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Machine Learning Approach to Predict Quality Parameters for Bacterial Consortium-Treated Hospital Wastewater and Phytotoxicity Assessment on Radish, Cauliflower, Hot Pepper, Rice and Wheat Crops

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Abstract: Raw hospital wastewater is a source of excessive heavy metals and pharmaceutical pollutants. In water-stressed countries such as Pakistan, the practice of unsafe reuse by local farmers for crop irrigation is of major concern. In our previous work, we developed a low-cost bacterial consortium wastewater treatment method. Here, in a two-part study, we first aimed to find what physico-chemical parameters were the most important for differentiating consortium-treated and untreated wastewater for its safe reuse. This was achieved using a Kruskal–Wallis test on a suite of physico-chemical measurements to find those parameters which were differentially abundant between consortium-treated and untreated wastewater. The differentially abundant parameters were then input to a Random Forest classifier. The classifier showed that 'turbidity' was the most influential parameter for predicting biotreatment. In the second part of our study, we wanted to know if the consortium-treated wastewater was safe for crop irrigation. We therefore carried out a plant growth experiment using a range of popular crop plants in Pakistan (Radish, Cauliflower, Hot pepper, Rice and Wheat), which were grown using irrigation from consortium-treated and untreated hospital wastewater at a range of dilutions (turbidity levels) and performed a phytotoxicity assessment. Our results showed an increasing trend in germination indices and a decreasing one in phytotoxicity indices in plants after irrigation with consortium-treated hospital wastewater (at each dilution/turbidity measure). The comparative study of growth between plants showed the following trend: Cauliflower > Radish > Wheat > Rice > Hot pepper. Cauliflower was the most adaptive plant (PI: −0.28, −0.13, −0.16, −0.06) for the treated hospital wastewater, while hot pepper was susceptible for reuse; hence, we conclude that bacterial consortium-treated hospital wastewater is safe for reuse for the irrigation of cauliflower, radish, wheat and rice. We further conclude that turbidity is the most influential parameter for predicting bio-treatment efficiency prior to water reuse. This method, therefore, could represent a low-cost, low-tech and safe means for farmers to grow crops in water stressed areas.

Keywords: hospital wastewater; bacterial consortium treatment; machine learning; Random Forest classifier; phytotoxicity; crop irrigation

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1. Introduction

Hospital wastewater production, unsafe disposal and management is a large-scale problem across the globe. Developed countries generate from 0.4 to 1.2 m^3/bed/day, while developing countries produce from 0.2 to 0.4 m^3/bed/day [1–6]. Pakistan is a developing country, but its untreated hospital wastewater accounts for ~0.4–0.8 m^3/bed/day [7,8], which is the usual range for developed countries. Much of this wastewater is discharged without treatment [9,10]. The Government of Pakistan (GoP) has set strict safety regulations to ensure safe hospital waste management as an extension to the Pakistan Environmental Protection Act, 1997 [11]. The GoP has also set specific ranges for the National Environment Quality Standards (NEQs) for the physicochemical characteristics of wastewater before discharge, i.e., pH (6.6–8.5), total suspended solids (TSS) (<500 mg/L), total dissolved solids (TDS) (1000 mg/L), chemical oxygen demand (COD) (150–400 mg/L), biological oxygen demand (BOD5) (80–250 mg/L), turbidity (5 NTU), As (0.05 mg/L), Cd (0.01 mg/L), Cr (0.05 mg/L), Pb (0.05 mg/L) and Ni (0.02 mg/L) [12]. Nonetheless, the discharge of untreated hospital wastewater that shows physicochemical characteristics beyond the range limits has been reported, due to poor enforcement of these laws [4,13–15]. Hospital wastewater is particularly harmful if discharged untreated due to the spread of pharmaceutical pollutants [16,17] and heavy metals [9,16] in the aquatic environment. These pharmaceutical pollutants and heavy metals are recalcitrant in nature [18–20]. Heavy metals, such as Arsenic (As), Chromium (Cr), Lead (Pb), Nickel (Ni) and Cadmium (Cd) have the tendency to accumulate in water bodies [15,19]. Due to acute freshwater shortage, local farmers are utilising raw hospital wastewater for crop irrigation [20,21]. This results in the accumulation of toxic pollutants in crop plants [4,15,18,19] that are comprised of pharmaceutic compounds, heavy metals and other toxic contaminants [6,19,22–25]. Subsequently, all these contaminants enter the food web [22–26] and harm aquatic [27,28] and land [26,27] animals, as well as human beings [26,27]. These heavy metals and pharmaceutic pollutants cause inexorable health problems [29–35]. They have been shown to impair the process of reproduction [36] and nervous and immune systems in humans [26,29]. Reducing their uptake is doable compared to overcoming their irrevocable hazards.

In our previous work, we have developed a simple, low-cost, biological method for hospital wastewater treatment using a consortium of three bacterial species (an *Alcaligenes faecalis* sp. and two *Bacillus paramycoides* spp.) isolated from hospital wastewater [37,38]. Our results highlighted that this biotreatment was effective for the degradation of heavy metals and pharmaceutical compounds and other parameters in raw hospital wastewater. For example, we demonstrated a biotreatment with this consortium that reduced heavy metal concentrations (Cr, Pb and Ni (100–86%)) along with pharmaceutical pollutants (caffeine, diazepam, naproxen, octadecene, phenol and salicylic acid (100–74%)) from hospital wastewater after biotreatment. Moreover, we observed the reduction of a range of wastewater quality indicators, i.e., turbidity, colour, BOD5, COD, biodegradability index (BI), electrical conductivity (EC), salinity, TSS and TDS [90–26%] (Table 1). However, testing a suite of wastewater parameters separately can be challenging and expensive in low–middle income countries, and even more challenging in rural farm settings [39].

COD and BOD are the most conventional quality parameters used in predicting treatment efficiencies [40]. However, these fail to consider emergent and toxic contaminants (heavy metals, for example). Whilst one is disposed to acquiring an exhaustive set of parameters to differentiate treatment groups, some parameters may not change at all. For acquiring a low-tech solution, the use of artificial intelligence (AI) and Machine Learning (ML) is convenient to predict what is the most important parameter for the biotreatment efficiency. Recently, authors have predicted biological wastewater treatment efficiency prior to irrigation using various ML models, e.g., Adaptive Neuro Fuzzy Inference System (ANFIS), Artificial Neural Networks (ANN), Fuzzy Logic (FL) and Neuro-Fuzzy (NF) [40–42]. The Random Forest (RF) classifier method is an emerging ML method that has been used previously for monitoring urban wastewater [43]. The method also successfully predicted the performance of the adsorption of six heavy metals (As, Cd, Cu, Ni, Pb and Zi) in biochar within real waters and wastewaters [44]. To explore this further, RF classifier methods are typically applied, and their accuracy is established either through quality of fit criteria or through importance measures that delineate which parameters lead to better fit.

Table 1. Hospital Wastewater Quality Parameters.

Quality Parameters	Units	Hospital Wastewater		NEQS [12]
		Untreated	Treated	
Temperature	°C	25	4	=<3
Turbidity	NTU	51 ***	5 ***	5
BOD	mg/L	246 ****	78 ****	80–250
Colour	PCU	188	55	-
TSS	mg/L	2300 ****	483 ****	<500
COD	mg/L	396 ****	260 ****	150–400
BI	-	0.62	0.3	-
EC	µs/cm	444 ****	267 ****	-
Salinity	pg/L	0.2 **	0.1 **	-
TDS	mg/L	296 ****	220 ****	1000
Chromium	mg/L	1.8	Nd	0.05
Nickel	mg/L	1.8	Nd	0.02
Lead	mg/L	0.17	Nd	0.05

Note: Nd = Not detected; Significance (Welch's test) between treated and untreated hospital wastewater is indicated by $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****.

The present study includes two interdependent analyses. First, we aim to predict the quality parameters in hospital wastewater treated with a bacterial consortium (an *Alcaligenes faecalis* sp. and two *Bacillus paramycoides* spp.) using a Kruskal–Wallis test to identify the wastewater parameters that differentiate the treatment groups, and apply a Random Forest classifier on these reduced parameters to provide feature-wise importance measures for these parameters, thus highlighting the important parameter/s to consider in differentiating treatment efficiency for treated hospital wastewater application. Second, we aim to provide a phytotoxicity assessment for two purposes: to see whether our predicted quality parameter/s align with the phytotoxicity results and to analyse the response of plants (growth) on the reuse of treated hospital wastewater. We assess the seedling lengths and biomass measurements, germination indices and phytotoxicity indices for five crop species (radish, cauliflower, hot pepper, rice and wheat) grown in the treated wastewater and compare their responses in terms of growth to see which crop plants are more adaptive for wastewater reuse.

2. Materials and Methods

2.1. Wastewater Treatment and Quality Parameters

A bacterial consortium (an *Alcaligenes faecalis* sp. and two *Bacillus paramycoides* spp.) was added to deionized water (10 mL) in a test tube (20 mL). A consortium suspension was hence formed with a set value of optical density (OD) of 1. OD was kept the same to ensure an equal amount of three bacterial species in suspension. Treatment consisted of adding 10% of the consortium suspension to raw untreated autoclaved hospital wastewater and incubation at 37 °C (optimal temperature for bacterial growth) in a shaking incubator for 48 h. The control of this treatment was distilled water. After 48 h of incubation, this sample was centrifuged for 15 min (speed = 8000 g/min). The supernatant was shifted to a polypropylene falcon tube (15 mL). This supernatant was considered as treated hospital wastewater. The quality parameters selected for untreated and treated hospital wastewater were as follows: temperature, turbidity, BOD, colour, TSS, COD, BI, EC, salinity, TDS and heavy metals (Table 1).

2.2. Machine Learning Approach

2.2.1. Kruskal–Wallis Test

The non-parametric data was autoscaled in such a way that all the features were of equal weights. The Kruskal–Wallis test was performed to see if these features discriminate between treated and untreated categories of hospital wastewater. Because the test was applied independently on all the acquired parameters, the Benjamini and Hochberg procedure was applied to adjust p-values for multiple comparisons [45]. Only those features were retained where the adjusted p-value was <0.05.

2.2.2. Random Forest (RF) Classifier

The RF classification method is a machine learning algorithmic technique that helps in classifying data with the help of decision trees [46]. The Random Forest classifier was applied on the discriminating features (quality parameters selected) from the previous step. After training the classifier, we utilized two measures: Mean Decrease in Accuracy gives an estimate of classifier performance if each feature is removed from training, resulting in reduced accuracy; whilst Mean Decrease in Gini looks at how pure class memberships are in terms of probabilities. A higher mean decrease in Gini index indicates that the classification is pure with the probability of achieving one category maximised.

2.3. Phytotoxicity Assessment

Phytotoxicity is a measure to assess any delay, hindrance or inhibition in plant growth caused by chemical compounds (pharmaceutics, phytotoxins, etc.), heavy metals or environmental factors (soil contamination, etc.) [47]. The phytotoxicity assessment comprised germination experiments that yielded seedling germination and phytotoxicity indices followed by statistical analyses.

2.3.1. Germination Experiments

Five different types of certified crop seeds were procured from the Seed Certification Department, Lahore, Punjab. The crop seed varieties were *Raphanus sativus* L. (Radish) var. Radish Minto Early, *Brassica oleracea* L. (Cauliflower) var. Cauliflower 2801, *Capsicum annuum* L. (Hot pepper) var. Seminis Hybrid Hot pepper SKY LINE 3, *Triticum aestivum* L. (Wheat) var. FSD-2008 and *Oryza sativa* L. (Rice) var. PS-2 (PK-112). The seeds were germinated in sterilised petri plates watered with four different dilutions (25, 50, 75 and 100%) of untreated and treated hospital wastewater, as described in Rashid et al. [37]. The controls for the experiment were distilled water and tap water.

2.3.2. Germination Indices (GI)

The germination indices of three crops were calculated by multiplying the relative seedling germination and root growth. Lower GI values indicate the presence of EC and heavy metals within the seedlings [37,48], whereas higher GI values show safe removal of these physicochemical parameters from the seedlings [37,48]. The values were calculated using the following equation [37]:

$$Germination\ index = \frac{(\text{Germinated seeds} \times \text{Average root length})\text{for sample}}{(\text{Germinated seeds} \times \text{Average root length})\text{for control}} \div 100 \quad (1)$$

2.3.3. Phytotoxicity Indices (PI)

The most sensitive parameter to assess the biotreatment efficiency is phytotoxicity index. A positive value indicates lower toxicity [49], whereas its negative value means the seedlings are contaminant-free and non-lethal [50]. Values of PI indices cause toxicity in four categories: low, medium, high, and very high [49,51]. These values range from 0 to

−0.25, −0.25 to −0.5, −0.5 to −0.75 and −0.75 to −1, respectively [49,51]. The values were calculated using the following equation [52]:

$$Phytotoxicity\ index = 1 - \frac{\text{Root length of sample}}{\text{Root length of control}} \quad (2)$$

2.4. Statistical Analysis

One-way ANOVA was used to analyse the data statistically using GraphPad Prism® 2020. The comparison of the obtained data was carried out by *t*-test using Welch's correction (with two-tailed *p*-values) [53]. The differences of comparisons were significant when *p*-values were <0.05. Associated statistical calculations were carried out using Sidak's multiple comparison testing. These comparisons were assessed between control (distilled water–DW) along with each treatment at varying concentration levels (Tap Water–TW as control, Raw wastewater 25% (R25), Treated wastewater 25% (T25), Raw wastewater 50% (R50), Treated wastewater 50% (T50), Raw wastewater 75% (R75), Treated wastewater 75% (T75), Raw wastewater 100% (R100) and Treated wastewater 100% (T100)). Similarly, the comparisons were made between raw wastewaters within each concentration level and the corresponding treated wastewaters (e.g., Raw wastewater 25% (R25) and Treated wastewater 25% (T25)).

3. Results and Discussions

3.1. Prediction of Quality Parameters

The results of the Kruskal–Wallis test (Figure 1) shows which wastewater quality indicator parameters discriminate between Treated and Untreated hospital wastewater categories, highlighting adjusted *p*-values. All the parameters were discriminating significantly (TSS padj = 1.3655×10^{-7}, BOD padj = 1.3655×10^{-7}, Turbidity padj = 1.3655×10^{-7}, Colour padj = 1.3655×10^{-7}, BOD_COD padj = 9.3264×10^{-6}, COD padj = 6.7712×10^{-5}, pH padj = 0.00014423, EC padj = 0.0016633, Salinity padj = 0.0049779, Chromium padj = 0.013416, Nickel padj = 0.018595, Lead padj = 0.040042, TDS padj = 0.049674). The values for heavy metals in treated wastewater were reduced to zero. Therefore, the upregulation for these abundance values in Figure 1 relates to *p*-values of comparison of the untreated and treated data. Next, using these parameters we applied a Random Forest classifier. A confusion matrix aids in evaluating the correctness of our classification model. The confusion matrix here showed that we have obtained nearly perfect results, i.e., where those that are labelled as "Treated" are correctly classified as "Treated" and vice versa (Figure 2). Turbidity, BOD and colour showed the highest mean decrease accuracy, with values close to 16, while TSS had a value of 15 (Figure 3a). The mean decrease in Gini index was highest for turbidity (3), colour (2.9), BOD (2.8) and TSS (2.5) parameters (Figure 3b).

In light of the standard methods for wastewater quality analysis, five wastewater quality predicting parameters have been used in machine learning, i.e., BOD [14], COD [41,54–56], TSS [41,52,56], TDS [41] and turbidity [57,58]. The present RF classification successfully used artificial intelligence to predict twelve quality parameters between wastewater that was treated with the bacterial consortium versus untreated wastewater. Up to an 88% accuracy has been obtained in predictive analysis for industrial wastewater in the past [59]. The confusion matrix of our RF classifier showed nearly perfect results based on decision trees, highlighting the clear differentiation between treated and untreated wastewater. The results also suggested that turbidity was the most influential parameter to predict the effective biotreatment. Our results agree well with previous ML analyses using turbidity as a quality parameter for drinking water [57] and pharmaceutical wastewater treatment [58]. Turbidity represents a low-cost and simple measure of water quality. Thus, overall, this low-cost consortium method for wastewater treatment could easily be implemented at a small-scale on individual farms using turbidity as a measure to assess treatment efficiency prior to irrigation. Furthermore, turbidity in wastewater (due to the presence of contaminants) directly harms plant growth [42,60]. These plants directly impact animal and human life through consumption [60]. Hence, the phytotoxicity assessment on crop plants was carried out further to see the impact of treatment efficiency as predicted by our RF classifier.

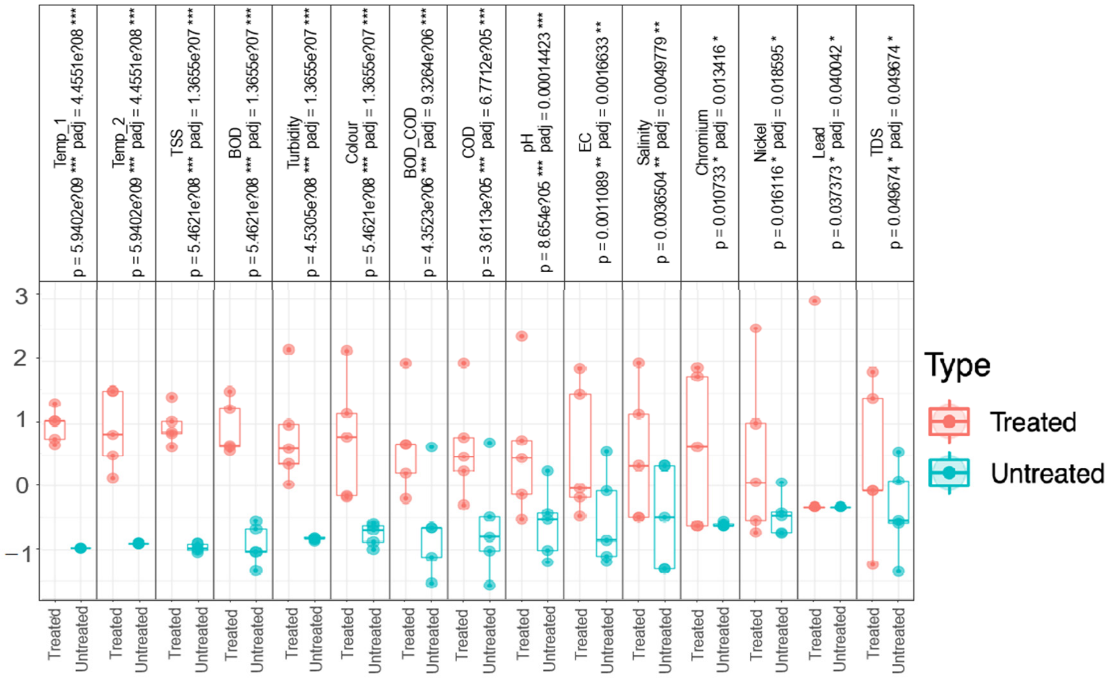

Figure 1. Box-plot graph showing the results of a Kruskal–Wallis statistical test to determine which of the water quality parameters (TSS, BOD, Turbidity, Colour, BOD_COD, COD, pH, EC, Salinity, Chromium, Nickel, Lead, TDS) were discriminating between the Untreated wastewater (in blue) and the Treated biotreated hospital wastewater (in red) on the *x*-axis and the autoscaled abundance on the *y*-axis. The statistical significance values are denoted within the box plots with symbol * and are indicated by $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****.

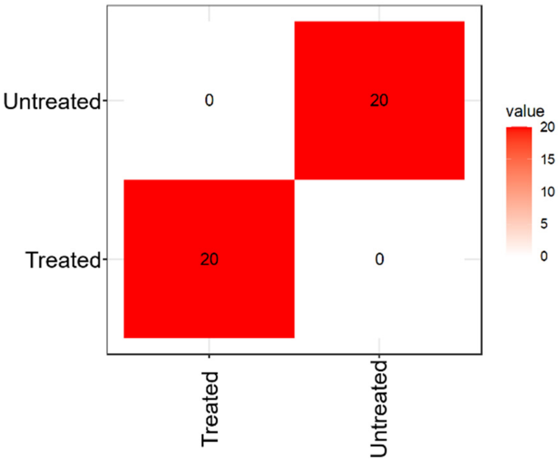

Figure 2. Confusion matrix for the water quality parameters in a Random Forest classifier between Treated and Untreated biotreated hospital wastewater.

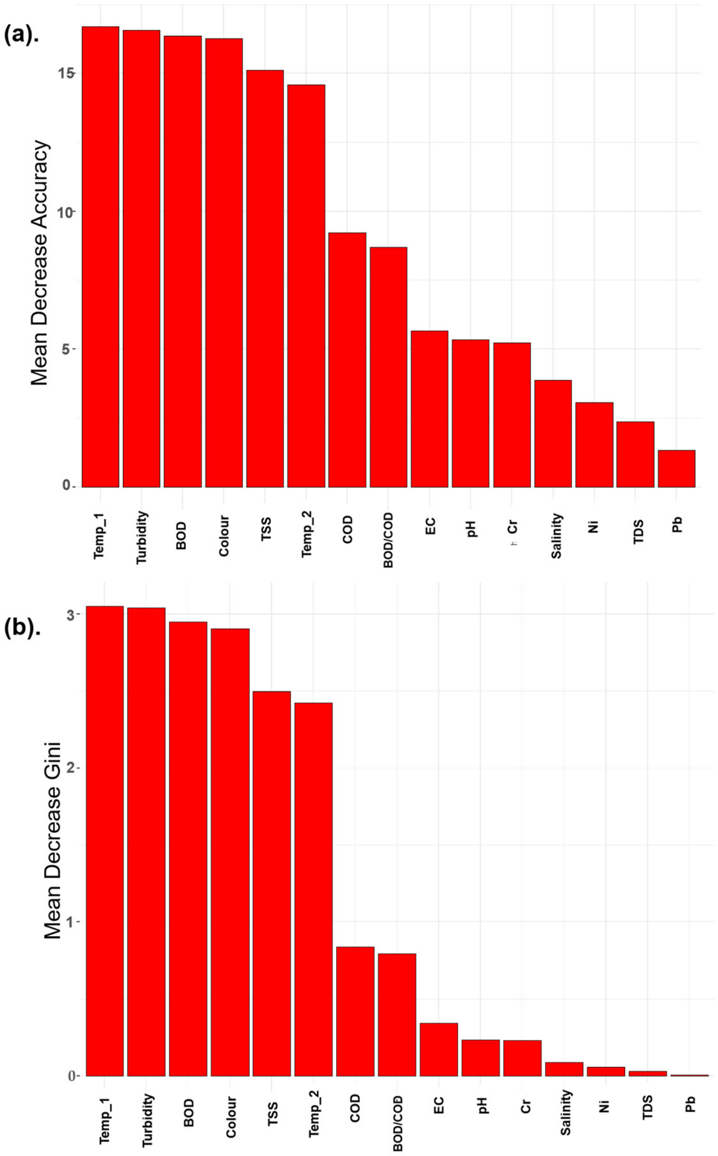

Figure 3. Bar-plots showing (**a**) the mean decrease in accuracy of water quality parameters sorted in terms of their importance; (**b**) the mean decrease in Gini of water quality parameters sorted in terms of their importance.

3.2. Phytotoxicity Assessment of the Re-Use of Treated Hospital Wastewater for Crop Irrigation

The lengths and biomass of seedling roots and shoots were measured on a daily basis and compared to controls after seedling germination (Figure 4a–e).

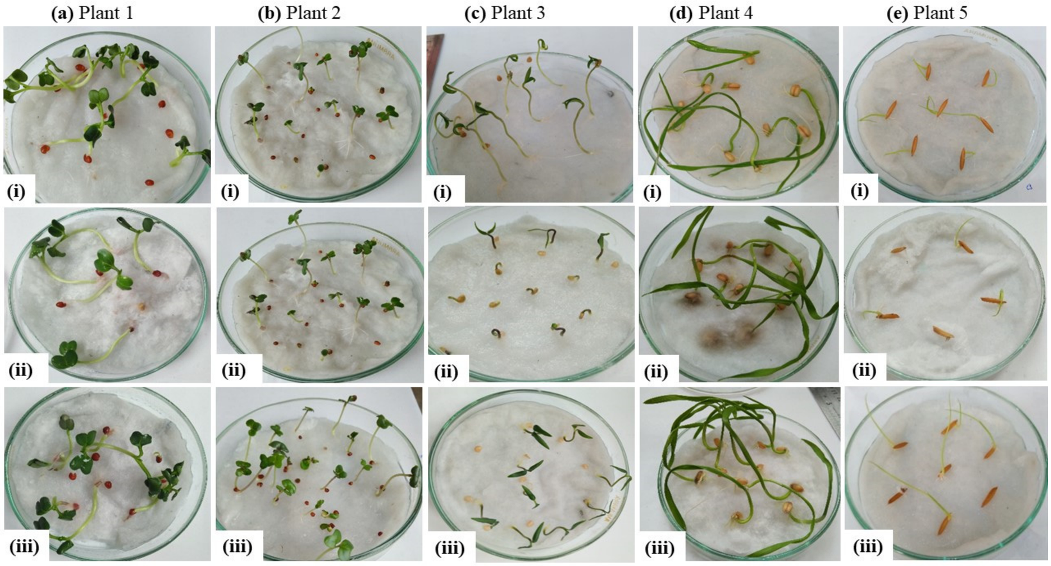

Figure 4. (**a**) Plant 1: *R. sativus* (Radish) seedlings grown in 100% concentration of (**i**) control (distilled water), (**ii**) untreated and (**iii**) treated hospital wastewater after two weeks; (**b**) Plant 2: *B. oleracea* L. (Cauliflower) seedlings grown in 100% concentration of (**i**) control (distilled water), (**ii**) untreated and (**iii**) treated hospital wastewater after two weeks; (**c**) Plant 3: *C. annuum* L. (Hot pepper) seedlings grown in 100% concentration (**i**) control (distilled water), (**ii**) untreated and (**iii**) treated hospital wastewater after two weeks; (**d**) Plant 4: *T. aestivum* L. (Wheat) seedlings grown in 100% concentration (**i**) control (distilled water), (**ii**) untreated and (**iii**) treated hospital wastewater after two weeks; (**e**) Plant 5: *O. sativa* L. (Rice) seedlings grown in 100% concentration (**i**) control (distilled water), (**ii**) untreated and (**iii**) treated hospital wastewater after two weeks.

3.2.1. Radish Crop

The seedling lengths and weights were compared between irrigation from untreated and treated hospital wastewater concentrations (25, 50, 75 and 100%) tested separately. It was found that the seedling length for both shoots and roots were elongated in treated hospital wastewaters at all concentrations (shoots: 3.1–4.7 cm; roots: 2.9–4.4 cm). Similarly, the seedling weights for both shoot and root (fresh and dry) were greater when grown in treated hospital wastewater concentrations (shoot fresh weight: 0.055–0.158 g; shoot dry weight: 0.02–0.089 g; root fresh weight: 0.011–0.043 g; root dry weight: 0.004–0.021 g) (Table S1). Increased lengths and biomass values with treated wastewater (comparable or greater to the control conditions) indicates that the radishes were not impacted by the treated wastewater but were impacted by the untreated wastewater.

In the present work, the radish seeds grown in treated hospital wastewaters (25, 50, 75 and 100%) showed an increase in GI values from 75–86% to 102–108%. (Figure 5a). The germination indices for all crop seedlings grown in four untreated hospital wastewater concentrations showed low values that reflect the presence of six organic toxic pollutants (caffeine, diazepam, naproxen, octadecene, phenol and salicylic acid) which were identified in our previous study [38]. These values also reflect the presence of three heavy metals in raw hospital wastewater (Cr, Pb and Ni) [38]. The reduced GI values hinder the relative germination of seedlings and root growth, accordingly [61]. Higher GI values for seeds grown in treated hospital wastewaters indicates the reduction or removal of heavy metals. High GI values with treated wastewater indicates that the radishes are not impacted by the treated wastewater in the same way as the untreated. This shows that the treated wastewater may be safe for irrigating radishes. Likewise, the decrease in EC and turbidity in hospital wastewater after biotreatment is an indication of treatment efficiency [37]. The present GI values for seeds grown in treated hospital wastewater endorse decreased EC and turbidity value, the same as those predicted by the RF classifier, and hence proves the treatment efficiency.

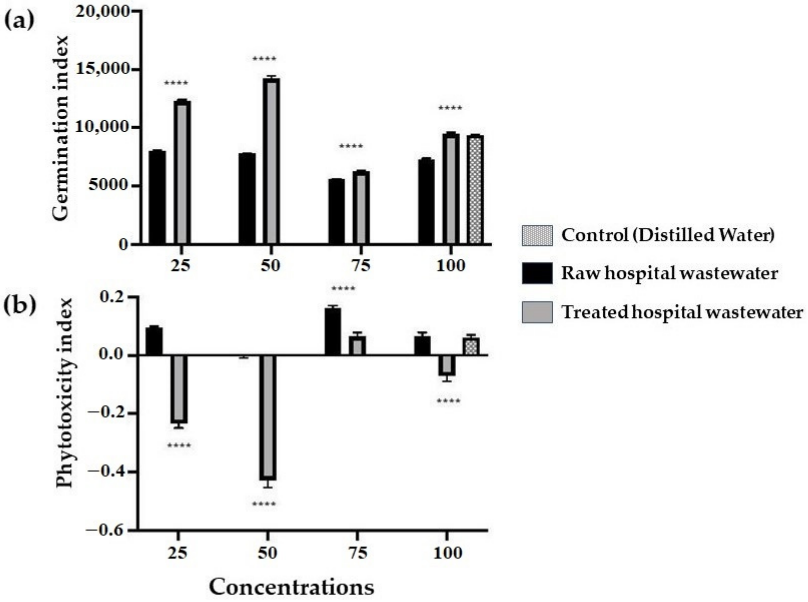

Figure 5. (**a**) Germination indices of Radish seeds grown in untreated and treated hospital wastewaters; (**b**) Phytotoxicity indices of Radish seeds grown in untreated and treated hospital wastewaters. Significance (Welch's test) between treated and untreated hospital wastewater is indicated by $p < 0.0001$ ****.

Previously, the phytotoxicity assessment in plants has been carried out using one growth predicting factor, i.e., GI [62]. The present study is significantly different as it chose two growth predicting factors, GI and PI. PI is a very sensitive indicator for assessing the toxicity level of heavy metals and pharmaceutic compounds among plants [50]. It shows to what extent the pharmaceutic pollutants, chemical compounds or heavy metals have either stunted or harmed plant growth. The PI values for radish seeds when grown in treated hospital wastewater were proven significant statistically in comparison to those grown in untreated hospital wastewater. The PI values of seeds grown in untreated hospital wastewater concentrations (25, 50, 75 and 100%) were positive (0.1, 0, 0.16, 0.06, respectively). This indicates a medium to large lethal effect on the growth of seedlings (Figure 5b). The positive values of PI for seeds grown in specific untreated wastewater concentrations may be ascribed to the occurrence of nitrogen (N), phosphorous (p) and potassium (K) [52]. In contrast, the seeds grown in three treated hospital wastewaters (25, 50 and 100%) exhibited negative values of PI (−0.23, −0.42 and −0.06). This indicates strong stimulatory effect on seedling growth. One treated hospital wastewater concentration (75%) showed positive value of PI (0.06) (Figure 5b). However, this PI value was reduced from 0.16 to 0.06, which shows a reduction in toxicity level. Previously, reduction in positive values of PI for the barley plant has been attributed to diluted concentrations of treated wastewater, which indicated removal of phenol and heavy metals [52]. In our work, crop irrigation with treated hospital wastewater leads to the reduction in PI value, which may also be due to various reasons: heavy metal reduction [63,64], reduction in phenols and supplementary organic complexes [65] and stress tolerances [66]. Radish roots are more sensitive towards the uptake of heavy metals and contaminants from the soil [67]; hence, the crop plant has more proximity towards inducing phytotoxicity. Therefore, lower (negative) PI values with treated wastewater in our results indicate that the radishes are not harmed by the treated wastewater in the same way as the untreated. Based on these findings, we conclude that the treated wastewater may be safe for reuse to irrigate radish crops.

3.2.2. Cauliflower Crop

The lengths and weights of cauliflower seedlings were compared statistically when grown in the untreated and treated hospital wastewater concentrations (25, 50, 75 and 100%). The statistical measurements also specified that the mean lengths and weights were significant. The lengths of seedlings (shoots and roots) were found to be longer in treated hospital wastewater concentrations (shoots: 3.2–3.9 cm; roots: 3.4–4.1 cm). Similarly, the seedling weights for both shoot and root (fresh and dry) were greater when grown in treated hospital wastewater concentrations (shoot fresh weight: 0.04–0.029 g; shoot dry weight: 0.006–0.031 g; root fresh weight: 0.006–0.029 g; root dry weight: 0.002–0.031 g) (Table S2). The cauliflower seeds grown in treated hospital wastewater (25, 50, 75 and 100%) showed a rise in GI values from 55–80% to 62–142%. (Figure 6a). The values for PI in seeds grown in untreated and treated hospital wastewater were significant statistically in comparison. The PI values for seeds grown in untreated hospital wastewaters (25, 50, 75 and 100%) were positive (0.09, 0.16, 0.14, 0.06, respectively). This indicates a medium lethal impact on the growth of seedlings (Figure 6b). Conversely, the seeds grown in three treated wastewater concentrations (25, 50, 75 and 100%) exhibited negative values of PI (−0.28, −0.13, −0.16, −0.06), which indicates a stimulatory effect on seedling growth. Cauliflower crop has shown low sensitivity towards accessing environmental pollution in the past [68]. Additionally, chromium has been proven to be the most harmful heavy metal for the growth of cauliflower plants [69]. In the present study, the reduction of chromium in treated hospital wastewater seems to contribute to the higher seedling length and biomass and higher GI values. Similarly, lower PI values with treated wastewater indicate that cauliflowers are not impacted by the treated wastewater in the same way as the untreated. This highlights the treatment efficiency and indicates that wastewater may be safe for irrigating cauliflowers.

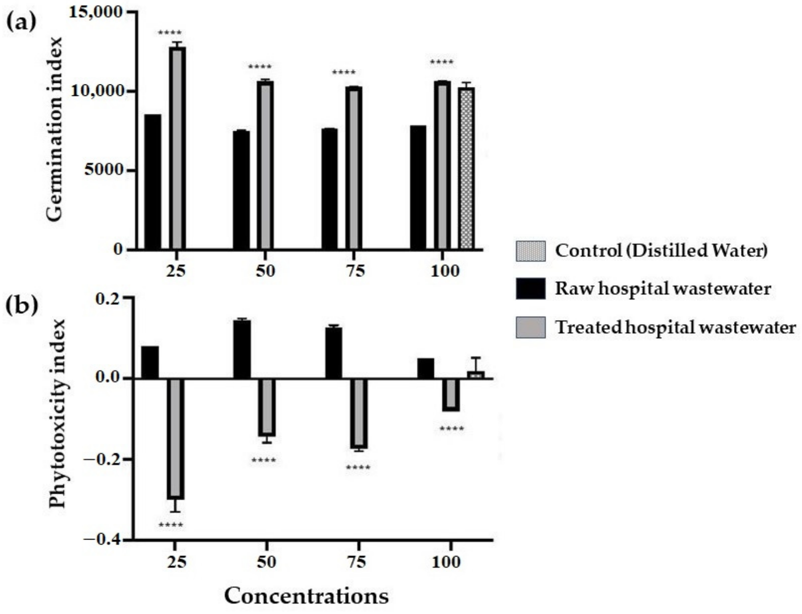

Figure 6. (**a**) Germination indices of Cauliflower seeds grown in untreated and treated hospital wastewaters; (**b**) Phytotoxicity indices of Cauliflower seeds grown in untreated and treated hospital wastewaters. Significance (Welch’s test) between treated and untreated hospital wastewater is indicated by $p < 0.0001$ ****.

3.2.3. Hot Pepper Crop

Hot pepper plants have been investigated in the past for phytotoxicity assessment of reusing treated industrial wastewaters, resulting in increased biomass [70–73]. The plant is more sensitive to lead concentrations than any other plant [74]. With reduced concentrations of lead in the wastewater, root and shoot lengths and the biomass of hot pepper plants were reported to be increased [74]. The present results endorse the previously reported findings. In the present study, the lengths of seedling (shoots and roots) were longer in treated wastewaters (shoots: 1.8–2.3 cm; roots: 2–5.2 cm). Additionally, the seedling weights for both shoot and root (fresh and dry) increased when grown in treated hospital wastewater concentrations (shoot fresh weight: 0.02–0.019 g; shoot dry weight: 0.006–0.05 g; root fresh weight: 0.011–0.019 g; root dry weight: 0.003–0.008 g) (Table S3). The hot pepper seeds grown in treated hospital wastewater (25, 50, 75 and 100%) showed an increase in GI values from 72–90% to 63–109%. (Figure 7a). The PI values for seeds grown in untreated wastewaters (25, 50, 75 and 100%) were positive (0.27, 0.15, 0.12 and 0.09, respectively). This indicates a medium lethal impact on the growth of seedling (Figure 7b). Conversely, the seeds grown in two treated hospital wastewater concentrations (25 and 100%) showed negative PI values (−0.09 and −0.58). This also indicates a stimulatory effect on seedling growth as the PI values were lower than the control. Another two treated hospital wastewater concentration (50 and 75%) showed positive values of PI (0.21 and 0.39) (Figure 7b). In light of the available literature, heavy metal reduction [63,64], reduction of phenols and supplementary organic compounds [65] may be the possible reasons that contributed to the positivity in phytotoxicity values.

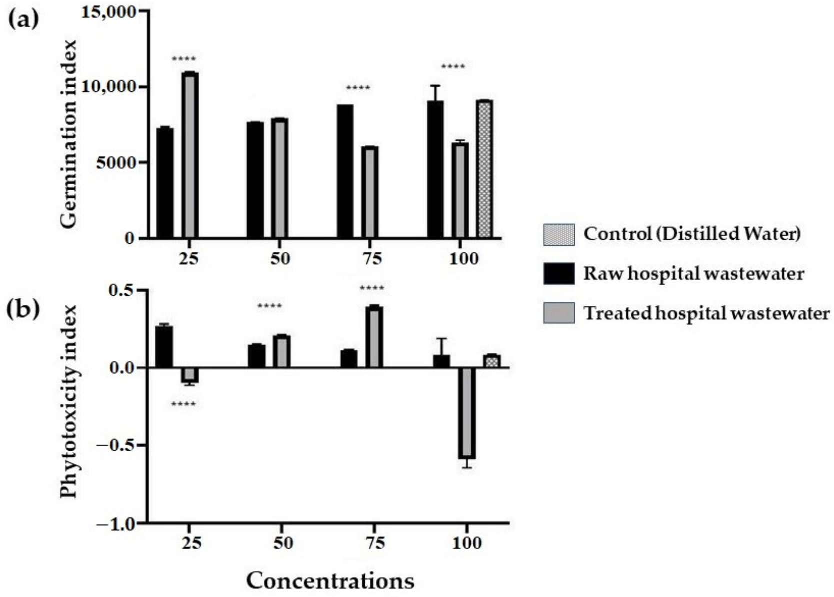

Figure 7. (**a**) Germination indices of Hot pepper seeds grown in untreated and treated hospital wastewaters; (**b**) Phytotoxicity indices of Hot pepper seeds grown in untreated and treated hospital wastewaters. Significance (Welch's test) between treated and untreated hospital wastewater is indicated by $p < 0.0001$ ****.

3.2.4. Wheat Crop

Wheat showed increased seedling length for both shoots and roots in treated hospital wastewater concentrations (shoots: 7.1–9.9 cm; roots: 8.6–10.2 cm). Additionally, the seedling weights for both shoot and root (fresh and dry) were raised when grown in treated hospital wastewaters (shoot fresh weight: 0.077–0.122 g; shoot dry weight: 0.019–0.053 g; root fresh weight: 0.068–0.089 g; root dry weight: 0.019–0.032 g) (Table S4). The wheat seeds grown in treated hospital wastewaters (25, 50, 75 and 100%) showed an increase in GI values from 79–106% to 106–126%. (Figure 8a). The PI values for wheat seeds grown in untreated hospital wastewaters (25, 50, 75 and 100%) were 0.10, 0.10, −0.19, 0.21, respectively, which indicates a medium to low toxic effect on seedling growth (Figure 8b). Conversely, the seeds grown in three treated hospital wastewater concentrations (25, 50 and 100%) showed negative PI values (−0.26, −0.10 and −0.12, respectively). Another treated hospital wastewater concentration (75%) showed a positive value of PI (0.06) (Figure 8b). Previously, wheat has showed positive PI values in phenol-containing industrial wastewaters [75]. Our work analyses a diverse range of pharmaceutical pollutants (caffeine, diazepam, naproxen, octadecene, phenol and salicylic acid) and heavy metals (Ni, Cr, Pb) which are present in untreated hospital wastewater. The positive growth of wheat plants after irrigation with treated hospital wastewater indicates treatment efficiency.

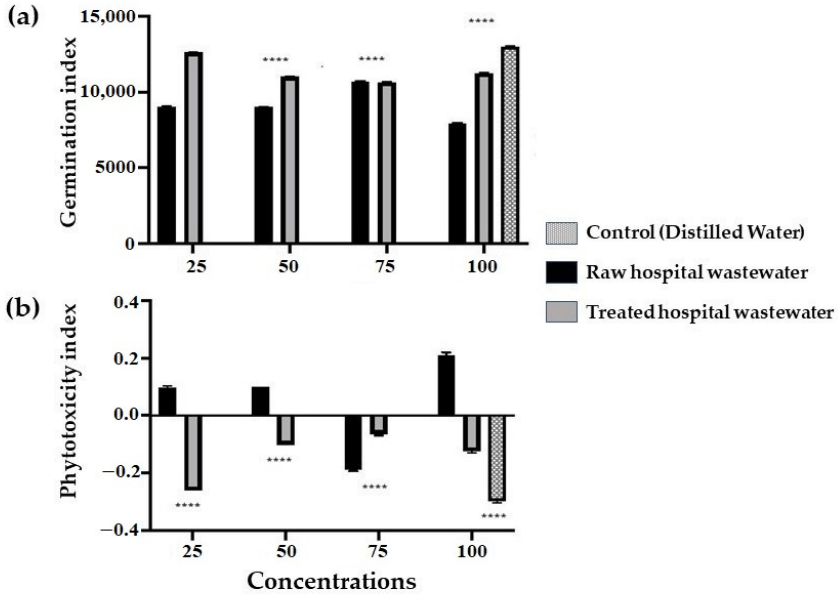

Figure 8. (**a**) Germination indices of Wheat seeds grown in untreated and treated hospital wastewaters; (**b**) Phytotoxicity indices of Wheat seeds grown in untreated and treated hospital wastewaters. Significance (Welch's test) between treated and untreated hospital wastewater is indicated by $p < 0.0001$ ****.

3.2.5. Rice Crop

The results showed that the lengths of seedlings (shoots and roots) were increased in treated hospital wastewaters (shoots: 3.1–5.1 cm; roots: 3.4–10.2 cm). Additionally, the seedling biomass for both shoot and root (fresh and dry) increased when grown in treated hospital wastewater concentrations (shoot fresh weight: 0.008–0.015 g; shoot dry weight: 0.001–0.006 g; root fresh weight: 0.052–0.05 g; root dry weight: 0.023–0.032 g) (Table S5). The rice seeds grown in treated wastewaters (25, 50, 75 and 100%) showed an increase in GI values from 36–90% to 90–144%. (Figure 9a). The PI values for seeds grown in untreated hospital wastewaters (25, 50, 75 and 100%) were positive (0.60, 0.27, 0.42, 0.42), which indicates a medium to high lethal effect on the growth of seedlings (Figure 9b). The seeds grown in three treated hospital wastewater concentrations (25, 50 and 100%) showed PI values of 0.07, 0.35 and 0.59, which were lower than the values of untreated hospital wastewater concentrations. Another treated hospital wastewater concentration (75%) showed negative values of PI (−0.23) (Figure 9b). Recently, researchers found rice to be a sensitive crop plant for reusing treated wastewaters [76,77]. They observed inhibitory effects on seed germination and plant growth. Our work agrees with the existing work, with stimulatory growth of rice seedlings in the presence of treated wastewater where there is no turbidity.

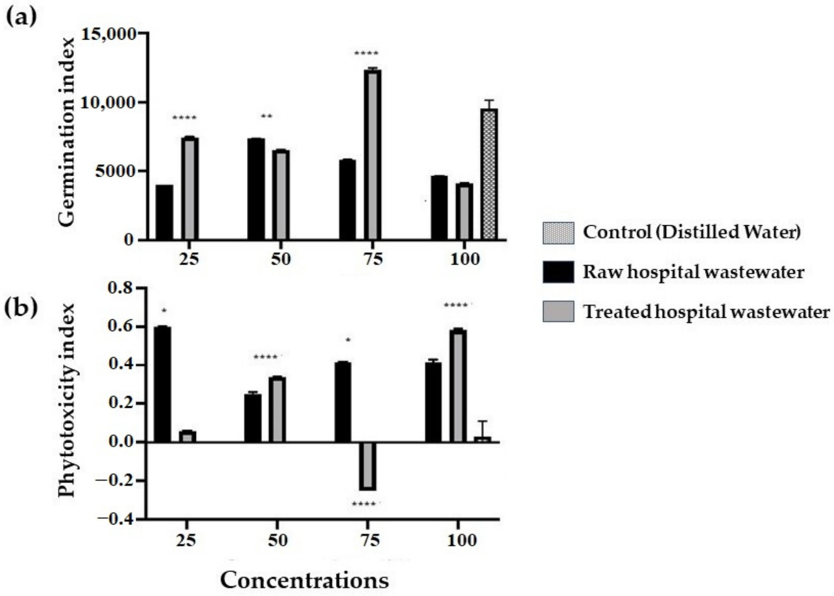

Figure 9. (**a**) Germination indices of Rice seeds grown in untreated and treated hospital wastewaters; (**b**) Phytotoxicity indices of Rice seeds grown in untreated and treated hospital wastewaters. Significance (Welch's test) between treated and untreated hospital wastewater is indicated by $p < 0.05$ *, $p < 0.01$ **, $p < 0.0001$ ****.

Many researchers have stated that higher phytotoxicity indices in plants grown in untreated industrial wastewaters indicate toxicity induction in plants due to polluted water [32,78–81]. The present study has shown a significant reduction in phytotoxicity for these five crop plants grown in consortium-treated wastewater, which in turn confirms the efficiency of biotreatment. The comparatively increased germination and reduced phytotoxic analysis of the five crops for the treated wastewater is as follows: Cauliflower > Radish > Wheat > Rice > Hot pepper. The capacity to accumulate heavy metals is different in various types of plants, both on land as well as in aquatic plants [82,83]. Leafy and root vegetable crops absorb these metals more frequently than other vegetables [82]. Cauliflower crop showed the highest adaptability towards treated wastewater; although it has sensitivity towards chromium [68,69], because chromium was absent in our treated wastewater, its GI was highest and PI values were lowest. Another contributing factor is the physiognomy of cauliflower as a flowering vegetable. Radish has more proximity towards inducing phytotoxicity [67], but as the treated wastewater neither had heavy metals nor pharmaceutic components beyond the acceptable NEQS range, it showed high GI values and low PI values. Wheat and rice showed improved growth and heavy metal tolerance with the application of reused wastewater [75,84,85]. Our work agrees with the previous study because our GI values were high and PI values were low, which are indicators of stimulatory plant growth. Hot pepper plants are vulnerable/sensitive to stress or pollutants [70–74]. A similar observation came to light in our work, where the plant showed sensitivity towards PI values even after the treatment. Because the other crops were fine, we can say that irrigation with treated wastewater in fact had a stimulatory effect on Cauliflower, Radish, Wheat and Rice. The phytotoxicity results highlight two key points: that the consortium-treated wastewater is safe for reuse for crop irrigation and that hot pepper plants are the most susceptible to growth under these conditions.

Our work shows the feasibility of the safe re-use of treated hospital wastewater for crop irrigation in water-stressed areas. Future work should include the following: a detailed assessment of any potential spread of antimicrobial resistance, translation of growth experiments to agricultural field sites, and research into how this consortium-treatment method could be implemented in practice. The environmental sustainability of the consortium treatment may be assessed via. comparing it with other methods such as electrochemical and advanced oxidation for advanced hospital wastewater treatment to remove the toxic pollutants and to see which of the method is more sustainable/low-cost or low-tech. Furthermore, a life-cycle analysis could be performed [86–89].

4. Conclusions

The present study supports the successful prediction of quality parameters in bacterial consortium-treated hospital wastewater using a Random Forest classifier. The confusion matrix of the RF classifier showed nearly perfect results based on decision trees, highlighting the clear differentiation between treated and untreated wastewater. The results also suggested that turbidity was the most influential parameter to predict the most effective biotreatment. Turbidity represents a low-cost and simple measure of water quality. Thus, overall, this low-cost consortium method for wastewater treatment could easily be implemented at a small-scale on individual farms using turbidity as a measure to assess treatment efficiency prior to irrigation.

Turbidity indicates the presence of heavy metals and pharmaceutic pollutants in the wastewater which are potentially harmful for plant growth. Unstable plant growth directly impacts human life. Therefore, the phytotoxicity assessment on five widely grown crop plants was carried out to see the impact. The crop plants showed increased seedling lengths and biomass, high GI values and low PI values when grown in the treated wastewater. The PI values were either similar to the control or lower, which indeed shows a stimulatory effect, and therefore, treated wastewater with this bacterium consortium may be a safe option for irrigation of these crop plants. The comparative analysis of the five crops for germination and phytotoxicity is as follows: Cauliflower > Radish > Wheat > Rice > Hot pepper. Our work recommends the reuse of quality-predicted biotreated hospital wastewater; however, there is a further need to analyse the products of these crop plants to ensure their safe consumption. Moreover, environmentally sustainable advanced hospital wastewater treatment would be a gateway to achieve Sustainable Development Goals 6, 14 and 15.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14010116/s1, Table S1: Comparison of untreated (R25, R50, R75, R100) and treated (T25, T50, T75, T100) hospital wastewater concentrations on seedling growth of R. sativus (Radish) var. Radish Minto Early (±Standard Deviation in brackets); Table S2: Comparison of untreated (R25, R50, R75, R100) and treated (T25, T50, T75, T100) hospital wastewater concentrations on seedling growth of B. oleracea (Cauliflower) var. Cauliflower 2801 (±Standard Deviation in brackets); Table S3: Comparison of untreated (R25, R50, R75, R100) and treated (T25, T50, T75, T100) hospital wastewater concentrations on seedling growth of C. annuum (Hot pepper) var. Seminis Hybrid Hot pepper SKY LINE 3 (±Standard Deviation in brackets); Table S4: Comparison of untreated (R25, R50, R75, R100) and treated (T25, T50, T75, T100) hospital wastewater concentrations on seedling growth of T. aestivum (Wheat) var. FSD-2008 (±Standard Deviation in brackets); Table S5: Comparison of untreated (R25, R50, R75, R100) and treated (T25, T50, T75, T100) hospital wastewater concentrations on seedling growth of O. sativa (Rice) var. PS-2 (PK-112) (±Standard Deviation in brackets).

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