

## Potential Toxic Elements Determined by ICP-MS in Tissues of *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from Tuticorin Coast

Koigoora Srikanth<sup>1,2</sup> and Janapala Venkateswara Rao<sup>2</sup>

- 1 CESAM-Centre for Environmental and Marine Studies & Department of Chemistry, University of Aveiro, Portugal
- 2 Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad, India

### Abstract

Three marine sponges *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* were collected from five different locations of Tuticorin coast, India. Concentrations of Potential toxic elements (PTEs) (As, Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn) were measured in the sponges using inductively coupled plasma mass spectrophotometer (ICP-MS). Except Cd, Cr and Ni rest of all PTEs among the studied stations were significantly different and the concentration of these PTEs followed a gradient along the collected stations. Among the three sponges *C. fibrosa* with high tissue content has accumulated significantly higher concentration of PTEs in comparison to *F. cavernosa* and *D. fragilis*. Contrasting interspecies difference in the accumulation of PTEs in three sponges is due to the difference in their tissue content. The high tissue content sponge *C. fibrosa* is seen to accumulate significantly higher concentration of all the PTEs in the coastal regions of Tuticorin and makes this sponge a promising bio monitor for the evaluation of PTEs in the contaminated sites.

**Keywords:** *Callyspongia fibrosa*; *Faciospongia cavernosa*; *Dysidia fragilis*; Potential toxic elements; Tuticorin

**Corresponding author:** Koigoora Srikanth

✉ koigooras@ua.pt

CESAM-Centre for Environmental and Marine Studies and Department of Chemistry, University of Aveiro, Portugal.

**Tel:** +351 919914260

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### Introduction

Gulf of Mannar (GoM) was declared as the first marine biosphere reserve of India and of the South East Asia in the year 1989 [1, 2]. The GoM has a coast line of approximately 10, 800 Sq. Km and consists of 21 uninhabited islands beginning from the holy town of Rameswaram to the industrial town of Tuticorin. It consists of diverse ecosystem constituting a number of flora and fauna especially dominated by corals and marine sponges [1-3]. Tuticorin is industrial city which is heavy threatened by pollutants from industries which usually include chemical processing industries, alkali plants, petrochemical industries, thermal power plant and sewage discharge from the local municipalities [2, 4]. The mangrove vegetation which is most prominent in Tuticorin is facing the threat of extinction due to the impact of the effluents released into the coast. Among all these industries, contribution from thermal power plant is seen causing significant enrichment of PTEs in the marine sediments of Tuticorin [2, 5]. The fly ash and slurry discharged from these units also resulted in the

significant enrichment of PTEs in the coast and resulted in the irreversible damage to the ecosystem [2]. The increased release of the contaminants from the surrounding industry into the coast may play a substantial role on PTEs pollution.

The pollution caused by PTEs has been studied in different coastal ecosystems of the world [6]. This is one of the major environmental problems being faced by a number of coastal ecosystems during the recent past due to rapid industrialization and anthropogenic activity posing a serious risk to the inhabiting aquatic biota. PTEs emitted from agriculture, industries and domestic sources have an impending effecting on the existing biota [2]. The concentration of the PTEs in seawater and sediments increases due to excessive release of effluents both from domestic and industrial sources [2]. Among the environmental contaminants PTEs are seen posing major ecological consequences [5]. A number of biota had been used to monitor the concentration of PTEs in different coastal regions of the world but still the most preferred are the marine invertebrates [7]. The use of

mussels in “Mussel Watch programmer” in North America to monitor the concentration of PTEs in coastal environments had given good results but with some limitations [8]. However, other researchers have emphasised the need to use other filter feeding organisms such as barnacles, polychaetes, tunicates and the sponges which are sedentary, filter feeding and sustain the fluctuation in environment have also been used as bio monitors for PTEs contamination [1, 2, 7-14]. Sponges are considered as good bio monitors by a number of researchers because of their unique features and their adaptations to a number of ecological niches [15]. The long life span, adherent natures, survival under extreme physical and chemical fluctuations, accumulation of contaminants both from dissolved and suspended phases all these features makes sponges an ideal bio monitor [1, 2, 7, 8, 14, 16]. PTEs concentration in the fish clams and gastropods may show significant difference [17]. In contrast, the sessile and benthic organism such as sponges significantly reduces this variation in the concentration of PTEs. This affirmation is based on the assumption that variation in PTEs concentration within the different sponge species of the same area are lacking? Here we measured the concentration of As, Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn in *C. fibrosa*, *F. cavernosa* and *D. fragilis* collected from Tuticorin coast. The concentration of nine PTEs in three species was compared at five stations (S1-S5) along the coast of Tuticorin.

## Materials and Methods

### Sample collection and identification

Marine sponges *Callyspongia fibrosa* (Ridley & Dendy, 1886, Family: Callyspongiidae, Order: Haplosclerida), *Faciospongia cavernosa* (Schmidt, 1862; Family: Thorectidae) and *Dysidia fragilis* (Family: Dysideidae, Order: Dictyoceratida) were collected along the coastal regions of Tuticorin, India. Sponge specimens were collected by snorkelling and skin diving from five different stations between 8° 30' and 8° 46' N latitude 78° 08' and 78° 15' E longitude in the state of Tamil Nadu, India and their morphological description is presented in **Table 1 and Figure 1**. The specimens were cut using a ceramic knife to avoid PTEs contamination, these samples were stored in zip lock bags and later washed thoroughly with natural seawater and the foreign matter such as mud particles, macro detritus and other organisms which are found in the sponges were removed by monitoring under the stereomicroscope with the help of plastic tweezers. The voucher specimens were submitted to National Institute of Oceanography (NIO) Goa, for depository purpose and were identified by Dr. Thomas as *C. fibrosa*, *F. cavernosa* and *D. fragilis*, at Central Marine Fisheries Research Institute (ICAR), Vizhinjam, Thiruvananthapuram, India.

### Sample preparation

The oven dried specimen samples of *D. fragilis*, *C. fibrosa* and *F. cavernosa* were used for the analysis of PTEs by the method of Mc Carthy and Ellis [18]. The samples were placed in Teflon beakers and digested with 10 ml of 70% nitric acid (HNO<sub>3</sub>) in a microwave vessel in an IFB (Indian Fine Blanks Ltd.) microwave oven, India. The programme was repeated once again to ensure

the total digestion of all samples. After completing the heating programme, the beakers were cooled to room temperature and carefully vented in fume hood. The contents of each beaker were quantitatively transferred to another Teflon beaker and evaporated to incipient dryness. The residues were re-dissolved in 5 ml of 1:1 HNO<sub>3</sub>: milli-Q water and brought to a final volume of 50 ml. Clear solutions were obtained in all cases, simultaneously blanks and standards were prepared. Potential toxic elements concentrations in the sponge samples were analysed by ICP-MS (Inductively Coupled Plasma Mass Spectrophotometer, Perkin-Elmer SCIEX, Model 6100 ELAN DRC II ICP-MS (Toronto, Ontario, Canada). For better operating conditions ICP-MS was used throughout. The sample introduction system consisted of a standard Meinhard nebulizer with a cyclonic spray chamber. All quantitative measurements were performed using instrument software (ELAN version 4.0). Several well-known isobaric interferences were programmed, and the corrections were automatically applied. The elements analysed were As, Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn. Measurements were calibrated using the provided standards solutions of all the tested PTEs, blanks and certified reference materials (NIST 1566 and 1575). The calibration of ICP-MS was continuously verified by standards measurement after every 10 samples. Even the acids used for the digestion of sponge tissues were also used as a control in measurements.

### Statistical analysis

All values are presented as mean ± SE. The statistical difference between PTEs concentration in the three sponge samples collected from different stations of Tuticorin coasts was determined using one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test using Origin 8.5 version software. Results were considered with significance levels of 5% on each PTE to test for the significant differences between the sponges among the PTEs.

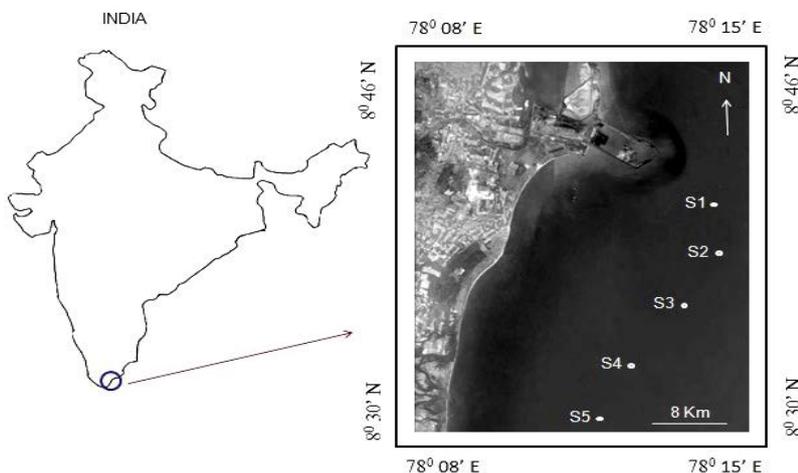
## Results

The concentration of PTEs in three species of sponges (*C. fibrosa*, *F. cavernosa* and *D. fragilis*) sampled from Tuticorin coast showed significant variation within the species and sampling stations (**Figures 2-6**). Spatial variation in the concentration of PTEs in all the three species of sponges was observed. Among all the five sampling stations the concentration of the PTEs was found to be highest in Station S1 followed by S2, S3, S4 and S5. The decreasing order of accumulation of PTEs in all the three species of sponges is of the following order Zn<Fe<Ni<Cr<Cu<Pb<Co<As<Cd. The accumulation of Zn in *C. fibrosa* in all the five stations was higher when compared to other PTEs. Accumulation of Zn was highest in *C. fibrosa* (274.24 µg g<sup>-1</sup>) and least in *D. fragilis* (13.25 µg g<sup>-1</sup>). Zn concentration in sponges decreased in the following order *C. fibrosa*>*F. cavernosa*>*D. fragilis*.

In station S1 (with exception of Cd and Ni) the PTEs were significantly different when compared with each other P ≤ 0.05. Moreover, in station S2 Cd and Cr among the sponges *F. cavernosa* and *D. fragilis* exhibited no significant variation in the

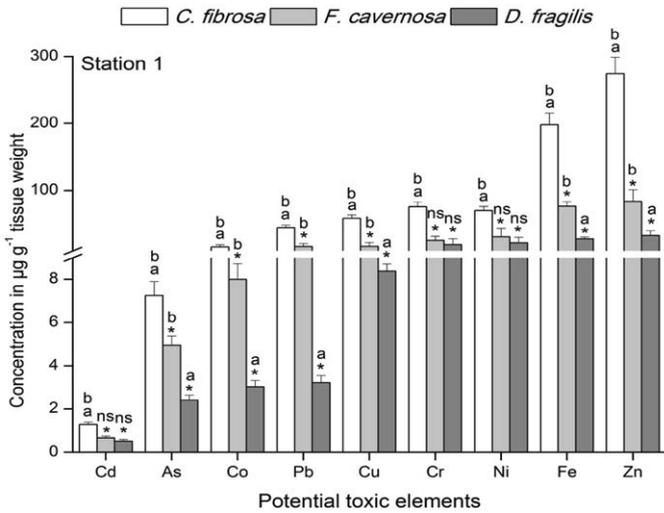
**Table 1** Morphology and key characteristics of the three marine sponge *Callyspongia fibrosa*, *Fasciospongia cavernosa* and *Dysidia fragilis* collected from Tuticorin coast of India.

<i>Callyspongia fibrosa</i>	Morphology description
	<p>Sponge is usually pale brown to yellowish in colour. <i>C. fibrosa</i> is composed of a number of branches on its surface with strong conules. The shape is irregular and has an uneven surface. At the growing tip of the sponge the conules are very prominent. Oscules irregularly distributed terminal, marginal, rounded or elliptical shallow and compound. <i>C. fibrosa</i> is hard and brittle</p>
<i>Fasciospongia cavernosa</i>	
	<p><i>F. cavernosa</i> is massive, rounded and tubular. The upper surface of sponge is usually dark brown in colour and the substratum or choanosome of the sponge is light yellowish in colour. Fleshy appearance of the body and the entire surface of the body is covered with conules giving a spiny appearance. This species always misunderstood to be as Iricinia species.</p>
<i>Dysidia fragilis</i>	
	<p><i>D. fragilis</i> usually appears in pale pinkish, greenish, reddish brown, brownish and greyish white. It is massive, lobe-shaped and encrusting. In most of our collections trips we could find only very small amount of this sponges. The entire surface of <i>D. fragilis</i> is covered with small openings. The dried specimen of <i>D. fragilis</i> is very brittle.</p>

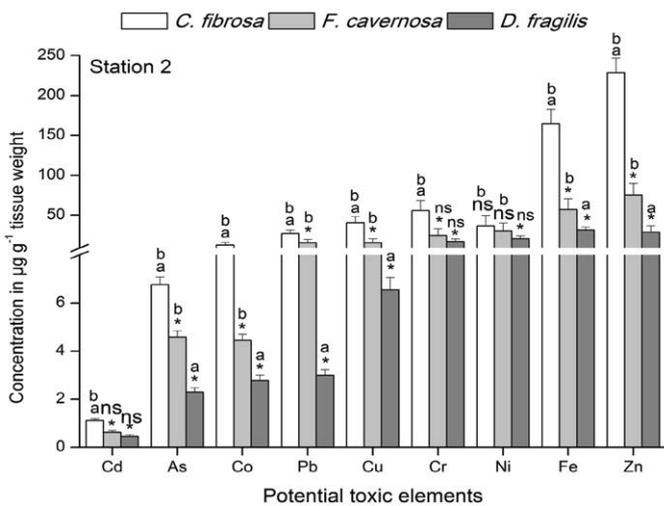


**Figure 1** Map showing the sampling locations of the three sponges along the five different stations of Tuticorin coast of India.

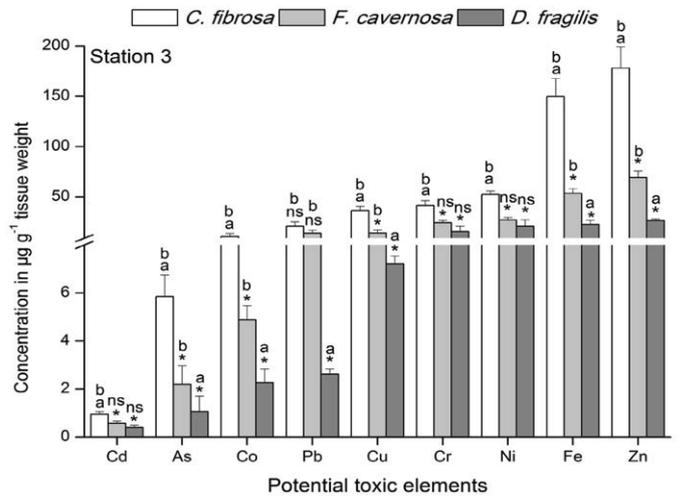
concentration of the PTEs. Similarly, the concentration of Ni in *C. fibrosa* when compared with *F. cavernosa* and *D. fragilis* exhibited



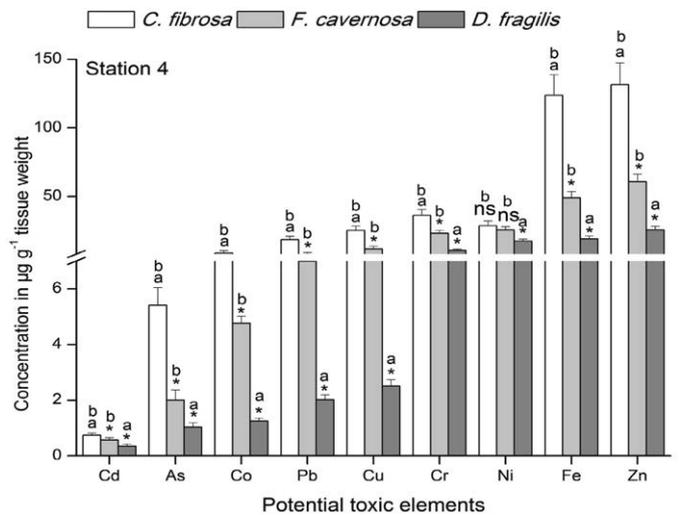
**Figure 2** Concentration of potential toxic elements concentration ( $\mu\text{g g}^{-1}$ ) in the three sponges *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from station S1 of Tuticorin coast, India. Data are expressed as mean  $\pm$  S.E of three independent experiments of five replicates each (n=15). \*Statistical significance of *C. fibrosa* with *F. cavernosa* and *D. fragilis*; a-Statistical significance of *F. cavernosa* with *D. fragilis* and *C. fibrosa* and b-Statistical significance of *D. fragilis* with *C. fibrosa* and *F. cavernosa*; ns-not significant).



**Figure 3** Concentration of potential toxic elements concentration ( $\mu\text{g g}^{-1}$ ) in the three sponges *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from station S2 of Tuticorin coast, India. Data are expressed as mean  $\pm$  S.E of three independent experiments of five replicates each (n=15). \*Statistical significance of *C. fibrosa* with *F. cavernosa* and *D. fragilis*, a-Statistical significance of *F. cavernosa* with *D. fragilis* and *C. fibrosa* and b-Statistical significance of *D. fragilis* with *C. fibrosa* and *F. cavernosa*, ns-not significant).

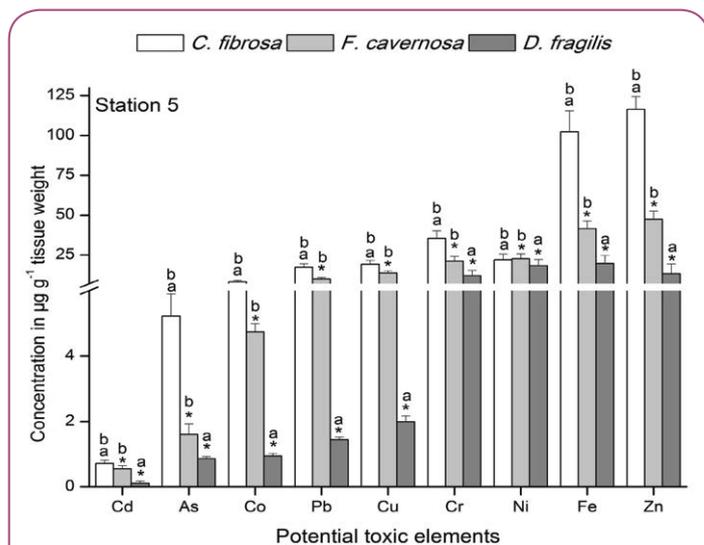


**Figure 4** Concentration of potential toxic elements concentration ( $\mu\text{g g}^{-1}$ ) in the three sponges *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from station S3 of Tuticorin coast, India. Data are expressed as mean  $\pm$  S.E of three independent experiments of five replicates each (n=15). \*Statistical significance of *C. fibrosa* with *F. cavernosa* and *D. fragilis*, a-Statistical significance of *F. cavernosa* with *D. fragilis* and *C. fibrosa* and b-Statistical significance of *D. fragilis* with *C. fibrosa* and *F. cavernosa*, ns-not significant).



**Figure 5** Concentration of potential toxic elements concentration ( $\mu\text{g g}^{-1}$ ) in the three sponges *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from station S4 of Tuticorin coast, India. Data are expressed as mean  $\pm$  S.E of three independent experiments of five replicates each (n=15). \*Statistical significance of *C. fibrosa* with *F. cavernosa* and *D. fragilis*, a-Statistical significance of *F. cavernosa* with *D. fragilis* and *C. fibrosa* and b-Statistical significance of *D. fragilis* with *C. fibrosa* and *F. cavernosa*, ns-not significant).

no significant variation in the concentration of Ni. Concentration of Ni in *F. cavernosa*, when compared in *D. fragilis* and *C. fibrosa*, showed no significant variation in their concentration.



**Figure 6** Concentration of potential toxic elements concentration ( $\mu\text{g g}^{-1}$ ) in the three sponges *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from station S5 of Tuticorin coast, India. Data are expressed as mean  $\pm$  S.E of three independent experiments of five replicates each (n=15). \*Statistical significance of *C. fibrosa* with *F. cavernosa* and *D. fragilis*, (a-Statistical significance of *F. cavernosa* with *D. fragilis* and *C. fibrosa* and b-Statistical significance of *D. fragilis* with *C. fibrosa* and *F. cavernosa*, ns-not significant).

Concentration of Cd, Cr and Ni in station S3 showed no significant variation in the sponges *F. cavernosa* and *D. fragilis*. However, the concentration of Pb among the sponges' *C. fibrosa* and *F. cavernosa* showed no significant variation. In station S4, except the concentration of Ni, the rest of all the PTEs were found to be significant  $P \leq 0.05$ . Moreover, in station S5, all the PTEs were significantly different when compared with each other.

## Correlation coefficient of PTEs in the three species of sponges along the five stations

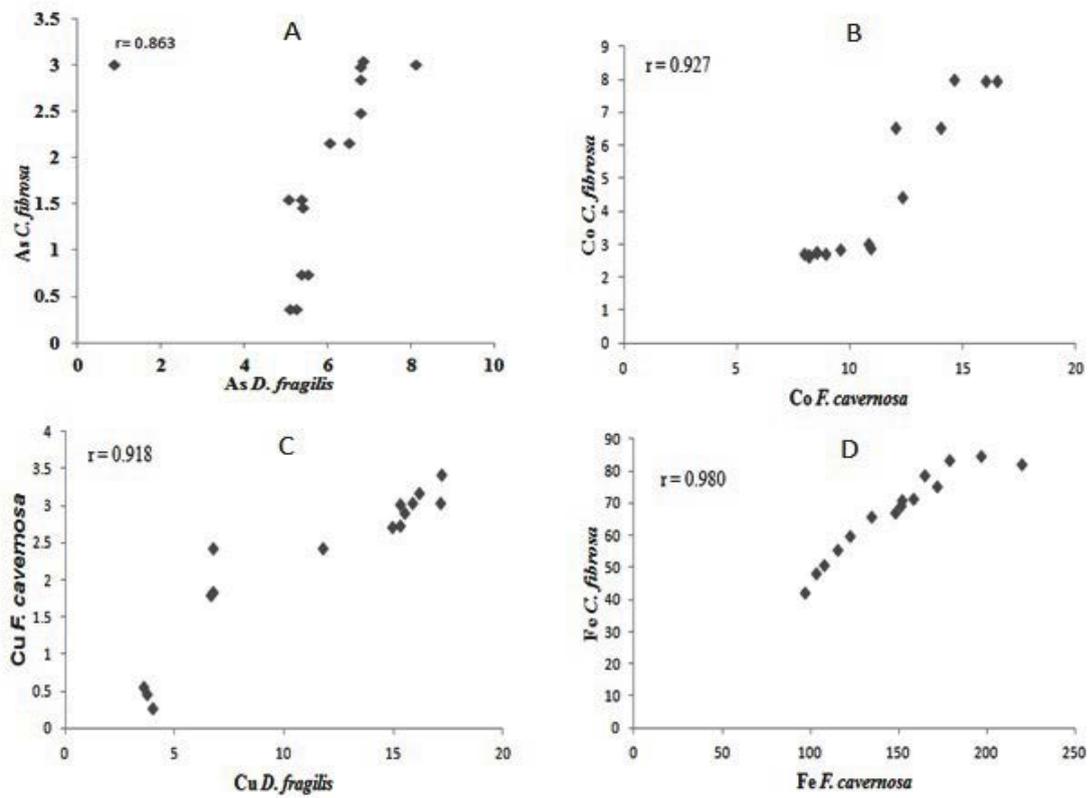
To find any significant correlation among the accumulated PTEs in three species of sponges collected at five stations the correlation analyses were performed. In our study as accumulation in *F. cavernosa* and *D. fragilis* showed a strong positive correlation. Similarly, Co in *C. fibrosa* and *F. cavernosa* showed a strong positive correlation, Cu in *F. cavernosa* and *D. fragilis* showed a strong positive correlation, Fe in *C. fibrosa* and *F. cavernosa* also showed a strong positive correlation, Ni in *F. cavernosa* and *D. fragilis*, Pb in *C. fibrosa* and *D. fragilis* and Zn in *C. fibrosa* and *F. cavernosa*, respectively (Figures 7 and 8).

## Discussion

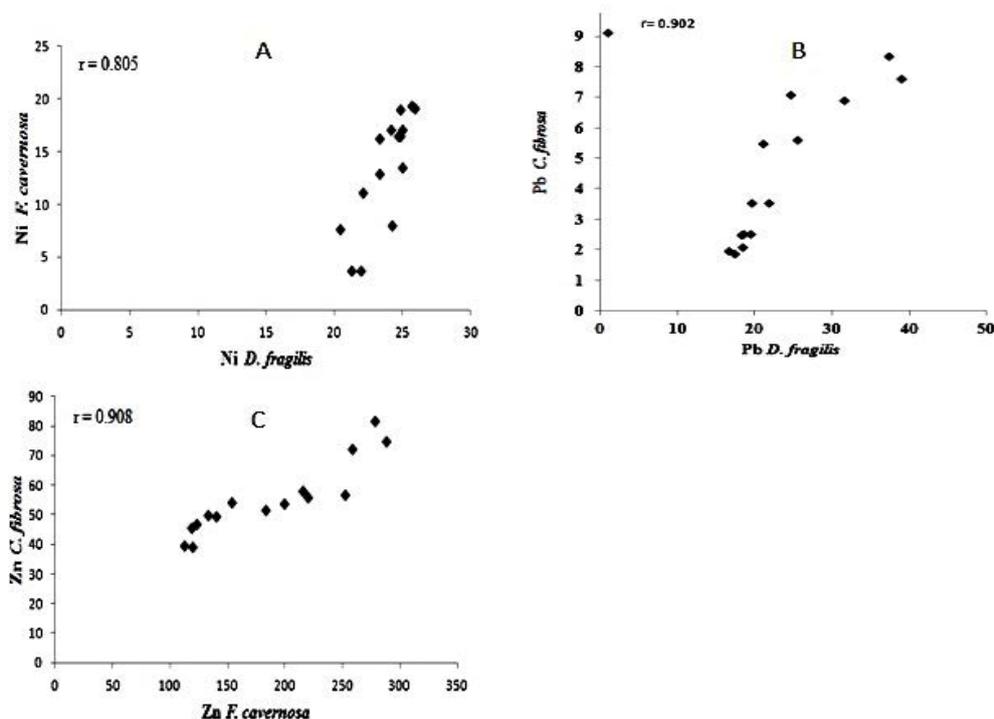
During recent past most of the researches have paid attention in the monitoring of PTEs contamination using marine sponges [1, 2, 8, 13, 14, 19]. However, among those species which are the best biomonitors of interest are still lacking. The most important

criteria for bio monitors are long life span, cosmopolitan distribution, sedentary nature, easy identification and dense population [1, 2, 19]. Current studies revealed that some of the sponge species have already been used for bio monitoring of PTEs and the results are encouraging [1, 2, 8, 12-14, 16, 20]. Sponges are highly tolerant to physiochemical fluctuations which make them ideal to be present in both pristine and polluted environments [1, 2, 8]. Although tissue level of organisation is absent in sponges with the help of massive choanocytes chambers they filter large quantities of water and are seen accumulating the contaminants in both dissolved and particulate forms. Hence, sponges from the field are known as potential bio monitors of hydrocarbons, organ chlorinated compounds, metals [1, 2, 12-14, 20-24]. In spite of a number of studies conducted on the bio monitoring of PTEs using sponges little attention has been paid towards the "Sponge Watch programme". Although, a number of sponges are used for bio monitoring of PTEs the ideal species for this purpose has yet to be identified. It should be noted that the concentration of PTEs found in the sponge species are much higher when compared to other marine organisms.

The current study is an attempt to present the difference in the accumulation of PTEs in the three species of sponges of Tuticorin coast. Our study supports the fact that the concentration of PTEs varies within species, between the species and among the stations [1, 12-14, 25, 26]. The inter species variation of the PTEs is believed to be related to its growth conditions, influence of local, seasonal environmental conditions and composition of their skeleton, life cycle, symbiotic organisms and their protein/tissues content [7, 27]. Out of the nine PTEs analyzed the concentration of all the PTEs were directly correlated to the tissue content in three species of sponges and also to the pollution gradient among the five stations of Tuticorin coast. Based on their tissue content the three species of sponges were used as potential biomonitoring organisms so as to evaluate the PTEs pollution in the five stations of Tuticorin coast. Our findings, however for the first time indicate that sponges with high tissue content can serve as a model species for bio monitoring of PTEs and serve as ideal biomonitors for the evaluation of PTEs. The concentration of PTEs in the five stations (S1-S5) followed the same trend with more accumulation of PTEs in *C. fibrosa* when compared to *F. cavernosa* and *D. fragilis*. As mentioned in one of our earlier manuscript from our group which clearly states that the tissue composition of the three species varies significantly and based on their tissue content the variation in the accumulation of PTEs elements occurred [28]. The massive, irregular, ramose and high tissue content sponge *C. fibrosa* displayed highest concentration of PTEs when compared to other two species. The high tissue content of *C. fibrosa* and high choanocytes chamber volumes which does facilitate much higher area for binding of the PTEs. The role of symbiotic organisms is also very important issue in relation to the variation of PTEs among the different species [29]. The high concentration of PTEs in *C. fibrosa* when compared to *F. cavernosa* and *D. fragilis* from the same site revealed contrasting mechanism of PTEs accumulation. High concentration of PTEs in the station S1 corresponds to the presence of coal fired thermal power plant and also the anthropogenic activities



**Figure 7** Correlation coefficient of potential toxic elements among the sponges *Callyspongia fibrosa* and *Dysidia fragilis* for Arsenic (A), in *Callyspongia fibrosa* and *Faciospongia cavernosa* for Cobalt (B), in *Faciospongia cavernosa* and *Dysidia fragilis* for Copper (C) and in *Callyspongia fibrosa* and *Faciospongia cavernosa* for Iron (D).



**Figure 8** Correlation coefficient of potential toxic elements among the sponges *Faciospongia cavernosa* and *Dysidia fragilis* for Nickel (A), in *Callyspongia fibrosa* and *Dysidia fragilis* for Lead (B), in *Callyspongia fibrosa* and *Faciospongia cavernosa* for Zinc (C).

occurring near the shore are also responsible for the increase in the concentration of PTEs around the S1. The pattern of accumulation of PTEs in three sponges is entirely different to that of the concentration of the PTEs observed in the seawater and sediment samples evaluated from the same coast [2]. As the distance from the origin of the thermal power plant increase the concentration of PTEs among the species also varied. The difference in the accumulation pattern might be due to several reasons one of which might be due to the high filtration rate of sea water by the corresponding sponges [20, 23, 29]. PTEs both in dissolved and suspended form are seen accumulated in the tissues of sponges as nearly 80% of the suspended materials are retained by the sponges [30]. Studies conducted by Pan et al. observed that different sponge species collected from the same site revealed significant difference in the accumulation of PTEs [20]. Significant variation in the concentration of PTEs observed in this study is not uncommon. Studies conducted by Patel et al. on *Spirastrella scupidifera* and *Prostylyssa foetida* collected from the same area the concentration of PTEs in *Spirastrella scupidifera* were significantly higher when compared to *Prostylyssa foetida* [12]. The observed difference in the accumulation of PTEs in the sponges is due to their difference in pumping physiology in the two species and also the volume of choanocytes chambers [31]. The criteria used for identifying a good bio monitor species include high tissue content [2, 7]. Our results clearly reveal that sponges containing high tissue content can serve as ideal biomonitor for monitoring the contamination of PTEs released from different sources. In the current study we conclude that sponge species with difference in their tissue content have complementary

profile of PTEs accumulation. For the first time in the Tuticorin coast the concentration of PTEs have been evaluated using three different marine sponges. Future studies on this aspect should focus on the variation of PTEs under different environmental conditions to see whether these factors could influence in the levels of PTEs in relation to their tissue content.

## Conclusion

This study found that the three marine sponges collected along the Tuticorin coast have significantly accumulated PTEs in the coastal regions of Tuticorin, India. The concentrations of As, Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn were seen differentially accumulated in *C. fibrosa*, *F. cavernosa* and *D. fragilis*. The initial findings support that *C. fibrosa* containing high tissue content is seen accumulating higher concentration of PTEs when compared to *F. cavernosa* and *D. fragilis*. The difference in the accumulation of PTEs in the three sponge samples collected at the five stations of Tuticorin is due to the difference in their tissue content. Thus sponge with high tissue content would be an ideal bio monitors for evaluating the contamination at particular location as revealed by *C. fibrosa*.

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