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Nutrients and isotopic composition in *Rhizophoraceae* mangroves of Kochi, South west coast of India

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The present study investigates the macronutrient and micronutrient elemental composition and isotopic composition in five Rhizophoraceae mangroves plants widely distributed along Kerala coast in order to verify the response and variations in these plants towards different nutrient elements and classify them accordingly. The lower C/N values of the leaves suggest that the leaves have high nutrient quality than bark. *Bruguiera* showing highest values of Na/K can be thus concluded to be the plants with least salt excluding capacity. The iron rich mangrove plant was found to be *B. gymnorhiza*. Zinc content was found to be highest in *K. candel*. Amount of Pb in all the leaf and bark samples under study in this research were found to be below the recommended levels while cadmium was found to be accumulated in the leaves and bark of *R. apiculata* as well as in the leaves of *B. cylindrica* and *K. candel*. The stable carbon isotope composition of leaves of the *Rhizophoraceae* mangrove under the present investigation matches well with the already established values of $\delta^{13}C$ for C3 plants and the present nitrogen stable isotope results fall within the range of plants that obtain inorganic nitrogen directly from seawater.

Keywords: Mangroves, Stable isotopes, Nutrients.

INTRODUCTION

Mangrove forests are ecosystems which produce organic carbon well in excess of the ecosystem requirements and contributing significantly to the global carbon cycle. Mangroves create typical ecological environments that host rich assemblages of species. They play a crucial role in the biogeochemical cycling of phosphorus, carbon, nitrogen and other nutrients (Ratheesh Kumar, 2011; Bunt, 1992). The *Rhizophoraceae* family of true mangrove plants is the most populated and contains widely distributed species. The *Rhizophoraceae* family of true mangroves have twenty four species in four genera which include *Bruguiera* containing seven species, *Ceriops* containing five species, *Kandelia* containing two species and *Rhizophora* having ten species (Nebula et.al., 2013). They mostly grow in the intertidal silty clay and loam soil, frequently inundated with high saline tidal seawater or brackish water; their salt resistance efficiencies are very high for several morphological and anatomical features of these true mangrove species (Naskar and Mandel, 1999).

Plants take up and accumulate mineral nutrients in their tissue from soil solution and also from air in some exceptional cases. They also release some nutrients back into the surrounding medium. The major fraction of the fresh weight of living plant organs, i.e. those displaying an active metabolism, consists on average of 85-90% water whereas the dry substance of the plant body is mainly composed of the following elements: carbon (44.5%), oxygen (42.%), hydrogen (6.5%), nitrogen (2.5%), phosphorus (0.2%), sulphur (0.3%) and the alkali and alkaline-earth metals potassium (1.9%), calcium (1.0%) and magnesium (0.2%) (Markert, 1992). On the basis of their higher concentration in the plant body the nine elements mentioned above are also termed macroelements. In addition, there are also so-called microelements present in the plant organism in lower concentrations and vital for most plants. These elements are chlorine

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(2000 mg/kg dry substance), silicon (1000 mg/kg), manganese (200 mg/kg), sodium (150 mg/kg), iron (150 mg/ kg), zinc (50 mg/kg), boron (40 mg/kg), copper (10 mg/kg), chromium (1.5 mg/kg), molybdenum (0.5 mg/kg) and cobalt (0.2 mg/kg) (Markert, 1992). Both macro and microelements are plant nutrients vital for the growth and normal development of the plant and their function cannot be replaced by any other element. Macro and microelements are therefore also known as macro and micronutrients.

Apart from the macro and micronutrients discussed above, a number of further chemical elements such as fluorine, iodine, nickel, selenium, tin and vanadium , also occur in plants (Adriano 2001; Bodeck *et al*., 1988; Hamilton 1979 and 1980; Caroli *et al*., 1989) which are regarded as essential for animal organisms. Further elements are under discussion including some which until recently were only considered from a toxicological point of view (e.g. cadmium and lead). There are currently indications that in a correspondingly low concentration these elements exercise metabolic functions in living organisms (Markert, 1992). Due to specific site conditions, element or organism specific accumulation processes frequently occur: sodium, bromine and chlorine are accumulated by many halophytes (Markert and Jayasekera 1987); copper, nickel, zinc, lead, cadmium and other heavy metals are taken up by metallophytes to an increased extent (Ernst 1974; Ernst and Joosse-Van Damme 1983). Admittedly, an accumulation is not to be equated with an increased physiological benefit from the element for the organism, indeed this probably often purely represents an adaptation to the respective site (Market 1992). Nutrients, thus taken up by the plants are translocated to other plant parts. Also, plants are able to take up elements via leaf surface both through stomata (gases) and through the cuticle (ions) (Marschner, 2012). With a defined nutrient, the uptake by plants depends on the reserves of the nutrient in the uptake medium and its availability.

Mangroves which grow in the saline environment must regulate ion uptake so as to maintain turgor, but at the same time protect sensitive metabolic sites from ion stress. They accumulate high concentration of inorganic ions which apparently function in osmoregulation of leaves and other tissues (Popp, 1984). Different taxa have different mechanisms for coping with high salt concentrations.

Carbon and nitrogen stable isotopic data from the primary producers in mangrove ecosystems are needed to investigate trophic links and biogeochemical cycling. Discrimination varies among plants using different photosynthetic pathways. The Calvin cycle (C3), Hatch–Slack cycle (C4) and Crassulacean acid metabolism (CAM) photosynthetic pathways differ so profoundly and so consistently (O'Leary 1981, 1988) that ecologists have used isotopic signatures in large-scale surveys of plant species (Teeri and Stowe 1976; Sage and Monson 1999).

Eventhough the *Rhizophoraceae* mangroves have proven potentials to use as source of novel compounds for food, pharmaceutical and agricultural use, there is a gap of information towards the chemistry of *Rhizophoraceae* mangroves from Kerala. In this perspective, the basic knowledge of the nutrient elments and isotopic compositions are of much importance towards asserting the toxic and non-toxic limits especially with respect to the heavy metal content. So such studies can pave a firm base for their use in pharmaceutical applications, chemotaxonomic studies as well as for the better management of the existing mangrove ecosystem. This paper is an attempt to characterise the *Rhizophoraceae* mangroves found in Kerala in terms of the nutrient minerals as well as carbon and nitrogen isotopic composition of their leaves and bark.

MATERIALS AND METHODS

Plant materials

Mangrove plant samples were collected in August 2011 during the monsoon period, the details of which are given in Table 1. Leaves and bark of five mangrove plants belonging to the *Rhizophoraceae* family of true mangroves were collected and transported to the laboratory.

All the plants were identified by Dr. Khaleel K.M. (Former Director, School of Enviromental Studies, Kannur University). Voucher specimens

Table 1. Details of mangrove plants selected for the present study

Species	Genus	Family	Order	Location
Bruguiera cylindrica	*Bruguiera*	*Rhizophoraceae*	*Malghiales*	Puthuvypu, Kochi
Bruguiera gymnorhiza	*Bruguiera*	*Rhizophoraceae*	*Malghiales*	Puthuvypu, Kochi
Kandelia candel	*Kandelia*	*Rhizophoraceae*	*Malghiales*	Valanthakkad, Kochi
Rhizophora apiculata	*Rhizophora*	*Rhizophoraceae*	*Malghiales*	Aroor
Rhizophora mucronata	*Rhizophora*	*Rhizophoraceae*	*Malghiales*	Puthuvypu, Kochi

(BCP8/2011, BGP8/2011, KCV8/2011, RAA8/2011, RMPP8/2011) were kept in at Inter University Centre for Marine Biotechnology, Cochin University of Science and Technology. The collected leaves and barks of the mangrove plants were washed with water and dried in an incubator at 40°C. Dried samples were ground to produce fine homogenous powders using an electric blender.

Macro and micronutrient composition

The elemental carbon, hydrogen, nitrogen and sulphur in the dried plant parts were determined using Vario EL III CHNS Analyser. Total phosphorous was estimated using vanadomolybdophosphoric acid method (Bhargava and Raghupathi, 2005).

Minerals (micro and macronutrients) present in the plant parts such as magnesium, iron, copper, zinc, manganese, cobalt and heavy metals, lead, cadmium in the plant part were estimated using Flame AAS (Perkin Elmer-311 0) after digestion using (di-acid mixture (1:5 $HCIO_3$:HNO_3) (AOAC, 1995). Accuracy of the analytical procedure was checked using standard reference material. Triplicate analysis of BCSS-1 showed a good accuracy and the recovery rate ranged between 82.7% for Mn and 103.9% for Zn (Table 2).

Table 2. Analysis of Standard reference material for heavy metals (BCSS-1)

Metal	Certified Value	Obtained concentration (n=3)
Co (μg g^{-1})	11.4±2.1	10.67±2.68
Cr (μg g^{-1})	123±1.4	112±0.65
Cr (μg g^{-1})	18.5±2.7	18.2±0.25
Co (μg g^{-1})	4.7±0.14	4.64±0.41
Fe (%)Mg	2.44±0.23	2.32±0.36
Mg (%)	229±15	189.47±10.75
Ni (μg g^{-1})	55.3±3.6	49.16±2.01
Pb (μg g^{-1})	22.7±3.4	24.9±0.08
Zn (μg g^{-1})	119±12	123.64±2.51

Isotopic analysis

The carbon and nitrogen isotope values were measured at the Marine Stable Isotope Lab (MASTIL) at National Centre for Antarctic & Ocean Research, Goa, India using an Isoprime Stable Isotope Ratio Mass Spectrometer in continuous-flow mode coupled with an EA (Isoprime, Vario Isotope Cube). The external precisions on $\delta^{13}C$ and $\delta^{15}N$ are ±0.04‰ and ±0.07‰ (1σ standard deviation) respectively obtained by repeatedly running Cellulose (IAEA-CH-3) & Ammonium sulphate (IAEA-N1) standards (n=21). $\delta^{13}C$ values are reported with respect to V-PDB and $\delta^{15}N$ values are reported with respect to air N_2. The reference standard used for normalising to V-PDB and air N_2 scale are Cellulose (IAEA-CH-3) & Ammonium sulphate (IAEA-N1).

RESULTS AND DISCUSSION

Macro and micronutrient elements

The macronutrient elements considered for the present study are C, H, N, S, P, K and Mg and the micronutrient elements analysed were Na, Fe, Cu, Zn, Mn and Co in the leaves and bark of the mangroves. Also, the two elements, Pb and Cd were considered in the present study.

In the present work, the carbon content in the leaves of the mangrove plants was found to be lower than the corresponding bark samples. *B. cylindrica* showed the lowest carbon content while *K. candel* was found to exhibit highest carbon content among the five *Rhizophoraceae* mangroves under investigation. The carbon content was found to be in the order *B. cylindrica*<*R. apiculata*<*B. gymnorhiza*<*R. mucronata*<*K. candel*.

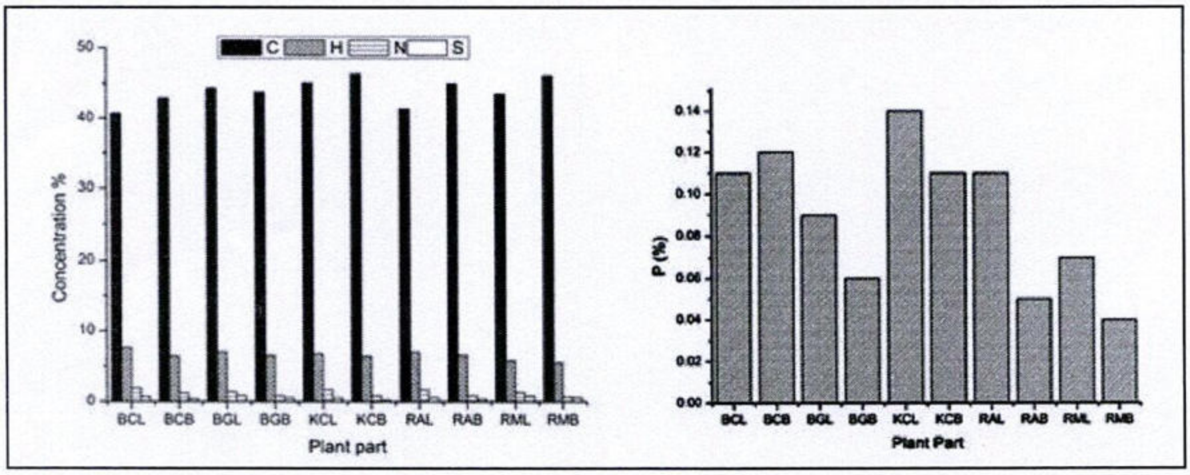

Fig. 1. a) CHNS composition and b) Phosphorus in the leaves and bark of *Rhizophoraceae* mangroves

As per a pilot study on carbon sequestration of mangrove forest, the average percentage of carbon in leaf samples of mangroves from Mumbai Coast was found to be 36.95% (Patil *et al*., 2012) which is lower than the values observed in this study. Mangroves are known to remove CO_2 from the atmosphere through photosynthesis. They fix greater amounts of CO_2 per unit area, than what the phytoplankton do in the tropical oceans (Kathiresan and Bingham, 2001). The mangroves have the capacity to accumulate and store carbon in the soil in large quantities. The present study indicates, the *Rhizophoraceae* mangroves from Kochi, thus contributes significantly to the carbon budget of this geographical location, *K. candel* being the major contributor. Because the mangroves fix and store significant amounts of carbon, their loss may have impact on global carbon budget and global warming.

In the present study, the bark of the mangroves showed comparatively lower percentage of hydrogen than the leaves. The genus *Bruguiera* showed higher hydrogen levels, followed by *Kandelia* and *Rhizophora* mangroves. *R. mucronata* showed lowest concentration of hydrogen in its bark and leaves than other mangrove plants under investigation. Similar to hydrogen levels, the nitrogen content also was observed to be higher in the leaves than their bark. Nitrogen and phosphorus availability influences primary production and growth in mangroves (Clough, 1992). The phosphorus levels was found to comparatively lower than N levels and found to be more concentrated in the leaves than in the bark. The sulphur content was found to least in *K. candel* leaves (0.48%) and bark (0.21%) while higher concentrations was observed in the leaves and bark of *B. gymnorhiza* (0.85% & 0.51%) and *R. mucronata* (0.80% & 0.56%). As observed for nitrogen and hydrogen, bark specimens showed lower sulphur percentage than the leaves. So it can be concluded that except carbon content, the macronutrient elements, H, N, P and S are more concentrated in the leaves and less in the bark of mangroves while C is more concentrated in their bark. No specific trend was observed according to genre. The elemental composition follows the order C>H>N>P>S.

The terrestrial vascular plants have C/N ratios more than 12 (Meyers 1994; Rumolo *et al.*, 2011). The observed C/N ratios for the mangroves are in the range 21.43 to 65.23 (Fig. 2a). The basic reason for these higher C/N ratios is simply due to the carbohydrate-rich (e.g., cellulose)/ protein-poor nature (Meyers, 1997) of these plant components. Also, decreased nitrogen invested in leaves and a concomitant increase in the carbon: nitrogen ratio of plant tissues can be attributed to adaptation of these plants to elevated levels of CO_2. The lower C/N in the leaves suggests that the leaves have high nutrient quality compared to bark and significantly higher nutritional quality was observed in *B. cylindrica* leaves than the other mangrove plants in this study. Mfilinge *et al.*, 2002 has reported higher N and lower C/N values for *K. candel* than *B. gymnorhiza* leaves in Okinawa, Japan indicating the higher nutritional quality of the former than latter. The current investigation is consistent with the aforesaid observation.

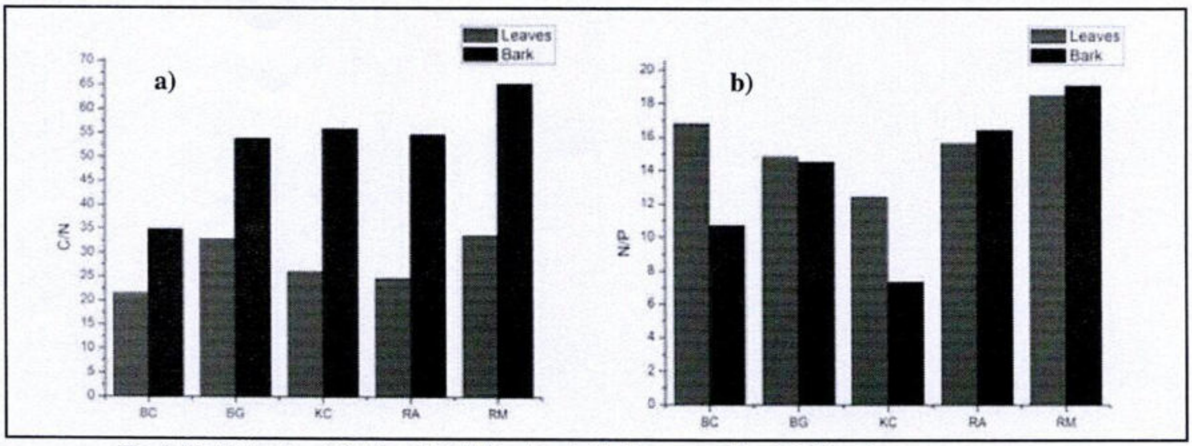

Fig. 2. Variation in a) C/N and b) N/P ratios in the leaves and bark *Rhizophoraceae* mangroves

Mangroves appear to be highly plastic in their responses to changes in nutrient availability, achieving high growth rates when nutrient limitations are relieved that are accompanied by associated reductions in nutrient-use efficiency and other nutrient conservation mechanisms (Reef *et al.*, 2010). The ratio N/P in plant tissue has also been used to infer N or P limitations to growth (Güsewell, 2004). Variation in leaf N/P, particularly where N/P is >32 (which is a global average for mangroves; Lovelock *et al.*, 2007), indicates that P may limit growth in many mangrove habitats (e.g., Malaysia, Kenya, China, Puerto Rico, Venezuela, Victoria, Australia, Florida and Honduras; reviewed in Lovelock *et al.*, 2007). The observed N/P value for the *Rhizophoraceae* mangroves; 10.68-19.05 (Fig. 2b), thus suggest that P is not a limiting factor in the ecosystems of the present study. The lower C/P ratio and hence the enrichment of phosphorous in the mangroves sediments of Puthuvypu (Ratheesh Kumar, 2011) supports this observation.

The plants belonging to *Rhizophoraceae* family exhibit relatively low concentration of metals due to the salt- exclusion mechanism that is operative in the species (Sarangi *et al.*, 2002). These mangrove plants have a well developed ultra filtration mechanism (McMillan, 1974; Scholander, 1968). When wetland plants translocate metals from root tissue to aerial tissue, they are accumulated in leaves and stems. The degree of upward translocation is dependent on the species of the plant, the metal and a number of environmental conditions (Weis and Weis, 2004).

The variations of the macronutrient elements K and Mg and the micronutrient element Na in the leaves and bark of mangrove plants is shown Fig. 3. Due to specific site conditions, element- or organism- specific accumulation processes frequently is reported to occur in mangroves leading to the accumulation of minerals like sodium, bromine and chlorine (Markert and Jayasekera, 1987). The presence of sodium in five *Rhizhophoraceae* mangrove plants is being reported in this study. Sodium which is grouped under electrolytic elements (Sansoni and Iyengar, 1978) present in plants is required for the construction of specific physiological potentials and is important for maintain defined osmolytic conditions in the cell metabolism. Except *B. gymnorhiza*, all the other plants studied are found to accumulate sodium in their leaves than in the bark. *Bruguiera gymnorhiza* possessed higher sodium content in the bark (56.5±1.20 mg g^{-1}) than in the leaves (22.5±0.74 mg g^{-1}). The highest sodium content was found in the *Bruguiera* plants; *B.cylindrica* and *B. gymnorhiza* having the sum of sodium concentrations in leaves and bark, 57.50 mg g^{-1} and 79 mg g^{-1} respectively. The next level was found in rhizophora mangrove plants, *R. mucronata* (37 mg g^{-1}) and *R. apiculata* (38 mg g^{-1}) followed by *K. candel* (12 mg g^{-1}).

Potassium, one of the primary nutrients, regulates many metabolic processes required for growth, fruit and seed development. It increases disease tolerance and drought tolerance, regulates opening and closing of stomata (Hasanuzzaman *et al*, 2018). The genus *Rhizophora* as well as *Kandelia* exhibited higher potassium content than the *Bruguiera* plants. Except *B. gymnorhiza*, all the other four plants were found to have potassium levels high in their leaves. In *B. gymnorhiza*, the bark tissue displayed higher potassium content than the leaves. The leaves of *Rhizophora* mangroves possessed three times the amount potassium than the bark. *Rhizophora* species are salt- tolerant and can normally exist in soil salinities as high as 60% (Cintron *et al.*, 1978).

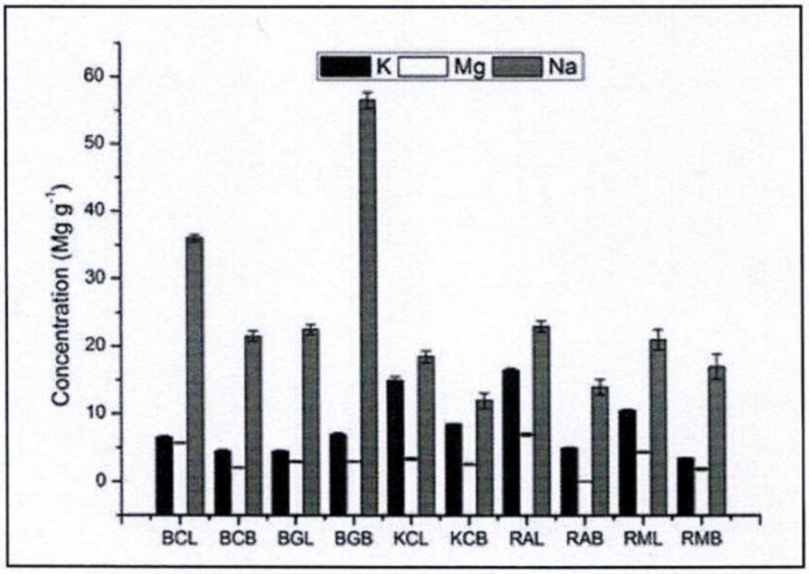

Fig. 3. Variations of Na, K, Mg in the mangrove leaves and bark

The salt tolerance results from the ability of the root membranes to exclude salts by developing a negative pressure in the plant conductive tissue due to leaf transpiration, a type of reverse osmosis process (Scholander *et al.*, 1965). Red mangroves exclude ions by this process (Scholander, 1968). It was shown that the this process was not an active transport mechanisms because electron transport uncoupling agents such as 2, 4 dinitrophenol do not interfere with salt exclusion in *Rhizophora*. In a healthy tree with salt excluding capacity, the ratio Na/K would be smaller than in a tree unable to exclude salt effectively. The values of Na/K in the leaves of mangrove plants under the present study for *B. cylindrica*, *B. gymnorhiza*, *K. candel*, *R. apiculata* and *R. mucronata* are 5.54, 5, 1.2, 3.3 and 2 respectively indicating that the salt exclusion mechanism is efficiently operative in *K. candel* followed by *Rhizophora* species. *Bruguiera* showing highest values of Na/K can be thus concluded to be the plants with least salt excluding capacity among the *Rhizophoraceae* plants under investigation. The values of Na/K given by Kotmire and Bhosale, 1979 for *R. mucronata* (4.5) and *B. gymnorrhizha* (14.1) also demonstrate that the former is better salt excluding species than latter. Page *et al.*, 1985 has demonstrated that the Na/K ratio of leaf tissue is a readily measured and potentially useful sublethal indicator of oil stress for *Rhizophora* and for other salt excluding halophytes.

In general, halophytes have lower K/Na ratios (< 1.0) while glycophytes have higher K/Na ratios (>1.0) (Albert and Popp, 1977; O'Leary and Glenn, 1984). The K/Na values obtained in this study also fall in the aforementioned range for halophytes ie; 0.15 for *B. cylindrica*, 0.22 for *B. gymnorhiza*, 0.066 for *K. candel*, 0.06 for *R. apiculata* and 0.10 for *R. mucronata*. Naidoo *et al.*, (2002) found that true mangroves *A. marina* and *Bruguiera gymnorrhiza* kept lower K/Na ratios in their leaves (0.1~ 0.25). This lower K/Na ratio is not attributed to the reduction in K+ uptake but apparently to the increase in Na+ uptake (Downton 1982; Parida *et al.*, 2004). Maintenance of efficient uptake of K under higher saline habitats is a key feature of salt tolerance (Glenn *et al.*, 1994; Niu *et al.*, 1995).

Magnesium is a constituent of the chlorophyll molecule, which is the driving force of photosynthesis. It regulates uptake of the other essential elements, serves as a carrier of phosphate compounds throughout the plant, facilitates the translocation of carbohydrates (sugars and starches), and enhances the production of oils and fats (Wilkinson *et al.*, 1990). In this work, among the five mangrove plants of the family *Rhizophoraceae, R. apiculata* leaves were found to be rich in magnesium content. At the same time its bark displayed the lowest Mg concentration. In *B. gymnorhiza*, it was observed that the Mg is uniformly distributed among the leaves and the bark. In rest of the plants leaves accumulate more Mg than their bark. In the *Rhizophora* mangroves studied, the difference observed in magnesium concentration between the leaves and the bark is very large when compared to the other genre studied, i.e. *Bruguiera* and *Kandelia*.

The composition of micronutrient elements Fe, Cu, Zn, Mn and Co is depicted in Fig. 3. Iron is an essential micronutrient element for chlorophyll synthesis in plants and plants could not complete its survival cycles without it. But high density of iron in plant has toxicity effects. Its recommended value in plant tissue is about 50-500 mg/kg (Pendias and Pendias, 2001). However, the results of the present study show considerably higher values of iron in the leaves and bark of the mangroves. Except *K. candel* all other plants show iron concentration more in the bark than in leaves. In case of *K. candel* the leaves are found to be iron rich than its bark. Among the leaves, the leaves of *B. gymnorhiza* (1315±1.41 mg kg^{-1}) and among the bark samples, the bark of *R. mucronata* (2880±4.24 mg/kg^{-1}) were found to be rich in iron content and these values are very higher than the recommended levels. Except these two plants under the present investigation, all others have Fe concentrations within the recommended levels. Thus, the iron rich mangrove plant was found to be *B. gymnorhiza* having high iron content in both leaves and bark. The results suggest that iron is taken up by the mangroves very efficiently. Higher values of iron have been reported earlier in the leaves of mangroves (Untawale *et al.*, 1980; Kotmire and Bhonsale, 1979; Thomas and Fernandez, 1997; Ramos e Silva *et al.*, 2006).

The physico-chemical characteristics of the soil have an influence on the nutritive value of plant organs (Assogbadjo *et al.*, 2012). The soil characteristics of the area Puthuvypu show a higher iron concentration (Ratheesh Kumar, 2011). Certain seaweeds are reported to have metabolic systems in which it is capable of directly absorbing elements from the seawater (Norziah and Ching 2000). Mangroves are also reported to have the capacity to take up heavy metals from the environment (MacFarlane *et al.*, 2007). May due to their specialised metabolic systems mangroves absorb elevated levels of iron from their iron rich environment and in the bark it is been stored as the amount of iron required for the normal growth of a plant is only 11 mg/100 g of the dry tissue (Epstein, 1972).

Manganese is one of the microelement essential for plants and it activate several important enzymes, involved in chlorophyll formation, increases the availability of P and Ca. Manganese deficient plants will develop chlorosis between the veins of its leaves. The availability of manganese is partially dependent on soil pH. Recommended value of manganese in the plant tissue is about 100-500 mg kg^{-1} (Pendias and Pendias, 1994). In the present study manganese was found to be more concentrated in the leaves than in the bark of *B. cylindrica*, *B. gymnorhiza* and *K. candel* while it was found to be more concentrated in the bark than in the leaves of *R. apiculata* and *R. mucronata*. The levels of manganese in *Rhizophoraceae* mangroves selected for the present study was from 15.5±0.42 mg kg^{-1} (*B. gymnorhiza* bark) and 235.00±1.41 mg kg^{-1} (*K. candel* leaves). Thus in the current investigation, except in the two *Rhizophora* mangroves, the manganese levels were found to be more concentrated in the leaves than in the bark.

While reporting the levels of manganese in the halophytes from two estuarine ecosystems on the central west coast of India, the *R. mucronata* and *B. gymnorhiza* leaves were reported to have its level at 80 mg kg^{-1} and 120 mg kg^{-1} respectively (Kotmire and Bhosale, 1979). Manganese found in the Indian mangroves is fairly high when compared to the 5mg/100g dry weight value given by Epstein (1972). So it appears that manganese is taken up by the plants very efficiently. All the mangrove plant parts investigated contained manganese within the recommended level.

The micronutrient, zinc participates in chlorophyll formation, and also activates many enzymes. The deficiency of zinc in plants leads to chlorosis and stunted growth. The recommended value in plant tissue is about 20-100 mg kg^{-1} (Pendias and Pendias, 1994). In the present investigation on the elemental composition of *Rhizophoraceae* mangroves, zinc content was found to be highest in *K. candel*. The concentration of zinc as total of leaves and bark concentration is in the order KC>BC>BG>RA>RM. The Zn concentrations in all the plants were below recommended values. As observed for copper, the zinc concentration in the leaves and bark are very much similar, so that it can be said that it is uniformly distributed in the plant. In a study carried out by Khafaji *et al.*, 1993, the values for mineral composition of *R. mucronata* matches with the present results for *R. mucronata*.

Copper is an essential component of some plant enzymes. In the absence of copper these enzymes are inactivated. Like iron, copper is involved in redox reactions in the mitochondria and in the light reactions of photosynthesis. About 70% of copper in leaves is contained in the chloroplast of land plants (Wilkinson, 1994). The recommended value of it in plant tissue is about 5-30 mg kg^{-1} and more than 20-30 mg kg^{-1} have toxicity effects in plants (Pendias and Pendias, 1994). In the present study, the highest copper content was found in *K. candel*. The distribution of copper in the leaves and bark is found to be somewhat similar. There is no drastic difference in values of copper concentration in the leaves and the bark of the same plant. All the samples contained copper concentrations below the recommended value. Higher values for copper are reported in *B. gymnorhiza* by Thomas and Fernandez, 1997. Low concentrations of Cu and Zn have also been reported in the leaves of Kandelia candel in Taiwan (Chiu and Chou, 1991).

The toxic metals Pb and Cd were detected in the mangrove plants (Fig. 4). Lead is available for plants from soil and aerosol sources. Recommended value of it in plant is 30- 300 mg/kg (Rahmani *et al.*, 2000). Amount of Pb in all the leaf and bark samples under study in this research were found to be below the recommended levels. The value of Mn, Cu, Zn and Pb were found be higher in *B. gymnorrhizha* collected from Kumarakom, Quilon and Veli in Kerala while the concentration of Fe was lower compared to the present values obtained for this plant (Thomas and Fernandez, 1997). In their studies high lead content was detected in the mangrove twigs.

Cadmium is an especially mobile element in the soil and is taken up by the plants primarily through the roots. Decisive for transfer into plants are cadmium levels, pH values and humus levels that determine the cadmium levels in the soil solution and plants availability to cadmium (Davami and Gholami, 2012).

Plant and animals are needless from this element.

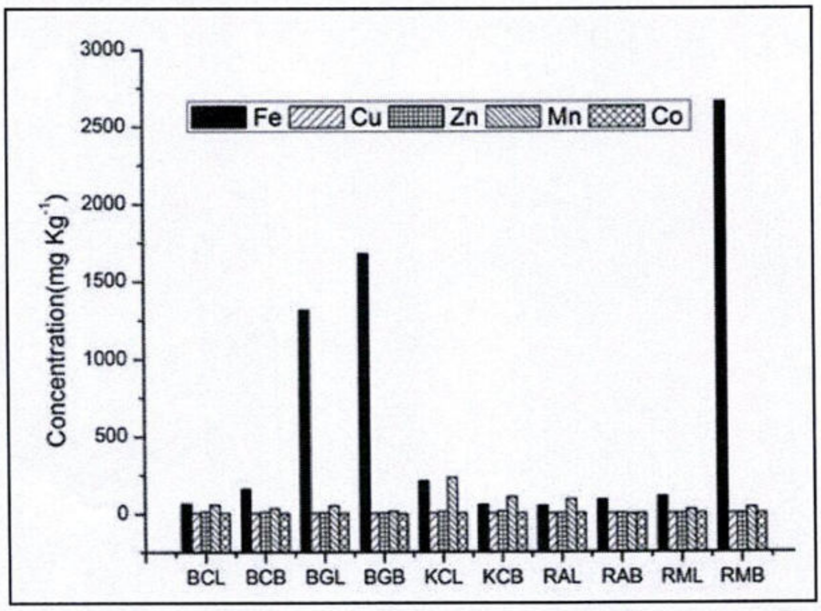

Fig. 4. Lead and Cadmium levels in the leaves and bark of *Rhizophoraceae* mangroves

In the this study, it was found that the leaves and bark of the mangrove plant *R. mucronata* is free from cadmium while it was found to be accumulated in the leaves and bark of *R. apiculata*. The leaves of *B. cylindrica* and *K. candel* were found to contain Cd while in their bark tissues it was not detected. The bark of *B. gymnorhiza* was found to accumulate cadmium whereas its leaves showed levels below the detection limits.

According to the specific environmental conditions accumulation processes specific to element or organism frequently occur in plants. An accumulation is not to be equated with an increase physiological benefit from the element for the organism; indeed this probably often purely represents an adaptation to the respective site (Markert, 1992). The reports of Utanwale *et al.*, 1980 suggests that the variation in the concentration of some heavy metals in the leaves of seven mangrove vegetation from goa, revealed that maximum concentration of iron and manganese, occurs during the monsoon season without any significant toxic effect on the foliage of mangroves while other metals like copper, nickel, cobalt and lead showed somewhat uniform concentration patterns. In their studies, the micronutrient elements, iron and manganese concentration were found to be much higher. The present study also is in line with these results. This is probably because of more availability and accumulating capacity of mangroves for these metals while the lowest levels was observed in the barks of *R. apiculata*.

Stable Isotopic composition

The values of δ^{13}C ranged from -32.52‰ (*R. apiculata* bark) to -29.32‰ (*K. candel* leaves) among the various plants of this study (Fig. 5). In the leaves, the maximum enrichment was found to be -29.32‰ (*K. candel)* and the most depleted was the leaves of *B. gymnorhiza* (-31.96%). The bark showed a lowest value of δ^{13}C in *R. apiculata* (-32.52%) and the enrichment showed a maximum of -29.75% in *K. candel*.

For δ^{15}N, the maximum was found to be 5.98 (*K. candel* leaves) and the minimum was found to be 2.79 (*R. apiculata* bark). In the leaves, the observed range was from 4.47 (*R. apiculata*) to 5.98 (*K. candel*). The bark samples showed δ^{15}N in the range from 2.79 (*R. apiculata*) to 5.47 (*B. cylindrica).*

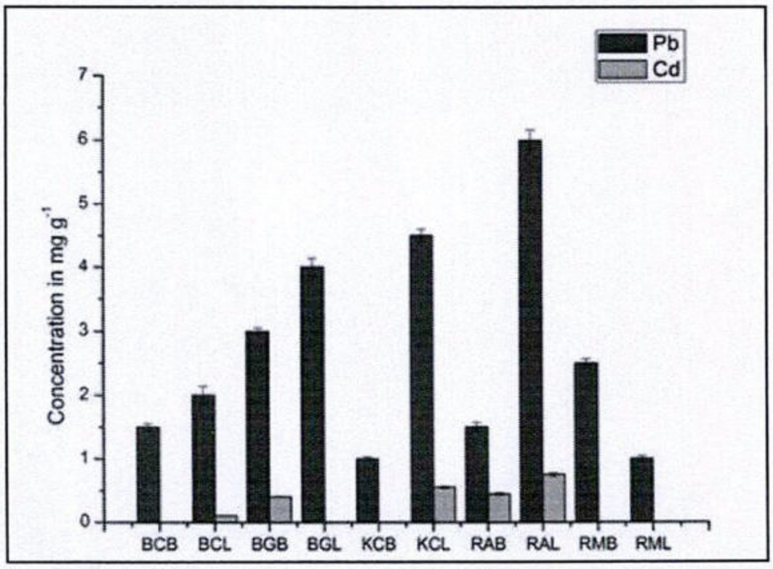

Fig. 5. The δ^{13}Cand δ^{15}N in the leaves (■) and bark (○) of *Rhizophoraceae* mangroves

The stable carbon isotope composition of leaves *Rhizophoraceae* mangrove under the present investigation shows a range from -32.52% to -29.32% which matches well with the all ready established values of δ^{13}C for C3 plants (Smith and Epstein, 1971; Rao *et al.*, 1994; Smallwood *et al.*, 2003). The plant components of *K. candel* were enriched in ^{13}C relative to other *Rhizophoraceae* mangroves by 1 to 2.5‰. The most depleted δ^{13}C was found in *B. gymnorhiza*. Lower isotopic values have been attributed to the contribution of CO_2 resulting from the decay of organic matter and respiration (Muzuka and Shunula, 2006). Stable carbon isotope composition also varies among plant tissues (O'Leary 1981). Some of this variation is due to differences among the chemical components of plant tissue. But in the present study, the leaves and bark specimens of same mangrove plant showed almost similar values for δ^{13}C (Fig. 3.3). The variability in δ^{13}C exhibited by C3 plants is primarily determined by variations in the concentration of CO_2 of the internal leaf space (Farqhuar *et al.*, 1982), primarily determined by the stomatal conductance to CO_2. A number of environmental factors can influence stomatal conductance, including salinity, humidity, soil moisture and temperature, which will subsequently influence stable isotope fractionation. The δ^{13}C values exhibited by *L. racemosa* from Florida and Belize are comparable to the average value for C3 plants (–27%) and would therefore seem to show that the *L. racemosa* studied are not experiencing physiological stress that would cause enrichment in ^{13}C (Wooller *et al.*, 2003).

Although the use of natural abundance ratios of N in plants is not as well established as that of C isotope ratios, there is a great deal to be learned from a comparison of δ^{15}N among plants within an ecosystem, between plants and their source of N, and among plant components. Some key applications using δ^{15}N in plant tissues include assessing contributions of various N sources to plant N uptake in the field, including symbiotic nitrogen fixation and atmospheric deposition, the role of mycorrhizal infection, uptake of dissolved N, and the interpretation of δ^{15}N profiles in soils. The δ^{15}N of plants reflects the net effect of many processes including the δ^{15}N of the source N, enzymatic fractionations within a plant, and plant-microbial interactions in soil (Dawson *et al.*, 2002). The majority of terrestrial plants have δ^{15}N near 0% in temperate zones, however, different species growing in the same environment have been found to vary by as much as 10% (Handley and Scrimgeour, 1997). Mangrove trees, in general, have N isotopic compositions that reflect the overall nutrient status of the ecosystem (Fry *et al.*, 2000). The present nitrogen stable isotope results are in accordance with various reported values that are greater than 4% and fall within the range of plants that obtain inorganic nitrogen directly from seawater (Chong *et al.*, 2001; Bouillon *et al.*, 2002). In this study, the $\delta^{15}N$ is found to be more enriched in the leaves than the bark samples (Fig. 3.3) by an average of 1.02%. $\delta^{15}N$ due to variation in nitrogen sources among the locations sampled is considered only due to the nutrient environment existing in the ecosystem as there is no direct external nitrogen sources found in the vicinity of the sampling locations. Organ-specific loss of nitrogen, different patterns of nitrogen assimilation, and reallocation of nitrogen can cause intra-plant variation in δ15N. Also, the loss of NH_3 could enrich leaves in $\delta^{15}N$ (Shearer and Kohl, 1986). The positive $\delta^{15}N$ indicates the fertility of the sediments.

CONCLUSION

The higher nutrient quality exhibited by the *Rhizophoraceae* mangrove plants under investigation can be used to assess and plan the ecosystem character. The values of $\delta^{13}C$ and $\delta^{14}N$ reconfirm them as C3 plants and characterise these plants as plants that obtain inorganic nitrogen directly from seawater. The present study reveals that the salt exclusion mechanism is efficiently operative in *K. candel* followed by *Rhizophora* species and least in plants of the genus *Bruguiera*. So it can be concluded that the species *K. candel* followed by *Rhizophora* mangroves are ideal species for shorelines prone to flactuations in salinity. Under the current conditions available in the sample sites the plants are free from lead but the presence of cadmium in tissues points out the need to quantify the toxic elements before using these plants for ethanopharmaceutical practices. Thus the present study Moreover, from the present results, it can be concluded that *K. candel* shows a greater tolerance for heavy metal stress than *Bruguiera plants.*

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Conflicts of Interest

The authors declare no conflicts of interest

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