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Study on the Ability of Duckweeds *(Lemna Spp.)* for Decreasing Bacterial Load and Residues of some Heavy Metals from Municipal Waste Water to Reuse in Aquaculture

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ABSTRACT

This study was carried out in the Central Laboratory for Aquaculture Research (CLAR, Abbasa, Egypt) during 2008 to determine the ability of aquatic duckweed; *Lemna spp.* for decreasing bacterial load and some heavy metals' residues from raw sewage water. The study was consisted of two parts. In the first, duckweed; *Lemna spp.* were cultured on different waters for 2 weeks. Nine glass aquaria (each with 30-l capacity) were used and divided into 3 groups. The 1st group was filled with de-chlorinated tap water (TW) as control, while the 2nd group was filled with mixed water (TW and raw sewage water; RSW) at a ratio of 1:1, and finally, the 3rd group was filled with 100% RSW. After 2 weeks, *Lemna spp.* at both 1st and 3rd groups were completely died, while that in the 2nd group (TW and SW) resulted in a good flowering case. Some heavy metals concentrations were measured at both waters and *Lemna spp.* tissues. The second for rearing *Oreochromis niloticus* and common carp; *Cyprinus carpio* fingerlings at a rate of 15 fish/aquarium for two weeks in two treatments. The 1st treatment, was stocked on water which resulted from the 2nd group (TW& RSW) at the first part of the study and was considered as water cultured with *Lemna spp*. (CWL) and fresh TW and SW without culturing *Lemna spp.* (WL), as 2nd treatment. Mean concentrations of Pb, Cd, Cu and Fe at fish organs had significant lower ($P<0.05$) levels at CWL treatment than WL treatment. *Lemna spp.* reduced the accumulation of the heavy metals in SW treatment by absorbing these metals in their tissues and this led to the reduction of these metals in the different organs of the reared fish. It is, therefore strongly, recommended to grow duckweed; *Lemna spp.* in sewage water before use in agricultural purposes especially in fish farming.

Keywords: *Lemna spp.*, bacterial load, heavy metals residues, waste water and aquaculture

INTRODUCTION

Drainage water above the Delta is returned to the River Nile, recycled downstream and reused. Drainage water, however, consists not only of irrigation return water but also in many cases industrial and domestic wastewater where huge volumes of untreated wastewater are discharged in agricultural drains daily. Drainage water is therefore contaminated with salts, agricultural chemicals such as heavy metal, and other pollutants as pathogens from the domestic sewage and industrial discharge (Al-Nagaawy, 2008).

Aquaculture-waste water systems have two purposes: treating waste water and producing a useful product. This means that the system must be able to produce an acceptable effluent as well as a product that does not contain pathogenic bacteria and viruses or unacceptable levels of harmful chemicals (Hejkal *et al*., 1983). Urban sewage contains toxic heavy metals, which are not removed properly during the traditional treatment of sewage (Chen *et al*., 2005). Therefore, removal of these toxic heavy metals from primary and secondary treated sewage has drawn the attention of workers (Sinha *et al*., 1996; Weis and Weis, 2004 and Brix and Arias, 2005).

Various aquatic macro-phytes have been tested as bio-filters to purify water by removing nitrogen, phosphorus and elements that cause eutrophication (Tripathi and Upadhyay, 2003 and Nahlik and Mitsch, 2006). However, duckweed such as *Lemna spp.* is the first choice of eco-toxicologists because it is wide spread, fast growing and reproduce faster. It is sensitive to many pollutants, which are assimilated from the growing medium (or aquatic environment) through the underside of the leaf (Greenberg *et al*., 1992; Becker *et al*., 2002 and Sharma *et al*., 2007).

However, Mara and Cairncross (1989) and Pillay (1992) mentioned that, fish reared in treated domestic sewage must be examined to ensure that it is suitable for human consumption. Fish found to be microbial contaminated could be used as fish meal for animal, fish, and poultry nutrition. At low concentrations, microorganisms are present on the surface of fish, gills, and general viscera, and this might represent a source for cross-contamination during fish processing. When present in low numbers, pathogens are not likely to penetrate into the fish muscles.

The objective of this work was to determine the ability of *Lemna spp.* to

decrease and/or remove bacterial load and some heavy metals (Pb, Cd, Cu and Fe) from raw sewage water.

MATERIALS AND METHODS

This study was carried out in the Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, governorate, Egypt during 2008. Aquatic duckweed; *Lemna spp.* was used to examine its ability for water purification by removing and/or decreasing heavy metals residues and the bacterial load from the treated sewage water.

Experimental design

Aquatic duckweed; *Lemna spp.* was grown in de-chlorinated tap water (TW) and raw sewage water (RSW) which was brought from Zagazig Domestic Sewage Water Station for tow weeks to study its ability for removing some heavy metals (Pb, Cd, Cu and Fe) and decreasing the bacterial load from these waters. Nine glass aquaria (each with 30-l capacity) were maintained in 3 replicates. The 1st group was filled with de-chlorinated tap water as control (TW), while the 2nd group was filled with mixed water (TW & RSW) at a ratio of 1:1, and finally, the 3rd group was filled with 100% RSW. All aquaria were inoculated with equal quantities of *Lemna spp.* (which was collected from irrigating canals) and left under the same conditions for two weeks. After that period, *Lemna spp.* in the 1st and 3rd groups was completely died, while that in the 2nd group (50% RSW) resulted in a good flowering case. Water from the 2nd group (50% RSW) was collected and reused for fish rearing (cultured with *Lemna spp.*; CWL) to complete the study. Another treatment used the same water of the 2nd group without culturing *Lemna sp* (WL). Nile tilapia; *Oreochromis niloticus* and common carp; *Cyprinus carpio* fingerlings were stocked at the tow treatments and the experiment was continued for tow weeks.

Bacterial analyses

Water samples were taken from the TW & RSW treatment before and after *Lemna spp.* growing with wide-mouthed 300-ml sterilized glass bottle disposal and taken to the laboratory in a thermo-insulated container for bacteriological analyses (APHA, 1985).

Samples of fish species were taken before and after *Lemna spp.* growing then placed in lapelled sterile polypropylene bags containing water from the aquaria and transported live to the laboratory. Fish surface was swabbed (1 cm^2) with a dry cotton swab. The swab was placed in 10 ml peptone water, agitated vigorously, and

squeezed dry on the inside of the media bottle. Serial dilutions were made to 10^{-5} with this resultant suspension and examined. Each fish was then killed and rinsed with de-ionized water for about 2 min, and the surface was decontaminated by dipping it in ethyl alcohol and flaming. Each fish was aseptically dissected and parts of the gills and muscles were weighed (1g for each) aseptically for analysis. Each tissue was homogenized separately in a blender in sterile peptone water (pH 7.2) to achieve a 10% (w/v) suspension of fish. All the microbiological examinations were carried out according to the technique recommended by Thatcher and Clark (1975).

Bacterial examination for total bacterial count was carried out using decimal dilution technique. Plates were incubated at 22 and 37°C for 48 hours after which colony forming units (cfu) were counted according to (Harrigan & McCance-Margart, 1976). The *coliform* and fecal *coliform* were counted according to Harrigan and McCance-Margart (1976) using ready-made McConkey medium. Plates were incubated at 37°C for 24 hours for *coliform,* while in case of fecal *coliform* plates were incubated at 44.5°C for 24 hours. *Salmonella spp.* and *Shigella spp*. were counted according to the methods described by Harrigan and McCance-Margart (1976) using *Salmonella and Shigella* medium agar (S.S. Agar). Plates were incubated at 37°C for 24 hours. *Staphylococcus spp.* was enumerated on Baird-Parker's medium (Oxoid CM 275; Baird-Parker and Davenport, 1965) at 37°C for 24 hours. *Streptococcus spp*. was isolated and identified using *Streptococcus* selective medium from Italian Bio-life Company and incubated at 37°C for 24 h. Fecal *Streptococcus* was identified by using Azide blood agar base supplemented with blood, for determining hemolytic reactions which showed β-hemolysis as the lysis of red blood cells, resulting in a clear zone surrounding the colony. Incubation was at 37°C for 24 h. *Pseudomonas spp.* was isolated and identified using medium from Becton, Dickinson and Company Sparks, MD 21152 USA, 38800 Le Pont de Claix, France. Incubation was at 37°C for 48 h. Thio-sulfate citrate bile salts sucrose agar (TCBS Agar) was used for the selective isolation of *Vibrio spp.* from Difco™ TCBS Agar. Incubation was at 37°C for 24 h. *Aeromonas spp.* was counted on *Aeromonas* selective agar base (HAVELAAR; Bio-life Company) at 30°C for 24 h.

Heavy metals residues

Before and after *Lemna spp.* growing, the residues of lead, cadmium, copper and iron in water and *Lemna spp.* tissues, also, the residues

of the same heavy metals were estimated in fish organs (skin, muscles and gills) either for fish rearing in water cultured with *Lemna spp.* (CWL treatment) or without *Lemna spp.* (WL treatment) by using atomic absorption spectrophotometer (Thermon Electron Corporation S series AA Spectrometer, UK) according to the method described by APHA (1985). Water samples were filtered and concentrated by evaporating suitable quantities to a constant volume. While, fish tissue and *Lemna spp.* samples were oven-dried at 115^{o}C until constant weight was reached. Afterwards, 1.0 g dry weight was burned in muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA). Ash was digested with concentrated HNO_3 using muffle furnace then diluted in 50 ml of 2 N HCl.

Statistical analysis

All data values were computed and subjected to statistical analysis of variance and significant differences between means were done using Duncan's multiple range by SAS (1996)

RESULTS AND DISCUSSION

Significant lower ($P<0.05$) mean concentrations of Pb, Cd, Cu and Fe of water (50% RSW) were observed at the CWL treatment, but inversely, higher ($P<0.05$) concentrations of Pb, Cd, Cu and Fe in duckweed tissues were observed at the same treatment (Tables 1 and 2). This may explain that *Lemna spp.* reduced the accumulation of the heavy metals in 50% RSW by absorbing these metals in their tissues as clearly noticed in Table (2) and this led to the reduction of these metals in the different organs of the reared fish as shown in Table (4)

Table (1): ***Concentrations of some heavy metals (Means ± SE, ppm) of mixed water (TW & RSW) at a ratio of 1:1 before and after growing of Lemna spp.***

Item	Pb	Cd	Cu	Fe
Before	**0.19 ± 0.01A**	**0.05 ± 0.001A**	**0.27 ± 0.05A**	**2.37 ± 0.58A**
After	**0.11 ± 0.04B**	**0.02 ± 0.002B**	**0.05 ± 0.01B**	**0.70 ± 0.17B**

Note: Means with the same letter in the same column are not significantly different (P ≥ 0.05),
TW = de-chlorinated tap water & RSW= raw sewage water.

Table (2):*Concentrations of some heavy metals (Means ± SE, ppm) in Lemna spp. tissues. before and after growing on mixed water (TW & RSW) at a ratio1:1.*

Item	Pb	Cd	Cu	Fe
Before	**0.066 ± 0.01B**	**0.047 ± 0.01B**	**4.88 ± 0.32B**	**148.78 ± 3.12B**
After	**0.546± 0.05A**	**0.275 ± 0.04A**	**10.13 ± 1.42A**	**200.54 ± 2.87A**

Note: Means with the same letter in the same column are not significantly different (P≥ 0.05),
TW = de-chlorinated tap water & RSW= raw sewage water.

A general trend of decreasing was observed for total bacterial counts under both 22 and 37^{o}C, some bacterial species in fish organs reared at CWL treatment and water, where *Lemna sp* was considered as bio-filter to many species of bacteria (Table 3).

In many fish organs, some bacterial species (fecal *coliform* and *Vibrio spp*.) were completely absent, while, many bacterial species were partially disappeared as *Coliform*, *Salmonella spp*., *Shigella spp*. *Pseudomonas spp* and *Aeromonas spp*. from fish musles of the CWL treatment. But, low numbers of bacterial species were noticed in other organs (skin and gills) as well as in water (Table, 3).

Regarding to the water samples, fecal *coliform*, *Vibrio spp*. and *Aeromonas spp*. were completely absent from CWL treatment (Table 3), while, a sharp decrease was noticed for *Streptococcus spp.* and fecal *Streptococcus* in the CWL treatment (Table 3).

Duckweed may serve as a reservoir for further transmission of some bacteria species in aquaculture-based sewage water treatment plants (Rahman *et al*., 2007). Generally, sewage water treatment plants reduce the numbers of microorganisms, but the sewage water effluents may still contain high numbers of fecal microorganisms (Yaziz and Lloyd, 1982 and Koivunen *et al*., 2001). The removal efficiencies for pathogenic and indicator organisms may vary according to the treatment type and may even vary among organisms (Koivunen *et al*., 2003).

It seems that no public health risk from fish reared in RSW from up-flow anaerobic sludge blanket (UASB)–duckweed ponds or fed on fresh duckweed grown in UASB

Table (3): ***Counts of total bacteria and some bacterial species (Log cfu/cm^2, g, ml) in skin, muscle and gills of Nile tilapia and common carp reared in mixed water (TW & RSW at a ratio of 1:1) which cultured with and without Limna spp***

Item			Water	Nile tilapia			Common carp		
			Cfu/ml	Skin	Muscle	Gills	Skin	Muscle	Gills
T. bacterial count	22 °C	WL	5.73	3.51	2.07	6.59	4.30	4.41	6.7
		CWL	3.58	3.60	2.95	6.26	3.04	2.41	5.6
	37 °C	WL	6.58	3.08	2.30	7.17	5.30	4.30	6.23
		CWL	5.04	3.08	2.38	4.60	2.75	3.45	4.6
Coliform		WL	4.00	3.46	1.15	5.89	2.38	3.11	5.53
		CWL	4.08	0.00	2.41	0.00	0.00	0.00	1.3
Fecal *coliform*		WL	3.91	3.08	0.00	4.76	2.20	0.00	4.93
		CWL	0.00	0.00	0.00	2.00	0.00	0.00	0
Salmonella spp. & Shigella spp.		WL	4.20	2.95	0.00	4.90	2.20	1.65	4.91
		CWL	3.30	2.70	0.00	3.26	0.00	0.00	2.9
Staphylococcus spp.		WL	4.65	1.90	0.00	4.60	2.30	2.54	4.58
		CWL	3.04	2.60	3.08	2.78	0.00	1.30	0
Streptococcus spp.		WL	4.38	2.30	1.60	2.11	3.32	2.54	4.53
		CWL	1.60	1.30	3.30	2.78	0.00	1.78	3
Fecal *Streptococcus*		WL	3.84	1.30	0.00	4.15	5.78	2.18	3.15
		CWL	1.60	0.00	0.00	0.00	0.00	0.00	0
Pseudomonas spp		WL	4.85	0.00	0.00	5.14	0.00	3.62	4.95
		CWL	2.48	3.30	0.00	0.00	0.00	2.30	0
Vibrio spp.		WL	3.60	1.00	0.00	3.86	2.45	0.00	3.78
		CWL	0.00	0.00	0.00	0.00	0.00	0.00	0
Aeromonas spp.		WL	5.42	3.30	0.00	5.93	0.00	3.81	5.98
		CWL	0.00	1.30	0.00	2.78	0.00	0.00	1.6

Note: WL = de-chlorinated tap water and raw sewage water without culturing Limna spp.
CWL = de-chlorinated tap water and raw sewage water with culturing Limna spp.
TW = de-chlorinated tap water & RSW= raw sewage water.

Table (4): ***Concentrations of some heavy metals (Means ± SE, ppm) in skin, muscle and gills of Nile tilapia and common carp reared in mixed water (TW & RSW at a ratio of 1:1) cultured with and without Lemna spp.***

Fish	Organ	Treatment	Pb	Cd	Cu	Fe
Nile tilapia	Skin	WL	3.24 ± 0.57A	0.12 ± 0.01A	7.85 ± 1.22A	293.02 ± 5.66A
		CWL	0.05 ± 0.01B	0.04 ± 0.001B	3.02 ± 0.34B	132.15 ± 4.02B
	Muscle	WL	0.12 ± 0.01A	0.05 ± 0.01A	3.66 ± 1.54A	212.67 ± 7.25A
		CWL	0.02 ± 0.001B	0.01± 0.001B	1.52 ± 0.34B	140.08 ± 3.49B
	Gills	WL	3.63 ± 0.48A	0.18 ± 0.01A	4.94 ± 0.66A	655.96 ± 9.21A
		CWL	0.08 ± 0.01B	0.06 ± 0.02B	1.02 ± 0.18B	155.01 ± 6.34B
Common carp	Skin	WL	1.44 ± 0.08A	0.86 ± 0.11A	3.12 ± 0.23A	514.04 ± 3.12A
		CWL	0.37 ± 0.10B	0.19 ± 0.02B	1.29 ± 0.13B	219.22 ± 3.23B
	Muscle	WL	1.22 ± 0.14A	0.04 ± 0.01A	2.01 ± 0.04A	354.31 ± 1.42A
		CWL	0.11 ± 0.05B	0.02 ± 0.01B	1.05 ± 0.33B	137.04 ± 5.63B
	Gills	WL	4.21± 0.47A	1.98 ± 0.24 A	6.42 ± 0.21A	724.12 ± 3.41A
		CWL	1.63 ± 0.21B	1.51 ± 0.12B	4.76 ± 0.55B	249.72 ± 4.39B

Note: Means with the same letter in the same column are not significantly different (P ≥ 0.05),
TW = 2de-chlorinated tap water & RSW= raw sewage water,
WL = de-chlorinated tap water and raw sewage water (1:1) without culturing *Limna spp. And*
CWL = de-chlorinated tap water and raw sewage water with culturing *Limna spp*.

effluent. Similar results have been reported by several demonstration projects (Pillay, 1992; Easa *et al*., 1995 and Eves *et al*., 1995).

Mean concentrations of Pb, Cd, Cu and Fe at fish organs had significantly lower levels (P<0.05) at

WCL treatment than WL treatment (Table 3).

These results agreed with those of Ozturk *et al*. (1994 and 2005). Korner *et al*. (1998) mentioned that the use of the free-floating duckweed in sewage water treatment systems has been advocated because it is fast growing and easy to harvest, has a low fiber and high protein content, and shows a high efficiency in removal of nitrogen and phosphorous from sewage water.

It could be concluded that raw sewage water not enough to reuse in aquaculture and it is strongly recommended that it should be retreated by using *Lemna spp.* prior to its use in fish aquaculture to ensure good production and good quality of fish.

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دراسة على قدرة نبات عدس الماء (*Lemna spp.*) على تقليل الحمل البكتيري ومتبقيات بعض العناصر الثقيلة من مياه الصرف الصحي لإعادة استخدامها فى الاستزراع السمكي

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1- المعمل المركزي لبحوث الثروة السمكية بالعباسة–مركز البحوث الزراعية

2- قسم الميكروبيولوجيا الزراعية -كلية الزراعة – جامعة الزقازيق

أجريت هذه الدراسة فى المعمل المركزى لبحوث الثروة السمكية بالعباسة، أبو حماد، شرقية لدراسة إمكانية استخدام نبات عدس الماء لتنقية مياه الصرف الصحي بمحطة الزقازيق لتقليل الحمل البكتيري وتخليصها من متبقيات بعض العناصر الثقيلة (الرصاص، الكادميوم، النحاس و الحديد) بغرض تحسين جودتها . استخدمت 9 أحواض زجاجية (30 لتر)، قسمت إلى 3 معاملات (3 مكرر/معاملة). وكانت المعاملة الأولي عبارة عن ماء صنبور منزوع الكلور، أما الثانية فكانت 50% ماء صنبور و50% ماء صرف صحي (أحضر من محطة رفع الصرف الصحي بالزقازيق), وكانت الأخيرة عبارة عن 100% ماء صرف صحي. زرعت جميع الأحواض بنبات عدس الماء الذي أحضر من إحدى الترع المجاورة للمعمل واستمرت التجربة لمدة 15 يوماً داخل المعمل الرطب. لوحظ موت جميع النباتات فى المعاملتين الأولى والثالثة، بينما ازدهرت فى المعاملة الثانية. تم تجميع مياه هذه المعاملة واستزرعت بأسماك البلطي النيلي والمبروك العادي ومقارنتها بمعاملة أخري عبارة عن نفس المياه إلا أنها لم تزرع بعدس الماء من قبل لمدة 15 يوماً أخري. تم أخذ عينات مياه وأسماك ونباتات قبل وبعد كلاً من المعاملتين بغرض إجراء الاختبارات البكتيرية المختلفة بالإضافة إلى قياس تركيزات بعض العناصر الثقيلة فى المياه والأسماك والنباتات.

وقد أسفرت الدراسة عن النتائج الآتية:

أظهرت الدراسة أن نبات عدس الماء المستزرع على نسبة التخفيف 50% صرف صحى له القدرة على تحسين جودة المياه وتخليصها من كمية عالية من متبقيات العناصر الثقيلة, وكذلك إختزال نسبة كبيرة من الأعداد البكتيرية في مياه الصرف والأسماك المستزرعة بها.

ولهذا توصى الدراسة إستخدام نبات عدس الماء فى عمليات معالجة مياه الصرف الصحي لما له من كفاءة عالية فى التخلص من كميات كبيرة من العناصر الثقيلة وتحسين جودة المياه قبل إعادة استخدام هذه المياه في الأغراض الزراعية المختلفة وخاصة الإستزراع السمكي.