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Elemental Composition of *Drulia browni* Collected in Negro River (Amazonas, Brazil)

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Abstract

Sponges, comprising not only organic chemical composition, but also the metals that they filter and accumulate, are aquatic animals that constitute one of the main hotspots in the search for new drugs, being the subject of many studies. The inorganic accumulation pattern from these sponges could be used for several purposes, from bio-remediation to chemo-taxonomic characterization. In the present study, the X-ray fluorescence inorganic analysis of *D. browni*, one of the most common species of freshwater sponge in the Negro River, was performed in order to approach its chemical composition. Silicon, aluminium and calcium are the most abundant elements in this species. In addition, the presence of S, Cl, K, Ti, V, Mn, Fe, Cu, Zn and Sr were also detected. Aluminium associated with Ca, K and S is a characteristic chemical profile that can be used as a bio-marker of this genus.

Keywords: Porifera; Demospongiae; X-ray fluorescence; Amazonia; Freshwater sponges

Introduction

Worldwide, only about 200 sponge species can be found in freshwater environments – all belonging to the class Demospongiae-of the approximately 8,500 species of phylum Porifera [1-3].

Due to the extensive research carried out during the last five decades with the Neotropical fauna of freshwater sponges at large and the Amazonian ones in particular [4-6], altogether a large array of recognized species and genera was exposed, with easy access to these organisms during the dry season. This body of knowledge is now available for the application of sophisticated procedures aiming to explore the chemo and bio-chemical characteristics of these continental representatives of the phylum Porifera. The chemical composition has been used in order to help the differentiation of species and genus, with some studies exploring the diversity of fatty acids [7,8] and sterols [9-13]. Even though several studies have been performed and the technique proved to be promising, the organic chemical relationships are not yet fully established.

The elemental composition of the sponges belonging to the Demospongiae class differs significantly according to the species, but is primarily silicon, oxygen and hydrogen. Silica (SiO_2) is the major constituent, about 85.2% (w/w) in spicules, in sponges of the Demospongiae and Hexactinellida classes [14]. Other elements present in low concentrations include aluminium, calcium, chlorine, copper, iron, potassium, sodium, sulfur, and zinc [14-17].

Regarding the elemental composition, the best known and more studied freshwater sponges are located in Russia, at Lake Baikal. Sponges of the Lubomirskiidae family were studied using inductive coupled plasma mass spectrometry (ICP-MS) allowing the detection of 19 elements: Mg, Al, P, Ca, Ti, Mn, Co, Ni, Cu, Rb, Sr, Y, Cd, Ba, La, Ce, Pb, Th and U. Some of these elements were detected in the sponges in concentrations of up to 1,000 times higher than observed in the Lake Baikal water, expressing the huge ability of these animals to accumulate certain elements [18-20].

The genus *Drulia* Gray, 1867, of the exclusively freshwater family Metaniidae, is endemic to South American waters and extremely abundant in Amazonian waters [6,21]. In Eastern Amazonia, two species of genus *Drulia*, *D. uruguayensis* and *D. cristata*, were analyzed by energy dispersive X-ray fluorescence (EDXRF) to determine their inorganic composition, presenting a high amount of silicon, between 35.01% and 37.17% [22]. These two sponges, collected in the Tapajós River, also showed various other elements and highlighting aluminium as the second most abundant, an unusual element that could be used as a bio-marker to the genus *Drulia*.

Among all species of freshwater sponges previously reported in the Amazon region, *Drulia browni* (Bowerbank, 1863) is the one with the largest distribution and is particularly abundant along the flooded areas of the Negro River [6,21]. The absence of chemical data for this species encouraged the present study, where analysis by EDXRF was performed to determine the elemental composition of a broad range of chemical elements, allowing its association with the composition of other freshwater sponges for chemo-taxonomy studies.

Materials and Methods

Two *Drulia browni* (Figure 1) specimens were collected from the Negro River, in the city of Iranduba (Amazonas, Brazil), on 5 September 2007 (03° 13’ 31.19” S 60° 00’ 09.89” W). On 17 October 2009, other specimens were collected at two points near to that of the original (3° 13’ 29.32” S 60° 00’ 14.38” W, and 3° 13’ 28.28” S 60° 00’ 17.32” W). The

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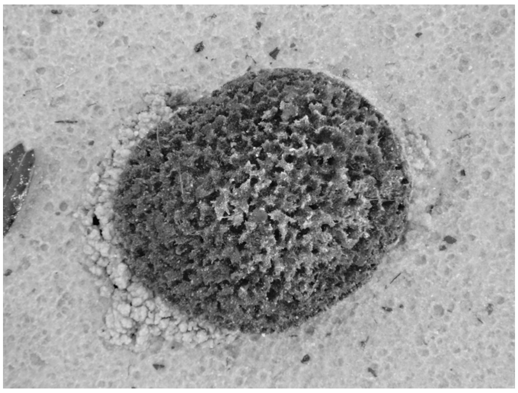

Figure 1: A *Drulia browni* specimen.

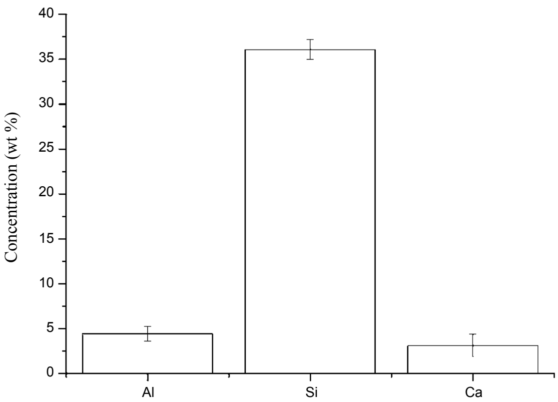

Figure 2: Concentration of major elements (> 3%) in *D. browni* sample.

two sampling dates were during the dry season, the period of low waters. The species were identified by microscopy techniques [23] and voucher specimens were deposited in the Porifera collection of the Museu de Ciências Naturais from Fundação Zoobotânica do Rio Grande do Sul with numbers MCN-POR 7996, 8366 and 8407, respectively.

Cleaning of the sponges was performed by mechanical removal of materials attached to the sponges (leaves, twigs and bark or small stones) [24]. Without any other pretreatment, the sponges were ground in an agate mortar for data collection preparation.

EDXRF analysis

Pellets were prepared in a 10,000 Kgf press, using 7.000 g of high purity H_3BO_3 for fixing a sample of 1.000 g that had been homogenized in an agate mortar to obtain a fine powder. Each sample was analyzed in triplicate, using Rh Kα radiation, a 10 mm collimator under vacuum atmosphere and without any pre-treatment using the EDXRF solid-state technique in a Shimadzu EDX 700 device. The irradiation time was 200 s for each sample.

Quantification of elements observed

Geological reference materials, such as GBW 3125, GBW 7105, GBW 7113, were employed in the equipment calibration, and also for determining the accuracy and precision thereof. Thus, the average error ranged between 1% and 12% for the elements quantified here. Aiming to measure the accuracy and precision of the method, the same patterns were analyzed five times each, reaching an average standard deviation of 7%. The detection limits were calculated using the formula suggested by Araújo et al. [24] reaching values of 10 ppm for the heavier elements and 150 ppm for the lower atomic number. The intensities found in the first analysis of the samples allow us to decide that sample dilution of 1:7 boric acids would be enough to compensate for the matrix effect.

Salts of known purity of Al, Si, Ca, S, K, Fe and Cu, diluted in boric acid, also of known purity, were used in six predetermined concentrations, which were subjected to the same analysis conditions as the samples. These patterns were analyzed under the same conditions as the samples, generating standard curves that were used for quantification of each element.

Results and Discussion

The sponge specimens were collected from the Negro River (black water) during the dry seasons of 2007 and 2009. The main objective of these two samplings was to identify the uniformity in *D. browni* specimen's composition over time and to check for possible taxonomic chemo-markers. The EDXRF analysis allowed the detection of Si, Al, Ca, S, K, Fe, common to all samples. Compared with a previous study, with sponges of the genus *Drulia* (*D. cristata* and *D. uruguayensis*) collected in Tapajós River (clear water), calcium and potassium were not previously detected in this genus. Both species from the Tapajós showed high amounts of silicon, together with Al, S, Cl, Ti, V, Mn, Fe, Cu, Sr and Se [22].

The family Metaniidae is found in rainforests on different continents, while the family Lubomirskiidae is endemic to Lake Baikal [1,3]. Although the phylogenetic relationships between these two families are not well established [3], the references reported in the literature concerning the elemental composition of freshwater sponges involve species of Lubormiiskidae family, what makes it the best models for comparison with the data obtained here.

The three elements observed at higher concentrations - Si, Al and Ca – have concentrations ranging from 36.1 to 3.1 wt% (Figure 2).

Silicon is the higher abundance element, representing 36.1 ± 1.1% of the mass of the samples. This value is similar to those reported for the species *D. cristata* (35.01 ± 1.82%) and *D. uruguayensis* (37.17 ± 0.61%) [22]. In studies with marine siliceous sponge spicules, the silicon amount is very close to that observed here [14]. Freshwater sponge, belongs to Demospongiae class, presents amorphous silica spicules [1-3,14-17], that explain Si as main element.

Aluminium was detected as the second most abundant element in *D. browni* samples (4.4 ± 0.8%). This pattern is similar to that observed in the two other studied species of the genus *Drulia* [22]. For freshwater sponges of the Lubomirskiidae family, aluminium is reported in concentrations lower than 0.13% in all studies [18-20]. As dissolved aluminium is present in the waters of both the Tapajós and Negro Rivers [25-27], sponges from the *Drulia* genus may have a particular aluminium absorption pattern in their metabolisms, suggesting that this element could be used as a bio-marker from this genus.

Calcium was also found in large quantities in samples of *D. browni*, representing 3.1 ± 1.3% of the mass of the samples. In *D. uruguayensis* this element was not detected while in *D. cristata* it was reported, but at a concentration below 0.05% [22]. Complementary studies are necessary to describe the concentrations of this metal in these three species in other environments, but the present result suggests that it could be a bio-marker of the species. In sponges of the Lubomirskiidae family, calcium is found in the highest concentration of 0.11% [18].

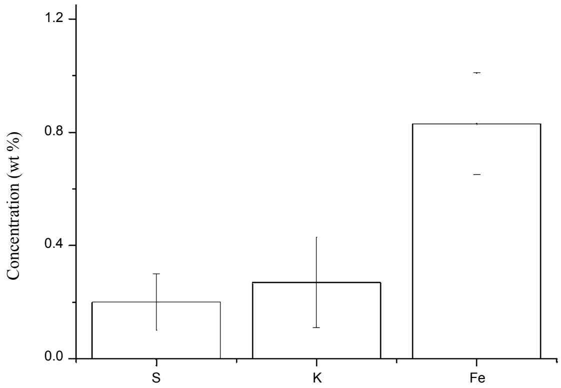

Figure 3: Concentration of minority elements (< 1%) in *D. browni* samples.

Sulphur, iron and potassium were detected in amounts varying from 0.2 to 1.0 wt % and quantified. As the scale is very different from the amount observed to Si, Al and Ca, they are showed at Figure 3.

In *D. browni* samples, iron represents 0.8 ± 0.2% of the composition of sponges. This amount is two times greater than that reported in other species of the same genus collected from the Tapajós River [22]. As this element is found in the highest concentration in suspended matter in the Negro River compared to the Tapajós River [27], this fact indicates the presence in this species of accumulation mechanisms of this element.

Sulfur is found to be frequent in marine Demospongiae sponges [14]. A different pattern, the absence of sulfur, was reported only in one study, involving ten marine species of this class [24]. *D. browni* from the Negro River presents about 0.2 ± 0.1% of sulfur, a value close to that reported for two other species of the *Drulia* genus from the Tapajós River [22]. The sulfur is reported in the form of the sulfate ion (SO_4^{2-}) in the Negro and Tapajós waters [28], but in concentrations lower than those reported in Lake Baikal [29]. It is interesting to observe that this element is not reported for sponges of the Lubomirskiidae family [18-20], suggesting that their presence is a characteristic of the genus *Drulia*, or at least common in some other Demospongiae genera and families.

Potassium was found at the concentration of 0.3 ± 0.2% (Figure 3), a concentration greater than that reported for the species *D. cristata* and not detected for *D. uruguayensis* [22]. It is an element commonly reported in the Lake Baikal waters, but was not detected in their sponges [18-20,29].

Similarly, zinc was detected in some samples of *D. browni* and was reported in the species *D. cristata* [22] and in purified spicules of *D. uruguayensis* [30]. However, it was not present in the *Lubomirskia baicalensis* species [18-20], although it was present in the waters of Lake Baikal [29].

The elements Cl, Ti, V, Cu and Sr were detected in at least one of the samples of *Drulia browni* analyzed. All of them were previously reported for species of the same genus [22]. Copper and titanium are also reported in sponges of the Lubomirskiidae family [18-20].

Copper was found in *Drulia browni* in a similar concentration to the other two species previously studied, 0.1% of its mass [22]. Sponges of the marine genera *Halichlona* and *Halichondria* were seen to have the ability to absorb metals selectively, including copper [31].

As all aquatic invertebrates, sponges accumulate trace metals in their bodies, being or not these elements essential to their metabolism [32]. Some sediment incorporation mechanisms based on mineral composition are known in marine sponges, but is not clear the reason of these behaviors [33].

Trace metal concentration in these organisms has pratical significance when employed in biomonitoring programs. Sponges presents some of the requests to act as a biomonitor (i.e. sessil, dense population, wide dispersion and longevity) [34]. Although to enable these use it is essential to know the elemental composition of these animals and the kinetic of trace metals accumulation.

Conclusion

Use of EDXRF showed it to be a versatile technique, allowing identification and quantification of chemical elements in complex matrices such as sponges. Although there are a very limited number of studies on the inorganic composition of freshwater sponges, apparently it is possible to differentiate the species already studied on such grounds. Aluminium, as the second most abundant element, appears to be a characteristic of the genus *Drulia*, a different pattern that distinguishes these sponges from those of Lake Baikal. The species *D. crista*, *D browni* and *D. uruguayensis*, may even be differentiated by the concentration of Ca, K and S.

The minority elements should be observed very carefully, because they are influenced not only by the species and the environment, but also by other factors, such as exposure time and size. To understand the dynamics of metal accumulation by these organisms, a study with a larger population and temporal assessment is necessary, thus enabling the concentration of specific elements to be assigned to human action.

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