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Phytoremediation potential of selected plants in Netravati and Gurupura estuary of Karnataka

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ABSTRACT

Unsustainable land use due to increasing human population, economic development and emerging global markets have resulted in contamination of soil, soil erosion and eutrophication in coastal regions. Freshwater needs of people are met almost entirely by precipitation on land, with a small though increasing amount by desalination. Hence, appropriate technologies need to be adopted to conserve and protect both soil and water. Use of plants to reduce environmental pollutants is considered as a non invasive technology. Mangrove ecosystem in the estuarine waters of Netravati and Gurupura rivers acts as a shield to absorb the heavy metals before it reaches the Arabian sea. The heavy metal Cu absorbed by *Acanthus ilicifolius* (AI) was 9.24±2.87 ppm, while Cd, Cr and Hg absorbed in the sedge plant *Cyperus malaccensis* (CM) *was* 0.40±0.47 ppm, 1.09±0.32 ppm, and 0.21±0.05 ppm, respectively. Out of the 7 different plants selected from the estuarine area near the bar mouth of Mangaluru, *Acanthus ilicifolius* and *Cyperus malaccensis* was observed to have greater potential to adsorb heavy metals. Treated, semi treated and untreated wastewater discharged from urban area into the Arabian sea through the estuary is rich in nutrients leading to eutrophication. Preliminary treatment studies were carried out to assess the removal of nutrient load from this effluent water in laboratory conditions using aquatic weeds. This revealed that *Hydrilla verticillata* reduced 42.8% ammonia in the effluent water while *Eicchornia crassipes* and *Pistia stratiotes* reduced 61.7% phosphate and 68.2% nitrite, respectively. The study reveals that aquatic weeds could be used to remove nutrients from wastewater before it enters the marine ecosystem and locally available mangrove and sedge plants aid in reduction of contaminants in the coastal ecosystem.

Key words:
Environmental contamination
Heavy metal
Phytoremediation
Soil
Water

1. INTRODUCTION

Land is used more intensively during the last 20 years, cities especially draw upon extensive rural hinterlands for water and disposal of waste, while their demands for food, fuel and raw materials have a global reach (UNEP, 2007). Indian agriculture is trapped in a complex situation of ground water depletion and energy subsidies, which earlier offered opportunities to India's small and marginal farmer to irrigate crops from the deep ground water table (Mohanty *et al.,* 2020). The focus was mainly on increasing crop water productivity ignoring the the cost of water (Meena *et al.,* 2021) and also the contamination from other sources. Many chemicals persist in the environment, circulating between air, water, sediments, soil and biota and travel long distances to supposedly pristine areas (De Vries and Others 2003). Climate change-induced higher water temperatures, reduced river flow, and increased precipitation intensity exacerbate water pollution (Vijay and Qiong 2021). Migration of heavy metal contaminants into non-contaminated areas as dust or leachates through soil are a few examples of events contributing towards contamination of ecosystem (Gaur and Adholeya, 2004). To reduce environmental contamination, there is a need to develop appropriate technologies to assess the presence and mobility of metals (Shtangeeva *et al.,* 2004) in soil, water and wastewater. The demand for water in Mangaluru city of Dakshina Kannada district is 162 MLD while the present supply is 140 MLD. There are several industries situated in the vicinity of the coastal areas that discharge the treated effluents into the Arabian sea. At present, the total effluents discharged is 80 MLD which is expected to increase with more industries to be commissioned in the special economic zone (SEZ). Recycling and reuse of wastewater is one of the ways forward to effectively use water. Phytoremediation is a term applied to a group of technologies that use plants to reduce, remove, degrade, transform or immobilize environmental pollutants, primarily those of anthropogenic origin, with the aim of restoring the area to better conditions (Peer *et al.,* 2005). Use of plants for remediation of soils and waters contaminated with heavy metals, has gained acceptance in the past two decades as a non-invasive technology (Mojiri, 2012). Sewage, municipal wastewater, coal pile runoff, landfill leachate, mine drainage, and groundwater plumes can be phytoremediated (Olguin and Galvan, 2010). The factors that influence phytoremediation include type and amount of contaminant, soil characteristics, water content, nutrient availability, species and plant growth. The screening of plant species for their ability to grow and establish in contaminated soils and water is one of the first steps in their selection for phytoremediation (Reeves, 2003). Previous studies in the region have focused on the occurrence and distribution of true mangrove (eumangrove) species, such as *Rhizophora mucronata*, *R. apiculata*, *Acanthus ilicifolius*, *Avicennia alba*, *A. marina*, *A. officinalis*, *Bruguiera cylindrica*, *B. gymnorrhzia*, *Lumnitzera racemosa*, *Excoecaria agallocha*, *Kandelia candel*, *Sonneratia alba*, *S. caseolaris*, *Aegiceras corniculatum*, and *Ceriops decandra*, along the estuaries of Karnataka (Rao and Suresh, 2001; Chandran *et al.,* 2012; Vijaya and Vijaya, 2012). In the present study different species of mangrove plants, namely *Rhizophora mucronata* (RM), *Acanthus ilicifolius* (AI), *Avicennia marina* (AM), *A. officinalis* (AO), *Excoecaria agallocha* (EA), *Kandelia candel* (KC) already growing in the vicinity of the bar mouth and estuary of the rivers Netravati and Gurupura were selected to assess its potential for phytoremediation based on the possibility of contaminants in the region. In addition, *Cyperus malaccensis* (CM), a perennial, salt tolerant sedge plant growing in association with mangroves was also selected.

David and Gentry (2000) pointed out that effluent from urban discharge, wastewater treatment plants, and agricultural fields are the primary source of phosphate and nitrogen contributing to eutrophication. Once the contaminated fresh water from land sources reach the marine ecosystem there is a change in salinity regime and increase in nutrient load to the marine ecosystem. In order to assess the ability of fresh water plants to reduce the nutrient load at source preliminary studies were conducted with the effluent under laboratory conditions using three plants, *viz*., *Hydrilla verticillata*, *Pistia stratiotes* and *Eicchornia crassipes*. *Hydrilla verticillata,* a native to India is a submersed rooted aquatic plant that can grow up to depth of 12 m in non-turbid water. The optimal growth temperature range for low salinity tolerant *Pistia stratiotes* is 22-30^{o}C. *Eicchornia crassipes* is a highly adaptive aquatic plant, which has recently been identified as a means of producing biofuel.

2. MATERIALS AND METHODS

Site Selection and Sample Collection

Mangaluru, an urban agglomeration (12°0.01′N, 74° 0.01′E), has a population of 6, 23,841 (Government of India, 2011) and covers an area of 132.45 km^2. Two major rivers, *i.e.,* the Netravati and Gurupura, form an estuary and drain into the Arabian sea. The city of Mangaluru has regions allocated as special economic zones (SEZs) for which fresh water is a primary requirement and treated effluent is discharged into the Arabian sea. Samples of six mangrove species and a salinity tolerant grass species growing in the estuarine area was collected for analysis from seven different stations (Fig. 1). For each species triplicate samples were prepared for analysis.

Extraction for Heavy Metals

Leaves and shoots of the plants collected from seven locations (72 samples) during August, 2021 in the estuarine area of Netravati river and Gurupura river was thoroughly washed and air dried. EPA 3052 method was adopted for the complete digestion of the sample as it is suitable for micro-wave assisted acid digestion (Kingston and Jassie, 1988) of complex matrices including siliceous and organic matrices. Anton Paar Multiwave Go 82100766 digester was used. Well mixed samples of 0.5 g were weighed to nearest 0.001 g and digested. Heavy metal Cu, Cd, Cr and Hg were determined using Varian AA240 Atomic Absorption Spectrometer. The Vapor Generation of mercury was carried out using Varian's continuous flow VGA-77. This allows determinations of mercury at part-per billion (ppb) levels which was then converted to ppm (mg kg^{-1} dry weight).

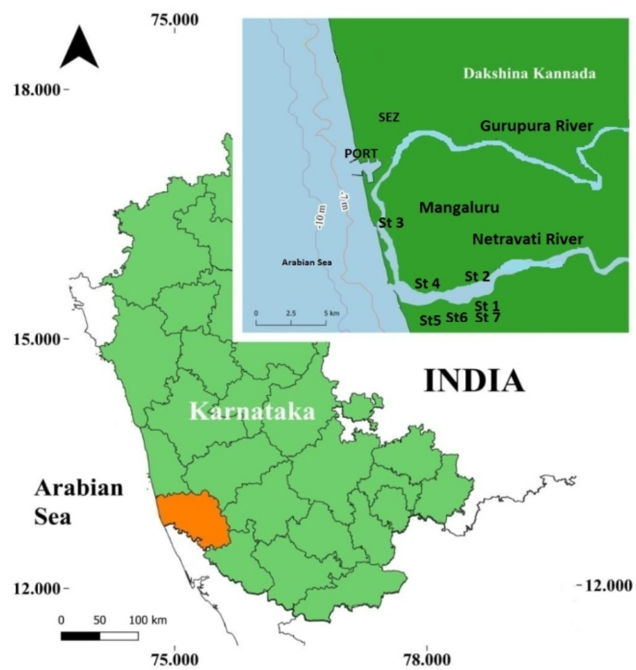

Fig. 1. Location of the study area

Statistical analyses comparisons of the means, and analysis of variance (ANOVA) were conducted using the SPSS v. 16 (IBM, New York, USA) and MS-Excel was employed for graphical representation of data.

Water Quality Analysis of Effluent in Laboratory

The estimation of nutrients in the effluent water were carried out using standard analytical methods (APHA, 1981) for dissolved orthophosphate, nitrite, nitrate and ammonia. Ammonium molybdate and potassium antimony tartrate react in an acid medium with dilute solutions of orthophosphate to form phosphomolybdic acid that is reduced to intensely coloured molybdenum blue by ascorbic acid. The intensity of the blue colour increases in proportion to the amount of phosphorous present thus dissolved orthophosphate was measured photometrically. The nitrite in the sample is allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts with N-(1-naphthyl)-ethylenediamine and forms a highly colored azo dye, the extinction of which is measured spectrophotometrically. The nitrate was reduced almost quantitatively to nitrite when the sample is run through a column containing cadmium filings loosely coated with metallic copper and thus estimated. For determining ammonia phenol and hypochlorite react in an alkaline solution to form phenyl quinone-monoimine, which in turn react with ammoma to form indophenol. Indophenol gives the solution a blue colour, the intensity of which is propotional to the concentration of ammonia present. Sodium nitroprusside is added to intensify the blue colour. This procedure gives an estimate of total ammonia nitrogen. The quantity of water taken for *Hydrilla verticillata*, *Pistia stratiotes* and *Eicchornia crassipes* was 4000 ml,1000 ml and 5000 ml, respectively based on the plant physiology. Three trials were conducted with three replications.

3. RESULTS AND DISCUSSION

ANOVA showed significant (P<0.001) difference in heavy metal levels between stations for Cd, Cr and Hg, while significant difference was observed between species for Cu and Hg metals (Table 1). Statistically significant difference (P<0.001) was also observed between heavy metal levels in plants collected from rivers Gurupura and Netravati for metal level of cadmium and chromium (Table 2). Higher levels were observed in plants from Gurupura river in comparison to that in Netravati river (Fig. 3) for all metals.

Heavy Metal Variation in Different Plants

Copper was found to be higher in *Acanthus ilicifolius*

Fig. 2. Experimental setup of (A) *Hydrilla verticillata* (B) *Pistia stratiotes* (C) *Eicchornia crassipes*

Table: 1
Heavy metal ANOVA for different stations and plant species

		Sum of squares	df	Mean square	F	Sig
Cu * Station	Between groups	97.61	6	16.269	1.596	0.162
	Within groups	662.51	65	10.192		
Cd * Station	Between groups	4.67	6	0.778	19.214	0
	Within groups	2.63	65	0.040		
Cr * Station	Between groups	11.39	6	1.898	38.975	0
	Within groups	3.17	65	0.049		
Hg * Station	Between groups	0.25	6	0.041	6.111	0
	Within groups	0.44	65	0.007		
Cu * Species	Between groups	466.42	6	77.736	17.204	0
	Within groups	293.71	65	4.519		
Cd * Species	Between groups	0.64	6	0.106	1.036	0.41
	Within groups	6.66	65	0.102		
Cr * Species	Between groups	3.05	6	0.508	2.870	0.015
	Within groups	11.51	65	0.177		
Hg * Species	Between groups	0.21	6	0.035	4.785	0
	Within groups	0.47	65	0.007		

Table: 2
ANOVA for heavy metal level of plants form Gurupura and Netravati river

		Sum of squares	df	Mean square	F	Sig
Cu * River	Between groups	19.15	1	19.15	1.809499	0.183
	Within groups	740.97	70	10.59		
Cd * River	Between groups	4.17	1	4.17	93.15679	0
	Within groups	3.13	70	0.04		
Cr * River	Between groups	6.78	1	6.78	61.05005	0
	Within groups	7.78	70	0.11		
Hg * River	Between groups	0.02	1	0.02	1.616877	0.208
	Within groups	0.67	70	0.01		

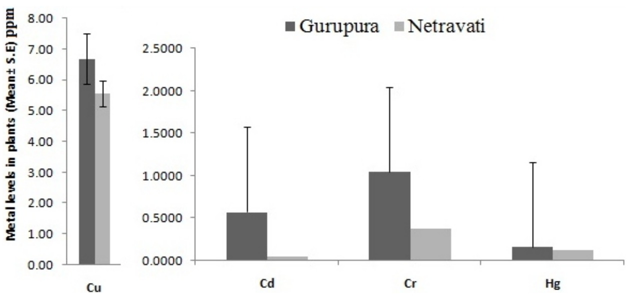

Fig. 3. Comparison of metal levels (ppm) in plants collected from Gurupura and Netravati rivers

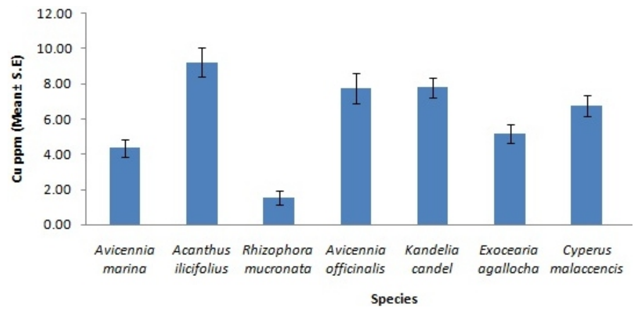

Fig. 4. Heavy metal Cu variation in different plants

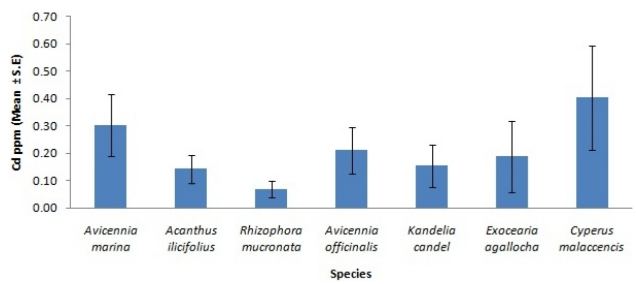

Fig. 5. Heavy metal Cd variation in different plants

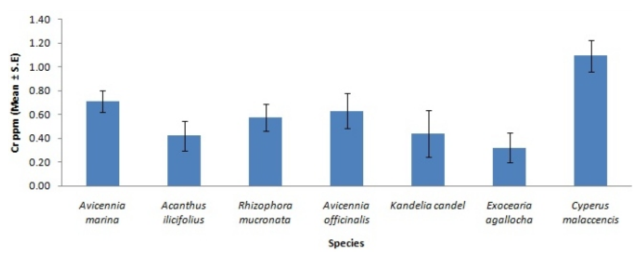

Fig. 6. Heavy metal Cr variation in different plants

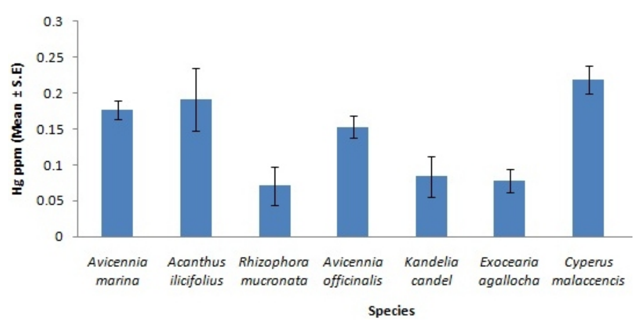

Fig. 7. Heavy metal Hg variation in different plants

(AI) in range of 2.53-6.69 ppm (Fig. 4). The absorption of copper metal in the plants was of the order AI (9.24±2.87 ppm) > KC (7.79±1.43 ppm) > AO (7.76±2.98 ppm) > CM (6.76±1.43 ppm) > EA (5.16±1.79 ppm) > AM (4.35±1.6 7ppm) > RM (1.52±1.32 ppm). The Cu accumulation found in the plants was higher than that reported by Kannan *et al.,* 2016 for the mangrove and associated plants of Ennore mangrove ecosystem. This is because the element concentration in plants depends on the physiological age of the plant and ability of various species to absorb the metal. In addition, Cu comes in the category of essential inorganic plant nutrient taken up by the root system as dissolved constituents in soil moisture.

The absorption of Cd in the plants selected was in the order CM (0.40±0.47 ppm) > AM (0.30±0.39 ppm) > AO (0.21±0.30 ppm) > EA (0.19±0.45 ppm) > KC (0.15± 0.19ppm) > AI (0.14±0.18 ppm) > RM (0.07±0.11ppm). The highest uptake ranged from below detectable level to 1 ppm in the sedge plant *Cyperus malaccensis* and (Fig. 5).

The absorption of Cr in the plants selected was in the order CM (1.09±0.32 ppm) > AM (0.71±0.32 ppm) > AO (0.63±0.51 ppm) > RM (0.57±0.40ppm > KC (0.44±0.48 ppm) > AI (0.42±0.43 ppm > EA (0.96±0.43 ppm). The highest uptake, ranging from 0.633 to 1.49 ppm was recorded in the sedge plant *Cyperus malaccensis* (Fig. 6).

The absorption of Hg in the plants selected was in the order CM (0.21±0.05 ppm) > AI (0.19±0.15 ppm) > AM (0.18±0.04 ppm) > AO (0.15±0.05 ppm) > KC (0.08±0.07 ppm) > RM (0.07±0.09 ppm) EA (0.07±0.06 ppm). The highest absorption of Hg was in the sedge plant *Cyperus malaccensis* ranging from 0.152 to 0.281 ppm (Fig. 7), followed by that of *Acanthus ilicifolius* ranging from below detectable level to 0.41 ppm.

In the east coast mangrove ecosystem, Kannan *et al.,* 2016 observed that the heavy metal level accumulation in *Avicennia marina* was greater than that in the associated plants. Here, it was observed that the sedge plant *Cyperus malaccensis* was a better accumulator of metals Cd, Cr and Hg while higher amount of Cu was accumulated by *Acanthus ilicifolius.* The presence of a contaminant in soils tend to naturally attract organisms such as bacteria, yeast and fungi which prefer chemical as a source of food and energy. It was observed by Sridhar *et al.,* 2010, that changes in chemistry of decomposing sedge *Cyperus malaccensis* coincided with diversity of fungal species, terrestrial fungi being replaced by mangrove or marine fungi as salinity changed in southwest coast estuary of India. A symbiotic relationship evolves between plants and soil microbes. The concentration of metals in a plant species is an indicator of the metal status of the site and the ability of the species to absorb metal from the soil (Massadeh *et al.,* 2009). Sun *et al.* (2004) underline in their study how tropical plants were selected for their ability to tolerate high salinity and remove specific hydrocarbons in coastal topsoil prior to further investigation of phytoremediation feasibility in deep contaminated soils.

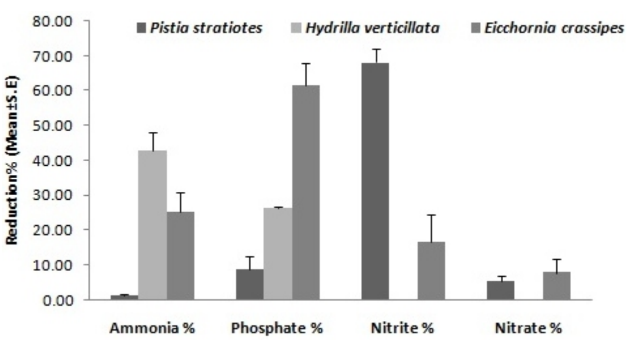

Fig. 8. Variation in reduction percent of nutrients for the different plants

Reduction of Nutrients in Effluent using Freshwater Plants

The phosphate in the effluent ranged from 0.263 to 0.406 mg L^{-1}, ammonia ranged from 0.505 mg L^{-1} to 1.03 mg L^{-1}, nitrite ranged from 0.012 mg L^{-1} to 0.086 mg L^{-1} while nitrate ranged from 0.859 to 6.153 mg L^{-1}. Reduction in phosphate, ammonia and nitrite was observed in the effluent within 6 days of treatment under *Eicchornia crassipes* after which the plant showed degradation. *Hydrilla verticillata* and *Pistia stratiotes* survived for 30 days and 26 days in laboratory though reduction in parameters was observed only within 10 days and 12 days, respectively. This could be due to the laboratory condition not being very favourable for the growth of the plant. The maximum reduction in ammonia (Fig. 8) was observed under *Hydrilla verticillata* and phosphate reduction, under *Eicchornia crassipes*, whereas *Pistia stratiotes* caused a higher reduction in nitrite. Statistically significant difference in reduction of nutrients (ammonia, phosphate and nitrite) was observed among the three plants (Table 3).

4. CONCLUSIONS

The changes in land and water use for urban and industrial growth, have impacts on freshwater and coastal ecosystems hence sustainable management of the resources is needed. It is evident that mangrove and sedge plants are capable of absorbing heavy metals in the estuarine area. The phytoremediation of treated effluent from industries or sewage from urban areas prior to release into the coastal ecosystem could further aid in reduction of nutrient load. The sedge plant *Cyperus malaccensis* was found to be a better accumulator of metals Cd, Cr and Hg while Cu was better accumulated by *Acanthus ilicifolius* in the estuarine area. Freshwater locally growing plants like *Hydrilla verticillata*, *Eicchornia crassipes* and *Pistia stratiotes* could be selected based on the need for remediation. A proper phytoremediation strategy to tackle soil contaminants, particularly those leading to heavy metal accumulation in soils would therefore necessitate the identification of appropriate plant species selected from among the rich diversity of plants in an area, depending on the type of contaminant in the area.

Table: 3
ANOVA for reduction in nutrients for the three plants

Reduction %		Sum of squares	df	Mean square	F	Sig.
Ammonia % * Plant type	Between groups	2627.01	2	1313.50	21.46422	0.002
	Within groups	367.17	6	61.20		
Phosphate % * Plant type	Between groups	4386.52	2	2193.26	43.40646	0
	Within groups	303.17	6	50.53		
Nitrite % * Plant type	Between groups	7577.54	2	3788.77	47.8423	0
	Within groups	475.16	6	79.19		
Nitrate % * Plant type	Between groups	95.57	2	47.79	2.651065	0.15
	Within groups	108.15	6	18.03		

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