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USE OF AZOLLA AND DUCKWEED FOR THE RECOVERY OF SOME TRACE ELEMENTS FROM WASTEWATER

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ABSTRACT: The capacity of two aquatic plant species (duckweed and azolla) was studied for removing trace elements (Fe, Cu, Zn, and Mn) from contaminated wastewater. For the doing so, wastewater samples were collected from Abu-Rawash sewage water treatment plant, El-Gabal El-Asfar sewage water treatment plant, Bahr El-Bakar drain, Mostorod drain, El-Sadat oxidation pond and 10th of Ramadan oxidation pond. 10th of Ramadan sample was selected for carrying out the experiment because of its high trace element content. The data showed that the concentration of Fe, Cu, Zn, and Mn in treated wastewater with duckweed and azolla decreased with increasing detention time. The corresponding decreases for duckweed were 36-85, 31-78, 48-94 and 50-94 per cent through 5 to 20 days detention time. The results of BCF also showed that both duckweed and azolla have a great tendency to accumulate many hundred times of trace elements in their tissues with respect to the initial concentration of these elements in wastewater. The study also revealed that the trace elements uptake, BCF, and rate of trace elements removal were increased progressively by increasing detention time for the studied aquatic plants.

Key words: Azolla, Duckweed, Zn, Mn, Fe, Cu, Bioconcentration Factor (BCF), Removal Rate, Uptake of Trace Elements and Detention Time.

INTRODUCTION

Heavy metals and trace elements are common environmental pollutants that are produced as the result of industrial, commercial and domestic activities. New pretreatment standards require some industrial discharges, to limit heavy metals and trace elements levels to very low residual concentrations. Studies in New York city show that heavy metals and trace elements can be found in municipal wastewater even when majour industrial sources are not part of the system (EPA 1991).

Environmental technologies suitable for XXI century are aimed at the recycling and recovery of nutrients or pollutants in contrast with early approach of treatment technologies. The use of aquatic plants for recovery of nutrients and trace elements from wastewater represents an alternative technology with a high potential for application in small and medium size cities (Olguin and Hernandez, 1998).

There are at least three different systems in which aquatic plants are utilized for the removal of nutrients and / or trace elements. The lagoons with floating plants such as water hyacinth and duckweed have been investigated since the seventies (Boyd, 1970) and are currently in use at a large scale for the treatment of municipal wastewater in Asia (Huub and Siemen, 1998). The constructed wetlands with emerging plants such as reeds and bulrush, have been investigated and applied more recently (Biddlestone and Gray, *1998*). Finally, the so-called Rhizofiltration system utilizes the roots of superior plants such as sunflower (Duskenkov *et al.*, 1995) to remove trace elements from contaminated soils or water. In this paper, only the system, which utilizes floating plants, will be discussed.

The aim of the present research is to quantify the capacity of two aquatic plant species viz., (Duckweed and Azolla) for removing trace elements from contaminated wastewater.

MATERIALS AND METHODS

Wastewater samples were collected after and before treatment from different locations as follows:

1-Abu–Rawash; treated waste water from the sewerage water treatment plant (Giza Governorate).

2-El-Gabal El-Asfar treated wastewater from sewerage water treatment plant (Qualubia Governorate).

3- Bahr El-Bakar drain at Saft El-Hana village (El-Sharkia Governorate).

4-Mostorod drain (Qualubia Governorate).

5-El-Sadat non treated wastewater before its entrance to oxidation pond (Menofia Governorate).

6-10 th of Ramadan non treated wastewater before entrance to oxidation pond – No. 3.

10th of Ramadan Sample was selected for experiment implementation because of its high trace element content. It is worthy to mention that this water is already used for irrigating Wady Elmollak agricultural area (Ismailia Governorate).

The experiment was carried out using aquatic plants in Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, Giza, during May to September 2000.

Duckweed (Lemna minor) was collected from Mostorod drain at Qalubia Governorate. This plant was defined according to Les *et al.* (1997), Zaki (1991) and Täckholm (1974). While, water fern, Azolla pinnata was obtained from Microbiology department, soil, water and environment research Institute, Agricultural Research center, Giza, Egypt.

The plants (duckweed and Azolla) were cleaned thoroughly under gentle running water to remove adhering algae and insect larvae. They were kept in plastic tanks containing tap-water for one week prior to starting the experiments. Five-g fresh weight sample of each plant was transferred to a plastic aquarium of 6L capacity containing industrial non treated wastewater obtained from 10th of Ramadan.

The treatments were left 5, 10, 15 and 20 days and each treatment was replicated five times. At the end of each treatment, 100 ml of the treated wastewater was dried on water bath and the residue was treated with digestion mixture (Greenberg *et al.,* 1985). Also plants were collected, cleaned by rinsing thoroughly with distilled water, blotted dry, and weighed for fresh weight then oven dried at 70 C^0 for 48 h for dry weight. The dried plant materials were ground and representative samples were taken for chemical analysis. The plant and wastewater samples were digested by perchloric, nitric and sulfuric acid mixture (1:5:0.5) (Stewart, 1989).

Trace elements Fe,Cu, Zn and Mn content in plant and wastewater samples were determined using an atomic absorption 'Theromo Jarrell Ash, Model: AA-Scan".

From the obtained data the following parameters were calculated:

(a)The bioconcentration factor (BCF) was calculated according to Zayed *et al.*, (1998) and Dushenkov *et al.*, (1995).

BCF= Trace- element concentration in plant tissues (µg/g) at harvest/ Initial concentration of the element in solution (mg/l).

(b) Rate of trace element removal by each plant species (µg/plant/day) was calculated according to Qian *et al.*, (1999).

[{Trace element concentration in plant after treatment (µg/g) x Final plant dry weight g/plant}-{Trace element concentration in plant before treatment (µg/g) x initial plant dry weight (g/plant)}] /Treatment period (d).

RESULTS AND DISCUSSION

Wastewater analysis

Data in Table (1) show some chemical properties and total content of trace elements in the studied wastewater samples.

The salinity ranged from 1.30 to 1.96 dS/m for El Gabal El Asfar and Bahr El Baker respectively. The samples of the other sites were in the range of above values. pH values of the collected samples indicated that the characterized as neutral to mildly alkaline. The highest values of trace element were recorded in the *10 th of Ramadan* wastewater sample. This may be attributed to the effect of the industrial activities in this region.

Plant growth as a function of trace elements accumulation in wastewater

Data in Table (2) demonstrate the periodic changes in growth of duckweed and Azolla as affected by trace elements accumulation. The results showed that the maximum increase in duckweed dry weight at the end of growth period (20 days) was 324 g over control compared with 304 g for azolla. It is worthy to mention that the rate of growth of azolla was higher than duckweed at 5 and 10 days growth periods. While the rate of growth duckweed was relatively higher than azolla at 15 and 20 days.

It could be stated that increasing growth and yield of duckweed and azolla was due to that the growth media of wastewater contain convenient and sufficient nutritive elements either macro or micro.

Effect of aquatic plants on trace elements recovery from wastewater

The date are presented in Table (3) and showed that the concentration (mean of 5 replicates) of Fe, Cu, Zn and Mn in treated wastewater with duckweed, and azolla plants decreased with increasing growth time (detention time).

Table (1) Some chemical properties of wastewater samples

Location	E C (dS/m)	pH	Trace elements concentration (ppm)			
			Fe	Cu	Zn	Mn
Abu-Rawash sewage water treatment plant	1.50	6.9	1.80	1.55	1.52	1.25
El-Gabl El-Asfar sewage water treatment plant	1.30	7.3	1.70	1.42	1.44	1.33
Bahr El-Baker drain	1.96	7.8	1.83	1.35	1.25	1.40
El-Sadat oxidation pond	1.45	7.2	1.92	1.52	1.35	1.32
10^{th} of Ramadan oxidation pond No. (3)	1.85	7.1	1.98	1.66	1.59	1.45
Mostord drain	1.52	7.3	1.73	1.43	1.36	1.34

Table (2) Mean Values of Aquatic Plant Yield (g) at Different Growth Periods as Affected by Wastewater

Aquatic plant	Detention Time, days													
	Zero time		After 5			After 10			After 15			After 20		
	Fresh	Dry	Fresh	Dry	Increase %	Fresh	Dry	Increase %	Fresh	Dry	Increase %	Fresh	Dry	Increase %
Duckweed	5.00	0.249	7.92	0.37	58.4	12.7	0.58	154.00	17.59	0.80	251.80	21.22	0.96	324.40
Azolla	5.00	0.300	8.31	0.46	66.20	13.3	0.71	167.20	16.86	0.88	237.20	20.22	1.04	304.40

Table (3) Effect of Aquatic Plants on Mean Values of Trace Elements Recovery (mg/l) from Wastewater

Element	Aquatic Plant								
	Duckweed					Azolla			
	Detention Time, days								
	Zero Time	5	10	15	20	5	10	15	20
Fe	1.98	1.269	0.801	0.493	0.291	1.110	0.610	0.323	0.159
Removal %		35.90	59.55	75.10	85.30	43.90	69.19	83.68	92.00
Cu	1.66	1.138	0.778	0.530	0.358	1.020	0.573	0.392	0.262
Removal %		31.44	53.13	68.07	78.43	38.55	65.48	76.38	84.22
Zn	1.59	0.821	0.480	0.214	0.100	0.648	0.261	0.103	0.370
Removal %		48.36	69.81	86.54	93.71	59.24	83.58	93.52	97.67
Mn	1.45	0.732	0.364	0.175	0.080	0.662	0.300	0.128	0.049
Removal %		49.51	74.90	87.93	94.45	54.34	79.31	91.17	96.62

The data showed that the removal of Fe, Cu, Zn and Mn as percent were about 36-85,31-78,48-94 and 50-94 percent respectively, in treated wastewater by duckweed at the duration of growth intervals (5 to 20 days). For azolla the corresponding data were 44 - 92,39-84,59-98 and 54-97 percent respectively. It is worthy to mention that the higher removal percent of trace elements was found in the treated wastewater by azolla. These results emphasized that azolla plant was more efficient for recovery of trace element than the duckweed. These results agreed with that obtained by Jain *et al.* (1989)

Efficiency of aquatic plants for accumulation of trace elements

Data in Table (4) show that mean concentration of Fe at the end of growth period varied from 402 to 1859 and 463 to 1973 (μg/g) in duckweed and azolla respect-ively. The mean values of copper concentration in duckweed ranged from 595 to 1280 (μg/g), while Cu concentration in azolla ranged from 702 to 1364 μg/g. The highest mean concentration of Zn and Mn accumulated in azolla were 767 and 1341 (μg/g) respectively, while they were 708 and 1287 μg/g in duckweed. It could be concluded that the Fe ,Cu , Zn and Mn content in azolla and duckweed plants increased with increasing growth period. Also, the results in Tables (1and 4) revealed that, both duckweed and azolla have a tendency to accumulate many hundred times of trace elements in their tissues with respect to the initial concentration of these elements.

These results emphasized that the accumulation of Fe ,Cu , Zn and Mn in azolla was higher than that accumulated in duckweed accumulation of metals or ions in azolla and duckweed plants may be explained according to Dushenkov *et al.*, (1995) and Raskin and Ensley, (2000) as they suggest that the elements or ions interact with poly- galacturonic acid and other negatively charged molecules within plant cell walls. The longer term component of metal removal such as cellular uptake and precipitation, require biological activity of living cells. Although little is known about the mechanisms that allow plants to accumulate metals intera-celluary or to export them to the shoot yet, the vacuole plays an important role in the storage of such metal ions. Inside the vacuole, it is thought that metals are chelated by organic acids, such as citrate or malate, or by enzymatically synthesized compounds, commonly called phytochelatins.

Trace elements uptake, Bioconcentration factor (BCF) and rate of trace elements removal

a) Duckweed

Data in Table (5) demonstrate the uptake, BCF and rate of trace elements removal by duckweed plant. The uptake (mean values) of Fe, Cu. Zn, and Mn was 148.7, 220.2, 88.1 and 144.7 μg/total dry weight at a detention time of 5 days, respectively. These values increased progressively by increasing detention time and reached to 1784.6, 1228.8 , 679.7 and 1235.5 μg/total dry weight, respectively at 20 days detention time.

These results are in harmony with those recorded by Jain *et al.*, (1989) for duckweed and water velvet grown in polluted water with trace elements.

BCF is considered as an index of the ability of the plant to accumulate the trace elements and to identify wetland plant species that are most efficient for removing trace elements from wastewater polluted with potentially toxic and trace elements (Zayed *et al.*, 1998 and Qian *et al.*, 1999).

Data in Table (5) illustrate the bioconcentration factor (BCF) of Fe, Cu, Zn, and Mn in duckweed plant at different detention times.

The results showed that the BCF of Fe, Cu, Zn, and Mn increased with increasing detention times. The recorded mean values of BCF Were 203 to 939 for Fe, 359 to 771 for Cu, 150 to 445 for Zn and 269 to 887 for Mn. The results showed that the lowest mean values of BCF were recorded in case of Zn at all detention times while, the highest mean values of BCF were recorded for Cu at 5 and 10 days detention times. While, the highest values were obtained for Fe at 15 and 20 days detention time.

Due to the large variation in the rate of biomass accumulation of plant species tested, it is important to take into account the plant biomass accumulation when different plant species are compared for their trace element removal efficiency. For this reason, the rate of trace element accumulation was calculated for plant as the amount of each trace element accumulated per plant per day (Qian *et al.* 1999). The data in Table (5) showed that the rate of trace elements accumulation by duckweed varied with respect to each element and detention time.

The recorded mean values for Fe ranged from 29 to 89, Cu from 44 to 61, Zn from 17 to 34 and Mn from 29 to 62.

Table (4) Mean Values of Trace Elements Concentration (µg/g) in Aquatic Plants Treated with Wastewater

Element	Aquatic Plant									
	Duckweed					Azolla				
	Detention Times, days									
	zero	5	10	15	20	zero	5	10	15	20
Fe	25	402	643	1563	1859	31	462	673	1639	1973
Cu	19	595	897	1023	1280	24	702	1012	1234	1364
Zn	12	238	397	514	708	16	257	413	588	767
Mn	21	391	603	1066	1287	26	433	642	1182	1341

Table (5) Mean Values of Trace Elements Uptake (µg/total dry weight), Bioconcentration Factor, and Rate of Trace Element Removal By Duckweed Plant

Trace Elements	Days											
	5			10			15			20		
	Uptake	BCF*	R**	Uptake	BCF	R	Uptake	BCF	R	Uptake	BCF	R
Fe	148.7	203	29	373.0	325	37	1250.4	789	83	1784.6	939	89
Cu	220.2	359	44	520.3	540	52	818.4	616	54	1228.8	771	61
Zn	88.1	150	17	230.3	249	23	411.2	323	27	679.7	445	34
Mn	144.7	269	29	350.0	416	35	852.2	735	57	1235.5	887	62

* BCF = Bioconcentration Factor

** R = Rate of Trace Elements Removal (µg /plant/day)

Table (6) Mean values of Trace Elements Uptake (µg/total dry weight), Bioconcentration Factor, and Rate of Trace Element Removal By Azolla Plant

Trace Elements	Detention Time, days											
	5			10			15			20		
	Uptake	BCF*	R**	Uptake	BCF	R	Uptake	BCF	R	Uptake	BCF	R
Fe	212.5	234	41	477.8	340	47	1442.3	828	96	2052.0	996	103
Cu	323.0	423	36	718.5	609	71	1086.0	743	72	1418.6	821	71
Zn	118.2	162	23	293.2	259	29	517.4	400	34	797.7	482	40
Mn	199.2	298	38	455.8	443	45	1040.2	815	69	1394.6	924	70

* BCF = Bioconcentration Factor

** R = Rate of Trace Elements Removal (µg /plant/day)

b) Azolla:

Results presented in Table (6) follow the same trend as in duckweed

Some slight variations in the magnitude of the obtained values for each parameter. However, the obtained values in case of azolla are higher than obtained in case of that duckweed.

The floating wetland plants seem to be an exception because they bioaccumulate Cu to higher levels of 300 to 1500 mg/Kg in duckweed (Jain *et al.*, 1989, Zayed *et al.*, 1998 and Qian *et al.*, 1999)

The results (Tables 5&6) emphasized that azolla is more efficient for accumulating elements in its tissues. This is attributed to that azolla exhibited the highest rate of biomass for accumulation and attained high tissue concentration of all studied elements compared with duckweed.

The results provide some of the available choices of appropriate plant species that may be used for the removal of trace elements studied based on their accumulation in plant tissues or their rate of removal by harvestable plant.

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استخدام الأزولا وعدس الماء لاسترجاع بعض العناصر النادرة من مياه الصرف

هشام إبراهيم القصاص

قسم العلوم الزراعية – معهد الدراسات والبحوث البيئية – جامعة عين شمس

تهدف هذه الدراسة إلى قياس قدرة نوعين من النباتات المائية (عدس الماء – الأزولا) على إزالة بعض العناصر النادرة من مياه الصرف .
ولتحقيق هذا الهدف تم عمل حصر وتجميع مياه صرف من كل من محطة معالجة مياه الصرف الصحى بأبو رواش ، محطة معالجة مياه الصرف الصحى بالجبل الأصفر مصرف بحر البقر ، مصرف مسطرد ، بركة الأكسدة بمدينة السادات ، بركة الأكسدة بالعاشر من رمضان.

أوضحت النتائج أن تركيز كل من الحديد , النحاس , الزنك و المنجنيز فى مياه الصرف المعالجة بكل من نباتى عدس الماء – الأزولا تناقص بزيادة فترة احتجاز النباتات فى مياه الصرف

وأوضحت النتائج أن نسبة تناقص هذه العناصر(الحديد، النحاس، الزنك، المنجنيز) فى حالة استخدام نبات عدس الماء كانت ٣٦ -٨٥ ، ٣١ – ٧٨، ٤٨ – ٩٤ ، ٥٠ – ٩٤ % على التوالى خلال فترة احتجاز نبات عدس الماء ٥ ، ٢٠ يوم على التوالى.

وبينت النتائج أن معامل التركيز الحيوى (BCF) للعناصر لكل من نباتى عدس الماء والأزولا نتيجة تراكم هذه العناصر داخل أنسجة هذه النباتات يقدر بمئات المرات عن التركيز الأصلى فى مياه الصرف.

كما بينت النتائج ان كل من الامتصاص و معدل التركيز الحيوى ومعدل الإزالة لهذه العناصر النادرة قد زاد باطراد بزيادة فترة احتجاز النباتات المائية بمياه الصرف .