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Research Article

Determination Of Trace Elements In Ascidians Composed In The Tuticorin Coastal Area (Tamil Nadu)

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ABSTRACT

In recent years reported that the children who die worldwide with nutritional deficiency related disease like malnutrition, anemia etc. Hence there is a need to promote an alternative and cheaper source of nutritive food from non-conventional sources to meet the needs of poor people. Ascidians are also consumed as food in the form of various preparations in many parts of the world including Chile (Probecho), France (Figueodemer), Korea (Meongge), Italy (Limone di mare, Uova di mare), Japan (Hoya, Maboya) etc., Thus the study focused on tunicates. Tunicates were collected with the help of Scuba divers from Tuticorin coastal area for a period of 6 months. The Powdered samples were used for the analysis of trace elements. Sodium (Na), Potassium (K) and Calcium (Ca) levels were estimated using Flame Photometer and Iron (Fe), Zinc (Zn), Manganese (Mn), Magnesium (Mg) and Selenium (Se) were measured by Atomic Absorption Spectroscopy (AAS). The observed results indicate clearly that the selected species of tunicates such as, *Phallusia arabica, Styela canopus, Microcosmus exasperates and Herdmania pallida.* The results showed that the significantly increase in the level of trace elements in four tunicates. Hence the study proved that the Ascidians could be used as a dietary source for the Thyroid disease, Polycystic kidney disease, Diabetic mellitus patients which would reduce the risk factors like hypertension, osteoporosis, cardiovascular problems, osteodystrophy, anemia etc.,.

INTRODUCTION:

The elements like calcium (Ca), sodium (Na), potassium (K) and heavy metals like manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and non-metal selenium (Se) are considered "Trace elements (TE)". In human Mn, Fe, Cu Zn and Se accomplish decisive functions to maintain human health. Veeramuthuamri and Isabel (2013) studied that such high blood pressure, anemia, weak bones and poor nutrition occurred due to variation in the level of elements like serum calcium (Ca), potassium (K) and decrease in the level of serum sodium (Na), Iron (Fe), zinc (Zn), manganese (Mn), and selenium (Se). So, it would be found as TE leads to undesirable pathological conditions that can be prevented or reversed by adequate supplementation (Fraga, 2005). Hence, the work aimed that the identification of natural sources which contains all the essential elements could be edible to human in south coastal area (Tuticorin), Tamil Nadu, India.

Trace elements include, at least, the transition metals vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu) zinc (Zn), and molybdenum. And the non-metals are selenium (Se), fluoride (F) and iodine (I). All of these are belong to the category of micronutrients, which are needed by the human body in very small quantities (less than 100mg/day). As opposed to elements considered macronutrients such as sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), chlorine (Cl) etc., which are required in larger quantities. Trace elements (TE) are essential components of biological structure and functions (Fraga, 2005). Trace elements like Copper, Manganese and Zinc are essential micronutrients with a human requirement of no more than a few milligrams per day. However, micronutrients such as cadmium and Pb may become harmful when their ingestion rates are too high or inhalation is excessive (WHO, 1996). Deficiencies, excess, or imbalances in the supply of inorganic elements from dietary sources have been reported to a have deleterious influence on human health (Santos *et al*., 2004).

In recent years reported that the children who die worldwide with nutritional deficiency related disease like malnutrition, anemia etc. Hence there is a need to promote an alternative and cheaper source of nutritive food to meet the requirements of needy people. According to earlier reports the ocean has vast storage of flora of flora and fauna which is found to be effectively utilized as food for the ever growing and ensuing population on earth. Abdul Jaffar Ali (2004); Tamilselvi *et al.,* (2010; 2011) studied that the biodiversity of ascidians in Tuticorin coast and they are also suggested that it could be used as non-conventional edible organisms especially in India.

Tunicates are also known as ascidians or sea-squirts which are marine invertebrates belonging to the phylum Chordata, sub-phylum Urochordata. Chordata is the same phylum that humans are in. Even though tunicates look like sacks of water or sheets of colorful jelly, they are under the invertebrate group but most closely related to us. During their tadpole-like larval phase they possess critical chordate characteristics such as a notochord, a dorsal tubular nerve cord, and pharyngeal slits. Although not all of these traits are retained in the adult stage, in their larval stage they look very much like other developing chordate embryos, including humans (Simkanin and Noble, 2011). Shenkar and Swalla (2011) reported that the tunicates are diverse and abundant members of benthic marine invertebrate communities and studies on their biology and ecology are providing critical insights into evolution and development, natural products chemistry, physiology, biogeography, the impacts of climate change and species invasions. Hence the present study also to be focused on tunicates and human physiology.

Van Campenhout *et al.,* (2006); Silverberg *et al.,* (2003) revealed that more than 30% of diabetic patients have cardiovascular problems, mostly in older age group, which consistent with other studies reporting that about 20-40% of all patients with cardiac problems have diabetes mellitus (DM), which constitutes a major risk factor for congestive heart failure, a situation that is worsened by anemia which may appear early in diabetes, even before the onset of chronic renal failure. In such case, anemia is associated with blood levels of erythropoietin that are in appropriately low for the level of Haemoglobin. The main causes of anemia in elder DM patient may be due to Fe deficiency anorexia and reduced Fe intake and absorption. Thus the combination of heart disease and premature anemia could partly explain the high prevalence of cardiac failure in diabetic patients and its severe prognosis.

Millar (1971) stated that the presence of minerals, iodine, vitamins, fat and digestible nature of proteins. Tamilselvi (2008) analyzed the seasonal variations of carbohydrates, proteins and lipids in commonly available tunicates at two stations in

Tuticorin coast. So, the present study is the follow up of Tamilselvi (2008). The study will provide the additional steps to minimize the malnutrition, awareness regarding the Tunicate as natural food in India and it also prove the medicinal value for certain recently found common metabolic and genetic diseases like hypo and hyper thyroidism, diabetes mellitus, kidney disease and cardiovascular problems. Hence this study might be the first study in finding out the level of trace elements in tunicates in Tuticorin coast of South India.

Materials and Methods:

Study subjects:

Ascidians were collected with the help of Scuba divers from Tuticorin coastal area situated along the South east coast of India for a period of every 3 months. The collected tunicates were washed with sea water to remove the sand and silt. The epibionts were removed by the light application of brush, and then the samples were dried in an oven at 110^{o}C for about 24 hours. The dried samples were grind into fine powder (Radhalakshmi *et al.,* 2014). The Powdered samples were stored at 4^{0}C.

Biochemical analysis:

The powdered samples were used for the analysis of trace elements. Calcium (Ca), Sodium (Na) and Potassium (K) levels were estimated using Flame Photometer (ELICO-SL-173) and Iron (Fe), Zinc (Zn), Manganese (Mn), Magnesium (Mg) and Selenium (Se) were estimated using Atomic Absorption Spectroscopy (AAS): (ELICO-SL173) (Dalman *et al.,* 2006; Veeramuthumari, 2007, Veeramuthumari and Isabel*.,* 2013).

Statistical analysis:

The mean and standard error was calculated for trace elements in three time analysis of ascidians sample. The significant differences between the levels of trace element among the ascidians were analyzed by *student t test.*

Results and Discussion:

The observed results indicate clearly that the selected species of tunicates such as, *Phallusia arabica, Styela canopus, Microcosmus exasperates and Herdmania pallida* contains the trace elements like Calcium (Ca), Sodium (Na), Potassium (K), Iron (Fe), Zinc (Zn), Manganese (Mn), Magnesium (Mg) and Selenium (Se) (Table:1). The results showed that the presence of trace elements in four tunicates. Among these four tunicates, the study also demonstrated that significantly increase (at $p<0.0001$) in the level of Calcium (Ca), Sodium (Na), Potassium (K), Iron (Fe), Manganese (Mn) and Magnesium (Mg) in *Herdmania pallida* when compared to other ascidians **(Table:1).** Hence the study proved that the Ascidians could be used as a dietary source for the thyroid disease, polycystic kidney disease, diabetic mellitus patients and would reduce the risk factors like hypertension, osteoporosis, cardiovascular problems, osteodystrophy, anemia etc.,.

Table: 1 The Levels of Calcium (Ca), Sodium (Na), Potassium (K), Iron (Fe), Zinc (Zn), Manganese (Mn), Magnesium (Mg) and Selenium (Se) in Ascidians:

Trace elements	*Phallusia arabica*	*Styela canopus*	*Microcosmus exasperates*	*Microcosmus squamiger*	*Herdmania pallida*
Calcium (Ca)	119.158 ± 0.069	110.201± 0.152	165.27 ± 3.57	153 ± 6.10	195.27 ± 3.57*
Sodium (Na)	127.699± 0.611	105.692± 0.906	140.44 ± 10.31	163 ± 6.10	183.12 ± 4.48*
Potassium (K)	44.386± 0.061	67.589 ± 0.077*	57.45 ± 1.43	66.589 ± 0.077*	57.45 ± 1.43
Iron (Fe)	164.776 ± 1.381	122.809 ± 0.700	79.73 ± 4.45	122.809 ± 0.700	179.73 ± 4.45*
Zinc (Zn)	32.028 ± 0.038	41.301 ±0.033*	28.08 ± 2.06	38.08 ± 2.06	40.61 ± 0.89
Manganese (Mn)	118.5± 1.9	110.6 ±0.6	75.36 ± 15.68	34.93 ± 19.11	177.84 ± 4.08*
Selenium (Se)	90.5 ± 0.640 *	79.1 ± 0.034	60.67 ± 0.71	24.22 ± 0.34	44.62 ± 1.22

***-** *Highly significant at p<0.0001*

Values are Mean ± standard error in mE/L.

Ascidians body is found to be covered by a substance called by tunic, which consists predominantly of polysaccharides and proteins. Cheney *et al.,* (1997) reported that ascidians accumulate heavy metals including manganese, magnesium, iron, molybdenum, niobium, tantalum,

chromium and titanium. Trace elements are reported to be either essential or beneficial to humans. All essential minerals are regulatory pathway that maintains optimal tissue concentration in spite of variations in dietary supply. Some elements, such as Cu, Mn, and Zn are essential micronutrients with a human requirement of the more than a few milligrams per day (WHO, 1996). Also deficiency, excess, or imbalance in the supply of inorganic elements from dietary sources found to be an important deleterious influence on human health (Santos, 2004).

Based on the functions of these TE, on their dietary origin and on the diseases and pathological situations developed because of TE deficiency or toxicity, an appropriate intake of TE is a relevant aspect of a health diet. The presence of TE in food is often determined by the availability of metals in the soil. Thus, within a geographical region with soils deprived of a TE, its population is at risk and TE supplementation becomes necessary. Such supplementation has been implemented or is being evaluated in several places around the world by adding the appropriate TE basic foods (milk, flour etc.) (De Romana *et al.,* 2005; Hurrell *et al.,* 2004). The present study suggested that ascidians also used as dietary sources for trace elements.

Locatelli *et al.,* (2004) and Munro (2000) reported that supplementation becomes necessary in several disease treatments like anemic conditions in kidney dialysis and physiological conditions like extensive blood loss during menstruation. Unfortunately, in recent years the avalanche of uncontrolled supplementation with TE has put some TE on the border of toxicity in several populations. Thus, it is a crucial priority to define the requirements for TE, based on essentiality and health promotion, and the limits for toxicity. Further, to define a value for a determined nutrient, it is necessary to consider different aspects in the metabolism of this nutrient. For example, the requirements for Fe are calculated from a factorial model that includes basal Fe losses, menstrual losses, fetal requirement during growth and or increased tissue and storage of Fe (Kennedy and Meyers, 2005). As a consequence the Fe requirement for women varies significantly depending on the age and condition of the women.

Fraga (2005) reported that Mn, Fe, Cu, Zn and Se accomplish functions essential to maintaining human health. Deficiency in any of these TE leads to undesirable pathological conditions that can be prevented or reversed by adequate supplementation. In sufficiently nourished persons, supplementation should be carefully controlled, given the toxic effects ascribed to TE when present at levels that exceed those required for accomplishing their biological functions. Trace elements perform functions indispensable for maintenance of life, growth and reproduction. Inadequate levels of some elements may impair cellular and physiological functions or cause illness while other elements, even though present in low concentration, may be toxic. In addition to the vital role that trace elements play in enzymatic reaction, they have been examined critically as potential key factors in varied diseases including cancer, cardiovascular disease and renal disease (Sullivan *et al.,* 2013). Sullivan *et al.,* (2013) also reported that the evaluation of the trace elements role in health and disease becomes difficult due to metabolic interactions among the trace elements themselves.

People with chronic renal failure are reported to develop complications such as high blood pressure, anemia, weak bones and poor nutrition due to increase in the level of elements like serum calcium (Ca), potassium (K) and decrease in the level of serum sodium (Na), and Iron (Fe). This might also increase the risk of having heart and blood vessel diseases. It might eventually lead to kidney failure (Fraga, 2005).Iron is a mineral needed for healthy blood cells and for overall good health. Iron helps the body function in many ways. One way is to help make red blood cells. Red blood cells carry oxygen from lungs to all parts of body, which gives the energy needed for daily activities. The kidneys signal the body to make enough red blood cells, and iron helps in making healthy red blood cells (www.kidney.org, National Kidney Foundation, 2003). Anemia is an almost invariable feature of progressive renal disease and its most important cause is decreased production of erythropoietin, but reduced iron intake might also contribute to anemia. The prevalence of anemia increases as kidney function decreases and certain subgroups are at increased risk of anemia. Several studies investigated the effects of preventing progressive renal anemia (McClellan *et al.,* 2004; Mafra *et al.,* 2008).

Trace elements and other associated diseases:

Thyroid dysfunction:

Sodium, potassium, calcium are next to each other in the periodic table and form a square on the left side (Edmonds and Smith, 1999; Veeramuthumari *et al.,* 2011). There are strong interactions between

these elements. The balance between these minerals seems to be critical to health and is very critical for thyroid health. Excess amounts or deficiencies of any one of the three may severely disrupt thyroid function (Disashi *et al.,* 1999). Copper and zinc are the two metallic minerals with critical thyroid functions. Copper deficiency has been reported to interfere with the proper functioning of both potassium and magnesium and zinc has been reported to be more related to sodium and calcium metabolism. It is also stated that all of these minerals are involved in the production, degradation or cellular response to thyroid hormone (Disashi *et al.,* 1999).

Obesity:

Obesity is a worldwide disease affecting population of all age groups and all socio-economic levels, in both developed and developing countries. It is known to be a contributory risk factor for several disease states, including diabetes mellitus (Al-Saleh *et al.,* 2006; Kahn and Flier, 2000). Trace elements are suggested to be the essential nutrients with regulatory, immunologic, and antioxidant functions resulting from their action as essential components or cofactors of enzymes throughout metabolism (Lobo *et al.,* 2010). Trace elements and minerals influence the pathogenesis of obesity and diabetes and their complications, mainly through their involvement in peroxidation and inflammation.

Yerlikaya *et al* (2013) reported that the levels of Zn (P<0.001), Mn (P<0.05), Fe (P<0.05) were significantly lower and the level of Cu (P<0.001) and Cu / Zn ratio (P<0.05) were significantly higher in the diabetic obese women than those of the healthy women. The levels of Zn and Fe were also shown to be significantly lower and the levels of Cu to be significantly higher in the non diabetic obese women than those of the healthy group. They have shown that the serum Zn levels negatively and serum Cu levels positively correlated with anthropometric values in diabetic and non diabetic obese women. Further, serum Zn, Mn and Cr levels were shown to be negatively correlated and a serum Se level was reported to be positively correlated with glycemia control parameters in diabetic obese women. In addition, a serum Zn level was reported to be negatively correlated with high-sensitivity C-reactive protein (hsCRP) in diabetic and nondiabetic obese females. Their findings showed significant association between Zn and Fe deficiencies and obesity. Obese women with diabetes are reported to be at a greater risk of developing imbalances and deficiencies of trace elements compared with obese women without diabetes (Yerlikaya *et al.,* 2013).

Diabetes mellitus:

Diabetes mellitus is a state of sustained hyperglycemia due to absolute or relative insulin deficiency or inactivity (Nsirim, 2000). There is progressive increase in the global prevalence of diabetes probably due to life style changes (Shaw *et al.,* 2010). The current estimate shows that more than 285 million people worldwide are affected by the disease representing 6.4% of the world's population (Shaw *et al.*, 2010). It has been predicted that the worldwide estimate of diabetes will reach 7.7% by the year 2030 (Shaw *et al.*, 2010). Nigerian diabetic population is 3.9% as shown in 2010 (Shaw *et al.*, 2010). Deficiencies of micronutrients may increase susceptibility of diabetic mellitus and the associated complications (Aliyu *et al.*, 2005; Wali *et al.,* 2011).

Although diabetes mellitus is defined in terms of high blood glucose level, it is associated with multiple metabolic, endocrine and haematological changes which are important in the pathogenesis of the disease and its complications (Carl and Burtis, 2001). Micro and macro vascular complications associated with diabetes mellitus are reported to be responsible for significant morbidity and mortality (WHO 1994. Nsirim, (2000) has stated that the diabetic population is characterized by higher rate of blindness, kidney disease, gangrene and coronary heart disease several times more than non-diabetics

Cardiovascular disease:

Trace elements play a vital role in maintenance of health and development of optimal physiological function (Saraymen *et al.,* 2003). Deficiencies of some trace elements have been shown to have marked alterations in lipid and lipoprotein metabolism. There are some contradictory findings regarding the relationship between serum trace elements with lipid and lipoproteins (Sulibursk*a et al*., 2011). Sulibursk*a et al*., (2011) have evaluated the serum concentrations of trace elements such as copper, zinc, and manganese and have correlated them with lipid profile of adult men in Mosul City, Iraq.

Chausmer, (1998) and Disilvestro (2000) reported that Zinc (Zn) is involved in the synthesis, storage, secretion and conformational integrity of insulin monomers and that Zn assembles to a dimeric form for storage and secretion as crystalline insulin. Lower levels of Zn might affect the ability of pancreatic islet cells responsible for the production

and secretion of insulin, such as in type II DM. Epidemiological studies have reported decreased plasma and intracellular Zn concentrations in conjugation with increased urinary Zn excretion in diabetic patients and have also stated that low Zn intake increases the risk of coronary heart disease (Singh *et al.,* 1998).

Polycystic kidney disease:

It has been reported that patients with chronic kidney disease have an abnormal metabolism of zinc, selenium and many other minerals (Kalantar-Zadeh and Kopple, 2003; Mafra *et al.,* 2008). El-Khawaga and El-sayed, (2012); Pietrzak *et al.,* (2002) have reported that, there is significant decrease in the concentration of Zn, Cu, and Mn in dialysis group compared to the control group. Zinc deficiency has also been reported to cause immune deficiency and susceptibility to other infections (Fischer and Black, (2004); Batista *et al.,* (2006)). Zima *et al.,* (1999) have stated that the deficiency of manganese leads to impaired growth and abnormal metabolism of glucose and lipids. Guo *et al.,* (2011) and Guo and Wang, (2013) have also reported low plasma Zn concentrations during dialysis treatment.

Essential trace elements are involved in a number of metabolic activities, including neuroconduction, transport, excretory processes and serving as cofactors for enzymes (Chawla 1999; Zachara *et al.,* 2000). Some of the trace elements like Selenium, Zinc, Manganese, Magnesium and Copper are cofactors or structural components of antioxidant enzymes (Rakhshanizadeh and Esmaeeli, 2014). Moreover, selenium and glutathione peroxidase play an important role in protecting cell membranes from oxidative damage and decreased blood selenium, and are common in chronic renal failure patients. However, chronic renal failure may result in impaired renal excretion and accumulation of some trace elements in the body. During dialysis few trace elements found to be accumulate in the body as dialysis fluid impurities and some may be removed from blood to dialysate leading to deficiency of those trace elements in the body (Grzegorzewska, 2004).

In humans, manganese (Mn) deficiency is associated with nausea, vomiting, poor glucose tolerance (high blood sugar levels), skin rash, and loss of hair color, excessive bone loss, low cholesterol levels, dizziness, hearing loss, and compromised function of the reproductive system (Guo and Wang, 2013). Severe manganese deficiency in infants can cause paralysis, convulsions, blindness, and deafness. Lee and Kim, (2011) have presented data on the association of manganese (Mn) level with hypertension in a representative sample of the adult Korean population who participated in the Korean National Health and Nutrition Examination Survey (Lee and Kim, 2011). Iron deficiency also increases blood Mn level in the general population (Lee and Kim, 2011; Kazi *et al.,* 2008). All patients undergoing hemodialysis presented elevated serum manganese levels (da Silva *et al.,* 2007).

The Manganese (Mn) is rich in liver and kidney. Mn serves as a cofactor for several enzymes and is required for the formation of bone, proper reproduction and normal functioning of nervous system and it inhibits lipid peroxidation. Conflicting results have been reported regarding serum levels of Mn in hemodialysis patients, like there are reports of low, normal and high levels of Mn in chronic dialysis patients (Zima *et al.,* 1999). Morever, this study showed that there is a significant decreased level of Mn in pre and post dialysis group when compared to the control groups which are in agreement to the findings of Pietrzak *et al* (2002). Impaired growth and abnormal metabolism of glucose and lipids have been reported to be due to the deficiency of Mn (Ramprasad and Mohammed, 2013).

Sullivan *et al.,* (2013) have stated that, low serum selenium values have been associated with low zinc, calcium, and magnesium levels in cirrhotic patients.Selenium is a trace mineral that our bodies used to produce glutathione peroxidase. Glutathione peroxidase is part of the body's antioxidant defense system. It also works with vitamin E to protect cell membranes from damage caused by dangerous, naturally occurring substances known as free radicals (Rakhshanizadeh and Esmaeeli, 2014).

China has very low rate of colon cancer, presumably because of the nation's low-fat diet. However, in some parts of China where the soil is depleted of selenium, the incidence of various types of cancer is much higher than in rest of the country. This fact has given rise to a theory that selenium deficiency is a common cause of cancer also and that the Se supplements can reduce this risk (Harrison *et al.,* 1997). It is required for a well-functioning immune system and could be suggested as a treatment for people with HIV (Constans *et al*., 1995; Baum *et al.,* 1997; Campa *et al.,* 1999).

Trace elements, Se, Cu, and Zn play an essential role in the antioxidant defense system. Free radicals damage different tissues or organs, hence the trace

and toxic elements have differently damaged various organs. Tonelli *et al.,* (2009) have shown that in patients with CKD, the levels of cadmium, chromium, copper, lead and vanadium are higher and the levels of selenium, zinc and manganese are lower than in healthy individuals. Korstanje, and DiPetrillo (2004), Bethesda (2003) and USRDS (2003) reported that chronic kidney disease also contributes indirect medical costs because it is a risk factor for cardiovascular disease, including myocardial infarction, atherosclerosis, stroke, and hypertension. Mortality from myocardial infarction, stroke, and coronary artery disease escalates with increasing urinary albumin levels, and the link between chronic kidney disease and cardiovascular disease is supported by numerous other studies

The study concluded that the trace elements are essential nutrients for metabolism, growth and neurological and immunological functions (Marriott *et al.,* 2007). There are 2,300 species of ascidians and three main types: solitary ascidians, social ascidians. Hence, ascidians would be consumed as food in various parts of the world due to its high nutritional and medicinal value. Tamilselvi *et al.,* (2008, 2010, 2011) and Radhalakshmi *et al.,* (2014) reported that the biochemical components like carbohydrate, protein, lipids and amino acids were quantitatively estimated in *H. pallida*. The present study also quantified the levels of trace elements such as Calcium (Ca), Sodium (Na), Potassium (K), Iron (Fe), Zinc (Zn), Manganese (Mn), Magnesium (Mg) and Selenium (Se). *Herdmania pallida* is found to be the reich source of above measured trace elements when compared to other ascidians.

Future focus:

The study also interested to find out the presence of thyroid hormones in *Phallusia Arabica*, *Styela canopus*, *Microcosmus exasperates*, *Microcosmus squamiger*, *and Herdmania pallida.* It would be very much applicable for the treatment of thyroid problems.

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