



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>  
Journal DOI: [10.21474/IJAR01](https://doi.org/10.21474/IJAR01)

INTERNATIONAL JOURNAL  
OF ADVANCED RESEARCH

## RESEARCH ARTICLE

## CLIONA VIRIDIS: MINERAL COMPOSITION AND CONTRIBUTION TO THE ASSESSMENT OF THE QUALITY OF THE MARINE ENVIRONMENT.

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### Manuscript Info

#### Manuscript History:

Received: 19 March 2016  
Final Accepted: 22 February 2016  
Published Online: April 2016

#### Key words:

Cliona viridis, mineral composition, heavy metals, XRF, ICP-OES, bioindicator.

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### Abstract

The lifestyle and aquiferous system of sponges are the main factors behind their structural characteristics. They are capable of filtering large volumes of water and have a high rate of retention for particles of 0.2 to 50 microns ( $\mu\text{m}$ ) and therefore a large capacity accumulation of pollutants. At the same time, members of all sponge classes (Demospongia, Sclerospongia, Hyalospongia) can secrete mineral elements called spicules of opaline silica. These siliceous spicules constitute their skeletons involved in several functions, namely in defense, support and the like. *Cliona viridis* is chosen for the study of mineral composition, and contribution to the evaluation of the quality of the marine environment by measuring the concentration of certain heavy metals in two different zones.

Silica  $\text{SiO}_2$  is 70.99% ( $\pm 0.23$ ) of the biomass of the sponge with other minerals such as sodium, chlorine and calcium. The zinc (Zn) is the most accumulated metal then Iron (Fe) and aluminum (Al).

A large study with several species collected from different areas will allow us to understand the process of accumulation of silica and heavy metals and have a good image of level of contamination of the marine environment.

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### Introduction:-

Most sponges are symbiotic microorganisms that provide a significant amount of dissolved organic material in their diet (Reiswig, 1981) and secrete a variety of calcite mineral skeletons, aragonite and (or) of amorphous silica which reinforce and protect the physical disturbance. However, siliceous spicules are formed from a medium that is under-saturated with silicon. Silicification is the dominant process of biomineralization in the current sponge (92% of species). (Maria J-Uriz, 2006).

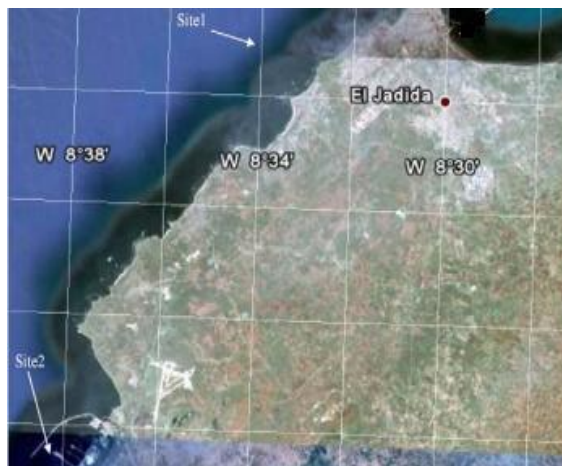
Their sophisticated aquiferous system gives them the ability to actively filter a water volume equivalent to their own volume in 3.7 to 20 seconds (Reiswig, 1974; Larsen & Riisgård 1994). They have excellent retention rates for particles from 0.2 to 50  $\mu\text{m}$ , thus including colloids and small procaryotic cells that are not retained by most other filter feeders (Reiswig, 1971; Thomassen & Riisgård, 1995; Ribes et al., 1999). With these properties, sponges can collect a very large variety of pollutants from the suspended or dissolved phases. Several studies have already demonstrated the potential of sponges for the monitoring of pollutant levels in the marine environment: hydrocarbons (Féral et al., 1979; Zahn et al., 1981), organochlorinated compounds (Verdenal et al., 1990, Arnoux et al., 1992), and metals (Patel et al., 1985; Verdenal et al., 1990; Richelle-maurer et al., 1994; Hansen et al., 1995).

In this paper we examine the mineral composition of sponges of the Moroccan Atlantic coast and we report their heavy metal concentrations regarding two different sites.

## Materials and methods:-

### Sample:-

The sponge *Cliona viridis* was collected in two different sites (Figure 1: Map showing the collect sites of the marine sponge):



**Figure 1:** Map showing the collect sites of the marine sponge.

**Site 1:** The open sea of El Jadida Sidi Bouzid, at low tide, to a depth of 10-18 m (open zone & less polluted).

**Site 2:** Under wave breaking blocks in Jorf Lasfar trading port to a depth of 3 to 5 m.

**Preparation of samples for analysis:-** In the laboratory and after the sample collection, the marine sponge was washed, frozen and lyophilized.

The freeze dryer is installed at the joint laboratory of the Department of Biology, College of Science El Jadida. The samples were frozen at  $-30^{\circ}\text{C}$  for 1 night and then placed in the freeze drying chamber equipped with a tray heater. The freeze drying was set up at  $-70^{\circ}\text{C}$  and at low pressure and it lasts several hours depending on the type of the sample.

The systematic identification of marine sponge was performed by Dr. Maria Jesús Uriz, research professor at the Center for Advanced Studies of Blanes (Centro de Estudios Avanzados of Blanes [BEAC]) and the Higher Council for Scientific Research (Consejo Superior Investigaciones Científicas of [CSIC]) in Spain.

**Determination of total mineral substances (or ash) by incineration:-** The water content is given by the gravimetric method (determination of the weight loss on drying). The ash content is obtained after a sample incineration in a muffle furnace at  $550^{\circ}\text{C} \pm 25^{\circ}\text{C}$  for 4 hours. The average rate of the amount of ash is determined by weight difference. However, the ash content does not correspond exactly to the mineral content (loss of substance by volatilization or significant increase in weight training carbonates or oxides) (Francis Rouessac et al., 2007).

**Determination of ash insoluble in Hydrochloric Acid 10%:-** Treatment of total ash with a hydrochloric acid solution 10%, filtration and incineration of the resulting residue.

**Study of the mineral composition:-** The methods of analysis of the mineral composition used in this study are ICP-OES for the samples of the two areas and for the XRF sample taken at the site 2: Analysis by ICP-OES is carried out according to ISO 22036: 2008, which involves the digestion of the sample by an acid etching followed by filtration and reading emission spectrometer ICP inductively coupled plasma -OES. While the analysis by XRF, is performed according to the standard XP P 15-467 by conducting a sponge **incineration** followed by fusion. The fused mixture is then poured into a mold for XRF analysis.

## Results:-

**The total ash content:-** The percentage by weight of the total ash obtained from the sample and in the dry matter is equal to:  $CT\% = (m_2 - m_0) / (m_1 - m_0) * 100$ . With:  $m_0$ : mass in grams of the empty capsule.  $m_1$ : mass in grams of the dish and the sample.  $m_2$ : mass in grams, of the dish and the total ash.

The percentage of the total ash *Cliona viridis* is: % Total ash = 65, 7% ( $\pm 0. 4$ )

**Insoluble ash Hydrochloric acid 10% (C.In/Acide):-** Ash insoluble in hydrochloric acid consists mainly of silica and silicates. This residue also called "sand" is likened to the earth and other mineral impurities. The percentage by mass of ash insoluble in acid obtained from the sample in the dry matter, is equal to:  $\% C.In/acid = (m_3 - m_0) / (m_1 - m_0) * 100$ . With:  $m_0$ : mass in grams of the empty capsule.  $m_1$ : mass in grams of the empty capsule and the sample.  $m_3$ : mass in grams of the dish and ash insoluble in acid.

The percentage of ash insoluble in acid obtained *Cliona viridis* is: C.In/Acide= 54,85% ( $\pm 0.2$ ) So the ash insoluble exhibit 36% relative to the dry matter.

## Study of the mineral composition:-

- a. **XRF method:** The table (Tab.1) below shows the percentage of oxidized minerals and materials present in the sample taken from the second site; analyzed by XRF method.

According to the obtained results, the mineral part of the sponge *Cliona viridis* represents 65.7% ( $\pm 0.4$ ) of its dry weight of which 36% ( $\pm 0.2$ ) consists of silica and silicates. On the other hand, the results of XRF (Tab.1) revealed the presence of various minerals and their oxidized forms in varying percentages with predominance of Silicon (Si), Sodium (Na), Chlorine (Cl) and Calcium (Ca). We also note that the high percentage of silica ( $SiO_2$ ) in the specie *Cliona viridis* in relation to other compounds with a value of 70.99% ( $\pm 0.23$ ) of the biomass of the sponge which 33,19% ( $\pm 0,11$ ) of the silicon element (Si).

**Table 1:** The percentage of oxidized minerals and materials present in *Cliona viridis*.

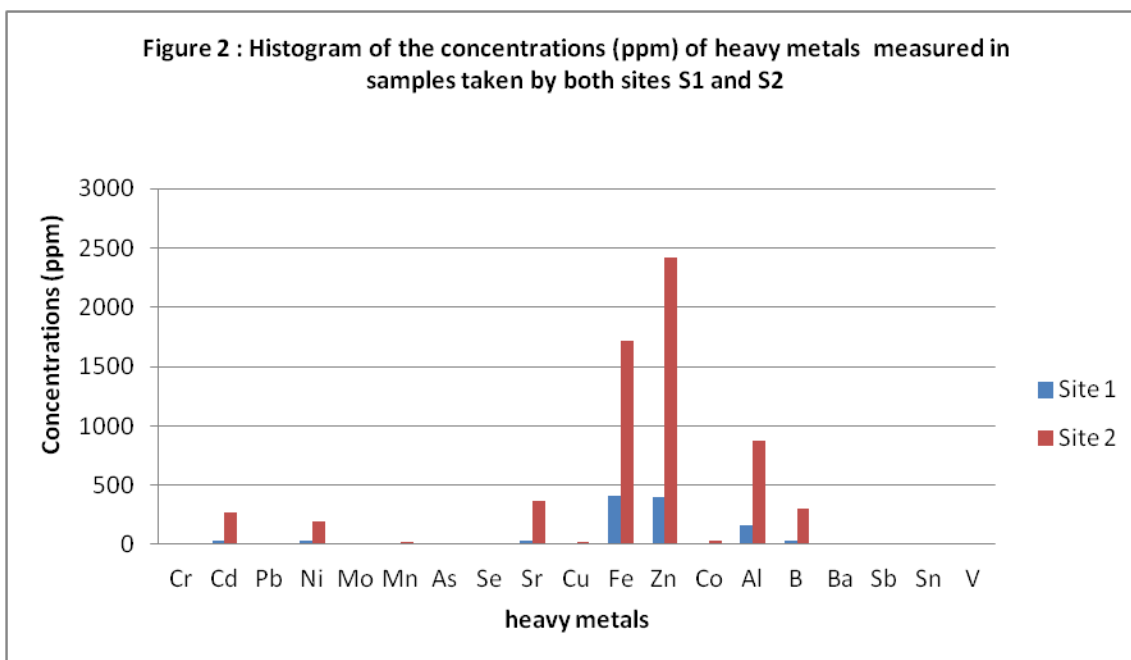
Compound	Percentage %	Element	Percentage %
$SiO_2$	70,99 $\pm$ 0,23	Si	33,19 $\pm$ 0,11
$Na_2O$	6,76 $\pm$ 0,13	Na	5,01 $\pm$ 0,09
CL	4,73 $\pm$ 0,11	CL	4,73 $\pm$ 0,11
$CaO$	4,43 $\pm$ 0,10	Ca	3,16 $\pm$ 0,07
$SO_3$	3,83 $\pm$ 0,10	Sx	1,53 $\pm$ 0,04
$MgO$	1,97 $\pm$ 0,07	Mg	1,19 $\pm$ 0,04
$P_2O_5$	1,88 $\pm$ 0,07	Px	0,822 $\pm$ 0,030
$AL_2O_3$	1,24 $\pm$ 0,06	AL	0,655 $\pm$ 0,029
F	0,92 $\pm$ 0,13	F	0,92 $\pm$ 0,13
$ZnO$	0,770 $\pm$ 0,038	Zn	0,618 $\pm$ 0,031
$K_2O$	0,762 $\pm$ 0,038	K	0,632 $\pm$ 0,031
Br	0,513 $\pm$ 0,026	Br	0,513 $\pm$ 0,026
$Fe_2O_3$	0,490 $\pm$ 0,024	Fe	0,343 $\pm$ 0,017
$CdO$	0,143 $\pm$ 0,007	Cd	0,125 $\pm$ 0,006
$SrO$	0,114 $\pm$ 0,006	Sr	0,0965 $\pm$ 0,0048
I	0,0784 $\pm$ 0,0039	I	0,0784 $\pm$ 0,0039
$NiO$	0,0713 $\pm$ 0,0036	Ni	0,0560 $\pm$ 0,0028
$Y_2O_3$	0,0535 $\pm$ 0,0027	Y	0,0421 $\pm$ 0,0021
$BaO$	0,0382 $\pm$ 0,0019	Ba	0,0342 $\pm$ 0,0017
$Cs_2O$	0,0306 $\pm$ 0,0015	Cs	0,0289 $\pm$ 0,0014
$TiO_2$	0,0271 $\pm$ 0,0014	Ti	0,0162 $\pm$ 0,0008
$RuO_4$	0,0209 $\pm$ 0,0014	Ru	0,0159 $\pm$ 0,0010
$CeO_2$	0,0152 $\pm$ 0,0027	Ce	0,0124 $\pm$ 0,0022
$Nd_2O_3$	0,0138 $\pm$ 0,0015	Nd	0,0118 $\pm$ 0,0013
$Rh_2O_3$	0,0131 $\pm$ 0,0012	Rh	0,0106 $\pm$ 0,0010
$ZrO_2$	0,0120 $\pm$ 0,0008	Zr	0,0089 $\pm$ 0,0006
$As_2O_3$	0,0113 $\pm$ 0,0031	As	0,0086 $\pm$ 0,0023
$PdO$	0,0111 $\pm$ 0,0011	Pd	0,0096 $\pm$ 0,0010
$Pr_6O_{11}$	0,0086 $\pm$ 0,0028	Pr	0,0071 $\pm$ 0,0023

Sb <sub>2</sub> O <sub>3</sub>	0,0077±0,0012	Sb	0,0064±0,0010
La <sub>2</sub> O <sub>3</sub>	0,0074±0,0026	La	0,0063±0,0022
Cr <sub>2</sub> O <sub>3</sub>	0,0070±0,0007	Cr	0,0048±0,0005
Ag <sub>2</sub> O	0,0068±0,0010	Ag	0,0063±0,0009
CuO	0,0061±0,0006	Cu	0,0049±0,0005
MnO	0,0056±0,0007	Mn	0,0043±0,0005
Dy <sub>2</sub> O <sub>3</sub>	0,0055±0,0023	Dy	0,0048±0,0020
PtO <sub>2</sub>	0,0047±0,0022	Pt	0,0040±0,0019
PbO	0,0037±0,0017	Pb	0,0034±0,0016
SeO <sub>2</sub>	0,0036±0,0011	Se	0,0026±0,0007
V <sub>2</sub> O <sub>5</sub>	0,0033±0,0006	V	0,0018±0,0004
Rb <sub>2</sub> O	0,0032±0,0007	Rb	0,0029±0,0006
Nb <sub>2</sub> O <sub>5</sub>	0,0031±0,0007	Nb	0,0022±0,0005
WO <sub>3</sub>	0,0030±0,0014	W	0,0024±0,0011

**b. ICP-OES method:** The Metal concentrations (ppm) measured in samples taken from two sites (S1 and S2) are shown in the following table (Tab.2) and Figure (Fig.2).

**Table. 2:** The Metal concentrations (ppm) measured in samples taken from two sites.

Site/Métal :	Site 1	Site 2
Cr	2,511 ±0,006	14,74 ±0,03
Cd	36,7 ±0,34	274 ±0,2
Pb	N.D	3,002 ±0,023
Ni	27,60 ±0,22	189 ±0,6
Mo	0,0268 ±0,0046	0,0985 ±0,0601
Mn	4,319 ±0,025	25,42 ±0,26
As	0,0015 ±0,034	1,409 ±0,1241
Se	N.D	3,967 ±0,074
Sr	31,83 ±0,04	362,2 ± 3
Cu	13,20 ±0,09	16,98 ±0,3
Fe	407,8 ±9,7	1714 ±12
Zn	402,3 ±4,1	2424 ±14
Co	0,9378 ±0,0124	36,71 ±0,34
Al	157,6 ±0,5	878,2 ±3,9
B	27,21 ±0,48	302,4 ±13
Ba	0,6936 ±0,0043	6,453 ±0,07
Sb	N.D	0,0028 ±0,3
Sn	4,716 ±0,331	4,868 ±0,119
V	1,256 ±0,087	2,895 ±0,108



The study of heavy metals in *Cliona viridis* shows that concentrations vary depending on the sampling site. We note that (i) used concentrations in this species, collected in site S2 (Jorf Lasfar Port of trading, which is a basin where the exchange with the ocean is very limited, and which boost the concentration of different polluted discharges) are higher than the concentrations of marine waters reported in the literature (**P.W. Ball, 2004**), and (ii) these levels are significantly higher compared to those observed in the same species collected at site 1 (El Jadida-Sidi Bouzid's wide, less polluted and open zone).

The analysis revealed a chemical bioaccumulation of several metals: Zn, Fe, Al, Cd in the case of *Cliona viridis*. It also indicates that these metals are related to the life cycle of these animals and others to marine pollution due to human activities that generate pollutants affecting water quality, threatening marine life and consequently affecting the food chain and human health.

### **Discussion and conclusion:-**

Indeed, the high value of the silica in the case of *Cliona viridis* can be explained by the fact that this marine sponge belongs to the class of Demospongiae whose backbone, more or less rigid, consists of spicules, siliceous or spongin. Some spicules can be large (megascleres). They are essential to the structure of the animal. Others, smaller (microscleres) are embedded in the parenchyma. They are often organized in the backbone with their pointed end into or projecting from the surface of the sponge.

According to the results, the mineral composition of *Cliona Viridis* mostly consists of silica ( $\text{SiO}_2$ ) with some trace elements. This is consistent with previous studies carried out on Demosponges. This composition varies slightly depending on the species and concentration of the water they contain. Other inorganic ions which do not take part in the spicules composition, such as  $\text{Fe}^{2+}$ , appear to be decisive in activating the enzymes that catalyze silica polymerization (**Le Pennec et al., 2003**). Also, it is outlined that silicon and  $\text{Fe}^{3+}$  contribute substantially to the formation of larger primmorphs (size of 10 mm) as well as of a canal system in primmorphs; canals are probably required for an improved oxygen and food supply. (**Le Pennec G, 2003**).

Sponges take up silicon in the form of soluble silicic acid. This silica absorbed by the sponges may vary depending on the silicon (Si) concentration in the water, temperature, and other environmental factors affecting the physiology and metabolism of the sponge (**Frohlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 1999**).

Spicules seem to represent harmful elements in the food, particularly if we consider that they may represent up to 75% of the sponge biomass (**Rutzler and Macintyre, 1978; Desqueyroux-Faundez, 1990**) and that they are often arranged in the skeleton with their sharp end towards or protruding the sponge surface. As a consequence, the siliceous skeleton of sponges has often been interpreted as an effective mechanism for deterring predation (**Randall and Hartman, 1968; Sara and Vacelet, 1973**). Spicule concentration appears to be a plastic trait that can be induced by damage in some morphotypes of *Antosigmella varians* Duchassaing and Michelotti (**Hill and Hill, 2002**). These authors found that sponges unprotected from predators increased spicule yields, and suggest that the large spicule rich cortex of these morphotypes is an inducible structural defense.

Several other functions, sometimes complementary to the main supporting role, can be envisaged for sponge spicules, although most of them appear exclusive of particular species. In extreme environments where an active filter feeder has a low yield, the family Cladorhizidae Dendy has developed particular tools for capturing living prey passively, which consist of long thin tentacles provided with a dense layer of protruding upraised hook-shaped microscleres. These carnivorous sponges capture small crustaceans (less than 1 mm in size), which are entrapped thanks to the sponge microscleres (**Vacelet and Boury-Esnault, 1995**).

Spicules also appear to play a role in gamete and larvae dispersal of some species. Certain larval spicules such as the discotriaenes that cover the hoplitomella larva of Alecionidae (**Vacelet, 1995**) are not present in the adults. Some of the larval spicules notably favor larval buoyancy and thus may increase larval dispersal, as reported for the styles that largely protrude through the armored hoplitomella of Alecionidae, which can be found among the oceanic plankton (**Tregouboff, 1942**).

Indeed Sponges absorb not only silica but thanks to their aquiferous system, these invertebrates are suspension-feeders that filter large amounts of water. However, the nature of the organic material and the size of the particles

that are retained differ significantly. Sponges have a high retention rate for particles of bacteria size, and appear to be able to meet their entire carbon requirements by feeding on particles smaller than 1  $\mu\text{m}$  (Stuart & Klumpp, 1984; Ribes et al., 1999). In contrast, sponges may live several decades and thus accumulate during a longer time.

The diversity of morphology of the aquiferous system may be related to their ability to up-take a wide array of particles and to their powerful pumping activity influencing the water volumes passing through the body (Reiswig, 1975), and thus the pollutant accumulation.

At the sponge *Cliona viridis* object of study, the Zinc (Zn) is the most accumulated metal, a result similar to that found by Thierry perez and all. 2004. Also it shows very high concentrations of the Iron (Fe) and the Aluminium (Al). This sponge, which contains a large mineral fraction, made of calcareous debris and of skeletal siliceous spicules, also has a very different mode of life. All clionid sponges (family Clionidae) excavate calcareous substrates.

The special behavior of *C. viridis* for accumulation of metals may be related to this ability to bio-erode calcareous substrates, which are frequently biological concretions made primarily by calcareous algae, which are themselves able to accumulate heavy metals mainly from the dissolved fraction. *Cliona viridis* is unique in its symbiotic association. It is associated with a large quantity of light-dependent zooxanthellae (Sarà & Liaci, 1964; Rosell & Uriz, 1992), and with only very few bacteria. It is possible that this unique association influences the accumulation capacity and the peculiarities found for this species. Like other unicellular algae (Riley & Roth, 1971; Bonin et al., 1986), zooxanthellae could be capable of accumulating metals from the dissolved phase.

The structure and biological composition of the sponge are probably the most important factors for its ability to retain or to eliminate the metals. The skeletal structure, whose size varies considerably in sponges, is able to concentrate metals differently from the living tissue (Verdenal et al., 1990).

In fact, Sponges are well suited for the monitoring of type of chemical contamination. More studies are needed with representative sampling for species and locations to collect samples and to determine the appropriate bioindicator species.

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