

Statistical Significance of Bio Monitoring of Marine Algae for Trace Metal Levels in a Coral Environment

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Samples of macro algae collected at random distances from the coral and near shore environment of Lakshadweep archipelago were analyzed for their trace metal content (Fe, Mn, Zn, Cu, Co, Cr, Cd, Pb, and Ni). All the species showed an affinity towards essential elements (Iron, Manganese, Zinc and Copper) indicating a clear distinction between the biologically active essential elements and non essential elements (Cobalt, Chromium, Cadmium, Lead and Nickel). Among the essential elements, highest concentration was exhibited by Fe in all the species irrespective of their classification. The trace metal content reported in this study were much lower than the limit prescribed by FAO in seafood for human consumption. In all the seven algal species studied concentration of lead was below the limit of detection (< 0.1 mg/kg). Metal Selectivity Index (MSI) calculated in statistical analyses emphasized the ability of algae to accumulate a particular metal from among the metals studied. To show the similarity between species/uptake of metals group linkage clustering technique was used and the results were depicted using dendrogram. The marked similarities in the levels of trace metals in algal species have supported the view that all samples originated from a common coastal habitat. This exemplifies the applicability of bio-monitoring studies of marine algae to forensic investigations.

Key Words: Macro algae; Coral reef; Metal selectivity index; Species selectivity index; Trellis diagram.

Introduction

Lakshadweep is an archipelago and is located between $8^{\circ} - 12^{\circ} 13''$ N latitude and $71^{\circ} - 74^{\circ}$ E longitude. It is 220 - 440 kms away from the coastal city of Kochi in Kerala, India (Figure 1). Lakshadweep has an area of 32 km^2 and has 10 inhabited islands. The Lakshadweep islands are India's only coral islands. Coral reefs, which are known as "*rain forests of the oceans*" are noted for their productivity, rich biodiversity and hence they need to be focused as centres of scientific research (Reichelt and Harrison, 2000; Anu *et al.*, 2004; 2005). They are one of the most sensitive ecosystems easily susceptible to destruction by pollution or other disturbing factors. Heavy metal contamination in the marine environment is of critical concern, due to the toxicity of metals and their accumulation in marine habitats. In an area such as Lakshadweep, where people have lived depending on their primary links to the ecosystem and productivity pattern were not so energy intensive, the concept of pollution is very new and is being known as the "no industry zone". Studies on the background values of heavy metal concentrations in the marine environment of these unique coral islands are very limited and are mostly focused on geochemical aspects (Muley *et al.*, 2000). In this context, sedentary organisms such as macro algae can be effectively employed to monitor the pollution of marine environments for the reason that trace metals, generally existing in low concentration levels in the water column, have a tendency to associate preferentially with organisms and attain considerable concentration.

Phillips (1990) has associated the use of macro algae as indicator organism for depicting the metal levels in a given milieu. Several investigators subsequently have shown that the use of marine algae as an indicator of heavy metal levels gives at least a qualitative picture of heavy metal contamination in the area of study. Sea grasses are becoming widely used as in situ indicators of the relative health and condition of subtropical and tropical estuarine ecosystems (Kemp, 2000).

This article reports the statistical significance of the base-line data obtained from the analysis of some marine macro algae for their heavy metal contents. Thus it paves a way to environmental forensics as it involves the combination of both analytical and data interpretation/modelling parts connected with the attribution of pollution events, if any to the most sensitive coral ecosystems, which are easily susceptible to destruction by pollution. Compilation of these types of forensic interpretations of environmental analytical data and their meanings on multiple features of coral

ecosystem would help regulatory agencies to control the costs of environmental damage and to implement clean-up strategies in time.

Analytical method

The species of macro algae randomly collected from the coral and near shore environments of Lakshadweep islands are given in Table 1. Collected samples collected were thoroughly washed with water to remove sediment, debris and associated fauna. Final washings were done by using deionised and Milli-Q waters and then dried at 105⁰ C for 24 hrs. Trace metals were determined by adopting the procedure of Say et al. (1986). Dried material was ground to fine powder and again redried for 3 hrs. All samples were analysed in triplicate by digesting 0.5 g sample in 10 mL 2M HNO₃ and by heating to near dryness. The residue was dissolved in 10 ml 2M HNO₃, filtered and made upto 25 mL with Milli-Q. Reagent blanks were also prepared. Samples as well as blanks were then analysed for trace metals (Copper, Cobalt, Cadmium, Chromium, Lead, Nickel, Zinc, Manganese and Iron) using a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer 3110). The precision of the analysis was determined by standard spiking technique of triplicates and expressed as coefficient of variation for each element: Cu 2.7%, Co 3.2%, Cr 2.8%, Cd 2.7%, Pb 4.2%, Zn 3.2%, Ni 2.1%, Mn 2.3% and Fe 2.7%.

Statistical approach

Statistical methods applied were 2 way analysis of variance (ANOVA) for testing the significance of the 2 way differences: differences between algal species and differences between trace metal contents in different species. Having found that these differences were significant, Student's *t* test was applied to determine the pair-wise differences between species and pair-wise differences between metals with respect to their concentrations. The significant value of *t* was represented by means of Trellis diagrams in cases wherever possible.

Bray Curtis similarity (Bray and Curtis, 1957) index was computed to study the similarity between species/metals using the non-transformed data of metal concentrations and using group linkage clustering technique for drawing the dendrogram which show the similarity between species and similarity between metals. Normalised Euclidean distance was also applied to study the departure between species as well as between metals. Multi Dimensional Scaling (MDS) analysis (Clarke and Warwick, 2001) is applied to depict the distance between the species as well as between the metals.

Capacity of algae to accumulate a particular trace metal from the 9 trace metals studied was calculated as Metal Selectivity Index (MSI) for the algae and it is calculated as

$$\text{Metal Selectivity Index (MSI}_{ij}(\%)) = \frac{(X_{ij} \times 100) / \sum_{j=1}^{j=s} (X_{ij})}{\sum_{j=1}^{j=s} (X_{ij})}$$

where X_{ij} was the concentration of the j^{th} trace metal in the i^{th} species.

Similarly, affinity for a metal to get accumulated in a particular algal species, called as Species Selectivity Index (SSI ij (%)), is calculated as,

$$\text{Species Selectivity Index (SSI}_{ij}(\%)) = \frac{(X_{ij} \times 100) / \sum_{i=1}^{i=r} (X_{ij})}{\sum_{i=1}^{i=r} (X_{ij})}$$

where X_{ij} is the concentration of the j^{th} trace metal in the i^{th} species.

Results and discussion

Biochemical Attributes

The determination of trace metal contents of marine macro algae in this study reveals that algae accumulates considerable amount of essential trace elements required for human and animal nutrition (Table 2). Generalizing the behaviour of trace metal bioaccumulation by different algal species, it can be seen that there exists a clear distinction between the biologically active or the so called ‘essential elements’ and the biologically inactive elements or nonessential elements. It seemed that the tendency of seaweeds to bioconcentrate Mn and Zn was exactly the same order in all the selected species, Fe > Mn > Zn. Seaweeds from other tropical areas also exhibited a similar trend (Lobban *et al.*, 1985; Ganesan *et al.*, 1991). Comparatively higher concentration of Mn in seaweeds confirmed the earlier findings of Zingde *et al.* (1976) who reported that seaweeds are better representatives of manganese in seawater than other organisms. The high Fe concentration encountered in all the species studied here pointed out to the cumulative effects of several factors: the established need of iron for normal growth of marine plants (Goldberg, 1952), ability of most algal species to biomagnifying iron from the surrounding environment and contamination from industrial and other operations (Eisler, 1981). It is possible that a major part of the trace metal enters is in complexation with algal polysaccharides in cell walls and a small amount is (actual requirement

for biochemical budget) stored in vacuoles, granules and inter and intra cellular fluid (Lozano *et al.*, 2003).

Mn, Fe, Zn and Cu are essential micronutrients, which may limit algal growth (Lobban *et al.*, 1985). It is found that tropical seaweeds tend to accumulate more Fe than Mn, Zn and Cu (Ganesan, *et al.*, 1991). In temperate regions, uptake of Zn is less because of reduction of photosynthesis due to short day length and low light intensity (Bryan, 1969; Farias *et al.*, 2002). Cu and Zn concentrations of the seaweeds analyzed in the present study are less than maximum permissible limits prescribed for seafood for human consumption (10 mg/kg, 50 mg/kg respectively) in India (Food And Agriculture Organisation [FAO], 1983). The levels of biologically active or essential elements like Fe, Mn, Cu and Zn were higher than the level of biologically inactive or non-essential elements like Cd, Ni, Cr, Co and Pb in which the last three metals were below the detection limit (< 0.1 mg/kg). Heavy metals are incorporated into seagrass leaves and vascular tissue from either the water column or the sediment (Lyngby *et al.*, 1982). Mechanisms of accumulation and the contribution of seagrass to heavy metal cycling have been documented by many studies (Lyngby *et al.*, 1982; Ward *et al.*, 1986), but less is known about the physiological effects of heavy metal accumulation (Macinnis-Ng and Peter, 2002). Metals like Zn, Cd, Hg, Pb and Cu interfere with pigment biosynthesis of seagrasses thereby reducing the chlorophyll content (Clijsters and van Assche, 1985; Prasad and Strzalka, 1999).

Statistical attributes.

2 way ANOVA applied to test the differences in the trace metal concentrations with respect to algal species and to metals showed significant differences between species ($F_{(6,48)} = 4.52$, $P < 0.05$) and between metals ($F_{(8,48)} = 10.48$, $P < 0.05$). From the Student's *t* test applied for pair-wise differences, it was observed that at 5% level of significance for algal species, significant difference was observed between *L. papillosa* and, the species (1) *T. hemprichii* ($t=2.31$), (2) *S. tenerimum* ($t=2.27$), (3) *G. cylindrica* ($t=2.94$) and (4) *H. musciformis* ($t=2.62$). Non-significant differences were more than that of significant differences, indicating that most of the algal species accumulated almost the same level of trace metals except for the few cases mentioned above. On comparing between trace metals, the concentrations of metals accumulated were highly significant in all cases of metals ($t > 3.05$) except that between nickel and cadmium ($t < 2.18$, $P > 0.05$) as indicated in the Trellis diagram for algal species (Figure 2) and for trace metals (Figure 3). Further, the trellis diagram emphasises that

L. papillosa is an algal species having a unique distribution of the metal accumulation that is quite different from that of the other four species mentioned. This identity was further explored using group linkage clustering drawn based on Bray Curtis similarity index (Figure 4 and Figure 5) in which two distinct clusters were used. Cluster 1 contains the species *L. papillosa* and *J. adhaerens* which link at 63% similarity level and cluster 2 contain the species *H. musciformis*, *G. crassa*, *T. hemprichii* and *S. tenerimum* which cluster at very high level of similarity, i.e. 83%. The latter cluster was found to be more associated with *G. cylindrica* (at 50% similarity level) than with cluster 1 (35% similarity level). Lesser extend of overlapping between clusters 1 and 2 is an indication of their distinctiveness in trace metal accumulation capacity (Figure 4 and Figure 5). Normalised Euclidean distance used as a measure of similarity gave a pattern showing the maximum distance between the species *G. cylindrica* and the rest of the 6 species unlike in the case of dendrogram which is based on Bray-Curtis similarity index (Field et al, 1982). The disparity observed in the species similarity between the Bray Curtis similarity method and the distance formula may be due to the weightage given for joint absences in the latter method. Further the pattern of similarity can be classified as a dispersal model in the case of Normalised Euclidean distance method, while it is more of a convergence model in the case of Bray Curtis Method. The MDS analysis (Figure 6) applied to depict the distance between species showed two distinct clusters of species as indicated by dendrogram.

Similarity between distributions of metals concentrations was also studied using the three methods given earlier. MDS showed high similarity between Cr, Co, Pb and Cd and to a lesser extent, Cu. Zn and Mn form the second cluster of similar distribution and its distribution pattern is different from that of the first cluster of the six elements.

SSI calculated for metals (Table 3) showed that *J. adhaerens* has the highest capacity for accumulating copper (28.86%), while *G. cylindrica* accumulated the highest percentage of Cr (99.43%). *G. crassa* (37.34%) and *G. cylindrica* (35.48%) showed almost the same capacity for accumulation of Co. *S. tenerimum* recorded highest accumulation of Cd (48.69%) and *G. cylindrica* recorded the highest for Fe (41.48%). This analysis further showed that among these algal species *G. cylindrica* accumulated the maximum concentration of Cr, Pb, Mn and Fe while *J. adhaerens* recorded the maximum of Cu and Zn.

MSI calculated for species (Table 4) showed that all the species consumed the maximum of Fe. The order of accumulation could be given as

Fe>Zn>Mn>Cu>Cd>Pb >Co>Cr for *L. papillosa*

Fe>Mn>Cu>Zn>Cd>Pb>Cr>Co for *T. hemprichii*

Fe>Mn>Zn>Cu>Pb>Cd>Cr>Co for *J. adhaerens*

Fe>Mn>Zn>Cu>Co>Cr~Pb~Cd for *G. crassa*

Fe>Mn>Zn>Cd>Pb>Cu>Co>Cr for *S. tenerimum*

Fe>Mn>Zn>Cu>Pb>Cd>Cr>Co for *G. cylindrica* and

Fe>Mn>Zn>Cu>Cr>Co>Pb>Cd for *H. musciformis*.

Environmental attributes

Metal levels accumulated in seaweeds represent the total environmental exposure. Seaweeds incorporate metals in soluble form from aqueous and sediment sources. Some of the algae samples were collected from rocky shores where the algae were not in direct contact with bottom sediments. Under such conditions, accumulation of metals is possibly by water turbulence. Since sediment acts as a sink for metals, it contains the highest levels of contamination within an aquatic ecosystem. In this study, all the metals studied were well below the limit prescribed by FAO, it is not at all a concern for its sources. Apart from the population factor, agro based activities can also contribute significantly to the built up of metal loading in the pristine environments of islands (Anu et al, 2010). Regarding the influence of agriculture on pollution, coconut constitutes 99% of the net sown area, and it is the mainstay in agriculture and economy of Lakshadweep. In addition to this other cultivations, such as vegetables, banana, rice and others are also practiced.

Lakshadweep archipelago remains unpolluted with respect to many elements. Anu et al, (2010) pointed out to a limited extent for Zn and Pb, for example, in some sediment samples. The surroundings of the islands including the lagoon were getting loaded with oil and metallic contents from ships, barges and boats. Being a centre of tourist attraction, activities related to tourism (eg., contribution of buildings and mode of transportations (boats/vessels) among these islands) play a significant role in the loading of some of the metals, such as Pb, Zn, Fe and Mn. For example. The lower productivity in coconut pklantations resulted from loss in soil fertility and forced the natives to

switch over to the usage of large quantities of fertilizers and chemicals, which in turn increases the availability of many metals such as Fe and Zn for example. Comparatively, higher values of Fe (40-700ppm) also reported in sediments of some of the islands of Lakshadweep (Anu et al, 2010) where mangrove vegetation is predicament, possibility by the precipitation of Fe as iron sulphides, which are common in mangrove ecosystem compared with the earlier reported background levels (40-700ppm) of Fe in sediment sources by Anu et al, (2010), the algae species bioconcentrated Fe to the extent of one fold only (25-560 $\mu\text{g.g}^{-1}$). Thus, Fe and most of the other trace elements which bioaccumulated in algae species had a common ambient source of sediments. The trace metal levels in sediments are influenced by both natural and anthropogenic activities mentioned previously. This demonstrated the usefulness of biomonitoring techniques as a tool to trace out the environmental pathways of trace metals.

Conclusion.

Statistical analyses like SSI and MSI further confirms the tendency of algal species' to have more affinity towards the biologically essential elements than the non essential ones. Iron shows a distinct distribution pattern in all the algal species as is shown by its unique cluster in the Dendrogram. Irrespective of species, Cr, Co and Pb are the least selective metals for accumulation in all algal species analysed in the coral reef ecosystem of Lakshadweep Archipelago. Statistical methods like MSI and SSI selected in this study seems to be highly useful in selecting bio indicators in pollution monitoring studies in the future scenario. Extrapolating of this types of environmental analytical data and their statistical interpretation to forensic applications would help regulatory agencies to control the cause of environmental damage and to implement clean up strategies in time.

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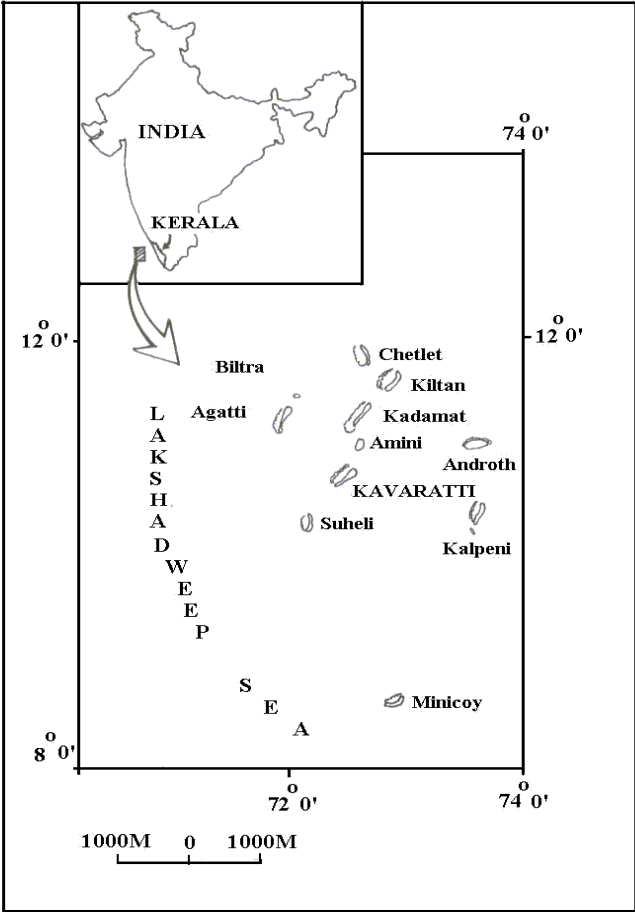


Figure 1. Location map of Lakshadweep Archipelago

		Cu	Cr	Co	Ni	Zn	Mn	Cd	Fe
Copper	Cu	-	●	●	●	●	●	●	●
Cromium	Cr	10.65	-	◐	●	●	●	●	●
Cobalt	Co	9.48	2.86	-	◐	●	●	●	●
Nickel	Ni	7.07	4.45	2.65	-	●	●	⊕	●
Zinc	Zn	6.32	9.26	8.98	8.49	-	●	●	●
Manganese	Mn	14.8	17.58	17.34	16.86	7.11	-	●	●
Cadmium	Cd	6.01	4.74	3.25	0.86	8.25	16.62	-	●
Iron	Fe	7.51	7.63	7.61	7.60	7.24	6.79	7.59	-

⊕ $t < 2.8$, ◐ $2.8 \leq t < 3.05$, ● $t \geq 3.05$

Figure 2

	Lp	Sg	Je	IGW	Sar	Cy	Mus
<i>Laurencia papillosa</i>	Lp	-	⊕	⊕	⊕	⊕	⊕
<i>Thalassia hemprichii</i>	Sg	2.31	-	⊕	⊕	⊕	⊕
<i>Jania adhaerens</i>	Je	0.61	2.14	-	⊕	⊕	⊕
<i>Gracilaria crassa</i>	IGW	2.10	0.32	1.91	-	⊕	⊕
<i>Sargassum tenerimum</i>	Sar	2.27	.004	2.10	0.32	-	⊕
<i>Gracilaria cylindrica</i>	Cy	2.94	2.12	2.88	2.26	2.11	-
<i>Hypnea musciformis</i>	Mus	2.62	.602	2.48	0.91	0.59	1.79

⊕ $t < 2.15$, ⊕ $2.15 \leq t < 2.98$, ⊕ $t \geq 2.98$

Figure 3

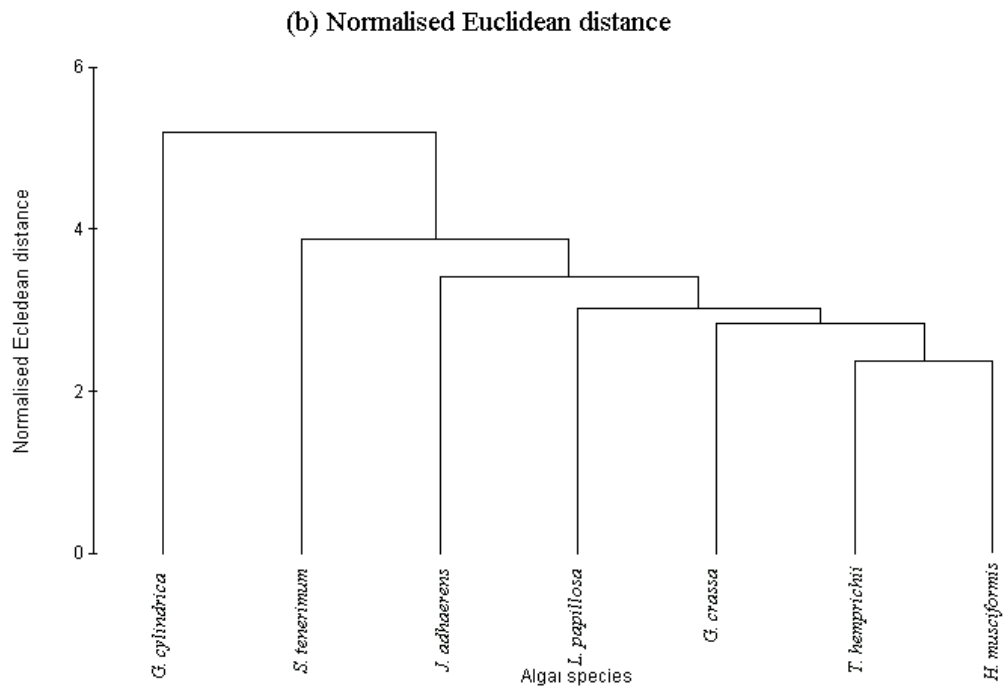
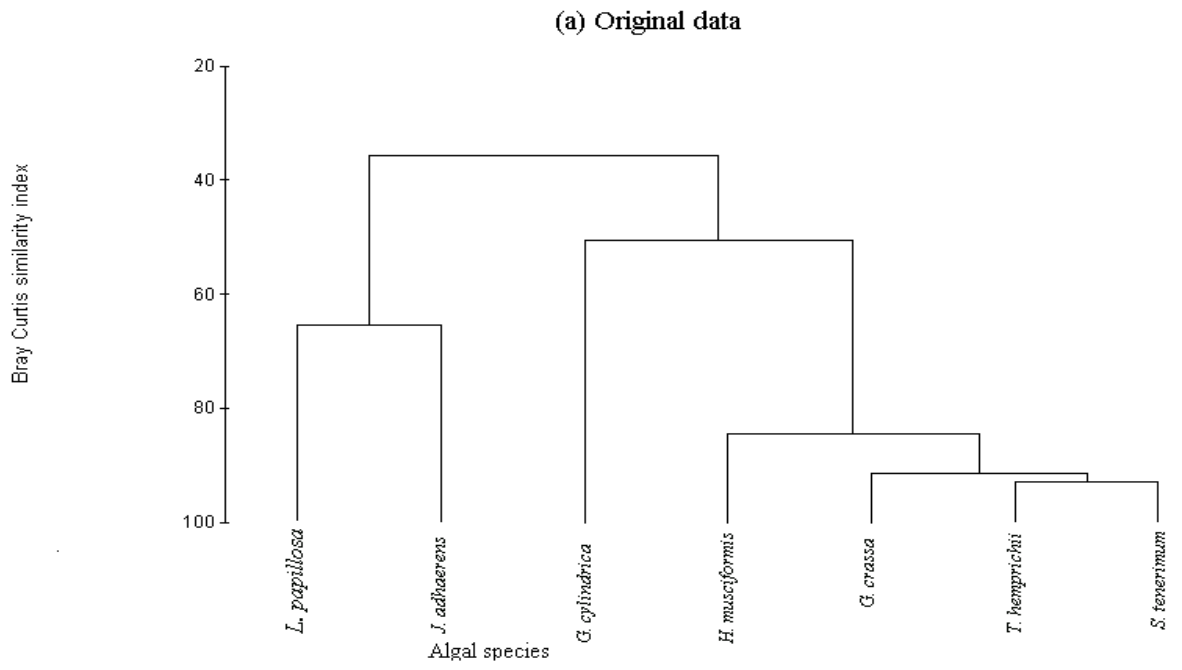


Figure 4

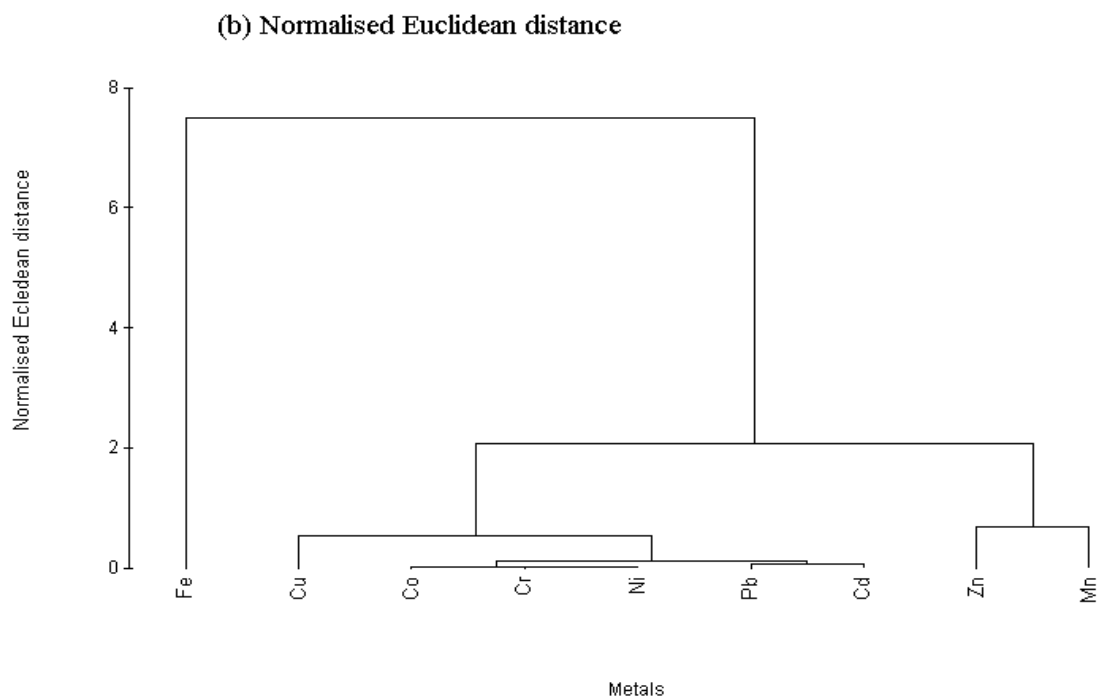
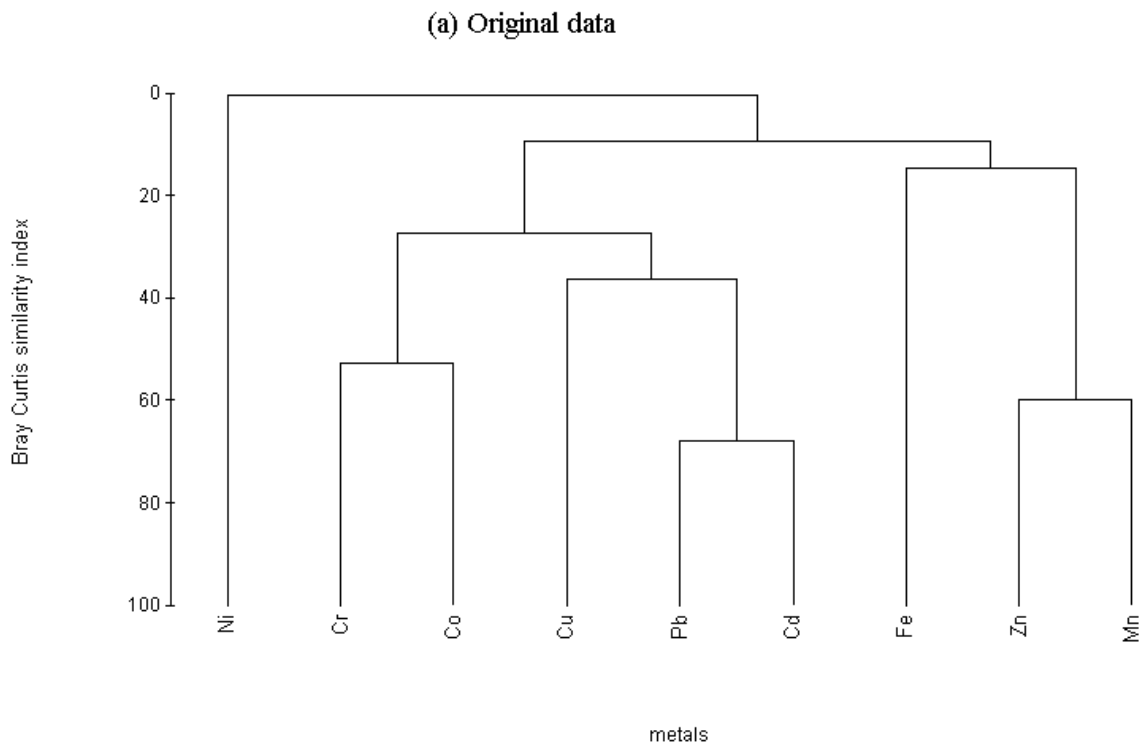
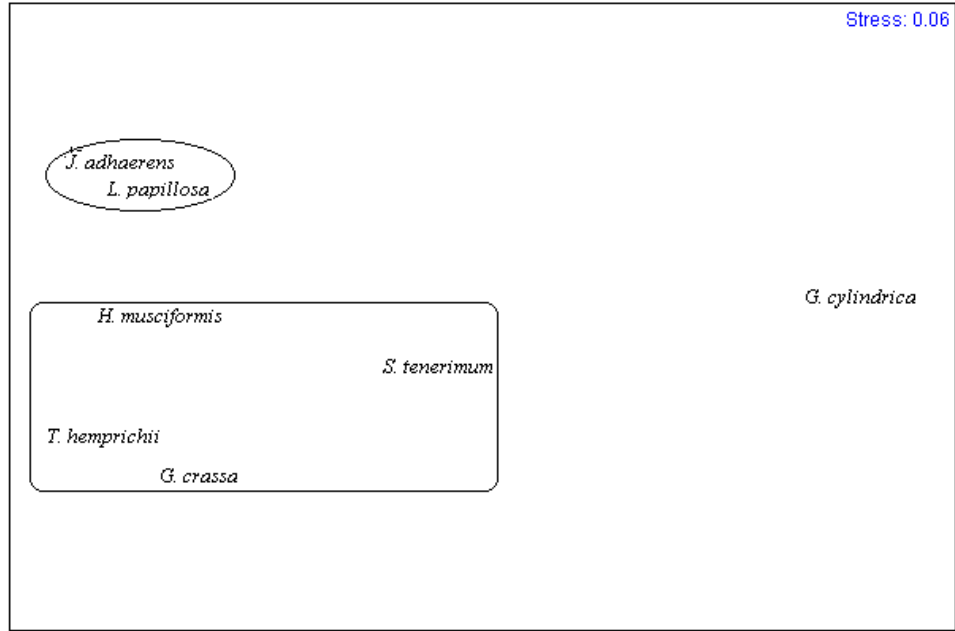


Figure 5

(a) Algal species



(b) Metals

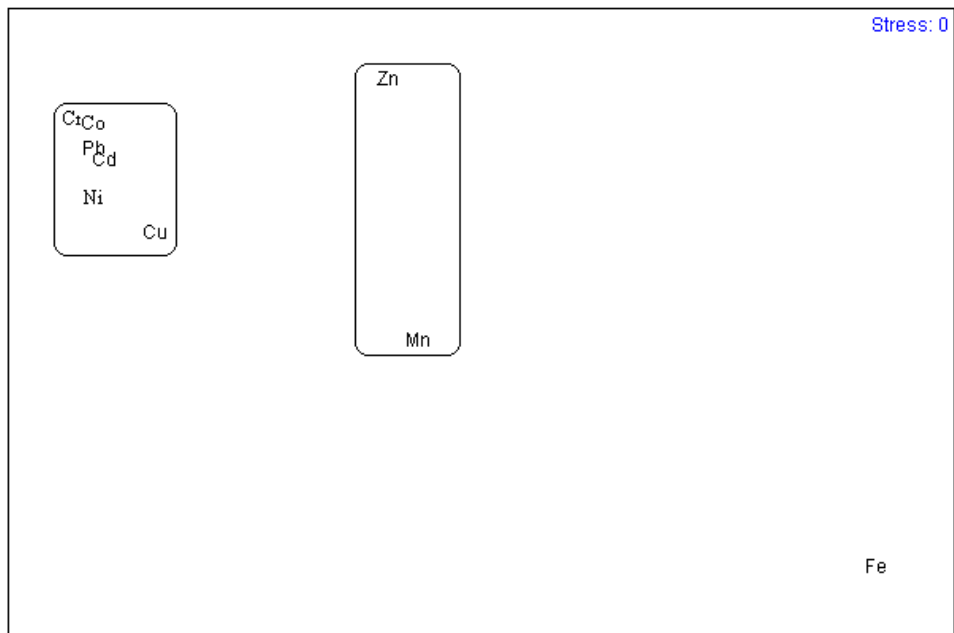


Figure 6

Figure Captions

Figure 1. Location map of Lakshadweep Archipelago.

Figure 2. Trellis diagram for student's t statistic to test the significance of the difference between trace metal concentrations in algal species.

Figure 3. Trellis diagram for student's t statistic to test the significance of the difference between algal species with respect to concentration of trace metals.

Figure 4. Dendrogram for algal species using (a) original data and (b) Normalised Euclidean distance based on non transformed data to show the similarity between species.

Figure 5. Dendrogram for metals using (a) original data and (b) Normalised Euclidean distance based on non transformed data to show the similarity between metals.

Figure 6. MDS for algal species (a) and metals (b) based on normalized Euclidean distance on non-transformed data to depict the distance between the species as well as between the metals.

Table 1. Species of macro algae collected at random from the coral and near shore environments of Lakshadweep islands.

No.	Species name	Family
	<u>Seaweeds</u>	
1	<i>Laurencia papillosa</i> (Forssk)	Rhodophyceae
2	<i>Jania adhaerens</i> (Lamour)	Rhodophyceae
3	<i>Gracilaria cylindrica</i>	Rhodophyceae
4	<i>Gracilaria crassa</i> (Harv)	Rhodophyceae
5	<i>Hypnea musciformis</i>	Rhodophyceae
6	<i>Sargassum tenerimum</i>	Phaeophyceae
	<u>Seagrass</u>	
	<i>Thalassia hemprichii</i> (Ehrenberg)	Hydrocharitaceae

Table 2. Average concentrations of heavy metals in seaweeds / seagrass collected from Lakshadweep islands ($\mu\text{g g}^{-1}$ dry wt)

Sp. collected	Cu	Cr	Co	Ni	Pb	Zn	Cd	Mn	Fe
<i>L. papillosa</i>	3.2	NDL	NDL	0.7	NDL	11.4	1.5	7.2	44.4
<i>J. adhaerens</i>	6.1	NDL	NDL	0.8	NDL	22.2	0.8	24.6	25.2
<i>G. cylindrica</i>	3.5	1.0	1.0	2.9	NDL	10.8	1.4	32.4	562.4
<i>G. crassa</i>	1.2	NDL	1.1	NDL	NDL	4.7	NDL	17.3	155.4
<i>H. musciformis</i>	1.9	NDL	NDL	NDL	NDL	14.2	NDL	24.6	218.1
<i>S. tenerimum</i>	0.9	NDL	0.8	1.7	NDL	4.5	3.7	15.5	177.6
<i>T. hemprichii</i>	4.4	NDL	NDL	0.1	NDL	0.6	0.1	26.2	173.0

Table 3. Species selectivity index (SSI (%)) for each metal (%)

Species	Co	Cr	Co	Pb	Zn	Mn	Cd	Fe	Ni
L. papillosa	14.9939	0.0949	0.0339	11.0221	16.5339	4.8881	20.3567	3.2713	14.2858
<i>T. hemprichii</i>	20.5405	0.0949	0.0339	1.5372	0.8200	17.7419	1.8626	12.7582	14.2858
<i>J. adhaerens</i>	28.8605	0.0949	0.0339	12.870	32.1926	16.6331	10.3963	1.8596	14.2858
<i>G. crassa</i>	5.4335	0.0949	37.3392	0.0164	6.8301	11.7071	0.0132	11.4582	14.2858
<i>S. tenerimum</i>	4.5043	0.0949	27.0481	27.5879	6.5093	10.4697	48.6922	13.0972	14.2858
<i>G. cylindrica</i>	16.4466	99.4307	35.4773	46.9501	15.6123	21.9251	18.6658	41.4730	14.2858
<u>H.</u>									14.2858
<u>musciiformis</u>	9.2208	0.0949	0.0339	0.0164	21.5019	16.6351	0.0132	16.0826	

Table 4. Metal selectivity index (MSI (%)) for each species (%)

Species	Co	Cr	Co	Pb	Zn	Mn	Cd	Fe	Ni
L. papillosa	4.6492	0.0015	0.0015	0.9857	16.6606	10.5664	2.2537	64.8815	0.0006
<i>T. hemprichii</i>	2.1306	0.0005	0.0005	0.0460	0.2764	12.8295	0.0690	84.6476	0.0006
<i>J. adhaerens</i>	7.6795	0.0013	0.0013	0.9877	27.8376	30.8547	0.9877	31.6504	0.0006
<i>G. crassa</i>	0.6412	0.0006	0.6139	0.0006	2.6194	9.6315	0.0006	86.4923	0.0006
<i>S. tenerimum</i>	0.4665	0.0005	0.3903	0.8241	2.1909	7.5595	1.8006	86.7675	0.0006
<i>G. cylindrica</i>	0.5666	0.1703	0.1703	0.4665	1.7478	5.2654	0.2296	91.3837	0.0006
<u>H.</u>									0.0006
<u>musciiformis</u>	0.7535	0.0004	0.0004	0.0004	5.7097	9.4763	0.0004	84.0590	