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Phytoremediation of Wastewater from Al-Hindiyah District Sewage Water Treatment Plant

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Abstract. This study was conducted with the aim of applying Phytoremediation technology in order to identify the efficiency of *Ceratophyllum demersum* L. for improving the quality of wastewater discharging from tank of Al-Hindiyah district sewage water treatment plant in Karbala Governorate and included treatment before entering the water to the plant and treatment after its exit from secondary sedimentation without sterilization and with sterilization.The results showed that the temperatures were lower in the water treated with plants compared to the water in the control treatment. As for the pH, it was observed to increase by increasing the time period of the alkaline side in all treatments. As for the total dissolved solids (TDS) an increase in their values was observed in the *C. demersum* containers reaching (2515.3, 2208.5 and 2451.6) mg / L., for the pre-entry treatment and the post-entry treatment without sterilization and the post-entry treatment with sterilization and they differed Significantly with the control treatment without plant. *C. demersum* plant did not record efficiency in reducing the values of Electrical conductivity in all parameters, but on the contrary it values increased in the end of treatment. As for total hardness, *C. demersum* plant recorded the highest reduction rate after 9 days of Phytoremediation, and for calcium hardness, it decreased after the Phytoremediation. As for the reduced rates of magnesium hardness it increased on days 3, 6 and 9, after that no reduction rate was recorded in days 12. And 15 for all treatments. The results also showed that the *C. demersum* plant has a high efficiency in reducing nitrite concentrations throughout the treatment period, and it recorded the highest reduction rate after 12 days of treatment. As for the reduction of nitrate values, the highest reduction rate was reached after 12 days of treatment with (82.58, 84.65 and 91.45)% for the pre-entry treatment and post-entry treatment without sterilization and post-entry treatment with sterilization, respectively and they differed Significantly with the control treatment without plant.As for the efficiency of the *C. demersum* plant in removing the Cadmium, the removal rate reached (89.64, 96.64 and 84.58)%, and for its efficiency in removing Nickel it reached (84.90, 85.65 and 60.77)% and for its efficiency in removing the Lead, it reached (83.93, 92.87 and 73.98) % After 12 days of plant treatment in pre-entry and post-entry treatment without sterilization and post-entry treatment with sterilization, respectively. *C. demersum* plant showed an effective role in reducing Cd, Pb and Ni based on the accumulation of these metals in the tissues of the plant at the end of the treatment, as the rate of Cd accumulation in the *C. demersum* plant reached (73.58, 52.74 and 51.34) μg /g, and the rate of accumulation of the Pb metal in the tissues of the *C. demersum* plant (90.52, 70.66, and 69.31) μg /g and for Ni metal, the rate of accumulation in the *C. demersum* plant was (93.43, 85.51 and 84.37) μg /g for each of plants treatment in pre-entry and post-entry treatment without sterilization and post-entry treatment with sterilization, respectively.

Keywords. Heavy Metal · Wastewater · *Ceratophyllum demersum* · Sewage water.

INTRODUCTION

Water shortage is an indication of a real threat to the environment, and climate change, water scarcity and drought that the world is witnessing, especially during hot seasons, makes it necessary to adapt projects to reuse

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wastewater, such as treated wastewater for irrigation at least, it is known that water covers nearly three-quarters of the Earth's area. However, the constant misuse and waste have made the world in urgent need of water, for example in Iraq, water is consumed around 7.8 million m3 / day, and 5.8 million m3 / day is returned to water bodies, drains and rivers, including the Tigris and Euphrates[1].

Global statistics showed that water use has increased three times since 1950, that one in six people has no access to safe drinking water, and that the lack of safe drinking water has affected the health of 1.2 million people annually[2]. And forecasts of the United Nations Environment Program (UNEP) United Nations Environment Program show that about a third of the world's population live in countries suffering from water scarcity at rates ranging from moderate to high water stress, as they consume 10% of renewable freshwater resources [3]. The water crisis that the world is witnessing today is the result of the poor distribution of this wealth and the lack of recycling in addition to the wrong disposal and many other factors that helped spread this crisis [4]. Sewage water consists of domestic wastewater, industrial wastewater, rainwater, and groundwater infiltration into the sewage network. Household wastewater consists of liquid discharges from homes, institutions, and commercial and industrial buildings, and the raw wastewater available from cities is a mixture of domestic, commercial and industrial activities [5]. The drainage of sewage water into rivers without treatment or inefficient treatment by water treatment plants leads to great damage to the water environment because that water contains high concentrations of harmful environmental pollutants, which is a general case in Iraq, as most of the reeds of cities are located on the banks of rivers. The damage caused by the disturbance of the environmental balance and the occurrence of nutrient enrichment in rivers, which is summarized by the growth of unwanted organisms at the expense of other important organisms such as fish, the spread of pathological microbes, and the increase in the concentrations of other determinants such as carcinogenic phenolic compounds, oxygen-consuming waste and other determinants of pollution and the sharp decline in river levels Its low flow rate exacerbates this damage [6].The term phytoremediation is known as green technology is the use of plants in the treatment of organic and inorganic pollutants by removing them, restricting their movement or reducing their toxicity, and it is an effective technique in removing heavy metals and storing them in plant parts such as roots, stems or leaves, or transforming them into less dangerous substances or gases that are liberated. To air by transpiration [7].

Plants possess latent cellular mechanisms that can include detoxification of heavy metals and then resistance to metal stress. Some plants also resist the influence of heavy metals by stimulating biochemical responses, which include peroxides and other enzymes that counteract the metabolic stresses caused by these metals [4].Also, some plant roots secrete specialized organic materials such as Citrate, Malate and Histidin that can form chelating compounds with some heavy metals and reduce the intake of toxic free metal ions from plant cells [8]. A laboratory study by [9] The efficiency of *C. demersum* plant in removing the cadmium metal by controlling the pH (pH 3-8) in the experimental ponds. They observed an increase in the ability of *C. demersum* to absorb cadmium metal by changing the pH, and the highest removal efficiency was obtained at pH = 7, reaching 99 %.

[10] indicated the efficiency of *C. demersum* plant and the European frog plant *Hydrocharis morsus-ranae* in the bioaccumulation of a number of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn) and were able to adopt them as a reliable biomarker for mineral water contamination. Heavy.

In the present study aimed to use *C. demersum* plant as Phytoremediation methods to investigating it effectiveness to treat the physical and chemical properties of wastewater and removing heavy metals in one of the sanitation stations in the Karbala Governorate, Iraq.

MATERIALS AND METHODS

Water Sample collection

Samples of wastewater were collected from the sewage treatment station located in Karbala Governorate, Al Hindiyah District on the road leading to Babylon Governorate- Iraq samples were taken 15/12/2019

FIGURE (1). The station is supplied with water from the city of Al-Hindiya through the conveyor line, meaning that the sewage water in this station contains household waste, restaurant waste, and industrial waste.

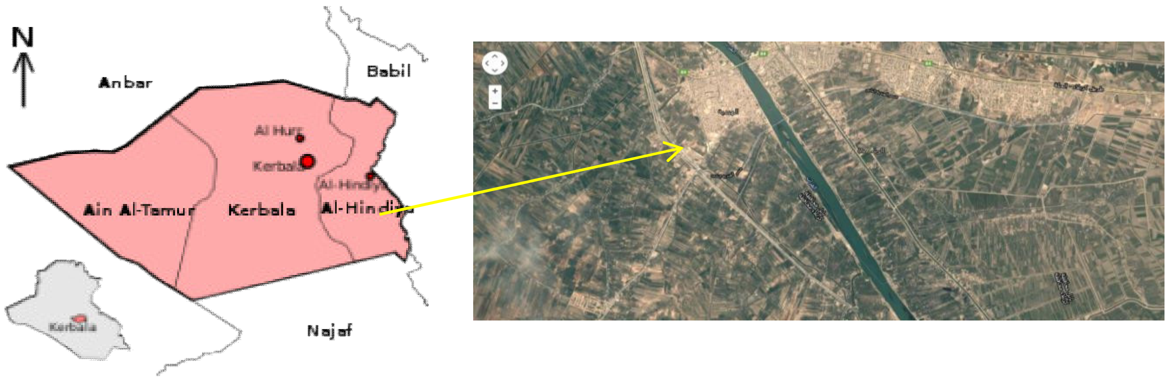

FIGURE 1.A map representing the site of the study taken from a sitehttps://www.google.com/earth

Plant Sample collection

The *C. Demersum* plant was collected using polyethylene bags that were marked with the addition of a little water from the same site from which it was collected, and in the laboratory it was washed well with tap water and then warm water to remove suspended materials and placed in containers with dimensions (35x30x70) and the plants were left for two weeks for the purpose of acclimatization and discarding the suspended pollutants. After that, the plants were placed at a rate of 10g/liter [11], with the addition of 20 liters of industrial and household wastewater by means of 20-liter polyethylene containers, leaving containers representative of control that included wastewater untreated by plants, the following treatments were carried out in three replicates for each treatment with leaving containers as a control treatment that includes wastewater without plants

1. Treating wastewater before entering a wastewater treatment station.
2. Treatment of wastewater after entering without disinfection of water
3. Treating wastewater after entering with sterilization of water

As for the sterile water treatment, it was sterilized with an Autoclave at a temperature of 121 ° C and a pressure of 15 pounds / inches for a period of (15) minutes. The purpose of the sterilization was to kill the micro-organisms present in the wastewater and to see the efficiency of the plant in removing the pollutants. Physical and chemical tests were carried out and the assessment of heavy metals (Cadmium, Lead and Nickel) By withdrawing a quantity of water after (3,6,9,12,15) days.

Physical and chemical measurements

1. **Temperature** the water temperature was measured by a Hanna multi-meter.
2. **Electrical conductivity(EC)**was measured with a Hanna multi-meter and the results were expressed in(µ/cm).
3. **pH** was measured using a multi-pH meter manufactured by Hanna.
4. **Total dissolved solid (TDS)** were measured using a multi-meter device manufactured by Hanna and results was expressed in units (mg /L) [12].
5. **Total hardness** were determined according to APHA [13].
6. **Hardness of calcium and magnesium** Calcium hardness was measured according to what was mentioned in [13]. As for magnesium, it was estimated in the sample according to what was mentioned by the computational method in [14], and it is based on both total hardness and calcium hardness.
7. **Nitrite** Were determined according to the method described by [15].
8. **Nitrate** were determined according to the Cadmium column reduction method described by [15].
9. **Extraction of Total Dissolved Heavy Metals** 50 ml of the sample water was taken from the three aforementioned treatments and digested by adding 5 ml of concentrated nitric acid $HNO3$ and the samples were heated on a hot plate at a temperature of 80 ° C and 1 ml of concentrated nitric acid was added to it and the solution was left until the dissolution is complete on the hot plate and then the volume was completed to 50 ml with water The ion-free distillate was stored in polyethylene bottles until the concentrations of heavy metal ions (Cadmium, Lead and Nickel) were measured using a flammable atomic absorption spectrophotometer, and the product was expressed in mg /L [16]. The removal percentage of the following heavy metals was calculated [4]

$$R\% = ((C0-C1) / C0) * 100 \quad (1)$$

whereas : **R** = Percentage removal% **C0** = concentration of heavy metal ions in the primary solution (mg / L). **C1** = concentration of heavy metal ions in the final solution (mg / L).

Extraction of heavy metals from aquatic plant samples

the plant samples were washed with tap water, then with warm distilled water at a temperature of 38 ° C to remove the small invertebrates trapped in them [17]. After that, the plant parts were washed with distilled water free of ions and dried at a temperature of (70) C, then milled and passed through a sieve with holes of (40) mesh. 2.5 ml of nitric acid to 0.5 g of the plant sample for 24 hours and then we put it at a temperature of 80 ° C for an hour on a thermal plate, after which it is air-cooled for a period of time, then we add 2.5 ml of pyrochloric acid (HClO4) at a temperature of 180 ° C for a period From 2 to 3 hours on a hot plate, until the color turns from dark brown to a clear colorless, then we filter the samples with Whatman No. 42 filter paper, then the volume is supplemented to 10 mL, after which Lead and Cadmium are measured by an atomic absorption spectrophotometer [18]. After that, the concentrations of heavy metals (Cadmium, lead, and Nickel) were measured using an atomic absorption spectrophotometer, and the product was expressed in µ/g dry weight. Heavy metals removal was calculated.

Statistical analysis

Data are presented as mean±SD. For all statistical analyses using the SPSS Version 9 program[19].Significant differences among treatments were tested by using CRD analysis In all cases, under probability $P \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Preliminary specifications of wastewater before treatment

Table 1 shows the preliminary specifications of the wastewater before treatment that was collected from the sewage plant in Al-Hindiya district / Karbala governorate, before entering the station and after entering the plant from the sedimentation basin without sterilization, in addition to the sterile water specifications after entry. It is noted from Table 1 when comparing them with the local standard specifications that most of the studied characteristics exceeded determinants, especially for the metals of Cadmium, Lead and Nickel compared to the permissible limits according to the Iraqi determinants for the year 2012 [20].

TABLE 1. Preliminary specifications for wastewater before treatment

The studied characteristics	pre-entry	post-entry treatment without sterilization	post-entry treatment with sterilization	The permissible limits
TDS mg/L	1550	1410	1380	2500
E.C. µs / cm	1780	1620	1610	3140-3600
pH	6.8	7.5	7.5	6.5-8.5
The temperature C°	34.7	34.4	34.1	less than 35
Total hardness mg /L	715	650	550	-
Calcium hardness mg/L	180	160	140	450
magnesium Hardness mg/L	63.624	60.046	48.008	80
Nitrates mg / L	11.614	21.351	20.271	50
Nitrite mg / L	2.171	0.895	0.794	-
Cadmium mg / L	1.928	0.545	0.536	0.01
Nickel mg / L	2.769	0.815	0.827	0.2
Lead mg/L	4.649	1.631	1.658	0.1

Physical and chemical properties of wastewater after treatment

Table (2) shows the specifications of wastewater after treatment with *C. demersum* plants in a treatment before entering the water to the station, where a decrease in the average concentrations of the studied traits was observed in plant ponds compared with the control treatment without plants, and significant differences were observed between plant treatment and the treatment without plants.

TABLE 2. The physical and chemical properties of wastewater treated with plants before entering the first line range and the second line (mean ± standard deviation)

The studied characteristics	without plant	with plant	L.S.D 0.05
TDS mg / L	1813.6 – 3911.6[a] 2776±147.96	1650 – 3613.3[b] 2515.3±177.02	362.8
E.C. µs / cm	2011.6 – 4283.3[a] 3272.6±210.6	18331.6 4214.6[b] 3110.2±252.65	480.8
pH	7.133 – 7.933[a] 7.613±0.144	7.433 – 8.733[b] 8.289±0.075	0.2177
Temperature C°	30.067 – 34.167[a] 31.853±0.124	28.800 – 33.100[b] 30.473±0.236	0.3731
Total hardness mg / L	803.33 – 1723.33[a] 1287.33±102.05	633.33 – 740.00[b] 604.33±25.13	142.9
Calcium hardness mg / L	195.33 – 367.67[a] 275.80±13.79	117.33 – 152.67[b] 136.13±6.75	21.17
Magnesium hardness mg / L	75.67 -194.20[a] 143.85±21.87	45.42 – 88.07[b] 63.48±8.23	35.32
Nitrate mg / L	12.704 – 20.815[a] 16.694±0.715	2.023 – 11.178[b] 5.949±0.493	1.132
Nitrite mg / L	2.575 – 3.850a 3.213±0.196	0.381 – 1.953b 1.058±0.201	0.412

Different letters mean a significant difference under probability $P \leq 0.05$

Table (3) shows the physical and chemical characteristics of wastewater treated with plants after entering without sterilization, and through it, high significant differences are observed between the treatments and for all the studied characteristics, as the average concentration of total soluble solids, total hardness, calcium hardness, magnesium hardness, nitrates and nitrites in the treatment of *C. demersum* (2208.5, 528.1, 112.53, 59.38, 9.890 & 0.109) mg/L, respectively, compared to the control treatment (no plant), as the mean concentrations were (2449.8, 1149.86, 256.93, 122.06, 26.852, & 0.596) mg/L, respectively.

It is also noticed from Table (3) that the values of electrical conductivity, temperature and pH were less in the plant treatment basins than the control basins, with clear significant differences.

It is noted from Table (4) the physical and chemical characteristics of wastewater treated with plants after entering with sterilization of sewage water that there are high significant differences between the treatment of the *C. demersum* plant and the treatment of the absence of the plant in all the studied characteristics, as the average concentration of total dissolved solids, Total hardness, calcium hardness, magnesium hardness, nitrates and nitrites in the *C. demersum* plant treatment (2451.6, 473.00, 108.73, 48.366, 8.029 & 0.242) mg /L, respectively, compared to the control treatment (no plant), as the average concentrations were (2622.6, 1055.33, 228.53 , 116.44, 20.213 & 0.790) mg /L, respectively.

It is noted from the foregoing that the physical and chemical properties studied in Table (3) were better than the specifications in Table (2) and (4) and this may be due to the lack of activity of micro-organisms analyzing the elements in treatment before entering Table (2) as well as to the absence of microorganisms. Because of the sterilization of water in the treatment after entry with sterilization, Table (4).

TABLE 3. The physical and chemical properties of wastewater treated with plants after entering without sterilization the first line range and the second line (mean ± standard deviation)

The studied characteristics	without plant	with plant	L.S.D
TDS mg / L	1471.6 – 3668.6[a] 70.99 ± 2449.8	1456.6 – 3242.3[b] 50.62 ± 2208.5	135.2
E.C. µs / cm	1746.6 – 4076.6[a] 101.45 ± 2839.0	1693.3 – 3630.0[b] 1108.12 ± 2475.0	211
pH	7.633 – 8.333[a] 0.058 ± 8.020	7.867 – 8.867[b] 0.066 ± 8.500	0.1077
Temperature C°	29.733 – 33.833[a] 0.277 ± 31.260	27.633 – 32.767[b] 0.213 ± 29.680	0.6388
Total hardness mg / L	722.66 – 1640.0[a] 78.51 ± 1149.86	420.0 – 632.6[b] 19.47 ± 528.1	114.9
Calcium hardness mg / L	178.67 – 328.67[a] 8.622 ± 256.93	95.67 – 133.33[b] 5.753 ± 112.53	13.76
Magnesium hardness mg / L	66.28 – 198.69[a] 22.21 ± 122.06	39.43 – 84.57[b] 5.23 ± 59.38	31.598
Nitrate mg / L	22.855 – 30.960a 0.667 ± 26.852	3.278 – 19.018b 0.672 ± 9.890	1.198
Nitrite mg / L	0.256 – 0.835a 0.057 ± 0.596	0.002 – 0.270b 0.025 ± 0.109	0.0715

- Different letters mean a significant difference under probability $P \leq 0.05$

TABLE 4.The physical and chemical properties of wastewater treated with plants after entering with sterilization the first line range and the second line (mean ± standard deviation)

The studied characteristics	without plant	with plant	L.S.D
TDS mg / L	1560.0 – 3843.3a 2622.6±95.86	1433.3 – 3563.3b 2451.6±86.092	192.4
E.C. µs / cm	1796.6 – 4176.6a 3008.6±212.08	1730.0 – 3796.6b 2806.6±84.85	332
pH	7.633 – 8.167a 7.940±0.089	7.833 – 8.800b 8.413±0.098	0.1645
Temperature C°	28.133 – 33.367a 30.307±0.088	27.600 – 32.633b 29.113±0.176	0.2675
Total hardness mg / L	588.33 – 1675.00a 1055.33±70.86	395.00 – 536.66b 473.00±11.46	118.9
Calcium hardness mg / L	158.33 – 292.00a 228.53±9.693	94.00 – 128.00b 108.73±3.412	14.35
Magnesium hardness mg / L	51.03 – 227.73a 116.44±20.44	35.011 – 62.988b 48.366±3.381	30.511
Nitrate mg / L	20.170 – 20.251a 20.213±0.088	1.733 – 17.561b 8.029±0.645	0.985
Nitrite mg / L	0.783 – 0.794a 0.790±0.005	0.068 – 0.546b 0.242±0.056	0.0698

- Different letters mean a significant difference under probability $P \leq 0.05$

It is also noted that there is a decrease in the concentrations of most of the studied traits compared to their concentrations before the plant treatment on the day (0) Table (1). Before entering the plant and after entering without sterilization and after entering with sterilization) its ability to remove many pollutants with different removal efficiency, while there was no reduction of the studied characteristics in the comparison treatment without plants, and the results also showed that the pH value in the basins containing plants was higher compared with the comparison basins. (Without vegetation) This may be due to the plant's photosynthesis process and the consumption of carbon dioxide in the form of bicarbonate and carbonate, which leads to an increase in the pH [21]. The results in Tables (2, 3 and 4) indicated an increase in the value of electrical conductivity in plant ponds compared to comparison basins. The reason may be because the electrical conductivity value depends on the concentrations of dissolved salts[22]. The reason may also be attributed to an increase in the amount of some unmeasured salts by experiment.

Heavy metals phytoremediation treatment

Cadmium *C. demersum* plant recorded high efficiency in reducing Cadmium metal concentrations from wastewater, as it is noticed from Table (5) in a treatment before entering a significant decrease in Cadmium concentrations before and after treatment, as its concentration before treatment in Table (1) was 1.928 mg / liter, and then its decrease was observed. At the first reading, after 3 days of treatment, it reached 1.249 mg / l with a reduced efficiency of 35.22%. The *C. demersum* plant continued to reduce the Cadmium metal concentrations to reach after 12 days of treatment at a concentration of 0.200 mg / liter, with a removal efficiency of 89.64%.

TABLE 5. Cadmium values and percentage of its reduction in treating water before entry

Treaetment	Transaction time period (in days) rate					Average
	3	6	9	12	15	
C. demersum	1.249 (%35.22)	0.940 (%51.23)	0.504 (%73.85)	0.200 (%89.64)	0.222 (%88.47)	0.623
Control	1.519 (%21.21)	1.429 (%25.90)	1.305 (%32.31)	1.199 (%37.81)	1.031 (%46.52)	1.297
Average	1.384	1.185	0.905	0.669	0.627	

- •L.S.D for treatments at the level of 0.05 = 0.1587 • L.S.D for the time at the level of 0.05 = 0.2510
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.3549
- Numbers in parentheses represent the Percentage removal%

Table (5) also shows that there is a decrease in the values of Cadmium concentrations in the control treatment without plants, as the lowest concentration of Cadmium was 1.031 mg /L after 15 days of treatment, with a reduction efficiency of 46.52%, and high significant differences were observed between the plant treatment and the control treatment.

It is noted from Table (6) the efficiency of the *C. demersum* plant in water after entering without sterilization, as it was noticed that the highest reduction efficiency reached 96.64% after 12 days of treatment, and the Cadmium concentration in the treated water was 0.018 mg / liter, which is considered within the recommended standards locally and internationally. He also notes rise in Cadmium metal after 15 days of treatment to reach 0.029 mg / liter, which may be due to the deterioration and decay of weak plants and their decomposition, and this in turn leads to the excretion of metals in plant tissues, including Cadmium, into the medium. Table (6) also shows that there was a noticeable increase in the reduction of Cadmium metal in the control treatment without plants, and to a lesser degree than what was observed in the treatment of plants, as the highest reduction efficiency reached 61.04% after 15 days of experimentation and that the Cadmium concentration in it was not within the Iraqi standard specifications as it reached 0.212 mg / L.

TABLE 6. Cadmium values and percentage of its reduction in treating water after entering without sterilization

Treatment	Transaction time period (in days) rate					Average
	3	6	9	12	15	
C. demersum	0.244 (%55.17)	0.167 (%69.30)	0.094 (%82.75)	0.018 (%96.64)	0.029 (%94.74)	0.110
Control	0.317 (%41.77)	0.295 (%45.81)	0.265 (%51.38)	0.203 (%62.81)	0.212 (%61.04)	0.258
Average	0.280	0.231	0.179	0.110	0.120	

- L.S.D for treatments at the level of 0.05 = 0.0365
- L.S.D for the time at the level of 0.05 = 0.0577
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.0817
- Numbers in parentheses represent the Percentage removal%

Table (7) shows the efficiency of the *C. demersum* plant in reducing the Cadmium values and the percentage of its reduction in treating water after entering with sterilization, as it shows the efficiency of the plant in isolation from the microorganisms analyzing the heavy metals, which were eliminated by thermal sterilization. 0.083 and 0.106 (mg /L) after (3, 6, 9, 12, & 15) days, respectively, compared to its concentration before treatment in Table (1), which reached (0.536) mg /L, and the highest reduction efficiency of Cadmium was 84.58% on the 12th day of treatment. As for the control treatment without plants, it was noticed that there was no removal rate in Cadmium concentrations due to the absence of microorganisms analyzing it.

TABLE 7. Cadmium values and percentage of its reduction in treating water after entering with sterilization

Treatment	Transaction time period (in days) rate					Average
	3	6	9	12	15	
C. demersum	0.315 (%41.29)	0.194 (%63.87)	0.112 (%79.04)	0.083 (%84.58)	0.106 (%80.16)	0.161
Control	0.536 (%0)	0.536 (%0)	0.536 (%0)	0.536 (%0)	0.536 (%0)	0.536
Average	0.425	0.365	0.324	0.309	0.321	

- L.S.D for treatments at the level of 0.05 = 0.0344
- L.S.D for the time at the level of 0.05 = 0.0544
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.0769
- Numbers in parentheses represent the Percentage removal%

Nickel It is noted from Table (8) the efficiency of the *C. demersum* plant in treating water before entering into the reduction of Nickel metal, as it was noticed that the highest reduction efficiency reached 84.90% after 12 days of treatment, and the Nickel concentration in it was 0.418 mg /L, and the rise of Nickel metal is also noted after 15 days. Of treatment amounting to 0.430 mg /L, which may be due to excretion by plant tissues due to deterioration and decay of weak plants and their decomposition in phytoremediation ponds. Table (8) also shows that there was an increase in the reduction of Nickel metal in the control treatment without plants, and to a lesser degree than what was observed in the treatment of plants, as the highest reduction efficiency reached 57.40% after 15 days of the experiment. The mean total Nickel concentration was (0.727 and 1.504) mg /L for each of the *C. demersum* plant treatment and the control treatment without plant, respectively. The results of the statistical analysis showed that there were significant differences between the control treatment on the one hand and the *C. demersum* treatment on the other hand.

TABLE 8.Nickel values and percentage of its reduction in treating water before entry

Treatment	Transaction time period (in days) rate					Average
	3	6	9	12	15	
C. demersum	1.176 (%57.54)	0.949 (%65.74)	0.662 (%76.10)	0.418 (%84.90)	0.430 (%84.48)	0.727
Control	2.073 (%25.13)	1.631 (%41.10)	1.359 (%50.92)	1.276 (%53.94)	1.180 (%57.40)	1.504
Average	1.624	1.290	1.010	0.847	0.805	

- L.S.D for treatments at the level of 0.05 = 0.2805
- L.S.D for the time at the level of 0.05 = 0.4436
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.6273
- Numbers in parentheses represent the Percentage removal%

C. demersum plant recorded high efficiency in reducing Nickel metal concentrations from wastewater, as it is noticed from Table (9) in a post-entry treatment without sterilization, a significant decrease in Nickel concentrations before and after treatment, as its concentration before treatment in Table (1) was 0.815 mg /L and then Its decrease was observed immediately and at the first reading after 3 days of treatment, to reach 0.512 mg /L with a reduction efficiency of 37.12%.

The *C. demersum* plant continued to reduce the concentrations of Nickel metal to the lowest rate after 12 days of treatment with a concentration of 0.117 mg /L, with a removal efficiency of 85.65%. Table (9) also shows that the concentrations of Nickel metal on days 9, 12 and 15 were within the local and international standards.

TABLE 9. Nickel values and percentage of its reduction in treating water after entering without sterilization

Treatment	Transaction time period (in days) rate					Average
	3	6	9	12	15	
C. demersum	0.512 (%37.12)	0.259 (%68.21)	0.180 (%77.86)	0.117 (%85.65)	0.125 (%84.71)	0.239
Control	0.603 (%26.03)	0.504 (%38.11)	0.396 (%51.43)	0.318 (%60.94)	0.315 (%61.30)	0.427
Average	0.558	0.382	0.288	0.218	0.220	

- • L.S.D for treatments at the level of 0.05 = 0.0424
- • L.S.D for the time at the level of 0.05 = 0.0670
- • L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.0947
- • Numbers in parentheses represent the Percentage removal%

Table (10) shows the efficiency of the *C. demersum* plant in reducing the values of Nickel and the percentage of its reduction in treating water after entering with sterilization, as it shows the efficiency of the plant in isolation from the microorganisms analyzed for heavy metals after (3, 6, 9, 12 and 15) days, respectively, compared to its concentration before treatment, Table (1), which reached (0.827) mg /L.

TABLE 10. Nickel values and percentage of its reduction in treating water after entering with sterilization

Treatment	Transaction time period (in days) rate					Average
	3	**6**	**9**	**12**	**15**	
C. demersum	0.598 (%27.70)	0.516 (%37.61)	0.429 (%48.08)	0.324 (%60.77)	0.331 (%60.00)	0.440
Control	0.827 (%0)	0.827 (%0)	0.827 (%0)	0.827 (%0)	0.827 (%0)	0.827
Average	0.712	0.671	0.628	0.575	0.579	

- L.S.D for treatments at the level of 0.05 = 0.0357
- L.S.D for the time at the level of 0.05 = 0.0564
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.0798
- Numbers in parentheses represent the Percentage removal%

The highest reduction efficiency of Nickel was 60.77% on the 12th day of treatment. As for the control treatment without plants, it was noticed that there was no removal rate in the concentrations of Nickel, which is due to the killing of all microorganisms analyzing the heavy metals by sterilization.

Lead *C. demersum* plant recorded high efficiency in reducing Lead metal concentrations from wastewater before entry, as it is noticed from Table (11) a significant decrease in Lead concentrations before and after treatment, as its pre-treatment concentration was 4.649 mg / liter and its concentration decreased in the phytoremediation basin. After 3 days, it reached 2.282 mg /L, with a reduced efficiency of 50.91%. The *C. demersum* plant continued to reduce the concentrations of Lead metal, and after 12 days of treatment, it reached the highest percentage decrease with a concentration of 0.747 mg /L with a removal efficiency of 83.93%.

TABLE 11. Lead values and percentage of its reduction in treating water before entry

Treatment	Transaction time period (in days) rate					Average
	3	**6**	**9**	**12**	**15**	
C. demersum	2.282 (%50.91)	1.553 (%66.59)	0.968 (%79.17)	0.747 (%83.93)	0.818 (%82.40)	1.274
Control	4.143 (%10.89)	3.214 (%30.86)	2.845 (%38.79)	1.902 (%59.08)	1.864 (%59.90)	2.794
Average	3.212	2.384	1.907	1.325	1.216	

- L.S.D for treatments at the level of 0.05 = 0.4209
- L.S.D for the time at the level of 0.05 = 0.6655
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.9412
- Numbers in parentheses represent the Percentage removal%

The results indicated in Table (12) the efficiency of the *C. demersum* plant in the treatment after entering without sterilization of water, as it was noticed that the highest efficiency of Lead reduction reached 92.87% after 12 days of treatment, and no significant differences were observed between the concentrations of Lead on days 9, 12 and 15 as it reached (0.177, 0.116 & 0.125)mg /L, respectively, and these concentrations are within the recommended standards locally and internationally.

TABLE 12. Lead values and percentage of its reduction in treating water after entering without sterilization

Treatment	Transaction time period (in days) rate					Average
	3	**6**	**9**	**12**	**15**	
C. demersum	1.099 (%32.60)	0.584 (%64.17)	0.174 (%89.34)	0.116 (%92.87)	0.125 (%92.35)	0.420
Control	1.481 (%9.18)	1.059 (%35.09)	0.791 (%51.50)	0.543 (%66.69)	0.540 (%66.86)	0.883
Average	1.290	0.882	0.482	0.328	0.334	

- L.S.D for treatments at the level of 0.05 = 0.3009
- L.S.D for the time at the level of 0.05 = 0.4757
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.6728
- Numbers in parentheses represent the Percentage removal%

Table (13) shows the efficiency of the *C. demersum* plant alone without microorganisms in reducing the values of Lead and the percentage of its reduction in treated water after entering with sterilization, as it shows the efficiency of the plant in isolation from the microorganisms that decompose heavy metals, which were eliminated by thermal sterilization, so the concentration of Lead reached (1,228, 1.030, 0.859, 0.431, and 0.444)mg / L after (3, 6, 9, 12 and 15) days, respectively, compared to its concentration before treatment, Table (1), which reached (1.658) mg /L, and that the highest Lead reduction efficiency was 73.98% on day 12. Of processing.

TABLE 13. Lead values and percentage of its reduction in treating water after entering with sterilization

Treatment	Transaction time period (in days) rate					Average
	3	6	9	12	15	
C. demersum	1.228 (%25.93)	1.030 (%37.86)	0.859 (%48.18)	0.431 (%73.98)	0.444 (%73.22)	0.799
Control	1.658 (%0)	1.658 (%0)	1.658 (%0)	1.658 (%0)	1.658 (%0)	1.658
Average	1.443	1.344	1.259	1.045	1.051	

- L.S.D for treatments at the level of 0.05 = 0.179
- L.S.D for the time at the level of 0.05 = 0.283
- L.S.D for the overlap between treatments and the time period at the level of 0.05 = 0.401
- Numbers in parentheses represent the Percentage removal%

From the above, it is noticed that the used *C. demersum* plant showed high efficiency in treating the tested heavy metals, and this may be due to the conditions surrounding the plant, such as temperature, pH, nutrients, in addition to the heavy metal concentration in the water, exposure period, plant age, as well as the plant's physiological and genetic characteristics [23]. [10] indicated the efficiency of the *C. demersum* plant in treating many heavy metals and were able to adopt it as a reliable bio-indicator of heavy metal contamination of water.

Estimation of heavy metals accumulated in the *C. demersum* plant

Table (14) shows the concentrations of Cadmium, Nickel and Lead metals accumulated in the C. demersum plant before and after treatment in the three treatments. C. demersum plant showed an effective role in reducing Cadmium metal, based on the metal accumulation in the plant tissues at the end of the treatment, as its concentration in the control plant reached 24.67 µg/g dry weight compared to its concentrations in the plants of the rest of the treatments, which increased in treatment before entry and treatment after entry without sterilization and treatment. After entry with sterilization, which were (73.58, 52.74 & 51.34) µg/g respectively. The results of the statistical analysis did not show any significant differences between treatment after entry without sterilization and treatment after admission with sterilization, while they differed significantly with treatment before entry.

TABLE 14. Concentration of heavy metals accumulated in *C. demersum* plants (mean ± standard deviation)

Treatment	Transaction time period (in days) rate		
	Cadmium, Cd	Lead, Pb	Nickel, Ni
Control	24.67^{c} ± 2.517	35.33^{c} ± 2.082	50.32^{c} ± 5.508
Pre-entry	73.58^{a} ± 6.506	90.52^{a} ± 1.528	93.43^{a} ± 4.041
Post-entry without sterilization	52.74b^{b} ± 2.082	70.66^{b} ± 3.512	85.51^{b} ± 3.512
Post-entry with sterilization	51.34^{b} ± 0.577	69.31^{b} ± 3.512	84.37^{b} ± 3.215
L.S.D 0.05	6.875	5.435	7.839

- Different letters mean a significant difference under probability $P \leq 0.05$

It was also noticed from Table (14) that there were high significant differences between the control treatment on the one hand and the rest of the transactions on the other hand.

Table (14) shows the efficiency of the reduction of Lead metal by the *C. demersum* plant, as its concentration in the control plant reached 35.33 µg/g while the accumulated concentration in the plant was recorded in the limits of (90.52, 70.66 & 69.31) µg/g after wastewater treatment before entry and after entry without sterilization. And after entry with sterilization, respectively. This increase in the concentration of Lead in plant tissues is evidence of the metal being taken by the *C. demersum* plant and its efficiency in reducing it from wastewater. It is also noticed that there is a significant difference between the plant in the control treatment and the rest of the other treatments, and no significant difference was noticed between Post-entry treatment without sterilization and post-entry treatment with sterilization. A significant difference was also observed between a pre-entry transaction on the one hand and the rest of the transactions on the other hand.

It is also noticed from Table (14) that the *C. demersum* plant was highly efficient in drawing Nickel from the water its concentration in plant treatment plants was (93.43, 85.51 & 84.37) µg/g for each of the treated plants before entry and after entry without sterilization and after entry with sterilization, respectively, while its concentration in the control plant was 50.32 µg/g, it is also noticed that there are high significant differences between a transaction before entry on the one hand, and the rest of the transactions on the other hand. Table (14) shows that the concentration of Nickel before treatment in the control plant was higher than the concentrations of the rest of the metals, and perhaps this indicates that the *C. demersum* plant tolerates high concentrations of Nickel metal and stores it in its tissues as in general the accumulation of heavy metal inside the plant is affected by the effect of the metal concentration in the water [23]. As for the presence of these heavy metals (Cadmium, Lead and Nickel) in plants before their use in treatment, it is a result of the organic wastes contained in the area from which the plant was collected, noting that there are no heavy industries in that region, but rather an agricultural area in which the use of fertilizers and pesticides is frequent.

Aquatic plants show more concentrations of heavy metals in their tissues in polluted areas, which indicates that these plants tolerate high levels of these metals, and this may be due to the accumulation and storage of these metals within the tissues of the plant in non-toxic forms or that they have a special mechanism to withstand high concentrations of them [24].

Aquatic plants possess many means of collecting and detoxifying these metals within their tissues, some of which are necessary for their growth and development, but can be toxic if present in high concentrations. The increase in heavy metals in the water is often associated with an increase in the amount of dissolved and suspended organic matter [25].

The biological removal of heavy metals by the *C. demersum* plant may be attributed to the presence of negatively charged ions on the cell wall of the *C. Demersum* plant that withdraws positive metal ions from the pond water. This conclusion was indicated by [26]. It carries negative charges (anion) belonging to the carboxyl groups of pectic acid and thus these negative charges attract the positive charges to it and prevent it from exiting again into the medium of external growth, whether this plant is grown in the middle of the soil or in the solutions of nutritious farms. [27] explained that the negative charge on the cell wall is based on the hypothesis of the difference in electrical potential, since the concentration of hydrogen ions (H^+) in the solution is less than their concentration on the cell wall, noting that the water in the experiment basins was mostly basic, and in this case a difference occurs in The electrical potential to make the cell wall negative and Lead to the attraction of positive cations represented by heavy metals, as well as that the presence of nitrates in the center of growth has an encouraging or supportive effect on the absorption of Lead by the plant. Feeding with nitrates has a basic physiological effect, that is, it leads to the isolation of hydroxyl groups on the surface of plant cells, which increases their negative charge, and thus the plant absorbs cation (cation) to equalize this charge and the current study believes that this opinion is likely to have negative charges on the plant cell wall Which leads to the biological removal of heavy metals by the *C. demersum* plant.

CONCLUSIONS

The current study showed the efficiency of *C. demersum* plant in reducing pollutants from wastewater except for the values of total dissolved solids and electrical conductivity, the plant was not efficient in its reduction. *C. demersum* is an aquatic plant that is able to remove heavy elements, cadmium, lead and nickel from wastewater, and is efficient in accumulating heavy elements in its tissues.Water sterilization does not affect the increase in plant efficiency in removing heavy elements.

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REFERENCES

1. Muhammad, Najla Ajil. (2019) Determinants of wastewater pollution in Karbala Governorate for the year 2016. Journal of the College of Basic Education, Al-Mustansiriya University. Volume (25) Issue (103): 900-930.
2. WHO (World Health Organization Sanitation safety planning: manual for safe use and disposal of wastewater, greywater and excreta) .(2019). GENEVA, SWITZERLAND. WHO, 156 PP ISBN 978 92 4 154924 0.
3. UNEP (United Nations Environment Programme) . (2002) . State of the Environment and policy perspective : Global Environment Outlook 3 . Division of Early Warning and Assessment (DEWA), Kenya ., pp.150-179 .
4. Al-Enezi, M. S. AK. (2014). Wastewater treatment in The use of life technologies with the evaluation of the efficiency of the Hamdan plant.Basra, Ph.D. thesis.Basra University, 120 pages.
5. Tilley, E. ; Ulrich, L. ; Lüthi, C. ; Reymond, P. and Zurbrügg, C. (2014) . Compendium of sanitation systems and technologies: Swiss Federal Institute of Aquatic Science and Technology (Eawag) ; Duebendorf, Switzerland 170:175.
6. Hassoon, H.A.(2015). The Adsorption of Some Trace Heavy Metals from Aqueous Solution Using Non Living Biomass of Sub Merged Aquatic Plant Ceratophyllum demersum. Iraqi Journal of Science, 56(4): 2822-2828.
7. Mukherjee ,S. and Chatterjee ,S.(2014). Assessment of *Nelumbo nucifera* and *Hydrilla verticillata* in the treatment of pharmaceutical industry effluent from 24 Parganas, West Bengal. Internat. J. Sci. Eng., Vol. 7(2):100-105.
8. Sengar, R. M. S.; Singh, K. K. and Singh,S.(2011). Application of phycoremedation technology in the treatment of sewage water to reduce pollution load . Indian J.Sci.Res.,2(4) : 33-39.
9. Golabia, M., Shokripoura, H., Moazeda, H., & Haghighib, N. J.(2019). Investigation of biosorption on Ceratophyllum demersum L. biomass: removal of cadmium (II) from aqueous solution. DESALINATION AND WATER TREATMENT, *157*, 118-128.
10. Polechońska, L., & Klink, A. (2020). Validation of Hydrocharis morsus-ranae as a possible bioindicator of trace metal pollution in freshwaters using *Ceratophyllum demersum* as a reference species. *Environmental Pollution*, *269*, 116145.
11. Taha, Nidal Tahsin, Ahmad, Hashem Abd al-Razzaq and Qasim, Thaer Ibrahim. (2011). Test the efficiency of Lemna spp. In reducing zinc and iron concentrations from wastewater when increasing the biomass. University of Baghdad Journal, 8 (1): 471-477.
12. APHA (2003). American public Health Association. Standard methods for examination of water and wastewater, 20th, Ed. Washington .DC,USA.
13. APHA (American Public Health Association) .(1999). Standard Methods for Examination of Water and Wastewater . 20th ed., 2672 p.
14. Lind, O.T. (1979). Handbook of Common Methods in Limnology 2nd ed. The C.V. Mosby Company. St. Louis, London. 199p.
15. Parsons, T.R. ; Mait, Y. and Laulli, C.M . (1984) . A manual of chemical and bilogical methods for seawater analysis pergamone press oxford/ U.K.
16. APHA(American public health association).(2005).Standard methods for examination of water and wastewater.21the Ed. Washington, D.C., U.S.A.
17. Lytle , C. M. and Smith B. N. (1995). " Seasonal Nutrient cycling in Potamogeton pectinatrs of the lower provo River " , Great Basin Naturalist . 55(2) : 164-168.
18. Jones ,J. Benton .(2001). Laboratory guide for conducting soil tests and plant analysis. CRC Press LLC.
19. IBM Corp. (2017). *IBM SPSS Statistics for Windows*. Armonk, NY: IBM Corp. Retrieved from https://hadoop.apache.org
20. Iraqi Determinants of the Use of Treated Wastewater. (2012). No. 3 of 2012. Pursuant to Clause Three of Article (80) of the Constitution and Clause One of Article (38) of the Law for the Protection and Improvement of the Environment No. (27) of 2009. Al-Waqi'i Al-Iraqiya - Issue 4260: 23-30.
21. Al-Wazni, W. S., & Al-Fatlawy, H. J. (2016). Physiochemical and bacteriological analysis of some drinking water samples in Karbala city, Iraq. *Al-Kufa University Journal for Biology*, *8*(3).
22. Mahdi, Enas Awni. (2018): Evaluation of the efficiency of two types of submersible aquatic plants in removing nickel and lead and treating wastewater. Master Thesis - College of Science - University of Basra, 117 pages.
23. Qadir,A., Muhammad A. H., Bin Zafar,M.S., Farooqi, Z. U. R., Fazila Y., Hussain,M.M. and Mohy-Ud-Din,W.(2021). Phytoremediation of inorganic pollutants: An eco-friendly approach, its types and mechanisms. *Plant and Environment*, *1*(02), 110-129.

24. Al-Sahari, M., Al-Gheethi, A. A. S., & Mohamed, R. M. S. R. (2020). Natural Coagulates for Wastewater Treatment; A Review for Application and Mechanism. In Prospects of Fresh Market Wastes Management in Developing Countries (pp. 17-31). Springer, Cham.
25. DalCorso, G., Fasani, E., Manara, A., Visioli, G., Furini, A., 2019. Heavy metal pollutions: state of the art and innovation in phytoremediation. Int. J. Mol. Sci. 20 (14), 3412.
26. Cameselle, C., Gouveia, S., 2019. Phytoremediation of mixed contaminated soil enhanced with electric current. J. Hazard. Mater. 361, 95–102.
27. Yang, Y., Xiong, J., Tao, L., Cao, Z., Tang, W., Zhang, J., Yu, X., Fu, G., Zhang, X. & Lu, Y. (2020). Regulatory mechanisms of nitrogen (N) on cadmium (Cd) uptake and accumulation in plants: a review. *Science of the Total Environment*, *708*, 135186.