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Aquatic macrophyte *Spirodela polyrrhiza* as a phytoremediation tool in polluted wetland water from Eloor, Ernakulam District, Kerala.

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Abstract: *This study involved a laboratory experiment on the efficiency of the plant duckweed Spirodela polyrrhiza in improving the quality of two polluted wetlands of Eloor industrial area, Ernakulam, Kerala. The efficiency was tested by measuring some of physicochemical characteristics of the control and plant treatments after each eight days. All the parameters show considerable rate of reduction. In wetland I, The highest rates of reduction after 8 days of treatment were for heavy metals, accounting 95%, 79%, and 66% for Lead, Copper and Zinc, respectively, followed by 53% for Chromium, 45% for Mercury, 26% for Cobalt, 20% for manganese and 7% for Nickel. Other factors like pH, BOD, COD, Nitrate, Phosphate , sulphate, TDS, TSS and Turbidity reduced by 12%, 37%, 49%, 100%, 36%, 16%, 53%,85% and 52% respectively. In wetland II also heavy metals were removed with Cd(100%), Fe(98%), Pb(91%), Cu(74%) Zn(62%) and Hg(53%) removed more efficiently. The results showed that this aquatic plant can be successfully used for wastewater pollutants removal. Other physiochemical parameters like pH, BOD, COD, Nitrate, Phosphate , sulphate, TDS, TSS and Turbidity reduced by 14%, 40%, 60%, 100%, 38%, 65%, 73%, 85%, and 51% after 8 days of treatment.*

Key words: *Phytoremediation, Spirodela polyrrhiza, Lemnaceae, Wetland, Heavy metals*

I. Introduction

Wetlands support a wide array of flora and fauna and deliver many ecological, climatic and societal functions. Scientists often refer to wetlands as the" kidneys" of the earth. . Kerala is well known for its wetlands.

Eloor, an island of 11.21 sq/km, on the Periyar River is home to more than 247 chemical industries and large number of wetlands. The soil, water bodies and the wetlands in and around Eloor have been contaminated with heavy metals. Duckweed based wastewater systems are promising to be used in effluent treatment considering organic matter, pathogen and nutrient removal (Smith and Moelyowati 2001). Besides, duckweeds are floater plants, which reduce suspended solids by blocking light penetration. Thus, light availability causes algae die off, which settle or disintegrate. Ran *et al*. (2004) points the advantages of using duckweeds due to its high production rate, easy manual harvest from the surface, high protein and low fiber content The aim of the present investigation was to evaluate the effectiveness of duckweed *Spirodela polyrrhiza* to remove all impurities as well as heavy metals from the water samples taken from two sites of polluted welands in Eloor. Among macrophytes, duckweeds are very small floating aquatic macrophytes belonging to the Lemnaceae family which grow on the nutrient rich surface and in fresh waters and they are known for their efficiency in nutrient uptake (Bal-Krishna and Polprasert, 2008). Likewise, Lemnaceae have the greatest capacity in organic matter removal and in absorbing the micro-elements such as potassium, calcium, sodium and magnesium among others. However, duckweed plants grow only in the upper water surface layer where mainly pollutant removal takes place (Dalu and Ndamba, 2003). In India phytoremediation techniques are on initial scale and detail investigations are necessary for further research.

II. Materials and methods

I. Sample collection

One sample (Wetland 1) was collected approximately 8 metres north of the Kuzhikundam Thodu creek, at a location approximately 10 m northwest of the HIL site boundary (Lat 10^0 04'51.76"N and Long 76^0 17'32.55"E) . (see table 1) The second sample (Wetland 2) was collected from the wetlands southwest of the "Amanthuruthu" wetland area, approximately 150 metres west of the HIL (Hindustan Insecticides Limited) site, and approximately 80 metres south of the Kuzhikundam Thodu creek (Lat 10^0 04'48.13"N Long 76^0 17'22.75"E). The samples taken from both sites were analysed for physiochemical parameters.

II. Duckweed treatment system

Spirodela polyrhiza is a floating aquatic macrophyte belonging to the family Lemnaceae and can be found worldwide on the surface of fresh and brackish waters (Zimmo, 2003). The Lemna and Spirodela are among the most standardized test organisms in aquatic ecotoxicology (EPA 1996; DIN2000, 2001; Eberius

2001; OECD 2002). The wetland water collected had undergone preliminary sieving step to get rid of large suspended solids. The transferred water was immediately collected into the aquariums in laboratory conditions (as replicates) . The treatment system with growing duckweed in three small glass aquariums (length18 inches) was constructed in laboratory set up .Each aquarium was 10 inches deep and 9 inches wide. These aquariums were arranged in such a way that light availability is maximum. The sides were covered to prevent light entering except at the top (Parr *et al*., 2002),. Duckweed (*Spirodela polyrhiza.*) plants were collected from an unpolluted natural pond near Fort Kochi, Kerala .The stocks were cleaned by tap water then washed by distilled water . .

Approximately 50g of fresh, wet *Spirodela polyrrhiza* plants were stocked into each of the three aquariums. Each aquarium was supplied sequentially with wetland water diluted with distilled water in 1:4 ratios. Each of the three aquariums was filled with same dilutions of wetland water . An aquarium is kept with same dilution but without macrophyte is considered as control. The experiment was kept under laboratory conditions of temperature (25+2) and lighting (8 light: 16 dark). Detention time of duckweed was 8 days in the first reactor, 4 days and 2 days in the second and third one. After harvesting, new and prewashed duckweed was inserted. Water volume reduction by volatilization was compensated by addition of pure water.

III. Analytical methods

A single sample collection has been done from the study area. The water collected from the site was analysed for physio chemical characteristics. The parameters of study are pH, BOD, COD, Nitrate, Phosphate, Sulphate,TDS, TSS and Turbidity before and three weeks after the experiment. Analysis revealed that wetland water is a cocktail of variety of metals including heavy metals. Metals like Copper, Lead, Zinc, Chromium, Cobalt, Manganese, Mercury and Nickel were present .

During the treatment process subsurface (under duckweed mat) water samples for physico-chemical, were collected in polyethylene bottles from all sides of each tank and then mixed. This procedure carried out every week. Initial and final measurements after three weeks of exposure were made. The percentage of removal or removal efficiency was also calculated. Physico-chemical analysis were carried out according to standard methods for examination of water and wastewater (APHA, 1992). Field parameters were measured in situ. The statistical analysis was done using STATISTICA software.

IV. Results and Discussion

The results of efficiency of *Spirodela* in scavenging contaminants indicate that the presence of this macrophyte was an important element for contaminant removal in wastewater. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in the present study, since the accumulation of heavy metals were higher in plants than water. Rhziofiltration, also referred to as phytofiltration, is based on hydroponically grown plants that have shown to be most efficient in removing heavy metals from water (Raskin *et al*., 1994).

In physio-chemical analysis different parameters (colour, pH, BOD, COD, Nitrate, Phosphate , Sulphate total dissolved solids, TSS and turbidity of wetland I and II were studied . During sample collection colour of the wastewater samples was turbid or slightly yellowish. The level of colour in the wastewater may be due to the presence of total dissolved solids.

The pH of water from wetland I was alkaline 8.2 and for wetland II was 8.4 were found to be in the optimum range for duckweed growth(Dalu & Ndamba, 2002). After 2 days of treatment it has reduce to 7.2 and 7.9 for WI and WII. In the remaining two treatment chambers the pH remains 7.2 and 7.4 respectively after 4days and 7.2 for both samples after 8 days of treatment (Figure 1&2) In the present experiment temperature ranged between 21.7^{o}C and 23^{o}C which was within temperature tolerance limit for duckweed growth as mentioned by Culley *et al.,* (1981) who found that the upper temperature tolerance limit for duckweed growth was around 34oC. Duckweed tolerance allows it to be used for year– round wastewater treatment in areas where tropical macrophytes, such as water hyacinths, can only grow in summer (Cheng *et al.,* 2002).

Figure I: Physiochemical parameters of wetland I measured after 2,4 and 8 days of treatment using duckweed Spirodela polyrrhiza:

Physiochemical parametrs	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
PH	8.2	7.42	10	7.34	10.4	7.2	12.1
BOD (mg/L)	110	96	13	87	21	69	37.2
COD(mg/L)	320	196	39	178	44.3	162	49.3
Nitrate(mcg/L)	27	15	46	4.3	84.0	0	100
Phosphate(mcg/L)	11	9.2	16.36	8.2	25.4	7	36.3
Sulphate(mg/L)	500.12	469	6.30	444.2	11.1	421	16
TDS (mg/L)	3210.3	3111	3.09	2928.11	9	1522	53
TSS (mg/L)	218.41	99	55	65.12	70.1	32	85.4
Turbidity(NTU)	29	22.4	23	19.7	32.06	14	52
Copper(mcg/L)	65	28.22	57	21.56	67	14	79
Lead(mcg/L)	26	17.31	33.4	9.43	64	1.3	95
Zinc(mcg/L)	212	177.21	16.4	92.4	56.4	72.3	66
Chromium(mcg/L)	118	101.23	14.2	82	31	56	53
Cobalt(mcg/L)	7.2	6.4	11.1	5.2	28	5.2	28
Manganese(mcg/L)	8	8	5	7.2	10	6.4	20
Mercury(mcg/L)	2	2	0	1.8	10	1.1	45
Nickel(mcg/L)	19.3	19.1	1.03	19	4	18	9

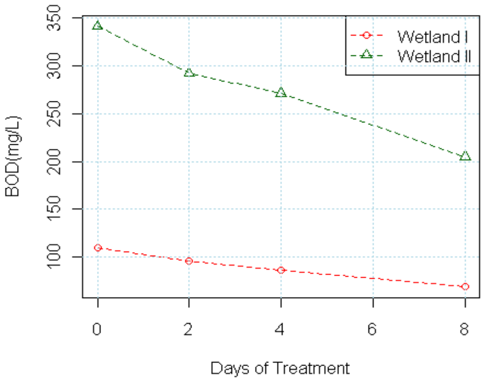
Figure II: Physiochemical parameters of wetland II measured after 2,4 and 8 days of treatment using duckweed Spirodela polyrrhiza:

Physiochemical parameters	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
PH	8.4	8	6.0	7.4	12	7.2	14.2
BOD (mg/L)	341	292.1	14.3	271.2	20.4	205.1	40.0
COD(mg/L)	679	511.2	25.0	464.4	32.0	268.3	60.4
Nitrate(mcg/L)	12.0	6.1	48.0	1.1	91.0	0	100
Phosphate(mcg/L)	13.1	12.0	10.0	10.4	21.0	8.1	38.0
Sulphate(mg/L)	133.0	15.0	89.04	27.4	79.3	46.2	65.1
TDS (mg/L)	593.1	477.0	20.0	266.3	55.1	158	73.3
TSS (mg/L)	359	181	50.0	126.0	65.0	53.0	85.2
Turbidity(NTU)	382	286	25.1	262	31.4	189	51.0
Copper(mcg/L)	63.3	36.0	44.0	22.0	66.0	16.4	74
Lead(mcg/L)	34.4	23.0	34.0	13.0	63.0	3.0	91
Zinc(mcg/L)	301	258	14.2	144.2	52.0	113.1	62.4
Chromium(mcg/L)	121	105.3	13.0	85.0	30	62.3	49.0
Cobalt(mcg/L)	8	7.2	10	6.3	21.2	5.0	40
Manganese(mcg/L)	7.3	7.1	3.0	6.4	12.3	5.1	30.1
Mercury(mcg/L)	3.4	3.1	9.0	3.0	18.0	2.0	53.0
Nickel(mcg/L)	22.3	21	6.0	18.1	19.0	18.0	22.0
Iron(mg/L)	5.3	5.1	4.0	4.2	21.0	0.1	98.1
Cadmium(mg/L)	3	3	0	3.0	7.0	0	100

Turbidity was reduced by 23% from 29 NTU to 22.4 NTU after 2 days for WI. It further reduced to 19.7(32 %) after 4 days of treatment . After 8 days it was 13.8 NTU which means almost half of the turbidity has been removed Fig (1) .In WII turbidity was reduced by 25.13% from 382 NTU to 286 NTU after 2 days. It further reduced to 262 (31.4) and to 189 NTU(50.5%) after 4 days and 8 days of treatment respectively(Fig:10).

This may be attributed to decrease the concentration of suspended material because of settlement on the bottom and adsorption on aquarium glass and this was shown in statistical analysis , as it recorded significant correlation between suspended solids and turbidity ($r = 0.94$; $p<0.05$).

Figure: 3



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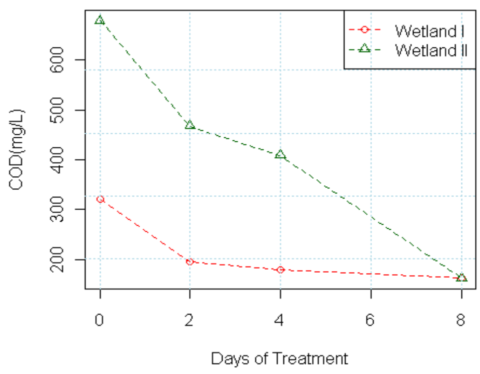

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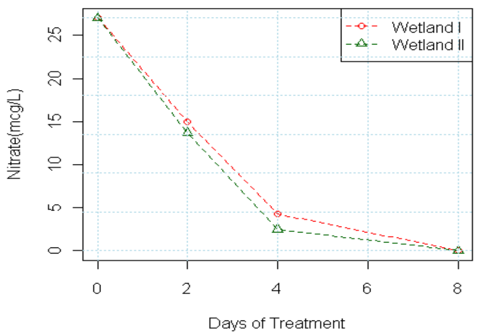

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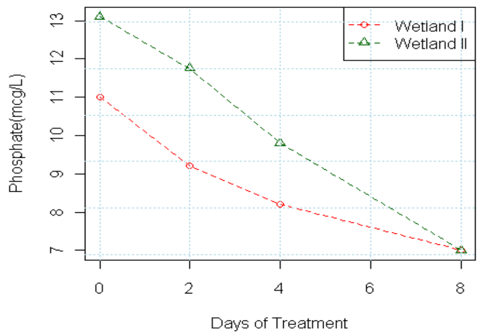

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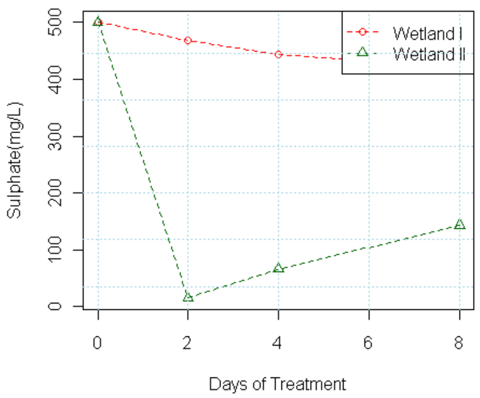

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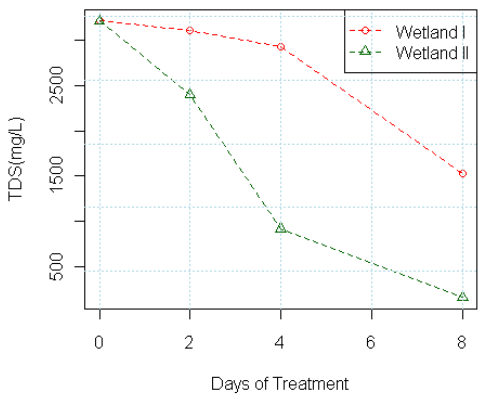

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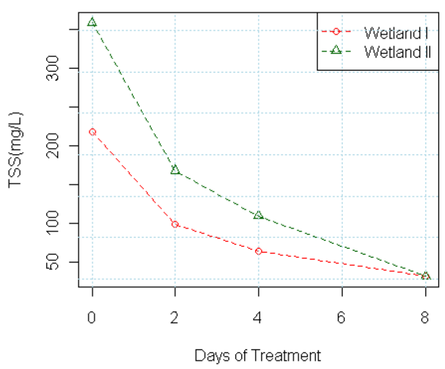

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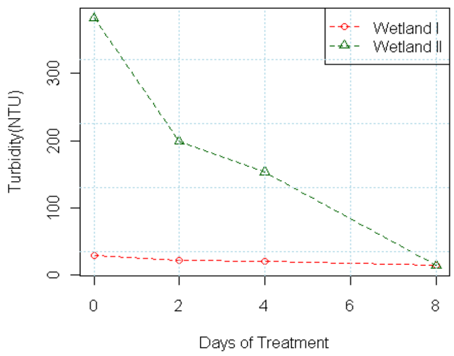

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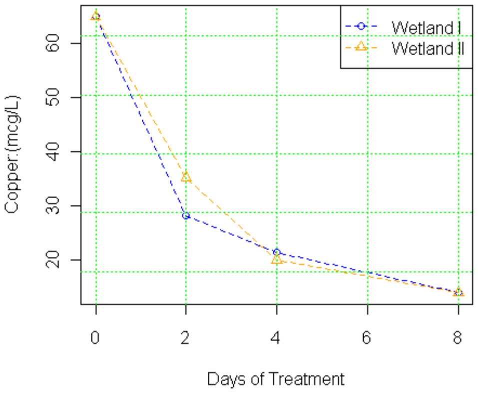

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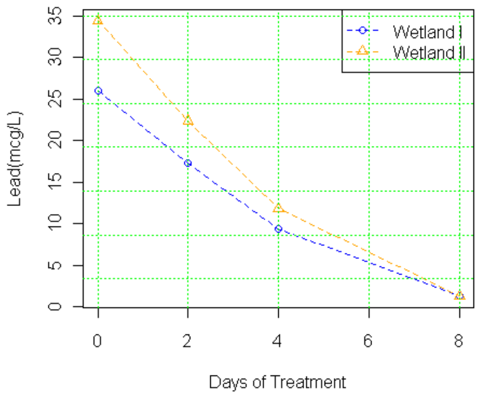

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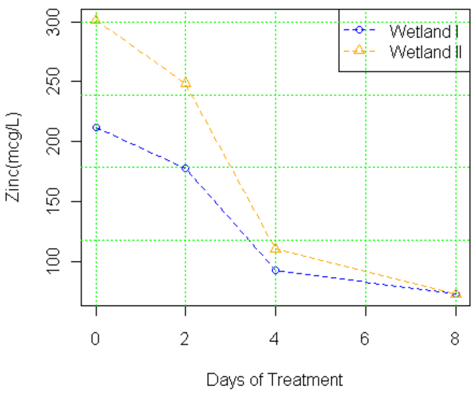

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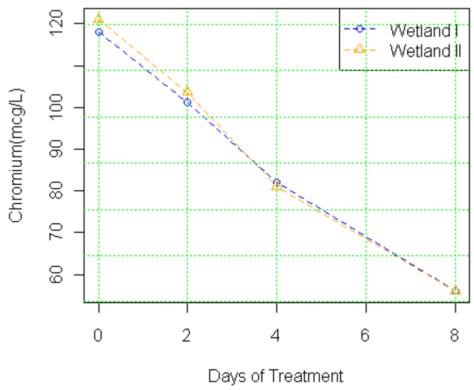

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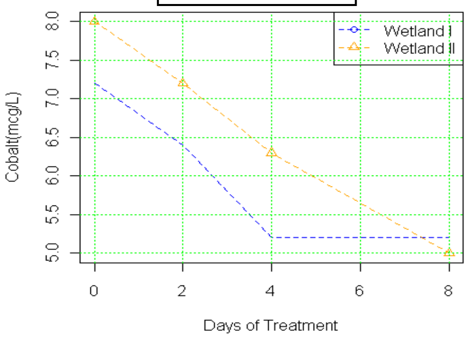

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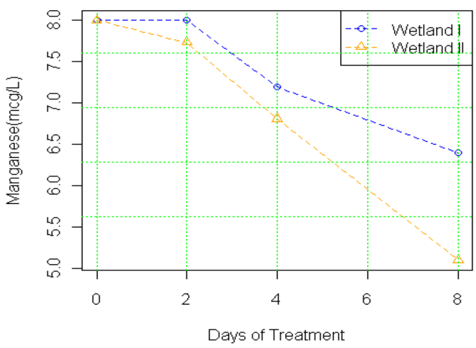

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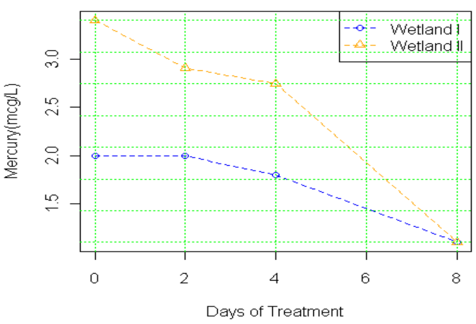

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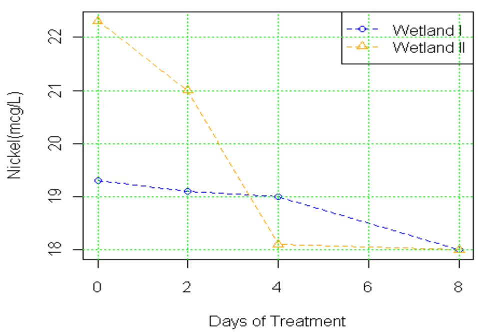

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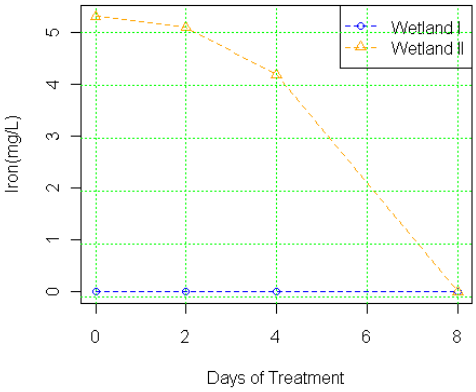

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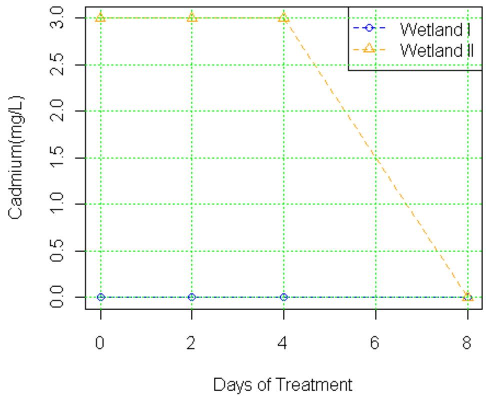

Figure : 9 shows total suspended solids (TSS) values decreased by increasing treatment periods, showing maximum concentration of 218.4 and 359 for WI and WII respectively before treatment. The concentration sides down to 98.66 and 181 mg.L-1after 2 days (55% and49.5% respectively) and further reduced to 65.12 mg/L (70.1%) and 125.6(65.01%)after 4 days and finally decreased by 85.4% and 85.2% (31.7 mg/L and 52.8 mg/L respectively) which corroborates the findings of Pandy, M.(2001) regarding discharged duckweed treatment system in Halisahar ,Likewise Huang, P.,Han,B and Lin,Z.(2007) record a clear reduction in resuspension of sediment in Taiho lake during 41 days which covered by floating aquatic plants , and this result agreed with the study of Al-Sabunji, A.A. and AlMarashi, A.M.(2002).

It was also revealed that total dissolved solids (TDS) of WI and WII recorded their minimum values of 1522 mg/L (52.5%) and 158 mg/L(73.3%) after 8 days treatment. It was 3111 mg/L (3 % reduction)and 476.5 mg/L(19.6% reduction) after 2 days of treatment .It was 2928.11 mg/L (8.7 % reduction) and 266.3mg/L (55.10%) after 4 days. Majority of TDS was reduced between 4 days to 8 days of treatment. This decrease was due to the plant capacity to take some organic and inorganic ions (Figure: 8).

Results in Figure (1and 2) shows that sulfate concentration in WI and WII recorded by 6.3% and 89.04% as reduction percentage during 2 days, 11.18% and 79.33% during 4 days and 15.8% and 65.15% after 8 days of Phytoremediation (Figure: 7). The cause of reduction may be due to plant ability to absorb different types of pollutants and accumulated in their tissues Mkandawire, M. and Dudel, E.G.(2007). But the sulphate reduction percentage after 8 days of treatment is found to be insufficient. It may be assumed that Spirodela polyrhiza is a poor tool for phytoremediation of sulphate from waste water. The phosphate content from the wetland water I and II were 11 mcg/L and 11.71 mcg/L respectively. After 2 days of treatment ,it has been reduced by 16.3% and 47.9% and after 4 days it has been reduced by 25.4% and 20.61% respectively.

After 8 days it has been reduced by 36.36% to 7.0mcg/L for WI and reduced by 37.6% to 8.17 after 8 days of growth (Figure:7).. The removal of phosphate is comparatively better than sulphate. Similarly Nitrate content was 27mcg/L for WI and 11.71 mcg/L for WII. It has been reduced to 14.7mcg/L (45.55%) and 6.1 mcg/L (47.9%) respectively with 2 days of treatment. It was further down to mere 4.3 mcg/L and 1.1mcg/L after 4 days (84% and 90.6% respectively). Eight days of treatment was enough to remove nitrate from the water completely from both samples (100%) See Figure:5.

Figure I and 2 also reveals the gradual reduction of factor like BOD, COD, Phosphate, Nitrate etc. with time. Data revealed that Spirodela polyrhiza mat effectively reduced BOD by 12.7% for WI and 14.34% for WII (reduced from 110 mg O2 L-1 at zero days reaching 96 mg O2 L-1 for WI and reduced from 341 mg O2 L-1 at zero days reaching 292.1 mg O2 L-1 2days treatment). After 4 days it further reduced by 20.9%(reduced to 87 mg/L) and 20.46% (reduced to 271.2 mg/L) for WI and WII respectively. After 8 days BOD stands at 69 mg/L (reduced by37%) for WI and stands at 205.1 mg/L (reduced by 39.85%) for WII. Zimmo *et al.* (2005) found that BOD removal efficiency was higher in duckweed based ponds than in algae based ponds. Pandey (2001) reported that in Delhi the duckweed ponds were operated at different flow rates giving hydraulic retention time from 5.4 to 22 days, a 30 - 50% reduction in phosphate, 56 - 80% reduction in ammoniacal nitrogen and 66 - 80% reduction in BOD (Figure:3). In concurrence with the present findings, Oron *et al.* (1988) mentioned that the duckweed contribution for the removal of organic material is due to their ability to direct use of simple organic compounds. The COD has been reduced by 38.75% for WI and 24.71% immediately after 2 days of phytoremediation (reduced from the initial concentration of 320mg/L to 196 mg/L and 679 mg/L to 511.2 mg/L). After 4 days it further reduced by 44.37% (reduced to 178mg/L) for WI and reduced by 31.60 % (reduced to 464.4 mg/L) and finally after 8 days, reduced to mere 162 mg/L (49.375%) for WI and reduced to

268.3 mg/L (60.4%) for WII (Figure: 4). Korner *et al.* (1998) mentioned that duckweed significantly enhanced COD removal in shallow batch systems. Pandey (2001) reported that COD removal was in the range of 70% - 80% in the discharged duckweed treatment system at Halisahar. However in the present study, the COD and BOD removal by the macrophyte were not up to the capacity of Lemna minor.

Ferrara *et al.* (1985) indicated the reliability of wastewater treatment by some aquatic plants including duckweed in adsorption of the heavy metals cadmium and zirconium. Viet *et al.* (1988) reported that duckweed plants proved to be an excellent bioaccumulator of various heavy metals, which allowed it to treat a variety of wastewaters including industrial and highly polluted wastes. Hammouda *et al.* (1995) evaluated the efficiency of duckweed aquatic treatment in heavy metals removal in various water systems data obtained suggested a maximum reliability of systems with mixtures containing high ratios of wastewater. In the present study metals like Copper, Lead, Zinc, Chromium, Cobalt, Manganese, Mercury and Nickel were found in the wetland water and removed by the plant by greater extend. The eight metals studied, showed Pb>Cu>Zn>Cr>Hg>Co>Mn>Ni pattern of absorbance. Pb concentration was 26mcg/L in the control solution. After 2 days of treatment it has been reduced by 33.4% to 17.3 mcg/L. After 4 days it further reduced by 63.7% to 9.43 mcg/L. Finally after 8 days Lead concentration is only 1.3mcg/L, which means 95 % removal (Figure: 12). After 8 days of treatment Copper content in the water has been removed by 78.76% which means a reduction from initial concentration of 65mcg/L to final concentration of 13.8 mcg/L (Figure: 11). Copper shows removal by 56% after 2 days and removal by 66.83 % after 4 days of treatment with Spirodela polyrhiza plant. Initial concentration of Zn was 212 mcg/L . Two days of treatment is only enough to remove 16.4% of Zn from the wetland water which means reduction to 177.21 mcg/L. Four days of treatment was enough to remove 56.4% of Zn (reduced to 92.4 mcg/L) See Figure: 13. Chromium concentration was 118 mcg/L before treatment. It has been reduced by 14.21% , 30.71% and 52.5% after 2, 4 and 8 days of treatment (Figure:14). Cobalt concentration was very low initially i.e.7.2 mcg/L. After 2 days it has been reduced by mere 11.11% . After 4 days the concentration was measured as 5.2 mcg/L i.e. 27.77%. Interestingly even after 8 days of treatment the cobalt concentration did not came down any further i.e. it remains at 27.77% removal (Figure:15). The Manganese concentration has been reduced by 20% from 8 mcg/L to just 6.4 mcg/L even after 8 days of treatment. This reveals that Spirodela polyrhiza is a poor accumulator of Manganese (Figure: 16). Mercury concentration remains the same even after 2 days of treatment i.e. 2.0 mcg/L. The concentration reduced to 1.1mcg/L after 8 days of treatment with the removal efficiency of 45% (Figure: 17). Nickel shows the least removal after the treatment regime of 8 days. It has been reduced from 19.3 mcg/L to 17.6 mcg/L with removal efficiency of just 8.80% that shows that Spirodela is a poor accumulator of Nickel(Figure: 18). Iron and Cadmium were present in Wetland II but were absent in wetland I. Fe were removed 98.1% and Cd removed completely (100%) after 8 days of treatment (Figure 19&20).

IV. Conclusion

In the current study macrophyte duckweed Spirodela polyrhiza was employed as effective phytoremediation agent in the polluted wetland water from Eloor. Constructed wetlands with Spirodela mat may help to prevent the spread of heavy metal contamination from land to the aquatic environment. High metal removal rates of close to 100% have been reported in both wetlands is quite promising. The advantage of lagoon treatment systems that use aquatic plants as productive 'sinks' for wastewater nutrients from a wide range of sources . It is easy and cheap to construct and operate suggests they are a suitable alternative for wastewater purification.

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