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Enhanced Degradation of Ciprofloxacin in Floating Treatment Wetlands Augmented with Bacterial Cells Immobilized on Iron Oxide Nanoparticles

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Abstract: Antibiotic contamination of water is an emerging global issue with severe implications for both public health and the environment. Ciprofloxacin (CIP) is a synthetic fluoroquinolone antibiotic, which is broadly used in human and veterinary medicines around the world to treat various bacterial infections. The presence of CIP in the aquatic environment poses serious health problems to human beings and other living entities. Floating treatment wetland (FTW) is a low-cost and eco-friendly wastewater remediation technology. In the current study, the *Canna indica*. (Indian shot) was vegetated in a floatable mat to develop FTWs. A consortium of three bacterial strains, *Acinetobacter lwoffii* ACRH76, *Bacillus pumulis* C2A1, and *Acinetobacter* sp. HN3, was immobilized on iron oxide nanoparticles (Fe_3O_4-NPs) and augmented in the FTWs for the remediation of CIP-contaminated (100 mg/L) water. The augmentation of bacteria (immobilized or free) in the FTWs significantly enhanced the removal of CIP from water. The maximum reduction in CIP (98%), chemical oxygen demand (COD; 90%), biochemical oxygen demand (BOD; 93%) and total organic carbon (TOC; 95%) was observed in FTWs that had Fe_3O_4-NP supported bacteria. This study reveals that FTWs have a great potential to remove the CIP from contaminated water, albeit its CIP removal efficiency was substantially enhanced by augmentation with Fe_3O_4-NPs supported bacteria.

Keywords: floating wetlands; contamination; bioaugmentation; nanoparticles; remediation

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1. Introduction

Pharmaceuticals have received growing attention in the last two decades due to their adverse environmental effects. Every year, thousands of tons of pharmaceuticals are used, not only to treat human and animal diseases, but in agriculture and aquaculture too, thereby posing a major threat to human health and the environment [1,2]. In particular, antibiotics have raised environmental concerns as their presence, even in small quantities, can be harmful and toxic to fauna and flora [2–5]. Ciprofloxacin (CIP) is a synthetic fluoroquinolone antibiotic that, due to its high bioavailability and low side effects, is widely used in human and veterinary medicine around the world to treat a wide range of bacterial infections [6,7]. Since humans and animals can only partially metabolize CIP, a major portion of it is excreted in urine (45–60%) and feces (15–20%) [8,9]. Hence, CIP is considered one of the top ten priority pharmaceuticals commonly found in aquatic environments [10]. Ciprofloxacin has been found in sewage and surface water [11] and in effluents from the pharmaceutical industry [12], hospitals and livestock [13]. According to statistics presented by health communities, the amount of CIP contamination discerned in surface and underground water were within the concentration range of 1< μg L^{-1}. However, the detected amount of CIP in the wastewater of hospitals and drug manufacturers is much

higher—up to 150 μg L^{-1} and 50 mg L^{-1}, respectively—which is extremely harmful to the health of human beings [14,15]. Concentration (ng/L) of ciprofloxacin detected in wastewater of different countries were 6900 Brisbane, Australia; Europe 3353; Valencia, Spain 3850, USA 320; UAE 1028; Mexico 2570; Kenya 1300, 28,000,000–31,000,000; and Pakistan 332.154.

Even at low concentrations, CIP in water encourages the production of antibiotic-resistant bacteria, posing a serious threat to human and animal health [16,17]. On account of several ecotoxicological and public health issues related to the presence of antibiotics in water, emerging treatment technologies are imperative to minimize and resolve the problem of environmental pollution. Until now, a variety of physical, chemical and biological approaches have been applied to remove these pollutants from water [18–21]. However, these approaches are not feasible due to their low efficiency, high investment cost, lack of guidance and problems associated with their maintenance and operation [20,22,23].

Floating treatment wetlands (FTWs) have evolved to become an effective approach, favored over traditional phytoremediation techniques for their unique abilities to remediate wastewater [24]. In FTWs, plants of aquatic or terrestrial origin are cultivated on self-buoyant mats that are fixed in wastewater reservoirs [25]. These mats allow plants to grow their roots deep into the contaminated water. The roots provide the large surface area that is required for the growth of microbes, resulting in the formation of biofilms. As such, these biofilms remove, degrade and detoxify organic contaminants present in the wastewater [25,26]. Plants provide nutrients and a safe haven for inoculated bacteria and the bacteria in turn promote plant health by reducing biotic and abiotic stresses by releasing phytohormones and nutrients, as well as assisting in the breakdown of pollutants [27–30].

Immobilized microbial cells have been widely used over recent years for a variety of biotechnological applications, including antibiotic synthesis, biodegradation and xenobiotic biotransformation in wastewater treatment plants [31,32]. The immobilized microbes have a number of benefits over traditional suspension systems such as high productivity, increased metabolic activity, and potent resistance to harmful substances [32,33]. These microbial cells can be retained on the surface of substrates with high surface area and an abundance of functional moieties on their surface such as iron oxide nanoparticles, clay minerals etc. Nanomaterials are innovative and fascinating matrices for microbial retention due to their unique physico-chemical properties such as large specific surface area, mass transfer resistance, high mechanical strength, and high absorption loading [34]. Ferric oxide nanoparticles such as magnetite (Fe_3O_4-NPs) have the additional benefit of being easily separated from the continuous phase by applying an external magnetic field. As a result of their ability to immobilize microbial cells on their surface, Fe_3O_4-NPs are more viable [35].

Canna indica (Indian shot) plant is a coarse perennial herb with heights ranging from 90 cm to 3 m. It has large leaves that resemble, but are not as large as, those of the banana plant. The flowers are red and can be found alone or in pairs. The plant can tolerate environments with high salinity and high concentrations of Cu and Cd^{2+} up to 5 mg/L. It is used in wetland systems to remove a range of contaminants from water and wastewater.

The potential of FTWs augmented with bacteria immobilized on NPs for the remediation of water contaminated with CIP has not been previously explored. The purpose of the current study was to assess the capability of FTWs vegetated with *C. indica* and augmented with a bacterial consortium immobilized on Fe_3O_4-NPs for the remediation of CIP-contaminated water. To the best of our knowledge this is the first study in which remediation of CIP-contaminated water was assessed with the FTWs having immobilize bacteria on nanoparticles. The water quality improvement was examined by analyzing treated water for important quality parameters such as pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total organic carbon (TOC).

2. Materials and Methods

2.1. Chemicals and Media

Ciprofloxacin tablets (250 mg) were purchased from a local pharmacy (Sami Pharmaceuticals, Private Limited, Karachi, Pakistan). All the other chemicals used in experiments were of analytical grade with desired purity, which were purchased from Merck, Germany, and Sigma-Aldrich, USA. A polystyrene sheet was purchased from Diamond Jumbolon Company and plastic tanks were purchased from the local market in Faisalabad, Pakistan.

2.2. Synthesis of Iron Oxide Nanoparticles

2.2.1. Preparation of Aqueous Extract

The leaves of *Azadirachta indica* (commonly known as neem) were picked and properly cleaned with distilled water several times to remove dust and were sun-dried for two days to remove any remaining moisture. The dried leaves were ground after being cut into little pieces and 10 g of fine powder was dissolved in 100 mL of deionized water. The solution was then heated for 1 h. The mixture was allowed to cool at room temperature before being vacuum filtered using Beckman filter paper to yield the neem leaves extract. The extract was kept at 4 °C until it was needed for synthesis of NPs [36].

2.2.2. Co-Precipitation Method for Fe Oxide NPs

Iron oxide nanoparticles were synthesized using the co-precipitation method. Ferrous chloride and ferric chloride were dissolved in distilled water in a 2:1 molar ratio under a nitrogen atmosphere. The mixture was heated to 80 °C for 10 min with gentle stirring. Thereafter, 5 mL of the aqueous leaf extract was gradually added to the reaction mixture followed by 20 mL of 25% ammonium hydroxide applied drop by drop using a burette while vigorously stirring for 30 min. The formation of Fe_3O_4-NPs was indicated by the quick black color appearance. The solution was put into a beaker and the supernatant was removed using magnetic decantation after 30 min. The vivid black Fe_3O_4-NPs were then rinsed with 15 mL deionized water and centrifuged for 10 min at 5000 rpm. After centrifugation, the product was washed again with 10 mL deionized water and methanol. The supernatant was discarded in each washing step. Ten mL deionized water was added to the pellet before it was transferred to a vial. The black powder was collected and dried for 24 h at 65 °C before being subjected to further characterization [36–38].

2.3. Characterization of Fe_3O_4-NPs via Atomic Force Microscope (AFM)

The atomic force microscope (AFM) is a valuable method for surface examination because it provides a high-resolution material topology (C-AFM Model ID: CA-2011, AFM Workshop 10 Capital Drive, Hilton Head Island, South Carolina, 29926). The test was carried out to conduct the surface morphology and particle size of Fe_3O_4-NPs using methanol dispersion. One mg of Fe_3O_4-NPs was dissolved in 1 mL of methanol. Spin cortar was used to coat glass beads with suspended Fe_3O_4-NPs. The drop was then let to dry overnight. The drop was used in an AFM to examine the morphology of Fe_3O_4-NPs [39].

2.4. Bacterial Strains

A consortium consisting of three bacterial strains, *A. lwoffiii* [40], *B. pumilus* [41], and *Mesorihizobium* sp. [42], was used in the current study. The strain *A. lwoffii* was isolated from the rhizosphere of *Accacia ampliceps*, whereas *B. pumulis* and *Mesorihizobium* sp. were isolated from pesticide-contaminated soil. These strains showed the potential to degrade CIP (100 mg L^{-1}) in a minimal salt medium (data not shown).

2.5. Immobilization of Bacterial Consortium on Fe_3O_4-NPs

In a buffer solution of sodium acetate (10 mL) with a pH of 4, a fine powder of Fe_3O_4-NPs was combined with 2 mL of bacterial consortium having 10^9 cells/mL of each strain. In a shaking incubator at 37 °C, the mixture was mixed for 30 min [43]. After immobilization, the magnet was attached to the flask and the immobilized cells were separated from the

rest of the medium. The immobilized cells were washed to remove any loose cells. The microbial retention on NPs was confirmed by serial dilution on LB agar plates [44].

2.6. Preparation of Stock Solution

The tablets of CIP were ground into fine powder with a pestle and mortar and dissolved in distilled water to make a 1000 mg CIP/L stock solution.

2.6.1. Wetland Plants

In this study, *C. indica* (Indian shot) was used to develop FTWs. *C. indica* has shown the potential to tolerate and grow in polluted wastewaters [45–48]. Moreover, this plant species is well-known for its rapid growth, extensive root system, and tolerance to contaminated waters [49,50].

2.6.2. Experimental Setup

The experiment was conducted in the month of May 2021 at the National Institute for Biotechnology and Genetic Engineering (NIBGE) in Faisalabad, Pakistan under natural environmental conditions. Polyethylene tanks (20 L capacity) were used to establish eighteen FTWs mesocosms. A polystyrene sheet with four-inch thickness was cut into a round shape (nine-inch diameter) with a hole in the center. A healthy plant of *C. indica* was planted in each hole. The mats with plants were placed in a container of tap water to develop roots. Tanks, mats, and roots were surface-sterilized with 5% sodium hypochlorite (NaCl) solution after one month by placing mats in the water.

Thereafter, 20 L of CIP-contaminated water (100 mg L^{-1}) was added in each tank. The following treatment design was used in the experiment, which was undertaken in triplicate:

control: CIP-contaminated water having only mat without vegetation,
control: Tap water without CIP having FTWs,
T1: CIP-contaminated water with FTWs,
T2: CIP-contaminated water with bacterial consortium only,
T3: CIP-contaminated water with FTWs and the suspension of bacterial consortium,
T4: CIP-contaminated water with FTWs and the bacterial consortium immobilized on Fe_3O_4-NPs.

A sample of contaminated water was taken every five days from each tank using a sequential fill-and-draw batch mode approach. The water samples were kept cool and dry until further analysis. The evapotranspiration losses were recovered by filling tanks with freshwater to a capacity of 20 L per tank. No rain was observed during the experimental period.

2.7. Water Quality Parameters

The water quality parameters such as pH, EC, turbidity, COD, BOD, and TOC were evaluated according to standard methods [51].

2.8. Determination of Ciprofloxacin

The residual amount of CIP in the water samples was determined according to Akram et al. (2015) [52]. Three mL of ferric chloride solution (8%, w/v) was added to an aliquot (3 mL) of each sample. Instantly, a red color complex was formed that was diluted to 10 mL with distilled water. To ensure the stability of the complex, it was allowed to stand at room temperature (25 ± 5 °C) for 30 min. Finally, the absorbance was recorded at 530 nm using a UV–Vis spectrophotometer.

2.9. Persistence of Inoculated Bacteria in FTWs

During the experiment, the persistence of the inoculated bacteria in water, root, and shoot samples was determined using a cultivation-dependent plate count method [53,54]. Briefly, the water samples were directly spread on LB agar plates. The plant roots and shoots were cleaned with distilled water before being washed with 70% ethanol for 10 and

5 min, respectively. Thereafter, the tissues were soaked in a 2% solution of NaCl for about one min. Lastly, sterile distilled water was used to wash the surface sterilize roots and shoots. The suspension was prepared by using a pestle and mortar of the sterilized roots and shoots in 10 mL NaCl solution (0.9% *w*/*v*). The suspension was plated on LB media containing 50 mg L^{-1} CIP. All of the Petri plates were kept in an incubator for 48 h at 37 °C for CFU observation [53,55,56]. Restriction fragment length polymorphism (RFLP) analysis was used to compare the identity of the isolates to the inoculated strains [25].

2.10. Plant Biomass

To evaluate the toxicity of CIP and the impact of inoculated bacteria on plant growth, the length of the roots and shoots, as well as the plant dry biomass, were recorded at the end of the experiment. A measuring scale was used to manually measure the root and shoot lengths. Plant roots and shoots were cut at the mat's surface and oven-dried for 72 h at 80 °C.

2.11. Toxicity Analysis

A phytotoxicity study was conducted in-vitro using wheat seeds to check the toxicity level of the treated water [57]. Briefly, five wheat seeds were placed in a Petri dish containing filter paper soaked in 5 mL distilled water, or in treated and untreated CIP-contaminated water. Seeds were allowed to germinate at room temperature (25 °C) in darkness. The test was conducted in triplets. After five days, germinated seeds were counted. Seeds were considered to have germinated when both the plumule and radicle were over 2 mm long. Plantlet growth (root length and shoot length) was measured.

2.12. Data Analysis

Microsoft Excel 2016 was used for all statistical analysis. One-way ANOVA was used to compare the treatments. Mean values (n = 3) and their standard errors were calculated for each treatment.

3. Results and Discussion

3.1. Characterization of Nano Particles by AFM

In this study, the surface symmetry of the synthesized Fe_3O_4-NPs was studied using an AFM. Iron oxide nanoparticles were 35 nm in size and had an average height and width of 4.83 nm and 0.22 µm, respectively. The roughness of the surface was 2.380 nm (Figure 1).

3.2. Remediation of CIP-Contaminated Water

The FTWs significantly reduced water pH, EC, turbidity, COD, BOD, TOC, and CIP concentration (Figures 2–4). The treatments with bacterial inoculation (T3 and T4) exhibited significantly more reduction in the level of pollutant than the treatments with only vegetation (T1), bacteria (T2), and mat (C). This may be linked to the efficient plant–bacteria interaction, as the bacteria had been previously isolated from the roots and shoots of plants and so may have evolved mechanisms of multiplication in the presence of CIP, allowing the bacteria to degrade the CIP while also symbiotically supporting the host plant's health while utilizing the bacteria's specific genes involved in CIP breakdown [57–59].

In this study, the FTWs with immobilized bacteria (T4) exhibited maximum reduction in COD (from 220 to 20 mg/L), BOD (from 90 to 9 mg/L), and TOC (from 130 to 11 mg/L). Other parameters such as pH, EC, and turbidity were also maximum reduced in this treatment (T4). This might be due to the fact that bacterial cells more efficiently proliferate on nanoparticles than their free cells, and ultimately enhance the degradation of CIP. Similar studies have been previously carried out in which immobilized bacteria exhibited more efficient degradation of organic contaminants compared to free cells [43,60].

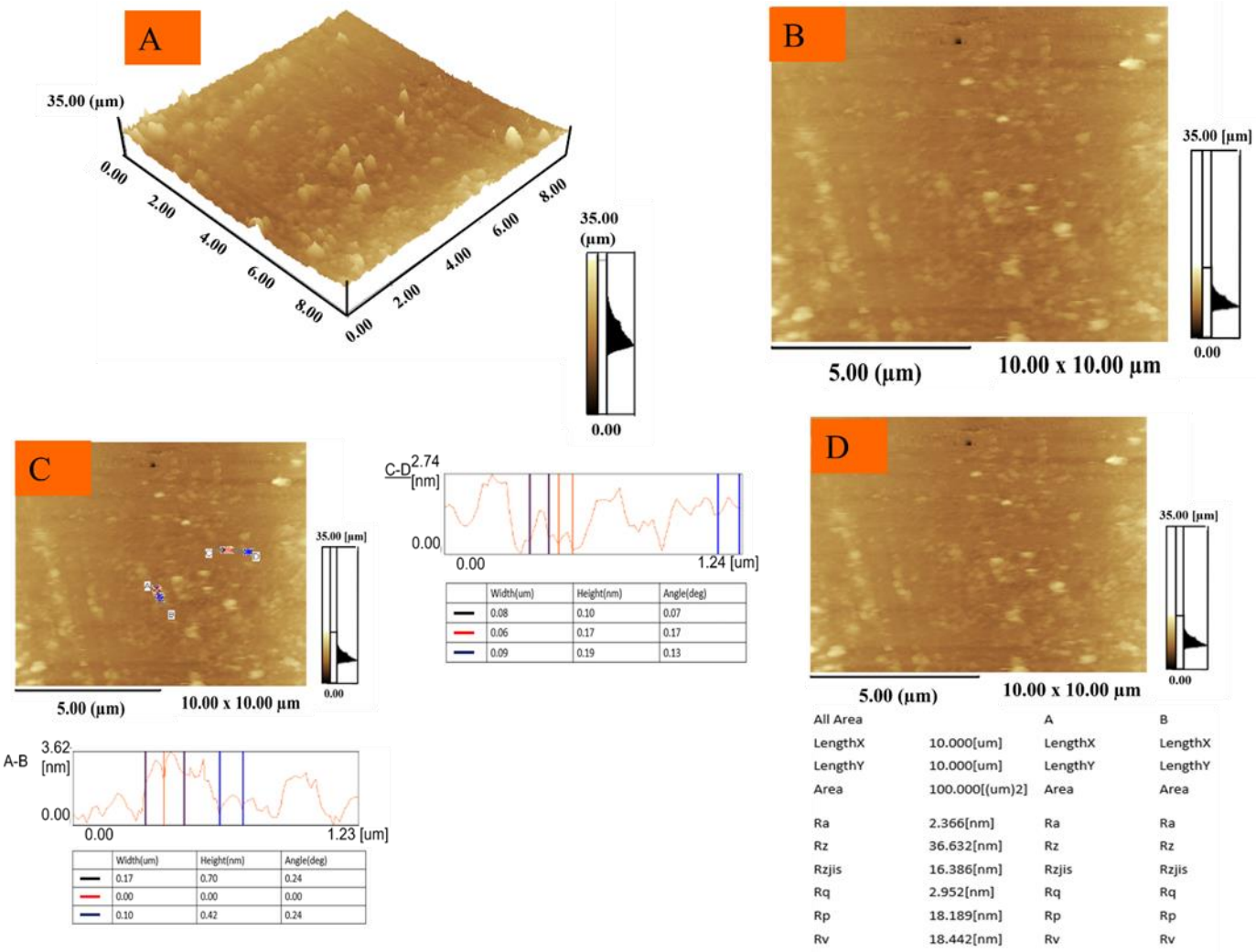

Figure 1. 3D image of iron oxide nanoparticles (**A**), size of iron oxide nanoparticles (**B**), height and width of iron oxide nanoparticles (**C**), and surface roughness of iron oxide nanoparticles (**D**).

The decrease in BOD, COD, and TOC could be attributed to bacterial enzymatic activity that degrades CIP and converts it into simpler substances, which are then taken up by plants as nutrients [58,61]. The reduction COD, BOD, and TOC indicates that the organic contaminants have been removed from the water [62]. Furthermore, in such an environment, a high oxygen concentration is crucial, and the availability of oxygen is responsible for effective biodegradation of pollutants [62–64]. In this study, vegetation could have provided oxygen in the water, allowing microorganisms to proliferate and eventually lead to the breakdown of pollutants [57,62,63].

The pH may be decreased as a result of the release of organic acids by the plant roots, as previously described [57,64,65]. Plant nutrient intake, as well as chemical and biological nutrients adhering to roots and soil particles, could all contribute to the reduction in EC [66,67]. Similar to EC, a reduction in turbidity was found in the current study. This shows that plants and bacteria play an important role in decreasing pH, EC, and turbidity [54,55,68]. Moreover, plant roots also act as filter, adsorbent and biosorbent and remove pollutants from the water [58,69,70].

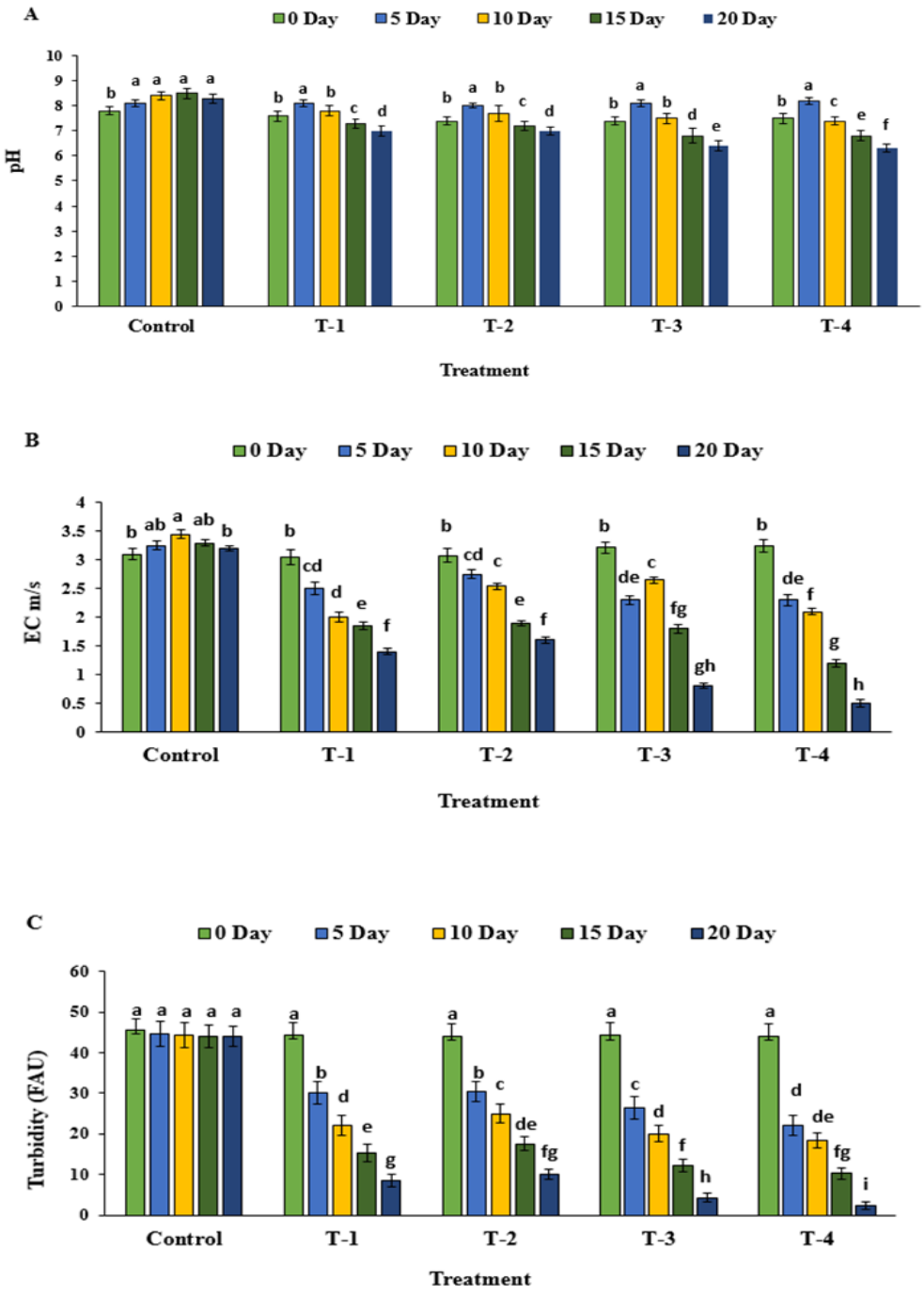

Figure 2. Remediation of ciprofloxacin (CIP)-contaminated water treated by floating treatment wetlands. Effect on pH (**A**), electrical conductivity (EC) (**B**), and turbidity (**C**). Only CIP-contaminated water (control), CIP-contaminated water treated by FTWs vegetated with *C. indica* (T1), CIP-contaminated water with only bacterial consortium (T2), CIP-contaminated water treated by FTWs vegetated with *C. indica* and bacterial consortium (T3), and CIP-contaminated water treated by FTWs vegetated with *C. indica* and augmented with bacterial consortium immobilized onto Fe_3O_4-NPs (T4). Each value is a mean of three replicates and error bars represent the standard deviation. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

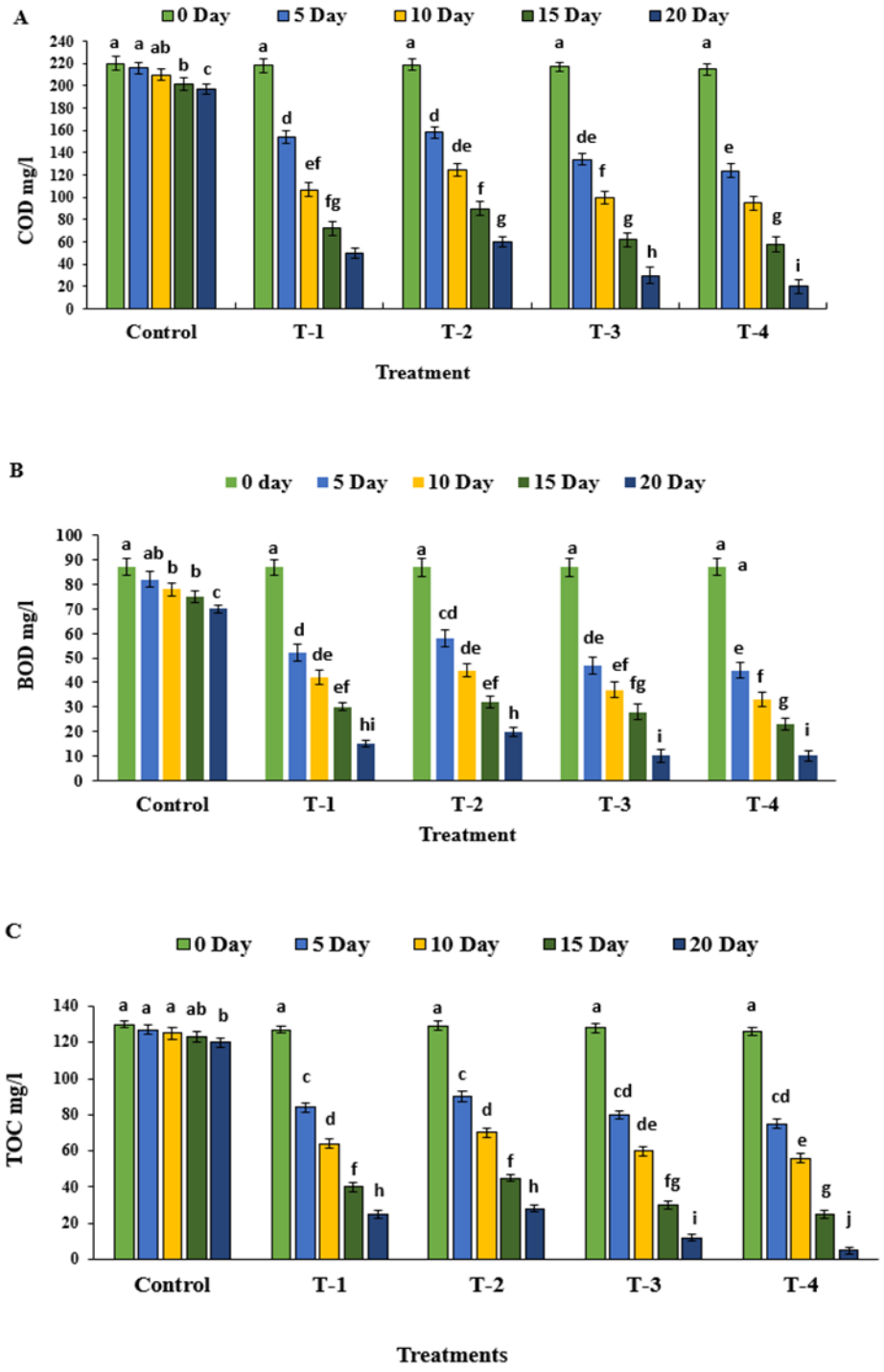

Figure 3. Remediation of ciprofloxacin (CIP)-contaminated water treated by floating treatment wetlands. Reduction in COD (**A**), BOD (**B**), and TOC (**C**). Only CIP-contaminated water (control), CIP-contaminated water treated by FTWs vegetated with *C. indica* (T1), CIP-contaminated water with only bacterial consortium (T2), CIP-contaminated water treated by FTWs vegetated with *C. indica* and bacterial consortium (T3), and CIP-contaminated water treated by FTWs vegetated with *C. indica* and augmented with bacterial consortium immobilized onto Fe_3O_4-NPs (T4). Each value is a mean of three replicates and error bars represent the standard deviation. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

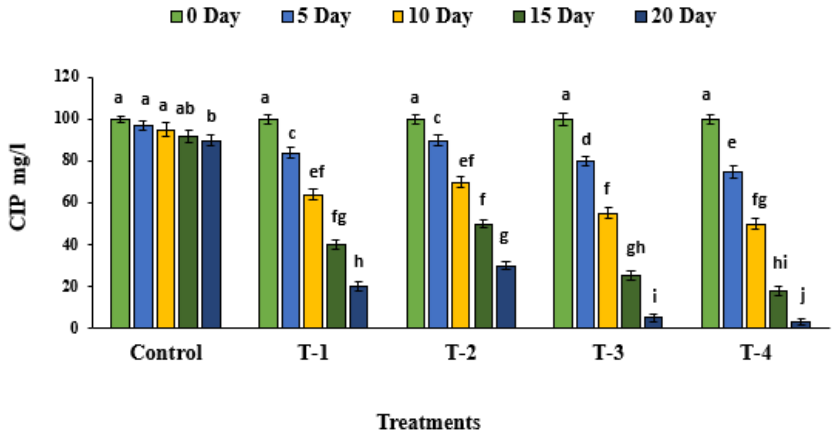

Figure 4. Remediation of ciprofloxacin (CIP)-contaminated water treated by floating treatment wetlands. Only CIP-contaminated water (control), CIP-contaminated water treated by FTWs vegetated with *C. indica* (T1), CIP-contaminated water with only bacterial consortium (T2), CIP-contaminated water treated by FTWs vegetated with *C. indica* and bacterial consortium (T3), and CIP-contaminated water treated by FTWs vegetated with *C. indica* and augmented with bacterial consortium immobilized onto Fe_3O_4-NPs (T4). Each value is a mean of three replicates and error bars represent the standard deviation. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

3.3. Ciprofloxacin Removal from Water

In this study, there was a significantly higher reduction in concentration of CIP in the water exposed to all the treatments when compared to the control (Figure 4). The elimination of CIP was speedy in the first five days followed by a slow reduction up to 20 days. The maximum (97%) removal of CIP from the water was observed in the treatment inoculated with bacterial consortium immobilized on nanoparticles and it was found to be significantly higher than other treatments. However, minimum removal (48%) of CIP from the water was observed in the FTWs (T1) without bacterial augmentation, followed (58%) by the treatment having only the bacterial consortium(T2). The bacteria have specific enzymes to degrade the organic chemicals in the water [69,71]. The accumulation of CIP in the roots and shoots of the plants was not observed in this investigation, though antibiotics have previously been reported to be transported to the stems and leaves of plants [70,72]. However, most of the CIP was reported to be accumulated in the roots [73].

3.4. Persistence of the Inoculated Bacteria in FTWs

The persistence of the inoculated bacteria is important in the breakdown of organic pollutants. The current study examined the persistence/existence of the inoculated bacteria in the water, as well as in the roots and shoots of the plants. The water from the unvegetated treatment (T2) contained a lesser number of inoculated bacteria than in the water of vegetated treatments (T3 and T4) (Table 1).

Table 1. Total bacterial population (CFU/mL) in the ciprofloxacin-contaminated water treated by floating treatment wetlands (FTWs) vegetated with *Canna indica*.

Treatment	Initial	5 Days	10 Days	15 Days	20 Days
T1	$0.7 \times 10^{2\,a}$ (0.1×10^2)	$2.8 \times 10^{2\,ab}$ (1.0×10^2)	$3.2 \times 10^{2\,ab}$ (1.0×10^2)	$2.5 \times 10^{2\,ab}$ (0.8×10^2)	$3.0 \times 10^{2\,ab}$ (0.7×10^2)
T2	$7.1 \times 10^{6\,de}$ (4.3×10^3)	$4.1 \times 10^{2\,bc}$ (1.3×10^2)	$3.9 \times 10^{2\,b}$ (1.1×10^2)	$4.4 \times 10^{2\,b}$ (1.2×10^2)	$3.8 \times 10^{2\,b}$ (1.0×10^2)
T3	$7.2 \times 10^{6\,de}$ (4.3×10^3)	$8.3 \times 10^{6\,de}$ (5.1×10^3)	$6.4 \times 10^{4\,cd}$ (2.6×10^3)	$4.7 \times 10^{4\,cd}$ (2.2×10^2)	$4.0 \times 10^{3\,c}$ (1.3×10^2)
T4	$7.2 \times 10^{6\,de}$ (4.3×10^3)	$8.7 \times 10^{8\,e}$ (4.3×10^3)	$8.3 \times 10^{8\,e}$ (3.9×10^3)	$7.5 \times 10^{6\,de}$ (3.3×10^3)	$6.8 \times 10^{5\,d}$ (3.1×10^3)

CIP-contaminated water treated by FTWs vegetated with *C. indica* (T1), CIP-contaminated water with only bacterial consortium (T2), CIP-contaminated water treated by FTWs vegetated with *C. indica* and bacterial consortium (T3), and CIP-contaminated water treated by FTWs vegetated with *C. indica* and augmented with bacterial consortium immobilized onto Fe_3O_4-NPs(T4). Each value is a mean of three replicates and error bars represent the standard deviation. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

This could be due to the lack of a symbiotic relationship between the bacteria and the plant [58,59,62]. The bacterial population was likewise much higher in the root interior of *C. indica* than in the shoot interior. Previous research has shown that the inoculated bacteria colonize more efficiently in the rhizosphere and root interior of wetland plants, which could explain the larger population density in the roots, suggesting that the root environment has a higher colonization potential than the shoot environment [40]. In previous research, the number of inoculated bacteria in the root interior was shown to be higher than in the shoot interior [40,59,74]. In this study, among the treatments, maximum bacterial population was found in the water, shoot, and root of the treatment (T4) with bacterial consortium immobilized on Fe_3O_4-NPs (Table 2).

Table 2. Population of the inoculated bacteria in the roots and shoots of *Canna indica*.

Treatment	Root/Shoot	Bacterial Population (CFU/g)
T1	RI	$6.9 \times 10^{4\,b}$ (0.8)
	SI	$5.5 \times 10^{4\,c}$ (1.1)
T2	RI	$7.5 \times 10^{4\,a}$ (1.3)
	SI	$6.4 \times 10^{4\,bc}$ (1.2)

Root interior (RI), shoot interior (SI), CIP-contaminated water treated by floating treatment wetlands (FTWs) vegetated with *C. indica* and bacterial consortium (T1), CIP-contaminated water treated by FTWs vegetated with *C. indica* and inoculated with bacterial consortium immobilized onto Fe_3O_4-NPs (T2). Values represent the means of three replicates and standard deviations are presented in parenthesis. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

3.5. Plant Biomass

The presence of toxic pollutants in water has long been known to limit plant growth [25,75–77]. In this study, the root and shoot length, as well as root and shoot dry biomass of the plant (Table 3) were measured at the end of the experiment. It was found that plants grown in CIP-contaminated water have lesser growth than the plants grown in tap water. However, plants inoculated with bacteria (T2 and T3) exhibited more growth and development (root and shoot length and biomass) than the plants without bacterial inoculation. This is because of the plant–bacteria symbiotic relationship, in which bacteria support plant growth and development by lowering the abiotic stress in contaminated water, such as the abiotic stress caused by the presence of CIP [78]. Bacteria also promote plant growth by producing phytohormones and essential nutrients [28,79,80]. Previous research has also reported that the inoculated bacteria can increase plant development by lowering pollutant-induced toxicity [59,68,81].

Table 3. Effect of ciprofloxacin and bacterial inoculation on the growth of *Canna indica*.

Treatment	Fresh Biomass (g)		Dry Biomass (g)		Length (cm)	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	110 ^{b} (7.85)	195 ^{c} (5.45)	35 ^{ef} (4.72)	118 ^{f} (3.46)	25 ^{f} (3.08)	30 ^{e} (5.18)
T1	100 ^{b} (9.23)	180 ^{c} (6.18)	25 ^{ef} (5.82)	95 ^{ef} (3.38)	16 ^{ef} (4.26)	25 ^{d} (6.28)
T2	104 ^{a} (8.54)	185 ^{b} (8.17)	28 ^{e} (5.62)	110 ^{ef} (4.61)	20 ^{ef} (5.47)	27 ^{d} (7.34)
T3	107 ^{a} (9.43)	190 ^{b} (8.07)	33 ^{e} (6.08)	112 ^{ef} (5.35)	23 ^{ef} (5.82)	28 ^{d} (7.78)

Tap water (control), CIP-contaminated water treated by FTWs vegetated with *C. indica* (T1), CIP-contaminated water treated by FTWs vegetated with *C. indica* and inoculated with bacterial consortium (T2), and CIP-contaminated water treated with FTWs vegetated with *C. indica* and inoculated with bacterial consortium immobilized onto Fe_3O_4-NPs (T3). Values represent the means of three replicates and standard deviations are presented in parenthesis. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

3.6. Phytotoxicity Evaluation of Treated Water

To evaluate the extent of improvement in the quality of treated water by FTWs, a wheat seed germination toxicity assessment was conducted at the end of the study. The radicle length was taken as an indicator for toxicity analysis, an approach consistent with the previous studies performed to assess the toxicity of organic or inorganic contaminants [82–84].

In this study, the relative data of radicle length (RL) and total length (TL) of plantlets are presented in Table 4.

Table 4. Effect of water treated by floating treatment wetlands on the growth of plantlets of wheat.

Treatment	Radicle Length (mm)	Total Length (mm)
Untreated water	10.5 ^{h} (0.74)	14 ^{g} (0.95)
Tap water	25 ^{d} (1.62)	42 ^{a} (2.27)
T1	21 ^{f} (1.41)	31 ^{c} (1.85)
T2	22 ^{e} (1.34)	28 ^{c} (1.67)
T3	24 ^{d} (1.13)	38 ^{b} (2.05)
T4	25 ^{d} (1.51)	40 ^{a} (1.71)

Ciprofloxacin-contaminated water treated with floating treatment wetlands vegetated with plant *C. indica* only (T1), CIP-contaminated water with only bacterial consortium (T2), CIP-contaminated water treated by FTWs vegetated with *C. indica* and bacterial consortium (T3), and CIP-contaminated water treated by FTWs vegetated with *C. indica* and augmented with bacterial consortium immobilized onto Fe_3O_4-NPs (T4). Each value is a mean of three replicates and error bars represent the standard deviation. Lettering shows that various treatments are significantly different at $p \leq 0.05$.

The plantlets produced from seeds exposed to water treated by FTWs showed more RL and TL than the seeds exposed to the water without treatment. This might be due to the reduction in the concentration of CIP in the water by the plant–bacteria partnership. However, the plantlets produced from the seeds exposed to the water treated by FTWs inoculated with bacterial consortium immobilized on Fe_3O_4-NPs showed maximum RL (25 mm) and TL (40 mm). The seeds exposed to untreated water developed minor radicle and no plumule. This is due to the toxic effects of CIP-contaminated water [85–88]. In an earlier study, Pan and Chu (2016) evaluated five major veterinary antibiotics, namely tetracycline, sulfamethazine, norfloxacin erythromycin, and chloramphenicol, for their phytotoxic effects on the germination of seeds of lettuce, tomato, carrot, and cucumber [89]. According to their results, these antibiotics significantly reduced root elongation, which is the most sensitive endpoint for the phytotoxicity study. They also found that tetracycline was the most toxic, and lettuce was the most susceptible to the tested antibiotics [89].

4. Conclusions

The application of immobilized bacteria onto Fe_3O_4-NPs significantly increased CIP remediation (97%) via FTW. To this end, a coarse perennial herb such as *C. indica* could be a suitable option due to its metabolic abilities to support microbial proliferation and enhance organics mineralization in the root zone. The bacterial strains, *A. lwoffii* ACRH76, *B. pumulis* C2A1, and *Acinetobacter* sp. HN3, were able to develop successful partnership with the plant, even in the presence of CIP, indicating abilities to survive in antibiotics stress. FTW supported by immobilized bacteria offer sustainable and scalable solutions to treat wastewater contaminated with antibiotics. Owing to the near-natural means of remediation and comparably small energy requirements, the technology is particularly attractive for countries with economic constraints such as Pakistan. Further studies are, however, needed to explore the metabolic activity of the bacteria during the degradation of CIP.

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