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Relevance of using portable X-ray fluorescence to identify gold hyperaccumulator plants

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ABSTRACT

Field Portable X-Ray Fluorescence (FPXRF) was investigated as an alternative analysis tool for the detection and quantification of gold in hyperaccumulator plants in the field. First, synthetic gold standards were prepared with materials chosen to best simulate plant material, using microcrystalline cellulose (mcc) or ash by adding known amounts of gold. Powder samples were obtained containing gold in amounts ranging from 10 to 1000 ppm of gold. They were analysed, as well as blank samples of mcc and ash, using a NITON x13t GOLDD+ FPXRF spectrometer in AllGeo and Soil modes with an analysis time of 60 s. Results were compared to Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis of the synthetic gold standards after digestion in *aqua regia*. Gold was detected and accurately quantified in synthetic gold standards containing more than 25 ppm of gold in AllGeo mode. At lower concentration, results were much more inaccurate, with no gold being detected at 10 ppm. In Soil mode, similar results to AllGeo mode were obtained with mcc synthetic standards. However, with ash synthetic standard gold quantification was very poor over the whole concentration range and gold was detected in the blank ash sample, which was confirmed as a false positive by the subsequent ICP analysis. Samples of *Phyllanthus stipulatus*, suspected gold hyperaccumulators, collected on gold-bearing sites in French Guiana were then analysed following the same protocol. The same false positive phenomenon was observed in some of them, with gold being detected by FPXRF in Soil mode but not by ICP analysis. Furthermore, analysis of the FPXRF spectra revealed that none of the characteristic gold emission ray was present. This false positive was attributed to an interference caused by zinc, present in significant amount in the ash of the synthetic standard and the collected plants.

1. Introduction

Hyperaccumulators (HAs) are plants able to accumulate high concentrations of specific elements, to a degree that would be toxic for other plants. The hyperaccumulation threshold had first been set at 1000 mg.kg⁻¹ of plant dry mass (van der Ent et al., 2013). However, in order to account for the great disparity in metal concentration both in plants and the soil on which they grow, another criterion has been proposed. It considers the difference between the metal concentration in plants growing on metal rich soils and normal soils and a comparison in metal concentration between the potential hyperaccumulator and the other plants growing on the same metal rich soil (van der Ent et al., 2013). This criterion leads to hyperaccumulation thresholds ranging from 100 mg.kg⁻¹ for elements like cadmium to thresholds as high as 10000 mg.kg⁻¹ for manganese (Reeves et al., 2018). For elements with no known natural hyperaccumulators such as gold, theoretical hyperaccumulation

thresholds have been proposed (1 mg.kg⁻¹ for gold) (Anderson et al., 1999). These theoretical values can be used to discover new hyperaccumulators through plants analysis during field campaigns. While first identified in the 1850s, hyperaccumulators have generated a growing interest in the last 50 years (Ent et al., 2018).

In recent years, these plants have been used to develop soil remediation and mining technologies known respectively as phytoremediation and phyto- or agromining, by taking advantage of their ability to accumulate trace elements present in their environment (Ent et al., 2018). Agromining is intended to exploit uneconomic ore deposits in a more environmentally friendly manner than traditional mining methods (Ent et al., 2018). The great majority of known HAs are nickel HAs (532 out of 721 species); they have been extensively investigated, as have the processes for recovering the metal (Ent et al., 2018, McCarthy et al., 2019). Some of these species can reach very high degrees of nickel accumulation, up to 10 000 mg.kg⁻¹ and are currently used for

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agromining of ultramafic soils (Nkrumah et al., 2018).

Nickel HAs have most often been discovered and identified by means of botanical surveys, carried out on areas known to be covered by ultramafic soils (McCartha et al., 2019). In the past, researchers used paper soaked in coloured indicators (such as dimethyl glyoxime, which turns pink in the presence of nickel), but nowadays they use field portable X-ray fluorescence (FPXRF) detectors (McCartha et al., 2019). Originally designed for mining and metallurgical applications, they are now widely used in environmental science to measure the concentration of certain elements of concern in a variety of matrices, ranging from sediments to marine litter (Turner and Solman, 2016, Bull et al., 2017, Parsons et al., 2013). The ability of this technology to detect and quantify metals and other elements in plants and algae has also been demonstrated (Bull et al., 2017, Reidinger et al., 2012, van der Ent et al., 2019, Turner et al., 2018, Block et al., 2007). Thus, in our field of interest, they allow rapid analysis of soil and plant samples. They can also be used to analyse metals in dry plants from herbarium collections, as the analysis is non-destructive (van der Ent et al., 2019).

This technique has the advantage of being very fast and simple compared to laboratory techniques, which involve transporting samples to the laboratory, pre-treating them by acid digestion and analysing them by atomic absorption spectroscopy (AAS) or inductively coupled plasma (ICP). This is especially true since botanical surveys are most often conducted in remote locations and difficult environments (mountain sites, tropical forests etc.).

Botanical surveys and/or herbarium investigations are also carried out to identify other HAs than nickel ones, and there is a growing interest in the hyperaccumulation of other elements, especially gold. Indeed, gold is present in low concentrations in mine tailings in the vicinity of mining sites and cannot be extracted by conventional techniques (Ent et al., 2018). It would therefore be particularly relevant to extract it with the help of plants. Research on the phytoextraction of gold has already been published, and field trials have been carried out in which plants have been grown on gold-bearing substrates (Anderson et al., 2005). Gold hyperaccumulation was induced artificially by treating the substrate with cyanide and thiocyanate. The plants were then harvested, and biomass analysis showed promising results, with gold accumulating at average concentrations up to 39 ppm in the dry biomass (Anderson et al., 2005).

However, gold hyperaccumulators have not yet been found in a natural environment. Indeed, if plants have been used as prospecting tools to identify gold-bearing sites, the concentrations measured have always been below the hyperaccumulation threshold of 1 mg.kg^{-1} (Wilson-Corral et al., 2012).

The objective of this study is to evaluate the ability of an FPXRF spectrometer to be used in the field to identify gold hyperaccumulators in their natural environment, or to rapidly analyse plant samples during field-induced gold hyperaccumulation trials. To this effect, a sampling campaign was carried out in French Guiana, with the objective to collect sample of tropical plants from gold mining sites. Samples collected were all specimen of the specie *Phyllanthus stipulatus*.

We started by investigating the use of an FPXRF spectrometer to detect and quantify gold in synthetic standards covering a range of concentrations. They were prepared using different substrates chosen to best mimic real biological matrices and analysed using a NITON FPXRF spectrometer. Minimal sample preparation prior to FPXRF analysis was used to imitates field conditions. The results were then compared to ICP analysis to assess the accuracy of the instrument. Finally, the samples of tropical plants collected in French Guiana were analysed by both FPXRF and ICP to evaluate the performance of the FPXRF analyser with real samples.

2. Materials and methods

2.1. Preparation of synthetic standards

To test the calibration of the FPXRF spectrometer for Au, two matrices were spiked with the ICP Au standard solution (PlasmaCAL SCP SCIENCE Au 1003 $\pm 4 \mu\text{g/mL}$ 10% HCl) to produce synthetic standards. Microcrystalline cellulose (mcc) (Fluka analytical, Avicel PH – 101) was used to simulate a biological matrix. Wood ash obtained from a wood chip boiler located in Blainville-sur-l'Eau, (Grand-Est, France), was used to simulate plant ash.

Samples of each matrix were weighed in glass tubes and an appropriate amount of Au solution was added to obtain powders containing 0 (blank), 10, 25, 50, 100, 250, 500 and 1000 ppm of Au. Glass tubes were chosen to avoid powder loss by electrostatic effect. The samples were then placed in the oven at 65°C for 72 hours.

2.2. Preparation of tropical plant ash

Samples of tropical plants (*Phyllanthus stipulatus*, leaves and branches) were collected from several gold-bearing sites in the French Guiana rainforest. Fractions of these samples of approximately 100 g of mixed branches and leaves were then washed with deionized water and dried at 65°C in an oven for 72 hours. The biomass was then incinerated in an oven at 550°C for 2 hours. The ash was stored in plastic containers prior to analysis.

2.3. FPXRF analysis of standards and of plant ash

The FPXRF spectrometer used in this study was a NITON XL3t GOLDD from Thermo Scientific equipped with a radiation backscatter shield (BACKSCATTER SHIELD ASSEMBLY, XL3, Thermo Scientific). Sample preparation before XRF analysis was minimal to best imitate field conditions for synthetic standards and tropical plant ash. A titanium plate (0.2 mm thick) was used to provide a uniform background, and the powder sample was dispersed onto a sheet of white paper placed on top of the titanium plate. The nose of the FPXRF spectrometer was then pressed lightly against the powder and the spectrometer trigger was pulled. The analysis took 60 s, using the main energy filter for 30 s and the high energy filter for 30 s. Analysis were carried out in triplicate with both Soil and AllGeo modes. These calibrations algorithms are respectively meant to be used with samples containing low amounts of elements (below 5000 ppm) and with samples in which the amount of elements is not well known (Manual, 2022).

2.4. ICP analysis

After FPXRF analysis, samples were digested in 10 mL *aqua regia* in glass tubes using a Milestone ultraWAVE digestion system, transferred to 50 mL plastic tubes, and the volume was adjusted to 50 mL with deionized water. Elemental analysis was then carried out using a Thermo Scientific ICP-OES ICAP 6300 DUO. The instrument was externally calibrated using the same ICP standard solution used for the synthetic standard preparation by dilution in 10% *aqua regia*.

2.5. Data exploitation

FPXRF data were transferred from the spectrometer to a computer and processed using the NITON Data Transfer (NDT) software (Version 8.5.1; Thermo Scientific Portable Analytical Instruments, Inc). Gold concentrations were given in ppm, with a 2σ counting error. FPXRF limits of detection (LOD) are expressed as 1.5 times counting error for a given element in a sample containing trace of this analyte. ICP-OES data were exported using Qtegra software, and the raw intensities were converted to dry weight concentration in ppm. Linear regressions were obtained using Microsoft Excel. Error bars on the figures represent a

95% confidence interval.

3. Results and discussion

3.1. FPXRF analysis of the standards

Table 1 shows the results of FPXRF analysis of synthetic standards mcc and ashes using the AllGeo mode and Soil mode with a total count time of 60 s. In AllGeo mode, no gold was detected in the blank and in the 10 ppm samples. For samples containing more than 25 ppm gold, the results were close to the expected values (within 15%). All measurements counting error were below 20% of the analysed value for samples above 25 ppm of gold, meaning that they can be considered as quantitative according to the NITON XL3 user manual (Manual, 2022).

In Soil mode, the results obtained for the synthetic mcc standards were comparable with the ones obtained in AllGeo mode. Gold was detected in the 10 ppm standard, which was not the case in AllGeo mode. For the synthetic ash standard however, the values were significantly different from the expected concentrations, despite the measurement errors being below the 20% of the gold concentration value, making them theoretically quantitative. A strong tendency of the FPXRF spectrometer to underestimate the amount of gold is observed at a concentration above 25 ppm, and an overestimation below 25 ppm. The poor performance of the FPXRF spectrometer could be due to the high concentration of other elements like iron in the ash analysed (between 7000 and 10 000 ppm) while Soil mode is intended to be used on samples containing less than 5000 ppm of a given element (Manual, 2022). This makes Soil mode poorly suitable for hyperaccumulator ash analysis while AllGeo mode appears more indicated for this purpose.

Gold was also detected in significant amount in the blank ash sample, the mean concentration value being 11.7 ppm. This unexpected result was most likely due to interference and will be explained in more detail in section 3.3. Soil mode analysis of the mcc blank sample was also performed, and no gold was detected.

3.2. Comparison between FPXRF and ICP-OES results for synthetic standards

In Fig. 1, the analytical results of the synthetic standards obtained by FPXRF (AllGeo mode) have been plotted against those obtained by ICP-OES at a wavelength of 242.795 nm. The linear regression gave a slope equal to unit value (within 1%) with a correlation coefficient greater than 0.99.

The ICP-OES analysis confirmed that the FPXRF results for the 10 ppm standards (no gold detected) were a false negative, but there were no FPXRF false positives in the AllGeo mode. These results demonstrate that FPXRF analyses in each matrix studied were in good agreement with ICP-OES results above 25 ppm. However, at lower concentrations, the gold concentration was either underestimated or not detected.

Fig. 2 presents the comparison between the ICP-OES and FPXRF in soil mode analysis of the mcc and ash synthetic standard. No gold was detected in the ash blank sample with ICP-OES, confirming that the

Table 1

Mean gold concentration in ash and synthetic mcc standards returned by FPXRF analysis in AllGeo mode and Soil mode.

Sample	AllGeo mode (ppm Au)		Soil mode (ppm Au)	
	mcc	ash	mcc	ash
Blank	<LOD	<LOD	<LOD	11.7 ± 6.38
10 ppm	<LOD	<LOD	5.59 ± 3.61	16.0 ± 6.37
25 ppm	17.9 ± 4.14	29.4 ± 11.4	23.0 ± 4.53	24.8 ± 6.89
50 ppm	47.9 ± 5.20	48.2 ± 12.1	32.6 ± 4.86	37.0 ± 7.40
100 ppm	99.0 ± 6.16	105 ± 14.5	102 ± 6.49	65.7 ± 8.55
250 ppm	270 ± 9.28	260 ± 19.3	270 ± 9.91	163 ± 11.70
500 ppm	522 ± 13.1	496 ± 24.9	525 ± 16.6	287 ± 14.8
1000 ppm	1070 ± 18.1	1040 ± 35.7	1180 ± 21.8	545 ± 20.4

FPXRF result in Soil mode was a false positive.

In this case, the slope coefficient of the linear regression with the ash synthetic standard was significantly lower than unity, confirming the tendency of the FPXRF spectrometer to strongly underestimate the gold concentration. However, as shown in Table 2, this tendency to underestimate was only true for high concentrations (above 25 ppm), while concentrations below this threshold were systematically overestimated.

3.3. Interference between gold and zinc

Regarding the false positives of the gold concentration measured by FPXRF with Soil mode in ash standard, we hypothesize that they may be caused by an interference with the zinc present in the samples.

X-ray irradiation of the atoms during FPXRF analysis creates an electron vacancy in their inner shell. This hole is then filled by an electron of the outer shell, forming a photon whose energy is equal to the difference between the energy of the shells. Depending on the shells, these electron transitions are named differently. Transition are named after the shell into which the electron falls to fill the vacancy. Traditionally, a suffix (α , β ...) is added to identify which shell the filling electron comes from. For example, a transition from the M shell to the K shell is named K_{β} , a transition from M to L is named L_{α} .

The photons released by these transitions have specific energy, and form the characteristic X-ray spectrum of the irradiated elements (Kalnicky and Singhvi, 2001).

In the case of gold, there is a possible overlap between the L emission lines of gold and the K emission lines of zinc, as shown in Table 3, because of their energy levels proximity. This would explain why interference was only observed with zinc-containing ash and not with mcc. The amount of zinc detected by FPXRF in the ash synthetic standard ranged between 200 and 300 ppm in both AllGeo and Soil modes, and ICP-OES analysis showed a zinc concentration of 154 ppm. This type of interference is already a well-documented pitfall of XRF analysis, some of which are even added in some XRF calibration software (e.g. As/Pb interference) (Laperche and Lemi re, 2021). It can occur when the difference between the energy level of the emission rays of two elements is too small to be accurately detected and properly resolved by the spectrometer detector (Gallhofer and Lottermoser, 2018). The resolution of the detector in the NITON xl3t GOLDD is around 185 eV according to its technical documentation, meaning it could not be able to properly resolve the L_{α} ray of gold and the K_{β} ray of zinc, as well as the L_{β} ray of gold and the $K_{\alpha 1}$ of zinc.

Another issue specific to FPXRF is the relatively low power delivered to the anode by the spectrometer battery, limiting its ability to excite the K shells of heavier elements. This leads to the quantification of elements above molybdenum, such as gold, being done solely using the L emission rays when performing the analysis with a spectrometer using a 40 to 50 kV X-ray source, which is very common in portable spectrometers (Gallhofer and Lottermoser, 2018). The NITON used in this work has a 50 kV anode for example, impeding the proper quantification of gold.

Regarding the FPXRF analysis of mcc, significant amounts of zinc were detected in the 250, 500 ppm and 1000 ppm samples using both AllGeo and Soil modes, as shown in Table 4.

These results were confirmed as being false positive to zinc by ICP-OES analysis, and they further clearly demonstrate the existence of an interference between gold and zinc in FPXRF when analysing plant ash.

3.4. Analysis of the tropical plant ash with FPXRF and ICP-OES

FPXRF analysis of tropical plant ash in AllGeo mode showed no gold in any of the measurements. Table 5 presents the results of FPXRF analysis in Soil mode. Gold was detected above the detection limit in three out of the six samples. However, these measurements could not be deemed quantitative, due to their high counting error relative to the measured value.

No gold was detected in the samples by ICP-OES analysis after

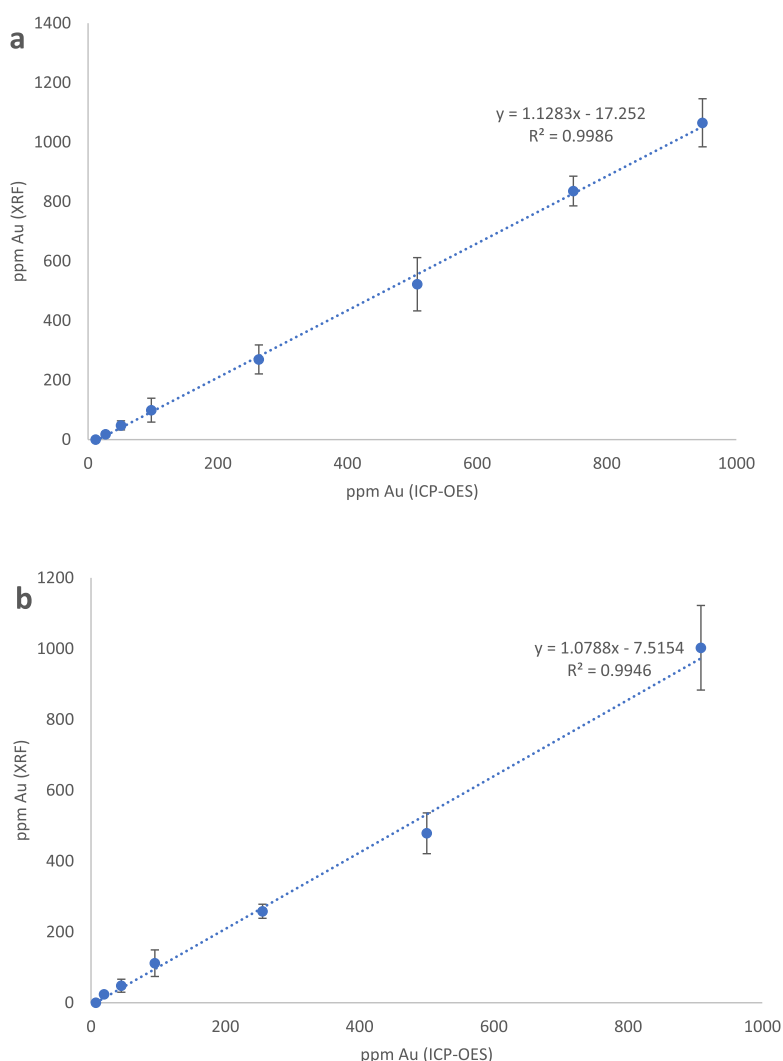


Fig. 1. Comparison of concentration of gold in mcc (a) and ash (b) synthetic standard measured by ICP-OES at 242.795 nm and FPXRF in AllGeo mode.

digestion in *aqua regia*. We hypothesize that these gold concentrations detected by FPXRF were caused by the same zinc interference as described in section 3.3. The tropical plant ash contained zinc in variable amounts, ranging from around 300 to 3 000 ppm (Table 6), measured by ICP-OES.

4. Conclusion

This work has shown that FPXRF can be used to quantify gold in samples containing both low and high amounts of other elements. However, satisfactory results could only be attained once gold has reached a sufficiently high concentration (50 ppm) and attention should be paid to the calibration mode chosen, as there is some difference between the modes pre-included in the NITON xl3t GOLDD (AllGeo and Soil).

While the AllGeo mode accurately quantified gold in both mcc and ash synthetic standards, Soil mode resulted in a strong underestimation of gold content in the synthetic ash standard, while the mcc results were comparable to AllGeo. In Soil mode, the impact of interference between gold and zinc in FPXRF also led to false gold positives in the synthetic ash standards and tropical plant ash samples, whereas this phenomenon was not observed with the AllGeo mode. The inaccuracy of the Soil mode could be explained by the high concentration of the analysed ash in other element such as iron, while Soil mode is designed to be used with

samples containing low amounts of elements.

FPXRF could therefore be used to perform *in-situ* or laboratory measurements of gold in order to identify gold hyperaccumulators, provided that the gold concentration is high enough. Gold concentrations of up to 63 ppm have been reported in field trials aiming at inducing gold hyperaccumulation, well above the 25 ppm threshold, making the FPXRF spectrometers a valuable tool for practically assessing the amount of gold accumulated in this case. However, interferences such as the one highlighted in this work need to be considered.

The use of more advanced spectrometers with better resolution or a more powerful X-ray source able to excite the gold K-shells could also allow to work around this interference. However, most FPXRF spectrometers use low-power X-ray sources in the 40 – 50 kV range, to increase portability and battery life (Cornaby and Kozaczek, 2016). Regarding the resolution of the spectrometer detector, some portable spectrometers can achieve a resolution of less than 140 eV under good analytical conditions, slightly better than the 185 eV resolution of the spectrometer used in this work but not low enough to properly resolve all the emission ray overlap identified here.

Another way to make the analysis more accurate would be to first validate the FPXRF results with another analysis technique such as ICP-OES and then develop an analytical method specific to the samples analysed in order to account for all the possible interferences and the matrix specificity. A zinc filter could also be used to eliminate the

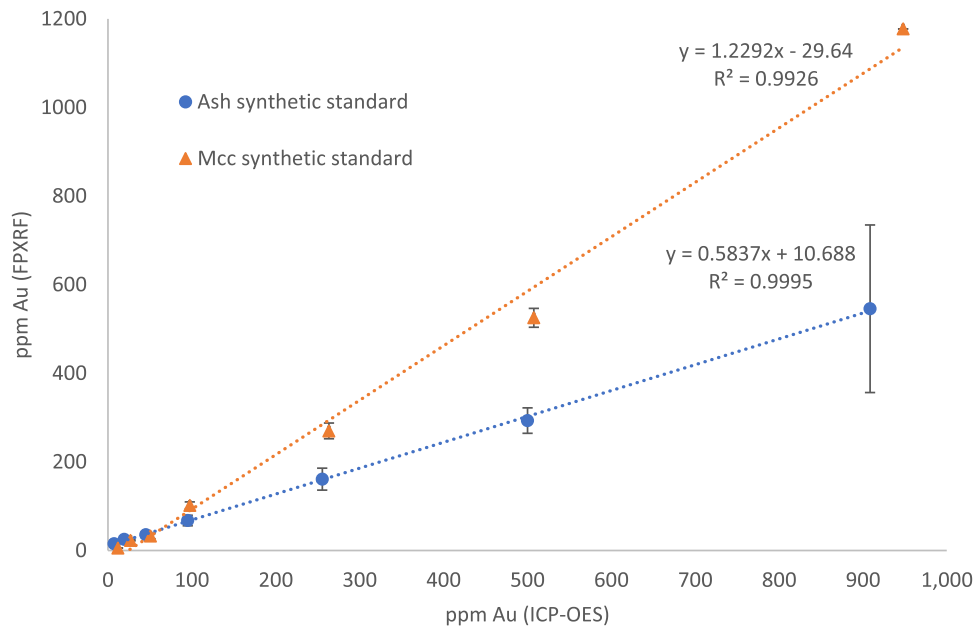


Fig. 2. Comparison of concentration of gold in mcc and ash synthetic standard returned by ICP-OES at 242.795 nm and FPXRF in Soil mode.

Table 2

Gold concentration returned by FPXRF in Soil mode and ICP-OES analysis in ash synthetic standard

Sample	Mean concentration (ppm Au)	
	FPXRF	ICP-OES
Blank	11.7 ± 6.38	0
10 ppm	16.0 ± 6.37	9.70
25ppm	24.8 ± 6.89	24.3
50 ppm	37.0 ± 7.40	51.5
100 ppm	65.7 ± 8.55	103
250 ppm	163 ± 11.70	252
500 ppm	287 ± 14.8	515
1000 ppm	545 ± 20.4	908

Table 3

Energy levels of the K_{β} and L_{α} X ray emission lines for gold and zinc

Element	X ray emission lines (keV)					
	$K\alpha$ 1	$K\beta$ 1	$K\beta$ 3	$L\alpha$ 1	$L\alpha$ 2	Ll
Au	68.804	77.984	77.580	9.713	9.628	8.494
Zn	8.639	9.572	9.572	1.012	1.012	0.884

Table 4

Zinc concentration in mcc standards returned by FPXRF in AllGeo and Soil modes.

Sample	AllGeo mode (ppm Zn)	Soil mode (ppm Zn)
Blank	<LOD	<LOD
10 ppm	<LOD	<LOD
25 ppm	<LOD	<LOD
50 ppm	<LOD	<LOD
100 ppm	<LOD	<LOD
250 ppm	<LOD	9.85 ± 5.27
500 ppm	<LOD	27.6 ± 8.10
1000 ppm	19.4 ± 6.51	23.9 ± 7.93

Table 5

Gold concentration returned by FPXRF analysis in Soil mode in tropical plant ash.

Tropical ash	Au (ppm)		
	Measure 1	Measure 2	Measure 3
Sample 1	<LOD	<LOD	<LOD
Sample 2	<LOD	10.26 ± 6.61	<LOD
Sample 3	<LOD	16.77 ± 8.43	14.71 ± 8.17
Sample 4	<LOD	<LOD	<LOD
Sample 5	<LOD	9.18 ± 6.07	<LOD

Table 6

Zn concentration in tropical ash returned by ICP-OES analysis

Tropical ash	Zn (ppm)
Sample 1	559
Sample 2	1190
Sample 3	2114
Sample 4	314
Sample 5	3604

interfering radiations if zinc quantification is of no interest to the operator.

CRediT authorship contribution statement

Thomas Monot: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Marie-Odile Simonnot:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Baptiste Laubie:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marie-Odile Simonnot and Baptiste Laubie reports a relationship with Econick company that includes: board membership.

Data availability

Data will be made available on request.

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