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Ulva lactuca and U. intestinalis as biomonitor of heavy metals contamination (Cu, Zn and Pb) in western Bay of Bengal

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ABSTRACT

Variation in the concentrations of heavy metals Cu, Zn and Pb were measured seasonally for two green seaweeds namely *Ulva lactuca* and *U. intestinalis* at two anthropogenically different sites (station 1: Vuda Park-selected to cover the expected polluted areas and station 2: Tenneti Park - less polluted region) along Visakhapatnam coast of Andhra Pradesh. Seawater and sediment samples were also analysed simultaneously to detect the metal contents in the ambient media. The order of metal accumulation were similar in both *Ulva* species and stations and was in the order Zn > Cu > Pb. Bioconcentration factors in *U. lactuca* and *U. intestinalis* is higher being a polluter lover than *U. lactuca*. Metal pollution index was also greater for *U. intestinalis* than *U. lactuca* proving *U. intestinalis* to be the biomonitor of heavy metal contamination.

Key words : *Bioaccumulation, Bioconcentration factor, Green algae, Heavy metals, Metal pollution index.*

Introduction

With rapid urbanization and industrialization, the pollution is increasing day by day, so as the environmental problems. Heavy metals are the most serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation (Tam and Wong, 2000). Metal containing industrial wastes are discharged into the environment directly or indirectly causing serious environmental pollution (Masindi and Muedi, 2018; Wang, 2002). High levels of heavy metals (e.g. cadmium, cobalt, mercury, copper, lead, vanadium and zinc) in aquatic ecosystems are regarded as serious pollutants; because they can be toxic and incorporated into the food chain (Kishe and Machiwa, 2003). Urban and industrial activities introduced large amounts of pollutants into the marine environment causing significant and permanent disturbances in marine systems and consequently, environmental and ecological degradation. This phenomenon is especially significant in coastal zones, as these are the main sinks of almost all anthropogenic pollutants. Recovery of heavy metals from industrials waste streams is becoming increasingly important as society realises the necessity for recycling and conservation of essential metals (Hashim and Chu, 2004).

Heavy metals are metallic elements with atomic weight greater than 55.8 g/mol or density greater than 4.5-5 g/cm^3. Biosorption of metals is not based on only one mechanism. It consists of several ones that quantitatively and qualitatively differ according to the type of biomass, its origin and its processing. Metal sequestration may involve complex mechanisms, mainly ion exchange, chelating, adsorption by physical forces and ion entrapment in capillaries and spaces of the structural polysaccharide cell wall network (Volesky and Holan, 1995). Due to the common occurrence of the raw biomass material and its high metal uptake capacity (Utomo *et al*., 2016; Schiewer and Volesky, 2000) recent studies has focused on marine macro algae otherwise known as seaweeds (Figueira *et al*., 2000; Kratochvil and Volesky, 1998; Conti and Cecchetti, 2003). The process of metal bioaccumulation in marine food chains is poorly understood because very little data is available on metal concentration at different trophic levels (Davis, 2000; Lanza, 1989)or their temporal (Talavera-saenz *et al*., 2007; Abdallah *et al*., 2006) or spatial variation (Rodriguez-Castaneda *et al*., 2006) and their effects on the photosynthetic process (Kalesh and Nair, 2006).

Seaweeds have been widely used to monitor and characterize the status of environmental pollution. They play an important role in the nutrient dynamics of coastal systems and reflect changes in water quality efficiently (Catriona *et al*., 2002). Hence, any change in the nature of the dynamics (like increased inputs of contaminants such as heavy metals) will likely to be reflected by these marine seaweed. These are able to accumulate trace metals, reaching concentration values that are thousands of times higher than the corresponding concentrations in seawater (Wilson, 2002). Algae bind only free metal ions, the concentrations of which depend on the nature of the suspended particulate matter (Bryan and Langston, 1992). The analysis of environmental matrices such as water or sediment provides a picture of the total contaminant load rather than that of fraction of direct eco-toxicological relevance. Thus, the use of biomonitors eliminates the need for complex studies on the chemical speciation (and hence presumptive bioavailability) of aquatic contaminants (Volterra and Conti, 2000).

Metal concentrations identify the bioaccumulation of trace metals that occur in high degrees, satisfying all the fundamentals requirements for bioindicators (Phillips and Segar, 1986; Campanella *et al*., 2001). In particular, seaweeds are recognized to concentrate metals up to levels many times larger than those found in the surrounding waters (Farias *et al*., 2002). The cell wall of algae consists of a verity of polysaccharides and proteins, some of them containing anionic carboxyl, sulphate or phosphate group that are excellent binding sites for metal retentions. The binding of metals by seaweeds was shown by (Murphy *et al*., 2007) to be strong, with only a minimal exchange between bound metals and ambient water. Seaweeds take up metal elements from the aquatic environment, depending on species, exposal time, type of metal and its oxidation states, pH, salinity and presence of organic pollutants (Jennett *et al*., 1980). Contamination of the seaweeds surface from simple contact with the elements dissolved in seawater has been observed in both unicellular and pluricellular algae. Metal ions (which are essential elements) are also taken up by algae through pores in their cell walls. Consequently, the cell components as well as the composition and structure of the cell walls are important factors in determining the ability of seaweed species to absorb metals (Utomo *et al*., 2016). Many studies of contaminants and their affects on marine seaweeds have been published since the beginning of the 1960's (Lobban and Harrison, 1994). Other data have shown that seaweeds can absorb metals such as Pb and Sr (Eide *et al*., 1980). Ho (1990), found that the seaweed *Ulva lactuca* is an important bio indicator of Cu, Zn and Pb present in seawater.

Many industrial and mining processes cause heavy metal pollution which can contaminate natural water system and become a hazard for human and other organisms. Therefore, colonization of macrophytes on the sediments polluted with heavy metals and the role of these plants in transportation of metals in shallow coastal areas are very important. The present investigation was planned and executed considering the potential of macrophytes as a biological litter of the aquatic environment.

Visakhapatnam coast of Andhra Pradesh in the east coast of India is characterised by many presence of many small and large scale industries like Visakhapatnam Steel Plants, Bharat Heavy Plates and Vessels, Hindusthan Zinc Limited, Hindusthan Petroleum Corporation Limited, Port Trust, Hindusthan Ship Yard and Fishing Harbour, Coramandel Fertilizers, L.G. Polymeres, Essar Shipping, Simhadri Project of National Thermal Power Corporation (NTPC), medium and small scale industries being developed by Andhra Pradesh Industrial Infrastructure Corporation. These industries realise large amounts of heavy metals to the adjoining waters. Hence heavy metal contamination is a very serious issue in the study area owing to the problems of bioaccumulation and then biomagnification in the marine food webs. Since heavy metals are conservative pollutants hence their removal by biological means is one of the binds although it is time consuming. Bioaccumulation of heavy metals by seaweeds not only helps in cleaning process of adjoining seawater but also helps in harvesting these seaweeds for local manure in agricultural fields.

The applicability of green seaweed biomass such as *Ulva* for metal removal has not been extensively investigated yet despite its large abundance in the world's shorelines (Morand and Birand, 1996).

Materials and Methods

Study area

The east coast of India comprises of four states (West Bengal, Odisha, Andhra Pradesh, and Tamil Nadu) and one union territory (Puducherry) with a total coastline of 2545.1 km. Visakhapatnam coast of Andhra Pradesh lies between 17°1423022N to 17°1724522N and 83°1622522E and 83°2123022E on the east-coast of India and provides a suitable environment for the growth and survivability of seaweeds. The coast line is known for its rich marine life especially the intertidal biota (Lakshmi and Rao, 2009). Visakhapatnam coast, which is about 10 km long, is characterised by rocky substratum interspersed with sand. The intertidal region, which is about 60-75 m, supports a rich growth of seaweeds which in turn harbours a variety of animals (Sowjanya and Sekhar, 2015).

Two stations (Figure 1) were selected in this geographical locale to understand the variation in the physico-chemical characteristics and heavy metal content in water, sediment and two selected seaweeds (*Ulva lactuca* and *U. intestinalis*). Sampling location was detected using GPS localization. The stations were as follows: Station 1: Vuda Park (Coordinates: 17°43′26.76′′N 83°20′22.2′′E) also known as the Visakhapatnam Urban Development Authority is a popular attraction in Visakhapatnam and is a favourite tourist spot. One of its main attractions here is a skating ring and musical waterfall. This station is anthropogenically stressed area with domestic garbage and receives the outfall of the city and discharges from nearby Textiles Mills (Sangam Textiles), organic chemical (Rashtriya Chemicals and Fertilizers Limited) and battery industry (HBL

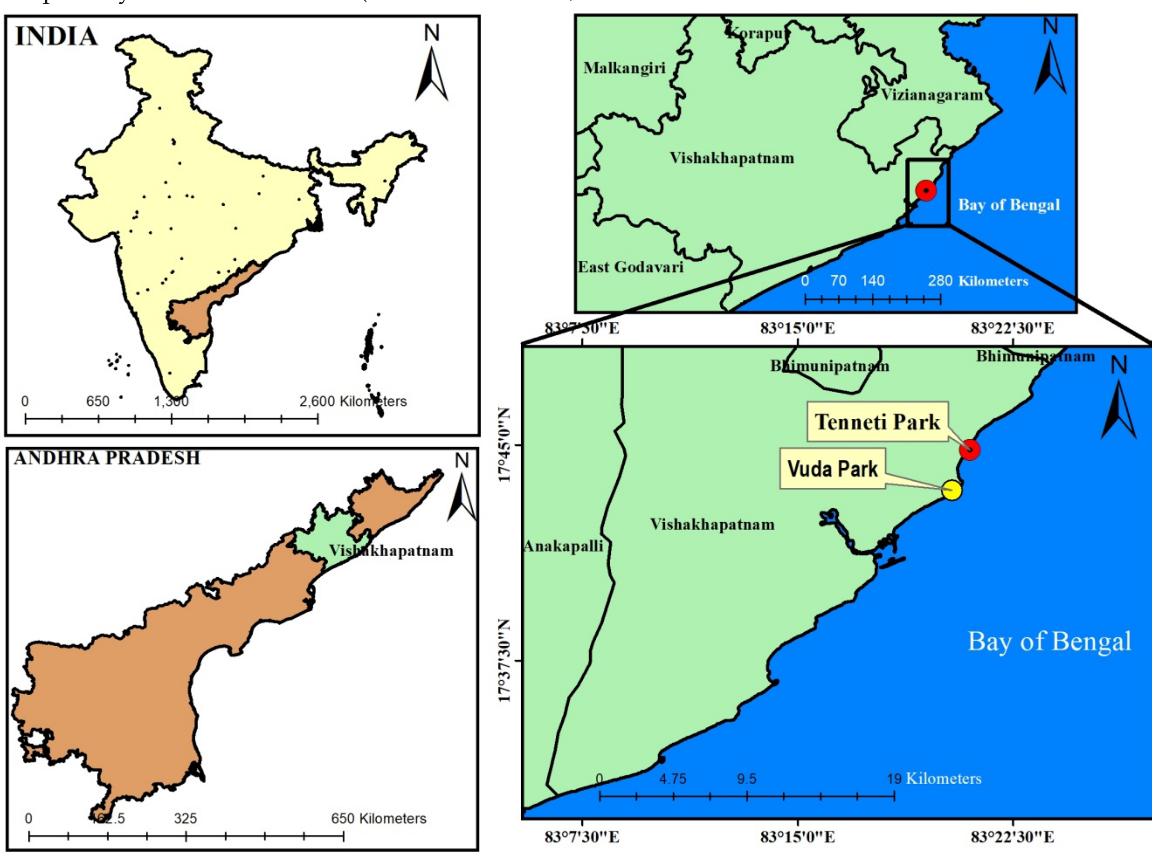

Fig. 1. Map of sampling stations.

Power Systems Limited) which generally releases such Cu, Zn and Pb and hence the adjacent waters have high heavy metal concentrations. Station 2: Tenneti Park (Coordinates:17°44′54.24′′N 83°20′58.2′′E) is located in the Vizag-Bheemili road. The park is situated along the beach with the picturesque view of the mountain and the sea. This area is a less stressed area with respect to anthropogenic pressure and distant from industry. Rocks of various sizes and rocky platforms are exposed at the selected stations in intertidal zone and offer a variety of habitats which supplies sufficient sunlight's, pigment and nutrient found along the shore of seawater leads to abundant growth of seaweeds.

Collection of seaweeds and identification

A field study was undertaken in the selected sampling stations of Visakhapatnam coast of Andhra Pradesh over a period of 9 months of August 2017 to April 2018. Seaweed samples were periodically collected whole fronds with holdfast by hand-picked during low tide by random sampling with a total of 5 quadrants, each plot was 25X25 cm^2 at both the field stations. After collection, seaweeds were washed with seawater to remove all extraneous matters such as epiphytes, shells, associated fauna and adhering sand particles at the sampling station, placed in plastic bags and transported to the laboratory in an icebox. At the laboratory, seaweeds were rinsed with distilled water, dried at 60°C for 24 hours to constant to weight in the oven, the dried samples were weighed and powdered with a porcelain mortar and pestle, sieved and stored in plastic bottle until further analysis of heavy metals (Zn, Cu, Pb) as per the standard methodologies. Seaweed species were identified by referring to authentic taxonomic keys (Kaliaperumal, 1995; Domettila and Jeeva, 2013).

Sampling and analysis of physico-chemical parameters of seawater and sediments

Surface water samples were collected using sampler bottles. For collecting seawater the sampler bottles were opened at the particular water level and samples were collected and preserved by adding 1 ml of concentrated nitric acid (HNO_3) to it thus, preventing microbial growth in the water sample. Water temperatures were measured using a digital thermometer (MEXTECH Multi stem handheld portable LCD digital thermometer with sensor probe -50 °C to 300 °C or -58 °F to 572 °F). The values for pH of water were measured using a pH meter (Oakton eco-tester pH 2 water proof pH tester 0.0 to 14.0 pH range). The salinity values presented in psu unit were measured using a refractometer (Hand-Held refractometer, ERMA).

Surface sediments samples were collected during low tide from two different points and stored in a pre-cleaned polythene bag in order to limit gaseous exchange and were brought back to the laboratory and kept refrigerated at 4 °C before analysis. Sediment samples were later spread on a flat tray inside the laboratory to be air dried for three days at room temperature after which it was been grinded and sieved for further digestion process.

Analytical procedures

Analysis of heavy metals in seawater

The seawater samples were filtered through a 0.45 mm Whatman no.1 filter paper. A 1000 ml sample of seawater was transferred to a pre-washed separating funnel and 1.0 ml of citrate buffer was added. The pH of samples was adjusted to 4.0 by using concentrated hydrochloric acid (HCl) or purified ammonia (NH_3), after adjusting the pH, add 2 ml ammonium pyrollidine dithiocarbamate (APDC) and 20 ml methyl isobutyl ketone (MIBK) solution, and shake vigorously for 2-3 minutes. The extracted organic layer was aspirated directly to the atomic absorption spectrophotometer (AAS) (Hitachi Zeeman Polarised, Z. 5000 series). A blank determination was done by using the same procedure but without water sample. Standard operating conditions of the instrument were set during the analysis of metals in seawater (Brewer *et al.*, 1969).

Analysis of heavy metals in sediments

The sieved sediments were dried completely in glass Petriplates using a hot air oven at a temperature of 90°C. The dried sediment samples were gently disaggregated with a glass pestle and mixed thoroughly in order to get a composite sample. For heavy metal determination, 5 g of the dried sample was digested using a HNO_3: H_2O_2: HCl mixture and analysed using atomic absorption spectrophotometer (AAS) (Hitachi Zeeman Polarised, Z. 5000 series) (Doshi *et al.*, 1969).

Analysis of heavy metals in green algae

The samples of *Ulva* thalli were washed thoroughly in tap water to remove sand, particulate matter and epiphyta. They were then rinsed with distilled water. The samples were dried at 60°C to get a constant weight, homogenized by using a glass pestle and mortar and kept away from metallic materials and dusty conditions to avoid contamination. Approximately 5 g of dried seaweeds powder sample was weighed and wet-digested using HNO_3: H_2O_2: HCl and the metals were analysed by using atomic absorption spectrophotometer (AAS) (Hitachi Zeeman Polarised, Z. 5000 series)(Doshi *et al*., 1969).

Calculation of Bioconcentration Factor (BCF) and Metal Pollution Index (MPI)

$BCF = C_R/C_S$

To access the ability of the studied seaweeds as heavy metal bioaccumulators, the Bioconcentration Factor (BCF) and Metal Pollution Index (MPI) were determined by the following formula (Black and Mitchell, 1952) as:

Where: C_R=Mean Metal Concentration in the macro algae tissue and C_S=Mean Metal Concentration in seawater sediment.

$MPI = (M_1 \times M_2 \times M_3 \times \_\_\_\_\_\_\_\_ \times M_n)^{1/n}$

The MPI (Usero *et al*., 2005) was use to compare the total accumulated metal in the two tested alga species from different study area.

Where: M_n=The concentration of metal and n = Metal no.

Statistical analysis

All values for physico-chemical parameters and heavy metals were expressed in terms of mean ± S.D. Pearsons correlation-coefficient was carried out using SPSS 13.1 in order to find out the interrelationship between the selected physico-chemical parameters of seawater and sediment with the selected seaweeds.

Results and Discussion

Marine pollution is a global environmental problem which is caused generally by human activity in the coastal and marine water areas. They are contributed to the ambient environment through discharge of various kinds of pollutants such as heavy metals into ecosystems (Pote *et al*., 2008). Recently heavy metal contamination being non-biodegradable became one of the most alarming environmental problems which led to harmful impact on land and sea living organisms (Wang *et al*., 2006). Seaweeds have been documented as important metal pollution bioaccumulators because of their size, abundant in aquatic system and their competent ability to uptake and accumulate metals (Maharana, 2010; Olivares, 2016). Brown *et al*., (1999) report significantly higher levels of Cu and Zn in *U. intestinalis* than in *U. lactuca*.

In high concentration, heavy metals e.g. Cu, Zn and Pb have detrimental effects on seaweed growth and metabolism, yet at low concentration Cu and Zn are essential for catalyzing enzymatic reaction. The highly toxic metal like Pb is associated as cofactor for activation of some algal enzymatic system (Manoj and Padhy, 2013). Seaweeds also produce antioxidants to face environmental stress such as temperature, salinity and heavy metal pollutants. These stresses lead to intermittent intracellular oxidative stress conditions developed by the accumulation of reactive oxygen species (ROS) (Pinto *et al*., 2003) which lead to lipid peroxydation, protein deformation, DNA damages and finally cell death (Manoj and Padhy, 2013; Collen *et al*., 2003).

Seasonal variation of physico-chemical parameters like surface water temperature, pH and salinity were monitored in both the stations. Water temperature values ranged from 27.2±0.4°C during post-monsoon at station 2 to 32.8±1.1°C during pre-monsoon at station 1. The values of pH ranged from 7.7±0.6 during monsoon at station 1 to 8.2±0.5 during pre-monsoon at station 2. Salinity values ranged from 21.8±0.5 psu during monsoon at station 2 to 34.2±0.5 psu during pre-monsoon at station 1 respectively (Figure 2).

Heavy metal concentration in seawater as well as sediment was monitored seasonally at both the stations and the values for Cu ranged from 12.01±0.45 mg/l during pre-monsoon at station 2 to 18.95±0.45 mg/l during monsoon at station 1 for seawater and 17.8±0.45 mg/kg during monsoon at station 2 to 29.4±0.45 mg/kg during pre-monsoon at station 1 for sediment respectively. The values for Zn ranged from 26.5±0.40 mg/l during pre-monsoon at station 2 to 37.9±0.55 mg/l during monsoon at station 1 for seawater and 23.6±0.45 mg/kg during monsoon at station 2 to 35.4±0.45 mg/kg during pre-monsoon at station 1 for sediment respectively. The values for Pb varied from 9.29±0.45 mg/l during pre-monsoon at station 2 to 15.70±0.40 mg/l during monsoon at station 1 for seawater and BDL during monsoon at station 2 to 6.9±0.45 mg/kg during pre-monsoon at station 1 for sediment respectively (Figures 2 and 3).

The heavy metal accumulations in seaweeds were monitored seasonally for two different species at the two different stations. The values of *Ulva lactuca* for Cu ranged from 12.5±0.45 mg/kg during pre-monsoon at station 2 to 67.0±0.40 mg/kg during monsoon at station 1; the values for Zn ranged from 53.7±0.46 mg/kg during pre-monsoon at station 2 to 84.1±0.40 mg/kg during monsoon at station 1 and the values of Pb ranged from BDL during pre-monsoon at station 2 to 10.5±0.40 mg/kg during mon-

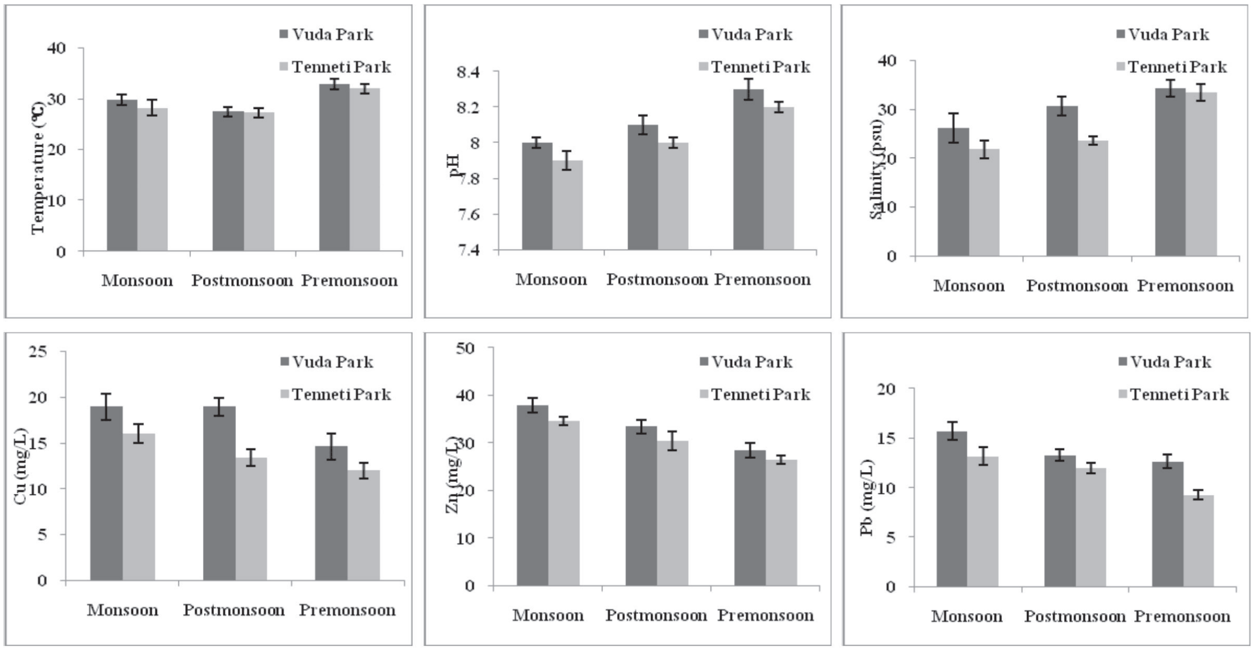

Fig. 2. Seasonal variation in physico-chemical parameters of seawater of selected stations.

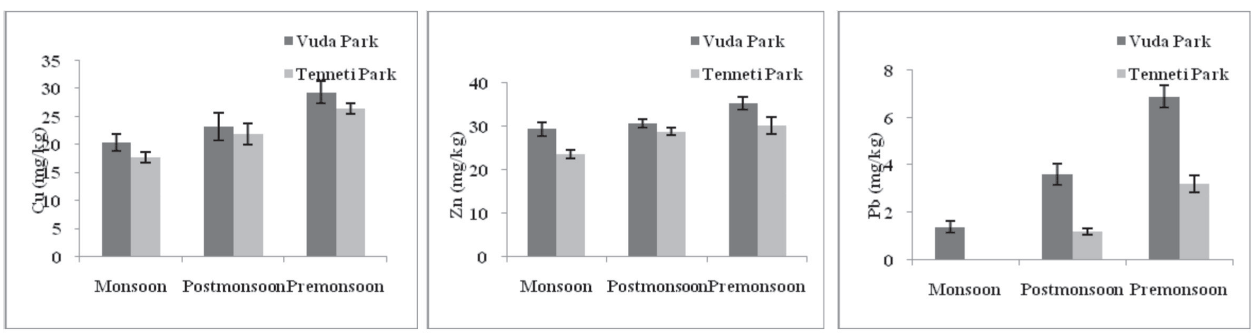

Fig. 3. Seasonal variation in heavy metals in sediment of selected stations.

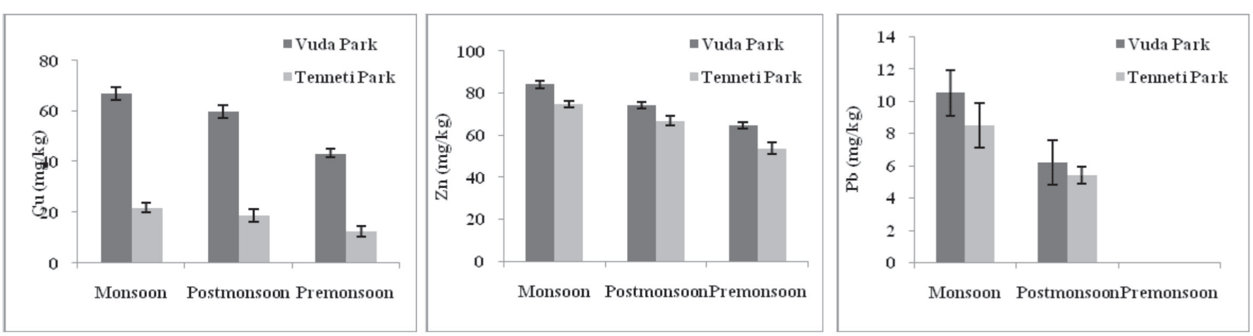

Fig. 4. Seasonal variation in heavy metals in *U. lactuca* of selected stations.

soon at station 1 respectively (Figure 4).

For *U. intestinalis* values for Cu concentration varied from 24.6±0.45 mg/kg during pre-monsoon at station 2 to 104.1±0.4 mg/kg during monsoon at station 1; the values for Zn varied from 100.4±0.35 mg/kg during pre-monsoon at station 2 to 150.8±0.46 mg/kg during monsoon at station 1 and the values for Pb varied from 6.0±0.35 mg/kg during pre-monsoon at station 2 to 28.0±0.35 mg/kg during monsoon at station 1 respectively (Figure 5).

The higher temperature, higher salinity and low pH at station 1 might probably be due to the location of the station being exposed to the open sea and lack of vegetation and heavy waste water discharge from the adjacent city of Visakhapatnam. This might probably be also the reason for high metal concentration in seawater and sediment. The heavy metal concentration in seawater was higher during monsoon in both the stations which might be probably due to the fact that the metals are more in dissolved form during monsoon owing to the cause of low pH in the seawater. On contrary, the heavy metal concentration in sediment was higher in case of pre-monsoon in all the stations which might probably be due to settlements of metal ions in the sediment at high pH.

The results of heavy metal concentration in the two selected seaweeds have proved the fact that *U. intestinalis* is a better bioaccumulator of heavy metals in comparison to *U. lactuca*. The relationship between the dissolved heavy metal and heavy metal in seaweeds for both the species have shown significant positive relationship ($p < 0.01$) proving the fact that both *U. lactuca* and *U. intestinalis* are good bioaccumulators of heavy metals. The relationship between temperature, salinity and dissolved Zn has shown significant negative relationship which proves that low temperature and salinity favours more Zn accumulation as Zn is an essential heavy metals for plants. Similar is the case for Pb accumulation except insignificant relation with *U. intestinalis*, although Cu did not show any relationship with the ambient physico-chemical parameters in the present geographical locale (Tables 1 and 2).

In case of bioaccumulation of heavy metals from sediment by seaweeds it has shown insignificant relationship for both *U. lactuca* and *U. intestinalis* which proves that seaweeds generally accumulates heavy metals from water rather than the sediments because heavy metals usually remain in complex form in sediment which cannot be uptake by the

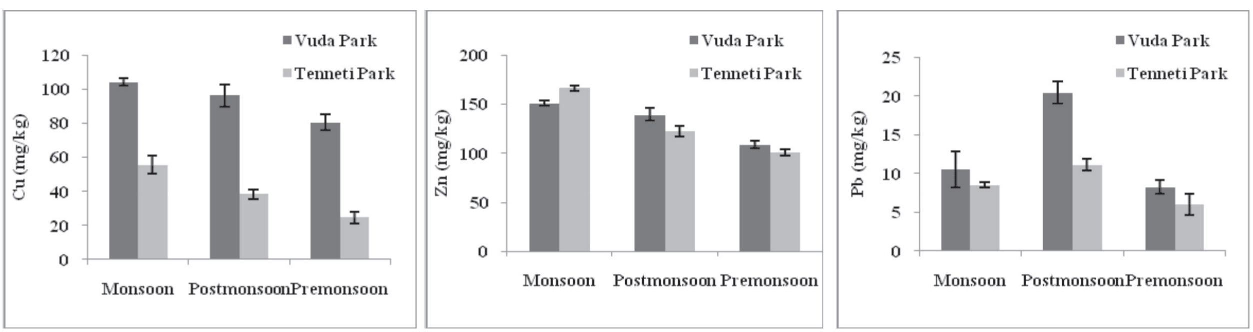

Fig. 5. Seasonal variation in heavy metals in *U. intestinalis* of selected stations.

Table 1. Correlations between physico-chemical parameters and value of heavy metals in *Ulva lactuca* (W = water; SD = sediment; SW = seaweed).

U. lactuca	Temperature (W)	pH (W)	Salinity (W)	Cu (W)	Zn (W)	Pb (W)	Cu (SW)	Zn (SW)	Pb (SW)
Temperature (W)	1.000								
pH (W)	0.474	1.000							
Salinity (W)	0.725	0.398	1.000						
Cu (W)	-0.298	-0.149	-0.308	1.000					
Zn (W)	-0.467	-0.161	-0.608	0.927	1.000				
Pb (W)	-0.279	-0.111	-0.396	0.943	0.895	1.000			
Cu (SW)	-0.090	-0.014	0.086	0.898	0.689	0.837	1.000		
Zn (SW)	-0.480	-0.179	-0.566	0.945	0.975	0.955	0.760	1.000	
Pb (SW)	-0.604	-0.136	-0.760	0.776	0.944	0.762	0.482	0.900	1.000

plants. There was a *significant* positive *correlation* of sediment Zn, Cu and Pb with temperature, pH and salinity in both the stations has proved the fact that with increase in temperature, pH and salinity the heavy metals get chellated in the sediment and vice-versa (Tables 3 and 4). Similar studies recorded from various parts of the world also show that our study values are well within the limits of studies made in other part of the world (Table 5).

The Bioconcentration Factor (BCF) is the ratio that describes the relationship between the concentration of metals in algal thalli and the ambient environment (seawater and sediments). This is often used to check the possibility of using algal species as bioaccumulator (Zayed *et al.*, 1998). The bioaccumulation factor of metals in both the tested seaweeds has shown higher accumulation capacity of *U. intestinalis* than *U. lactuca* in both the stations.

Table 2. Correlations between physico-chemical parameters and value of heavy metals in *Ulva intestinalis*.

U. intestinalis	Temperature (W)	pH (W)	Salinity (W)	Cu (W)	Zn (W)	Pb (W)	Cu (SW)	Zn (SW)	Pb (SW)
Temperature (W)	1.000								
pH (W)	0.474	1.000							
Salinity (W)	0.725	0.398	1.000						
Cu (W)	-0.298	-0.149	-0.308	1.000					
Zn (W)	-0.467	-0.161	-0.608	0.927	1.000				
Pb (W)	-0.279	-0.111	-0.396	0.943	0.895	1.000			
Cu (SW)	-0.079	-0.023	0.074	0.896	0.676	0.860	1.000		
Zn (SW)	-0.577	-0.306	-0.759	0.761	0.895	0.718	0.439	1.000	
Pb (SW)	-0.406	-0.148	-0.399	0.952	0.950	0.873	0.779	0.744	1.000

Table 3. Correlation between physico-chemical parameters and values of heavy metals in sediments in *Ulva lactuca* stations.

U. lactuca	Temperature (W)	pH (W)	Salinity (W)	Cu (SD)	Zn (SD)	Pb (SD)	Cu (SW)	Zn (SW)	Pb (SW)
Temperature (W)	1.000								
pH (W)	0.474	1.000							
Salinity (W)	0.725	0.398	1.000						
Cu (SD)	0.575	0.483	0.758	1.000					
Zn (SD)	0.565	0.421	0.824	0.850	1.000				
Pb (SD)	0.634	0.500	0.885	0.821	0.934	1.000			
Cu (SW)	-0.090	-0.014	0.086	-0.183	0.257	0.184	1.000		
Zn (SW)	-0.480	-0.179	-0.566	-0.610	-0.318	-0.400	0.760	1.000	
Pb (SW)	-0.604	-0.136	-0.760	-0.764	-0.595	-0.661	0.482	0.900	1.000

Table 4. Correlation between physico-chemical parameters and values of heavy metals in sediments in *Ulva intestinalis* stations.

U. intestinalis	Temperature (W)	pH (W)	Salinity (W)	Cu (SD)	Zn (SD)	Pb (SD)	Cu (SW)	Zn (SW)	Pb (SW)
Temperature (W)	1.000								
pH (W)	0.474	1.000							
Salinity (W)	0.725	0.398	1.000						
Cu (SD)	0.575	0.483	0.758	1.000					
Zn (SD)	0.565	0.421	0.824	0.850	1.000				
Pb (SD)	0.634	0.500	0.885	0.821	0.934	1.000			
Cu (SW)	-0.079	-0.023	0.074	-0.170	0.264	0.218	1.000		
Zn (SW)	-0.577	-0.306	-0.759	-0.854	-0.729	-0.690	0.439	1.000	
Pb (SW)	-0.406	-0.148	-0.399	-0.601	-0.260	-0.354	0.779	0.744	1.000

Table 5. Comparison of current study results with other researchers for different *Ulva* sp.

Species name	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Country	Season	Reference
U. lactuca	37.18 (2.05-11.16)	69.66 (8.95-14.01)	5.1 (0-1.75)	India	annual cycle	current studies
U. intestinalis	66.53 (4.1-17.35)	131.13 (16.73-27.7)	14.88 (1-4.66)			
U. australis	no data	6.95	no data	Australia	annual cycle	Farias *et al*., 2019
U. fasciata	9	55.5	no data	Brazil	annual cycle	Lacerda *et al*., 1985
U. intestinalis	15.13 (1.50-38.95)	31.95 (7.20-68.22)	4.91 (2.49-8.02)	Turkey	annual cycle	Yozukmaz *et al*., 2018
U. intestinalis	0.28	73.32	0.10	Romania	rainy	Trifan *et al*., 2015
U. intestinalis	no data	57.14	no data	Russia	no data	Castanedal *et al*., 2006
U. intestinalis	0.0075	0.0299	no data	Spain	Summer	Villares *et al*., 2002
U. lactuca	6.65	16.59	7.99	Egypt	rainy	Mourad *et al*., 2019
U. lactuca	12.38	51.86	13.17	Ireland's	rainy	Wan *et al*., 2017
U. lactuca	2.44	0.96	5.11	Algeria	rainy	Laib and Leghouchi, 2012
U. lactuca	0.46	3.18	1.82	Algeria	summer	Allam *et al*., 2016
U. lactuca	4.6-9.3	24.7-44.4	no data	Australia	summer	Talbot and Chegwidden, 1982
U. lactuca	9.48 (1.65-12.31)	77.58 (50.61-90.60)	no data	Sweden	no data	Hagerhall, 1973
U. lactuca	6.2-11.1	10.2-19.9	no data	Cuba	no data	Ramirez *et al*., 1990
U. lactuca	9-170	20-360	no data	Norway	no data	Stenner and Nickless, 1974
U. lactuca	5.5-26	59-160	no data	S.W. Iberian peninsula	spring	Stenner and Nickless, 1975
U. lactuca	13.07-24.75	31.22-66.92	no data	Bosporus	autumn and winter	Guven *et al*., 1993
U. lactuca	0.67	9.91	3.34	USA	summer	Chaudhuri *et al*. (2007)
U. lactuca (mg/g)	64.5	no data	68.9	Egypt	spring	Ibrahim *et al*., 2016
U. pertusa	11.68	51.74	2.18	China	rainy	Pan *et al*., 2018
U. reticulata	12.2	15.8	0.1	India	no data	Gomathi and Sheba, 2018
U. reticulata	13.85	8.89	no data	India	no data	Agadi and Bhosle, 1978
Ulva rigida	8	81	no data	Croatia	autumn	Munda and Hudnik, 1991
Ulva rigida	4.49-5.67	6.0-11.9	no data	N. Turkey	summer and autumn	Guven *et al*., 1992
U. rigida	9.25-4.65	25.1-14.3	no data	Spain	winter and summer	Villares *et al*., 2002
Ulva sp.	90.22 (3.55-962)	30.5 (6.15-77.7)	no data	Spain	summer	Puente, 1992
Ulva sp.	1.5	24	no data	S. Australia	no data	Maher, 1998

Table 6. Bioconcentration Factor (BCF) in the selected *Ulva* species (W = water; SD = sediment; SW = seaweed).

Seasons	Name of the sample	Vuda Park (Station-1)						Tenneti Park (Station-2)					
		CF_{Cu}		CF_{Zn}		CF_{Pb}		CF_{Cu}		CF_{Zn}		CF_{Pb}	
		SW/W	SW/SD	SW/W	SW/SD	SW/W	SW/SD	SW/W	SW/SD	SW/W	SW/SD	SW/W	SW/SD
Monsoon 2017	*U. lactuca*	3.53	3.28	2.21	2.86	0.66	7.5	1.36	1.23	2.15	3.16	0.62	0
	U. intestinalis	5.49	5.10	4.07	5.12	1.83	20	3.46	3.11	4.88	7.07	1.18	0
Postmonsoon 2018	*U. lactuca*	3.50	2.57	2.22	2.40	0.46	1.72	1.43	0.85	2.19	2.31	0.45	4.5
	U. intestinalis	5.69	4.14	4.14	4.5	1.53	5.66	2.91	1.74	4.01	4.23	0.92	9.25
Premonsoon 2018	*U. lactuca*	2.96	1.46	2.27	2.04	0	0	1.04	0.47	2.02	1.77	0	0
	U. intestinalis	0.98	2.73	3.81	3.06	0.64	1.18	2.04	0.93	3.78	3.32	0.64	1.87

Table 7. Metal Potential Index (MPI) for seawater, sediment and *Ulva* speciesin the selected stations.

Seasons	Vuda Park (Station-1)				Tenneti Park (Station-2)			
	Seawater	Sediment	*U. lactuca*	*U. intestinalis*	Seawater	Sediment	*U. lactuca*	*U. intestinalis*
Monsoon 2017	22.424	9.434	38.966	76.033	19.402	20.495	24.036	52.402
Postmonsoon 2018	19.609	13.701	30.187	64.792	16.980	9.123	18.928	37.333
Premonsoon 2018	17.387	19.293	52.888	41.515	14.352	13.664	25.908	24.562

The bioaccumulation factor values of station 2 (Tenneti Park) were slightly higher than station 1 (Vuda Park) in except for Cu which is shown to be higher than Zn in station 1 proving the fact that there is no specific pattern for metal bioconcentration (Table 6). The accumulation of metal ranged in the order Zn > Cu > Pb in the both species. The higher absorption power of *U. intestinalis* in comparison to *U. lactuca* might be probably due to chemical groups (carboxyl, sulphonate, hydroxyl and amino groups) on their cell walls causing metal biosorption and chelation (Wang *et al*., 2006; Olivares *et al*., 2016) and related to the morphology of the algae since *U. intestinalis* thallus is highly coiled providing more surface area for biosorption than *U. lactuca*.

Metal Pollution Index (MPI) was calculated seasonally for both the stations and both the seaweeds respectively. The Metal Pollution Index of seawater is maximum in station 1 in comparison to station 2 and vice-versa with the sediment proving the fact that low pH in the station leads to more accumulation of heavy metals by seaweeds of station 1 (Table 7). The MPI is also greater for *U. intestinalis* than *U. lactuca* proving *U. intestinalis* to be the effective biomonitor of heavy metal contamination.

Conclusion

The result of BCF and MPI suggests that *U. intestinalis* is far better bioaccumulator of heavy metal in comparison to *U. lactuca*. The bioaccumulation capacity of *U. intestinalis* is superior being a polluter lover. The data over the world suggests the average accumulation capacity of *U. intestinalis* to be of the order: Cu=215 μg^{-1}; Zn=950 μg^{-1}; Pb=0.08 μg^{-1}; similarly*U. lactuca*: Cu=90.22 μg^{-1}; Zn=90.65 μg^{-1}; Pb=0.008 μg^{-1}. Hence, *U. intestinalis* is considered valuable indicator because of their accumulation capacity of heavy metals which can be used in the phytoremediation system for cleaning the marine environment from pollutant.

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References

Abdallah, A.M.A., Abdallah, M.A., Beltagy, A. and Siam, E. 2006. Contents of heavy metals in marine algae from Egyptian Red Sea coast. *Toxicological and Environmental Chemistry.* 88 : 9-22.

Agadi, V.V., Bhosle, N.B. and Untawale, A.G. 1978. Metal concentration in some seaweed of Goa, India. *Botanica Marina*. 21 : 247-250.

Allam, H., Aouar, A., Benguedda, W. and Bettioui, R. 2016. Use of sediment and algae for biomonitoring the coast of Honine (Far West Algerian). *Open Journal of Ecology.* 6 : 159-166.

Black, W.A.P. and Mitchell, R.L. 1952. Trace elements in the common brown algae and in seawater. *Journal of the Marine Biological Association, UK.* 30 : 575-579.

Brewer, P.G., Spencer, D.W. and Smith, C.L. 1969. Determination of trace metals in seawater by atomic absorption spectroscopy. *American Society for Testing and Materials Special Technical Publication*. 443 : 7-77.

Brown, M.T., Hodgkinson, W.M. and Hurd, C.L. 1999. Spatial and temporal variations in the copper and zinc concentrations of two green seaweeds from Otago Harbour, New Zealand. *Marine Environmental Research*. 47 : 175-184.

Bryan, G.W. and Langston,W.J. 1992. Bioavailability accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental Pollution.* 76 (2) : 89-131.

Campanella, L., Conti, M.E., Cubadda, F. and Sucapane, C. 2001. Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean. *Environmental Pollution*. 111 : 117-126.

Castanedal, R.A.P., Sanchez, R.I., Shumilin, E.N. and Sapozhnikov, D. 2006. Element concentrations in some species of seaweeds from La Paz Bay and La

Paz Lagoon, south-western Baja California, Mexico. *Journal of Applied Phycology.* 18 : 399-408.

Catriona, M.O., Macinnis-Ng, C.M.O. and Peter, J.R. 2002. Towards a more ecologically relevant assessment of the impact of heavy metals en the photosynthesis of the seagrass, *Zostera capricorni*. *Marine Pollution Bulletin*. 45 : 100-106.

Chaudhuri, A., Mitra, M., Havrilla, C., Waguespack Y. and Schwarz,J. 2007. Heavy metal biomonitoring by seaweeds on the Delmarva Peninsula, east coast of the USA. *Botanica Marina.* 50 : 151-158.

Collen, J., Pinto, E., Pedersen, M. and Colepicolo, P. 2003. Induction of oxidative stress in the red macroalga *Gracilaria tenuistipitata* by pollutants metals. *Archives of Environmental Contamination and Toxicology*. 45 : 337-342.

Conti, M.E. and Cecchetti, G. 2003. A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. *Environmental Research*. 93 : 99-112.

Davis, T.A., Volesky, B. and Vieira,R.H.S.F. 2000. *Sargassum* seaweed as biosorbent for heavy metals. *Water Resources*. 34 (17) : 4270-4278.

Domettila, C. and Jeeva, S. 2013. *Gracilaria idinthakaraiensis* in Rasthacaud coastal water, Kanyakumari district, Tamilnadu, India: a Rediscovery. *Science Research Reporter*. 3 (1) : 01-03.

Doshi, G.R., Sreekumaran, C., Mulay, C.D. and Patel,B. 1969. Ashing procedures for biomaterials. *Current Science*. 9 : 206-207.

Eide, I., Myklestad, S. and Melson,S. 1980. Long-term uptake and release of heavy metals by *Ascophyllum nodosum* (Phaeophyceae) in situ. *Environmental Pollution*. 23 : 19-28.

Farias, D.R., Hurd, C.L., Eriksen, R.S. and Macleod,C.K. 2019. *Ulva australis* as a tool for monitoring metal-polluted estuarine system; spatial and temporal considerations. *Global NEST Journal*. 21 (4) : 449-454.

Farias, S., Arisnabarreta, S.P., Vodopivezc, C.P. and Smichowski, P. 2002. Levels of essential and potentially toxic trace metals in Antarctic macro algae Spectrochim. *Acta Part B*. 57 : 2133-2140.

Figueira, M.M., Volesky, B. and Ciminelli,V.S.T. 2000. Biosorption of metals in brown seaweed biomass. *Water Resources*. 34 : 196-204.

Gomathi, K. and Sheba,L.A. 2018. Phytochemical screening and heavy metal analysis of *Ulva reticulate*. *Asian Journal of Pharmaceutical and Clinical Research*. 11 (4): 84-88.

Guven, K.C., Saygi, N. and Ozturk, B. 1993. Survey of metal contents of Bosphorus algae, *Zostera marina* and sediments. *Botanica Marina*. 36 : 175-178.

Guven, K.C., Topcuoglu, S., Kut, D., Esen, N., Erenturk, N., Saygi, N., Cevher, E., Guvener, B. and Ozturket, B. 1992. Metal uptake by Black Sea algae. *Botanica Marina*. 35 : 337-340.

Hagerhall, B. 1973. Marine botanical-hydrographical trace element studies in the Oresund area. *Botanica Marina*. 16 (2) : 53-64.

Hashim, M.A. and Chu,K.H. 2004. Biosorption of cadmium by brown, green, and red seaweeds. *Chemical Engineering Journal*. 97 : 249-255.

Ho, Y.B. 1990. Metals in *Ulva lactuca* in Hong Kong intertidal waters. *Bulletin of Marine Science*. 47 : 79-85.

Ibrahim, W.M., Hassan, A.F. and Azab,Y.A. 2016. Biosorption of toxic heavy metals from aqueous solution by *Ulva lactuca* activated carbon. *Egyptian Journal of Basic and Applied Science*. 3 (3) : 241-249.

Jennett, J.C., Hassett, J.M. and Smith, J.E. 1980. The use of algae to control heavy metals in the environment. *Minerals and the Environment*. 2 : 26-31.

Kalesh, N.S. and Nair, S.M. 2006. Spatial and temporal variability of copper, zinc, and cobalt in marine macroalgae from the southwest coast of India. *Bulletin of Environmental Contamination and Toxicology*. 76 : 293-300.

Kaliaperumal, N., Kalimuthu, S. and Ramalingam, J.R. 1995. *Economically Important Seaweeds.* Dr. M. Devaraj (Ed.), Special publication 62. Central Marine Fisheries Research Institute, Indian Council of Agricultural Research, Post Box. No. 1603, Cochin-682014, India, pp 1-36.

Kishe, M.A. and Machiwa, J.F. 2003. Distribution of heavy metals in sediments of Mwanza Gulf of Lake Victoria, Tanzania. *Environment International*. 28 : 619-625.

Kratochvil, D. and Volesky, B. 1998. Advances in the biosorption of heavy metals. *Trends in Biotechnology*. 16 : 291-300.

Lacerda, L.D., Teixeira, V.L. and Guimaraes, J.R.D. 1985. Seasonal variation of heavy metals in seaweeds from Conceicao de Jacarei (R. J.), Brazil. *Botanica Marina*. 28 : 339-343.

Laib, E. and Leghouchi, E. 2012. Cd, Cr, Cu, Pb, and Zn concentrations in *Ulva lactuca*, *Codium fragile*, *Jania rubens* and *Dictyota dichotoma* from Rabta Bay, Jijel (Algeria). *Environmental Monitoring and Assessment*. 184 : 1711-1718.

Lakshmi, K.P. and Rao, G.M.N. 2009. Some numerical studies on marine algae of Visakhapatnam Coast. *Journal of Algal Biomass Utilization*. 1 (1) : 60-85.

Lanza, D.L.G., Ortega, M.M. and Laparra, J.L. 1989. Chemical analysis of heavy metals (Hg, Pb, Cd, As, Cr and Sr) in marine algae of Baja California. Annals of the Institute of Biology of the National Autonomous University of Mexico (Bot). 59 (1) : 89-102.

Lobban, C.S. and Harrison, P.J. 1994. *Seaweed Ecology and Physiology.* Cambridge University Press, Cambridge, pp xi + 366.

Maharana, D., Jena, K.B. and Pise, N.M. 2010. Assessment of oxidative stress indices in a marine macro brown alga *Padina tetrastromatica* (Hauck) from comparable polluted coastal regions of the Arabian Sea, west coast of India. *Journal of Environmental Science*. 22: 1413-1418.

Maher, W.A. 1998. Trace metal concentrations in marine organisms from St. Vicent Gulf, South Australia. *Water Air Soil Pollution*. 29 : 77-84.

Manoj, K. and Padhy, P.K. 2013. Oxidative stress and heavy metals: an appraisal with reference to environmental biology. *International Research Journal of Biological Science*. 2 : 91-101.

Masindi, V. and Muedi, K.L. 2018. Environmental contamination by heavy metals. *Heavy Metals*. pp 115-133.

Morand, P. and Birand, X. 1996. Excessive growth of macroalgae: a symptom of environmental disturbance. *Botanica Marina*. 39 : 491-516.

Mourad, F.A. and El-Azim, H.A. 2019. Use of green alga *Ulva lactuca* (L.) as an indicator to heavy metal pollution at intertidal waters in Suez Gulf, Aqaba Gulf and Suez Canal, Egypt. *Egyptian Journal of Aquatic Biology & Fisheries Zoology Department*. 23 (4) : 437-449.

Munda, I.M. and Hudnik, V. 1991. Trace metal content in some seaweed from the Northern Adriatic. *Botanica Marina*. 34 : 241-249.

Murphy, V., Hughes, H. and McLoughlin 2007. Cu (II) binding the dried biomass of red, green and brown macroalgae. *Water Research*. 41 : 731-740.

Olivares, H.G., Lagos, N.M., Gutierrez, C.J., Valenzuela, G.L. and Lillo, M.E.H. 2016. Assessment oxidative stress biomarkers and metal bioaccumulation in macroalgae from coastal areas with mining activities in Chile. *Environmental Monitoring and Assessment.* 188 : 125.

Pan, Y., Wernberg, T., Bettignies, T.D., Holmer, M., Li, K., Wu, J., Lin, F., Yu, Y., Xu, J., Zhou, C., Huang, Z. and Xiao, X. 2018. Screening of seaweeds in the East China Sea as potential bio-monitors of heavy metals. *Environmental Science and Pollution Research*. 25 : 16640-16651.

Phillips, D.J.H. and Segar, D.A. 1986. Use of bioindicators in monitoring conservative contaminants: programme design imperatives. *Marine Pollution Bulletin.* 17 : 10-17.

Pinto, E, Sigaud-kutner, T.C.S., Leitao, M.A., Okamoto, O.K., Morse, D. and Colepicolo, P. 2003. Heavy metal-induced oxidative stress in algae. *Journal of Phycology*. 39 : 1008-1018.

Pote, J., Haller, L., Loizeau, J.L., Sastre, V. and Wildi, W. 2008. Effect of a sewage treatment plant outlet pipe extension on the distribution of contaminants in the sediments of the Bay of Vidy, Lake Geneva. *Switzerland Bioresource Technology*. 99 : 7122-7131.

Puente, X. 1992. Metais pesados en organismos bentónicos dos esteiros de Galicia. Tesis de Licenciatura. University of Santiago de Compostela, 58.

Ramirez, M., Gonzalez, H., Ablanedo, N. and Torres,I. 1990. Heavy metals in macroalgae of Havana's northern littoral, Cuba. *Chemical Ecology*. 4 (2) : 49-55.

Rodriguez-Castaneda, A.P., Sanchez-Rodriguez, I., Shumilin, E.N. and Sapozhnikov, D. 2006. Element concentrations in some species of seaweeds from La Paz Bay and La Paz Lagoon, south-western Baja California, México. *Journal of Applied Phycology*. 18 : 399-408.

Schiewer, S. and Volesky, B. 2000. Biosorption by marine algae. In: Ed. J.J. Valdes, *Bioremediation*, Kluwer, Dordrecht, The Netherlands, 139-169.

Sowjanya, V.I. and Sekhar, P.S.R. 2015. A comparative study of seaweed flora over a period of time with reference to climate change in Visakhapatnam Coast, Andhra Pradesh, India. *Advances in Applied Science Research*. 6 (6) : 187-193.

Stenner, R.D. and Nickless, G. 1974. Distribution of some heavy metals in organisms in Hardangerfjord and Skjerstadfjord, Norway. *Water Air Soil Pollution*. 3: 279-291.

Stenner, R.D. and Nickless, G. 1975. Heavy metals in organisms of the Atlantic coast of S.W. Spain and Portugal. *Marine Pollution Bulletin*. 6 : 89-92.

Talavera-saenz, A., Gardner, S., Riosmena-Rodriguez, R. and Vargas, B.A. 2007. Metal profiles used as environmental markers of Green Turtle (*Chelonia mydas*) foraging resources. *The Science of the total environment*. 373 : 94-102.

Talbot, V. and Chegwidden, A. 1982. Cadmium and other heavy metal concentrations in selected biota from Cockburn Sound, Western Australia. *Australian Journal of Marine and Freshwater Research*. 33 : 779-788.

Tam, N.F.Y. and Wong,Y.S. 2000. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. *Environmental Pollution*. 110 : 195-205.

Trifan, A., Breaban, L.G, Sava, D., Bucur, L., Toma, C.C. and Miron, A. 2015. Heavy metal content in macroalgae from Roumanian black sea. *Revue Roumaine Chimie*. 60 (9) : 915-920.

Usero, J., Morillo, J. and Gracia, I. 2005. Heavy metal contamination in molluscs from the Atlantic coast of southern Spain. *Chemosphere*. 59 : 1175-1181.

Utomo, H.D., Tan, K.X.D., Choong, D., Yu, J.J., Ong, J.J. and Lim, Z.B. 2016. Biosorption of heavy metal by algae biomass in surface water. *Journal of Environmental Protection*. 07 (11) : 1547-1560.

Villares, R., Puente, X. and Carballeira, A. 2002. Seasonal variation and background levels of heavy metals in two green seaweeds. *Environmental Pollution*. 119 : 79-90.

Volesky, B. and Holan, Z.R. 1995. Biosorption of heavy metals. *Biotechnology Progres*. 11 : 235-250.

Volterra, L. and Conti, M.E. 2000. Algae as biomarkers, bioaccumulators and toxin producers. In: Conti, M.

E., Botre, F. (Eds.), The Control of Marine Pollution: Current Status and Future Trends. *International Journal of Environmental Pollution*. 13 (1-6) : 92-125.

Wan, A.H.L., Wilkes, R.J. and Heesch, S. 2017. Assessment and characterisation of ireland's green tides (*Ulva* species). *PLoS ONE*. 12 (1) : 1-23.

Wang, J.J. 2002. Biosorption of Cu(II) by chemically modified biomass of *Saccharomyes cerevisiae*. *Process Biochemistry*. 37 : 847-850.

Wang, X.J., Chen, L., Xia, S.Q., Zhao, J., Chovelon, J.M. and Jafrezzic-Renault, N. 2006. Biosorption of Cu(II) and Pb(II) from aqueous solutions by dried activated sludge. *Minerals Engineering*. 19 : 968-971.

Wilson, S. 2002. Nutritional value of detritus and algae in blenny territories on the Great Barrier Reef. *Journal of Experimental Marine Biology and Ecology*. 27 : 155-169.

Yozukmaz, A., Yabanli, M. and Sel, F. 2018. Heavy metal bioaccumulation in *Enteromorpha intestinalis,* (L.) Nees, macrophytic algae: The example of Kadin creek (Western Anatolia). *Brazilian Archives of Biology and Technology.* 61 : 1-12.

Zayed, A., Lytle, C.M., Qian, J.H. and Terry, Y.N. 1998. Chromium accumulation, translocation and chemical speciation in vegetable crops. *Planta*. 206 (2) : 293-299.