PERFORMANCE OF FREE WATER SURFACE FLOW CONSTRUCTED WETLAND FOR LEAD AND CADMIUM IONS REMOVAL USING AZOLLA PINNATA PLANT

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ABSTRACT

Phytoremediation is the ability of some plants to bio accumulate, decompose, or transformation of pollutants in soils, water, or air. In the current study, an effort is made to study the effectiveness of free-floating plants to treat simulated industrial wastewater polluted with Lead and Cadmium ions using Azolla Pinnata plant in a lab condition. Free water surface flow constructed wetland were fed with simulated Cd and Pb ions in different concentrations (5, 10 and 20) mgl^{-1}. Different operating conditions were studied such as; pH, dissolved oxygen, electrical conductivity, temperature, and sodium adsorption ratio to find out the possibility of using treated wastewater for irrigation. Results showed that, Azolla plant had a higher removal efficiency to extract Lead ions (90.1%, 86.67%, and 81.3%) than Cadmium (79.3%, 78.4%, and 69.7%) when the initial concentration is (5, 10, 20) mgl^{-1} respectively, during 5 days. A slight reducing in pH (8.3 to 7.4) and dissolved oxygen (8.7 to 7.2) mgl^{-1} observed, all these values mentioned satisfied with the Iraqi standards and World Health Organization. From values of electrical conductivities (250- 750) (µScm^{-1}) and the sodium adsorption ratios (0- 10) it was found that treated wastewater falls into class C2S1 which mean that water has good quality and can be used to irrigate moderately salt-tolerant crops on soils with good permeability.

Keywords: heavy metals, Phytoremediation, irrigation water, Azolla Pinnata.

أداء الجريان السطحي الحر للأراضي الرطبة المنشأة لإزالة ايونات الرصاص والكادميوم باستعمال نبات الازولا

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باحث أستاذ

قسم الهندسة البيئية / كلية الهندسة / جامعة بغداد

المستخلص

المعالجة النباتية هي قدرة بعض النباتات على التراكم الحيوي أو التحلل أو تحويل الملوثات الموجودة في التربة أو الماء أو الهواء. في الدراسة الحالية ، تم العمل على دراسة فعالية النباتات العائمة الحرة لمعالجة مياه الصرف الصناعي باستعمال نبات ***Azolla Pinnata*** في ظروف مختبرية. تمت تغذية الأراضي الرطبة المُنشأة ذات التدفق السطحي للمياه بعنصري الكادميوم والرصاص بتراكيز مختلفة هي (5 , 10 , 20) ملجم لتر$^{-1}$. تمت دراسة الصفات المختبرية المختلفة مثل ؛ درجة الحموضة والأكسجين المذاب والتوصيل الكهربائي ودرجة الحرارة ونسبة امتزاز الصوديوم لمعرفة إمكانية استخدام المياه المعالجة للري. وبحسب النتائج ، فإن قدرة نبات الأزولا على ازالة الرصاص أعلى (90.1٪ , 86.67٪ , 81.3٪) من الكادميوم (79.3٪ , 78.4٪ , 69.7٪) عندما يكون التركيز الأولي (5 , 10 , 20).) مجم / لتر على التتابع خلال 5 أيام. انخفاض في درجة الحموضة (8.3 إلى 7.4) والأكسجين المذاب (8.3 إلى 7.2) ملغم / لتر ، كل هذه القيم المذكورة تتوافق مع المعايير العراقية ومنظمة الصحة العالمية. من التوصيل الكهربائي (250–750) (µS / سم) ونسبة امتزاز الصوديوم (0–10) وجد أن مياه الصرف الصحي المعالجة تقع ضمن فئة (C2S1) مما يعني أنه يمكن استخدام الماء لري المحاصيل التي تتحمل الاملاح بدرجة معتدلة في تربة ذات نفاذية جيدة.

الكلمات المفتاحية: العناصر الثقيلة، المعالجة النباتية، مياه الري، نبات الازولا.

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INTRODUCTION

Mining operations result in increasing discharges of various types of pollutants including heavy metals, which may threaten the water quality of receiving water bodies (2, 11). Various industries, such as metal processing plants, tanneries and batteries, have produced a large amount of heavy metals wastewater (41). Heavy metal pollution has already seriously threatened the global human health and ecosystems (7, 25). Lead and cadmium are common heavy metals in water systems (12). Lead can cause serious damage to the reproductive system and brain function of humans according to the report proposed by D. Bulgariu (12). M. Rechberger reported that cadmium could cause renal dysfunction and even death and has been included in the list of carcinogens by the US Environmental Protection Agency (35). It is necessary to remove Lead and Cadmium from wastewater before discharge into the environment. A cleaner and sustainable development could be accomplished by introducing efficient and cost-effective technologies for environmentally friendly treatment of industrial wastewater (43). The effluent from domestic activities and industrial sources contain wastewater with a high proportion of heavy metals, which is continuously increasing in past decades (1, 46). Various techniques are available for the purpose of heavy metals removal from environment. Some of most common methods used in wastewater include ion exchange, filtration, precipitation, reverse osmosis and adsorption. However, these methods are costly and have detrimental effects on the affected area (19). One must take into account the availability of good management to the water (33) .Therefore, there is a need for new alternative treatments using low-cost materials. Constructed wetlands (CWs) have been widely used in wastewater for removal of many contaminants, such as suspended solids, organic compounds, nutrients, pathogens, metals, and emerging contaminants (10, 28). CWs are gaining popularity in many countries as a wastewater treatment process due to its effectiveness (4). It is not only cost-effective but also an operation-flexible solution compared to conventional wastewater treatment processes (14). Constructed wetland is a sustainable treatment method which causes a minimum threat to the downstream water bodies (45). As suggested by Water, Sanitation and Hygiene Institute, constructed wetland includes a high degree of aerobic biological improvement and can be used as secondary treatment citation (44). Constructed wetland are of two types i.e. free water surface flow constructed wetland (FWSCW) and sub-surface flow constructed wetland. Subsurface flow is divided into vertical flow (VF) CW, horizontal flow (HF) CW, French vertical flow (FVF) CW and hybrid type CW (44). Free water surface constructed wetland is a natural wetland in which wastewater flows over the surface. It is helpful for flood prevention and shoreline erosion control along with wastewater quality improvement (16). A wide range of plants could be used in the free water surface constructed wetland as emergent plants (Typha, Phragmites, Scirpus), submerged plants (Potamogeon, Elodea) and floating plants (Eichornia, Lemna) (13). In this context, current study aimed to assess the short-term efficiency of passive biological treatment using CW for heavy metals removal from simulated industrial wastewater. *Azolla pinnata* plant was selected because of its simplicity, high efficiency, low cost, and sustainable. This study is divided into two main sections. The first section is the removal of Lead and Cadmium ions from polluted wastewater. while the second section is measuring the most important determinants of irrigation water.

MATERIALS AND METHODS

Preparation of Plants: In this study, *Azolla pinnata* was selected for the heavy metal removal because of their Simplicity, high efficiency, and low cost, this plant brought from northern Iraq/Mosul in very small quantities; In period of growing of the Azolla, it needs to place under it a layer of soil suitable for cultivation (21, 22). A domestic water was used for plant growth after it is left exposed to the atmosphere and the sunlight for 48 hours to get rid of chlorine that may harm the plant. Approximately a tablespoon of Azolla was grown, growth phase started in November 2021 and lasted for three months until an adequate amount was obtained to start experiments. Figure (1) shows Azolla pinnata in CWs units during phytoremediation.

Preparation of Constructed Wetland Units

Tow glass basins were used the dimensions of each container were 60* 30 and 30 cm in depth. plant was distributed evenly between containers with a water level of 15 cm. It also turned out that these plants do not tolerate direct sunlight, so a mesh cover was placed above to block a proportion of direct sunlight as shows in Fig (2). Tap water used in this research is tap water after leaving it for 48 hours to be ensure getting rid of chlorine that may harm the plant.

Fig 1. *Azolla pinnata* in CWs units for various Lead and Cadmium concentrations during phytoremediation

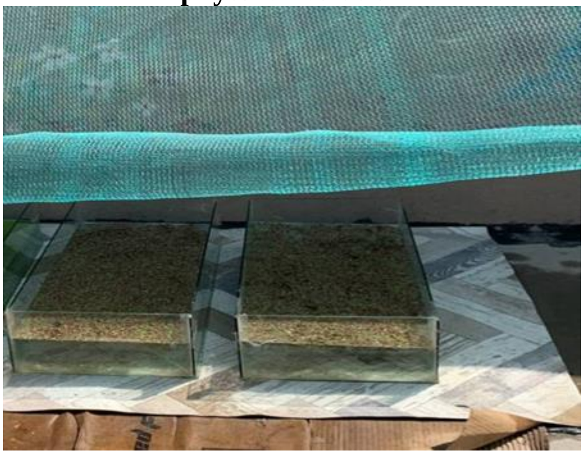

Fig 2. CWs units installing and the mesh cover that placed on them

Simulated wastewater Sampling

The stock solutions of Lead and Cadmium (manufactured by HIMADIA, India) was prepared with stock solution of (1000 mgl^{-1}) by dissolving $Pb(NO_3)2H_2O$ and $Cd(NO_2)_2.4H_2O$ respectively, in distilled water. The required mass pb(NO3)2H2O and Cd(NO2)$_2$.4H2O was calculated as follows (33):

$$W = V \times C_t \times \frac{M.Wt}{At.Wt} \quad \ldots.. 1$$

Where:

W: weight of Pb(NO3)2 and Cd(NO3)$_2$.4H2O (g)

V: volume of solution (L)

Ct: initial concentration of Lead and Cadmium (mgl^{-1})

M.Wt: Molecular wight of Pb(NO3)2 and Cd(NO3)$_2$.4H2O (g)

A.Wt: Atomic wight of Lead and Cadmium and they stored at room temperature, then diluted to the ranges; (5,10, and 20 mgl^{-1}). The main characteristics of the lead and cadmium salts are summarized in Table (1). Plant containers watered with 5 $mg1^{-1}$ of heavy metals. Monitoring the removal percent for a period of five days, then the plant gets break time for five days to restore its vitality. After that, watered with concentration of 10 mgl^{-1} and so on in the same cycle. The measurement for the Lead and Cadmium ions were carried out using Atomic Adsorption Spectroscopy (AAS) (PERKEN Elmer,1100, USA) in College of Science department of Biology/ University of Baghdad.

Table 1. Main characteristics of Lead and Cadmium salts used in the experimental tests of the present constructed wetland units

Property	Lead	Cadmium
formula	$pb(NO_3)_2H_2O$	$CdN_2O_6.4H_2O$
appearance	White crystal	White crystal
Molecular wight	331.21	308.48
Atomic weight	207.2	112.41
company	HMEDIA, India	HMEDIA, India

Microbial Study: The biological samples were measured and isolated at the Al Raya Lab/ in Bab Al-Muadhem, Baghdad, Iraq. The biological isolate from the roots were collected from Azolla. Roots of plant were separated randomly from the surface. Using a sterile scoop to transport the samples to laboratory for further experiments. the selected rhizobacteria were identified using Vitek® 2 Compact systems (bioMérieux, France). The result of all analyzed set obtained within 4 to 8 hours. Figure (3) shows the preparing of agar plate for bacterial isolation of plant roots.

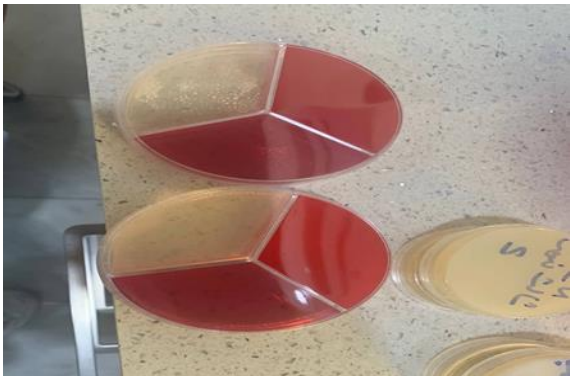

Fig 3. Agar plate for the bacterial isolation of plant roots.

Analysis for Irrigation Water and Salinity Classification: There are reliable specifications and limits to determine if the water is suitable for irrigation. Most important of these determinants are pH, electrical conductivity (EC), sodium adsorption rate (SAR), Temperature, and dissolved oxygen (DO). Table (2) shows the analysis that were carried out, as well as the instrument and their details.

Table 2. analysis for the selected irrigation water and instrument

Parameters	Instrument, Model, Company, and Origin
Water temp °C	Multi-430SETF, WTW, Germany
pH (Standard unit)	Multi-pp-206 pH/mV/Temp Meter Extech, US
EC µscm^{-1}	Multi-430SETF, WTW, Germany
DO (mgl^{-1})	DO meter (WTW 340i, Germany)
Na (mgl^{-1})	Atomic Adsorption Spectroscopy (AAS)
Ca (mgl^{-1})	Atomic Adsorption Spectroscopy (AAS)
Mg (mgl^{-1})	Atomic Adsorption Spectroscopy (AAS)

Sodium absorption ratio (SAR) is an important irrigation water quality parameter. It is a measure of amount of Sodium relative to the amount of Calcium and Magnesium in a water sample. Specifically, SAR is ratio of the Sodium concentration divided by the square root of half the sum of the Calcium and Magnesium concentration (5):

$$\mathrm{SAR} = \frac{\mathrm{Na}}{\sqrt{\frac{\mathrm{Ca+Mg}}{2}}} \quad \ldots\ldots \quad 2$$

High levels of Sodium ions in water affect the permeability of soil and could lead to water infiltration issues, while the impact severity of high SAR water depends on many specific soil quality factors (such as soil type, texture, drainage capacity, etc), typically the higher SAR, less suitable water for irrigation (18).

RESULTS AND DISCUSSION

Heavy metals Removal Efficiencies

The results showed the efficiency of azolla to remove the heavy metals. Different concentrations of Lead and Cadmium ions (5, 10, and 20) mgl^{-1} were used. The best removal efficiencies observed at 5 mgL^{-1} and it was 90.1% and 79 % for Lead and Cadmium ions respectively. The reason for this is that initial concentrations were low and the plant can easily adsorb it from the wastewater. The efficiencies orders; Lead > Cadmium this attributed to that Lead is an active metal redox that destroys the metabolic equilibrium of the cellular antioxidants pool (38). Also, the fact that lead is removed more than cadmium agrees with Z. Stepniewska (40). It became clear from the results that the removal started effectively from first day of the treatment process. It also turned out that removal rate decreases as the initial concentration increase due to the load of heavy metals on plant. Figure (4), (5), and (6) shows the comparison between the removal efficiencies of Lead and Cadmium ions during five days in different influent concentrations of heavy metals.

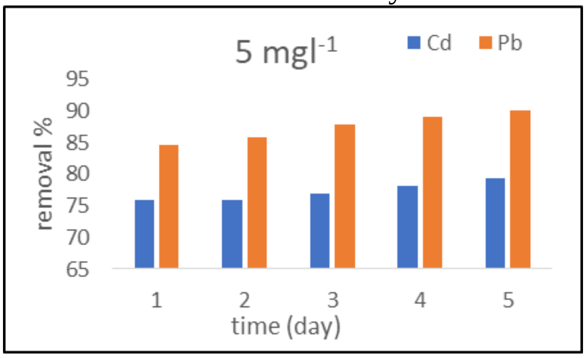

Fig 4. Removal efficiencies of Lead and Cadmium ions with constant initial concentration (5 mg/L) in CW of Azolla

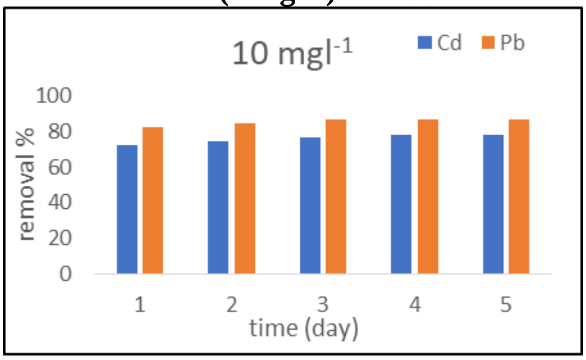

Fig 5. Removal efficiencies of Lead and Cadmium ions with constant initial concentration (10 mg/L) in CW of Azolla

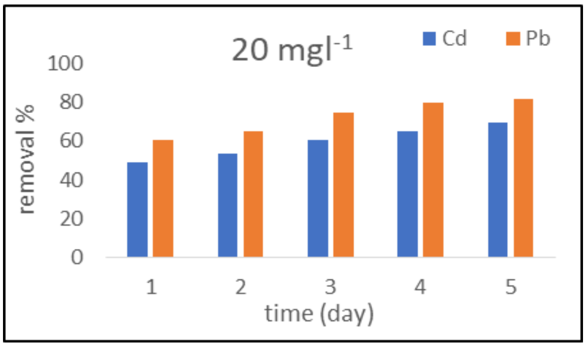

Fig 6. Removal efficiencies of Lead and Cadmium ions with constant initial concentration (20 mg/L) in CW of Azolla

Bacterial Isolation: The selected rhizobacteria were identified to know the genus of bacteria that may control the treatment process. The roots were separated randomly from surface of Azolla plant. Results shows that the main bacteria founded in root of plant was *klebsiella pneumoniea ssp pneumonia*. *Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium as shows in Figure (7). It appears as a mucous lactose fermenter on MacConkey agar as present in Figure (8) (26).

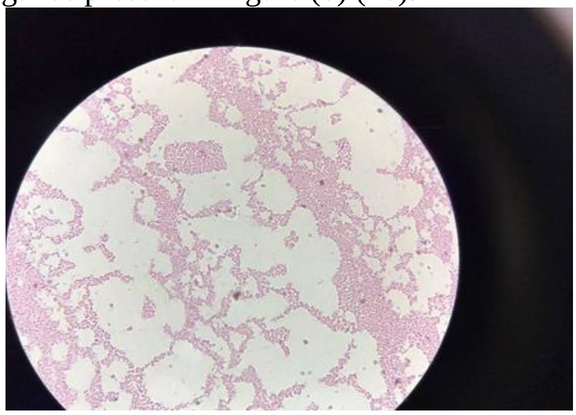

Fig 7. sectional image *klebsiella pneumoniea ssp pneumonia* as identified in Vitek® 2 Compact systems

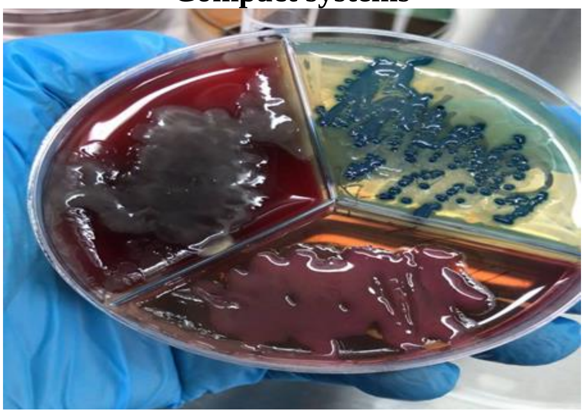

Fig 8. *Klebsiella pneumoniea* bacteria a mucous lactose fermenter on MacConkey agar

Chemical and Physical Parameters

Monitoring pH levels is crucial due to their delicate effects on water quality, particularly dissolved ions in CWs, where pH may affect the microbial capacity during the breakdown of contaminants. It was indicated that pH was slightly lower than pH of the influent. Note that influences of plants on pH was not significant and the changes were ranged from 0.10 to 7 unit. As shows in Table (3). This reduction could be due to the phytoremediation; during the sunlight hours of the day, the plants remove CO_2 from the water for use in photosynthesis and release O_2, this process is reversed during the dark hours of day which is lead to secreted of organic acids that decrease pH values (32, 29). All final values of pH satisfy the Iraqi standards for treated wastewater (15, 30), as well as, World Health Organization, between 6.5-8.5 (34). Figure (9), (10), and (11) represents the variation of pH values of effluent solution in CWs unit during 5 days. Concentration of DO in wastewater directly affects degree of redox reactions and microbial activities, which in turn affects heavy metals removal efficiencies (23). The suitable amount of DO make the adsorption, and precipitation favored for retention of heavy metals, with higher redox potentials causing most of the heavy metals precipitate or adsorb as metal oxides (31). Value of DO shows slightly reduction after the first day of plant watering. Figure 12, 13, and 14 shows the variation of DO values of effluent solution in CWs units during 5 days.

Aerobic conditions enhance microbial activities, with the release of carbon dioxide forming carbonic acid, which is beneficial for the treatment of heavy metals in alkaline wastewater (32). Rhizobacteria use a lot of DO to oxidize the heavy metals, which is the cause of the decline in DO levels (32). Table (4) shows variation of DO values of different heavy metals concentration during 5 days.

Slight changes in the water temperature could be recognized in the range varied from 22 to 24.4 C^o for all CWs units under different values of heavy metals concentration. It seems that the measured temperature in each unit is approached the atmospheric temperature because the CWs units are kept open.

Table 3. Variation of pH value of different influent heavy metals concentration during 5 days

	Cd		Pb	
Conc. (mg/l)	**1th day**	**5th day**	**1th day**	**5th day**
5	**8.3**	**8.2**	**8.2**	**8.2**
10	**7.8**	**7.8**	**8.1**	**7.8**
20	**7.8**	**7.4**	**7.7**	**7.6**

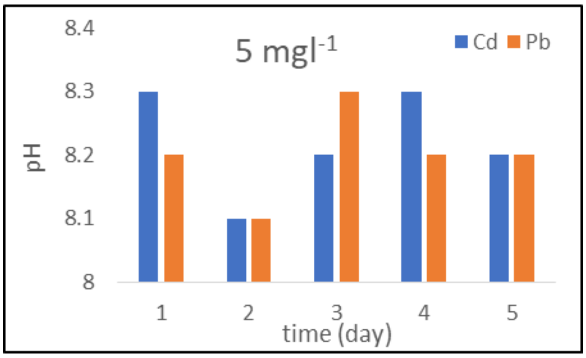

Fig 9. pH values of effluent solution in constant initial concentration (5 mgL^{-1}) in CW of Azolla

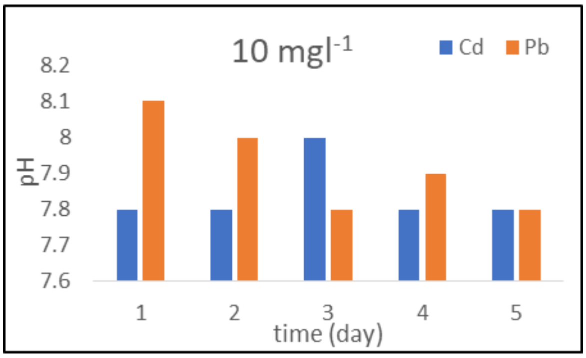

Fig 10. pH values of effluent solution with constant initial concentration (10 mgL^{-1}) in CW of Azolla

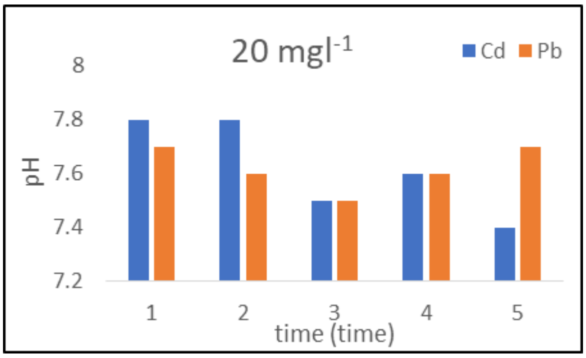

Fig 11. pH values of effluent solution with constant initial concentration (20 mgL^{-1}) in CW of Azolla

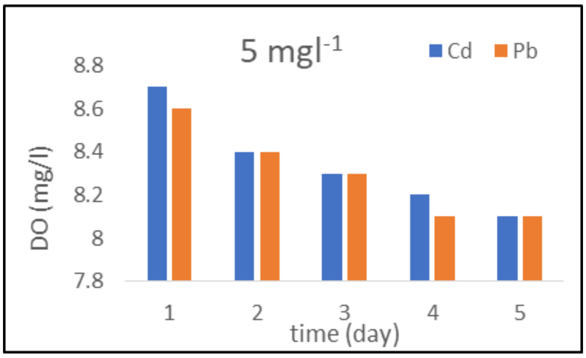

Fig 12. DO values of effluent solution with constant initial concentration (5 mgL^{-1}) in CW of Azolla

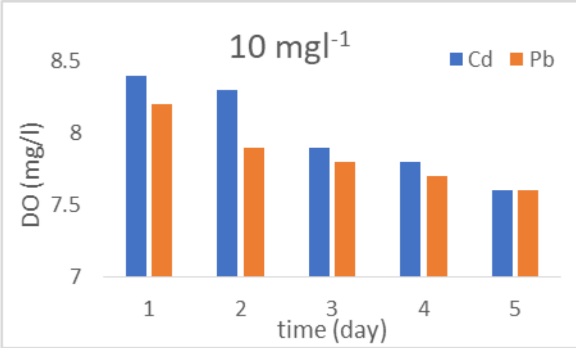

Fig 13. DO values of effluent solution with constant initial concentration (13 mgL^{-1}) in CW of azolla

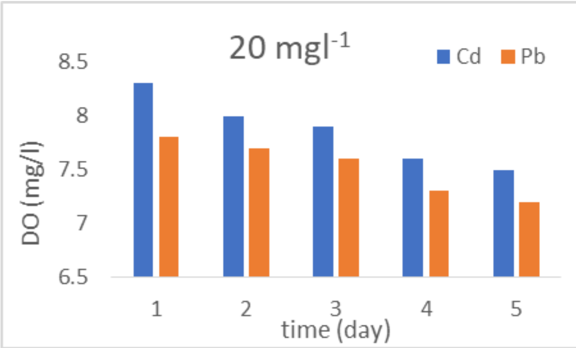

Fig 14. DO values of effluent solution with constant initial concentration (20 mgL^{-1}) in CW of Azolla

	Cd		Pb	
Conc. (mgl^{-1})	**1th day**	**5th day**	**1th day**	**5th day**
5	**8.7**	**8.1**	**8.6**	**8.1**
10	**8.4**	**7.6**	**8.2**	**7.6**
20	**8.3**	**7.5**	**7.8**	**7.2**

Table 4. Variation of DO value of different influent heavy metals concentration during 5 days

Variations of EC, SAR, Ca, Mg, and Na ions: EC is the most important factor in determining the suitability of water for irrigation because it reflects the dissolved solids in wastewater (37). The primary effect of elevated EC reduces the osmotic activity of plants and thus interferes with the absorption of water and nutrients from soil (42) . It has been observed that the electrical conductivity values decrease with time as shows in Table (5), also, EC reduced as the influent concentration of heavy metals increased. Results indicated that EC levels were efficiently reduced by using CWs Which is in consistent with (27). It was turn out that EC of tab water about 1003 and 1142 (μScm^{-1}) for Cadmium and Lead CWs unit, respectively. In Cadmium CWs unit; After one day the EC level dropped to 749 (μScm^{-1}) which is in turn reduced to 497 after 5 days. These final EC value (i.e., 497 μScm^{-1}) will rise to 680 which considered as a references value to next experiments as showed in table (5), the increasing in EC value could be due to the addition of heavy metal solution or due to the addition of tab water to the CWs units to keep water level suitable for the plant vitality and going on to other EC values of Cadmium and Lead solutions. It was found that EC values were eliminated with time between the 1th and 5th of the all CWs experiments for different heavy metals concentrations. Noted that the removal efficiency of heavy metals reduced as the initial concentration increase, also efficiency of Lead CWs unit is higher than Cadmium. This observation may indicate that the reduction of EC was partially due to the removal of not only NaCl but also heavy metals (27). The most convenient procedure for assessing water salinity is the measurement of EC. The high value of EC is harmful to plants, and affects growth by reducing water absorption, and affects osmotic pressure. Water with high EC content changes soil structure, aeration, and permeability, affecting the crop. These negative effects are called salinity hazards (36).

Table 5. Variation of EC values of different influent heavy metals concentration during 5 days

	Conc (mgl^{-1})	EC(μScm^{-1}) of ref. water	EC (μScm^{-1}) 1th day	EC (μScm^{-1}) 5th day	Removal efficiency %
Cadmium	5	1003	749	497	50.4
	10	680	585	491	27.8
	20	700	684	574	18
Lead	5	1142	741	564	50.6
	10	779	605	533	31.5
	20	690	547	510	26

Calcium is so essential and plays a major role in plant metabolism and formation of cell membranes as well, as the salt of the fatty substance lecithin is included in the composition of the plant cell membrane (6, 9). Results showed a very slightly decrease in Magnesium ions, The reason is that Mg^{2+} is considered as a cofactor for enzymes in the photosynthesis process and an essential component of the chlorophyll molecule, whose high concentrations harm the plant. It is one of the components of the green pigment chlorophyll in plants. Therefore, the use of aquatic plants (Azolla) helped to reduce Mg^{2+} in wastewater, where the results of the current study agree with the mentioned results by H. Azarpira (9). The reason is that Na^+ concentration is an important element for plants that are highly sensitive because it increases salinity. Its high concentration causes great harm to most plants because high salinity affects normal growth and metabolism (9). Sodium adsorption ratio (SAR) is an important irrigation water quality parameter. High Sodium content, may Lead to deflocculating (i.e., breakdown) of soil clay particles, leading to a severe reduction of the soil aeration and water infiltration and percolation, which means that soil permeability is decreased through the irrigation with the water that is high in sodium (20). Thus, the optimal water measure likely effect on soil permeability is water's SAR and its EC. Table (6) shows the values of SAR and Ma^{+2} Na^{+} Ca^{+2} in (mgl^{-1}) at different heavy metal concentration after treatment duration 5 days. This result presents the slight increase in SAR values as the initial concentration of heavy metal increase. All these values are occurrence within the global determinants of irrigation water (24).

Table 6. SAR, Ca, Mg, and Na ions value at different initial concentration of heavy metals during 5 days

cadmium				
Conc.(mgl^{-1})	Ca	Na	Mg	SAR
5	68	10.7	30	1.53
10	64	12	26.3	1.79
20	52	14.3	43	2.07
lead				
Conc.(mgl^{-1})	Ca	Na	Mg	SAR
5	70	12	32	1.68
10	64	12.4	34	1.77
20	65	12.5	38.5	1.74

US Salinity Laboratory Classification

EC and SAR are responsible for salinity and Sodium risk, respectively. Therefore, the US Salinity Laboratory proposed a classification, regarding SAR as an indicator of Sodium hazard and electrical conductivity (EC) as an indicator of salinity hazard (24, 3). From the nomograph figure (15) using SAR and EC as a coordinate, locating the corresponding point on the diagram, the position of the point determines the quality classification. The classification and laboratory results for salinity are presented in Table (7). The treated simulated wastewater of the floating aquatic plant units is of (C2S1) class. However, water that belongs to this class is also useful for almost all plants provided that a moderate amount of leaching takes place or for plants with reasonable salinity tolerance (3).

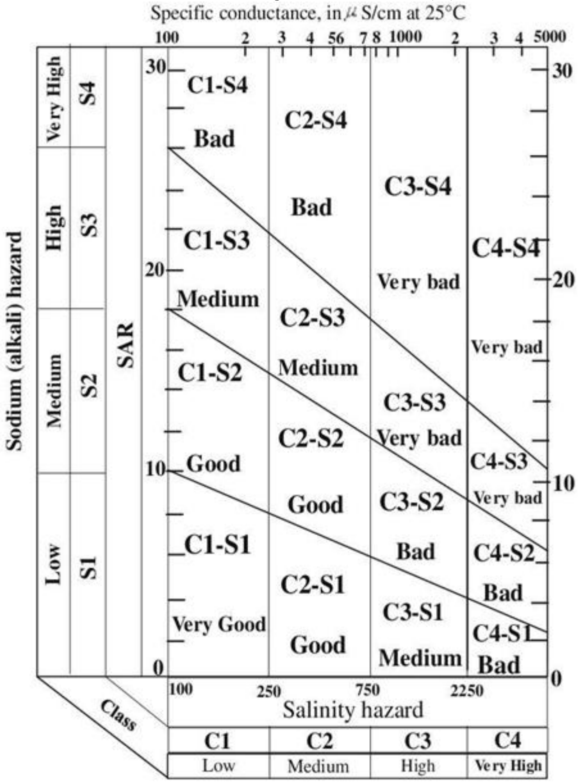

Fig 15. U.S. Salinity Laboratory (USSL) classification chart (Kumar et al. 2009)

Table 7. Treated industrial wastewater quality classification for irrigation

Element	Quality	Class	SAR	EC (µS/cm)	Possibilities of utilization
Cd	good	C2S1 -Medium Salinity -Low Sodium	0- 10	250- 750	In general, water can be used without special measures to irrigate moderately salt-tolerant crops on soils with good permeability
Pb	good	C2S1 -Medium Salinity -Low Sodium			

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