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A Study of the Phytoremediation Process Using Water Lettuce (*Pistia Stratiotes*) in the Removal of Ciprofloxacin

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ABSTRACT

The use of antibiotics has become imperative and unavoidable in medicine to Figureht against microbes, but the majority of these antibiotics are found in environmental ecosystems. It is revealed that the presence of these in the environment, intoxicates the bacterial ecological medium.Then, this investigated the phytoremediation abilities of Water lettuce (*Pistia Stratiotes*). Adolescent plants were placed in two different concentrations of Ciprofloxacin solution for 7 days. The aim was to see if the plant could remove the Ciprofloxacin, what amount of it and the effects of the drug on the plant thereafter. The concentrations were 50ppm and 10ppm of Ciprofloxacin. The result was that at 50ppm, the plants developed necrosis within 3 days and died. At 10ppm solution, water lettuce managed more than 70% removal efficiency, and also a steady growth of the plant was maintained at 0.1606 g/day. For the concentration of Ciprofloxacin, analysis of sample water was done using UV-Visible Spectroscopy and plant extract was analyzed by HPLC. The study proved that water lettuce can be used as a remediation technique for surface waters, or can be an end-of-pipe measure for pharmaceutical wastewater treatment facilities before discharge into surface waters.

INTRODUCTION

Freshwater reservoirs are being depleted and ocean temperatures are rising, causing water pollution. As a result of anthropogenic activities, water is polluted 1. Adverse effects have been observed on the water supply. These include lakes, rivers, oceans, aquifers, reservoirs, and groundwater. When contaminants are introduced into bodies of water, it leads to pollution. The pollution control industry has seen an increase in the last few years as a result of rising concerns. Untreated wastewater and industrial effluents. There are a number of ways in which antibiotics enter the environment. Among them are direct human or animal excretion, animal manure applied to crops as fertilizer, municipal wastewater treatment plants, hospitals, and manufacturing plants (Balarabe & Maity, 2022; Booth *et al.*, 2020; Kraemer *et al.*, 2019). With the rapid development of pharmaceutical waste, a growing threat is posed to surface and groundwater resources with adverse effects on aquatic ecosystems (Balarabe *et al.*, 2022; Jin & Aslam, 2019). As most antibiotics have active ingredients that dissolve in water, they can be transmitted into aquatic food webs as well. Bioaccumulation poses a threat to public health, destroys aquatic flora and fauna, and leads to drug-resistant waterborne diseases. Several pharmaceutical wastes end up in the environment, including antibiotics, hormone wastes, and analgesics, from inappropriately disposed of pharmaceuticals, unused or expired tablets, and unprescribed pills. Tetracycline, Oxytetracycline, Ibuprofen, Ciprofloxacin, and Norfloxacin are some of the antibiotics that persist in wastewater after treatment (Shikha & Gauba, 2016). To classify fluoroquinolones, it is necessary to examine their spectrum of activity as well as their pharmacokinetic profile. A fluoroquinolone-type antibiotic known as Ciprofloxacin is an antibiotic that has broad antibacterial activity against both Gram-positive and Gram-negative bacteria (Wu *et al.*, 2008). With well-established safety features, Ciprofloxacin is a promising and effective antibiotic. Having effectively treated over 250 million people globally, its safety profile has been extensively documented in a large number of scientific articles. Ciprofloxacin inhibits DNA gyrase, which is needed for disease replication. After oral treatment, ciprofloxacin is rarely absorbed completely. Ciprofloxacin has an absolute bioavailability of 70–80 percent, with no significant loss due to first-pass metabolism (Sharma *et al.*, 2009). Many traditional cleanup procedures do not provide adequate solutions to pollution in water and soil today. Pharmaceutical and industrial waste products accumulate in the land, air, and water, destroying plants and causing health problems. Heavy metal toxins, antibiotics, hormonal wastes, and pharmaceuticals are among the pollutants. As part of the phytoremediation process, plants are utilized in soil, sediment, and water to remove, transport, stabilize, and decompose pollutants deposited in them through the use of plants (Shikha & Gauba, 2016). Restoration of the environment with plants is centuries old and cannot be credited to any individual. A phytoremediation method is environmentally friendly, cost-effective, and promising. An example of phytoremediation is the use of plants to treat contaminated environments when they are naturally occurring or genetically modified. There is a growing

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interest in phytoremediation, in which macrophyte plants are used in constructed wetlands and stormwater detention ponds to treat eutrophic waterways (Tanmay Sanyal & Saha, 2022) Phytoremediation has evolved recently. The concept of phytoextraction was developed by Singh & Santal, an approach that uses plants to absorb pollutants into their biomass. Pollutants are absorbed by plants and stored in their aerial portions, after harvesting, the plant is discarded (Kumar *et al.*, 2018) . Rhizofiltration involves the root system of plants interacting with toxins to remove pollution. This technology has the potential to reduce the bioavailability of organic and inorganic contaminants. Rhizofiltration leaves the pollutant on/in the root. In phytovolatilization, toxins are absorbed from the soil, converted to a volatile form, and released into the atmosphere (A. Yan *et al.*, 2020). Then, plants involved in process requires a dense root system (Radziemska *et al.*, 2017) (A. Yan *et al.*, 2020). As well as the degradation of contaminants in soil, groundwater, and surface waters, phytodegradation is the enzyme-mediated uptake and breakdown of pollutants within plants. Plants and accompanying microbes digest organic pollutants to transform them into harmless forms. Plant roots absorb a considerable amount of contaminants. There are thousands of *Pistia stratiotes* (water lettuce) floating in the ocean. It spreads rapidly in nutrient-contaminated water. Due to its availability and ability to withstand temperatures up to 30°C, water lettuce can treat wastewater. It grows in massive colonies on water as an Araceae macrophyte. If left unchecked, these colonies can be invasive. While a dense root network absorbs/adsorbs contaminants from water, hydrophobic leaf surfaces keep it afloat (Galal & Farahat, 2015) and (Mustafa & Hayder, 2021). Phytoremediation, therefore, is an environmentally friendly, cheap, efficient, and effective way to remove antibiotics from contaminated water (Ansari *et al.*, 2020). Industrial, household and agricultural wastewater have been treated with *Pistia stratiotes*. This plant is widely used because of its availability, durability in toxic environments, bioaccumulation potential, and invasive properties (Mustafa & Hayder, 2021). In a lab test, (Gowri *et al.*, 2020) found that water lettuce can be used to purify eutrophic surface water, but not for drinking. To name a few, water lettuce reduced or increased BOD, COD, pH, Nitrates, Phosphates, and TDS. A study by (Kumar *et al.*, 2018) found that Water Lettuce (*P. Stratiotes*) can remove heavy metal contamination. A 75% maximum extraction of heavy metal was from the water. According to (Upadhyay & Panda, 2009), copper on water lettuce could be a bioindicator for copper levels in surface water. (Odjegba & Fasidi, 2004) tested the effectiveness of Water lettuce for the removal of heavy metals was tested. It was found that the rate of leaf growth was found to be reduced when metal type, concentrations, and exposure time were increased. This study aims to remove Ciprofloxacin from a hydroponic nutrient solution by water lettuce.

MATERIAL AND METHOD

All the materials and solvents were purchased from commercial sources (Finer Chemicals, India, Sisco research laboratories Pvt. Ltd., India, Sigma Aldrich, and Abhishek Enterprise Pvt. Ltd.) and used as received without purification. Distilled water was used as the solvent for Ciprofloxacin and the Hoagland solution in which the plants were grown. For standardization in HPLC, Milli-Q water was used as a solvent as required by the HPLC protocol. Milli-Q water and spectroscopic grade solvents were used for all measurements.

Water Lettuce (*Pistia stratiotes*) plants

The adolescent water lettuce plants were obtained from Umarose Nursery and Farm, Gandhinagar, Gujarat. The plants were washed thoroughly and grown in a hydroponic solution for 1 week prior to exposure to a Ciprofloxacin solution.

Preparation and characterization of Hoagland Solution

Pistia stratiotes plant life was sustained in a hydroponic system by using a Hoagland solution which is prepared based on the modified protocol of Hoagland and Amon in 1950 (Seth *et al.*, 2011). The nutrients were made separately into stock solutions and the working solution was mixed accordingly (Table 1).

Table 1: Hoagland Solution Composition: The stock and working solutions.

	Nutrient	Stock solution (g/100mL)	Working solution (mL/L)
Macro-nutrients	Calcium nitrate	23.61	2.50
	Potassium Nitrate	5.02	2.50
	Magnesium sulfate	24.64	1.00
	Monopotassium phosphate	1.31	1.00
Micronutrients	Boric acid	2.86	1.00
	Manganese sulfate	1.54	1.00
	Zinc sulfate	0.22	1.00
	Copper (2) sulfate	0.08	1.00
	Molybdic acid	0.09	1.00
	EDTA-K salt	2.50	1.00
	Ferric Sulfate	2.50	1.00

Preparation and Characterization of Ciprofloxacin HCL

The Ciprofloxacin HCL used was obtained from Abaris Healthcare Pvt. Ltd., Mehsana, India. Ciprofloxacin is insoluble in water, therefore, the study utilized Ciprofloxacin Hydrochloric powder. The study targeted the degradation of a 10ppm solution of Ciprofloxacin.

UV-Visible Spectroscopy was used to analyze a 10ppm solution of Ciprofloxacin and single distilled water at full-spectrum analysis (200-800nm) to find the absorbance peak for Ciprofloxacin. The same analysis was also done using 10ppm Ciprofloxacin and Hoagland solution. High-Performance Liquid Chromatography (HPLC) was used to Characterized Ciprofloxacin according to the protocol described by (Wu *et al.*, 2008).

Development of Ciprofloxacin Calibration curve

To develop a standard for Ciprofloxacin, 2 calibration curves were plotted using results from UV-Visible spectrometry and HPLC analysis. The standard determined the key concentration to use in characterization studies. A sample of 1mg/10ml was used to create a 100ppm stock solution, from which 2, 4, 6,8, and 10ppm working solutions were derived to create the calibration curve. The absorbance for Ciprofloxacin was determined at 271nm.

Preparation of Citrate - phosphate buffer

To check the availability of Ciprofloxacin in the plant, the plant extract was derived using a Citric-dihydrogen phosphate buffer called McIlvaine buffer after its creator, Theodore McIlvaine in 1921. Development and use were done following the protocol by (McIlvaine, 1921; Y. Yan *et al.*, 2021).

Experimental procedure

Pistia stratiotes plants were grown in two different concentrations, 50 ppm, and 10 ppm concentrations. The first was to introduce plants to a slightly high concentration, to determine the level of toxicity water lettuce can withstand. The second concentration was primarily the focus of the study, to see if and what amount of the Ciprofloxacin could be removed by the plant from water. It was a test of its phytoremediation capability. Plants were monitored for 7 days for both parameters. Water lettuce was grown at ambient temperature. The pH was monitored as the plant needs a pH of 6.5-7.5 to grow. Water loss through evapotranspiration was refilled with distilled water and Hoagland solution. Readings for UV- Visible spectroscopy were taken initially from Ciprofloxacin solution prior to transplanting the plants. On the 7th day, another UV-Visible reading was done to check the amount of Ciprofloxacin left. The pH reading was carried out every day because Ciprofloxacin HCL is acidic and acidity could kill the plant. Foil paper was used on samples to reduce photodegradation of Ciprofloxacin as shown in the experimental set-up. The *Pistia stratiotes* resilience by taking initial and final growth fresh weights and calculating the growth per day. The growth was monitored in the 7 days the plant was exposed to Ciprofloxacin.

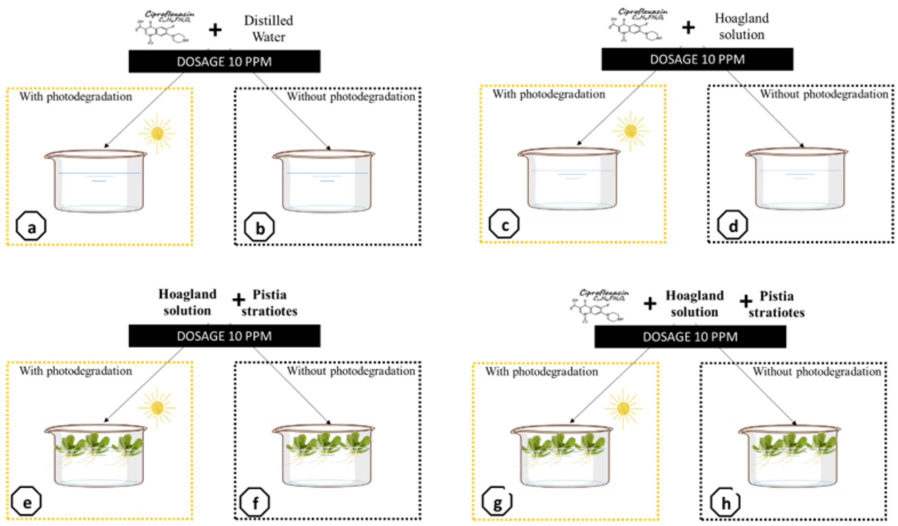

Figure 1: Experimental setup:
(a) Distilled water mixed with Ciprofloxacin (at 10 ppm) under the sun; (b) Distilled water mixed with Ciprofloxacin (at 10 ppm) without the sun; (c) Distilled water mixed with Holang solution (at 10 ppm) under the sun; (d) Distilled water mixed with Holang solution (at 10 ppm) without the sun; (e) Holang solution (at 10 ppm) with *Pistia stratiotes* under the sun; (f) Holang solution (at 10 ppm) with *Pistia stratiotes* without the sun; (g) Ciprofloxacin, Holang solution (at 10 ppm) and *Pistia stratiotes* under the sun; (h) Ciprofloxacin, Holang solution (at 10 ppm) and *Pistia stratiotes* without the sun.

RESULTS AND DISCUSSION

Ciprofloxacin concentrations of 5, 10, 20, 30, 40, and 50ppm were tested. Figure. 2a shows that the higher the concentration of ciprofloxacin, the more difficult it is for the plant to survive. The plant grows normally up to 10pmmIn the Ciprofloxacin solution, plants developed chlorosis and necrosis within the first 3 days and died. Plants suffer toxicity from absorbing fluorine, which explains this. Plant growth is illustrated in Figure. 2a&b at 10ppm and 50ppm, respectively. Fluorine is a determining factor in the structure of Ciprofloxacin, which is a fluoroquinolone (Sharma *et al.*, 2009). Plants sensitive to fluorine are susceptible to necrotic lesions, burning, chlorosis, leaf damage, and development and reproductive suppression (Banerjee & Roychoudhury, 2019). It became apparent that the water lettuce samples that contained 50ppm will be effective as bioindicators in the future (Galal & Farahat, 2015).

UV- Vis spectroscopy indicated a lambda max of 271nm for 10ppm ciprofloxacin solution and a maximum absorbance of 0.8573111 (Figure. 3a). The lambda max was also found to be 271nm for Ciprofloxacin and

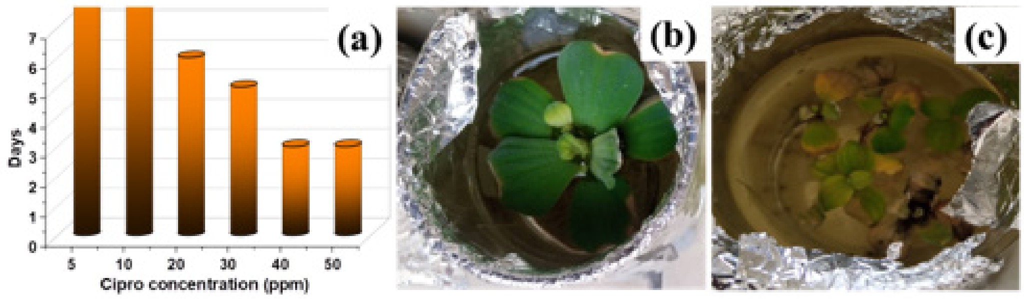

Figure 2: (a)-Water lettuce survival analysis, (b)-Normal growth of Water lettuce in 10ppm Cipro solution, (c)-Dying of Water lettuce plants in 50ppm Cipro solution.

Hoagland solution at 10ppm, showing almost the same absorbance. In Figure. 3c, the calibration curve at different concentrations (2, 4, 6, 8, and 10ppm) was plotted and the equation was y= 0.0844x + 0.0136 with an R2 of 0.9992. The UV-Visible reading of day 7 indicated that there had been a significant decrease in UV absorption. The degradation efficiency of the can be defined as Degradation efficiency (%) = (C0-Ct)/C0 × 100% 2, where: C0 is the Cipro concentration at 10ppm, and Ct is the residual concentration of Cipro after 7 days. In Figure. 3d, the degradation efficiency of the treatments S1, S2, S3, S4, S7, and S8 has been shown. It is evident from Treatments 1 and 2 that light contributes to ciprofloxacin degradation. 3.62% of Cipro removable was attributed to light. Furthermore, when Hoagland's solution was added (S3 treatment), the degradation rate increased from 3.62% to 5.69%. Iron present in Hoagland's solution may act as a reducing agent. Combined with light + Hoagland's solution + Water lettuce, significant degradation occurs. Therefore, Water lettuce is able to absorb 71.92% (treatment S7) of ciprofloxacin compared to 66.60% without light (treatment S7). In addition, this illustrates how light influences ciprofloxacin degradation. After this, on the 7th day, Water lettuce from treatment S7 was harvested and dried at ambient temperatures. This took 4 days for the plants to be completely dry. The dried

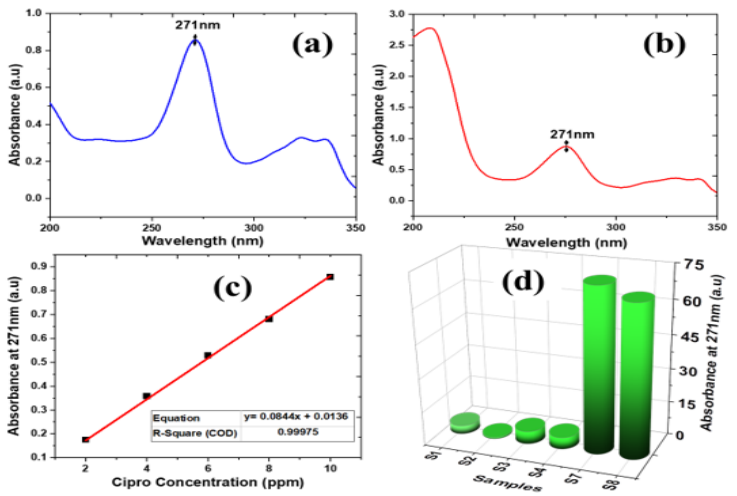

Figure 3 : (a)-UV- Vis Spectroscopy of Cipro solution, (b)-UV- Vis Spectroscopy of Cipro and Hoagland solution, (c)-Calibration Curve of Cipro solution in UV-Vis Spectroscopy and (d)-Cipro removable efficiency per treatment.

plant was then prepared for HPLC using the protocol which uses McIlvaine buffer to get plant extract (A. Yan *et al.*, 2020). A mortar was used to fine-grind dried plant samples. The plant powder was then sifted and placed in a centrifuge tube. 0.1 molar of McIlvaine Buffer at pH 3 was prepared up to 20 ml, then added to the plant sample. This was sonicated for 10 min and placed in a centrifuge for 10 min with extraction done 3 times. The extract was filtered using Whatman's filter paper. The clear plant extract was analyzed by HPLC to determine Ciprofloxacin content. Bypassing the plant extract through HPLC, it was observed that a peak synonymous with Ciprofloxacin was observed (Figure. 4a&b). This proved beyond doubt that Water lettuce had the ability to absorb Ciprofloxacin from water. The peak observed in HPLC is shown in Figure. 3a&b. The time of the peak during calibration is the same as the time observed from the plant extract. This observation means that the same compound (Ciprofloxacin) was retained, eluted, and detected in both instances. From the standard curve of the 2, 4, 6, 8, and 100 in Figure. 4c with equation y= 33519x-34322 and R^2= 0.9963, the peak from the water lettuce plant extract in Figure. 4b corresponded with the peak for 8ppm concentration. Therefore, the concentration of Ciprofloxacin in the water lettuce plant extract can be calculated from the standard curve equation. According to HPLC, the amount of Ciprofloxacin present in the plant after 7 days was 7.78 ppm. This supports the hypothesis that Water lettuce can be used as a phytoremediation strategy to cleanse wastewater that has Ciprofloxacin. The removal efficiency indicates 77.8%, which is similar to the efficiency percentage obtained by UV-Visible spectroscopy. There was also confirmation from a mass spectrometry analysis of degraded Cipro in the dye solution that no significant smaller fragments are present as a result of this degradation process (Figure. 4d&e). The following table 2 presents a brief summary of the previous studies, the methods used, and the results derived from these studies. During the 7-day period, the water lettuce plant had accumulative growth

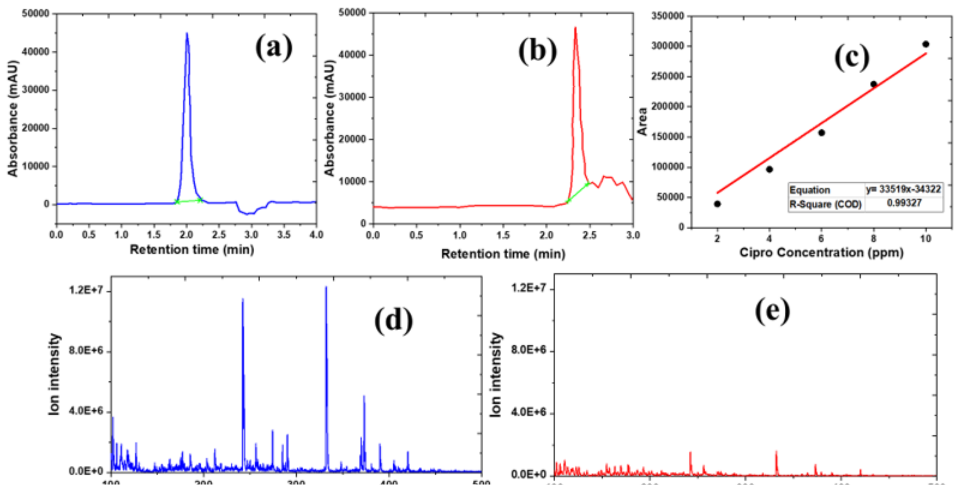

Figure 4: (a)-HPLC peak of 10 ppm Cipro concentration, (b)- Plant Extract analysis in HPLC, (c)-Calibration Curve of Cipro in HPLC and Mass Spectrometry analysis of Cipro (d)-before and (e)-after 7 days.

of 0.1606grams each day. This growth was not deterred by the effects of Ciprofloxacin, which means that it is a hyperaccumulator. The Relative growth rate (g/d) = (W2-W1/ T2-T1) (Kumar *et al*., 2018).

Where W1 (9.53167g) is the initial mass of fresh plants, W2 is the final mass of fresh plants (8.5676g); T1 is day 1 and T2, is the last day. The Relative growth rate is 0.1606grams/day.

The Bioconcentration factor or bioaccumulation factor is calculated to determine if a plant is a hyperaccumulator. This means that the plant biomass will not be disturbed by the amount of pollutant accumulation at a particular concentration of said pollutant. In other words, it is a ratio of the contaminant in the plant in relation to its concentration in the water. For hyperaccumulators, the BCF is more than 1. The BCF = CHPLC / CUV.

Where CHPLC (7.78ppm) is the contaminant concentration in plant tissue (HPLC result) and CUV (2.69ppm) is the contaminant concentration in wastewater (UV result).

For this particular study, the BCF for water lettuce was 2.89. BCF is more than 1 means that Water Lettuce is a hyperaccumulator and can be used to reduce bio-availability of Ciprofloxacin in affected waters. BCF is also important as it shows the impact or risk to the ecosystem under threat from a contaminant.

Table 2: A summary of previous studies

Serial No.	Location	Experimental Parameters	Analytical Technique	Summary	References
1	Uttarakhand, India	Removal of selected metals Copper, Iron, and Mercury using Water Lettuce	Absorbances recorded using UV-Vis Spectroscopy	Water lettuce managed to effectively remediate synthetic and industrial wastewater	(Kumar *et al*., 2018)
2	Vanarasi, India	5 heavy metals (Cu, Cr, Fe, Zn, Cd) in 3 different concentrations (1.0, 2.0, 5.0mg-L	Atomic absorption spectrophotometer, UV-Vis, Extraction air acetylene flame method	Water lettuce along with two other aquatic plants showed that it was highly effective as a phytoremediator, without damage from toxicity.	(Mishra & Tripathi, 2008)

3	Lagos, Nigeria	Exposure of live plants to crude oil (0–100 ppm) for 28 days at a normal temperature of 30 ± 2C.	Total Hydrogen content (THC) and metal ion concentration were measured using AAS.	Crude oil was toxic to the plant. Using growth and cell division Water lettuce can be used as a bio-indicator in water.	(Akapo *et al.*, 201 C.E.)
4	Ankara, Turkey	Water Lettuce exposed to different concentrations of Cadmium and Lead	ICP-MS used to analyze plant extract	Water lettuce was successful in removing heavy metals at moderate concentrations	(Ali *et al.*, 2020)
5	Fort Pierce, USA	2 plots in 2 different stormwater detention plots. 1plot with water lettuce plants, Analysis of water samples weekly for 22 months	ICP-OES	20% reduction in metals in water. The highest accumulation was of Cr	(Lu *et al.*, 2010)
6	Prague, Czech Republic	8 variants were set up. Plans were grown in Hoagland solution. Harvesting of plants for analysis on days, 2 4 and 8	ICP-OES, UV-Vis Spectroscopy	Pb accumulation by rhizofiltration. Chlorosis due to increased Pb accumulation	(Veselý *et al.*, 2013)
7	Alexandria, Egypt	3 experimental units with water lettuce. Growth monitored for 7 days.	Physicochemical parameters of wastewater analyzed	High removal rate of Fe, Cu, Zn. Reduction of TN and TP and removal of HNO3	Gaballah *et al.*, 2019)
8	Nigeria	Using Water lettuce to treat wastewater from rubber industry effluent for 3 years	AAS	Successful in reduction of water perimeters to WHO permissible limits	(Owamah *et al.*, 2014)
9	Shanghai, Bangkok,	Weekly sampling of physicochemical properties of water under study- 3 macrophytes. 6months in 3 separate tanks. Analysis after every 10 days	Water parameters analyzed	Water lettuce exhibited the highest efficiency removal of Phosphorus. High nitrogen removal was attributed to its dense root system which encouraged microbial activity for denitrification	(Lu *et al.*, 2010)
10	Thailand China	Water lettuce grown and analyzed for 7 days with different Chlorpyrifos concentrations	GC-ECD	Water lettuce growth and removal efficiency was dose dependent. Img + concentration of the pesticide was toxic.	(Prabakaran *et al.*, 2019)
11	Gujarat India	Water lettuce grown and analyzed for 7 days with different 10ppm Ciprofloxacin	UV-Vis, HPLC, GC-MS	Water lettuce growth and removal efficiency was approx. 70%	This work

CONCLUSION

Phytoremediation of Ciprofloxacin using Water lettuce was achieved in the study. The study supports earlier work mentioned above that macrophytes can remediate surface waters. The study was done under ambient temperatures. The variable that was maintained was the pH. The plants need pH of between 6.5 - 7.5. As the study was done during the month of May, one of the hottest months for Gujarat, India, it showed resilience for high temperatures. In order to maintain the sustainability of contaminated large-scale landscapes and damaged aquatic ecosystems, phytoremediation is a practical and economical method of cleanup that uses macrophytes like water lettuce. Water lettuce is an invasive macrophyte which grows in most tropical regions. By harnessing macrophytes to remediate surface water, not only do ecosystems benefit, it is also an investment in future environmental sustainability. Research is needed to find out if Water lettuce can remediate more pharmaceutical waste. The performance of the plant in a field study on pharmaceutical waste water needs to be studied. In the above study, Ciprofloxacin interactions with the rhizosphere and plant tissue were not explored.

Compliance with ethical standards.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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