

Quantitative Estimation of Heavy Metals in Colonial Ascidian *Eudistoma Viride*

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Abstract

Heavy metals are natural constituents of the earth's crust. Prolonged exposure to heavy metals such as cadmium, copper, lead, arsenic, chromium, mercury and zinc can cause deleterious health effects in humans. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage and are also classified as human carcinogens. Most recently, ascidians belonging to the sub-phylum urochordata are used as food with lot of proteins and lipids. In India, the study on accumulation of metals in ascidians is meagre. The present study aims at analysing the heavy metals in the marine colonial ascidian *Eudistomaviride*. Accumulation of heavy metals like copper, cadmium, lead, arsenic, zinc and mercury in the selected animal sample was performed by atomic absorption spectrophotometer (AAS). Measurements were made using a hollow electron discharge lamp (EDL) at wavelengths of 220.62 nm, 228.80 nm, 283.31 nm, 193.70 nm, 240 nm and 253.7 nm respectively. This study indicated that the risk of heavy metal contamination in this ascidian appears low.

Keywords: Atomic absorption spectroscopy, *Eudistomaviride*, heavy metal.

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. This coast is ecologically important as it receives untreated urban effluents containing significant loads of organic matter and anthropogenic metals. It is one of the least studied areas in the Bay of Bengal which is polluted by metals to a greater degree. Concentration of heavy metals in the sea water and animal tissues may not be high enough to kill the organisms, but are presumably sufficient to destroy their food and have physiological, teratogenic, mutagenic, carcinogenic effects on organisms. Ascidians, commonly called as “Sea Squirts” are filter feeding organisms. They are marine sedentary

S.Sankaravadivu and H.Kohila Subathra Christy organisms belonging to biofouling community found in piers, pilings, harbour installations, materials used in aquaculture operations etc. *Eudistomavirideis* a colonial ascidian belonging to the family polycitoridae and it is available all over India. *Microcosmussulcatus*, *Styelaplicata* and *Polycarpapomaria* are taken as food in the Mediterranean.^[1] *Halocynthiaroretzi* in Japan, is cultured in the North of Honsyu^[2] for human consumption and *Pyura chilensis* is popular in South America^[3] as a food source. Margalino and Destefano found that the flesh of *Microcosmussulcatus* is almost as digestible as whole egg and the protein content higher.^[4] Previous studies show that *Eudistoma viride* possesses antioxidant and antifouling activity^[5-6]. Earlier reports are available for the determination of heavy metal indifferent ascidians. Metal accumulation by ascidians and their role as bioindicators of water pollution was reported by Meenakshi 1997, 2010^[7, 8]. Heavy metal studies in some selected tunicates were investigated by Jafarali *et al.*, 2004; Tamilselvi *et al.*, 2010, 2011; Selva prabhu *et al.*, 2012; Radhalakshmi *et al.*, 2014 and Jaffar ali *et al.*, 2015^[9-12]. Determination of heavy metal in simple ascidian *Phallusia nigra* was carried by Shanmuga Priya *et al.*, 2016^[13]. The objective of this work is to investigate the magnitude of heavy metals contamination in the animal under study by Atomic Absorption Spectroscopy.

Materials and Methods

Collection and Identification

Eudistoma viride (Fig.1) was collected from the undersurface of calcrete rocks from Thoothukudi North Break water and identified using Key to identification of Indian ascidians.^[7] A voucher specimen 3047 is deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamilnadu, India.



Figure: 1 Colony of *Eudistomaviride*

Systematic Position

Eudistomaviride belongs to Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Aplousobranchia, Family: Polycitoridae, Genus: *Eudistoma* and Species: *viride*. The metals selected for AAS were ^[14] copper, cadmium, lead, arsenic, zinc and mercury.

Sample Preparation

For estimation of heavy metals, 1 g of sample was digested with a 1:4 ratio of nitric: perchloric acid over a hot plate. The samples were digested till a white-colored solution was formed. When the samples were completely digested, distilled water was added and the solutions were allowed to cool. These samples were filtered, the solute part was separated and the volume was made up to 25 ml with distilled water. These filtered samples were directly used to determine heavy metal concentration by AAS. For blank either 0.1% or 1% nitric acid or demonized water was used. The blanks were run in triplicate to check the precision of the samples.

Standard Preparation

For each of the selected metals a standard linear calibration curve of various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm were analysed by AAS. To ensure that the method has wide adaptability and good accuracy.

Instrumentation

Analysis of the heavy metal in the selected animal sample was performed by (Elico-SD 164 India) (LithnorandGoran, 1975) in SDMRI atomic absorption spectrophotometer (AAS). Measurements were made using a hollow electron discharge lamp (EDL) for copper, cadmium, lead, arsenic, zinc and mercury at wavelengths of 220.62 nm, 228.80 nm, 283.31 nm, 193.70 nm, 240 nm and 253.7 nm respectively.

Atomic Absorption Spectrophotometer - Instrumentation

Analysis was performed by testing samples at three different concentrations 0.5000 ppm, 1.0000 ppm and 1.5000 ppm to ensure that the method has wide adaptability and good accuracy.

Results and Discussion

The results of heavy metals determination in a selected colonial ascidian *Eudistomaviride* is presented in Table 1.

Table1. Level of heavy metals in *Eudistomaviride*

Copper	Cadmium	Lead	Arsenic	Zinc	Mercury	MDL ^a
ND	ND	ND	ND	ND	ND	0.01mg/kg

ND: Not detected; MDL: Minimum detection limit; ^an=3.

Atomic absorption spectrometry detection was carried out on positive ionisation mode because this mode gives sharp and sensitive signals. It was optimised by using a standard linear calibration curve for various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm (three points). The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each compound. The heavy metals (copper, cadmium, lead, arsenic, and mercury) were analysed at their particular wavelength and the ion with the uppermost intensity was selected as the basic ion. The study revealed that no resultant spectral peaks of Cu, Cd, Pb, As and Hg in *Eudistomaviride* was observed (Table1). The accumulation of metals may be due to direct uptake of polluted water and contaminated food from the marine environment by the filter feeding mechanism. The results of the present study indicate that species of ascidians differ in their capacity to accumulate metals. This may be due to the variations in the availability of metals and their role in metabolism of species ^[15,11]. Many researchers have reported inter specific variations of metal accumulation in ascidians ^[9].

Conclusion

It can be concluded that heavy metals was not found in the colonial ascidian *Eudistomaviride*. Ascidians act as bioindicator of metals in marine ecosystems. Hence it is advisable to take this kind of ascidians in our regular diet.

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