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Wastewater remediation by using *Azolla* and *Lemna* for selective removal of mineral nutrients

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Abstract

At global level safe disposal and management of municipal wastewater is a serious threat to environment and human health. Wastewater remediation using aquatic plants is now a universal solution accepted worldwide. Present investigation was focused on phytoremoval of different nutrients and chlorides from municipal wastewater of Pune city. The experiment was performed at Environmental Sciences Department, University of Pune, India, during the year 2012-13 using *Azolla* and *Lemna* with dilution of wastewater in different proportions. The results showed that K^{+}, Ca^{+2}, Mg^{+2}, Na^{+} and Cl^{-} contents were highly reduced due to the activities of both the plants in diluted wastewater (1: 3). However *Lemna* had shown slightly better performance in removing all above nutrients in 75 % dilution when compared with *Azolla*. From the results it can be concluded that *Lemna* will be highly preferred candidate for phytotreatment on large scale.

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Introduction

The wastewater quantity generated in urban areas is very huge and unmanaged, inviting the problems of human health and deterioration of environment (Obek and Sasmaz, 2011). The purification and removal of nutrients, organic pollutants with convenient method is not cost effective and sustainable (Deval *et al.,* 2012). Therefore pytoremediation technology is accepted throughout the world as it is user friendly and affordable also. Since the inception of this methodology, several plants have been selected viz *Pistia, Wolffia, Lemna, Azolla, Hydrialla, Typha, Salvinia, Potamogeton, Ceratophyllum,* Water hyacinth, etc (Lu *et al.,* 2011; Priya *et al.,* 2012). The suitability of plants depends on the type of wastewater and the nature of pollutants in it (Ra *et al.,* 2007). In general *Azolla* and *Lemna* had shown wide application in phytoremediation as these plants are small in size, free floating and growing at faster rate, having heavy rates of absorption and uptake of nutrients and different pollutants (Dixit *et al.,* 2011). This green technology to clean up the wastewater is the best and highly accepted method. Extensive studies have been conducted on uptake, bioaccumulation and adsorption of mineral elements using hydrophytes which reduce the concentration of Mn^{+2}, Mg^{+2}, Ca^{+2}, K^{+}, Na^{+} and Cl^{-}.

The mechanisms for these phenomena were deposition or adsorption on the external root surface of aquatic macrophytes (Mishra *et al.,* 2008). However some elements like Na^{+} and their salts (Cl^{-}) are persistent in recycled water and most difficult to remove but some plants have capacity to uptake these elements and reduce their concentrations (Hegedus *et al.,* 2009). About 90 % reduction in the concentrations of metal ions by aquatic plants was reported by Mishra and Tripathi, (2008). These aquatic plants have shown better performance in natural water bodies as compare to laboratory experiments (Gupta *et al.,* 2012).

The survey of literature indicated that the conventional physical or chemical treatments were not feasible for the remediation of non point source pollution at the large scale (Taylor and Crowder, 1983).The application of *Azolla* and *Lemna* is a very common practice in phytoremediation, because they have very good potential for hyper accumulation of different pollutants, minerals and heavy metals, restoring polluted aquatic resources. They have ability for altering water quality by regulating oxygen balance and nutrient cycles (Sood *et al.,* 2012). The treatment of municipal wastewater becomes most effective if it is diluted in different proportions and the uptake of nutrients by aquatic plants is increased with progressive increase in dilution ratio. This type of research is less attempted, hence require in depth investigation.

The under-management of municipal wastewater in metropolitan city like Pune presents a major challenge and management of wastewater generated is a difficult task. The unsafe disposal of wastewater generates pollution of water as well as terrestrial ecosystem and various health problems, epidemics due to serving the contaminated water. Therefore, present investigation was undertaken with following objective i.e removal of nutrients from municipal wastewater by using *Azolla* and *Lemna* after diluting the wastewater in different proportions as it is eco-friendly and sustainable technology.

Materials and methods

Collection of wastewater samples

Wastewater samples (120 litters) were collected from sewage treatment plant of Pune Municipal Corporation during the year 2012-13 and brought to the laboratory in plastic containers as per experimental requirements.

Collection of aquatic plants

The selected plants viz *Azolla pinnata* and *Lemna minor* were collected from natural pond at Horticulture Research Station, Ganesh Khind, Pune, and brought to the laboratory in plastic bags, cleaned carefully to remove dirt and dust and stabilized in laboratory conditions.

Experimental design and treatment details

Factorial arrangement with randomized complete block design with three replications was used to conduct the experiments at Department of Environmental Science, University of Pune, India. Treatments included P0: no plant, P1: *Azolla pinnata* and P2: *Lemna minor* and ratios for dilution of wastewater with distilled water were as follows: R0: 100 % wastewater, R1: (3:1), R2: (1:1) and R3: (1:3). The wastewater after dilution was poured into rounded, transparent plastic trough (18×20×20cm) having surface area 254 cm^2. The capacity of each plastic trough was about 5 litters. Selected plants, 5 g each of *Azolla pinnata* and *Lemna minor* were inoculated separately in above containers as per the experimental layout and treatments formulated. The laboratory conditions were maintained uniform throughout the experiment.

Physicochemical analysis of wastewater

All the samples (pre and post treatments) were analyzed for sodium (Na^+), potassium (K^+), calcium (Ca^{+2}), magnesium (Mg^{+2}) and chlorides (Cl^-) by using standard methods (APHA, AWWA, WEF, 2005).

Statistical analysis

The results were analyzed statistically by using MSTATC computer software and a comparison of recorded data was done on the basis of Duncan's multiple range tests at Alfa level 5 %.

Results and discussion

Potassium

The mean comparison of plants revealed that K^+ was maximum in absence of plants (7.8 mg/L) which was reduced to the minimum (2.1 mg/L) in presence of *Lemna* at final stage (Fig. 1). The reduction in K^+ content was observed at final stage with increasing ratios of dilution and the lowest content was noted in 1: 3 dilutions as compare to 100 % wastewater (Fig. 2). The influence of dilutions and plant species together showed that K^+ was comparatively high (11 mg/L) in absence of plants and no dilution but it was drastically decreased with dilution of 75 % and presence of *Lemna* (1 mg/L) followed by *Azolla* (1.5 mg/L) (Table 1).

Table 1. Interaction effect of plant species and dilution ratio on potassium, calcium and magnesium content of municipal wastewater.

Treatment	K^+ (mg/L)		Ca^{+2} (mg/L)		Mg^{+2} (mg/L)	
	Initial	Final	Initial	Final	Initial	Final
P0R0	13.0 a	11.0 a	532.0 a	519.0 a	198.3 a	190.0 a
P0R1	11.3 b	9.0 b	406.3 b	394.0 b	153.3 b	147.0 b
P0R2	9.0 c	7.3 c	283.0 c	280.0 c	85.0 c	75.3 e
P0R3	6.0 d	4.0 e	136.0 d	131.0 e	51.3 d	45.3 f
P1R0	13.0 a	5.0 d	532.0 a	287.3 c	198.3 a	99.0 c
P1R1	11.3 b	3.8 ef	406.3 b	82.3 f	153.3 b	30.0 g
P1R2	9.0 c	2.3 g	283.0 c	29.3 g	85.0 c	12.0 i
P1R3	6.0 d	1.5 i	136.0 d	8.0 g	51.3 d	5.0 j
P2R0	13.0 a	3.3 f	532.0 a	246.0 d	198.3 a	89.0 d
P2R1	11.3 b	3.7 e	406.3 b	73.0 f	153.3 b	25.0 h
P2R2	9.0 c	2.1 h	283.0 c	19.0 g	85.0 c	7.4 j
P2R3	6.0 d	1.0 i	136.0 d	5.0 g	51.3 d	3.0 j

Means with different letters are significantly different at P=0.05, using Duncan's Multiple Range Test.

Potassium is major element essential for the plants but its high concentration is toxic, causing different types of cellular damages to the plants and inducing metabolic abnormalities in them. The wastewater usually containing very high potassium content (Elumalai *et al.*, 2013; Deval *et al.*, 2012).

Phytoremediation plays crucial role in reduction of K^+. In present investigation K^+ concentration was drastically reduced in the presence of both the plants when municipal wastewater was diluted up to 75 %. Dipu *et al.,* (2010) recorded significant decrease in K^+ in dairy effluent using *Typha* and other aquatic macrophytes. They further noted that dilution was more helpful to reduce K^+ content. Deval *et al.,* (2012) indicated that phytoremedial capacity of floating aquatic macrophyte like *Azolla* was having good potential for removal of K^+ in zinc plating effluents. According to Elumalai *et al.,* (2013) *Eichhornia* was a promising candidate for phytoremediaion of wastewater polluted with different metals. Lu *et al.,* (2011) indicated slight reduction in K^+ by using *Pistia*. The results of present study are in agreement with above workers. The reduction in K^+ can be attributed to its uptake and adsorption by *Lemna* and *Azolla*.

Table 2. Interaction effect of plant species and dilution ratio on Na^+ and Cl^- content of municipal wastewater.

Treatment	Na^+ (mg/L)		Cl^- (mg/L)	
	Initial	Final	Initial	Final
P0R0	54.3 a	51.0 a	971.7 a	952.3 a
P0R1	40.3 b	37.0 b	739.0 b	712.3 b
P0R2	24.0 c	21.0 d	486.3 c	477.7 c
P0R3	14.0 d	12.0 e	232.0 d	227.3 e
P1R0	54.3 a	26.3 c	971.7 a	501.0 c
P1R1	40.3 b	9.2 f	739.0 b	154.0 f
P1R2	24.0 c	3.5 g	486.3 c	52.0 h
P1R3	14.0 d	1.6 gh	232.0 d	11.7 i
P2R0	54.3 a	23.0 d	971.7 a	433.7 d
P2R1	40.3 b	8.3 f	739.0 b	111.0 g
P2R2	24.0 c	3.0 gh	486.3 c	43.0 hi
P2R3	14.0 d	1.0 h	232.0 d	9.0 i

Means with different letters are significantly different at P=0.05, using Duncan's Multiple Range Test.

Calcium

The calcium content at final stage was higher in absences of plants and it was significantly reduced in presence of both the plants (Fig. 3). The dilution of wastewater showed similar trend and lowest calcium content was recorded in highest ratio of dilution (1:3) (Fig. 4). In the treatment of interactions of dilution along with plants, highest calcium content (518.7 mg/L) was recorded in absence of plants and no dilution. However it was drastically reduced (5 mg/L) in presence of *Lemna* and 75 % dilution which was followed by *Azolla* (8 mg/L) (Table 1).

Calcium is highly essential and playing major role in plant metabolism, structural material of cell wall and signaling (Patel and Kanungo, 2010). However its high concentration is phytotoxic, therefore appropriate Ca^{+2} content is needed, which can be achieved through phytotreatment. In present study calcium was brought to the minimum level by *Azolla* and *Lemna* in 75 % dilution. Similar trend was noted by El-Khier *et al.,* (2007) with *Lemna* for wastewater. They further claimed that *Lemna* was the best biological material for removal of different pollutants and metal ions from wastewater. Lu *et al.,* (2011) recorded reduction in Ca^{+2} content by using *Pistia*. Chavan and Dhulap, (2012b) proposed that phytoremediation with the appropriate assortment of locally available aquatic plant is more trust worthy. The better results obtained in the present study regarding removal of high content of Ca^{+2} might be due to the use of locally adapted *Azolla* and *Lemna* for wastewater purification and the treatment efficiency increased with dilution.

Magnesium

Magnesium was 68 and 73 % reduced in presence of *Azolla* and *Lemna* respectively at final stage (Fig. 5). The decrease at final stage in Mg^{+2} was very high dilution of wastewater (1:3) ratio as compare to 100 %

wastewater (Fig. 6) and the removal efficiency was very high in combination of diluted wastewater and *Lemna* (3 mg/L) followed by *Azoll* (5 mg/L) as compare to control (Table 1).

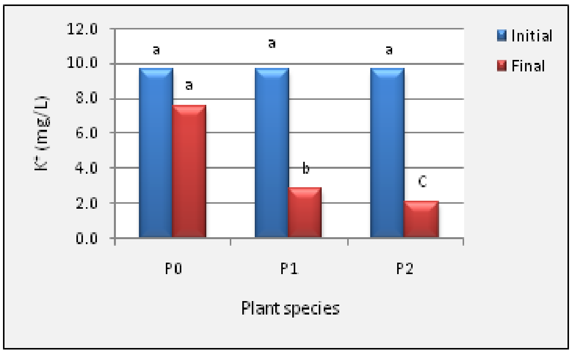

Fig. 1. Effect of plant species on potassium content of municipal wastewater.

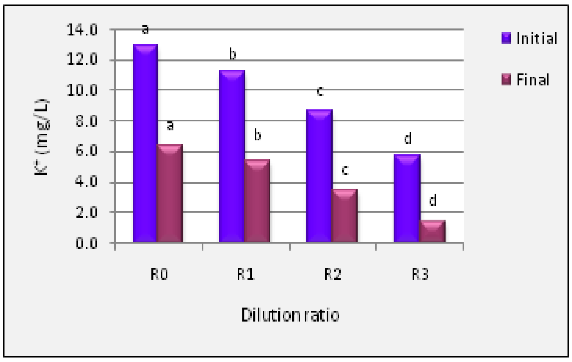

Fig. 2. Effect of dilution ratio on potassium content of municipal wastewater.

The content of Mg^{+2} greatly lowered down during phytoremediation of wastewater by both the plants in highest dilution ratios. Hegedus *et al.,* (2009) reported similar range of Mg^{+2} in effluent water of fish farming. Considerable reduction was noted in Mg^{+2} by Lu *et al.*, (2011) using *Pistia*. Similar trend was noted for wastewater treatment using *Lemna* by El-Khier *et al.,* (2007). They also recorded about 50 % reduction in Mg^{+2} with different ratios of dilution. The micro element Mg^{+2} is a cofactor for photosynthetic enzymes and a constituent of chlorophyll molecule which its high concentrations are injures to plant health and functioning; therefore it should be available to the plants in traces. The minimum concentration can be achieved through phytoremediation with different macrophytes (Dixit *et al.,* 2011). The results of present study are in agreement with above findings.

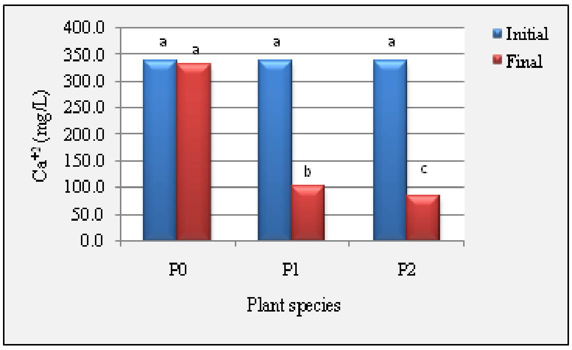

Fig. 3. Effect of plant species on calcium content of municipal wastewater.

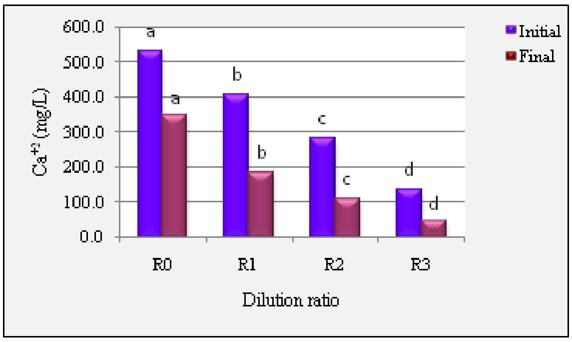

Fig. 4. Effect of dilution ratio on calcium content of municipal wastewater.

Sodium

The mean comparison of plants indicated that the maximum content of Na^{+} (30.3 mg/L) recorded in absence of plants and it was brought to the minimum (8.8 mg/L) in presence of *Lemna* (Fig. 7). The comparison of dilution ratios at final stage revealed that with increasing dilution Na^{+} content was decreased progressively by 85.7 % as compare to 100 % wastewater (Fig. 8). Sodium content influenced by different plant species and dilution ratios showed highest content (51 mg/L) in absence of both the plants and no dilution. Sodium was drastically lowered down (1 mg/L) in presence of *Lemna* and 75 % dilution of wastewater followed by *Azolla* (1.6 mg/L) (Table 2).

Sodium is an important trace element for halophytes and the glycophytes are highly sensitive to it as it increases the salinity and its high concentration is highly injuries to most of the plants as it creates salinity stress (Elumalai *et al.,* 2013). For normal growth and metabolisms its high concentration should be lowered down by using phytotreatment (Dipu *et al.,* 2010). The aquatic macrophytes used in present study have significantly reduced the Na^+ content of wastewater. However the highest efficiency and potential of both the plants was seen in dilution of wastewater (1:3) and presence of *Lemna minor*. The results recorded by Dixit *et al.,* (2011) showed the reduction in Na^+ using different aquatic plants such as *Eichhornia, Typha, Justcia*. Results of El-Khier *et al.,* (2007) showed similar trend for Na^+ using *Lemna*. Dipu *et al.,* (2010) also reported removal of Na^+ using macrophytes. Present finding with *Lemna* and *Azolla* corroborate with above results.

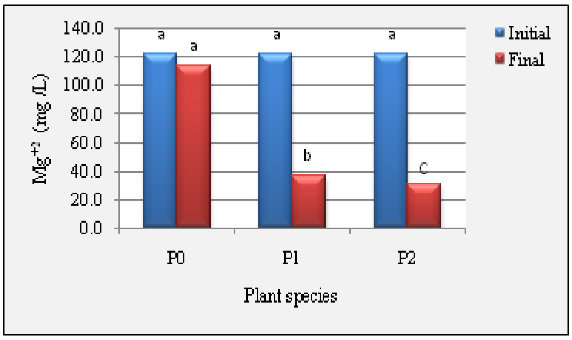

Fig. 5. Effect of plant species on magnesium content of municipal wastewater.

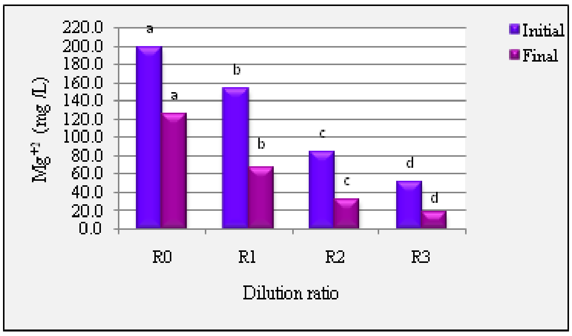

Fig. 6. Effect of dilution ratio on magnesium content of municipal wastewater.

Chlorides

The mean comparison of plants very clearly indicated that at final stage maximum content of Cl^- (592.4 mg/L) recorded in absence of plants but it was brought to minimum (149.4 mg/L) in presence of *Lemna* (Fig. 9). The mean comparison of dilution ratios at final stage indicated progressively reduction in Na^+ with increasing dilution and the reduction by 86.8 % was noted in 75 % dilution when compared with no dilution (Fig. 10). The interactions of dilution ratio and plants together revealed that highest Cl^- (952.3 mg/L) was in absence of plants and without dilution. However it was greatly reduced (9 mg/L) in presence of *Lemna* and 75 % dilution which was followed by *Azolla* (11.7 mg/L) (Table 2).

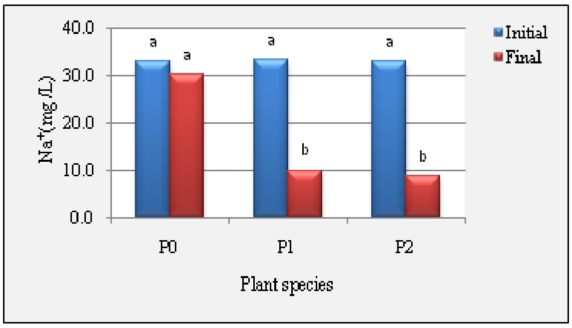

Fig. 7. Effect of plant species on sodium content of municipal wastewater.

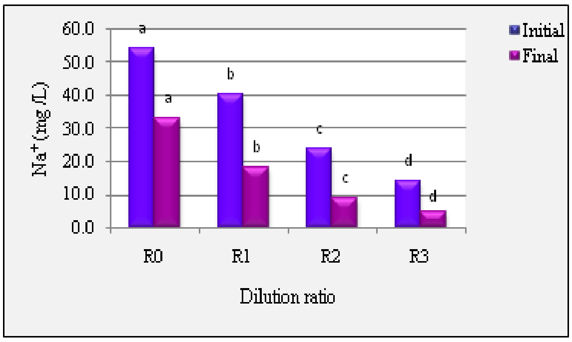

Fig. 8. Effect of dilution ratio on sodium content of municipal wastewater.

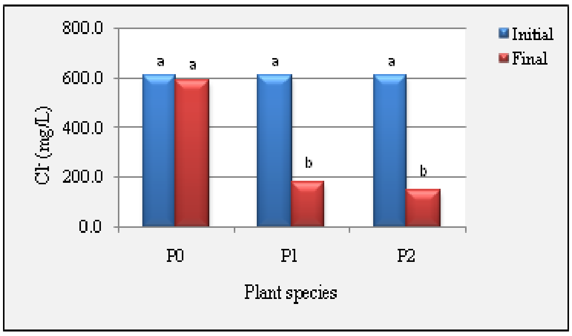

Fig. 9. Effect of plant species on chloride content of municipal wastewater.

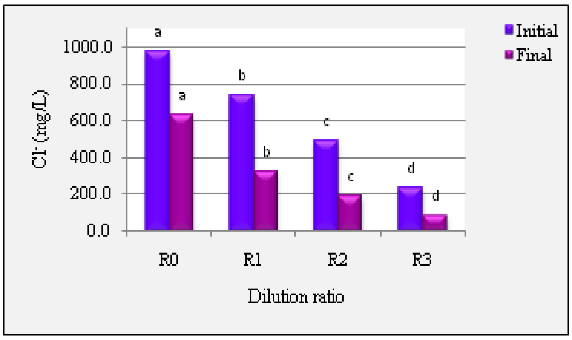

Fig. 10. Effect of dilution ratio on chloride content of municipal wastewater.

The chloride content in wastewater dropped down to lowest level by using highest dilution (75 %) in presence of both the plants but *Lemna* was superior to *Azolla* in phytoremediation. El-Kheir *et al.,* (2007) studied the assessment of *Lemna* in wastewater and reported reduction in chlorides up to 21 %. The reduction in Cl^- content was also reported by Elumalai *et al.,* (2013). According to Tripathi and Upadaya, (2003) there was high Cl^- content in dairy effluent which was brought to the minimum level with different aquatic macrophytes such as *Eichhornia crassipes, Azolla* and *Lemna*. Similar trend was also observed by Hegedus *et al.,* (2009) using phytoremediation technology. Sood, *et al.,* (2012) explained that aquatic plants remove Cl^- and other nutrients from wastewater using mechanism of bioaccumulation or bioabsorption. The results of present investigation are in conformity with above reports.

Conclusion

Municipal wastewater contains different types of nutrients like Ca^{+2}, K^+, Mg^{+2}, Na^+ and Cl^- in very high concentration. The high level of macro elements is toxic to plants and microorganisms. They enter in metabolic chains and block these reactions leading to impairments in their growth and functioning. Therefore removal of the metals and Cl^- ions is necessary for which phytoremedation is the most convenient and highly suitable method. Considering this, the free floating aquatic macrophytes such as *Azolla* and *Lemna* were selected for wastewater remediation. Both of them have successfully removed all the nutrients and brought them to minimum level. The phytotreatment was highly effective under the influence of dilution with distilled water in different proportions. The efficiency of both macrophytes for reducing the contaminants progressively increased along with increasing dilution percentage. Among the plants selected *Lemna* was performing better than *Azolla* in highest dilution treatment. The level of reduction was quite satisfactory and hence it can be concluded that for removal of mineral nutrients from wastewater *Lemna* will be the best candidates for its purification.

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