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Bioremediation of industrial effluent at source using aquatic macrophytes

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Original Article

Abstract

Kerala State in India has 4.3% of the total number of small-scale industries in the country which is around 4,52,826 and this number is increasing per annum. Even though industrialization and urbanization are considered as the steppingstone to a country's development, the effluents released into the water bodies may lead to unrepairable damage to the aquatic environment thereby affecting livelihood of coastal people. The present investigation was done in Cochin inshore waters (9° 59′ 12. 56″ N and 76° 16′ 20. 74″ E) polluted due to effluent discharge into the canal water connecting to the sea through the lake. The chemical parameters showed wide variation in ammonia content in effluent water which was found to be very high with a value of 4.05 mg/l compared to the normal estuarine water (0.14 mg/l) and was 96% higher in effluent water. Similarly, the phosphate and silicate content of the effluent water was about 84.3 and 87.6% higher than the normal estuarine water. The presence of heavy metals like zinc as well as copper was also observed in the effluent. Experiment conducted in the laboratory using aquatic plants like *Eichhornia crassipes*, *Pistia stratiotes* and mangrove such as *Rhizophora mucronata* and *Bruguiera gymnorrhiza* for 5 days showed increase in DO and BOD level with decreasing TSS and nutrients. The absorption of ammonium ion was quite low, ranging from 4.0 -10.5%, but toxicity of ammonia was observed to affect the aquatic plant and mangrove used for treatment. The biosorption study was carried out for the removal of heavy metals from the effluent by passing through a column packed with seaweed powder of *Sargassum wightti* and *Kappaphycus alvarezii*. *Sargassum* could accumulate zinc 17.6 (mg/kg) and copper (1.96 mg/kg) whereas *Kappaphycus* could accumulate 76.1 (mg/kg) of zinc and 16.64 (mg/kg) of copper in different effluent collected from outfall area leaving the elute below the detection level. The main aim of this study is to bring forth a new concept of eco-friendly processes to counter contamination of water bodies by phytoremediation using various aquatic plants and suggest the industries to implement this cost effective technology of effluent treatment. This will ensure reuse of freshwater and protect the fragile aquatic system from pollution besides mining the heavy metals from the seaweed through phyto-mining.

***Keywords**: Bioremediation, phyto mining, aquatic weeds, mangroves, heavy metals*

Introduction

Human progress paved the way for different innovations in scientific and technological areas. Global development, however, raises new challenges, especially in the field of environmental protection and conservation (Bennett *et al.,* 2003). Though the Water Act of India, 1974 provides for the prevention and control of water pollution and maintaining or restoring of wholesomeness of water through various management guidelines and restrictions, ironically, economic, agricultural and industrial developments are often linked to environment pollution (Ikhuoria and Okieimen, 2000). Kerala has a landmass of 1.18% with a total area of nearly 38,863 km^2 and a coastline of 580 km, 44 rivers and 27 backwater bodies which covers 4720 km of the total land area. Of the 14 districts in Kerala, 9 districts have coastline and population density range from 604 to 1492/km^2 (Coastal Kerala Development Council) which is much higher than the National average. According to the Government of India report under Ministry of Micro, Small, Medium Enterprises (MSME, 20th March 2020) Kerala has 4,52,826 small scale industries which is 4.3% of the country's total SSI. Ernakulam is the most densely populated and this district contributes the State's highest GDP from construction, manufacturing, ship building, seafood, spices, health services, agro based, chemical, fertilizer industries and tourism. Kochi backwater is directly connected to sea through canal, creeks and lake. Eloor, is the industrial belt in Ernakulam District having more than 280 industries situated in the vicinity of backwater and around 110 are chemical industries (Anjusha *et al*., 2020). Even though there are stringent guidelines by the Central Pollution Control Board for industries (effluent treatment plant in every industry and zero discharge to natural environment), at certain discharge point violation of such guidelines has been observed in the creek and canal waters leading to occasional change in colour of water and also fish mortality. Industrial pollution has contributed to water quality deterioration by the discharge of untreated or partially treated effluent from factories or industries in the district (Sumangala *et al*., 2013). Physico-chemical parameters like pH, temperature, phenolphthalein alkalinity, acidity, free CO2, chloride, hardness, phosphate, dissolved oxygen, BOD, COD and total alkalinity were analysed from the Mangalavanam mangrove ecosystem caused due to industrial pollution (Madhusudhanan and Jayesh, 2011). Many of the industries such as paint, textile industries use large amount of zinc oxide for manufacturing purposes (Moezzi *et al.,* 2012). It has been reported that water bodies in the region are biologically polluted with the presence of Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb, Bi, As, Hg (Sobha and Anish, 2003). Several conventional methods are available for treatment of these inorganic and organic chemicals, but they are extremely costly. So, it is high time to consider and focus on the most important green movement three R's; Reduce, Reuse and Recycle to protect the water resources by using the process of "Phytoremediation". Aquatic plants play an important role in recycling and absorbing nutrients and heavy metals from aquatic ecosystem (Pip and Stepaniuk, 1992; Boyd, 1970; Brix and Schierup, 1989; Rao, 1986; Noemi *et al*., 2004). Several aquatic plants like *Eichhornia crassipes, Pista stratiotes, Lemna minor* exhibits an impressive level of phytoremediation of nutrient rich waste water (Manjunath and Kousar, 2016; Reed *et al.,* 1995) and seaweeds like *Sargassum* sp. and *Kappaphycus alvarezii* have the capacity to remediate heavy metals (Bina *et al.,* 2006; Costa *et al.,* 1996; Holan and Volesky, 1994; Davis *et al.,* 2000; Kang *et al.,* 2011; Pandya *et al.,* 2017; Rafiq *et al.,* 2013). This work is aimed to determine suitable solution to bridge the gap between development and environmental sustainability by treating hazardous waste at the source by a cost effective method of bioremediation using aquatic plants like *E. crassipes, P. stratiotes* and mangrove such as *Rhizophora mucronata* and *Bruguiera gymnorrhiza* and seaweed like *Sargassum wightti* an*d K. alvarezii.* Implementation of this method by industries will help them to work together on a single platform towards environmental protection from pollution.

Material and methods

For the present investigation, water samples were collected from the outfall area (latitude 9° 59' 12. 56" N and longitude 76° 16' 20. 74" E) located close to the Cochin inshore waters. Colour of water in the area was noticed to change from fluorescent blue to navy blue (Fig.1 & Plate 1). Sampling was done twice, in the month of December, 2018 and January, 2019. Effluent water was collected from the discharge point and analysed for its odour, colour, heavy metals like copper, zinc, cadmium and lead, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Total Suspended Solid (TSS), nutrients like nitrate, nitrite, phosphate and silicate. The main aim of this study is to bring forth a new

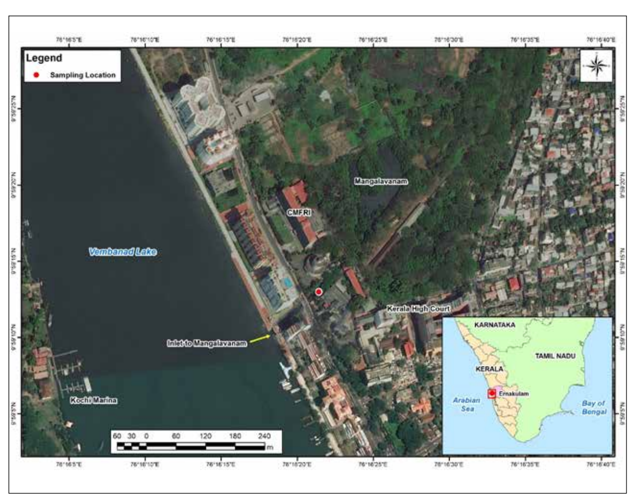

Fig. 1. GPS location of the site

Plate 1. Effluent collected from the outfall area during sampling period 2018 (a & b) and 2019 (c & d).

concept of eco-friendly processes to counter contamination of water bodies by phytoremediation using various aquatic plants like *E. crassipes, P. stratiotes* collected from Cochin backwaters. Healthy plants were selected and washed in tap water and distilled water to remove all the organisms and particles adhering to the plant before they were used for the experiment. The mangrove saplings of *R. mucronata* and *B. gymnorrhiza* were collected from Mangalavanam bird sanctuary (latitude 9°59'17.74"N and longitude 76°16'22.0"E). Saplings of each species were collected and transported to the laboratory in sampling zip lock cover. In the laboratory they were washed thoroughly in tap water followed by distilled water before using them for treatment of the effluent water. *S. wightti* was collected from the intertidal area of Alappuzha (9°31'10.5"N, 76°18'42.7"E) and *K. alvarezii* from Mandapam coast. Algal samples were rinsed thoroughly with seawater on-site and placed in plastic bag, transferred to the laboratory. The sample was washed using freshwater to remove the salt content, any mineral particles and organisms adhering to the plant tissue followed by double distilled water without soaking for a long period and dried in a preheated oven at 75°C in the case of *S. wightti* and 90°C for *K. alvarezii*. The dried seaweeds were ground using mortar and pestle to a fine powder to be used as packs with glass beads in glass column.

Experiment was conducted in the laboratory with effluent water collected from the discharge point. They were subject to different treatments like T1, T2, T3, T4 and T5. Treatment 1 was provided only with aeration whereas treatments T2, T3, T4 and T5 were treated with different aquatic plants and mangrove seedlings like *E. crassipes* (T2), *P. stratiotes* (T3), *R. mucronata* (T4) and *B. gymnorrhiza* (T5) for five days (Plate. 2) along with aeration. Effluent water without aeration was maintained as Control. Water quality parameters were analyzed using standard analytical procedures like BOD, DO by Winkler (1888), ammonia by Solarzano (1969), nitrate by Grasshoff (1964), nitrite by Bendschneider and Robinson (1952), phosphate by Murphy and Riley (1962) and silicate by Mullin and Riley (1955). pH, salinity and TSS were also recorded for each sampling. Effluent was passed through a glass column to remove the excess heavy metals from the effluent water by using dried seaweed powder mixed with glass beads (Plate. 3). Two different seaweeds such as *S. wightii* and *K. alvarezii* were used in the column separately with a constant flow rate. The elute, effluent and the seaweed

Plate 2. Experimental design of effluent treatment & ammonia toxicity on plants after 5 days of treatment a) Experiment I b) *Eichhornia crassipes* c) *Pistia stratiotes* d) *Bruguiera gymnorrhiza* before e) after experiment f) Experiment II *Eichornia* before g) after experiment.

sample were taken for estimation of heavy metals by using an ICP analyzer (APHA, 2017) by standard test method. All the analysis was done in triplicate. The data generated from the experiment were analyzed statistically by multivariate bootstrap Hotelling's T square tests.

Results

Analysis of physicochemical parameters of normal estuarine water (ESW), effluent water (EFW) and the treated effluent water (TEFW) were carried out and are presented in this paper. Physical observations at the time of collection showed tiny white suspended particles and colour of the water was blue to fluorescent blue. The effluent had very pungent odour from the chemicals. Salinity of the EFW sample ranged between 6-15 ppt compared to the ESW which was always 19 ppt and above. TSS value of the EFW was 124.0 mg/l compared to ESW with 86mg/l. pH of the EFW was found to be slightly alkaline in nature (8.1) compared to ESW which was 7.9 (Fig. 2).

Upon treatment for 5 days, the TSS value declined from 124 to 60mg/l in T4 by using mangrove *R. mucronata* and to 65.0mg/l in T3 when treated with *P. stratiotes,* almost the same value of estuarine water. The pH of EFW did not show much variation than ESW before or after treatment with aquatic plants. There

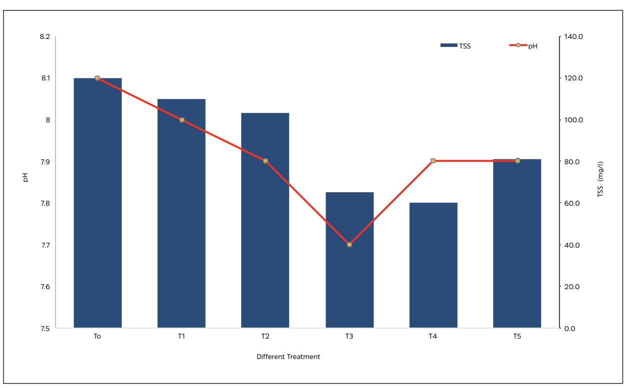

Fig. 2. TSS, Salinity and pH in effluent and normal estuarine water

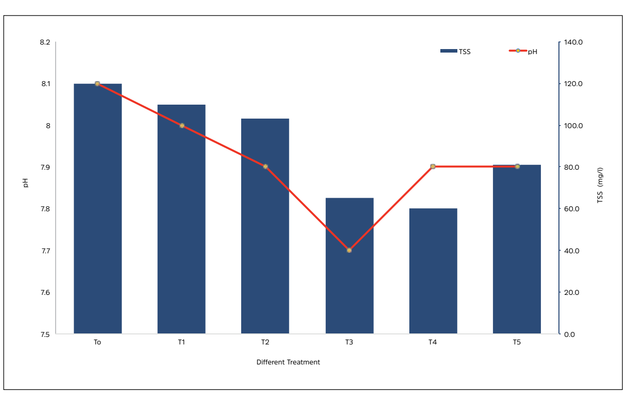

Fig. 3. TSS and pH effluent after 5 days of different treatment

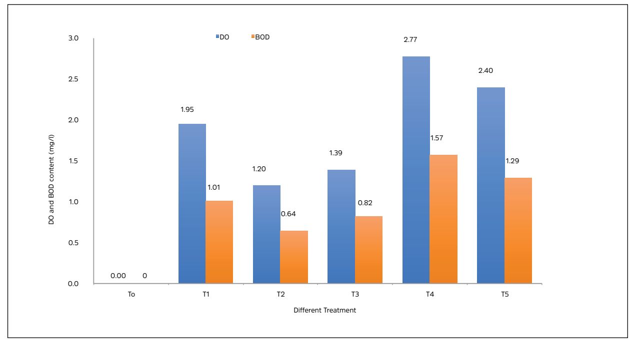

Fig. 4. DO and BOD value of effluent under different treatment

was marginal decline of pH by 5% from 8.1 to 7.7 when treated with aquatic weed *P. stratiotes* (Fig. 3). The second collection of effluent from the same outfall area showed very high value of TSS (419.6 mg/l) which significantly declined to 68mg/l when treated with *E. crassipes* in T2. Highly significant variation was observed in the TSS of effluent sample under different treatment ($p<0.05$).

DO and BOD value was found to be zero just after collection. The DO as well as BOD values increased to 1.2-2.7% after the treatment, where the maximum increase was exhibited when treated with mangroves. The DO value increased from 0 to 2.77 mg/l when treated with *R. mucronata* (T4) whereas it was 2.4 mg/l in *B. gymnorrhiza* (T5). Similarly, the BOD value also showed an increase in a similar way with 1.57mg/l in T4 and 1.29 mg/l in T5. The effluent treated with aeration also had a DO value of 1.95 mg/l and BOD value of 1.01 mg/l whereas in controlled condition both the values remained zero even after 5 days (Fig.4). During the second collection of effluent, DO and BOD showed significant increase from zero to 2.38 and 1.44mg/l respectively when treated with *E. crassipes* (T2). There was a highly significant variation in BOD value of effluent sample ($p<0.05$) but DO did not show any statistical significance.

Ammonia content of the effluent water was higher compared to nitrate and nitrite. While comparing with different treatments ammonia reduced marginally ranging from 4.0 to 10.5%. Maximum decline was found in *R. mucronata* and *B. gymnorrhiza* from an initial value of 4.05 to 3.62mg/l and 3.63mg/l respectively (Fig.5). The ammonia content in ESW (0.14mg/l) was very low compared to the EFW (4.05mg/l). Highly significant variation was observed in the ammonia content of effluent sample under different treatment ($p<0.05$).

Nitrite and nitrate content of the effluent water were 0.038 and 0.049mg/l compared to the normal estuarine water with a value of 0.026 and 0.045mg/l. Nitrite content under different treatment ranged between 7.89 and 44.5% and was effectively removed in *P. stratiotes* (0.021mg/l) followed by *E. crassipes* (0.024 mg/l). The mangrove plants also exhibited

Plate 3. Effluent passed through seaweed granules a) *Sargassum* granules b) *Kappaphycus* granules c) Elute collected after the effluent passed through column of *Sargassum wightti* d & e) colour of *Kappaphycus* granunles before and after effluent pass through glass column f) Elute collected after the effluent passed through column of *Kappaphycus alvarezii*.

decline in the nitrite content to 0.031 mg/l (T5) and 0.033 mg/l (T4). Nitrate content was not as high in the effluent water sample compared to the ESW. Decrease in nitrate to 0.037mg/l was noticed effectively by *E crassipes* and *B gymnorrhiza* (0.037 mg/l). Nitrate and nitrite content did not show any statistical significance.

Phosphate content declined significantly from 2-90.5% with different treatments and the maximal reduction was observed in *R. mucronata* (T4) with a value of 0.033mg/l from 0.346 mg/l followed by *B. gymnorrhiza* (0.053mg/l) and *P. stratiotes* (0.063 mg/l). The phosphate content of ESW was 0.054mg/l. Silicate content in the ESW was 0.371mg/l. Silicate present in the effluent water was found to be higher similar to ammonia and phosphate with a value of 2.990mg/l. Silicate content declined from 4 to 63.9 % under different treatment and the diminishing value of silicate was found higher in *E crassipes* (1.07mg/l) followed by *R. mucronata* with a value 1.78 mg/l (Fig. 6). Highly significant variation was observed in the phosphate and silicate content of effluent sample under different treatment ($p<0.05$).

The effluent sample was passed through the column packed with seaweed powder of *S. wightii*. The transmittance of the eluted water showed the colour change from blue to light blue (Plate. 3). Analysis of heavy metals, showed presence of zinc and copper and the absence of cadmium and lead in the effluent water. The amount of zinc present in the EFW sample was higher (0.71 mg/l) compared to copper (0.16 mg/l) whereas the elute did not show any detectable levels of copper and zinc. Prior to treatment the seaweed powder which lacked heavy metals like zinc and copper showed accumulation of zinc (17.60 mg/kg) and copper (1.96mg/kg) after the treatment. The effluent collected from the same

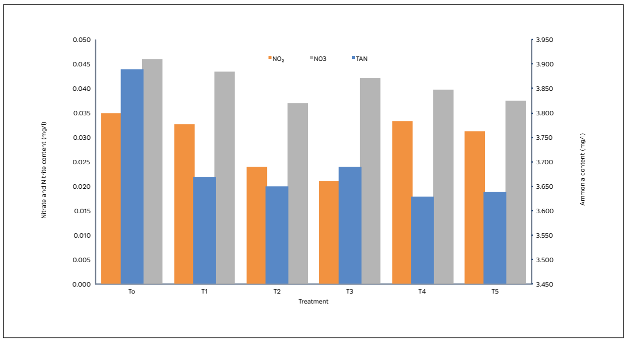

Fig. 5. Ammonia, Nitrite and Nitrate content of effluent under different treatment

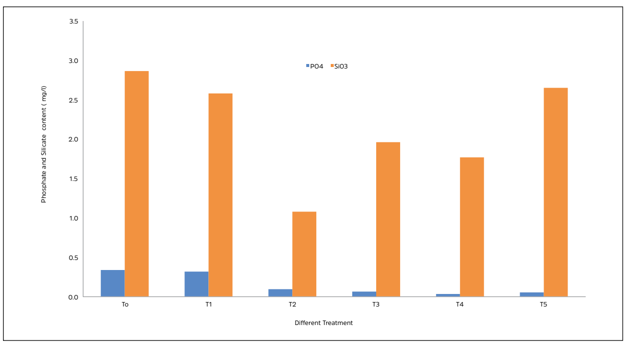

Fig. 6. Phosphate and silicate content of effluent under different treatment

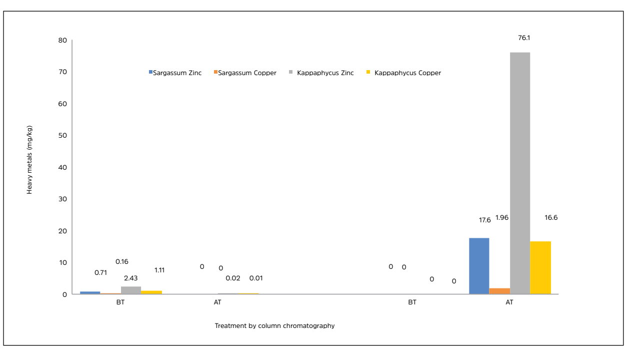

Fig. 7. Biosorption of heavy metals by seaweeds

outfall area for the second time was also passed through the column packed with seaweed granules of *K. alvarezii*. The amount of zinc present in the EFW sample was higher (2.43 mg/l) compared to copper (1.11mg/l). The amount of zinc present in the effluent reduced to 0.02 mg/l and copper to 0.01 mg/l after the treatment (Fig. 6). The elute did not show much variation in the color rather was converted to pale ash color due to the pigment present in the dried seaweed powder of *K. alvarezii.* Thus, transmittance of the elute treated with *K. alvarezii* was 25.11% compared to 63.39% in effluent sample. Seaweed samples in the column was dried in a preheated oven which showed almost a dark brown color compared to pale yellow in the untreated sample (Plate. 3). Initially the seaweed powder which did not contain any zinc or copper could accumulate 76.1 mg/kg zinc and 16.6 mg/kg of copper respectively after the treatment (Fig. 7).

Discussion

The salinity of effluent water was less compared to the normal estuarine water which may be due to the frequent release of fresh effluent from the nearby industry getting mixed with the canal water, where the salinity is influenced by the tide. There is not much variation in the pH of effluent water and normal estuarine water, which may be due to chemical pollution rather than organic pollution. It can also be emphasized that no strong acid or alkaline chemicals were released with the effluent. Dissolved oxygen is the major factor in determining water quality. DO exhibited the clear-cut evidence of chemical pollution in water bodies which did not allow any plants and bacteria to grow in the outfall area and making the area a dead zone. Low oxygen concentrations are generally associated with heavy contamination by organic matter (Madhusudhanan and Jayesh, 2011). But here the area was not contaminated with organic pollutant as evidenced by the zero level of BOD during collection. After the effluent was exposed to aeration and treatment, there was very negligible recovery of the effluent with a minimal value of DO and BOD allowing the bacteria to grow in the system and thus helping in nitrogen cycle.

Ammonia is used either directly or indirectly in industries and agriculture and if discharged to natural water sources without treatment, are hazardous (Sheela and Beebi, 2014). There are different methods to treat effluents like adsorption, chemical precipitation, advanced oxidation processes (AOPs) such as direct photo oxidation (Wang *et al.,* 1994). These methods have disadvantages because of continuous input of expensive chemical which is not very cost effective whereas the process of bioremediation is simple, eco-friendly, cost effective and can be used in any aquatic environment for removal of excessive ammonia or heavy metals present in the industrial effluent. In the present experiment, the effluent discharged without proper treatment from nearby industries to natural water had strong pungent smell of ammonia. In the presence of high level of ammonia and zero oxygen, nitrogen assimilation was negligible thereby showing very low concentrations of nitrate and nitrite in effluent water. Thus, the nitrogen cycle of the effluent was not taking place in the strong anaerobic condition, to convert the ammoniacal nitrogen to nitrite and nitrate and made available to the plants. Report states that during such condition the aerobic bacteria will be washed off from the effluent and ammonium would be converted to mainly nitric oxide and nitrous oxide (Sliekers *et al.*, 2005; Keluskar *et al.,* 2013). Rittmann and Mc Carty (2001) stated that the most common genus of bacteria that carries out ammonia oxidation is *Nitrosomonas* species (beta subdivision of proteobacteria) whereas conversion of nitrite to nitrate by alpha subdivision of proteobacteria, *Nitrobacter*.

Even though plants have the potential to absorb the required nutrients for their growth and development, they are unable to absorb these nutrients due to very high dose of ammoniacal nitrogen which affect the root and shoot system of aquatic plants showing symptom of wilting. Similar damage was also observed in the cortical layer of the mangrove plant (Plate 2). Reduction of ammonia from the effluent water after treatment was quite low which is contrary to the work of Sooknah and Wilkie (2004) where the reduction in ammoniacal nitrogen was more than 99% when aquatic macrophyte were cultured in anaerobically digested flushed dairy manure wastewater. When pollution is caused due to heavy metals and inorganic ammonical nitrogen, it completely damages the system making it a dead zone. Such condition can gradually affect the nearby mangrove ecosystem. Untreated wastes when channelled into aquatic environment causes extensive damage to water quality and ecology of the environment specifically when microbial degradation activities cannot cope up with the fast removal of the toxic pollutants (Obire *et al.,* 2008).

Effluent treated with mangrove plant exhibited decline in the value of nitrogen and phosphorus content which may be due to efficient uptake of nutrients by mangroves for their metabolic activities. Lack of DO and BOD with a higher value of 96% ammonia, 84.3% phosphate and 87.6% silicate content observed in the effluent with reference to the normal estuarine water show the gravity of the situation and its impact on the aquatic flora and fauna. Deshmukh *et al.* (2011) observed 69.9% phosphate removal by *Eichhornia* sp which is similar to the present experiment (71.9%) and 47.27% by *Pistia* sp which is almost half to that reported in this study (81.8%).

Heavy metals like copper and zinc was also detected in the effluent. *S. wightii* could accumulate zinc (17.6 mg/kg) and copper 1.96 (mg/kg) whereas *K. alvarezii* could accumulate 76.06 (mg/kg) of zinc and 16.64 (mg/kg) of copper from the effluent. Lead and cadmium were within permissible limit. Earlier reports also explained that *K. alvarezii* is a good bio sorbent for the removal of nutrients (Rathod *et al.*, 2014) and heavy metals (Kang *et al.*, 2011) from the aquatic environment. The bio adsorption efficacy of heavy metals was found to be better in *K. alvarezii* than *S. wightii* because of the high phycocolloid (carrageenan) present in the plant than the algin content in *S. wightii*. The bio adsorption efficacy can further be improved in *K. alvarezii* by reducing the particle size of the plant used for experiment.

Bioremediation is a suitable solution and cost effective method for treating hazardous waste at the discharge point. It is suggested that industries adopt this cost effective technology of effluent treatment so as to reap benefit from the approach. This can lead to the reuse of an important resource like freshwater and also mining the heavy metals can be accomplished through phytomining. Simultaneously, it will safeguard natural water bodies from pollution and maintain synergy with the aquatic flora and fauna.

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