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ORIGINAL RESEARCH PAPER

Exploring the Potential of Alternative Methods to Monitor the Impact of Nanomaterials on Corals Using *Gorgonia Ventalina* as a Model Organism

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ABSTRACT
The environmental fate and biological effects of nanomaterials in marine ecosystems are of increasing concern, yet monitoring techniques remain limited. This study explores alternative methods to evaluate the influence of Gold Nanoparticles (AuNPs) on corals using Gorgonia ventalina as a model organism. Through energy dispersive X-ray spectroscopy (EDAX), we traced AuNP accumulation within coral tissues. Raman spectroscopy revealed an elevation in polyene content, signifying a stress response attributable to nanoparticle exposure. Furthermore, Fourier transform infrared spectroscopy demonstrated a reduction in calcium levels, while the Bradford assay indicated a decrease in protein concentration, suggesting a disruption in the calcification process vital to coral health. These findings highlight the utility of these alternative analytical techniques in providing comprehensive insights into the effects of nanomaterials on corals. The integration of these methods offers a more robust framework for environmental monitoring, with the potential to inform conservation strategies for marine ecosystems amid growing nanoparticle use.

Keywords: *Gold Nanoparticles, Gorgonia Ventalina, Ftir, Raman, Bradford.*

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INTRODUCTION

Nanomaterials (NMs) are one of the novel technological breakthroughs in recent decades. In general, NMs are materials molded into a specific shape, ranging between 1 nm and 100 nm in size and thus displaying a relatively high surface-to-volume ratio [1]. Their sizes, shapes, and materials yield distinctive NMs properties and physicochemical behaviors, making them a highly versatile technology [2-4]. NMs have gained interest mainly due to their biosensing and chemical detection capacity [5, 6]

Despite their use in some daily-life applications and the forecasted raises in their everyday use, NMs are poorly regulated. Instrumentation and methodologies for the detection, quantification, and remediation NMs in environmental and biological matrices remain in their early stages [2, 7, 8]. These limitations call for a refining of existing technologies, aiming not just for enhanced

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detection accuracy and precision of nanomaterials but also for an integrative analysis of their physiological consequences.

Consequently, NMs have emerged as an environmental issue as their ecological impacts are not fully understood [2, 9]. Research evidence has shown that NMs could enter the human body through the skin, airways, and digestive system with potentially harmful consequences [10, 11], while studies of the impact of Au-NPs in aquatic organisms, such as the marine invertebrate worm *Gammarus pulex* [12] and the sewage nematode *Caenorhabditis elegans* [13], show adverse behavioral effects, decreased reproduction, and increase in abnormalities in the reproductive organs.

Coral reefs constitute less than one percent of the marine environment. They are indispensable to marine biodiversity, supporting nearly a quarter of all oceanic species within their compact and complex habitats [14]. However, their profound diversity is at constant risk because of their high sensitivity to environmental disturbances. Global threats to these critical ecosystems are multi-faceted, encompassing climate change, disease, overfishing, and the influx of anthropogenic pollutants, including NMs [15]. These materials initiate a cascade of adverse effects, inducing bleaching, upregulating heat-shock proteins, inhibiting growth, and changing aggregation properties of microalgae, which may negatively affect the uptake of these indispensable symbiont organisms by coral reef [16-19]. Unraveling the complexities of nanomaterial impacts on coral vitality is thus critical, being necessary to monitor their health and adaptability to changing conditions [20, 21].

Herein we present an exploratory study centered on the assessment of alternative analytical methodologies for monitoring the impact of nanomaterials on coral health, leveraging *Gorgonia ventalina*, a prominent octocoral in the subtropical and tropical Atlantic, as a key biological model due to its ecological significance and resilience to environmental stressors. This sea fan coral is an established sentinel species due to its widespread presence in Caribbean and Atlantic ecosystems, its pivotal role in reef architecture, and its demonstrated resilience to environmental stressors, making it an optimal reference organism for methodological assessment [22, 23].

The resilience of *G. ventalina*, evidenced by its recovery from fungal infections and its dominance in regions where scleractinian corals are declining, allows for a more straightforward interpretation of nanomaterial impacts absent of confounding factors from disease susceptibility [24-26]. Its complex defensive strategies, such as the variability in semivolatile organic compounds including polyenes, proteins, and calcification between healthy and diseased states in *G. ventalina* offer a unique metric for gauging coral health in the presence of nanomaterials [23]. In contrast, conventional methodologies for coral health assessment, such as total protein analysis and gas chromatography-mass spectrometry (GC-MS), though informative, are often laborious, invasive, and less suited for expeditious field monitoring, potentially inducing stress in coral populations [23].

In our investigation, we scrutinize the physiological responses of octocorals to gold nanoparticle (Au-NP) exposure through the use of various analytical modalities. We apply Resonance Raman (RR) spectroscopy for metabolic response profiling, Fourier-transform infrared spectroscopy (FTIR) for assessing calcification dynamics, quantify proteins with the Bradford assay, and localize Au-NPs within the coral matrix using energy dispersive X-ray spectroscopy (EDAX).

The intent is for this research to be instrumental in equipping conservation efforts with advanced, efficient tools to safeguard marine biodiversity. In light of the scarcity of related studies, our pilot study, directed to qualitatively assess the impact of nanoparticles on octocorals, emerges as an invaluable approach to identify strategies that may be adopted. The findings from this study are expected to deepen our understanding of coral physiology, yielding vital data to refine monitoring approaches and develop adaptive strategies that are crucial for maintaining the integrity of coral ecosystems in the face of environmental change.

MATERIALS AND METHODS

Gold nanoparticle (Au-NPs) synthesis and characterization

In a 50 mL flask, 20 mL of 1 mM chloroauric acid trihydrate solution was heated and stirred. After a soft boiling, 5 mL of sodium citrate tribasic solution (1%, w/vol) was added under heating and stirring until a color change occurred. Once the solution turned to an intense red, the flask

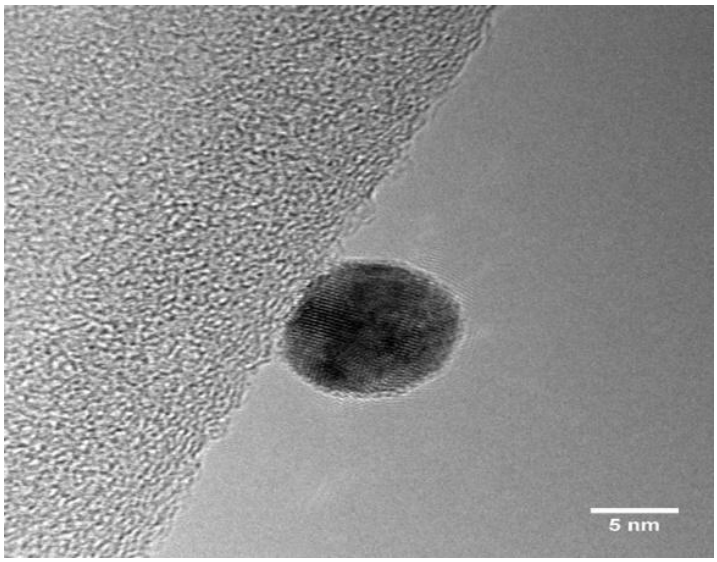

Fig. 1: Bright field TEM image of the synthesized gold nanoparticles, with a FEI F20 S/TEM (200 kV field emission, 1 Å monochromator) at 71,000× magnification.

was removed from heating after 2 min. After cooling, the flask was labeled, covered, and stored at 4 °C. The synthesized Au-NPs solution was analyzed with UV-Vis spectrophotometry to confirm the size of 10 to 20 nm, and the spectrum correspondingly showed a peak at 522 nm [27]. Transmission Electron Microscopy (TEM) was used to characterize the morphology of the Au-NPs (Fig. 1). The image shows the characteristic spherical morphology and an average size of 10-15 nm was confirmed by distribution analysis performed with ImageJ.

Coral collection, acclimation period

Three different colonies of *G. ventalina* were collected from "La Ocho" at Escambron Beach in San Juan, Puerto Rico. Each colony spanned an area of nearly 100cm² and showed no visible signs of stress. A sea fan colony comprises numerous interconnected but semi-autonomous polyps. These polyps are embedded in a gelatinous-like substance supported by calcium-carbonate sclerites at the outer surface of the colony, and an endoskeleton made of gorgonin infiltrated with sclerites. Immediately after collection, the colonies were brought to the laboratory, where they were placed in individual 10 L tanks with recently collected seawater. Colonies were kept for a fourteen-day acclimatization period at 28.0°C, salinity between 35 – 37 g/L, and a pH of 7.00. Seven days after being placed in the tank, 50% of the seawater from each tank was replaced with recently collected from the same colony fragment collection site and under similar environmental conditions. This was repeated on day 14, considering the colonies were already adapted to the study environment (Fig. 2).

Au-NPs exposure and data collection

After the acclimatization period, AuNPs were introduced gradually into two of the three tanks, reaching a final concentration of 2.5 mg/L, by Borase [28] study, which identified significant toxicity in *Moina macrocopa* at concentrations around 1.47 and 2.95 mg/L. This decision sought to strike a practical balance while ensuring a meaningful evaluation of the ecological impact on the coral. The colonies were monitored and named accordingly - experimental colony 1 and experimental colony 2 for the coral colonies exposed to Au-NPs, and the third colony was kept as the control, which was free of Au-NPs. The tank's temperature, pH, and salinity were monitored continuously. During days 3, 7, 14, and 21, the treatment of replacing the water and adding nanoparticles was repeated correspondingly. Fig. 2 illustrates the Coral acclimation, AuNPs exposure, and monitoring method design used in the study.

For the data collection, one fragment of 1 cm² was cut at different time intervals from each colony, that is, after acclimatization (day 1) and at day 3, 7, 14, and 21 after exposure to Au-NPs. Each sample was left overnight in the oven for drying and then

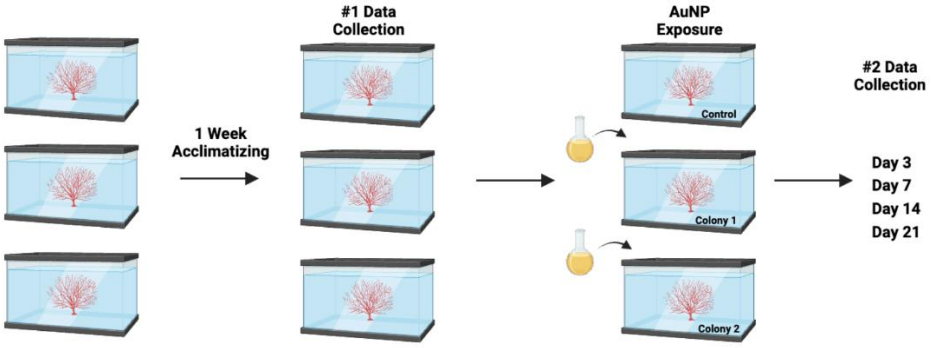

Fig. 2: Schematic Diagram Illustrating the Acclimatization of Corals, Exposure to Gold Nanoparticles (AuNPs), and the Monitoring Methodology Employed in the Study.

used for the different analyses, starting with the non-destructive ones.

Resonance Raman (RR) Analysis

RR analysis is an exciting technique because it does not require preparation before the study, is non-destructive (the sample could be reused in other analyses), is versatile, and is capable of detecting metabolic traces in small quantities without the need to be isolated from the study organism [29]. The resonance enhancement of carotenoids favors detecting them in biological systems for diagnostic analyses [30]. Therefore, RAMAN, by developing in situ measurements, reduces the need for laboratory analysis to minimize coral alteration, providing real-time evaluations. Consequently, RR was used to identify and evaluate the carotenoid content as a stress sensor in the corals. The RR spectra were obtained using a Thermo Scientific DXR Raman Imaging Microscope at room temperature. A laser wavelength of 532 nm and power of 10 mW was used, with a magnification objective of 10x and a resolution of 4 cm^{-1}.

ATR-FTIR analysis

The sensitivity of corals to Au-NPs exposure in the bio-mineralization process was investigated using Attenuated Total Reflectance Fourier Transform Infrared (FTIR) spectroscopy. Fragments utilized in the RR spectroscopic analyses were employed for this technique. Thin membranes for measurement were prepared by pressing the sea fan fragments using a mini hand press. The spectra were acquired using a Bruker Tensor 27 attenuated total reflectance (ATR) FTIR spectrometer [31]. The spectral range covered 4000–500 cm^{-1}, with 32 scans accumulated at a resolution of 4 cm^{-1}. All measurements were conducted at room temperature.

SEM-EDAX analysis

The sea fan fragments previously used in the ATR FTIR analyses were subjected to EDAX analysis using a scanning electron microscope (SEM). This analysis aimed to determine the presence of Au-NPs in the fragments' tissue matrix and skeleton matrix. Image acquisition and spectral element detection were performed using a JEOL 6480LV SEM operating under low vacuum conditions and an energy-dispersive X-ray spectroscopy (EDAX) capability at 20 KV. The EDAX analysis was conducted on solid samples at a magnification of 10,000x.

Protein analysis

A modified Bradford assay was used to quantify the total soluble protein content in the coral colonies, control, and experimental sea fan samples as a proxy for stress [31]. Initially, soft tissue was separated from the skeleton and collected in 1.5 mL sterilized tubes. Then, the Allegra X-30R centrifuge was used at 10000 rpm and room temperature to separate soluble proteins from any remaining coral fragments. The remaining supernatant was then collected to perform the Bradford assay. Standard solutions were prepared by serial dilution with the following concentrations: (0.063, 0.125, 0.250, 0.500, 0.750, 1.000, 1.500, 2.000) mg/mL. Bovine serum albumin at 200 mg/mL concentration was used as a stock solution. To measure protein concentration, 5 µL of each extracted sample was placed in triplicate in 96-well plates along with a buffer-only control. Then, 250 µL of Bradford reagent was added and left to incubate for 5 minutes at room temperature. Absorbance was

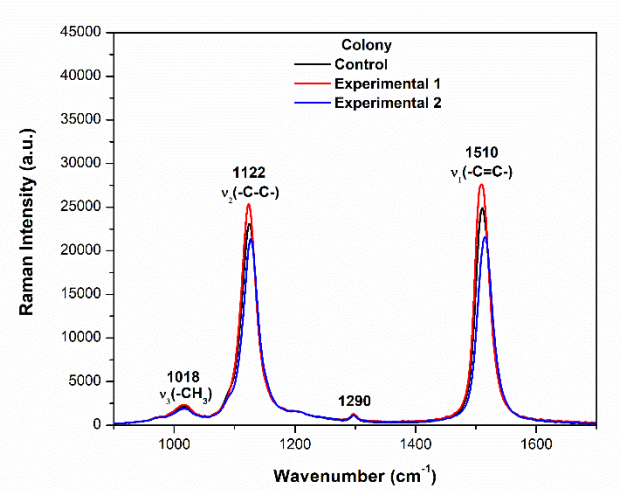

Fig. 3: RR spectra of three G. ventalina colonies on day 1 of exposure show no significant difference.

recorded at 595 nm using a Scientific Multiskan GO spectrophotometer. Total protein concentration was then expressed in mg/mL.

RESULTS

Our results provide valuable insights into the effects of gold nanoparticles on *G. ventalina*, encompassing metabolic shifts identified through RR spectroscopy, changes in calcium levels assessed using infrared spectroscopy (FTIR), alterations in protein content determined by the Bradford assay, and the confirmation of Au-NPs presence within the coral matrix through energy dispersive X-ray spectroscopy (EDAX) analysis.

Raman Resonance (RR) Spectroscopy

RR has proven to be a valuable tool for investigating the physiological state and stress response in sea fans (understanding Coral Metabolism), as noted in prior studies [32]. Previous studies on *G. ventalina* have identified the presence of conjugated polyenes, including a purple pigment, as a defense mechanism against various insulting agents [33]. This research with *G. ventalina* began with this non-invasive technique, seeking to deepen the changes induced by Au-NPs. The RR spectra (Fig. **1**) unveiled two prominent bands at 1122 cm^{-1} and 1510 cm^{-1}, corresponding to the stretching vibrations (ν_2 and ν_1, respectively) of single (-C-C-) and double (-C=C-) carbon backbone bonds, indicative of polyene compounds (Maia et al., 2014). Notably, a peak observed at around 1018 cm^{-1} (ν_3) represents a vibrational bending mode of the attached methyl ($-CH_3$) groups in the polyene chain, further supporting the presence of conjugated polyenes, often associated with carotenoids derived from isoprene units [34]. The vibrational mode at 1290 cm^{-1} is associated with other vibrations within the polyene chain [35]. Initial evaluations of the maximum RR intensities of the three samples on the first day (Table **1** in the supplementary) did not reveal significant variations; as we observed in Fig. **3**, we can establish a baseline for subsequent observations. However, the following examinations revealed a different behavior of the experimental samples. First, it is observed that the control colony showed a gradual decrease in its characteristic peaks, being able to observe lower intensities on days 3 and 14 (Fig. **4-A**). Experimental colony 1 showed a slight reduction in peak intensities after day 3, although not to the extent observed as did the control colony. In contrast, experimental colony 2 followed a different behavior, maintaining and even amplifying the maximum intensities on days 7 and 14. In particular, experimental colony 2 did not survive beyond day 14.

Finally, qualitative descriptions derived from RR results provide information on changes in the production of conjugated polyenes within *G. ventalina* corals, particularly in response to stressors such as gold nanoparticles, with a significant difference observed between the three samples. The identification and study of the pigment produced by the coral can be scanned and compared, which is helpful for future diagnoses that affect the behavior of the coral.

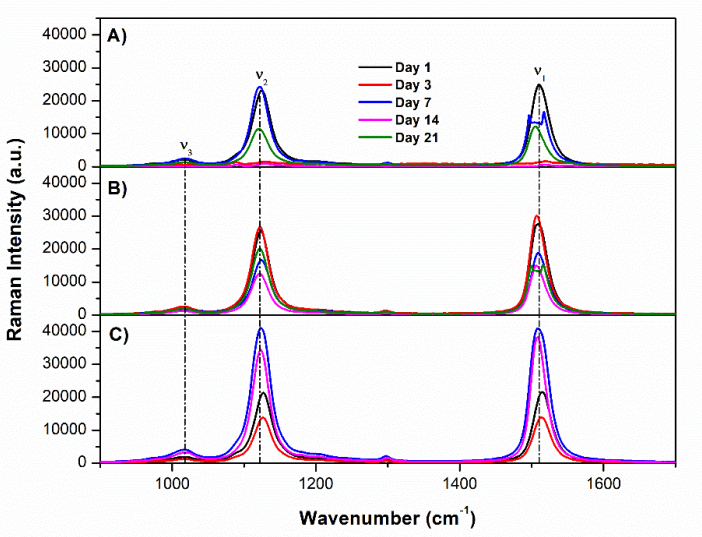

Fig. 4: RR Spectra of G. ventalina depicting (A) a control colony, (B) experimental colony 1, and (C) experimental colony 2. The spectra feature distinct prominent peaks at approximately 1510 and 1120 cm^{-1}, corresponding to the vibrational modes (ν_1 and ν_2) of backbone double and single carbon bonds, respectively. Furthermore, two comparatively weaker peaks are discernible: one near 1018 cm^{-1} (ν_3), signifying the vibrational mode of the methyl group, and another around 1300 cm^{-1} associated with coupled vibrations.

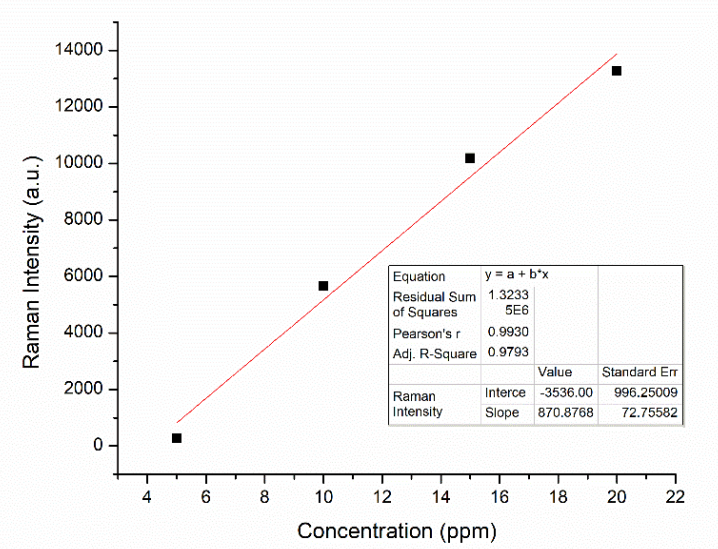

Fig. 5: Raman spectroscopy to monitor changes in signal intensity with changes in carotenoid concentration on coral.

Table 1: RR intensity of selected vibrational modes compared to the three samples on day 1.

Average Peak Intensity (cm^{-1})	Coral colony		
	Control	Experimental 1	Experimental 2
1510	*24889*	*27618*	*21570*
1121	*23065*	*25353*	*21371*
1018	*2131*	*2346*	*1900*

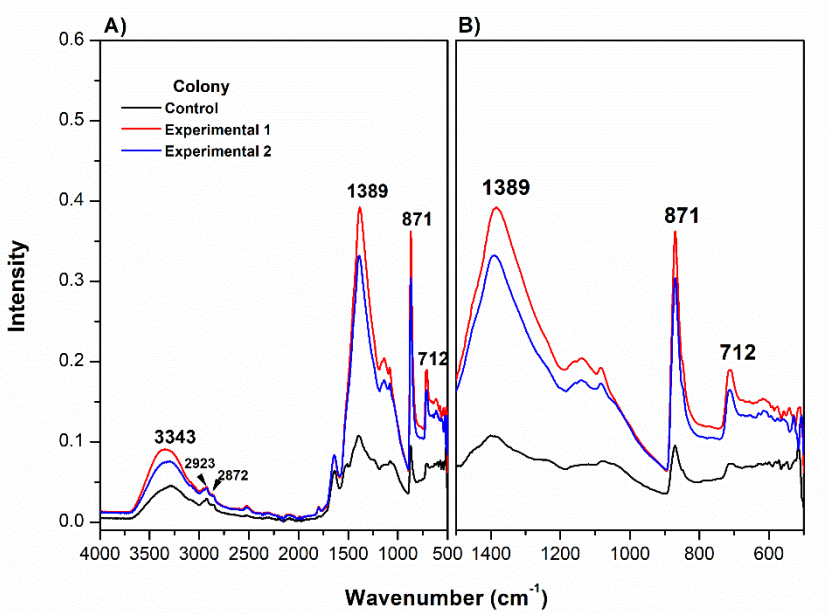

Fig. 6: Comparison of the infrared spectra among control and experimental colonies of G. ventalina on day 1. (A) FTIR spectra in the range 4000 cm^{-1} to 500 cm^{-1} show vibrational modes of CO_3^{2-} at 1394, 1080, and 870 cm^{-1} and of Ca^{2+} at 712 cm^{-1}. (B) Extended plot of the fingerprint region from 1500 to 500 cm^{-1}.

Calibration Curve RR spectroscopy (Fig. **5**)

To evaluate the possible response of carotenoids to the concentration of Au-NPs, through RR, a calibration curve is meticulously constructed through a series of experiments with β-carotene (conjugated polyene). Five different concentrations of β-carotene (ranging from 1 to 20 ppm) were prepared in nanopore water. The *G. ventalina* coral was sectioned into 1 cm^2 fragments and five fragments were assigned to each sample. Subsequently, the *G. vetalina* coral fragments were immersed in 10 mL of a β-carotene solution of different concentrations prepared in 20 mL vials, allowing an immersion period of 45 min, and finally lyophilized before analysis.

FTIR spectroscopy

Infrared spectroscopy (FTIR) was instrumental in understanding the relationship between coral health and calcification. We focused on calcium carbonate ($CaCO_3$), the predominant component of coral skeletons, to understand coral calcification. FTIR spectra as shown in Figs. **6 and** Fig. **7** presented characteristic vibrational bands of carbonate (CO_3^{2-}), particularly around 1390 cm^{-1} and 871 cm^{-1}, indicative of the C=O bond in carbonate [1]. The 871 cm^{-1} band may overlap with minerals such as strontium (Sr) and magnesium (Mg), confirming the presence of these minerals by EDAX analysis [36]. Furthermore, the 1080 cm^{-1} band, characterized by the symmetrical stretching mode of CO_3^{2-}, is predominantly associated with aragonite spectra characteristic of these soft corals [37]. It is worth noting that a peak around 712 cm^{-1} means the presence of calcium ions (Ca^{2+}), an intensity attributed only to the aragonite phase [38]. These spectroscopic signals offer a qualitative insight into the complexities of the carbonate network hidden within the coral exoskeleton. Bands at 3340, 2968, and 2931 cm^{-1} (Fig. **5-A**) correspond to the stretching vibrations of O-H, C-H, and C-C bonds, respectively, while the peak around 1645 cm^{-1} corresponds to silicates [38]. In the following, we will focus on the signals caused by $CaCO_3$, specifically the 1080 and 712 cm^{-1} peaks.

Table 3 (suppl) shows the intensity values of the band at 712 cm^{-1} for the different samples. This peak representing the calcium ion helps to identify the amount of inorganic material, and it will be employed to compare calcium carbonate levels in control coral samples versus those exposed to Au-NPs. The IR absorbance of the control for this signal indicates calcium production across the study period. Experimental colony 1 shows only a slightly variable signal. In contrast, experimental colony 2 showed substantial variations in the signal. These results suggest that the behavior of the carbonate

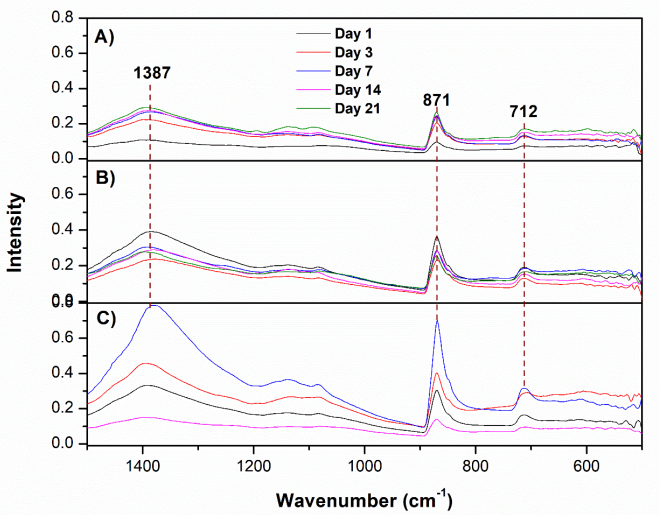

Fig. 7: Comparative FTIR spectra for coral G. ventalina colonies after Au-NPs exposure a) control, b) experimental 1, and c) experimental 2. The peaks 712 cm^{-1}, 871 cm^{-1}, and 1387 cm^{-1} correspond to the presence of calcium carbonate in the aragonite phase.

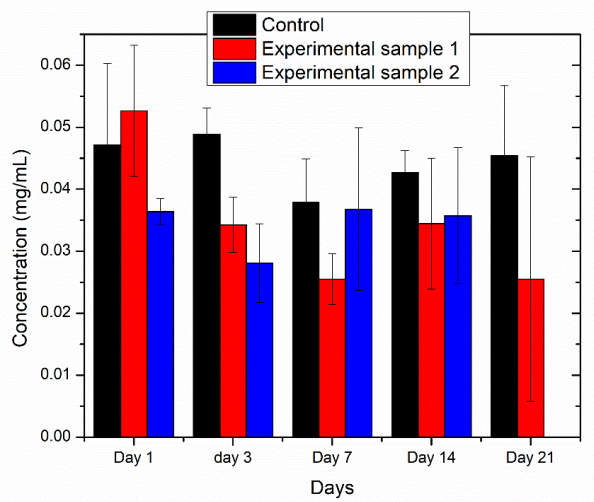

Fig. 8: Comparative Analysis of Total Protein Contents in Soft Tissues of G. ventalina using the Bradford Method.

Table 2: Variation in RR intensity of the bands at 1122 cm^{-1} and 1510 cm^{-1} for different *G. ventalina* colony samples across experimental days.

Coral / Average signal (cm^{-1})		Peak intensity per day				
		1	3	7	14	21
Control	1121	*23065*	*1506*	*24244*	*973*	*11470*
	1510	*24889*	*1655*	*16636*	*640*	*12227*
Experimental 1	1121	*25353*	*26732*	*16633*	*12486*	*20095*
	1510	*27618*	*30083*	*18798*	*14932*	*15128*
Experimental 2	1121	*21371*	*13830*	*41004*	*34380*	*No data*
	1510	*21570*	*13913*	*40929*	*38241*	*No data*

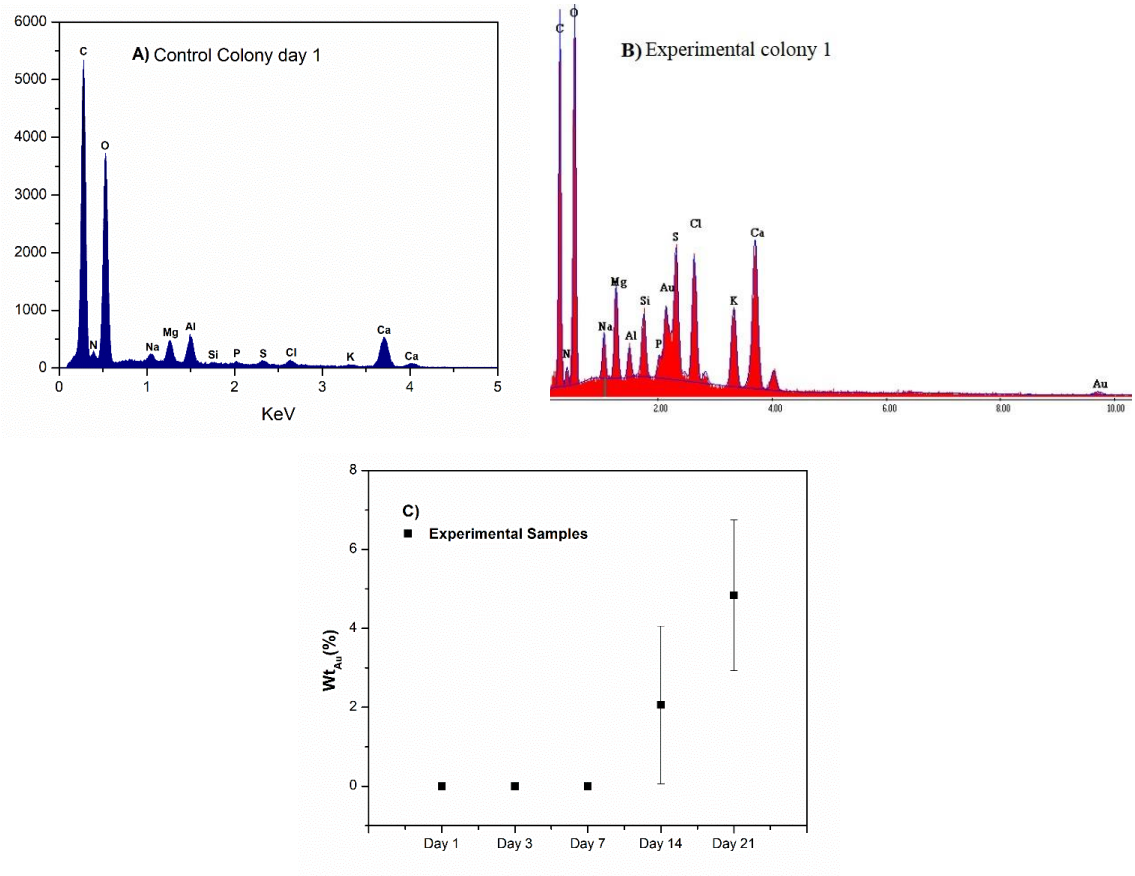

Fig. 9: EDAX spectrum for standardless quantitative data of G. ventalina, at 20 kV and 10000x magnification (A and B), and Au in the coral through the time (C).

measured in the experimental colonies differed from the control colony.

Protein concentration

Protein concentration, a critical tool for coral well-being, showed variable patterns throughout our study (Fig. **8**). The control colony mainly maintained higher levels of protein concentration. In contrast, samples exposed to Au-NPs showed a reduction in protein content, indicating a change in the behavior of the experimental colonies in response to this exposure, shedding light on the health of the corals.

SEM-EDAX

Considering the intricate nature of coral systems and the possible overlap of specific stronger signals, we employ energy-dispersive X-ray spectroscopy (EDAX). This analysis aimed to identify elements within the coral matrix beyond the scope of FTIR and RR analyses (Fig. **9**). The analysis of the control revealed elemental signatures within the coral exoskeleton, dominated by carbon (C), oxygen (O), and calcium (Ca), coming mainly from calcium carbonate (CaCO3). Carbon is also present in polyene chains and possible alkaloids. Magnesium (Mg) confirmed the existence of the aragonite phase, a crucial component of the coral's crystalline carbonate network [38]. In addition, trace elements such as sodium (Na), nitrogen (N), and others emerged, representing various compounds from skeletal, residual components of salt water, or traces of microorganisms. Significantly, the analysis provided qualitative evidence of the integration of Au-NPs in the experimental samples within the coral matrix.

DISCUSSION

Our investigation leverages the ecological significance of *G. ventalina*, a pivotal species within the marine ecosystems of the Caribbean Sea and the Atlantic Ocean, to assess the utility of diverse

Table 3: Infrared Analysis of Coral Segments via Absorbance of the 712 cm^{-1} Infrared Band to Determine the Presence of Aragonite State Calcium Carbonate.

	Coral colony		
Day	Control	Experimental 1	Experimental 2
1	*0.07*	*0.18*	*0.16*
3	*0.13*	*0.13*	*0.28*
7	*0.13*	*0.19*	*0.31*
14	*0.15*	*0.17*	*0.09*

analytical techniques in monitoring the impact of nanomaterials, specifically gold nanoparticles (Au-NPs). These techniques were chosen for their ability to provide insights into different aspects of coral health and response to stressors. Previous research on the octocoral *G. ventalina* shows its ability to produce specific metabolites as a defense mechanism, like the antioxidant activities attributed to conjugated polyenes or alkaloids [39]. These conjugated polyenes, characterized by polyene chains linked to methyl, represent a protective quality against photooxidation and free radicals [40, 41]. This unique insight into *G. ventalina* biochemical arsenal underscores the intricate strategies within coral ecosystems. In our research, we employed Resonance Raman (RR) spectroscopy as a principal analytical technique to investigate whether polyenes in *G. ventalina* are altered as a response to gold nanoparticle (Au-NP) exposure. This method proved instrumental in detecting subtle biochemical changes, offering a non-invasive and sensitive approach to monitor the coral's response to nanomaterial stressors. Notably, an increase in the RR spectrum around 1018 cm-1 (Fig. **3** & Fig. **4**) indicates a shift in polyene concentrations under stress conditions, as supported by previous studies [39], which identified these molecules as part of the coral's defense mechanism. The changes observed in experimental colonies samples, particularly in the marked rise of polyenes and subsequent colony death, underscore a potential link between Au-NP exposure and oxidative stress—a phenomenon documented in other organisms [42] and echoed in our findings through both RR and EDAX analyses. The difference between the control and experimental colonies is shown in Fig. **4**. Accordingly, experimental colony 2 showed a notable increase in polyenes on day 14, which could respond to a stressful stimulus due to possible oxidative stress in the coral due to the Au-NPs found in the coral matrix, as reported with the EDAX analysis (Fig. **9**). Studies have shown that in other organisms, such as *E. coli*, Au-NPs cause toxicity due to oxidative stress [42]. Hence, the results of the spectra of the experimental samples show instability in the production of conjugated polyenes, and these alterations could be attributed to the exposure of Au-NPs. As an observation, experimental colony 2 died after day 14, presenting a detachment of the external tissue. Although both experimental colony samples showed concentrations of gold in their organic matrix, as demonstrated by the EDAX analysis, the death of experimental colony two cannot be attributed entirely to the interaction with the Au-NPs due to the need for more specific studies to identify another stressful coral event. However, this colony showed in the RR analysis a high content of conjugated polyenes, compounds that the organism secretes as a means of defense or stress. The studies by [43] have documented that oxidative stress does not always result in coral mortality, even more so considering that the conditions in the aquarium were identical for the three colonies under study.

In addition to this study, other coral studies use the RR spectroscopy method to identify effects against different invaders, such as metabolic studies of the corals *Tubastrea coccinea* and *Tubastrea tagusensis* located in the South Atlantic (Brazil) and Indo-Pacific (Hawaii) regions, respectively. In these studies, carried out with RR spectroscopy, conjugated polyenes were identified, where these polyenes are present in metabolites produced in corals as a defense response against external agents [32]. Likewise, other studies by *G. ventalina* determined the presence of purple pigments in coral sclerites produced in response to an insulting agent such as *Aspergillus sydowii* and slime mold.

This purple pigment in coral had a conjugated polyene structure [33]. Our study is based on the implications that RR has for the conservation of corals that are increasingly threatened by environmental changes and pollution, where this technique is ready to become a valuable tool, by facilitating rapid and non-invasive evaluations. on coral health, allowing researchers to quickly respond to stressors, including emerging contaminants such as NMs. Complementing RR spectroscopy, our use of Fourier-transform infrared spectroscopy (FTIR) provided a window into the calcification processes by identifying variations in calcium carbonate levels, an essential component in coral structure and health. In Fig. **6** and Fig. **7**, the control shows an increase in the intensities that correspond to calcium carbonate, in contrast to the experimental colonies that offer a varied behavior of these intensities. These variances, as observed in experimental versus control groups, highlight potential disruptions in *G. ventalina's* calcification, which may be further elucidated by protein concentration measurements indicative of metabolic and immunological health [44].

The concentration of total proteins in the coral *G. ventalina* was measured to observe the response to external stressors due to possible decalcification due to photothermal protection capacity and enzymatic roles for the immune response, which promote a series of metabolic pathways that provide a defense [44]. It can be seen in Fig. **8** that the control colony showed a higher protein concentration than the experimental colonies; therefore, it also has a greater intensity in the calcium carbonate peaks. Our studies coincide with previous research that elucidated the impact of metals such as Cadmium (Cd) and Nickel (Ni) that are inhibitors of calcifying enzymes in other coral species, such as *Stylophora pistillata* [45]. Other studies on the effects of calcification of metallic particles in different corals vary depending on the exposure time in the study and show that calcification is not affected but does cause an alteration [46]. Finally, the alterations observed in the conjugated polyenes of the corals, together with the variation in the intensities of calcium carbonate, could be associated with changes in the concentrations of the enzymes (proteins) obtained in the biomineralization process, signs of possible stress or disorder in the coral. Using primarily RR spectroscopy, we identified anomalies in corals affected by gold nanoparticles. With this technique, we aim to quickly detect the harmful effects that the coral is going through. Being a rapid technique with portable equipment, it is shown as an alternative to identifying alterations to the corals to protect them promptly [47]. As there are only few reports related to *G. ventalina* that quickly identifies the state of the coral, we consider that more specific metabolomic techniques are required for a more detailed knowledge of the coral protection mechanism to identify the characteristic polyene, the presence of alkaloids and the behavior of the calcifying enzyme. It is necessary and urgent to implement a rapid biomarker method through these study techniques to identify possible conditions in these organisms affected by pollutants or climate change.

CONCLUSIONS

In summary, our investigation highlights the promising application of Resonance Raman (RR) spectroscopy in detecting the biological impact of Au-NPs on corals. Despite the constraints of our experimental framework, our study assessed the efficacy of Raman spectroscopy as a rapid, non-invasive, non-destructive, and field-compatible method for monitoring coral health, with potential applications for *G. ventalina* and beyond when compared to traditional assessment techniques. It offers a pragmatic balance between methodological rigor and the ethical consideration required for studying vulnerable coral ecosystems. Although this study is an exploratory step, it underscores the need for additional research to fine-tune the efficacy of RR spectroscopy in monitoring and potentially forecasting coral health. Advancing this technique could substantially bolster our approach to marine conservation, opening new avenues for proactive environmental stewardship in the face of burgeoning nanomaterial pollutants and climatic shifts.

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