International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 22-12-2021, Accepted: 06-01-2022, Published: 22-01-2022
Volume 7, Issue 1, 2022, Page No. 416-424

Phytoremediation of domestic waste water by aquatic macrophytes and its effect on growth and composition of plants

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Abstract
Municipal wastewater treatment and its properdisposal are major problems being faced by all the municipalities of growing cities throughout the country. The high price of treatment plant has forced many of the municipalities to discharge domestic waste water without any treatment. Remediation of nutrients from wastewater is an emergingtechnique of environmental biotechnology in which various living organisms used for environmental clean up and pollution control. The objectives of the present study was to evaluate the potential of three different aquatic macrophytes for treatment of municipal waste water and are recommended to its use for gardening and irrigation purposes after dilution. Maximum fresh matter and dry matter yield at 7, 15 and 30 days growth of *Cladophora* and *Lemna* was observed at 60 percent level of municipal waste water and of *Azolla* was observed at 40 percent level of municipal waste water. Maximumvalues for chlorophyll, ascorbic acid, catalase and peroxidase activities was observed with the range of 20 to 60 percent level of municipal waste water at 7, 15 and 30 days growth and heavy metals Iron, Manganese, Zinc and Chromium accumulationwas observed at 30 days growth of *Cladophora*, *Azolla* and *Lemna* plants.

Keywords: municipal waste water, phytoremediation, aquatic macrophytes

Introduction

Although the history of civilization is as old as the men knowledge of fire and tools to alter his environment, but its wholesome impact on nature has never been as grave as during recent decades. The present day speedy socio-economic development of humanity, which is the highest form of vital activities, has most vigorously stimulated changes in nature. Technological advancement, if on one hand, has bettered the human life on this planet, it has sharply intensified the pollution of environment and posed still severe threats for future generation, on the other hand. Increasing population growth, extermination of a number of life forms because of man's activity, dwindling natural resources that man has been consuming for centuries, and deterioration of overall environmental quality, are the primary ecological problems that man is creating and facing today. This renders it undisputed that there is an acute need to develop an ecological approach for all development planning.

A quick rise in population, rapid industrialization, increasing urbanization, growth of science, technology and advanced agricultural practices aimed to increasing the food production etc. are all feature of a modern society. In this process of development, however, little thought was given to the effect of waste, which such a society generates on the environment. In slow degrees wastes have been polluting the environment and threatening the very existence of healthy life. In addition, we have also been overexploiting our material resources which have now resulted in severe problems of degradation of agricultural fields, pasture, forests, soil erosion, floods etc. and have endangered the existence of many useful plant and animal species.

Today, the cry of "Pollution" is heard from all the nooks and corners of the globe, and the pollution has become a major threat to the very existence of mankind on this earth. When man and other higher animals began their life on this earth there was absolutely no sign of pollution. There was perfect balance in various natural processes. As human population increased, there was increase in our working sphere and with this also increased the pollution. On one hand, the advancement of science and technology have added to the human comfort by giving us automobiles, electrical appliances, supersonic jets, spacecrafts, better medicine, better chemicals to control harmful insects and other pests etc. But on the other hand, they have given us a very serious problem to face "Pollution".

Pollution of biosphere with toxic metals has accelerated dramatically, since the beginning of the industrial revolution (Nriagu, 1979). Extraction of minerals is associated with generation of large quantity of wastes, which creates serious environmental problem with potentially harmful consequence to agriculture and human. One of such waste is the tailing. Tailing are structureless matrix frequently with unsatisfactory porosity, aeration, water infiltration and percolation. They suffer deficiencies of essential nutrients and organic matter, lack soil organisms and are biologically hostile because of extreme of pH, and high levels of salts and toxic metals. During dry season, the finer particles of the tailing are subjected to wind erosion and not only create air pollution but may also affect adjacent croplands by deposition of fine particles of heavy metals and silica. Heavy metals, dissolved salts and undissolved suspension are carried out into streams and rivers either due to release of water from tailing dam or due to heavy rain fall. Further, water percolating through metalliferous waste pollutes the ground water. All these demand for the immediate remediation of the metalliferous wastes. This can be achieved either by proper stabilization or by detoxification of the area. Stabilization for the proper management of the tailings is done by any of the following methods: Physical Stabilization, Chemical Stabilization, and Biological Stabilization.

Material and Methods

Sampling of Water

The samples of Municipal Waste Water were collected from drainage of Sujatgang, Kanpur in wide mouth large plastic bottles between 7-8 AM and bottled cork immediately and all the samples were brought to settle in an open cemented pond of college garden for one week to allow microorganisms to break down solid organic waste. The waste water then filtered through an 80 µm plankton net filter and brought the laboratory and stored at 4^{0}C temperature in a refrigerator. Care was taken to prevent under shaking of the samples and also against sun light while transporting them to the laboratory.

Plant Analysis

Fresh matter yield was determined by weight first washing with running tap water, rinsing with distilled water and absorbing surface with clean white blotting paper.Dry matter yield was determined by drying and finely chopped and mixed plants samples in a forced draught oven at 65^{O}C for 24 hours to constant weight. The samples were taken out from the oven and placed in a desiccators, cooled for about an hours and weighed for the determination of yield. Since the dry matter was required for the estimation of heavy metals (Fe, Mn, Zn and Cr), fresh matter kept for drying was thoroughly cleaned against any surface contamination by first washing with running tap water, rinsing with distilled water and absorbing surface water with clean white blotting sheets.Chlorophyll was determined by the method of Petering *et al.* (1940) [48].Ascorbic acid content was estimated titrimetrically by the method of Harris and Roy (1933) [23].Catalase was assayed in crude tissue extracts by the permanganate titration method of the Euler and Josephson (1927) [15] and estimation of Peroxidase activity was done by the method of Gahagen *et al.* (1968) [17].
Estimation of heavy metals Iron and Manganese was done by the method described by Humphries (1956) [24] and Nicholas and Fischer (1950) [42]. Zinc and Chromium was done with the methods described in B.I.S. (2003).

Statistical Analysis

Data were subjected to the analysis of variance according to Steel and Torrie (1960) [61]. The significance of the differences between control and each treatment was determined using the value of least significant difference. The mean values with least significant difference (L.S.D.) at P = 0.05 and P = 0.01 have been presented in table 1.

Results (Table 1 & 2)

Fresh Matter Yield

With the increase in percent level of municipal waste water fresh matter yield increases up to 60 percent level of 7, 15 and 30 days growth of *Cladophora* and *Lemna* and up to 40 percent level of 7, 15 and 30 days growth *Azolla* plants. Further increase in percent level of municipal waste water, beyond 60 percent in 7, 15 and 30 days growth of *Cladophora* and *Lemna*, and beyond 40 percent in 7, 15 and 30 days growth of *Azolla* showed decrease in fresh matter yield of plants.
As compared to control, all the levels up to 60 percent level in *Cladophora* and *Lemna*,and up to 40 percent level in *Azolla* showed highly significant (P=0.01) increase in fresh matter yield of 7, 15 and 30 days growth.
Increase in fresh matter yield was found to be to be highly significant (P=0.01) at 10 percent over control, 20 over 10 percent and 40 over 20 percent level at 7, 15 and 30 days growth of *Cladophora, Azolla* and *Lemna* plants, and at 60 over 40 percent level of municipal waste water at 7, 15 and 30 days growth of *Azolla.*
Decrease in fresh matter yield was found to be highly significant (P=0.01) at 60 over 40 percent level of municipal waste water at 7, 15 and 30 days growth of *Azolla*, and at 80 over 60 percent, and 100 over 80 percent level of municipal waste water at 7, 15 and 30 days growth of *Cladophora, Azolla* and *Lemna* plants, except at 100 over 80 percent where value reach the level of significant (P=0.05) in 7 days growth of *Azolla*, and fails to reach the level of significance at 15 and 30 days growth of *Azolla* and 7 days growth of *Lemna* plants.
Maximum fresh matter yield at 7, 15 and 30 days growth of *Cladophora* and *Lemna* was observed at 60 percent level of municipal waste water and of *Azolla* was observed at 40 percent level of municipal waste water.

Dry Matter Yield

With the increase in percent level of municipal waste water, up to 60 percent level, increase in the dry matter yield of *Cladophora* and *Lemna*, and up to 40 percent level increase in the dry matter yield of *Azolla* plant were observed. Further increase in percent level of municipal waste water beyond 60 percent in *Cladophora* and *Lemna* and beyond 40 percent in *Azolla* decrease in dry matter yield of 7, 15 and 30 days old plants were observed.
At 7, 15 and 30 days growth increase in dry matter yield of *Cladophora*, *Azolla* and *Lemna* plants was found to be highly significant (P=0.01) at 10, 20 and 40 percent level of in *Cladophora* and *Azolla* and at 60 percent level of in *Cladophora* and *Lemna*, over control.
Increase in dry matter yield of 7, 15 and 30 says growth was found to be highly significant (P=0.01) at 10 percent over control, 20 over 10 percent, 40 over 20 percent municipal waste water level in *Cladophora, Azolla* and *Lemna* and at 60 over 40 percent in *Cladophora* and *Lemna* plants, except in 30 days of *Cladophora* where value showed only significant (P=0.05) increase and in 30 days growth of *Lemna* where increase was insignificant.
Decrease in dry matter yield of 7, 15 and 30 days growth was found to highly significant (P=0.01) at 60 over 40 percent in *Azolla* and at 80 over 60 and 100 over 80 percent municipal waste water level in *Cladophora*, *Azolla* and *Lemna* plants, except at 100 over 80 percent level in 15 and 30 days of *Azolla* where value fails to reach the level of significance.
Maximum dry matter yield of 7, 15 and 30 days growth of *Cladophora* and *Lemna* was observed at 60 percent and of *Azolla* at 40 percent level of municipal waste water.

Chlorophyll

Chlorophyll content increased with the increase in municipal waste water levels, up to 60 percent level at 7 days growth of *Cladophora up* to 40 percent level at 15 days growth of *Cladophora*, and 7 days growth of *Azolla* and *Lemna,* and up to 20 percent level at 15 and 30 days growth of *Azolla* and *Lemna* and 30 days growth of *Cladophora*. Beyond these respective levels, further increase in municipal waste water levels decreased the chlorophyll content of *Cladophora, Azolla* and *Lemna* plants.

As compared to control, increase in chlorophyll content was found to be to be highly significant (P=0.01) at 10 percent level in 30 days growth of *Azolla* and *Lemna*, at 20 percent level in all the stages of growth i.e. 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 40 percent level in 7 days growth of *Cladophora*, *Azolla* and *Lemna* and 15 days growth of *Cladophora,* and at 60 percent level in 7 days growth of *Cladophora*; significant (P=0.05) at 10 percent level in 7 days growth of *Azolla* and *Lemna*, 15 days growth of *Azolla* and 30 days growth of *Cladophora*;and fails to show any significant increase at 10 percent level in 7 days growth of *Cladophora* and 15 days growth of *Cladophora* and *Lemna*.

Increase in chlorophyll content was found to be highly significant (P=0.01) at 10 percent over control at 30 days growth of *Azolla* and *Lemna*, 20 over 10 percent at 7 days growth of *Cladophora* and 30 days growth of *Cladophora* and *Azolla*, and 40 over 20 percent at 7 days growth of *Cladophora,* 15 days growthof *Azolla* and *Lemna*, and 30 days growth of *Cladophora*, *Azolla* and *Lemna*; significant (P=0.05) at 10 percent over control in 7 days growth of *Cladophora* and *Azolla* and 15 days growth of *Azolla*, at 20 over 10 percent in 7 days growth of *Azolla*, 15 days of *Cladophora* and *Azolla*, at 40 over 20 percent in 7 days growth of *Azolla* and *Lemna*, 15 days growth of *Cladophora*; and insignificant at 10 percent over control in 7 days growth *Cladophora* and 15 days growth of *Cladophora* and *Lemna,* at 20 over 10 percent in 7, 15 and 30 days growth of *Lemna*, and at 60 over 40 percent in 7 days growth of *Cladophora*.

Decrease in chlorophyll content was found to be significant (P=0.05) at 80 over 60 percent at 15 days growth of *Cladophora* and *Azolla*, and at 100 over 80 percent at 7 days growth of *Cladophora* and *Lemna*; and highly significant (P=0.01) at 40 over 20 percent in 30 days growth of *Cladophora* and in 15 and 30 days growth of *Azolla* and *Lemna,* at 60 over 40 percent in 15 and 30 days growth of *Cladophora* and 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna,* and 80 over 60 percent in 7 and 30 days growth of *Cladophora* and *Azolla* and 7, 15 and 30 days growth of *Lemna* and at 100 over 80 percent in 7 days growth of *Azolla* and 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna* plants.

Maximum chlorophyll content was observed at 60 percent level in 7 days growth of *Cladophora*, at 40 percent level in 15 days growthof *Cladophora* and 7 days growth of *Azolla* and at 20 percent level in 30 days growth of *Cladophora* and 15 and 30 days growth of *Azolla* and *Lemna* plants.

Ascorbic Acid

With the increase in percent level of municipal waste water ascorbic acid content of *Cladophora*, *Azolla* and *Lemna* increased up to 60 percent level in 7 days growth of *Cladophora*, up to 40 percent level in 15 days growth of *Cladophora,* and 7 days growth of *Azolla,* and 7 and 15 days growth of *Lemna,* and up to 20 percent level in 30 days growth of *Cladophora* and *Lemna* and15 and 30 days growth of *Azolla.* Beyond these respective levels, further increase in municipal waste water levels, decreased the ascorbic acid content of *Cladophora, Azolla* and *Lemna*.

As compared to control, increase in ascorbic acid content was found to be significant (P=0.05) at 10 percent over control in 7 days growthof *Cladophora*; and highly significant (P=0.01) at 10 percent in 15 and 30 days growth of *Cladophora*, and 7, 15 and 30 days growth of *Azolla* and *Cladophora* plants, at 20 percent in 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 40 percent in 7, 15 and 30 days growth of *Cladophora* and *Azolla*, and at 60 and 80 percent in 7 days growth of *Cladophora*.

Except 10 percent over control in 7 days growth of *Cladophora*, where increase ascorbic acid content was found significant (P=0.05), at all other levels i.e. 10 percent over control, 20 over 10 percent level was found to highly significant (P=0.01) at 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna* plants, at 40 over 20 percent in 7 and 15 days growth of *Cladophora* and *Lemna* and 7 days growth of *Azolla*, and at 60 over 40 percent in 7 days growthof *Cladophora*.

Decrease in ascorbic acid content was found to be highly significant (P=0.01) at 40 over 20 percent in 30 days growth of *Cladophora*, *Azolla* and *Lemna* and 15 days growth of *Azolla*, 60 over 40 percent in 7 days growth of *Azolla* and *Lemna* and 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 80 over 60 percent at 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna* and 100 over 80 percent at 7 and 15 days growth of *Cladophora*, 7 and 30 days growth of *Azolla* and 7, 15 and 30 days growth of *Lemna* plants; and significant (P=0.05) at 100 over 80 percent in 30 days growth of *Cladophora* and 15 days growth of *Azolla*.

Maximum ascorbic acid content was observed at 60 percent in 7 days growth of *Cladophora*,at 40 percent in 15 days growth of *Cladophora* and *Lemna*, and 7 days growth of *Azolla* and *Lemna*, and at 20 percent level in 30 days growth of *Cladophora*, *Azolla* and *Lemna* and 15 days growth of *Azolla* plants.

Catalase Activity

Up to 60 percent, in 7 days growth of *Cladophora*, up to 40 percent in 7 days growth of *Azolla* and *Lemna* and 15 and 30 days growth of *Cladophora*, and up to 20 percent in 15 days growth of *Azolla* and 30 days growth of *Azolla* and *Lemna*, increase in municipal waste water level, increased the catalase activity of *Cladophora*,*Azolla* and *Lemna* plants. Beyond these respective levels of municipal waste water, further increase in levels, decreased the catalase activity of *Cladophora*, *Azolla* and *Lemna* plants.

As compared to control, increase in catalase activity was found to be insignificant at 10 percent in 7 days growth of *Cladophora*,*Azolla* and *Lemna* and 30 days growth of *Azolla*;significant at (P=0.05) 15 and 30 days growth of *Cladophora*, 15 days growth of *Azolla* and *Lemna*; and highly significant (P=0.01) at 30 days growth of *Lemna*. Highly significant (P=0.01) increase in catalase was also observed at 20 percent level in 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 40 percent in 7, 15 and 30 days growth of *Cladophora*, 7 days growth of *Azolla* and 7 and 15 days growth of *Azolla*, at 60 percent in 7 days growth of *Cladophora*.

Increase in catalase activity was found to be highly significant (P=0.01) at 10 percent over control in 30 days growth of *Lemna*; and significant (P=0.05) at 10 percent over control in 15 days growth of *Cladophora*, *Azolla* and *Lemna*, 30 days growth of *Cladophora*, *Azolla* and 15 days growth of *Lemna*, at 40 over 20 percent in 7 days growth of *Cladophora*,and fails to show any significant increase at 10 percent over control in 7 days growth of *Cladophora*, *Azolla* and *Lemna* and 30 days growth of *Azolla*, at 40 over 20 percent in 7 days growth of *Lemna*, at 20 over 10 percent in 30 days growth of *Lemna*, and at 40 over 20 percent in 15 and 30 days growth of *Cladophora*.

Decrease in catalase activity was found to be insignificant at 80 over 60 percent in 15 and 30 days growth of *Azolla*, at 100 over 80 percent in 7 days growth of *Cladophora*, *Azolla* and *Lemna*, 15 and 30 days growth of *Cladophora*; significant (P=0.05) at 40 over 20 percent in 15 and 30 days growth of *Lemna*; and highly significant (P=0.01) at 40 over 20 percent in 15 and 30 days growth of *Azolla*, 7 days growth of *Lemna*, at 60 over 40 percent in 7 days growth of *Azolla* and *Lemna* and 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 80 over 60 percent in 7 days growth of *Cladophora*, and at 100 over 80 percent in 30 days growth of *Lemna*.

Maximum catalase activity was observed at 60 percent in 7 days growth of *Cladophora*, at 40 percent in 15 and 30 days growth of *Cladophora*, and 7 days growth of *Azolla* and *Lemna*, and at 20 percent in 15 days growth of *Azolla* and 15 and 30 days growth of *Azolla* and *Lemna* plants.

Peroxidase activity

Peroxidase activity of plants increased with the increase in municipal waste water levels, up to 60 percent in 7 days growth of *Lemna*, up to 40 percent in 7 days growth of *Cladophora* and *Azolla*, at 15 days growth of *Azolla* and *Lemna*, and up to 20 percent levels in 15 days growth of *Cladophora* and 30 days growth of *Cladophora*, *Azolla* and *Lemna* plants. Beyond these respective levels, further increase in municipal waste water levels, decreased the peroxidase activity of *Cladophora*, *Azolla* and *Lemna* plants.

As compared to control, increase in peroxidase activity was found to be highly significant (P=0.01) at 10 percent level in 30 days growth of *Azolla*, at 20 percent in 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 40 percent in 7 days growth of *Cladophora* and *Azolla*, 15 days growth of *Azolla* and *Lemna*, and at 60 percent in 7 days growth of *Lemna*;significant (P=0.05) at 10 percent in 7, 15 and 30 days growth of *Cladophora*, 15 and 30 days growth of *Lemna*; and insignificant in 7 days growth of *Azolla* and *Lemna* and 15 days growth of *Lemna*.

Increase in peroxidase activity was found to be significant (P=0.05) at 10 percent over control in 7, 15 and 30 days growth of *Cladophora*, 15 days growth of *Azolla* and 30 days growth of *Lemna*, at 20 over 10 percent in 15 and 30 days growth of *Cladophora*, 7 and 30 days growth of *Azolla* and 7, 15 and 30 days growth of *Lemna*, at 40 over 20 percent in 7 days growth of *Cladophora* and at 60 over 40 percent in 7 days growth of *Lemna*; highly significant (P=0.01) at 10 percent over control in 30 days growth of *Azolla*, and 40 over 20 percent in 7 and 15 days growth of *Azolla*; and fails to show any significant increase at 10 percent over control in 7 days growth of *Azolla* and *Lemna*, 15 days growth of *Lemna*, 20 over 10 percent in 7 days growth of *Cladophora*, 15 days growth of *Azolla*, at 40 over 20 percent in 7 and 15 days growth of *Azolla*.

Decrease in peroxidase activity was found to be insignificant at 80 over 60 percent in 15 days growth of *Lemna*, and at 100 over 80 percent in 7 days growth of *Cladophora*, 7 and 15 days growth of *Azolla* and 30 days growth of *Lemna*; significant (P=0.05) at 80 over 60 percent in 7 days growth of *Cladophora* and *Azolla*, and 30 days growth of *Azolla*, at 100 over 80 percent in 15 days growth of *Cladophora* and *Lemna*; and highly significant (P=0.01) at 40 over 20 percent in 15 and 30 days growth of *Cladophora*, 30 days growth of *Azolla* and *Lemna*, at 60 over 40 percent in 7, 15 and 30 days growth of *Cladophora* and *Azolla* and 15 and 30 days growth of *Lemna*, at 80 over 60 percent in 15 and 30 days growth of *Cladophora*, 15 days growth of *Azolla*, and 7 and 30 days growth of *Lemna*, and at 100 over 80 percent in 30 days growth of *Cladophora* and *Azolla*, and 7 days growth of *Lemna* plants.

Maximum peroxidase activity was observed at 60 percent in 7 days growth of *Lemna*, at 40 percent in 7 days growth of *Cladophora* and *Azolla*, and 15 days growth of *Azolla* and *Lemna*, and at 20 percent in 15 days growth of *Cladophora* and 30 days growth of *Cladophora*, *Azolla* and *Lemna* plants.

Iron, manganese, zinc and chromium (table 2):

As compared to control, 100 percent municipal waste water showed increase in heavy metals contents in each of *Cladophora*, *Azolla* and *Lemna* plants. Iron, manganese, zinc and chromium contents of each *Cladophora*, *Azolla* and *Lemna* plants growth in 100 percent municipal waste water increased with the age of plants. The maximum value for these metal contents was observed at 30 days growth of *Cladophora*, *Azolla* and *Lemna* plants.

Table: 1 Effect of different concentrations of Municipal Waste Water on Growth, Dry matter yield, Chlorophyll, Ascorbic acid, Catalase and Peroxidase activities of *Cladophora*, *Azolla* and *Lemna*

Plants	Days	Percent Municipal Waste Water							LSD	
		C	10	20	40	60	80	100	P=0.05	P=0.01
		g Fresh matter yield/treatment								
Cladophora	7	10.73	10.86	10.98	11.07	11.13	10.69	10.52	0.05	0.07
	15	10.76	10.98	11.19	11.28	11.34	10.70	10.54	0.05	0.07
	30	10.81	11.31	11.56	11.93	12.04	10.78	10.62	0.05	0.07
Azolla	7	10.52	10.58	10.69	10.78	10.50	10.46	10.41	0.05	0.07
	15	10.58	10.89	11.03	11.23	10.51	10.48	10.46	0.05	0.07
	30	10.62	10.95	11.13	11.35	10.55	10.50	10.48	0.05	0.07
Lemna	7	10.43	10.49	10.58	10.69	10.76	10.41	10.37	0.05	0.07
	15	10.53	10.73	10.81	10.92	10.98	10.45	10.39	0.05	0.07
	30	10.54	10.73	10.87	11.06	11.16	10.49	10.42	0.05	0.07

g Dry matter yield/treatment										
Cladophora	7	1.427	1.446	1.462	1.474	1.484	1.422	1.398	0.005	0.007
	15	1.431	1.460	1.488	1.502	1.507	1.421	1.401	0.005	0.007
	30	1.436	1.503	1.534	1.586	1.602	1.432	1.413	0.005	0.007
Azolla	7	1.284	1.291	1.303	1.314	1.280	1.276	1.272	0.005	0.007
	15	1.291	1.327	1.345	1.370	1.286	1.279	1.275	0.005	0.007
	30	1.294	1.335	1.358	1.385	1.288	1.281	1.278	0.005	0.007
Lemna	7	1.305	1.313	1.323	1.337	1.346	1.303	1.296	0.005	0.007
	15	1.316	1.342	1.352	1.365	1.372	1.305	1.298	0.005	0.007
	30	1.317	1.346	1.358	1.382	1.395	1.312	1.303	0.005	0.007
mg Chlorophyll/g FM										
Cladophora	7	0.71	0.73	0.76	0.81	0.83	0.72	0.68	0.03	0.05
	15	0.74	0.77	0.81	0.85	0.78	0.66	0.58	0.04	0.06
	30	0.75	0.78	0.84	0.79	0.67	0.51	0.32	0.03	0.05
Azolla	7	0.52	0.55	0.59	0.62	0.53	0.48	0.42	0.03	0.05
	15	0.54	0.58	0.63	0.57	0.46	0.41	0.33	0.04	0.06
	30	0.54	0.59	0.65	0.53	0.39	0.28	0.21	0.03	0.05
Lemna	7	0.60	0.63	0.65	0.69	0.61	0.56	0.51	0.03	0.05
	15	0.62	0.65	0.68	0.60	0.54	0.45	0.36	0.04	0.06
	30	0.62	0.67	0.69	0.56	0.43	0.33	0.22	0.03	0.05
mg Ascorbic acid/g FM										
Cladophora	7	0.016	0.017	0.019	0.021	0.023	0.018	0.015	0.001	0.002
	15	0.018	0.020	0.022	0.025	0.019	0.016	0.014	0.001	0.002
	30	0.020	0.022	0.025	0.023	0.016	0.013	0.012	0.001	0.002
Azolla	7	0.025	0.027	0.030	0.031	0.026	0.024	0.023	0.001	0.002
	15	0.027	0.029	0.032	0.027	0.024	0.022	0.021	0.001	0.002
	30	0.028	0.030	0.033	0.025	0.021	0.019	0.017	0.001	0.002
Lemna	7	0.033	0.036	0.038	0.041	0.035	0.033	0.031	0.001	0.002
	15	0.035	0.038	0.040	0.043	0.034	0.028	0.026	0.001	0.002
	30	0.035	0.039	0.043	0.038	0.030	0.026	0.023	0.001	0.002
unit Catalase/g FM										
Cladophora	7	1.20	1.30	1.50	1.70	1.90	1.40	1.30	0.20	0.30
	15	1.30	1.50	1.70	1.80	1.40	1.20	1.10	0.20	0.30
	30	1.40	1.60	1.80	1.90	1.30	1.10	1.00	0.20	0.30
Azolla	7	1.60	1.70	1.90	2.10	1.70	1.50	1.40	0.20	0.30
	15	1.70	1.90	2.10	1.80	1.50	1.40	1.20	0.20	0.30
	30	1.90	2.00	2.20	1.70	1.40	1.30	1.10	0.20	0.30
Lemna	7	2.10	2.20	2.40	2.50	2.20	2.00	1.90	0.20	0.30
	15	2.20	2.40	2.60	2.70	2.10	1.90	1.70	0.20	0.30
	30	2.30	2.60	2.70	2.50	2.00	1.80	1.50	0.20	0.30
n mole Peroxidase/g FM/min.										
Cladophora	7	39	41	42	44	40	38	37	2	3
	15	41	43	45	42	37	34	32	2	3
	30	42	44	46	40	35	31	26	2	3
Azolla	7	26	27	29	32	28	26	25	2	3
	15	27	29	30	33	27	24	23	2	3
	30	27	30	32	28	24	22	19	2	3
Lemna	7	35	36	38	39	41	37	34	2	3
	15	37	38	40	41	36	35	33	2	3
	30	37	39	41	35	32	29	28	2	3

Table: 2 Heavy metal contents in aquatic macrophytes grown in municipal waste water

Plants	Days	mg / g dry biomass							
		Iron		Manganese		Zinc		Chromium	
		Control	100% MWW	Control	100% MWW	Control	100% MWW	Control	100% MWW
Cladophora	7	0.01	0.20	0.001	0.008	0.00	0.08	0.00	0.03
	15	0.02	1.75	0.001	0.036	0.00	0.44	0.00	0.20
	30	0.04	3.26	0.002	0.060	0.02	0.87	0.01	0.44
Azolla	7	0.01	0.14	0.001	0.005	0.00	0.15	0.00	0.05
	15	0.03	1.60	0.002	0.024	0.01	0.83	0.00	0.25
	30	0.03	3.18	0.001	0.046	0.02	1.43	0.01	0.45
Lemna	7	0.01	0.16	0.001	0.012	0.00	0.10	0.00	0.01
	15	0.02	1.77	0.001	0.045	0.00	0.60	0.00	0.13
	30	0.03	3.14	0.001	0.073	0.01	1.16	0.01	0.40

Discussion

Waste water is gaining popularity as a source of irrigation water in different countries around the world. This is especially true in India, where it has been in use for a long time. Its economic benefits and its importance as a coping strategy for the poor have had title recognition. The lack of alternative source of water has generated viable markets for waste water. Increased disposable incomes have resulted from the catalytic use of waste water that was formerly not socially acceptable, i.e. the farmers considered it unhealthy and unclean. The use of waste water to grow food crops poses uncertain risks to the health of both, the consumer and to those who actually handle the waste water. It is useful in the discussion to differentiate between unplanned use of waste water resulting from poor sanitation and planned use which tries to address matters such as economic benefits but also institutional challenges and risks which require different management approaches and ideally different guidelines. This diversity makes the current WHO guidelines, which try to be global in nature, complex to understand and apply (Dwivedi *et al*., 2012) [13].

Total chlorophyll content and photosynthetic rate were significantly enhanced by the application of waste water as compared to ground water. It may also be due to the presence of many important nutrients in the waste water in surplus amount, e.g. Mg, which is an important constituent of chlorophyll and K has a role in photosynthesis as a co-factor for many photosynthetic enzymes (Devlin and Witham, 1986 and Kanenyal *et al.*, 1994) [9, 29]. Earlier reports have also indicated the beneficial effect of waste water on chlorophyll contents (Kanenyal *et al.*, 1994) [29].

Where vegetables are the main commodity produced with waste water there can be more balanced diet. In the case of *Accra*, for example more than 200000 people eat vegetable produced with waste water every day (Qadir *et al.*, 2007) [51]. On the other hand this is also the group potentially at risk as the possible adverse health effects to farmers and consumers are well established (WHO, 2006) [65].

Few studies have quantified the aggregate contribution of waste water to food supply. In Pakistan about 26 percent of national vegetable production is irrigated with waste water (Ensink *et al.*,2004) [14], while in Hanoi, Vietnam which is much better than Pakistan about 80 percent of vegetable production is urban and peri-urban areas irrigated with diluted waste water (Lai, 2002) [38]. Major cities in West Africa between 50-90 percent of vegetable consumed by urban dwellers are produced with in or close to the city (Drechsel *et al.*, 2006) [11].

Waste water recycles organic matter and a large diversity of nutrients than any commercial fertilizer can provide. It is estimated that 1000 cubic meters of municipal waste water used to irrigate 1 ha can contribute 16-62 kg total N_2, 4-24 kg P, 2-69 kg K, 18-208 kg Ca, 4-110 kg Mg and 27-182 kg Na (Jimenez, 2006) [28]. It therefore can reduce the demand for chemical fertilizers especially where the waste water is not diluted i.e. make crop nutrient more accessible to poor farmers. On the other hand excessive concentration of nitrogen in waste water can lead to over fertilization and cause excessive vegetative growth, delayed or uneven crop maturity and reduced quality (Jimenez, 2006 and Kumar *et al.*, 2010) [28, 37]. Phytotechnologies involving use of plants for pollutant removal is relatively a new approach and has gained importance during the last two decades (Dhir, 2010) [10].

Macrophyte utilization for wastewater nutrient removal had been already reported and discussed frequently (Fisher, 1988; Kumar and Garde, 1989; Bishop and Eighmy, 1989; DeBusk *et al*., 1989; Oron, 1990) [16, 36, 4, 7]. Use of floating aquatic macrophytes to reduce also the concentration of noxious phytoplanktons in the effluent from stabilization ponds and to remove nitrogen and phosphorous from the water (Steward, 1970) [62]. Members of free floating duckweeds (Lemnaceae), namely *Lemna minor*, *Lemna gibba*, *Wolffia arrhiza*, and *Azolla pinnata* have shown potential usefulness in the treatment of eutrophicated water system (Sutton and Ornes, 1975) [63].

The influence of levels of municipal waste water was studied with growth of 7, 15 and 30 days of *Cladophora*, *Azolla* and *Lemna*. In general it was observed that below 50 percent level of municipal waste water is safe to use for growth of aquatic plants *Cladophora*, *Azolla* and *Lemna*. The results observed for growth and composition of aquatic plants revels that:

With the increase in percent level of municipal waste water fresh matter yield increases up to 60 percent level of 7, 15 and 30 days growth of *Cladophora* and *Lemna* and up to 40 percent level of 7, 15 and 30 days growth *Azolla* plants. The results are in agreement with results of Muvea *et al.* (2019) [41]with *Lemna* and *Azolla* in a constructed wetland, Yin *et al.* (2015) [66], Pavani *et al.* (2008) [46] with *Pistia* in canteen waste water, Patel *et al.* (2008) [45] with *Hydrilla verticillata* in municipal waste water, Shakya *et al.* (2008) [56] with *Cladophora* in waste water, Abou El-Kheir *et al.* (2007) [1] with *Lemna gibba* in sewage water, Arora and Saxena (2005) [2] reported good growth potential with *Azolla microphylla* in sewage effluent, Goopy and Murray (2003) [20] with duckweed. However Khuantrairong and Traichaiyaporn (2012) [35] with *Cladophora* reported that no effect on biomass production in canteen waste water and in disagreement with Singh and Singh (2006) [59] with *Lemna minor* in industrial and sewage water and Khosravi *et al.* (2005) [34] with *Azolla*. Biomass yields of small-leaf floating macrophytes are quite lower than for large leaf floating aquatic macrophyte such as *Eichhornia crassipes* or *Pistia stratiotes* (Pena *et al*., 2017) [47].

Maximum dry matter yield of 7, 15 and 30 days growth of *Cladophora* and *Lemna* was observed at 60 percent and of *Azolla* at 40 percent level of municipal waste water. The results are in agreement with results that Shakya *et al.* (2008) [56] with *Cladophora* in waste water, Abou El-Kheir *et al.* (2007) [1] with *Lemna gibba* in sewage water, Khedkar and Dixit, (2004) [33] with spinach in polluted water, Dutta (2002) [12] with rice in paper mill effluent.

Fresh and dry weights increased with corresponding increase in treatment time. These results are in accordance with Hammouda *et al*. (1995) [22] who found that wastewater support higher growth rates for duckweed with increasing treatment periods. Tripathi and Misra (1990) [64] reported that duckweed grown in domestic wastewater indicated higher nutritional values than those grown in natural water.

As compared to control, increase in chlorophyll content was found to be to be highly significant (P=0.01) at 10 percent level in 30 days growth of *Azolla* and *Lemna*, at 20 percent level in all the stages of growth i.e. 7, 15 and 30 days growth of *Cladophora*, *Azolla*and *Lemna*, at 40 percent level in 7 days growth of *Cladophora*, *Azolla* and *Lemna* and 15 days growth of *Cladophora*, and at 60 percent level in 7 days growth of *Cladophora*; significant (P=0.05) at 10 percent level in 7 days growth of *Azolla* and *Lemna*, 15 days growth of *Azolla* and 30 days growth of *Cladophora*. The results are in agreement with the results of Khuantrairong and Traichaiyaporn (2012) [35] with *Cladophora* in canteen waste water, Deval *et al.* (2012) [8] with *Azolla* in zinc plating industry effluent, Khedkar and Dixit, (2004) [33] with spinach in polluted water Iqbal and Mehta (1998) [25] with wheat in sugar factory effluent, Chaturvedi *et al.* (1995) [5]with wheat in industrial effluent, Sharma and Gaur (1995) [58] with duckweed, Mishra and Behra (1992) [39] with rice in paper industry effluent and in disagreement with Chris *et al.* (2011) [6] with *Azolla filiculoides* in pesticide (monocrotophos) containing water, Singh and Singh (2006) [59] reported increases in chlorophyll content of *Lemna minor* at primary concentration, then significantly decreases in industrial and sewage water and chlorophyll content of *Wolffia globosa* was not much affected at lower levels of chromium and cadmium. Chlorophyll is an important pigment of the photosynthetic activity and responsible for maintaining primary productivity. Industrial wastewater not only affects the chlorophyll content but the chloroplast activity also (Song and Huang, 2001) [60].

With the increase in percent level of municipal waste water ascorbic acid content of *Cladophora*, *Azolla* and *Lemna* increased up to 60 percent level in 7 days growth of *Cladophora*, up to 40 percent level in 15 days growth of *Cladophora*, and 7 days growth of *Azolla*, and 7 and 15 days growth of *Lemna*, and up to 20 percent level in 30 days growth of *Cladophora* and *Lemna* and15 and 30 days growth of *Azolla*. The results are similar with the findings of Raja *et al*. (2013, 2012) [52, 53] with *Azolla microphylla* in different concentrations of endosulfan insecticide reported significant increase in ascorbic acid content. Ascorbic acid an important antioxidant, which react not only with H_2O_2 but also with O_2, OH^- and lipid hydroperoxydases (Reddy *et al*., 2004).

Maximum catalase activity was observed at 60 percent in 7 days growth of *Cladophora*, at 40 percent in 15 and 30 days growth of *Cladophora*, and 7 days growth of *Azolla* and *Lemna*, and at 20 percent in 15 days growth of *Azolla* and 15 and 30 days growth of *Azolla* and *Lemna* plants. Results are also on parallel lines with those of Prasad *et al*. (2005) [50], Pflugmacher *et al.* (2004) [49] stated that the peroxidase activity of the free floating aquatic plant *Lemna minor* and the filamentous macro alga *Cladophora fracta* increased significantly. Raja *et al.* (2013) [53] also reported that the catalase increases up to certain concentration, and then it decreases.

As compared to control, increase in peroxidase activity was found to be highly significant (P=0.01) at 10 percent level in 30 days growth of *Azolla*, at 20 percent in 7, 15 and 30 days growth of *Cladophora*, *Azolla* and *Lemna*, at 40 percent in 7 days growth of *Cladophora* and *Azolla*, 15 days growth of *Azolla* and *Lemna*, and at 60 percent in 7 days growth of *Lemna*;significant (P=0.05) at 10 percent in 7, 15 and 30 days growth of *Cladophora*, 15 and 30 days growth of *Lemna*. Similar results were also reported by Chris *et al.* (2011) [6] with *Azolla filiculoides* in pesticide (monocrotophos) containing water, Sharma (2002) [57] with crop plants.

The results presented that increase in Fe, Mn, Zn and Cr are in agreement with Kara(2010) [30], Mohan *et al.* (2009), Singh and Singh (2006) [59], Garg and Chandra (1994) [18] and Ghazvini and Mashkani (2007) [19] for chromium in *Azolla*; Kara *et al.* (2003) [31] and Jain *et al.* (1989) [27] in *Lemna* and *Azolla*, Kay *et al.* (1984) [32] in water hyacinth for Fe and Cu. Jain *et al*. (1992) [26] with *Azolla* and *Lemna* for Fe, Mn and Zn. Similar results for Zn accumulation was observed by Deval *et al.* (2012) [8], Khosravi *et al*. (2005) [34] and Grant *et al.* (1998) [21] with *Azolla*. Khosravi *et al*. (2005) [34] reported 1260 mg/kg Zn accumulation in *Azolla*, Sela *et al* (1989) [55] also reported uptake of zinc by *Azolla*.

Conclusions

The main idea behind this present study is to develop the general awareness of using municipal waste water as gardening and irrigation in crop fields to economise to heavy expenditure of chemical nutrients and also to solve environmental pollution problem.On the basis of the results of present study it was found that the range of 20 to 60 percent municipal waste water level shows maximum values of growth and composition of *Cladophora*, *Azolla* and *Lemna* plants and municipal waste water can be used for culture of aquatic plants as bioremediation treatment. Among three test plants tried, *Lemna* has greater potential to reduce the pollution load of the municipal waste water. *Lemna* can be recommended for bioremediation treatment of municipal waste water and for this proper experiment design in field condition has to be innovated.

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