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Decontamination procedure for trace elements determination in coral skeleton samples

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ABSTRACT

The three most used decontamination procedures for coral samples were evaluated in order to subsidize the development of a simpler, faster and more efficient cleaning procedure for decontaminating coral skeleton for trace element determinations using ICP OES. The procedures tested involved a sequence of ultrasonic cleaning with deionized water, 0.2 mol.L^{-1} HNO_3 and/or an oxidizing mixture of 30% H_2O_2 and 0.2 mol.L^{-1} NaOH in an ultrasonic bath to remove particles and residues from saw blades used during the collection and pretreatment of coral samples. The main contaminants identified were Fe, Cu and Zn. The three decontamination procedures tested were efficient, but indicated that the decontamination steps with deionized water are unnecessary. The procedure proposed in this study proved to be more efficient, as only one extracting agent was used, the number of steps required to decontaminate the coral samples was reduced, consequently saving time and increasing analytical frequency.

1. Introduction

Corals are widely used as biomonitors for tropical ecosystems due to their sensitivity to physical and chemical changes in the marine environment [1–8]. Long-lived corals, working as proxy tools, record ocean surface water changes over long periods of time. This is one of the advantages of corals as compared to other biomonitors [9]. Variations over time are recorded in coral skeletons, which grow continuously, by simple accretion along the surface of the colony [10].

The incorporation of several trace elements to coral skeleton is well documented. Several elements, such as toxic metals, can substitute Ca in the skeleton or can be associated with particulate organic matter within skeletal pores [11–13]. Since Howard and Brown's review [11] of trace elements in coral reefs, many studies have used corals as biomonitors for metal contamination. Both long-term [14–16] and short-term evaluations of several metals in skeletons have confirmed the potential of coral as an environmental impact proxy [13,17–22].

Differently from other widely employed biomonitors such as oysters and mussels, with coral the collection and preparation of samples are potentially important sources of contamination. In most cases, clean practices are not employed throughout sampling and the early stages of the pretreatment of coral samples. In fact, coral samples are usually collected as individual heads or cores retrieved using diver operated drills and other metal tools which potentially cause superficial contamination. In addition, corals have to be sawed in the laboratory to obtain sample slices which are thin enough for chronological studies.

As a result, Shen and Boyle [2] developed an exhaustive cleaning procedure for coral sample decontamination prior to chemical analysis. They also found that lattice-bound concentrations of trace elements were three orders of magnitude lower than any previous report [11,2]. According to Shen and Boyle [2], low metal concentrations resulted from the elimination of particulate matter and metal oxides during the cleaning procedure. Several studies have used the procedure developed by these authors [23,24,13] or adaptations of it [25,26]. Other studies have proposed alternative cleaning procedures [27,28], but none of them quantified and/or identified the metals eliminated by each cleaning step and/or process involved to justify their use. Inoue [16] was the only exception, showing that Cu and Sn were eliminated during a decontamination procedure.

The objective of this study was to evaluate and compare the most used decontamination procedures for trace element analyses in coral samples, and to develop a simple, fast and efficient cleaning procedure to decontaminate coral skeleton for trace element determinations.

2. Experimental procedure

2.1. Sample preparation

All experiments were conducted with skeleton samples of the coral *Siderastrea stellata VERRILL, 1868*, collected at Todos os Santos

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Bay, Bahia, Brazil (12°48′51,37″S; 38°34′16,72″W). The sample was washed with seawater and scrubbed with a plastic brush in order to clean off all algae. Then the sample was divided into blocks of 1.0 cm^2 with a prosthetic diamond saw, washed with deionized water and dried at 60 °C for 6 h. For each decontamination experiment around 4 g of corals was used.

An analytical balance (Sartorius Ag Gottiengen, Germany), an agate pestle and mortar, an ultrasonic bath (Benc top Cleaner VWR, 75D, and a centrifuge (Eppendorf, Harburg, Germany), were employed in sample preparation.

2.2. Reagents and solutions

All labware was soaked in an HNO_3 acid bath (65% w/v, diluted 1/10 with high purity water) for 24 h and rinsed with high purity water. Subsequently, all materials were dried under clean-air conditions at ambient temperature. All plastic containers, polyethylene flasks, pipette tips and PFA Teflon digestion vessels (Milestone SRL, Sorisole, Italy) were cleaned prior to use. All solvents and reagents used were of the highest commercially available purity.

Deionized water with a resistivity of ≥18 MΩ cm^{-1} (Milli-Q Plus, Millipore Molsheim, France) was employed to prepare all standard and sample solutions. Analytical grade reagents were used for sample dissolution. Mono-elemental, high-purity grade 1 g L^{-1}, stock solutions of trace elements (Titrisol®, Merck, Germany) were used daily to prepare the multi-elemental analytical reference solutions. Diluted acid solutions of HNO_3, HCl and CH_3COOH (1.0, 2.0 and 3.0 mol L^{-1}) were prepared by dilution with pure water.

2.3. Instrumentation

An inductively coupled plasma optical emission spectrometer (ICP OES) with axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) equipped with a solid state detector, a cyclonic spray chamber and a concentric nebulizer was employed for the determination of trace elements. The operating conditions are summarized in Table 1.

2.4. Decontamination procedures

Fig. 1 shows the three procedures tested [29,25,26]. The decontamination procedures were a stepwise sequence of ultrasonic cleaning in 10 mL of ultrapure water, and 10 mL of 0.2 mol.L^{-1} HNO_3 (Fig. 1). The simplest cleaning procedure was proposed by David [26]. The procedure proposed by Bastidas and Garcia [25] not only uses successive ultrasonic cleaning with ultrapure water and HNO_3, but also complementary ultrasonic cleaning in a 10 mL 50%–50% oxidizing mixture of 30% H_2O_2 and 0.2 mol.L^{-1} NaOH. The procedures proposed by Bastidas and Garcia [25] and Guzmán and Jarvis [29] also included a coarse comminuting step. Between the cleaning steps, samples were centrifuged at 3000 rpm for 10 min and 7 mL of the supernatant was then transferred to a previously decontaminated tube. The supernatant from the water cleaning steps was acidified with concentrated ultrapure HNO_3. All experiments, including procedural blanks, were prepared in triplicate. Trace and major elements in the supernatant solution were analyzed using ICP OES, employing yttrium as the internal standard. The use of Y is already a routine recommendation in various standard methods for the analysis of biological and environmental samples, to improve the overall precision and/or to compensate error due to matrix effects.

After the decontamination procedures, the samples were dried and crushed in an agate mortar and pestle followed by sieving through a 500 μm nylon mesh. Aliquots of 0.50 g of coral samples were transferred to 50 mL centrifuge tubes and dissolved in 9.0 mL of 3.0 mol L^{-1} HNO_3 at ambient temperature. An aliquot of the coral that did not undergo any decontamination treatment was also dissolved in 9.0 mL of 3.0 mol L^{-1} HNO_3 at ambient temperature. The major and trace elements in coral skeletons were determined using ICP OES.

The detection limits (LOD) were estimated using the RSD obtained from ten experimental blanks and a background equivalent concentration (BEC) according to the following equation [30]:

$$LOD = 3 \times BEC \times RSD/100$$

2.5. Optimization of decontamination procedure

After analyzing the results of the three procedures tested, an alternative procedure was tested and evaluated. It consisted of a sequence of ultrasonic cleaning with 10 mL 0.2 mol.L^{-1} HNO_3. Between cleaning steps, samples were centrifuged at 3000 rpm for 10 min (Fig. 1D). Trace and major elements were determined using ICP OES, as described earlier.

3. Results and discussion

The trace element concentrations of the supernatant analyses for the three procedures tested allowed for their separation into two groups.

Firstly, the elements that were extracted in the treatment steps with deionized water: Fe, Ni, and Cu (Fig. 2), and secondly the elements that were only extracted with diluted acid solutions: V, Mn and Ba (Fig. 3).

In general, the elimination of Fe, Ni and Cu with water was only efficient during the initial steps, with the concentrations of Fe, Ni and Cu decreasing after the beginning of the cleaning procedure (Fig. 2). In the treatment involving acid, the levels of these elements decreased throughout the procedure. Similar behavior was observed by Inoue [16] who studied the incorporation of Cu and Sn to the coral skeleton.

The extraction of Fe, Ni and Cu with deionized water indicates a weak association between these elements and the skeleton, suggesting that the main source of these elements was contamination during sample collection, handling and/or pretreatment steps.

The diamond saw blade used in the sampling procedure is made of austenitic stainless steel, which contains Fe, C, Si, Mn, Cr, Ni, Mo, Cu and N in relatively high concentrations [31]. Furthermore, the diamonds are fixed on the blade with electrolytic Ni containing Fe, Cu,

Table 1
Operational parameters used in axial view ICP OES.

Characteristics	Instrument conditions
RF generator	40 MHz
Power	1.3 kW
Spray chamber	Cyclonic chamber
Nebulizer	Sea Spray
Plasma gas flow	15.0 L min^{-1}
Auxiliary gas flow	1.5 L min^{-1}
Nebulizer gas flow	0.7 L min^{-1}
Injector tube diameter	2.4 mm
Emission lines (nm)	Al II 167,019; Mn II 257,610; Zn I 213,857; Ba II 455,403; Cu I 324,754; Co II 238,92; Fe II 259,940; Se I 196,026; Ni II 221,648; V II 292,464

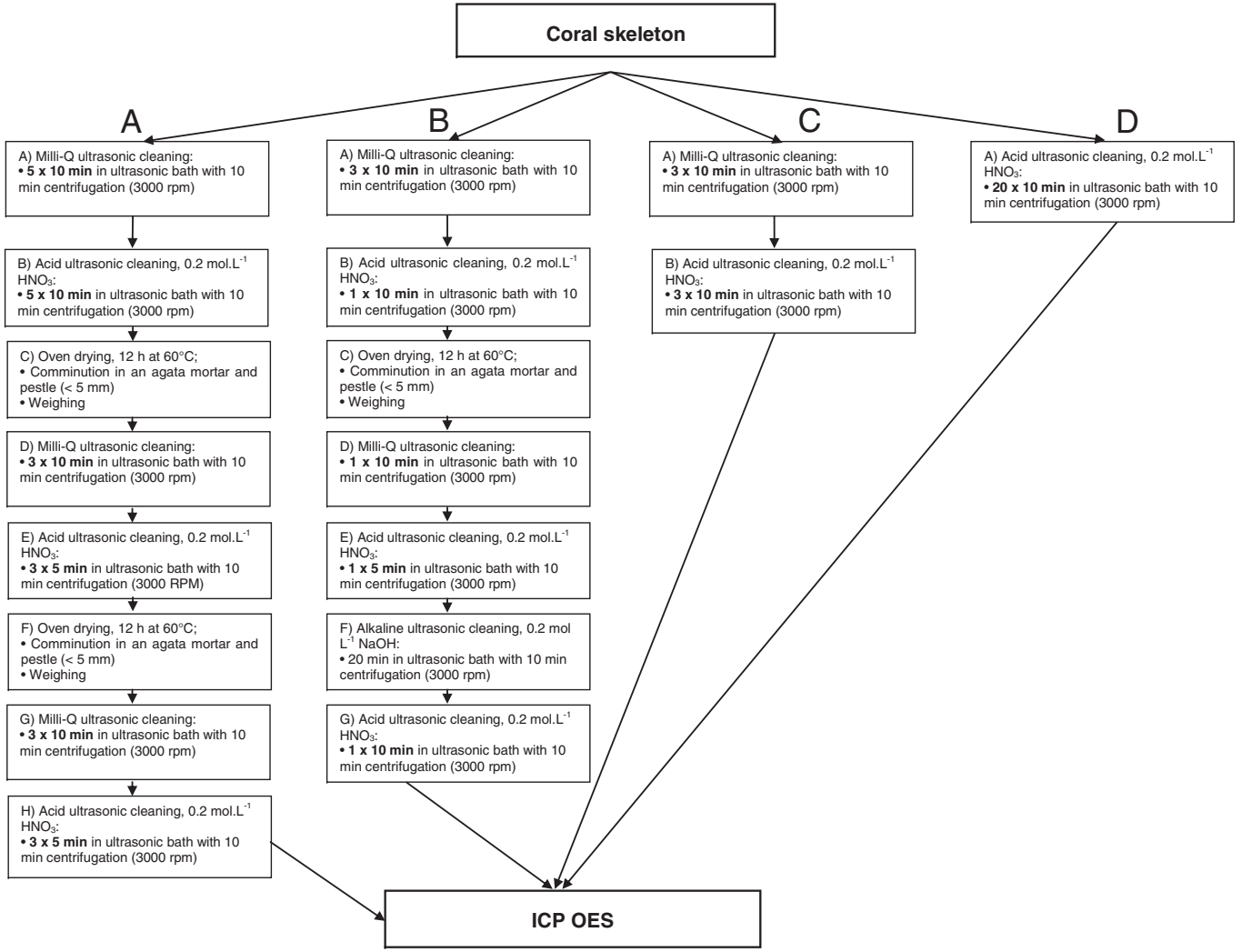

Fig. 1. Flow diagram of cleaning procedures. A. Guzmán and Jarvis [29]; B. Bastidas and Garcia [25]; C. David [26]; D. procedure tested in this study.

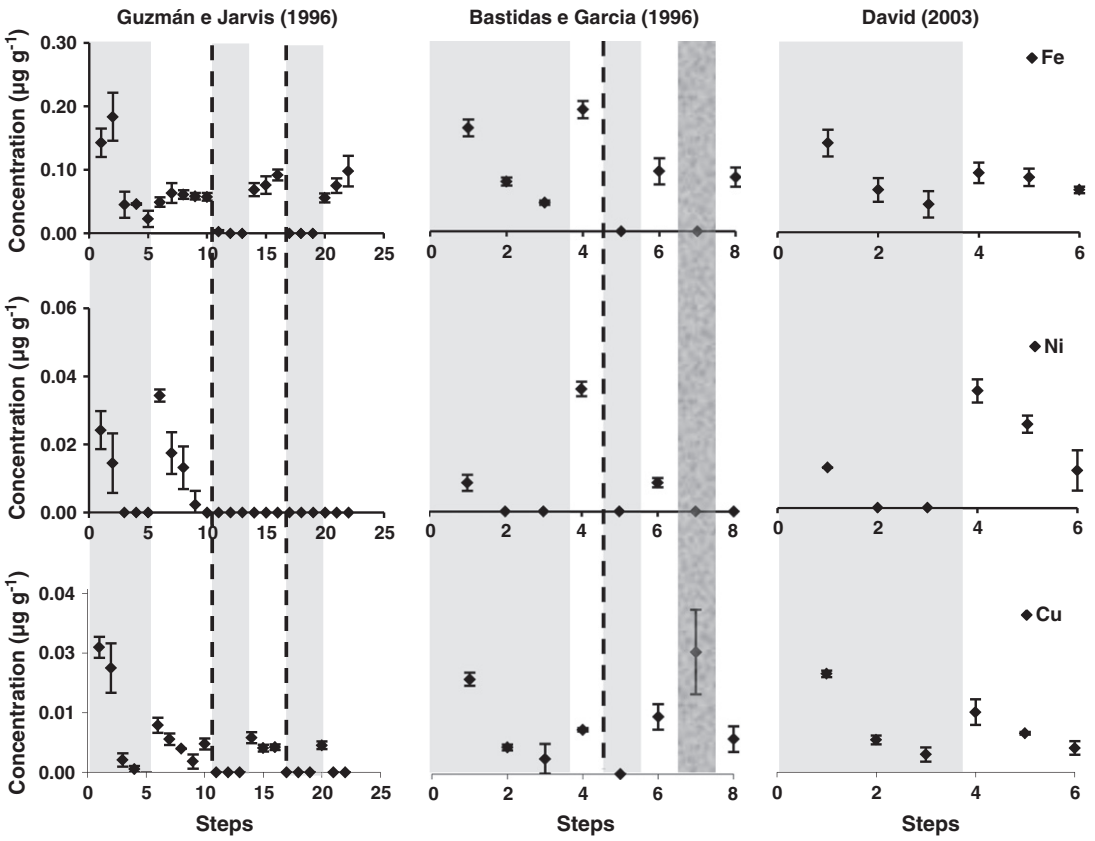

Fig. 2. Concentrations of Fe, Ni and Cu in supernatant solutions for the Guzmán and Jarvis [29], Bastidas and Garcia [25] and David [26] procedures. The areas in light gray indicate treatment steps with deionized water; areas in white indicate treatment steps with HNO_3 0.2 mol.L^{-1} and the patterned area corresponds to 30% solution of H_2O_2 + 0.2 mol.L^{-1} NaOH 1:1. Dotted lines correspond to sample comminution steps.

Co and Zn impurities. A comparison of the elemental composition of the diamond saw with the trace metals that were extracted with water indicates that the cutting process of the samples is an important source of potential contamination with Fe, Ni and Cu. As a result, alternative procedures and materials for both sample collection and slicing need to be developed.

On the contrary, the behavior of V, Mn and Ba showed that these elements are more strongly attached to the crystalline structure of the coral skeleton, since acid solution was necessary to mobilize them.

Barium and Mn in the supernatant solution peaked during the initial treatment step with acid, followed by a decrease in the subsequent steps (Fig. 3). This pattern suggests that contamination due to the handling of samples may result in an increase in the concentrations of these elements.

Vanadium, however, behaved differently. Overall, the concentrations resulting from the initial treatment step with acid caused no significant extraction, showing the same behavior as during water procedures (Fig. 3). It was also observed that the V concentrations tended to increase (Fig. 3) with the procedures which included sample comminution between successive steps [29,25]. Clearly the grinding step was an important way to expose more sample surface and enhance the efficiency of V elimination. Compared to other elements however, the effect of sample handling on V was much smaller.

The estimated LOD values ($\mu g.g^{-1}$) of the supernatants for Al, Ba, Co, Cu, Fe, Mn, Ni, V and Zn were respectively, 1.25×10^{-1}, 3.0×10^{-4}, 7.9×10^{-2}, 3.0×10^{-4}, 1.7×10^{-2}, 9.0×10^{-3}, 2.0×10^{-1}, 1.1×10^{-1} and 2.0×10^{-2}. The concentrations obtained for Ni (Fig. 2) were very close to the estimated LOD, and should only be used to indicate that Ni was extracted from coral samples to some degree in the decontamination procedure. However, one cannot state about the absolute value that was extracted.

The concentrations of trace metals in the skeleton samples also indicate that the coral sample surfaces were contaminated, especially by Fe, Ni, Al, Cu and Zn (Table 2). The concentrations of these elements in the untreated sample (i.e. sample that did not undergo any decontamination procedure) were significantly higher than the concentrations of the same elements in decontaminated samples (Figs. 4 and 5; ANOVA at 95%, $p>0.05$). These results corroborate the findings obtained from the supernatant solutions. However, when the untreated sample was compared to the samples submitted to different decontamination procedures, there was no significant difference ($p<0.05$) in element concentrations. This result illustrates that the efficiency of all the procedures tested is the same, independent of the number of steps or complexity of treatments.

The pattern of the Fe, Ni and Cu concentrations in the supernatant solutions (Fig. 2), compared to the significant differences shown by the variance analysis for the decontaminated and non-decontaminated skeleton samples, indicates that the handling, collection and pretreatment processes of the coral samples have a high contamination potential. However, for V, Ba, Co, Se and Mn this contamination did not seem to be representative, since there was no significant difference between the samples which underwent decontamination and those which did not (Figs. 3 and 5).

Fig. 6 shows the concentrations of trace metals obtained after the successive cleaning steps employing only HCl (Fig. 1D). Results showed that in the fourth washing step the concentration of Ni was below the detection limit of the method, remaining constant until the

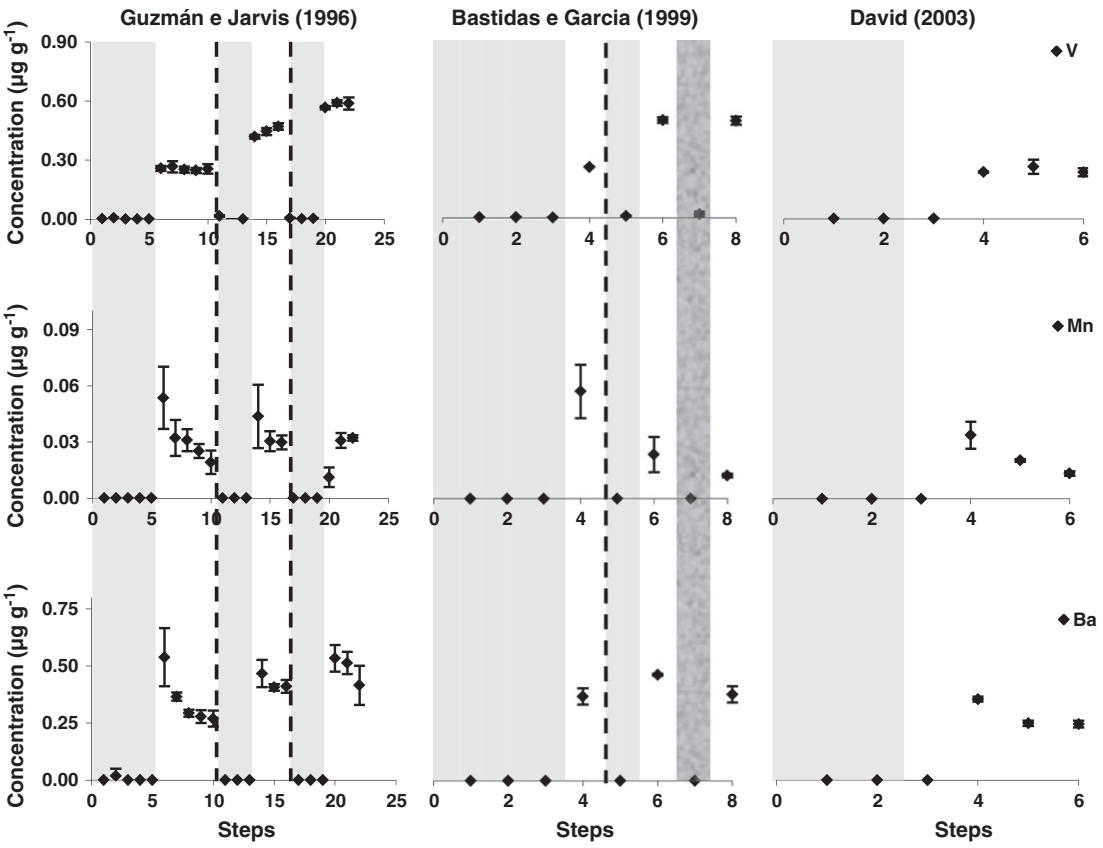

Fig. 3. Vanadium, Mn and Ba concentrations in the supernatant solutions for the Guzmán and Jarvis [29], Bastidas and Garcia [25] and David [26] procedures. The areas in light gray indicate treatment steps with deionized water; areas in white indicate treatment steps with HNO_3 0.2 mol.L^{-1} and the patterned area corresponds to 30% solution of H_2O_2 + 0.2 mol.L^{-1} NaOH 1:1. Dotted lines correspond to sample comminution steps.

last step. For Mn, however, this behavior was observed from the fifth step. For Ba, the concentration remained stable after the third step.

The evaluation of the decontamination procedures tested showed that water was only efficient in remobilizing some trace metals in the initial treatment steps. These results suggest that it could be substituted by the diluted acid solution which is more efficient throughout all the steps and for all elements evaluated. Comparing the concentration results of Ni and Mn in the supernatants of the three procedures evaluated to the results obtained after cleaning only with HCl, it is clear that the cleaning procedure proposed in this study is simpler and more efficient, since only 5 washing steps were necessary to remobilize the metals attached to the coral surface, which are the result of sample handling contaminations.

4. Conclusions

The decontamination procedures for the skeletal samples of *Siderastrea stellata* are necessary since the collection, handling and sampling procedures currently used around the world can cause substantial sample contamination. The main contaminants identified were Fe, Ni, Cu and Zn. The three decontamination procedures tested [29,25,26] were efficient, no matter the number of steps. The results however, indicated that the decontamination steps with deionized water are unnecessary. Therefore, the procedure proposed in this study using only one extracting agent proved to be more efficient, reducing the number of steps necessary to decontaminate the coral samples, consequently saving time and increasing analytical frequency. Nevertheless it is necessary to point out that new material and procedures should be developed to avoid sample contamination during collection. Moreover, compared to other biomonitors, the amount of time and work involved in decontamination procedures is a disadvantage of working with coral matrix, but if long-term contamination is to be determined the use of coral is still highly recommended.

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Table 2
Average concentration and standard deviation of measured analytes (µg.g^{-1}) in skeleton samples of the *Siderastrea stellata*.

	Co	Fe	Ni	V	Al	Mn	Ba	Zn	Cu
SWT	0,21 ± 0,02	3,19 ± 0,05	0,38 ± 0,01	3,44 ± 0,02	3,50 ± 0,46	0,64 ± 0,04	10,39 ± 0,5	0,42 ± 0,04	0,24 ± 0,02
P1	0,21 ± 0,01	2,96 ± 0,07	0,15 ± 0,03	3,51 ± 0,14	3,49 ± 0,28	0,70 ± 0,11	9,95 ± 0,89	0,23 ± 0,02	0,06 ± 0,00
P2	0,15 ± 0,05	1,76 ± 0,28	0,13 ± 0,01	3,50 ± 0,14	1,83 ± 0,05	0,46 ± 0,04	9,43 ± 1,07	0,23 ± 0,005	0,07 ± 0,01
P3	0,18 ± 0,00	1,95 ± 0,12	0,17 ± 0,09	3,51 ± 0,13	2,58 ± 0,8	0,56 ± 0,03	10,03 ± 0,43	0,31 ± 0,02	0,14 ± 0,00

SWT: Sample without treatment; **P1:** Guzmán and Jarvis [29]; **P2:** Bastidas and Garcia [25]; **P3 :** David [26].

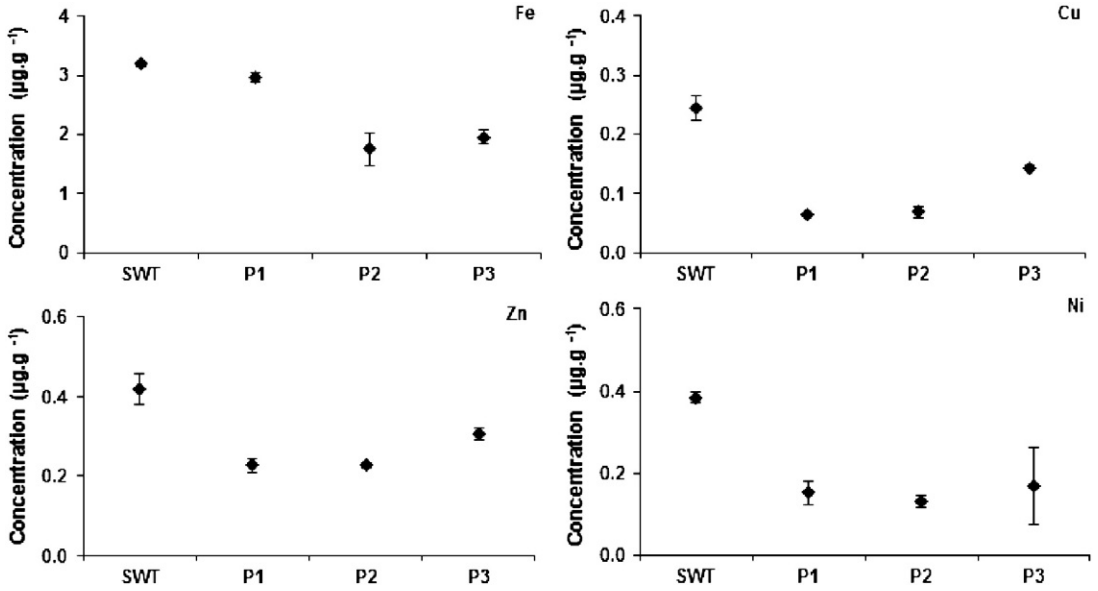

Fig. 4. Iron, Cu, Zn and Ni concentrations in the skeletal matrix of the *S. stellata*. SWT: Sample without decontamination treatment; P1: Guzmán and Jarvis [29]; P2: Bastidas and Garcia [25]; P3: David [26].

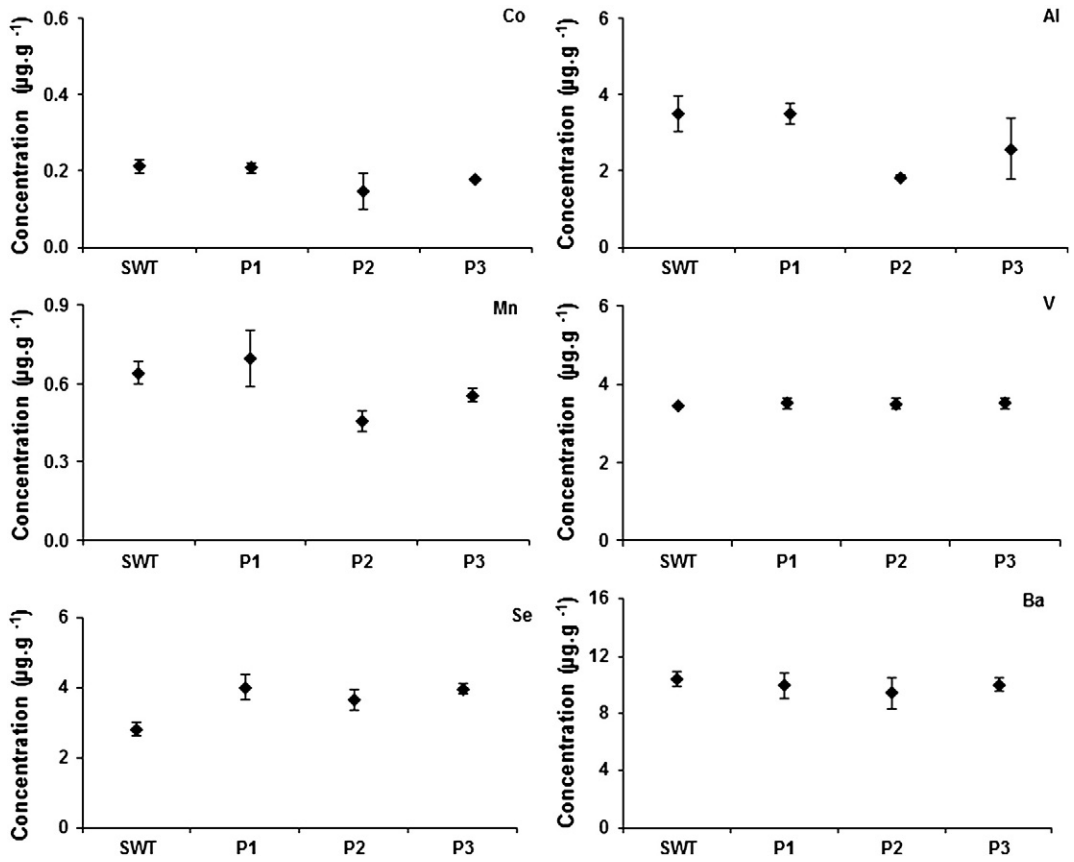

Fig. 5. Cobalt, Al, Mn, V, Se and Ba concentrations in the skeletal matrix of the *S. stellata*l. SWT Sample without decontamination treatment P1: Guzmán and Jarvis [29]; P2: Bastidas and Garcia [25]; P3: David [26].

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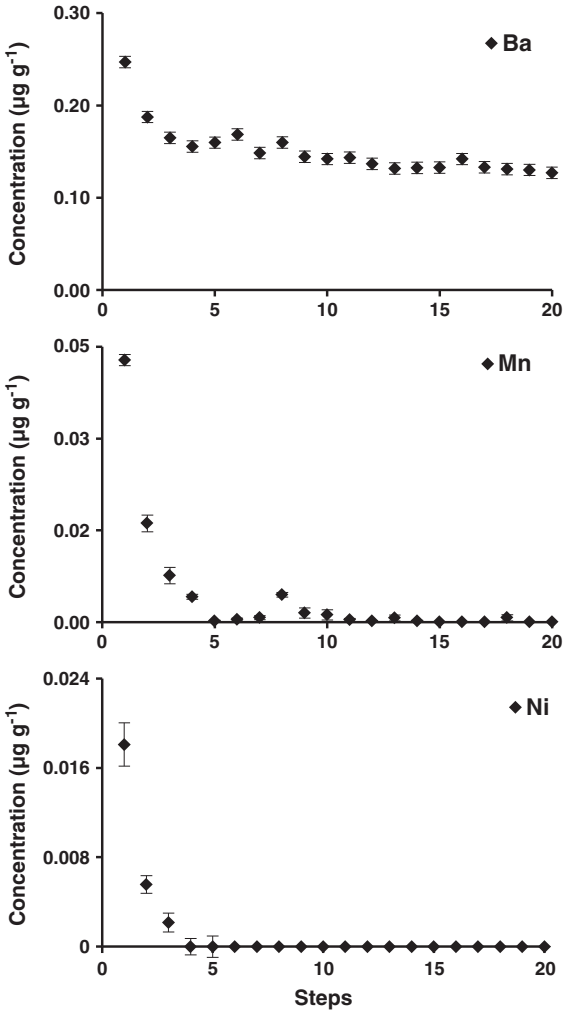

Fig. 6. Concentrations of the elements Ba, Mn and Ni in the supernatants for the decontamination procedure proposed in this study.

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