor B) with actual factor coding.

As can be seen in Figure 3, the TPC increased markedly in TSWP-D_0 (433.632 mg/GA/g FW) as compared to control pots (277.396 mg/GA/g), USW-D_0 (304.634 mg/GA/g FW), and TSW-D_0 (376.954 mg/GA/g FW).

Regarding USW-irrigated plants, the TPC content did not differ significantly in all other treatments according to the dilution factor. Meanwhile, pots amended with diluted reclaimed water (TSW-$D_{1/4}$ and TSWP-$D_{1/4}$) showed decreased TPC close to the control values. Moreover, the results revealed that treatments with dilution > $D_{1/4}$ markedly reduced the TPC, and its minimum values were observed in response to the application of TSW D1/16 (79.285 mg/GA/g FW) and TSWP-$D_{1/16}$ (81.037 mg/GA/g FW). Therefore, the suitable dilution for reclaimed water irrigation is $D_{1/4}$.

Our results demonstrated that irrigation with SW causes abiotic stress for maize plants, especially in the presence of detrimental effects of stress like heavy metals, as recorded through the physicochemical composition of the raw SW (USW) and the treated SW by STP (TSWP).

Similar findings were reported where plants irrigated by SW saw potential secretion of bioactive compounds, like phenolic substances, occurring in all plant parts (roots, shoots, and fruits) as a plant defense strategy against abiotic stresses, such as heavy metals, acidity, pollution, and soil composition [55].

Our results provide evidence for the beneficial impact of SW application to maize plants to fight stress. Additionally, the pre-treatments of SW simultaneously allow for a reduction in the high inputs of organic waste and metal(oid)s and the creation of stimulating preparations, which improve the plant's development and tolerance to abiotic stress.

Along the same line, Khan et al.'s [56] study showed the efficiency of micronutrients, especially Nitrogen (N), as plant fertilizers involved in abiotic stress management.

Similar studies demonstrated that the natural preparations made from active substances like protein hydrolysates, organic acids, and compounds containing nitrogen, known as biostimulants, might improve plant growth and protect them against biotic and abiotic stress [57,58].

b. Response R2 and R3: Total Chlorophyll Chll (a + b) and carotenoids

Some physio-biochemical markers, such as photosynthetic pigment synthesis regarding the total chlorophyll and carotenoids, assessed the impact of SW and clean water irrigation of maize plants.

From the data in Figure 4, it can be observed that SW irrigation positively influenced the total Chll(a+b) over the corresponding values recorded in water-irrigated plants (3.956 mg/g/FW), with a percent increase of 152.47%, 131.95%, and 47.05% in plants shoots irrigated with USW-D_0, TSW-D_0, and TSWP-D_0, respectively.

Unlike chll(a+b) flocculation, carotenoids showed heterogeneous values, and they have kept the baseline of control along with diluted SW application (Figure 5).

It is worth mentioning that the amount of carotenoids in shoots of maize growth under SW irrigation was adversely affected by the high load of organic and inorganic matters in USWD0 (0.365 mg/g/FW) as compared to control plants (1.208 mg/g/FW).

Dilutions of $D_{1/2}$ and $D_{1/4}$ showed an improvement of carotenoids, with a maximum content (1.420 mg/g/FW) observed in response to the application of TSW-$D_{1/4}$. In contrast, in the dilution of $D_{1/2}$, carotenoid values maintained the control values (1.281 mg/g/FW) in response to the application of TSW and TSWP. Thus, total carotenoids expressed on fresh weight (FW) gradually increased by irrigating with treated sewage water (TSW and TSWP).

In our study, the treated sewage water by fungal consortia (TSW) and by STP (TSWP) was characterized by high amounts of Sodium Na of about 375 $\pm$ 8.026 mg/L and 472 $\pm$ 0.54 mg/L compared to the USW (168 $\pm$ 4.15 mg/L), which may explain the increase in carotenoid contents in the TSW and TSWP-irrigated plants. This observation agrees with the results found by Yavuz et al. [59], who reported that irrigation with saline water improves plants' carotenoid content and antioxidant activity.

Overall results, these physiological observations could be explained by the deleterious impacts of water and nutrient uptake, as well as responses of various defense strategies by the plant against environmental stresses. Photosynthetic pigments are critical in light harvesting and dissipation of excess energy. It is known that the content of Chll(a+b) changes under environmental stress [59]. Carotenoids involve energy dissipation and support plant resistance against stressful conditions [60].

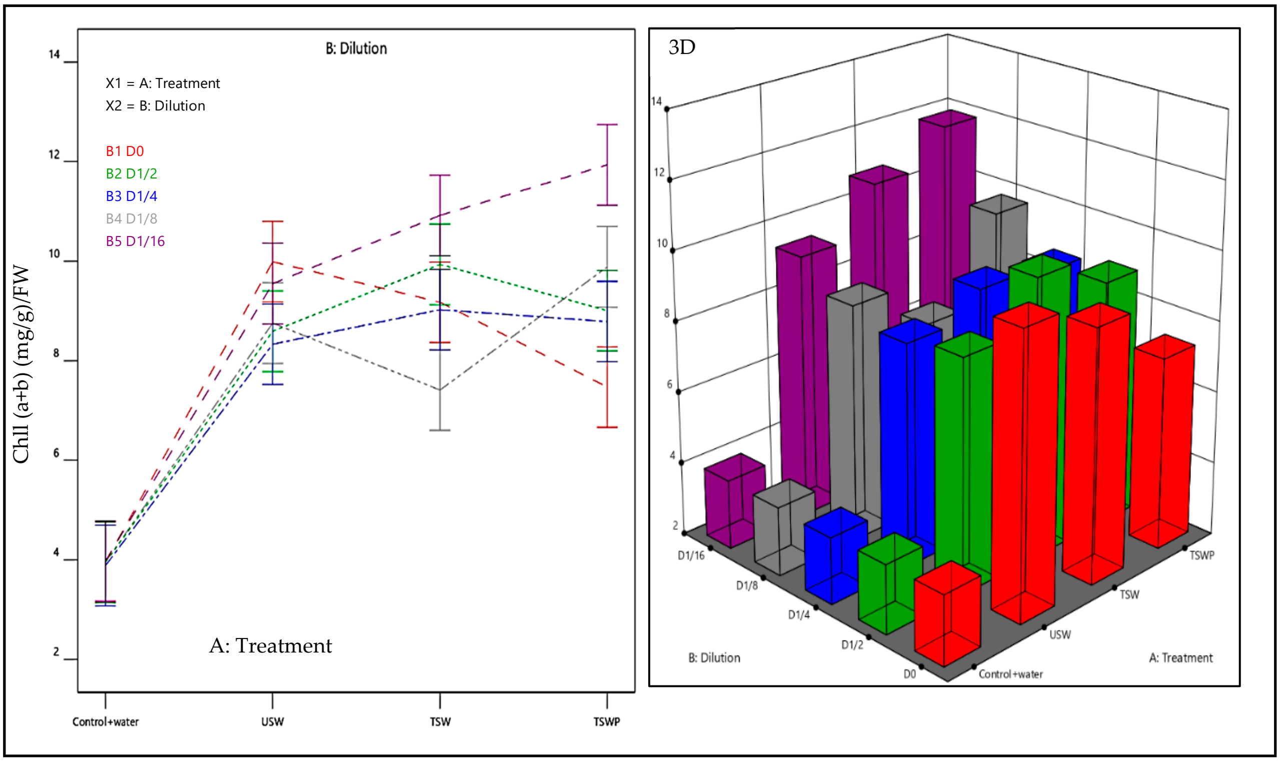

Figure 4. Effect of SW irrigation on chll(a+b) (of maize plants grown in the function of SW treatment (factor A) at different dilutions (factor B) with actual factor coding.

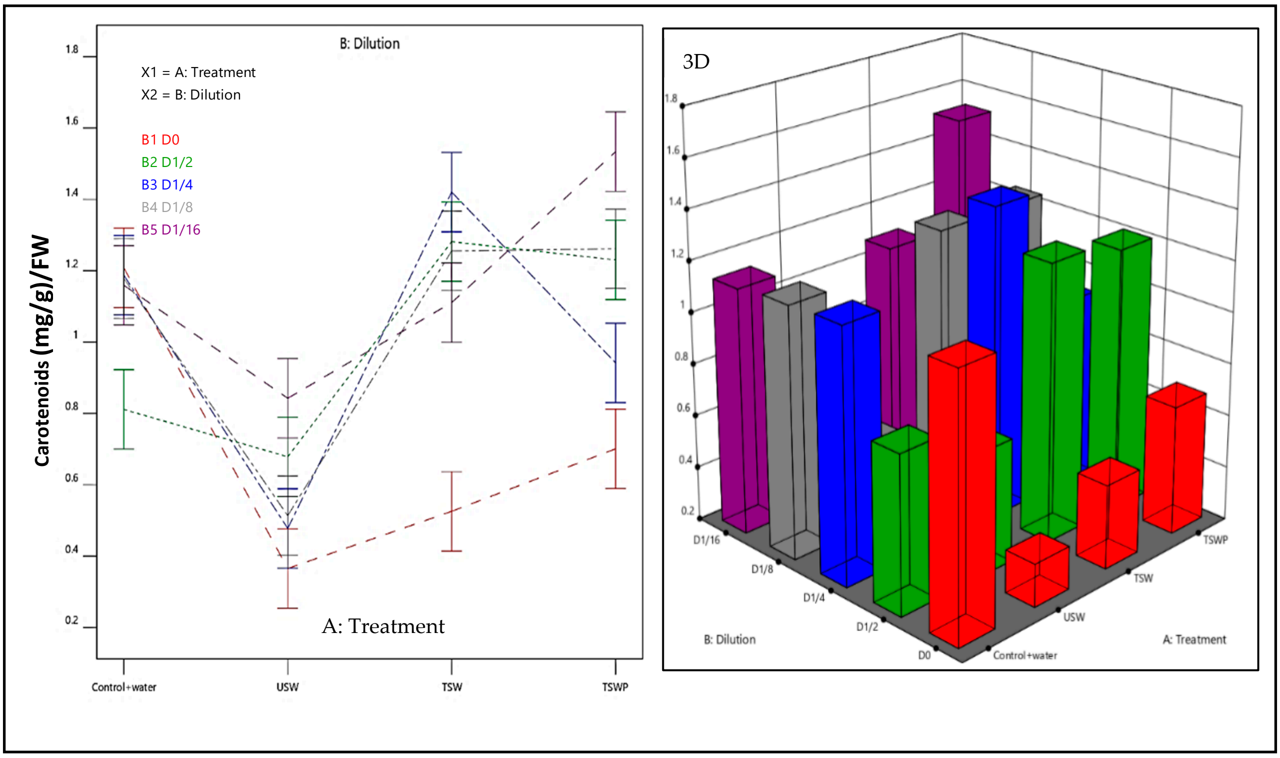

Figure 5. Effect of SW irrigation on Total Carotenoid of maize plants grown in the function of SW treatment (factor A) at different dilutions (factor B) with actual factor coding.

Also, some studies reported that the increase in biochemical parameters, such as total chlorophyll and total carotenoid contents, may also be due to the microbial activities present in SW, which may transform organic matters into by-products like CO_2, NO_3, PO_4, SO_4, and CH_4 [61,62].

c. Responses R4 and R5: Peroxidase POD and Catalase CAT Enzymes Activities

The changes in activities of antioxidant enzymes (POD and CAT) in maize shoots grown under the irrigation with different types of SW (USW, TSW, and TSWP) compared to clean water irrigation (control) are illustrated in Figure 6a,b.

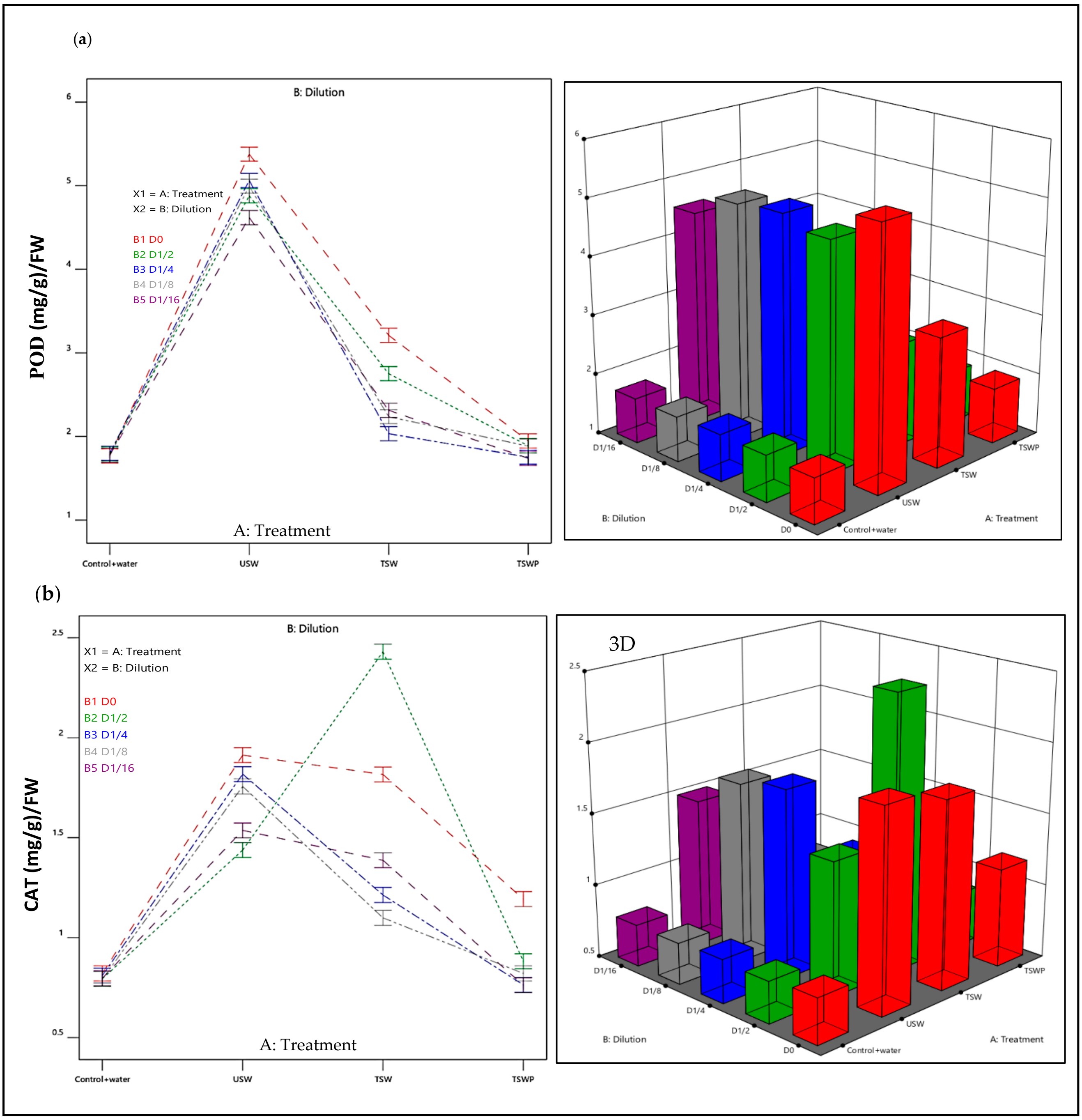

Figure 6. Effect of SW irrigation on (**a**) peroxidase activity and (**b**) catalase activity of maize plants grown in the function of SW treatment (factor A) at different dilutions (factor B) with actual factor coding.

The maize plant had initial POD and CAT enzyme activities of 1.766 mg/g FW and 0.821 mg/g FW, respectively. For plants irrigated with USWD$_0$, POD enzyme activities were higher than the controls, ranging from 5.376 to 4.618 mg/g FW at different dilutions. Additionally, POD activity was lower than in the USW-irrigated plants, ranging from 3.21 to 2.03 mg/g FW in TSW-D$_0$–irrigated plants. Meanwhile, for TSW-irrigated plants, POD values at different dilutions ranged from 1.946 to 1.738 mg/g FW, comparable to the controls. Furthermore, CAT activity was 2-fold enhanced in pots irrigated with USW-D$_0$ (1.913 mg/g FW) and TSW-D$_0$ (1.816 mg/g FW) overvalues in water-irrigated pots. For plants irrigated with TSWP-D0, CAT enzyme activities were comparable to those of water-irrigated pots. According to the given results, it was observed that maize plant irrigation with USW caused a significant increase in the activities of antioxidant enzymes (POD and CAT). This may be due to the high contents of organic waste and pollutants in the raw SW, which caused oxidative damage in maize plants. The irrigation with treated SW (TSW and TSWP) caused a significant decrease in both enzymatic and non-enzymatic antioxidants, indicating that the treatment lowered the stress of sewage water. Previous work has given similar findings [53,60].

3.9.2. Desirability Function (DF)

The optimization plot displays the DF and the optimum predicted values for the maximum growth and phytochemical contents of the *Zea mays* L. plant. The main goal of the experimental setup is to provide the perfect conditions that would maximize the responses.

Each variable's desirability function (DF) for the program ranged from zero (undesirable) to one (desirable). The numerical optimization detects the points at which the DF denote the maximum growth and phytochemical contents of *Zea mays* L. plants that were irrigated by different sewage water treatments and dilutions. Results in Table 5 represent the maximum DF at 0.531 with USW-D$_{(1/16)}$. These results demonstrate the stress conditions of the organic matter in the SW elevated antioxidant enzymes (catalase and peroxidase) and also phenol contents.

Table 5. Desirability function and the optimum predicted values for the maximum growth and phytochemical contents of *Zea mays* L. plant.

N	Treatment	Dilution	Total Phenol/FW	Chll(a+b)/FW	Carotenoids/FW	POD/FW	CAT/FW	Desirability
1	USW	D1/16	333.358	9.549	0.843	4.619	1.537	0.531
2	USW	D1/2	328.216	8.592	0.678	4.879	1.438	0.513
3	USW	D1/8	344.954	8.757	0.514	4.993	1.757	0.498
4	USW	D1/4	304.390	8.332	0.478	5.062	1.818	0.463
5	TSW	D0	376.954	9.176	0.526	3.210	1.817	0.445
6	TSW	D1/2	123.679	9.936	1.282	2.752	2.431	0.442
7	USW	D0	304.634	9.989	0.365	5.377	1.913	0.387
8	TSW	D1/4	331.786	9.025	1.420	2.033	1.213	0.373
9	TSWP	D0	433.632	7.472	0.701	1.947	1.193	0.317
10	TSWP	D1/2	319.966	9.008	1.230	1.890	0.882	0.280
11	TSW	D1/8	107.529	7.411	1.256	2.237	1.099	0.260
12	TSW	D1/16	79.285	10.920	1.111	2.313	1.387	0.224
13	TSWP	D1/8	102.077	9.885	1.261	1.885	0.821	0.182
14	Control	D1/4	278.386	3.883	1.188	1.800	0.810	0.143
15	Control	D0	277.396	3.956	1.208	1.767	0.821	0.138

Table 5. *Cont.*

N	Treatment	Dilution	Total Phenol/FW	Chll(a+b)/FW	Carotenoids/FW	POD/FW	CAT/FW	Desirability
16	Control	D1/8	278.526	3.954	1.178	1.775	0.793	0.129
17	TSWP	D1/4	323.205	8.787	0.942	1.752	0.764	0.127
18	Control	D1/16	277.745	3.977	1.159	1.777	0.797	0.125
19	Control	D1/2	278.101	3.944	0.811	1.791	0.794	0.124
20	TSWP	D1/16	81.038	11.937	1.534	1.738	0.762	0.070

4. Conclusions

Although the appropriateness of using treated wastewater for crop irrigation remains a topic of discussion among government authorities and policy-makers, in light of the above, our study presents a novel method that uses fungi consortium of *Aspergillus niger* KB5, *Sordariomycetes* sp. D10, and *Coniochaetaceae* sp. LB3 to remove organic load and metals from non-sterile sewage water for further reuse in the irrigation of *Zea mays* L. plants. On the other hand, there is a lack of studies about bacterial and fungal interactions in fungal treatments for the biodegradation. For plant responses towards SW irrigations, our research work compared and assessed the impact and the difference between USW, TSW, and TSWP-irrigated maize plants over water-irrigated plants in terms of morphological and physiological parameters. The results showed that the fungal consortia improved the BOD_5, COD, and metals (such as Cd, Cu, Ni, and Pb) percentage removal. Additionally, the application of treated and untreated SW showed that SW-irrigation supports the required qualities and quantities of microelements and water for plant growth. Oxidative stress assessed with TPC and antioxidant enzyme activities (Peroxidases POD and catalases CAT) showed that irrigations with treated SW (TSW and TSWP) caused a significant decrease in both enzymatic and non-enzymatic antioxidants, indicating that the treatment lowered the stress of sewage water.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal14040257/s1, Table S1: Growth traits determination after 40 days of maize cultivation and irrigation with Sewage water (treated in the lab scale or in the STP or untreated) and clean water as control. Table S2: Experimental responses in the randomized complete block (RCB) design. Table S3: Models final equations in terms of coded factors for all the tested responses.

Author Contributions: D.D. and A.N.H.: data curtain, methodology, revision, supervision; L.J.H.A.: methodology, writing; D.D. and R.A.H.: validation, resources, funding acquisition; R.A.H.: supervision, visualization. D.D: writing—review and editing. S.N.A.:resources, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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