Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
ISSN NO. 2320-5407
Article DOI:10.21474/IJAR01/ 9491
DOI URL: http://dx.doi.org/10.21474/IJAR01/9491

RESEARCH ARTICLE

THE ASCIDIAN MICROCOSMUS EXASPERATUS AS BIOINDICATOR FOR THE EVALUATION OF WATER QUALITY IN ESTUARIES.

Rafael Metri[1], Gésica da Costa Bernardo Soares[2], Pablo Damian Borges Guilherme[1] and Luis Fernando Roveda[1].

1. Departamento de Ciências Biológicas, Universidade Estadual do Paraná, campus de Paranaguá, Rua Comendador Correia Junior, 117, CEP 83203-280, Paranaguá, PR, Brasil.
2. Programa de Pós-Graduação em Ciência e Tecnologia Ambiental, Universidade Tecnológica Federal do Paraná - Campus Curitiba.

……………………………………………………………………………………………………....

Manuscript Info

……………………….

Manuscript History
Received: 05 June 2019
Final Accepted: 07 July 2019
Published: August 2019

Key words:-
Ascidiacea, Chemical elements, Metal concentration, Microcosmus exasperates, Pollution

Abstract

………………………………………………………………

Anthropogenic activities cause a great impact in marine biota. In estuarine environments, the sea squirts, sessile marine invertebrates, can be used as bioindicators and environmental quality biomonitors. The ascidian *Microcosmus exasperatus* was used in this study to compare two sites with different pollution stress. In the laboratory the individuals were dissected separating the inner soft body and the tunic. This body parts were weighed and processed to determine the concentration of various chemicals, including heavy metals. The water content of tissues showed significant differences between sites, with higher values in antropized site, indicating stress of pollution. Similarly, the condition factor presented differences between sites, which may be related to factors such as hydrodynamics, tidal cycle, but also indicate environmental pollution. In chemical analysis, it was observed that the ascidian retains higher concentration of certain chemicals in inner body than in tunic. Some chemical also showed different concentrations between places. The species studied is able to absorb a significant percentage of highly toxic elements, showing to be a good biomonitor for some metals.

……………………………………………………………………………………………………....

Introduction:-

The Estuary Complex of Paranaguá (ECP), which bathes the city of Paranaguá and other municipalities of the region (South of Brazil), connects the ocean to the Paranaguá Port, one of the largest port complexes in Latin America [1]. ECP is considered an estuary area with a high degree of complexity due to its pattern of multiple entrances and compartments, with three major entrances and interconnected and well developed internal water bodies. Therefore, it shows a high diversity of coastal ecosystems such as mangroves, marshes, restingas and islands with rocky outcrops, which support a rich biodiversity [2]. It is one of the main biodiversity hotspots in the country and it is considered as a priority area for conservation [3]. ECP is also a scenario of activities in conflict with the conservationist principles and consequently with the sustainable development, altering and negatively impacting the environment and the traditional communities that use the natural resources of the regionn [4,2].

In the ECP is located the Paranaguá Port, the second largest Brazilian port and the largest bulk carrier port in Latin America, also handling many other types of containerized and palletized cargoes. Then, with the port expansion

Corresponding Author:-Rafael Metri.
Address:-Departamento de Ciências Biológicas, Universidade Estadual do Paraná, campus de Paranaguá, Rua Comendador Correia Junior, 117, CEP 83203-280, Paranaguá, PR, Brasil.

plans, causes inherent impacts with the massive presence of companies and industries that interact daily with the port complex, making urgent the need of environmental monitoring in its area of coverage [5].

The high traffic of small, medium and large vessels, boats and ships in port areas [6], together with the effluents from the maritime terminals or household waste and also the local agriculture, which can be dumped with no treatment at the sea, these are routine situations observed in the region. Such waste, and especially oils and fuels, may contain organic compounds and other toxic elements, including heavy metals. These substances are present among the most abundant classes of pollutants in the estuarine areas [7] and massively compromise the quality of the water resources in the world [8].

Some organisms are able to answer or record the presence and concentration of pollutants in the water, not only at a precise time but over a long period of time in which they have been exposed to them due to the bioaccumulation in their tissues. Sessile aquatic animals, which remain at the same recruitment place under the influence of environmental conditions, can be used to monitor local water quality [9]. Thus, filtering animals can be used as representative bioindicators of water quality, as they may reflect the conditions present in the water and the suspended material used as food and accumulating the elements in their tissues [10].

Some ascidians are used as environmental bioindicators [11, 12, 13], since they are sessile marine invertebrates that perform several fundamental ecological functions, thus forming an important link in the food chain [14]. These animals have a larval phase when they live free and the tadpole larva selects a suitable place for fixation. In their youth phase, the ascidians adopt a sedentary life, losing the tail, and then become adult, fixed in consolidated substrates. Additionally, the ascidians have a large internal portion of their body extremely permeable and in intimate contact with a constant water flow. The gill sac of the ascidians is like a net of blood vessels and lashes that move the water through its body and this makes that many elements in the water can be absorbed directly. They remove from water more than 90% of bacteria and phytoplankton [15], which also makes them bioaccumulating substances. Thus, the study of the concentration of chemical elements with toxic potential in the body of the ascidians, can reflect the local conditions and allows an indirect evaluation of the water quality. Due to this capacity, recent studies point to the bioremediation potential of these organisms, which can remove significant amounts of water pollutants such as cadmium and lead [16].

In ECP, more than 18 species of ascidians were recorded, among them, 12 are considered cryptogenic, with wide distribution worldwide [17] and among them *Microcosmus exasperatus* Heller, 1878. This wide distribution and the restriction to port areas in Brazil suggest that the species are introduced to western Atlantic. Considering that these organisms are available in several regions that suffer anthropic impacts, they would be therefore, great models for ecotoxicological studies.

Therefore, the aim of this study was to evaluate the potential bioindicator of the ascidian *Microcosmus exasperatus* in order to monitor the environmental conditions, measuring the concentration of several chemical elements for this species. The species *M. exasperatus* was chosen because it is frequent both in the port area of ECP and in areas more distant from the port, presumably with better water quality and also because there are no formal studies available in the literature with this species, being this the first study considering several chemical elements.

Material And Methods:-

Microcosmus exasperatus is a solitary ascidian of the Pyuridae family, with broad global distribution in tropical and subtropical waters. The specimens of M. exasperatus were collected from natural or artificial substrates located in two different areas in the Estuary Complex of Paranaguá (ECP), Southern of Brazil. The specimens were obtained on the outskirts of a marina in a highly anthropized region, characterized by a large number of marinas and high traffic of small and medium boats. This point is located in the arm of the sea called Itiberê River (IT), adjacent to the urban area of the of Paranaguá and near the Paranaguá Port as seen in the Figrue 1 (25º 31' 1"S and 48º 29' 55"W). The other point, about 14km away, was Ilha da Banana (Banana Island) (IB), considered in the context of this study less impacted, it is far from urban areas and from the port (25° 24'48"S and 48° 25'1" W) (Figure 1). These locations were chosen because they may represent different water quality situations and because they have already been identified as places of great occurrence of this model species.

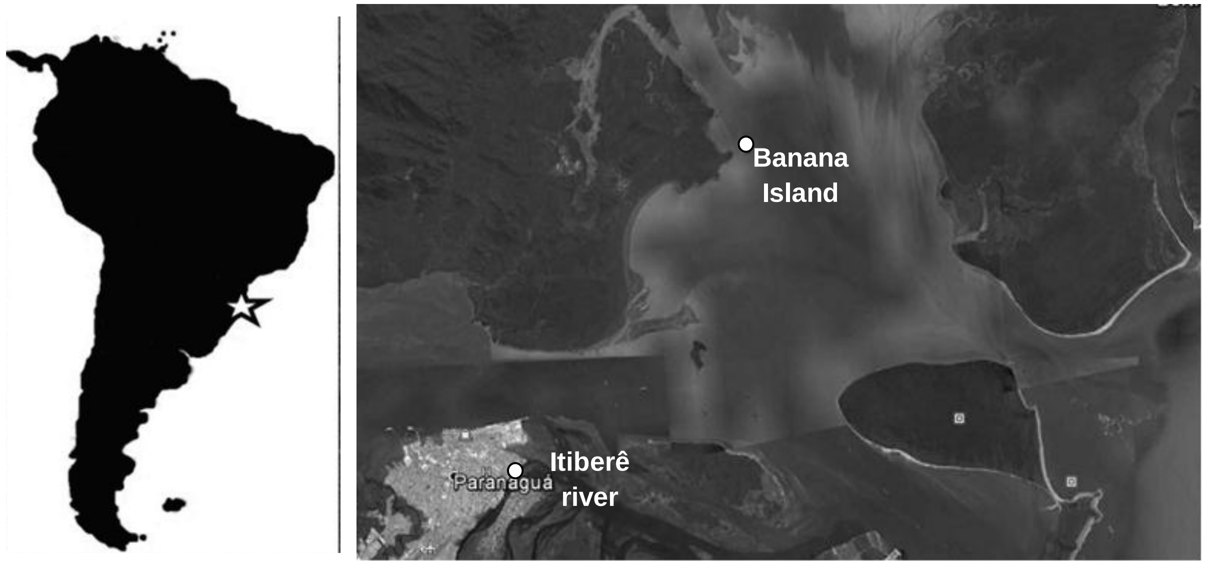

Figure 1:-Location of the *Microcosmus exasperatus* collection points in the Estuary Complex of Paranaguá. Source: Google Earth.

The specimens were collected manually or by scraping the substrate with a spatula in the same tide cycle with no more than 30 minutes interval. At each collection point, at least ten individuals were obtained. The collected animals were individually allocated in identified plastic bags and kept cool until the laboratory processing. In the laboratory the main fouling organisms in the tunic of the ascidians were identified and removed. Afterwards the samples were washed and rewashed with deionized water in order to remove possible contaminations.

Each individual had his tunic dissected and opened and, with the aid of tweezers and scalpel, the tunic was separated from the rest of the body delimited by the body wall. Each region of interest (called tunic and body) was transferred to porcelain crucibles previously identified and weighed (g) with a precision analytical balance. The material was kept in an oven at 60°C until get the constant weight for gauging the dry weight (g). The difference between wet weight and dry weight results in the tissue water content. It was also calculated the condition factor for each individual, which consists in the relation between the body weight without the tunic and the total weight.

In order to determine the concentration of the chemical elements in each part of the body, a dry digestion was carried out. It was initiated with the transfer to a muffle at 500°C for 4 hours. Subsequently, 4 drops of the hydrochloric acid solution (3 mol L-1) were added, this set was held for 4 hours in the muffle at 500°C for complete digestion of the material. After the completion of the digestion, the material was diluted in 10 mL of hydrochloric acid (3 mol L^{-1}) and placed on a hot plate for 3 minutes at a soft temperature (60 ° C). Then the material was filtered and transferred to a volumetric flask having its volume checked with distilled water to 25 mL, which was stored in a refrigerator until the reading.

The elements were read in duplicates by atomic absorption spectrophotometry with plasma coupled inductively (ICP, AES) after calibration with known concentration standards. Readings were carried out with aluminum, barium, boron, cadmium, calcium, lead, cobalt, copper, chromium, iron, phosphorus, magnesium, manganese, molybdenum, nickel, potassium, vanadium and zinc.

The water content in the tissue and the condition factor may indirectly indicate the health of the animal and were compared between the areas by the t-test for the two parts of the body. The medium values of the element concentrations were transformed (log (x+1) or Square Root) when necessary in order to guarantee the homogeneity of the data and submitted to an analysis of variance with two factors (collection points) x (body parts). When it was found significative difference, the averages were compared by the Tukey test at the level of 5%. Considering the multivariate nature of the data, all the elements were ordered by a linear discriminant analysis and graphically represented. Additionally, a multivariate analysis of permutational variance (PERMANOVA) was applied in an attempt to detect distinctions between the factors (collection points) x (body parts) and generate a hypothesis test.

Results:-

Several taxa of encrusting epibiont organisms have been observed in the tunic of the collected ascidians and may be related to the chemical data or the tissue water content obtained. In the individuals from Rio Itiberê (Itiberê River) (IT), the mussel *Mytella* sp., the bryozoan *Hippoporina indica*, the barnacle *Amphibalanus* sp., the octocoral *Stragulum bicolor*, and nematodes were registered. The individuals from Ilha da Banana (Banana Island) (IB) showed lower degree of epibiosis in the tunic, being observed especially Ophiuroidea and Polychaeta.

The tissue water content presented significant differences between the animals collected in the two areas (Figure 2A), with higher values at Itiberê River for both the tunic and the body (tunic: $t = 10.0$, $p < 0.05$; body: $t = 34.3$, $p < 0.05$). Following the same pattern of variation than the water content, the condition factor was different between the sites (Figure 2B), with higher values for the animals located at Itiberê River ($F = 10.9$, $p < 0.05$).

The concentration of 18 chemical elements was measured and compared between the sites and the body parts of the ascidians. The joint analysis of the elements by the Linear Discriminant Analysis (Figure 4) indicates the differentiation especially between the parts of the body of the ascidians (LD1 with 75% of the observed variation). The elements Ni, Ca, K, V among others, characterize especially the tunics, whereas B, P, Fe and Mg are typically found in higher concentrations in the body. The LD2 axis (16.5%) differentiates collection sites, but not as strongly as the anterior axis, demonstrating that the ions Mg, Fe, Ni and Ca are common at Banana Island and Mn, V, P, Zn and Pb at Itiberê River.

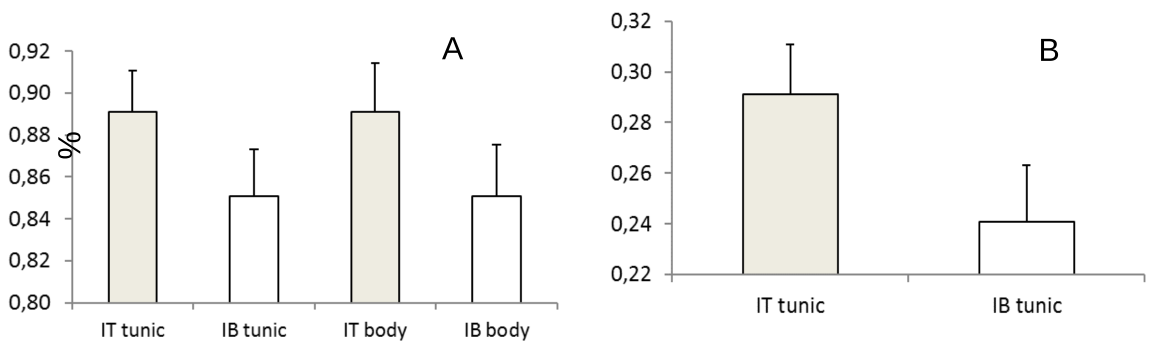

Figure 2:-Average values of Tissue Water Content (average + standard deviation) of tunic and body (A) and condition factor (average + standard deviation) (B) of ascidians collected Itiberê River (IT) and Banana Island (IB).

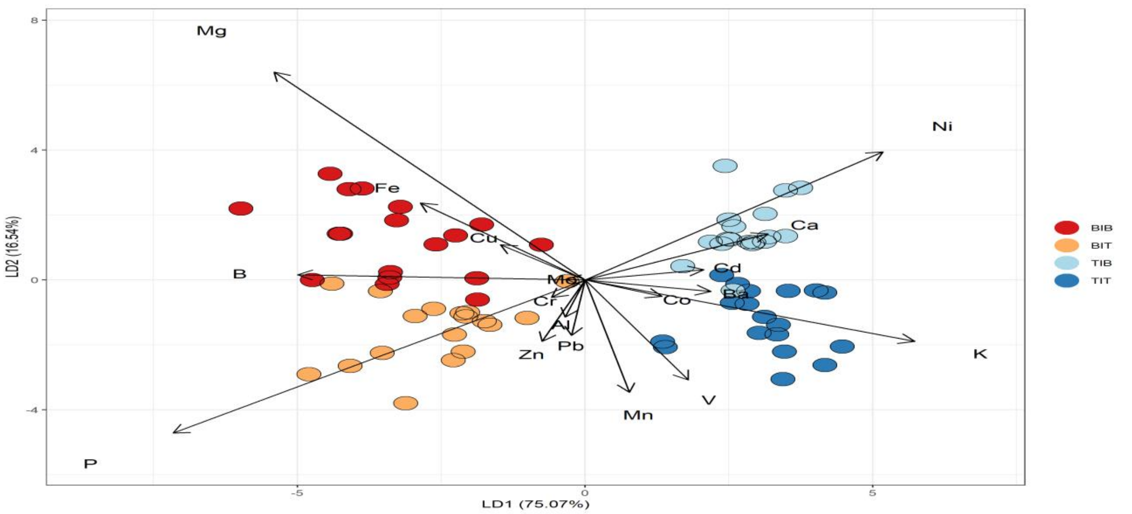

Figure 3:-Linear Discriminant Analysis (LDA) of the concentration of 18 chemical elements in the body (B) and tunic (T) of the ascidians *Microcosmus exasperatus* from Itiberê River (IT) and Banana Island (IB), in the Estuary Complex of Paranaguá.

In the univariate analyzes (Table 1), the elements as potassium (K), manganese (Mn) and copper (Cu) showed no difference (p> 0.05) among the factors. Other chemical elements analyzed showed distinctions (p <0.05) of concentration both between the collection sites and between the parts of the body. The elements boron (B), nickel (Ni), magnesium (Mg), cobalt (Co) and lead (Pb) were found in higher concentration in IB. In contrast, a higher zinc concentration (Zn) was obtained in the CI. In all cases there was a higher concentration of these elements in the body of the animals compared to the tunic.

However, the elements as phosphorus (P), barium (Ba), chromium (Cr) and molybdenum (Mo) showed no difference between the collection sites. Even so it is possible to notice a greater concentration in the body compared to the tunic. Calcium (Ca) showed the inverse pattern, being found in greater concentration in ascidians tunic.

For other chemical elements, the results showed an interaction between the two analyzed factors - collection sites and parts of the body. Iron (Fe), cadmium (Cd) and aluminum (Al) followed this pattern and they were found in higher concentration in the body of the individuals collected at the Banana Island. On the other hand, vanadium (V) was found in higher IC concentration, only for the tunic.

Tabela 1:-Average concentrations (mg.kg-1) observed for 18 chemical elements in the *Microcosmus exasperatus* in the Itiberê River and Banana Island.

	Body	Tunic	Média
		K*	
Itiberê	3258.3aA	3649.5aA	3453.9a
Banana	3073.9aA	3726.2aA	3400.0a
Mean	3166.1A	3453.9A	
		Cu*	
Itiberê	13.3aA	13.1aA	13.2a
Banana	15.4aA	9.9aA	12.7a
Mean	14.4A	11.5A	
		Mn*	
Itiberê	204.9aA	247.6aA	226.3a
Banana	213.3aA	202.5aA	207.9a
Mean	209.1A	225.1A	
		P**	
Itiberê	3584.2aA	1829.2aB	2706.7a
Banana	3434.1aA	1850.6aB	2642.4a
Mean	3509.2A	1839.9B	
		Ba**	
Itiberê	38.9aA	12.8aB	25.8a
Banana	47.5aA	26.7aB	37.1a
Mean	43.2A	19.7B	
		Cr**	
Itiberê	10.3aA	8.1aB	9.2a
Banana	15.5aA	6.2aB	10.9a
Mean	12.9A	7.2B	
		Mo**	
Itiberê	16.3aA	7.5aB	11.9a
Banana	11.1aA	6.1aB	8.6a
Mean	13.7A	6.8B	
		Ca**	
Itiberê	4659.1aB	9150.1aA	6904.6a
Banana	5016.6aB	12816.2aA	8916.4a
Mean	4837.9B	10983.1A	
		B***	
Itiberê	35.4bA	16.6bB	26.0b

Banana	52.3aA	24.1aB	38.2a
Mean	43.8A	20.3B	
		Pb***	
Itiberê	21.0bA	10.1bB	15.6b
Banana	34.1aA	13.5aB	23.8a
Mean	27.6A	11.8B	
		Zn***	
Itiberê	161.3aA	53.1aB	107.2a
Banana	98.5bA	31.0bB	64.7b
Mean	129.9A	42.0B	
		Ni***	
Itiberê	5.8bA	5.0bA	5.4b
Banana	10.0aA	6.9aB	8.5a
Mean	7.9A	6.0B	
		Mg***	
Itiberê	3709.9bA	2683.2bB	3196.6b
Banana	4769.0aA	3335.1aB	4052.1a
Mean	4239.5A	3009.2B	
		Co***	
Itiberê	3.0bA	2.8aA	2.9a
Banana	3.8aA	2.1bA	2.9a
Mean	3.4A	2.4A	
		Al****	
Iate Clube	5216.8 bA	2446.1 aB	3831.4 b
Ilha da Banana	8989.2 aA	3168.5 aB	6078.8 a
Mean	7103.0 A	2807.3 B	
		Cd****	
Iate Clube	0.28 bA	0.18 aA	0.2 b
Ilha da Banana	0.60 aA	0.28 aB	0.4 a
Mean	0.4 A	0.2 B	
		Fe****	
Iate Clube	3660.0 bA	2075.7 aB	2867.8 b
Ilha da Banana	6995.9 aA	2959.5 aB	4977.7 a
Mean	5327.9 A	2517.6 B	
		V****	
Iate Clube	7.1 aB	13.3 aA	10.2 a
Ilha da Banana	5.4 aA	5.78 bA	5.6 b
Mean	6.2 B	9.58 A	

Different letters represent statistical difference at a level of 5% by the Tukey test. Small letters denote differences between the sites, in the columns, and capital letters denote differences between the parts of the body, in the lines. *elements that did not show significant differences for any factor; ** elements with significant differences only between the parts of the body; ***elements with differences in both factors (part of the body and location); ****elements with significant interaction among the analyzed factors.

Discussion:-

The results of this study demonstrate that the ascidian *Microcosmus exasperatus* has population distinctions among the sites sampled (water content, condition factor and fauna epibionts) in addition, there is concentration of elements explored here. These differences may be related to the osmoregulation of the individuals, and may also be related to the life stages, salinity and hydrodynamism of the place besides the tidal cycle. This species is able to record by different parameters the environmental conditions of the areas where it occurs, showing to be a potential bioindicator for the water quality [10, 11].

Additionally, in the most impacted area, there was a greater richness and abundance of embedded epibionts, which may help the host ascidian in its protection against external agents [18]. It can be hypothesize that in this way the ascidian would not need to have a thick tunic for protection, investing more in the body mass, which results in a high condition factor, as it was observed. Another possibility related to the presence of epibionts and the thickness of the tunic may involve the hydrodynamics of the places. It is possible that at the Banana Island forces of the sea current and tidal forces are higher, making the individual to invest more in the tunic in order to secure the fixation, similar to what occurs with mussels [19] and also creating a barrier of colonization for the epibionts - which makes the condition factor minor at this site.

This species of ascidian has a broad and thin ciliated pharynx that is in constant contact with the sea water and the elements contained in it, being probably the main route for the incorporation of some dissolved elements [20]. This intimate contact with water may also be related to the level of hydration of the tissues. The results of the water content variation observed (Figure 2) may be related to the osmoregulation of the individuals, and consequently to the variable salinity of the environment. In this region, the Estuary Complex of Paranaguá, the salinity can vary from 12 to 29 in the summer, and from 20 to 34 in the winter time during tidal cycles [21]. During the high tide, characterized by high salinity, the animal could lose water to the environment by simple diffusion. Or, on the other hand, gain water in periods of lower salinity. The collections made during the same tides period and in the same sector of the estuary served to guarantee the equality of these conditions and, therefore, the observed differences must be related to other factors. The hydration of the tissues is controlled by the cellular metabolism of the animal and some environmental factors are able to change this factor, as well as the biological effects of the environmental signs that ascidian is registering in its habitat [22].

The accumulation of some chemical elements carried along with the water into the body of the ascidian can make some changes in physiological patterns. Such phenomenon was described in crabs tissues of *Carcinus maenas* [23]. Detailed information on the osmoregulation of *M. exasperatus* is not available and it could elucidate this question. However, the osmoregulation of this species exhibits a clearly populational pattern, since Nagar and Shenkar [24] describe *M. exasperatus* from the Mediterranean sea as highly sensitive to salinities below 33. Again, in the Estuary Complex of Paranaguá the species occurs in fluctuating salinities according to the tides, but that can reach 12 or less on days with heavy rainfall and rarely exceed 30.

In addition, the fact that Itiberê River is an environment with high traffic of boats and an urban waste disposal of several types of domestic and industrial effluents and it can lead these animals to accumulate more water in the body, in order to dilute pollutant present in the water, demonstrating that there are correlations among the concentrations of the trace elements found in the body of the ascidians with the levels of hydration in the tissues [25]. The physiological mechanisms are not well known, but the higher percentages of water in the tissues, actually occur in individuals from contaminated sites [26], corroborating with the observed results. However, most of the chemical elements quantified in this study had a higher concentration in the body of the ascidians from Banana Island, indicating that this effect should be promoted by other elements, by non-measured compounds, or by the specific effect of Zn and V, elements that were saw in higher concentration in animals from at Itiberê.

It is noteworthy in the results obtained, the fact that for the most parte of the elements, the concentrations measured were higher in Banana Island. Itiberê River is historically [27] and visibly more impacted by pollution than Banana Island. It is possible that the method of organisms collection has influenced in this result. At Itiberê River the animals were caught in the pillars of the pier where the boats stop, between 0.5 and 1m of the sand-muddy bottom. At Banana Island, the animals were collected on the sides and under rocks, near the sediment and, therefore, with a greater probability of contact with some elements present in the sediment.

Comparing the results observed for the body of *M. exasperatus* with those obtained from other similar organisms - in order to compare similar structures - it was verified that the ascidian accumulates relatively less potassium (3509.1 ± 1168.4 mg kg^{-1}) in comparison with mussels (7043.0 ± 1250.6 mg kg^{-1}) from a nearby estuary [28]. Considering the presumed high availability of this chemical element in Paranaguá, due to the proximity to the port where one of the main products transported and that are leached to the sea are the fertilizers, it is considered that ascidian is not a specially bioaccumulating organism for this element.

Concentrations of manganese (209.1 ± 102.9 mg kg-1) and copper (14.4 ± 5.9 mg kg-1) exceeded the values reported in the literature for mussels. The concentration average of manganese found in mussels *Mytilus* *galloprovincialis* collected in Morocco did not exceed (25.3 mg kg-1) [29]. The concentration of copper in the mussel *Perna perna* collected in Arraial do Cabo, Southeastern of Brazil, did not exceed 0.002 mg kg-1 [30]. Ali et al. [13] observed in ascidians *Phallusia nigra* copper values in the tunic and body, ranging from 0.3 to 0.5 and 1.7 to 4.2 mg kg-1, respectively. High concentrations of these elements tend to be recorded by filtering organisms [31, 32], and for these cases *M. exasperatus* has proved to be a more powerful bioaccumulator.

Differences in the concentration of some chemical elements among the body parts of the animal were expected. The external tunic is composed mostly of extracellular material, while the part bounded by the body wall is composed of soft fleshy tissues, internal organs, gonads and large gills [1]. The elements boron, cobalt, lead, magnesium, nickel and zinc were found in high concentrations in the body of the animal compared to the tunic.

The concentration levels of nickel (7.9 ± 3.5 mg kg-1) and zinc (129.9 ± 73.1 mg kg-1), considering the high deviations were similar to the levels found in the mussels *P. perna* (Ni = 7.9 ± 8.4 and Zn = 95.8 ± 148.9 mg kg-1) collected in the Itapocoroy cove, further south in Brazil [33]. It was verified that Magnesium (4239.4 ± 1494.6 mg kg-1) present in the body of the individuals was higher than that found in mussels (2121.0 ± 5699.0 mg kg-1) in another estuary nearby [28].

According to Rezende and Lacerda [30] the concentrations of lead did not exceed 0.02 mg kg-1 in *P. perna* collected in Guanabara Bay, southeast of Brazil. In another study with this mussel further south, the concentration of lead reached 0.1 ± 1.7 mg kg^{-1} [34]. In this study, concentrations of lead in the body were significantly higher (27.5 ± 15.1 mg kg^{-1}). Ali et al. [13] observed in the ascidians *Phallusia nigra* the amount of lead in the tunic and body varies from 12 to 19 and 21 to 45 mg kg^{-1}, respectively, indicating higher values than those found in this study. Lead is considered a highly toxic metal to humans when absorbed at high concentrations [35].

Phosphorus is one of the most important elements for life because it is an essential constituent of the cell membrane, nervous tissue and serveral biomolecules. *Microcosmus exasperatus* is able to record large concentrations of phosphorus, both in the tunic and in the body of the animal, indicating that the species is a potential bioaccumulator of this element as well as other filtering organisms [10, 11].

The concentration of barium detected in this study (43.2 ± 41.1 mg kg-1) exceeded the results obtained in the oysters *Crassostrea* sp. (4.1 ± 0.4 mg kg-1) in the south of Brazil [34]. Cadmium showed an inverse behavior, being more concentrated in oysters (0.5 ± 2.8 mg kg-1) when compared to the data of *M. exasperatus* (0,4 ± 0,3 mg kg-1) [36, 37]. Ali et al. [13] observed in the ascidians *Phallusia nigra* amounts of cadmium in the tunica and body ranging from 0.6 to 6 and 5 to 20 mg kg-1 respectively.

Chromium analysis also indicated high concentration in ascidians (12.9 ± 8.9 mg kg-1) in relation to the mussels (0.1 ± 0.02 mg kg-1) [34]. This is an indication of the need for future evaluation works in the area, and also considering the presence of metals in the sedimentation phase of the studied areas [27].

The presence of iron in alive animals has been known for a long time, and this element is considered essential [38]. The values found for iron concentration (5327.9 + 2819.0 mg kg-1) were higher when compared to the oysters *Crassostrea* sp. [30, 39, 34]. Similarly, the amounts of aluminum (7103.0 + 3373.2 mg kg-1) and vanadium (6.3 + 5.1 mg kg-1) also outperformed those obtained for oysters in the same studies.

Some species of tunicates accumulate high concentrations of vanadium in their body, especially in the tunic [1]. Ali et al. [13] observed in the ascidians Phallusia nigra values of vanadium in the tunic and body varying from 85 to 110 and 274 to 413 mg kg-1 respectively. *M. exasperatus* did not show such high values, corroborating with other studies [18]. It should be pointed out that the vanadium function is still uncertain for these animals [40], but the fact that it was found in higher concentrations at the Iate Clube (Yacht Club) may be an indicative that the port industrial activities are impacting in the environment in the surroundings of Paranaguá Port, since this element is an important indicator of oil pollution [41].

It is important to point out that at least four of the chemical elements studied in *M. exasperatus* exceed the tolerable limits mentioned by FDA (Food and Drug Administration Guidance Document) for mussels and oysters and in other monitoring programs created in the international scenario (Table 3). Other elements analyzed are under the maximum limits.

Table 3:-Tolerance limits for the concentration of some metals and semi-metals in molluscs used in feeding (values in mg kg^{-1} of dry weight) and in the body of *Microcosmus exasperatus.*

Elements	Certified Value**	*M. exasperatus*
Mn	12**	209,1 ± 102,8
Pb	1,7**	27,55 ± 15,1
Cr	13**	15,5 ± 8,9***
V	4**	6,3 ± 5,1
Cd	4**	0,46 ± 0,3
Cu	150*	14,4 ± 5,9
Ni	70**	7,9 ± 3,5
Zn	250*	129,9 ± 73,1

(**) Tolerance limits for bivalve molluscs indicated in accordance with (FDA) "Food and Drugs Administration Guidance Document [42].
(*) ABIA (Brazilian Association of Food Industries) [43]
(***) Data for the body of the non-anthropized area

Although this ascidians are not considered a food item in Brazil, in other regions and countries such as Europe and Asia, and also Chile, several species are collected or cultivated for consumption [44]. This calls attention to *M. exasperatus* and other similar species, which can serve as food. Thus, in places of collection or cultivation of these ascidians, there must necessarily be a good control of water quality, considering its high capacity to accumulate potentially toxic elements, especially lead, manganese, chromium and vanadium.

These ascidians are known for tolerance and occurrence in regions with low water quality and high contamination rates [18]. The data analyzed show that this type of ascidian can adapt well to sites with high pollution evidence, making it possible to use them as indicators of environmental pollution, both current and remote, and also providing knowledge of the main sources of pollution within a given water system [10, 11].

The Estuary Complex of Paranaguá is subject to exposure to various metallic and toxic elements [27], due to the domestic and industrial waste that is discarded in the bay, related to the intense urban and port activities. As studies with the concentration of chemical elements in *M. exasperatus* and even other species of ascidians are rare, the data showed here can be used as the first reference for the species in the region. In other organisms with similar characteristics as filtering habit, size and lifestyle, such as mussels and oysters, better known to be common sources of human food, these data are available.

It is investigated that different situations of this species occurrence can bring variations in the concentrations of chemical elements and should be considered in future studies as the substrate of ascidians fixation and distance of the sediment. It is also suggested to reconcile studies with the dosage of these elements and histological approaches in the different tissues and in the tunic, which may indicate tissue changes related to the pollutants. Studies considering the toxic organic compounds may also highlight the role of the ascidians in the environmental monitoring of the region.

Conclusion:-

This study represents the first wide assessment of the concentration of chemical elements, several potentially toxic, in solitary ascidians. The results reveal the potential of the species *Microcosmus exasperatus* for studies of biomonitoring of these elements and also indicate the care with the ingestion of elements above the limits safe for the health of these and similar organisms. Further studies are also suggested to elucidate the mechanisms of absorption and the effects of toxic elements on organisms.

Acknowledgment:-

The authors wish to thank Fundação Araucaria for scholarship granted during the research.

References:-

1. S.A. Rodrigues, R.M. Rocha, and T.M.C. Lotufo, Guia ilustrado para identificação das Ascídias do estado de São Paulo. Instituto de Biociências, USP, São Paulo. http://dx.doi.org/10.2983/0730-8000(2008). 1998.
2. P.C. Lana, R. Christofoletti, J.R. Gusmão, T.L. Barros, D. Spier, T.M. Costa, A.S. Gomes, and C.S.C. Santos, "Benthic Estuarine Assemblages of the Southeastern Brazil Marine Ecoregion (SBME)". In: P.C. Lana, and A. Bernardino (Eds), Brazilian Estuaries. Brazilian Marine Biodiversity. Springer, Cham. 2018.
3. UNESCO, http://www.unesco.org/new/pt/brasilia/culture/world-heritage/list-of-world-heritage-in-brazil/atlantic-forest-south-east-reserves/#c1465030. 2019.
4. R.M.B. Castella, P.R. Castella, D.C.S. Figueiredo, and S.M.P. Queiroz (Orgs.), Mar e Costa: Subsídios para o ordenamento das áreas estuarina e costeira do Paraná. SEMA. Curitiba. 2006.
5. C. Barcelos, N.H. Gruber, M. Quintas, and L. Fernandes, " Complexo Estuarino de Paranaguá: Estudo das Características Ambientais com Auxílio de um Sistema de Informação Geográfica". Colóquio Brasileiro de Ciências Geodésicas, 3, p. 62-83, 2003. http://geodesia.ufsc.br/Geodesiaonline/arquivo/GeoColoq_2003/artigos/T124.pdf.
6. A. Pletsch, M. Beretta, T. Tavares, "Distribuição espacial de compostos orgânicos de estanho em sedimentos costeiros e em Phallusia nigra da Baía de Todos os Santos e litoral norte da Bahia – Brasil." Quimica Nova. 33(2), 451-457. 2010.
7. S. Caralt, S. López-Legentil, I. Tarjuelo, M. Uriz, and X. Turon, "Contrasting biological traits of Clavelina lepadiformis (Ascidiacea) populations from inside and outside harbours in the western Mediterranean". Mar. Ecol. Prog. Ser., 244: 125-137. 2002.
8. F. Fu, and Q. Wang, "Removal of Heavy Metal Ions from Wastewaters: A Review." Journal of Environmental Management, 92, 407-418. 2011.
9. H. Michibata, T. Terada, N. Anada, K. Yamakawa, and T. Numakunai, "The accumulation and distribution of vanadium, iron, and manganese in some solitary ascidians." BioL Bull. 171: 672-681. 1986.
10. L. Carballo, and S. Naranjo, "Environmental assessment of a large industrial marine complex based on a community of benthic filter-feeders". Mar. Pol. Bul. 44:605-610. 2002. http://dx.doi.org/10.1016/S0025-326X(01)00295-8.
11. R. Beiras, J. Bellasa, N. Fernandeza, J.I. Lorenzoa, and A. Cobelo-García, "Assessment of coastal marine pollution in Galicia (NW Iberian Peninsula); metal concentrations in seawater, sediments and mussels (Mytilus galloprovincialis) versus embryo–larval bioassays using Paracentrotus lividus and Ciona intestinalis". Mar. Envir. Res. 56:531-553. 2003.
12. F.O. Marins, R.L.M. Novaes, R.M., and A. Junqueira, "Non indigenous ascidians in port and natural environments in a tropical Brazilian bay". Intern. J. Zool. 27:213-221. 2010.
13. H.A.J. Ali, M. Tamilselvi, A,S. Akram, M.L.K. Arshan, and V. Sivakumar, "Comparative study on bioremediation of heavy metals by solitary ascidian, Phallusia nigra, between Thoothukudi and Vizhinjam ports of India". Ecotox. and Env. Saf. 121: 93–99. 2015.
14. G. Lambert, "Ecology and natural history of the protochordates." Can. J. Zool. 83: 34-50. 2005.
15. J.K. Petersen, and I. Svane, "Filtration rate in seven Scandinavian ascidians: implications of the morphology of the gill sac." Marine Biology 140: 397-402. 2002.
16. N. Colozza, M.F. Gravina, L. Amendola, M. Rosati, D.E. Akretche, D. Moscone, and F. Arduini, " A miniaturized bismuth-based sensor to evaluate the marine organism Styela plicata bioremediation capacity toward heavy metal polluted seawater." Sci Total Environ. 2017. http://dx.doi.org/10.1016/j.scitotenv.2017.01.099.
17. R.M. Rocha, and L.P. Kremer, "Introduced ascidians in Paranaguá Bay, Paraná, southern Brazil." Revista Brasileira de Zoologia 22 (4): 1170-1184. 2005.
18. D. Stoecher, "Defenses of ascidians against predators." Ecology, 61, 1327-1334. 1980.
19. M. Brenner, and B.H. Buck, "Attachment properties of blue mussel (Mytilus edulis L.) byssus threads on culture-based artificial collector substrates". Aquacultural Engineering 42: 128–139. 2010.
20. A. Viarengo, "Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level." Rev. Aquatic Sci., 1,295-317. 1989.
21. P.C. Lana, E. Marone, R.M. Lopes, and E.C. Machado, "The subtropical estuarine complex of Paranaguá Bay, Brazil.". Ecological Studies, Coastal Marine Ecosystems of Latin America. Springer-Verlag Berlin Heidelberg, 144, p. 131-145. 2000.
22. P. Agre, A.M. Saboori, A. Asimos, And B.L. Smith, "Purification and partial characterization of the Mr 30,000 integral membrane protein associated with the erythrocyte Rh(D) antigen". J. Biol. Chem. 262:17497–17503. 1987.

23. M.H. Depledge, and P. S. Rainbow, “Models of regulation and accumulations of trace metals in marine invertebrates: a mini-review”. Comp. Biochem. Physiol., 97C, 1-7. 1990.
24. L. Nagar, and N. Shenkar, “Temperature and salinity sensitivity of the invasive ascidian *Microcosmus exasperatus* Heller, 1878”. Aquatic Invasions 11(1): 33–43. 2016.
25. E.K. Hoffmann, I.H. Lambert, and S.F Pedersen, “Physiology of cell volume regulation in vertebrates”. Physiol. Ver. 89:193–277. 2009.
26. J. Bellas, R. Beiras, and E. Varques, “Subtethal effects of trace metals (Cd,Cr, Cu, Hg) on embryogenesis and larval settlement of the ascidian Ciona intestinalis”. Arch Environ. Contam. Toxical. v.46 n.1, . 61-66. 2004.
27. F. Sá, “Distribuição e fracionamento de contaminantes nos sedimentos superficiais e atividades de dragagem no Complexo Estuarino da Baía de Paranaguá, PR.” Master dissertation Curso de Pósgraduação em Geologia – UFPR, 92p. 2003.
28. D. Seo, “Avaliação dos teores de Br, Cl, K, Mg, MN e V em mexilhês *Perna perna* (Linnaeus, 1758: Mollusca, Bivalvia) coletados no litoral do estado de São Paulo, Brasil.” Master disertation Curso de Pósgraduação em Ciências na Àrea de Tecnologia Nuclear – USP, 127p. 2012.
29. M. Maanan, “Biomonitoring of heavy metals using Mytilus galloprovincialis in Safi costal waters, Morocco”. Env. Toxicol. 1: 525-531. 2007.
30. C.E. Rezende, L.D. Lacerda, “Metais pesados em mexilhões *Perna perna* no litoral do estado do Rio de Janeiro.” Revista Brasileria de Biologia, 46, 239-247. 1986.
31. D. Hocckett, P. Tngramb, and A. Lefurgey, “Strontium and manganese uptake in the barnaclae shell: Electron probe microanalysis imaging to attain fine temporal resolution of biomineralization activity”. Mar. Env. Res. 43(3): 131-143. 1997.
32. M.I. Powell, and K.N. White, “Heavy metal accumulation by barnacles and its implications for their use as biological monitors”. Mar. Env. Res. 30: 91-118. 1990.
33. V.R. Bellotto, P.C. de Brito, G. Manzoni, and E. Wegner, “Biomonitoramento ativo de metais traço e efeito biológico em mexilhões transplantados para área de influência de efluente de indústria de beneficiamento de aço- fase I”. Braz. J. Aquat. Sci. Technol. 9(2):33-37. 2005.
34. A.J. Curtius, and J.F. Ferreira, “Avaliando a contaminação por elementos traço em atividades de maricultura. Resultados parciais de um estudo de caso realizado na Ilha de Santa Catarina – SC” . Quimica Nova, Vol. 26, No. 1,44-52. 2003.
35. D.S. Vaitsman, J.C. Afonso, and P.B. Dutra, Para que servem os elementos químicos. Rio de Janeiro - 20.710-290. p. 286. 2001.
36. T.P. O’Connor, “Mussel watch results”. Mar. Environ. Res., 41: 183. 1996.
37. T.P. O’Connor, “Mussel watch results”. Mar. Pollut. Bull., 37: 14. 1998.
38. K.S. Nielsen, Fisiologia Animal – Adaptação e Meio ambiente. Ed Com. Imp. Ltda. 5ª ed. 2002.
39. C.E.V. Carvalho, M.P.O. Cavalente, M.P. Gomes, V.V. Faria, and C.E. Rezende, “Distribuição de metais pesados em mexilhões (*Perna perna*, L.) da Iha de Santana, Macaé, SE, Brasil”. Ecotox. and Env. Rest. 4(1): 1-5. 2001.
40. H. Michibata, N. Yamagushi, T. Uyama, and T. Ueki, “Molecular biological aproaches to the accumulation and reduction of vanadium by ascidians.” Coord. Chem. Rev. 237: 41-51. 2003.
41. M. Roozbeh, F. Ali, F. Iraj, and I. Ali, “A Investigation of Nickel and Vanadium Ratio from Oil Pollution in Sediments and Rocky Shore Oysters (Saccostrea cucullata) in Bushehr Coasts (Persian Gulf)”. Oceanography, 4(14):5-5. 2013.
42. FDA, Food and Drugs Administration Guidance. http://www.cfsan.fda.gov, 2016.
43. ABIA, “Compêndio da Legislação de Alimentos. (Associação Brasileira das Indústrias de Alimentação - Brazilian Food Industry Association)”. Atos do Ministério da Saúde, São Paulo. https://www.abia.org.br/vsn/, 1996.
44. G. Lambert, R.C. Karney, W.Y. Rhee, and M.R. Carman, “Wild and cultured edible tunicates: a review.” Manag. Biol. Inv. 7(1): 59–66. 2016.

Appendix 1: -

Averages of concentrations of chemical elements (mg.kg^{-1}) analyzed in the present study.

Chemical elements	Anthropized Tunic	Non-anthropized Tunic	Anthropized Body	Non-anthropized Body
Aluminum	2446,0	3168	5216	8989
Barium	12,7	26,68	38,92	47,53

Boron	16,56	19,24	22,44	23,30
Cadmium	0,17	0,28	0,27	0,63
Calcium	9150	12816	4659	5016
Lead	10,09	13,52	21,04	34,06
Cobalt	2,79	2,06	3,04	3,78
Copper	13,07	9,92	13,33	15,44
Chromium	8,12	6,23	10,28	15,52
Iron	2075	2959	3659	6995
Phosphorus	1829	1850	3584	3434
Magnesium	2683	3335	3709	4768
Manganese	247	202	204	213
Molybdenum	7,50	6,07	16,33	11,12
Nickel	4,97	6,94	5,78	10,01
Potassium	3649	3726	3258	3073
Vanadium	13,4	5,8	7,1	5,4
Zinc	53,13	30,95	161,28	98,51