Dear Editor,

First and foremost, we would thank you and the referees for your effort in revising our paper. We believe the insights required gave us the opportunity to improve our work.

We have revised the manuscript and updated the abstract, introduction, methods, results and discussion, according to reviewers' comments. In particular, the methods, the results and discussion and the figures have been improved, in order to provide the additional information required by the reviewers and, in our opinion, improve the overall quality of the manuscript. Please find enclosed the updated version of the paper, that has been revised to address the points raised by the referees. In the text, the major changes made on the base of the reviewers' comments are highlighted in yellow. Our answers to the referees' comments are reported in the Q&A format, where the reviewers' original comments have been reported in italic.

Thank you for your consideration.

Yours sincerely,

Paolo Postorino

Reviewer 1:

This is a very interesting project, in which authors aim to characterize the purple color found in a "mineralized textile fragment" in the Archeological Museum of Naples (MANN). The authors aim also at identifying the sea snail species (mollusks species) used to dye this fragment.

However, there are several fundamental issues that must be carefully addressed for this paper to be accepted in Molecules.

i) In the introduction, authors must describe how it is possible to identify the species used to dye a textile as well as the limitations of this approach (consider that you are extracting the dye from the textile, and not from the murex). In its present form, it is only vaguely stated that is was done in literature. Please explain precisely the data necessary to assess the species used to dye an historical textile (analyzing extracts from the dyed textile). Why is this important? Because, it will be very difficult to ascertain accurately the species used, unless you have data on the original dyeing methods. Adding to this, it would be necessary to know or to predict the ageing of the textile: this issue is never addressed in this manuscript. Authors should be aware that the bromine bond may be cleaved in certain conditions, such as exposure to sun, and certainly to high temperatures. Scission of the bromine bond will lead to mono-bromo derivatives and indigo.

Response: We thank the Reviewer for his/her comment, which resulted useful to revise the paper and to provide further important information in the text on the complexity of the issue. We agree that the identification of the specific mollusc species from the textile extract is very complex, considering factors as the dye bath, possible exposure to sun and light and ageing. For this reason, we only hypothesized the source mollusc, based on the experimental data and with reference to the literature, but we highlighted that further studies are necessary to answer the question. For this reason, taking into account of the Reviewer suggestion, we added a part in the introduction to explain the major factors which make the identification of the dye source complex and to clarify our final hypothesis.

ii) Raman spectra: authors must add to their Figure 3, the SERS spectra of indigo, indirubin, 6,6'-dibromoindigo and a monobromo derivative. The main peaks attributed to CBr, NH, CNC, CNO for the various compounds should be added to the spectra. A table with the more relevant bands should be prepared for each of these compounds. This table will allow the reader to compare data published in the literature with the data obtained in this work.

Response: We added the reference spectra, extracted from literature, to the text. In order not to crowd the figure with the peak labels, a table with tentative assignations, based on the literature, was also added in order to allow the identification of the main vibrational bands.

iii) If authors could display a DAD chromatogram it would be important, as quantification of teh chromophores is more easy to perform.

Response: We agree with the referee about the fact HPLC-DAD is a technique of choice for the analyses of dyes and colorants in cultural heritage. However, in this work, we used HPLC-HRMS that it is comparable from the

chromatographic point of view. Moreover, we considered that the approach we adopt can be considered more informative, especially because structural information may be obtained even without standards. Based on these considerations, we think that additional information can be obtained by HPLC-DAD analyses.

iv) It is essential to separate indigo (that the authors refer as indigotin) from indirubin. If this is not carried out, the paper cannot be accepted. This is very straightforward using HPLC-DAD.

Response: We thank the reviewer for his/her observation on this somehow delicate topic. Actually, we tried to obtain the separation between indirubin and indigo, however even with different gradients and mobile phases a single peak was obtained by the analysis of our sample. Considering that the two compounds are isomers it is not possible to distinguish the two species based on the exact mass, for this reason we used the expression "indigotin/indirubin" in the text. We hypothesized that only indigo is present in the sample while indirubin is in a concentration <LOD; this hypothesis is supported by literature data, where the relative concentration of indirubin is always very low in comparison to the other species both in mollusc extracts and original samples (see references 15, 16, 18). Moreover, in the Raman spectrum of the sample, the specific peaks attributable to indirubin are not observable. For sake of clarity we add the following statement in the text:

"…In particular, the non-brominated compounds present in the dye should be indigotin and indirubin, which are isomers. However, a single chromatographic peak was observed for the relative value of accurate mass. Considering the relative concentrations typically reported in the literature on extracts from the molluscs and from the textiles, it can be stated that the amount of indirubin is often very low in comparison to indigotin. Moreover, the Raman spectrum does not show specific peaks for indirubin. Based on these considerations, it can be hypothesized that the dominant non-brominated species is indigotin, while indirubin concentration is likely below the limit of detection. The alternative hypothesis, i.e. that the separation was not performative, seems unlikely, taking into account the structural differences between the two compounds."

v) How was quantification performed by HRMS? By comparison with calibration curves using standards? Please add this information into the experimental sections. The information on how samples were extracted for HPLC-HRMS analysis must be completed. 2 mg of sample were dissolved in xxx ml of DMSO and heated at 70 ◦ C for 5 min? How was the extract filtered, prior to be suspended in methanol?

Response: We thank the reviewer for the observation. As discussed above, an advantage of the HRMS analysis is that the use of standards is not needed. For this reason, an accurate quantification could not be carried out. However, relative quantification was performed by comparing the area of the peaks of interest to the sum of the area of all the peaks: this was clarified in the text. We are aware that the use of XIC peak areas for the relative quantification has some shortcomings because of possible matrix effects and differences in ionization efficiencies that may lead to differences in ion intensity. However, because of the structural similarity of the species of interest, ionization efficiency can be supposed to be similar.

Regarding the requested data about extraction, we added the information in the Materials and Methods section.

Other minor points that should be clarified and corrected are described below.

1) Please, use purple for naming the color throughout your manuscript (and not violet)

Response: We changed "violet" in "purple", with the exception of two case where the word "violet" refers to particular shades obtained from the use of the dye.

2) line 46: first time MAAN is used, please give its full name; explain how it is know that it is a "luxury fabric"?

Response: We added the full name and reformulated the sentence to clarify our consideration.

3) line 50: please, add reference to "taking into account of the current scientific discussion"

Response: The references were added to the text.

4) line 54: "identification of the purple dyed sources"

Response: We corrected the sentence.

5) line 59: please, add reference

Response: We added a reference.

6) line 65: please, add reference; consider, for example, "La porpora. Realtá e immaginario di un colore simbolico". Or other that you consider more suitable.

Response: We added the suggested reference to the text.

7) lines 70-75: please, explain precisely in which conditions and how it is possible to make "the identification of shellfish purple "

Response: Taking into account of the previous suggestions from the reviewer and the added part in the introduction, we reformulated the sentence.

8) line 81: please summarize the information on references 24 and 25 that allow to conclude that "the archaeological evidence suggests that this fabric may be regarded as an example of purple and gold tapestry "

Response: We added the requested information to the text.

9) In Figure 2 can you please help the reader to see "z-twisted in an angle of 35-50°", by drawing in a part of the SEM image, for example. It is based on this SEM image that it is concluded that "the mineralized textiles found in Pompeii has been made of wool fibers"? This is stated in line 104, in which it is also concluded that the textile "dyed in shellfish purple". This conclusion is not possible to be reached based on SEM analysis: please correct

Response: We thank the reviewer for his comment: the z-twist feature is observable at the stereo-microscope and it can be related to the use of wool, as highlighted in the text and shown by an added image. We also corrected the "dyed in shellfish purple" expression.

10) Figure 4 should be prepared to be published in a scientific journal; as it is presented now, it would be acceptable for a report

Response: We modified the Figure accordingly.

Reviewer 2:

The article does not present any major innovative aspect from the scientific point of view. Nevertheless, it represents a significant case study mostly because of the relevance of the archaeological site and what the findings represent from a cultural point of view.

Response: We thank the referee for taking the time for careful reading the manuscript and provide us with his/her useful feedback. Beside the historical relevance of our case study, we believe that the present work introduces aspects of innovation in the contemporary research on mollusc purple dyes. The SERS spectra obtained from the sample show a great reproducibility, which is usually a critical factor in analysis through the used technique. Moreover, they do not evidence anomalous interfering bands attributable to the colloid reducing agent, a phenomenon tipically observed in other studies (see ref. 39): consequently, a proficient protocol for SERS analysis of mollusc purple is presented, while the SERS spectrum in the paper represents a reference high quality spectrum for the investigated dye mixture, available for other studies. Another important aspect is related to the use of High Resolution Mass Spectrometry: the combination with HPLC for the analyses of indigoids in Cultural Heritage materials is not common, especially for the purple dyes, whose analyses are usually performed through DAD detection. In particular, the possibility of obtaining High Resolution structural information could be considered a great advantage, which opens new perspectives to further studies for the characterization of these matrices.

Moreover, some major differences between the aims presented in the introduction/abstract and the results/findings highlighted in the the discussion/conclusion make the overall aim of the manuscript unclear. This aspect should be revised to make the article acceptable for publication. Other modifications (sentences rephrases, clarification of techniques and findings, ...) would also be required before considering publication of the manuscript. Please, refer to the annotated PDF for more detailed remarks.

Response: We modified the text with reference to the comments by the reviewer, with particular attention to his comments in the attached pdf document.

Taking into consideration the aforementioned remarks, I would recommend submitted to a more archaeologically-focused journal such as Archaeometry, Journal of archaeological science reports or Archaeological and Anthropological Sciences rather than Molecules.

Response: As stated in the previous answers, we consider that the combination of the multi-technical approach, the high quality of SERS protocol and spectra and the application of High-Resolution Mass Spectrometry to mollusc purple dye, whose composition represents a complex issue in the actual scientific research in Cultural Heritage chemistry, represent important aspects which make this paper suitable for the publication on the Natural Dyes special issue of Molecules.

Reviewer 3:

This work presents the study of a unique purple mineralized textile found in Pompeii. Raman results showed the presence of Tyrian purple as the main colorant resposible to give its characteristic color and thaks to chromatographic results it was determined the relative contents of different mono-, di- or non brominated compounds. From my point of view this last approach is the most relevant part of the work, showing that the used dye is related mainly to non brominated compounds. However, some minor revisions should be perfomed before the publication of this paper. First, I want to remark the good interpretation of the Raman and SERS results that led to discussion of the presence of the different brominated compounds and the subsequent chromatographic analysis,. In my opinion, the most relevant and novel part of the work is the chromatographic result and its discussion. However, the 3 chromatograms presented in this paper should be eddited in a better way, such as:

Add the x axis scale (retention time). There is lot of information useless, mainly in the right part (probably because you just copied and pasted the chromatograms from the software). The peaks cannot be seen easyly, only the main peak. I encourage to perform a good quality chromatograms to present in the paper, such as the one attached, with the retention times over each peak, and starting from minute 0, without empty spaces that only difficult the observation of peaks.

Response: We thank the reviewer for his/her positive feedback on the manuscript. We added the requested image to the updated version of the work.

Moreover, you do not explain the procedure that gives to the quantitative results of 80% indigotin/indirubin, 19% monobromo and 1% dibromo compounds. How did you do it? An explanation of how you get these contents is crucial since these results are the most novel issue of the work. Please add it.

Response: As discussed in the response to the Reviewer 1, the relative quantification of the compounds was performed by comparing the area of the peaks of interest to the sum of the area of all the peaks. This has been clarified in the modified version of the text. We are aware that the use of XIC peak areas for the relative quantification has some shortcomings because of possible matrix effects and of differences in ionization efficiencies that may lead to differences in ion intensity. However, because of the structural similarity of the species of interest, ionization efficiency may be supposed to be similar, hence supporting the relative quantification results.