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Phytoremediation of some antibiotics in wastewater of Al- Sadr medical city, Al-Najaf Al-Ashraf, Iraq

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Abstract:

The study was done in Autumn 2024, which included phytoremediation of wastewater coming out of the wastewater treatment unit in Al-Sadr Medical City (Al-Najaf Ashraf) using three aquatic plants: Ceratophyllum demersum, Lemna minor and Azolla sp.

A sample of that wastewater was taken to investigate pharmaceutical materials, the following antibiotics were found:Amoxicillin, Ampicillin, Azithromycin and Ciprofloxacin 112.5, 62.5, 80.9, 124.5 mg/l respectively. Then, a treatment experiment is designed using aquatic plants, each separately, and antibiotics and heavy elements are measured in addition to some water properties, such as pH, EC, Ca and Mg- hardness, total hardness, total dissolved solids, chlorides, nitrates, nitrites and phosphates, after 5, 10, 15, 20 and 25 days of plant treatment. The results showed that the pH values increased slightly after plant treatment, while the plants did not record a clear role in reducing calcium and magnesium hardness, total hardness, chlorides, electrical conductivity and total dissolved solid. On the contrary, the percentages of some of these indicators such as total hardness, magnesium hardness, total dissolved solids and electrical conductivity increased during plant treatment. The experimental plants also proved their effectiveness in removing antibiotics, and the highest removal rate was by C. demersum for the four antibiotics: Amoxicillin 40%, Azithromycin 91%, Ampicillin 91.7%, Ciprofloxacin 93.6%. While Azolla sp. is the least effective in removing antibiotics, as it recorded the highest removal rate of 82.9% for Azithromycin on the 25th day of the experiment.

Keywords: Treatment wastewater, antibiotics, phytoremedation,aquatic plants.

Introduction

Hospital wastewater has gained enhanced scientific and public attention in the last decade because it is a source for undesirable compounds such as multi antibiotic and heavy metals that cause toxicity for all sources of water [1]. The occurrence of pharmaceuticals in the water is considered an emerging, pollution and problem in the developed countries where the these pollutants and micro pollutants to the sources of water as drinking water[2]. Because of the occurrence of pharmaceutical waste such pathological, chemical, infectious, radioactive could be dangerous to public health and to ecological balance, where they excreted mainly with faces, can leading to diarrhea epidemics outbreaks of communicable disease, water contamination and radioactive pollution [3] . The found of micro pollutants such as pharmaceuticals in the water or wastewater lead to greater awareness to search of effective methods to treatment and removal them from water specially they might cause serious health consequences for many organisms such harmful effects on the functioning of the endocrine system of organisms, so removal these pharmaceutical from water considered important step to treated hospital wastewater, also the separation treatment of the pharmaceuticals lead to reduce the load of them in the wastewater treatment plants [4]. The antibiotics such as amoxicillin, ampicillin, azithromycin, and ciprofloxacin recorded high values especially Ciprofloxacin and Amoxicillin. And hese antibiotics identified and recorded as hazardous pollutants released into water bodies by many studies [5].

The traditional methods of wastewater treatment plants are not sufficiently effective in elimination these compounds especially biological methods that usually used in the wastewater treatment plant of hospital or municipal which results in their presence in natural waters [4]. Phytoremediation is a technology that use different types of plant to reduce the concentrations or toxic effects of contaminates in the many types of environments such as surface water, ground water, soil…etc , It is relatively recent technology and is perceived as cost-effective , ecofriendly , efficient ,novel and solar-driven technology with public acceptance [6]. Several processes are used in phytoremediation, including phytoextraction, also known as phytoaccumulation, which entails the uptake of heavy metals in plant roots and their subsequent transfer into parts of the plant that are above ground level, such as shoots, etc [7]. The characteristic that must available in the plants to use it in the phytoremediation are native, a quick growth rate and high biomass yield these characteristics enhance ability of plant to uptake of large amount of heavy metals and transports it in above ground parts of plant and a mechanism to tolerate metal toxicity, others factors like salinity greatly, solar radiation, nutrient availability and pH, influence the phytoremediation potential and growth of plant ,In general, phytoremediation low costly and effective green emerging technology, for successful phytoremediation the selection of plant species is the most significant aspect where some aquatic plants improve the efficiency for removal of organic and inorganic pollutants [8] . In this work, we aimed studied the ability of three aquatic plants, C. demersum, L. minor, and Azole sp, to treat four antibiotics present in the wastewater of Al-Sadr Medical City Hospital.

Material and methods

a- Hydrophytes

Aquatic plants The *L. minor* and *C. demersum* plants collected from multiple places in Najaf Governorate and confirmed the species and classified in the College of Science /University of Babylon, While the *Azolla sp.* was purchased from its own farms in Balad District, Salah Al-Din Governorate. These plants washed well with tap water and placed in plastic containers with dimensions of (70 x 30 x 35), and left Plants with tap water for a period of two weeks for the purpose of acclimatization. After, the plants are placed in 20 liters of wastewater at a rate of 10 g / liter with leaving containers that contain only untreated hospital wastewater only which represented the negative control [9]. And leaving other containers containing tap water with *L. minor* plant, *C. demersum* plant and *Azolla sp.* Separately to represent agroup of positive control for aquatic plants extracts experiment. An amount of water (250 ml) is withdrawn every 5 days until the 25thday for estimating the concentration of some antibiotics and conducting some physical and chemical tests.

b- Physical and chemical analysis

pH, EC (µs/cm) and TDS (mg/l) of water was measured using a pH – meter (multi - parameters), Oakton - U.S.A after calibrate a device with solutions that used to calibrate it.

The alkalinity was estimated by using the titration method according to [10]. Total hardness was measured according to method [10], which included titrating the sample with Na2-EDTA. Both Calcium hardness and magnesium were measured according to the titration methods , while Chloride was determined by titration [11].

c- Antibiotic concentrations

Samples were analyzed by high performance liquid chromatography HPLC model (SYKAM) Germany .The mobile phase is = (acetonitrile : methanol : buffer solution) and detector UV – 230 nm at flow rate 1ml/min[12].

Statistical analysis

Data was analyzed using SPSS (version 20, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean ± standard deviation (SD)). Statistical analysis was carried out using t-test student test for comparing between two groups, followed factorial experiments by using least significant difference (LSD). The value of $p \leq 0.05$ was considered to be statistically significant.

Results

1- Characteristics of Al-Sadr clinical city hospital wastewater before treatment

1-1 The physical and chemical characteristics

The physical and chemical characteristics of the wastewater collected from the Al-Sadr clinical city that collected in 16 / 10 / 2023 shows in table (1).

Table (1): Some physical and chemical properties and Antibiotic concentrations of Al-Sadr clinical city wastewater before phytoremediation.

Measured factors	Values
pH	7.1
EC (µs/cm)	985
TDS (mg/l)	690

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Total hardness(mg/l)	1200
Ca^{+2} (mg/l)	328.65
Mg^{+2} (mg/l)	90.7
Total alkalinity(mg/l)	1020
Chloride (Mg /l)	309.90

1-2 Antibiotic concentrations

Table (2): Antibiotic concentrations in Al-Sader clinical city wastewater before phytoremediation.

Antibiotic	**Concentration (mg/l)**
Amoxicillin	112.5
Ampicillin	62.5
Azithromycin	80.9
Ciprofloxacin	124.5

2-AfterPhytoremediation

Some physical and chemical characteristics of wastewater after phytoremediation

The statistical results showed significant differences ($p \leq 0.05$) in the pH, EC, TDS, Total hardness, Ca hardness, and Mg hardness values in the treated water among both of days and plants as explained in the following table.

Table (3) : some physical and chemical properties and nutrients of Al-Sader clinical city wastewater after phytoremediation.First line (mean ± standard deviation), second line (Range)

Days	**Treat.**	**pH**	**EC µs/cm**	**TDS mg/l**	**Total hardness**	**Ca hardness**	**Mg hardness**
		Mean ±S.D Range					
5	Control	8.27±1.1 (8.2-8.4)	932.33±12.5 (924-944)	766.67±8.8 (757-774)	1393.33±18.5 (1300-1450)	378.08±15.1 (360.7-388.8)	107.83±10.7 (96-116.8)
	Azolla sp.	8.47±1.3 (8.4-8.5)	914.33±9.9 (887-936)	760.33±20.6 (753-766)	1323.33±68.1 (1270-1400)	351.37±27.2 (320.6-372.7)	107.43±22.4 (81.5-120.4)
	Lemna minor	8.33±0.9 (8.2-8.5)	924.67±13.2 (923-928)	743.33±13.2 (742-745)	1376.67±18.2 (1300-1430)	398.13±16.1 380.8-412.8)(	92.07±10.1 (84-103.4)
	Ceratophyllum demersum	8.50±1.4 (8.4-8.6)	948.33±11.7 (942-955)	751.67±9.1 (748-755)	1383.33±15.7 (1300-1500)	380.76±17.4 (360.7-392.8)	104.20±35.8 (76.7-144.7)
10	Control	8.30±0 .7 (8.3-8.3)	1226.00±20.4 (1212-1243)	848.00±11.2 (823-865)	1343.33±40.4 (1300-1380)	428.85±8.1 (420.8-436.9)	108.25 ±9.2 (54.8-71.8)

	Azolla sp.	8.47±2.1 (8.4-8.5)	1230.33±11.2 (1220-1237)	867.00±5.6 (862-870)	1460.00±17.3 (1450-1480)	414.16±14.1 (400.8-428.9)	103.00±10.2 (91.2-109.5)
	Lemna minor	8.07±1.9 (8-8.1)	1244.00±15.7 (1209-1311)	852.33±20.3 (844-857)	1363.33±47.2 (1310-1400)	415.49±6.1 408.8-420.8)(	103.27±8.5 (69.4-86.4)
	Ceratophyllum demersum	8.00±1.3 (7.9-8.1)	1252.67±10.1 (1212-1318)	859.33±12.2 (855-867)	1406.67±22.6 (1360-1460)	410.15±10.1 (400.8-420.8)	104.76 ±9.4 (80.3-97.3)
15	Control	9.03±1.7 (8.9-9.2)	1253.33±30.9 (1242-1261)	842.67±6.6 (820-863)	1323.33±15.2 (1310-1340)	348.69±8.2 (340.7-356.7)	108.67±3.1 (105.8-111.9)
	Azolla sp.	8.43±1.4 (8.4-8.5)	1325.67±14.4 (1302-1353)	912.67±5.9 (900-921)	1536.67±5.8 (1530-1540)	419.50±10.1 (408.8-428.9)	117.90±7.6 (110.7-125)
	Lemna minor	8.27±1.2 (8.2-8.3)	1263.00±18.8 (1261-1267)	880.33±17.2 (873-886)	1493.33±15.1 (1480-1510)	408.81±8.1 (400.8-416.8)	113.57±7.1 (108.3-121.7)
	Ceratophyllum demersum	8.30±1.1 (8.2-8.4)	1285.00±14.3 (1279-1291)	861.67±11.5 (804-895)	1463.33±16.3 (1450-1480)	414.16±8.1 (408.8-420.8)	105.00±6.9 (97.3-110.7)
20	Control	8.93±1.3 (8.8-9.1)	1298.67±16.2 (1297-1301)	913.67±7.7 (910-918)	1433.33±15.3 (1420-1450)	336.67±6.9 (328.7-340.7)	142.30±8.5 (136.2-152)
	Azolla sp.	8.93±1.1 (8.8-9.1)	1366.00±13.3 (1335-1388)	947.00±12.2 (930-966)	1690.00±6.2 (1620-1740)	394.11±11.1 (384.8-404.8)	165.80±10.1 (154.5-173.9)
	Lemna minor	8.40±0.7 (8.2-8.6)	1339.00±11.7 (1298-1409)	913.67±15.3 (910-917)	1570.00±20 (1550-1590)	394.12±2.3 392.8-396.8)(	141.50±6.1 (135-147.2)
	Ceratophyllum demersum	8.50±1.9 (8.4-8.6)	1310.67±10.6 (1305-1315)	921.67±14.7 (905-940)	1543.33±17.8 (1520-1570)	398.13±3.4 (396.8-400.8)	136.25 ±7.3 (125.3-139.9)
25	Control	8.83±1.0 (8.7-8.9)	1390.33±14.1 (1385-1396)	962.67±11.8 (954-969)	1516.67±5.7 (1510-1520)	324.64±6.8 (320.6-328.7)	161.30±3.5 (159.3-165.3)
	Azolla sp.	8.83±1.2 (8.8-8.9)	1390.33±15.6 (1385-1396)	986.33±12.3 (969-995)	1823.33±5.5 (1770-1880)	364.72±3.6 (360.7-368.7)	218.67±11.6 (206-229)

2-2 Antibiotic concentrations

The wastewater of Al-Sadr Medical City before the bio treatments recorded elevated concentrations of four antibiotics: Amoxicillin, Ampicillin, Azithromycin and Ciprofloxacin as showed in table (2) and after the treatment with aquatic plants under study all were deceased significantly as illustrated in the tables (4).

Table (4) Antibiotic concentrations in the Al-Sadr Medical city wastewater after phytoremediation.

Days (B)	**5**	**10**	**15**	**20**	**25**	**Mean of plant (A)**
Plant (A)	Mean±S.D					
Amoxicillin						
Control	90.40±1.4	0	0	0	0	18.08±7.9
Azolla sp	74.13±1.6	0	0	0	0	14.83±13.2

Lemna minor	71.27±0.7	0	0	0	0	14.25±9.6
Ceratophyllum demersum	67.43±1.3	0	0	0	0	13.49±7.7
Mean of days (B)	75.81±9.2	0.00	0.00	0.00	0.00	
LSD (0.05)	(A)		(B)		(A*B)	
	0.339		0.379		0.758	
Ampicillin						
Control	51.07±0.7	38.03±0.9	31.33±0.5	21.80±0.3	16.40±0.6	31.73±12.3
Azolla sp	46.00±1.1	30.63±0.5	21.47±1.2	17.27±0.4	13.00±0.2	25.67±10.2
Lemna minor	41.83±1.1	21.37±1.2	15.43±0.6	11.97±0.4	8.00±0.9	19.72±8.8
Ceratophyllum demersum	32.10±1.2	15.77±1.0	10.17±0.6	7.70±0.4	5.17±0.3	14.18±6.2
Mean of days (B)	42.75±7.3	26.45±8.9	19.60±8.2	14.69±5.6	10.64±4.6	
LSD (0.05)	(A)		(B)		(A*B)	
	0.468		0.523		1.047	
Azithromycin						
Control	67.13±0.8	53.70±2.3	40.20±0.4	31.30±0.6	22.33±1.1	42.93±12.3
Azolla sp	55.97±0.8	43.77±2.2	32.43±1.3	23.50±1.2	13.83±1.1	33.90±5.9
Lemna minor	54.10±1.5	38.20±1.4	21.13±0.7	15.30±0.3	8.50±0.5	27.45±7.8
Ceratophyllum demersum	43.43±1.7	31.10±1.4	19.23±0.5	13.83±0.3	7.23±0.6	22.96±8.4
Mean of days (B)	55.16±8.8	41.69±8.7	28.25±8.9	20.98±7.3	12.97±5.2	
LSD (0.05)	(A)		(B)		(A*B)	
	2.728		2.814		3.629	
Ciprofloxacin						
Control	99.07±0.7	73.77±0.8	51.87±0.9	42.63±0.7	32.57±0.6	59.98±17.4
Azolla sp	75.07±0.7	58.83±1.0	41.17±3.1	33.30±0.6	24.87±0.3	46.65±18.2
Lemna minor	72.03±0.8	50.97±0.4	35.07±1.7	24.93±1.0	15.53±0.6	39.71±20.1
Ceratophyllum demersum	59.10±1.0	43.00±1.8	23.50±0.9	15.60±0.3	7.90±0.4	29.82±19.3
Mean of days (B)	76.32±15.0	56.64±11.9	37.90±10.7	29.12±10.4	20.22±9.7	
LSD (0.05)	(A)		(B)		(A*B)	
	0.530		0.592		1.185	

Discussion

In the current study, Al-Sadr Medical city hospital wastewater treatment plant recorded many water indicators before starting the treatment period with aquatic plants, where, the water is hard and has avery bad smell and grey in color with un increase in EC, TDS, T.H , T.A, Cl. Similar evaluate study of the wastewater treatment plant in Al-Sadr teaching hospital in Basrah recorded high levels in these indicators [13]. In the current study High pH levels lead to alkalinity and poor water quality, resulting in water mixed with sewage, medical and pharmaceutical chemicals, and other organic solutions, where the highest value recorded (8.43 - 8.93) by *Azolla* sp. The increasing pH in wastewater may be due to the free CO_2 is below its equilibrium with air, thus, the reduction of CO_2 tends to cause a shift in the form of alkalinity present from bicarbonate to carbonate, and from carbonate to hydroxide plants decreasing carbon dioxide through photosynthesis [14]. *Azolla sp.* shows a slight reduce rate (7.2%) of electrical conductivity during treated water of Al-Sadr medical city hospital in the fifth day of the experiment, this results similar to another study where *Azolla sp.* recorded a slightly reduced rate of electrical conductivity [15]. Also, *L.minor* plant recorded a slight removal rate (6.1%) of the electrical conductivity on the fifth day of the experiment, while, the electrical conductivity values increased during the 10,15, 20, and 25 days of the experiment. similar results recorded increase of the conductivity during the treated period by *Azolla sp*. [16]. The reason may by attributed this increase to the role of plants in the analysis of compounds containing mineral elements in the process of (phytodegradation). the total dissolved solids increase as period of treatment increase where this shows the inefficiency of plants in reduce TDs, this finding is similar to the results of the study that included increase in the percentage of TDs during phytoremediation for hospital wastewater [17]. The breakdown processes exceed the plants' capacity to mitigate them, particularly as the current findings reveal the plants' ineffectiveness in lowering magnesium and chloride levels., which are considered basic components of dissolved solids These results are consistent with previous study [18]. The result showed that the *L. minor* recorded reduction rate (15.6 %) on the fifth day of the experiment ,thus recorded higher efficiency in reducing total alkalinity compared to the rest of the plants and this study was in agreement with Raju *et al*., [19]which showed the efficiency of L. minor in treating such as total alkalinity .The reduction in total alkalinity may be due to the absorption of some dissolved solids, while the increase in total alkalinity concentrations in the last days of treatment with plants may be due to the formation of acidic compounds resulting from the decomposition of organic materials such as carbonic acid, which works to dissolve calcium carbonate and convert it Around the world, hard water can lead to a variety of issues, such as increased scaling on boilers and water pipes, strange-tasting drinking water, Minerals that have dissolved, mostly calcium and magnesium compounds, are what give natural water its hardness, the United States Geological Survey (USGS) and the Water Quality Association (WQA) classify hard water as follows: 0–60 ppm is considered soft; 61–120 ppm is considered moderately hard; 121–180 ppm is considered hard; and more than 180 ppm is considered very hard water, these classifications are based on the $Ca^{+2}$and Mg^{+2} ion content in the water [20] into calcium bicarbonate. The results in the table (3) indicates that the plants under study are not efficient in reducing the values of both total hardness and calcium and magnesium concentration, this is consistent with the study of kadhim, (2017) which that both the *C. demersum* and *L. Minor* did not record any reduction rate during the treatment period. The inefficiency of used plants in reducing total hardness Ca^{+2} and Mg^{+2} may due to the fact that the water is loaded with organic materials in high concentrations, in addition to the transformation of large amounts of calcium into dissolved carbonates, leading to an increase in total hardness, this is agreement with another study that showed hardness increased after using the phragmites plant to treat polluted water in some wells [21]. Also *C. demersum*, is less efficient in reducing the ions of calcium and magnesium because it is very small if compared to rooted hydrophytes because roots of the plant are the most important tissues for aquatic plants to absorb and accumulate contamination [22]. The used plants in current study are not efficient in reducing chloride values in wastewater, this may be due to the fact that chlorine does not have a distinct role in the metabolism of plants, so it is absorbed in very small quantities, and Apte *et al*., [23]showed that the ideal degree of chlorine absorption lies between 6.9-7.4 mg/l, it is explained that the removal process is completed by increasing the concentration of the element and

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increasing the time of contact of the plant with the element. Research on using phytoremediation to clean up antibiotic pollution in waterways is growing, because aquatic plants and medicines interact complexly in the aquatic environment, an increasing amount of research suggests that aquatic plants can transport antibiotics within themselves, and that roots are the most crucial tissues for antibiotic absorption and accumulation [22]. Plants used in the current study *C. demersum, L. minor* and *Azolla* sp. have been shown to be common plants in their ability around the globe to treat water and removal antibiotics [24]. Also, aquatic plants that used in the treatment are influenced by plant species, antibiotic concentration, running time, antibiotic kinds and biomass, have a major impact on the elimination of antibiotics [25].

Conclusions

The effluents of treated wastewater of Al-Sadr medical city were polluted with elevated concentration of antibiotics amoxicillin, ampicillin, azithromycin and ciprofloxacin. Each of *C. demersum, L. minor and Azolla,* were affecting in remove antibiotics from wastewater. Best removing to antibiotics was by *C. demersum*, then by *L. minor* and *Azolla*.

Author Contributions Statement

The authors were all associated in this work by collecting samples, preparing them and planting the plant, as well as the analysis and writing.

Declaration of competing interest

The authors declare that there is no competing interest

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