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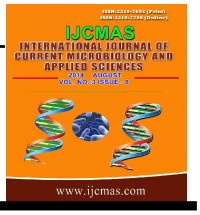

Original Research Article

Analysis of selected species of ascidians as bioindicators of metals in marine ecosystem

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Keywords

Ascidians, bioindicators, metals, bio-accumulation, pollution

A B S T R A C T

More recently, ascidians belonging to the sub-phylum Uro-chordata are used as potential model organisms in various parts of the world for biosorption of metals. In India, the study on accumulation of metals in ascidians is meagre. Hence the present study is aimed to analyze the concentration of metals in selected five species of ascidians and their environment in Thoothukudi coast, India. Among the four metals studied in seawater, Cu accumulated in highest concentration (0.0028 ppm) followed by Pb (0.0026 ppm), V (0.0023 ppm) and Cd (0.0021 ppm). Biosorption of four metals was in the order of Cu>V>Pb>Cd in *Microcosmus squamiger, Microcosmus exasperates* and *Herdmania pallida*. But, in *Phallusia arabica* and *Styela canopus*, the order was V>Cu>Pb>Cd. The concentration factors were in the following order Cu >V >Pb> Cd in all ascidians studied except in *P. arabica* where, it was V > Cu>Pb>Cdand BAF was 9665, 2176, 592 and 7.1 for V, Cu, Pb and Cd respectively. The BAF is high in *P.arabica* followed by *H. pallida, S. canopus, M. squamiger* and *M. exasperates.* Thus, it is concluded that ascidians could be used as bioindicator of metals in seawater.

Introduction

The coastal environments have always attracted the focus of scientific research as they have rich biodiversity. The increase of anthropogenic pollutants in the past two decades have caused extensive damage to the life and activities of living marine organisms and even to mass mortality. Among the pollutants the analysis of metals in the marine forms is of global importance as it helps to predict the pollution status of marine habitat and the significant risks to species. These metals gain entry into the sea through atmospheric deposition, erosion of geological matrix or due to the discharge of industrial effluents, domestic sewage, mining wastes etc~~,~~. The marine organisms in particular the benthic species accumulate metals several times above ambient levels. These tolerant species in polluted coastal areas could serve as bioindicators.

Though there are plenty of methods

available, these processes may be ineffective or expensive, (Volesky, 1990). Therefore, the research for new cost effective technologies for the removal of heavy metals from the environment has been directed towards biosorption. The use of absorbents of biological origin has emerged in the last decade as one of the most promising alternatives for the control of environmental pollution caused by heavy metals. The major advantages of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, regeneration of biosorbents and possibility of metal recovery (David Kratchovil et al. 1998).

Fishes and mussels have been used widely to analyse the accumulation of metals. More recently,the study of metal analysis in various parts of the world have reported that members of the class Ascidiaceae called ascidians accumulate metals. These sessile benthic forms also called tunicates are found in the tropic oceans and also in the polar region. The sedentary nature, filter feeding habits presence of vanadocytes and the absence of kidneys(expect the family Molgulidae) facilitate them to accumulate metals. These species are commonly available in Thoothukudi coast of South India. So Thoothukudi harbour is selected as study area.

This coast is of ecologically important as it receives untreated urban effluents containing significant loads of organic matter and anthropogenic metals. This coast is one of the least studied areas in the Bay of Bengal which is polluted by metals to a greater degree. Hence this study focuses on the biomonitoring of metals using tunicates in Thoothukudi coast.

Materials and Methods

Study area

For the present investigation, namely Thoothukudi coast situated along the south east coast of India, was chosen. This station situated in the geographical coordinates of $8^0$48'N and $78^0$11'E, is one of the most important cargo as well as fishery harbours with full-fledged year-round mariculture activities. Establishment of chemical and fertilizer industries, copper smelter and thermal power stations near the Thoothukudi coast has led to the discharge of effluents from most of these industries into coastal water of Thoothukudi and hence considered to be ecologically significant station.

Study animals

For the present study, five solitary ascidians such as, *Phallusia arabica*, *Styela canopus*, *Microcosmus exasperates*, *Microcosmus squamiger* and *Herdmania pallida* have been chosen. They have hitherto been recorded from the Indian waters ie. from Thoothukudi (South East coast). These common large solitary ascidians breed throughout the year.

Preparation of water sample for metal analysis (APHA, AWWA and WEF, 21st edition, 2005)

250ml of water sample was taken in a beaker and 15ml of HNO_3 was added to it and heated until the water and acid mixture was reduced to 25ml. This was then made up to 50ml with distilled water and this solution was sent to analyze the metals by Atomic Absorption Spectrophotometer.

Sampling and Preparation of Samples of ascidians

Specimens of chosen five solitary ascidians were sampled in Thoothukudi coast, India. The epibionts, sand and silt adhered on the surface of the samples were removed and then oven dried at 110^0C for 24 hrs. The dried samples were made into powder and stored in polythene pockets for further analysis.

Metal analysis

Known quantity of tissue of each species was subjected to acid digestion in a crucible using a mixture of 3ml of concentrated sulphuric acid and 60% perchloric acid. The digested sample was then evaporated to near dryness. The crucible was then cooled at room temperature and the residue was dissolved in 20ml of 2N hydrochloric acid . This solution was subjected to centrifugation and filtered. The clear filtrate thus obtained was transferred to a clean screw capped tubes and used for further analysis.

The metals copper (Cu), vanadium (V), lead (Pb) and cadmium (Cd) were measured using Atomic Absorption Spectrophotometer (model name : AA-6300) at specific wavelength for each metals.

Bioconcentration factor (BCF)

Bioconcentration factor was calculated to estimate the amount of heavy metal input from the surrounding environment . Bioconcentration is defined as the net result of the absorption, distribution and elimination of a substance in an organism, after an exposure via water. The bioconcentration factor is the ratio between the chemical concentration in the organism and the chemical concentration in water at equilibrium:

$$BCF_{biota} = \frac{C_{biota}}{C_{water}}$$

Where,

C_{biota} is the chemical concentration in test organism in mg/kg (preferably wet weight), C_{water} is the chemical concentration in water, in mg/l, and BCF_{biota} is the bioconcentration factor for the test organism.

Results and Discussion

Bioaccumulation of metals in the five solitary ascidians is represented in the Figure No. 1 Biosorption of four metals in *Microcosmus squamiger* was in the descending order of Cu>V>Pb>Cd. Similar trend was observed in both *M. exasperates* and *Herdmania pallida.* But, in *Phallusia arabica* and *Styela canopus,* the order was V>Cu>Pb>Cd.

Maximum biosorption of V (22.23 ppm) was observed in *P. arabica* and minimum (0.44 ppm) was in *M. exasperates*. Cu accumulation was high (8.45 ppm) in *H. pallida* and low (1.1 ppm) in *M. exasperates* whereas, Pb showed maximum accumulation (1.74 ppm) in *S. canopus* and minimum (0.33 ppm) in *H. pallida*. Cd showed maximum concentration (0.0199 ppm) in *P. arabica* and minimum (0.0035 ppm) in *H. pallida.*

In all the ascidian species studied, *P.arabica* showed maximum biosorption of V with 21.23 ppm which is followed by Cu (4.57ppm), Pb (1.54 ppm) and Cd (0.002 ppm). Least accumulation of Cd was observed next to Pb. The range of Cd accumulation was from 0.0035 (*H. pallida*)to 0.032 in (*S. canopus*) whereas, Pb was accumulated in the range of 0.33 ppm (*H. pallida)* to 1.74 ppm (*S. canopus*).

Figure.1 Concentrations of metals in selected species of tunicates

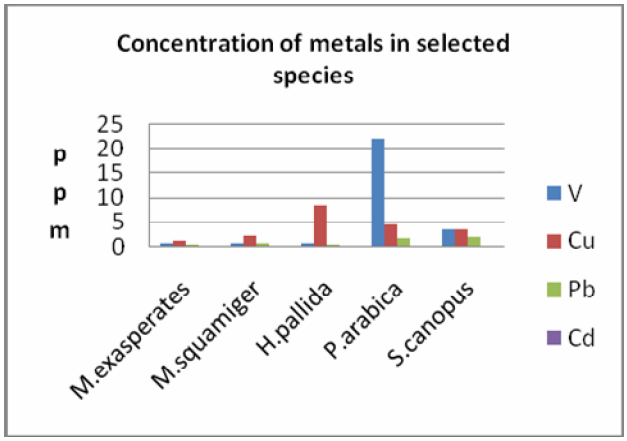

Table.1 Concentration of metals in water and ascidian species in ppm
Bioaccumulation factors are given in parenthesis

Metals	V	Cu	Pb	Cd
Seawater	0.0023	0.0028	0.0026	0.0028
M.exasperates	0.44	1.11	0.38	0.0109
	(191)	(529)	(146)	(3.9)
M.squamiger	0.56	2.07	0.53	0.0064
	(243)	(985)	(204)	(2.28)
H.pallida	0.55	8.45	0.33	0.0035
	(239)	(4023)	(127)	(1.25)
P.arabica	22.23	4.57	1.54	0.0199
	(9665)	(2176)	(592)	(7.1)
S.canopus	3.55	3.38	1.74	0.0322
	(1543)	(1609)	(669)	(11.5)

Table.2 Permissible levels of metals as prescribed by World Quality Standards

S.No	**Name of the metal**	**Permissible level(mg/kg)**
1.	Lead	**2**(FEPA, 2003).
2.	Copper	3(FEPA, 2003).
3.	Cadmium	0.005(FEPA, 2003).
4.	Vanadium	1.8(CAC,1993)

Seawater in Thoothukudi coastal region was also found to be contaminated with metals such as Cu, V, Pb and Cd. Concentration of metals in seawater around Thoothukudi coast were found relatively below recommended value (Table. No: 1). Among the four metals studied, Cu accounted for highest concentration (0.0028 ppm) followed by Pb (0.0026 ppm), V (0.0023 ppm) and Cd (0.0021 ppm).

The metal accumulation factor in the chosen ascidians was calculated by making use of the average concentration of metals observed in the seawater. The concentration factors were in the following order Cu >V >Pb> Cd for *H. pallida, S. canopus, M. exasperates* and *M. squamiger* whereas, it was V > Cu>Pb> Cd for *P. arabica(*Table No.1).

The coastal areas are preferred by mankind for both habitation and industries. The cities located near coasts are highly populated and millions of tons of domestic wastes and industrial effluents are generated. These untreated wastes are finally discharged into the sea. The study area Thoothukudi coast in Gulf of Mannar receives organic compounds from sewage, fly ash from thermal power station and chlorinated hydrocarbons from nearby industries

The presence of metals in Thoothukudi coastal water was contributed from variety of natural and anthropogenic activities and this could be substantiated with fact that this station is one of the most important cargo handling ports as well as fishing harbour with year round mariculture operations. Wastes from naval installations, unloading of bulk cargo such as coal, coke, sulphur, zinc-lead concentrations etc. at different periods of the years, the operation of anthropogenic inputs such as industrial effluents and domestic wastes all these might have polluted the medium with different pollutants including heavy metals. Further this area is protected within two parallel arms of North and South break waters resulting in the inability of substantial dilution of these metals with the open sea and is also considered to be highly industrialized area.

Among the pollutants, metals are significant as they accumulate in marine animals, enter the food chain and affect the health of marine animals and cause loss of biodiversity. In particular sedentary organisms are much affected. In the present study, the accumulation of metals in the five chosen species of ascidians was in the decreasing order of V>Cu>Pb>Cd.The present study clearly indicates that metals such as copper, cadmium, lead and vanadium are accumulated in the tissues of selected species of ascidians.

The accumulation of metals may be due to the direct uptake of polluted water and contaminated food from the marine environment by the filter feeding mechanism. The sedentary nature, filter feeding habits, presence of vanadocytes and the absence of kidneys (expect the family Molgulidae)of ascidians may also favors bio accumulation(Badsha,1991). When the metals could not be metabolized by the body, they accumulate in the soft tissues and they become toxic. The study area (Thoothukudi Harbour) is continuously under ecological stress due to the mixing of industrial and municipal run-off, untreated domestic pollutants, oil spills, leaching from solid-waste dumps and urban storm-water run-off. The urban effluents of Tuticorin thermal power

station (Asha,1999) and Southern Petrochemical Industries(Jeyaraju et al.,1996) play a significant part in polluting the coastal waters.The metals from land sources and the air borne particulate matter also gets deposited into the marine environment and contributes to metal contamination in the tunicates as they are closely attached to benthic surfaces.

Among the four metals analyzed in the five selected species, accumulation of Vanadium is higher in *Phallusia arabica.* This can be correlated with presence of vanodocytes, the special blood cells that contain the vanadobin which accumulates vanadium in the +3 and +4 oxidation states in the ratio of 45:55. Vanadobin maintains vanadium ions as vanadyl cations and has affinity for vanadium ion. The vanadium binding proteins in vanadocytes are named vanabins. The presence of vanodocytes and vanabins could be justified with the suggestions of Michibata et al.,(1986, 1990).The Vanadyl cations (IV) and V(III) are accumulated mainly in the two suborders of ascidians Aplousobranchia and Phlebobranchia.

Vanadium was first discovered in ascidians by Martin Henze in 1911. Vanadium exists in sea water as Vandate CH2 VO4 /HVO4-2) vanadium (+5) and it gets reduced to vanadium (+4) and then stored as vanadium (+3) (Dingley et al.1982; Brand et al. 1989 and Michibata et.al 2003).Vanadium in vanadocytes is also reported by many researchers (Kustin et.al, 1976; Brand et.al, 1989; Hirose, 1999, 2000; Frank et.al, 2003). Vanadocytes provide immune response to the ascidians as they maintain cells natural oxidation state and engulfs foreign bodies. Thus, vanadium is an essential trace element in ascidians. Vanadium provides anti-predatory defense for ascidians and hence sedentary ascidians survive better as they are deterred by predators. This may be the reason for the better survival of this species of ascidian. The invasive nature of Phallusia species reported by many researchers could be justified with this result. The presence of high concentration of vanadium in a sessile, invasive ascidian, *Ciona intestinalis* proves the anti-predatory and immune defense of vanadium which helps the invasive nature of species.

Next to vanadium, copper is observed to have high value of accumulation in *Herdmania pallida*. Copper is an essential component of metallo enzymes of living organisms and plays a crucial role in the synthesis of haemoglobin and catalysis of metabolic reactions. It is required in many biological enzyme systems that catalyze oxidation or reduction reactions. When the concentration of copper is relatively high in the environment, aquatic organisms are affected. Fish is found to be affected by the ionic forms of Cu such asCu^{2+}, Cu_2OH^+ and $CuOH^+$. The toxic effects of Copper include the increase in the rate of free radical formation, teratogenicity and chromosomal aberrations. The antifouling paints used on small vessels,(Claisse and Alzein, 1993), sewage discharges,(El-Shebly,1994; Reichelt-Brushett and Harrison, 2000 EI Gendy et al., 2003), fertilizers and herbicides used in coastal agricultural areas (Lena and Rao, 1997)are the major sources of copper. Free cupric ion is highly toxic compared to organic and inorganic copper complexes (Borgmann and Ralph, 1983). High concentration of copper is toxic to living organisms (Davis et al., 2000). Though copper is required for the normal growth and development of marine animals (Rainbow, 1998), it interferes with the metabolism and physiology of animals

when its concentration is high (Wright and Welbourn, 2002).The toxic effect of copper might have affected the immune system of larvae of Tunicates and hence its survival. Thus it might be the reason for decline in the number of adult Tunicates (Cima et al., 1998).

Next to vanadium and copper, the accumulation of lead is observed to be high in the present study.The combustion of petrol additives in automobiles and effluents discharged from battery manufacturing industries pollute soil with lead by atmospheric precipitation (Amusan et.al., 2002). Inorganic lead from these industrial sources, atmosphere and terrestrial sources entering the sea is present in the 2^{+} oxidation state. Lead interferes with the transport processes by binding to cell membranes of organisms. It prevents the normal functioning of enzymes and proteins binds with sulfur groups in enzymes and amino groups in proteins. Thus lead can interfere with metabolic activities of organism.The effect of lead on the reproductive mechanism of *Ciona intestinalis* has been reported to reduce the oocyte voltage gated sodium currents significantly by the inhibition of post-fertilization contraction.

In the present analysis of four metals, accumulation of Cadmium is observed to be lowest. Lowest value of cadmium is found in the solitary species, *Herdmania pallida*. This is in accordance with report of Tamilselvi et al(2010).Cadmium is widely distributed at low levels in the environment and does not play an essential role in living organisms including man. Cadmium is found to be toxic even in trace amounts. The toxic effect of Cadmium has caused a significant reduction in the success of fertilization of a coral species, *Aeropora tenuous* (Reichelt- Brushett and Harrison, 2000).The gradual increase in discharge of pollutants containing Cadmium might have caused accumulation of this metal and have affected the immune system and the reproductive rate of ascidians. This might have been the reason for the decline of diversity of ascidian species which are sensitive to Cadmium. This may have resulted in the limited availability of *Herdmania pallida* observed in the present study though they were reported to be abundant in the last decade.Tamilselvi et.al, (2012) also opined that the number of species of ascidians has been decreased due to increased ecological stress in Thoothukudi coastal area. The adverse effect of cadmium affecting the development and settlement of ascidian larvae reported by Zega et.al, (2009) coincides with the same result.

The results of the present study indicate that species of ascidians differ in their capacity to accumulate different metals (Heath, 1987). This may be due to the variations in the availability of metals and their role in metabolism of species. The suggestion of Heath (1987) and Selvaprabhu et al., (2012) also coincides with this result. Many researchers have reported inter specific variations of metal accumulation in ascidians (Abdul Jaffar Ali, 2004; Tamilselvi et.al,2011).

The Bioaccumulation factors (BAF) were in the following order Cu >V >Pb> Cd in all ascidians studied except in *P. arabica* where, it was V > Cu>Pb> Cd and BAF was 9665, 2176, 592 and 7.1 for V, Cu, Pb and Cd respectively. The BAF is high in *P.arabica* followed by *H. pallida*, *S.canopus, M. squamiger* and *M. exasperates.* Thus, it is concluded that ascidians could be used as bioindicator of metals in marine ecosystems.

The permissible level of lead in animal food is 2 mg /Kg (2ppm) (FEPA, 2003). The level of lead in the tissue of ascidians is low in all the 5 selected solitary species in present study. The permissible level of copper is 3 mg/kg in animal foods. (FEPA,2003). The present study indicates that the level of Copper exceeds the permissible level in 3 species *H.pallida, P. arabica* and *S.canopus* and the level of Copper is below the permissible levels in *M. exasperates* and *M. squamiger*,. The permissible level of Cadmium in animal foods is 0.005 mg/kg. (FEPA 2003, WHO,1985). Among the 5 selected species, the level of Cadmium is below the permissible level in *H.pallida* and is above the permissible level in all the other four selected solitary species. The permissible limit of Vanadium in foods is 1.8 mg (CAC, 1993). Among the 5 chosen species, the level of V is below the permissible level in *Microcosmus squamiger* and *Herdmania pallida*, and is above permissible level in *Phallusia arabica* and *Styela canopus*.

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