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## Reply to the ‘Comment on “Limitations in using the Cu isotopic composition of minerals from ancient copper mines for archaeometric purposes – a case study” by T. Rose, A. Wittke and S. Klein, *J. Anal. At. Spectrom.*, 2025, 40, DOI: 10.1039/D4JA00260A

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This reply to the comment by Rose *et al.* demonstrates the validity of the results reported and conclusions drawn in DOI: [10.1039/d3ja00150d](https://doi.org/10.1039/d3ja00150d).

### Introduction

At the time the research described in “Limitations in using the Cu isotopic composition of minerals from ancient copper mines for archaeometric purposes – a case study”<sup>1</sup> was carried out, all authors were active in a research group at Ghent University (Belgium) or at the University of Oviedo (Spain). The groups involved in this work are both “analytical chemistry” groups that develop analytical methodologies for a variety of applications. Both groups have already addressed archaeometric applications, based on element fingerprinting and isotopic analysis, since before 2000. The motivation to report the findings in this “case study” paper was the high and sometimes unrealistic expectations that some archaeologists have of the application of elemental and isotopic analysis as a source of information on the artifacts investigated. By means of a specific case study (and not an evaluation of the entire range of archaeometric application of Cu isotopic analysis, as explicitly indicated by the inclusion of “a case study” in the title of the manuscript), we wanted to make the readers aware of the limitations of such an approach, *e.g.*, in a case like this, where the intra-ore Cu isotope ratio variation is larger than the inter-ore one.

At the time, our manuscript was evaluated by three independent reviewers who apparently found both its quality and relevance high enough for publication in *JAAS*. We understand that Rose *et al.*,<sup>2</sup> being employed at an institution that offers Cu

isotopic analysis of archeological samples in a fee-for-service model,<sup>3</sup> feel the need to question the validity of our conclusions, but we would have rather appreciated counter-examples of successful use than the casting of doubt on the quality of our data, which for analytical labs that are fully aware of the need for validation is an allegation that cannot be overlooked. In the following paragraphs, we will therefore refute all of the statements that Rose *et al.*<sup>2</sup> made and demonstrate that there is no reason whatsoever to mistrust our data.

### Reliability of copper isotope ratio data

Although not necessarily organized in the most appropriate way, we have adhered to the sequence of allegations in the comment of Rose *et al.*<sup>2</sup> and explain point-by-point why their interpretation is incorrect.

#### Replicate measurements vs. analysis of different sub-samples

Already in the first sentence, it becomes clear that Rose *et al.* have either not carefully read or misinterpreted the information presented in our paper and/or are imprecise in their terminology. They refer to “each sample was measured in three replicates” and point out that the difference between data points for the same ore sample deviate more from one another than what should be expected from the measurement uncertainty (2SD value). Therefore, we want to explicitly repeat that the data are not calculated based on the results of 3 replicate measurements, but on the basis of analysis results for 3 sub-samples (each one individually powdered/ground and subject to the acid digestion and chromatographic Cu purification). Due to additional contributions to the total uncertainty and aggravated by potential heterogeneity in the material, this obviously leads to a spread in the data points that is larger than the measurement uncertainty. The measurement uncertainty of

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the  $\delta^{65}\text{Cu}$  value (shown in Table S3 of ref. 1) obtained for 3 measurement replicates ranged between 0.006 and 0.091‰ (2SD,  $N = 108$ ) only.

### Mortar cleaning

Surprisingly, even the method of cleaning the mortar is questioned, despite the use of a standardized cleaning protocol when a pestle and mortar is used for ore/mineral grinding. In introductory analytical chemistry textbooks, cleaning the mortar by grinding of an abrasive “cleaner” is described as a standard protocol.<sup>4</sup> In our case, after 3-fold thorough rinsing of the mortar and pestle with high-purity water, an amount of each sample itself was used as an abrasive cleaner and subsequently discarded after the cleaning. For one of the Cu-ores, 13 sub-samples were ground, providing  $\delta^{65}\text{Cu}$  values ranging from  $-0.29$  to  $-1.42\text{‰}$ . Such variation can simply not be attributed to an alleged “cross-contamination” between samples.

### Chromatographic Cu isolation

The next topic addressed is the ion exchange chromatography used for Cu isolation. Rose *et al.* question the protocol used simply because it was first developed for and applied to whole blood samples.<sup>5</sup> They point out the obvious difference between the matrix of whole blood and the ores analyzed and therefore call our approach “unorthodox”. The only reason why this paper<sup>5</sup> was referred to is that it describes the chromatographic protocol used in the greatest detail, thus providing readers with a maximum of information. But, apparently, Rose *et al.* overlooked that in the lab of the corresponding author, the same chromatographic protocol has also been systematically evaluated and successfully used for Cu isolation (preceding its high-precision isotopic analysis) from bronzes in a paper entitled “Copper and tin isotopic analysis of ancient bronzes for archaeological investigation: development and validation of a suitable analytical methodology”.<sup>6</sup> We assume that Rose *et al.* agree that, as a matrix, bronze is close enough to that of Cu ore to accept that the isolation method is feasible for addressing Cu ores.

### Quantitative recovery of Cu

Next, Rose *et al.* criticize the  $102 \pm 12\%$  Cu chromatographic recovery that we mentioned. They point out that the recovery should be as close as possible to 100% to avoid any effect of on-column fractionation on the isotope ratio results, which is common knowledge and obviously correct. On the basis of the value reported, Rose *et al.* conclude that some recoveries were lower than 100%. Obviously, this is a mathematically valid observation, but the interpretation that this points towards on-column Cu loss is not. Mathematically, with a  $102 \pm 12\%$  recovery, some recoveries must also have been  $>100\%$  and unless Rose *et al.* believe that Cu can be created on-column or we were able to contaminate the samples containing Cu as a major element to such an extent,  $102 \pm 12\%$  indicates quantitative recovery with the 12% uncertainty being a result of accumulated uncertainty as quantification relied on simple external calibration rather than on more powerful approaches

such as the method of standard additions or isotope dilution. It may be important to make the community aware that reported uncertainties  $<1\%$  are simply not realistic and fail to include certain contributions.

### Cu isotopic analysis without prior chromatographic Cu purification

Next, Rose *et al.* suggest bypassing column separation. Such approach is far from new, in the paper referred to above dating back to 2013,<sup>6</sup> the group of the corresponding author reported on a systematic comparison between the use of the chromatographic protocol for Cu isolation from bronze samples and the omission of such protocol and it was concluded that “For archaeological bronzes, the Cu isotope ratio can be directly measured without prior column isolation with a precision equal to that for the corresponding purified solution obtained using AG-MP-1 anion exchange resin (reproducibility in the range of  $\pm 0.02\text{‰}$  in the delta scale, expressed as  $2s$  for  $n = 5$ )”. Other elements typically present in bronze Cu alloys (*i.e.*, Sn, Zn, Pb, Ni) did not affect the results of Cu isotopic analysis at their usual concentration levels. There was no evidence for spectral interference or a matrix-induced effect on the extent of mass bias with the measurement methodology used. This makes it obvious that such an approach is not new to the authors of ref. 1 at all, on the contrary. Please note that our paper on omission of Cu chromatographic isolation in this context was published in 2013 and the paper Rose *et al.* are referring to<sup>7</sup> in 2020.

At the same time, we would like to point out that (i) the conclusion of ref. 6 refers to “elements typically present in archaeological bronzes at their usual concentration levels”, while (ii) next to bronzes, also Cu ores were the subject of investigation in ref. 1. As a result, taking into account the proverb “Haste is waste”, we have opted and would always opt for and recommend a measurement protocol including target element isolation exactly as to avoid spectral overlap or an effect of the matrix on the extent of instrumental mass discrimination.

### Use of reference materials for validation

We agree with Rose *et al.* that for validation purposes use of a reference material with a matrix composition and target element content as close as possible to those of the sample is preferred. However, in many cases this ideal situation does simply not exist. When consulting the widely used GeoRem database<sup>8</sup> for reference materials for which the literature reports  $\delta^{65}\text{Cu}$  values, 6 materials are mentioned only. Two of them consist of pure Cu (including NIST SRM 976 used in our work as the external standard), three are basalts and one is an andesite. Of course, we have also processed pure Cu: “A Cu single-element standard solution, previously characterized for its isotopic composition, was also used as an in-house standard for quality assurance/quality control”, but in any case, we also always run reference materials with “a matrix”, even if the composition of that matrix is deviating far from that of the sample as it is a more powerful approach, capable of flagging more problems, than using pure Cu only.



### MC-ICP-MS isotope ratio measurement

Also the measurement protocol itself is not approved by Rose *et al.* as the use of a higher mass resolution is criticized. Apparently, it is unclear to them which spectral interference has been avoided by measuring at (pseudo) medium mass resolution at the plateau at the left side of the peak center. It is true that this has not been explicitly mentioned (and maybe it should have been), but in the method development paper referred to ref. 5, it has been made abundantly clear (and is common knowledge among practitioners) that at low mass resolution the signals from  $^{63}\text{Cu}^+$  and  $^{65}\text{Cu}^+$  coincide with those from  $^{40}\text{Ar}^{23}\text{Na}^+$  and  $^{40}\text{Ar}^{25}\text{Mg}^+$ , respectively. As the Ar-based polyatomic ions show a slightly higher mass, the left side of the peaks as obtained at medium mass resolving power (“pseudo” high resolution) needs to be targeted for interference-free  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  monitoring. Even after chromatographic Cu purification, small amounts of other elements can still be present and practitioners realize that even a small extent of spectral overlap can have a dramatic effect on MC-ICP-MS isotope ratio data.

### Standard deviations of experimental data & literature values

Rose *et al.* are also not impressed by the “standard deviations” we report in ref. 1 and they mention values of 0.07 and 0.08‰ that we allegedly have reported. They conclude that such values “may indicate unstable measurement conditions”. There is little use in debating this as the values referred to are not even experimental data obtained by us, but values taken from the literature. The values we have observed experimentally are, of course, also included in our paper:

- “ $\delta^{65}\text{Cu}$  reference value =  $0.22 \pm 0.07\text{‰}$  (2SD)...”. The experimental  $\delta^{65}\text{Cu}$  value was  $0.23 \pm 0.05\text{‰}$  (2SD) calculated on the basis of different measurement days ( $N = 67$ , Fig. S3<sup>1</sup>).
- “For BHVO-2, our result of  $0.16 \pm 0.04\text{‰}$  fits the 0.08–0.27‰ literature range...” The value corresponds to the mean  $\pm$  2SD obtained for  $N = 6$  sample preparation replicates.
- “For BIR-1, our result of  $0.11 \pm 0.02\text{‰}$  agrees very well with the value of  $0.09 \pm 0.08\text{‰}$  reported...” The value obtained corresponds to the mean  $\pm$  2SD.

So our standard deviations were not 0.07–0.08‰, but 0.010–0.025‰.

To further endorse the reliability of our results, we have also systematically evaluated the  $\delta^{71}\text{Ga}$  values obtained (Ga was used as an internal standard) across all measurements. Based on a total of 856 measurements of samples and standards, a value  $0.00 \pm 0.16$  was found for  $\delta^{71}\text{Ga}$ , demonstrating that (i) there is no interference of  $^{138}\text{Ba}^{2+}$  affecting the signal obtained for  $^{69}\text{Ga}^+$  and (ii) there were no unstable conditions along measurement sessions.

### Contextual information on the material

Rose *et al.* claim that “very little contextual information is provided”. It seems that this especially pertains to the origin of the ores and the processes they underwent during ore formation and later reworking. Ore samples from these mines have been

previously analysed for their Pb isotopic composition and the corresponding results have been published by the Oviedo lab in an archaeometric journal for each relevant mine.<sup>9–11</sup> Obviously, all three papers were included as references in ref. 1 and they do provide much more information on the mines and the samples taken (even including detailed maps of the inner part of the mines). Additionally, in the current paper, the mineralogical nature of the samples, azurite and/or malachite (copper carbonates, thus, secondary minerals), have been specified. For a manuscript published in an analytical journal, a reference to the earlier papers providing contextual detail instead of recycling information already published elsewhere was considered sufficient and a detailed discussion on the origin of the variation in the Cu isotope ratio for ore samples from the same mine is still considered beyond the scope of this JAAS paper.

### Lead isotope ratio data

We agree with Rose *et al.* that ratios against  $^{204}\text{Pb}$  – the only non-radiogenic isotope – can provide relevant information. And that is exactly why the  $^{206}\text{Pb}/^{204}\text{Pb}$  ratios have been included in the ESI accompanying ref. 1. However, this paper focuses on Cu isotopic analysis and the Pb isotope ratios were mainly referred to for pointing out the “radiogenic” character of the Pb present in the ores. For this purpose, plotting the  $^{208}\text{Pb}/^{206}\text{Pb}$  ratio as a function of the  $^{207}\text{Pb}/^{206}\text{Pb}$  (Fig. S1 and S2 in the ESI)<sup>1</sup> suffices and ratios involving  $^{204}\text{Pb}$  are not strictly required.

### Scope and argumentation

#### Cu isotope ratio variations in Cu ores

Next to questioning the reliability of the data and thus, the analytical expertise of the labs involved, throughout the entire text, Rose *et al.* also suggest (both between the lines and more directly) that the authors lack background on the topic reported on. Already in a previous paragraph, we have documented investigation of a protocol without chromatographic Cu isolation *via* a paper<sup>6</sup> pre-dating ref. 7 provided by Rose *et al.* by 7 years to illustrate that the authors of ref. 1 may not be as ignorant as the authors of the comment seem to assume. The same is true for Cu isotope ratio variations within an ore material, for which Rose *et al.* inform us of the existence of papers over a decade old that address Cu isotope ratio variations in ore samples. This seems to suggest that Rose *et al.* assume that this has escaped our attention. However, in 2009 (more than a decade ago, indeed), the corresponding author’s lab carried out the Cu isotope ratio measurements that formed the foundation for the paper “Cu isotope ratio variations in the Dikulushi Cu-Ag deposit, DRC: of primary origin or induced by supergene reworking?”,<sup>12</sup> also referred to in our paper. The existence of intra-ore variations is thus not new to us, in ref. 1, we have only illustrated the consequence thereof in this specific case study.

### Conclusion

In general, the authors’ field might be more wide or less focused than that of the authors of ref. 2 but this does not make them



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necessarily completely unaware, as a more careful reading of the original manuscript including attention to the references cited and the ESI would have revealed. We conclude that there is no reason whatsoever to cast a doubt on the reliability of the measurement results presented in ref. 1. Whether or not the paper is also of interest for future research is a conclusion we would like to leave up to the readers.

## Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this article.

## Conflicts of interest

There are no conflicts to declare.

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