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Introduction to micro-residues analysis: Systematic use of Scanning Electron Microscope and Energy Dispersive X-rays Spectroscopy (SEM-EDX) on Patagonian raw materials

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ABSTRACT

Residue analysis is commonly applied to stone tools functional identification and is often used in conjunction with micro-wear analysis for understanding their past function and also early human behaviour. In spite of their important contribution in archaeological contexts, these studies present drawbacks in their application, since they require an exceptional state of conservation, as well as a correct identification by the observer. In this sense, actualistic studies are needed for a better residues characterization. Therefore, the following work explores the usefulness of scanning electron microscopy (SEM) as a tool to provide a better morphological and compositional identification of micro-residues on Patagonian raw materials. The residue analysis is a methodology rarely used on the studies of lithic technologies in Argentina so this paper constitutes a first approach to the main micro-structural features; distribution and elemental composition of micro-residues on stone tools using EDX spectra. The main results obtained from analytical experiments using SEM-EDX analysis improves the worked material identification and explore the possibility to apply this methodology at the Deseado massif's archaeological record.

1. Introduction

The micro-wear analysis has been the most used approach in the functional determination of different lithic technologies (Semenov, 1964; Yamada, 1993; Keeley and Newcomer, 1977; Plisson, 1985; Unrath et al., 1986; Rots, 2005; Miller, 2014; Yerkes et al., 2014; Ollé et al., 2016). However, in archaeological artefacts where micro-polishes with diagnostic features have not been developed, functional identification is difficult. Nowadays, the advances generated in different optical media and non-destructive chemical detection instruments allowed increasing the information on this respect (Sepúlveda, 2011; Prinsloo et al., 2014; Lemorini and Nunziante, 2014; Ollé and Vergès, 2014; Pedergnana and Ollé, 2017). The residue analysis is an alternative and integrative methodology to the micro-wear analysis and consists of the organic and inorganic elements identification on stone tools whose origin may be due to the different materials use, as well as to the contact with other substances after its deposit (Anderson, 1980; Clemente et al., 2002; Fullagar et al., 1996, 1998, 1999, 2006; Wadley et al., 2004; Lombard and Wadley, 2009; Langejans, 2011; Langejans and Lombard, 2015; Croft et al. 2016; Pedergnana and Ollé, 2017). The early researches focused on distinguishing microscopic structures of starch grains, phytoliths or blood cells. However, Loy (1983) in particular promoted molecular, biochemical and genetic analyses. While these analyses received series critical responses (Custer et al., 1988) were focus on determine whether a residue was present; its location on the artifact; main features that can attribute the residue to a plant or animal source; discern any tissue type; and provide a taxonomic designation if preservation is ideal. The combination of chemical and biochemical analytical methods can be employed and this provides a means of confirming the results obtained from microscopic analysis.

This methodology has an strong development in South Africa and mainly includes residues analysis in situ, this mean that the residues are recorded and located minutely on the instruments under a reflected light microscope, allowing to see different molecular structures (Wadley et al., 2004; Lombard, 2006, 2007; Lombard and Wadley, 2009; Langejans and Lombard, 2015).

Currently, procedures such as liquid chromatography-mass spectrometry (LC-MS), Fourier transform infrared spectroscopy (FTIR), Raman and the use of electron microscopy (SEM-EDX), are being systematically applied to the residues identification on stone tools (Anderson, 1980; Yamada, 1993; Boëda et al., 1996; Grünberg, 2002; Guiliano et al., 2007; Pawlik, 2000; Pawlik and Thissen, 2011; Monnier

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et al., 2012; Matheson and Veall, 2014; Ollé and Vergès, 2014; Lemorini and Nunziante, 2014). However, in Argentina these analyses were discontinuously developed until the mid-1990s (Mansur-Franchomme, 1983) and it is from that moment that the residues analyses with diverse analytical approaches are recorded (Mansur et al., 2009; Babot, 2002; Briz et al. 2014). Even so, its application is often skewed by the availability of suitable optical media and by the costs of applying these methodologies in a systematic way.

Therefore, the following study is part of a larger project carried out in the Deseado massif area (Santa Cruz Province, Argentina). The human occupation in this area began in the late Pleistocene/Holocene transition (about 11.000 years old BP) and the production and use of stone tools recovered at the area are main objectives in this project. In this sense, this paper continues the functional studies developed in Cueva Maripe site and aims to generate advances through specific micro-residues analysis on experimental stone tools. The study included a first morphological characterization of the residue microstructures in situ and a comparative study of chemical elements composition obtained from the use of electron microscopy with energy dispersive X-ray spectroscopy (Energy Dispersive Spectrometer-EDX). The results at a morphological and chemical levels allowed exploring the potential advantages and limitations of this method, as well as possibilities of application on the archaeological record at the study area.

2. Experimental design and methods

As we said before the main goal of this paper is to perform a systematic register of micro-residues (elemental and morphological composition) in experimental tools, in order to generate a reference collection from controlled experiments. This collection is destined to serve as a basis for comparing with archaeological residues that may be present in the Deseado massif lithic assemblages (Santa Cruz, Argentina).

The experimental activity is still on-going, that means that the present available experimental collection is not complete. However, all the activities have been divided into variables categories, including different varieties of raw materials, worked materials and motions (Table 1).

Although several flint varieties are present in the Deseado massif area we started employing the most common ones identified in Cueva Maripe archaeological record. This was a fine grain silicified ignimbrite and the cores were recovers from a secondary raw materials source (La Primavera-Pedimento 1 - LP-P1) (Hermo and Lynch, 2015; Lynch, 2016; Lynch and Hermo, 2015; Lynch and Miotti, 2016). In order to avoid contact with other materials, the experimental tools were placed into individual plastic bags immediately after knapping. Prior to the tool-use experiments, tools were photography and observed under low power microscope, before any use.

Diverse materials as fresh bone, unseasoned soft wood, dry hide and mineral pigments (pigments alone and paint mixtures) were worked on the experimental series (Table 1). These materials were selected based on the micro-wear analysis results previously obtained from the Deseado massif's archaeological record (Lynch, 2016). In many cases, the functional analysis only identified non-differentiable hard material use; so we believe that the micro-residues analysis could be a complementary analytical approach to obtain more information about the production and use of the massif's lithic technology.

The activities developed involved transversal (scraping, smoothing) and longitudinal (cutting) motions, sensu Mansur-Franchomme (1983) (Table 1).

After use with latex gloves without dust, the tools were not cleaning so the residues related to use could be recorded in situ and also their location. However, in those cases where the micro-traces were registered, we used the reference framework of heterogeneous materials (matrix and crystal analysis) for the characterization of these traces (Mansur-Franchomme, 1983).

The microscopic analysis was performed by applying two different but highly complementary approaches: the description of use-wear and the morphological and chemical characterization of the residues adhered on lithic surfaces. So we employed low and high-power microscopy: a binocular loupe Nikon SMZ 800 (10–63 × magnification) and a metallographic microscope Nikon Epiphot200 (magnification 100 ×–500 ×), both with a

Table 1
Mains features of experimental stone tools used and details of the experiments carried out.

Artifact N°	Raw material	Type of artifact	Use time	Length	Width	Thickness	Edge Angle	Edge length	Worked material	Kinematics
EU164	ISG1	Flake	60′	80,0	71	14	45	64	Unseasoned soft wood	Scraping
EU167	ISG1	Flake	60′	57,1	48,84	11,54	48	53,69	Unseasoned soft wood	Scraping
EU171	ISG1	Flake	60′	48,7	38,43	8,98	40	43,07	Unseasoned soft wood	Scraping
EU172	ISG1	Flake	60′	57,4	29,12	8,48	38	36,43	Unseasoned soft wood	Scraping
EU166	ISG1	Flake	60′	67,1	42,72	19,67	57	37,82	Fresh bone	Scraping
EU173	ISG1	Flake	60′	75,0	48,45	11,52	38	42,91	Fresh bone	Scraping
EU183	ISG1	Flake	60′	49,3	26,54	14,19	48	41,26	Fresh bone	Scraping
EU169	ISG1	Flake	60′	41,4	62,72	16,69	37	42,16	Dry hide	Scraping
EU170	ISG1	Flake	60′	39,0	30,1	6,9	46	34,26	Dry hide	Scraping
EU174	ISG1	Flake	60′	41,8	43,79	14,36	50	32,66	Dry hide	Scraping
EU182	ISG1	Blade	60′	73,5	26,93	12,97	71	66,19	Fresh bone	Cutting
EU178	ISG1	Flake	60′	40,7	38,87	11,69	32	37,97	Fresh bone	Cutting
EU175	ISG1	Flake	60′	58,2	32,83	10,99	46	34	Fresh bone	Cutting
EU176	ISG1	Blade	60′	63,9	35,09	10,08	60	42,04	Unseasoned soft wood	Cutting
EU177	ISG1	Flake	60′	44,9	30,16	9,96	40	43,63	Unseasoned soft wood	Cutting
EU179	ISG1	Flake	60′	52,1	30,63	8,93	22	47,46	Unseasoned soft wood	Cutting
EU181	ISG1	Flake	60′	61,3	33,41	9,16	49	44,37	Fresh bone	Cutting
EU168	ISG1	Flake	60′	37,5	29	11,28	48	37,53	Unseasoned soft wood	Cutting
EU180	ISG1	Flake	60′	36,4	27,74	11,07	39	26,52	Unseasoned soft Wood	Cutting
EU165	ISG1	Flake	60′	51,07	30,57	14,59	62	37,62	Fresh bone	Cutting
EU184	ISG1	Flake	60′	33,9	38,48	11,64	34	30,69	Dry hide and S9 pigment alone	Scraping
EU185	ISG1	Flake	60′	44,2	42,48	9,74	38	47,3	Dry hide and S4A pigment alone	Scraping
EU186	ISG1	Flake	60′	64,7	48,1	14,1	56	37,38	Dry hide and inorganic black pigment (paint mixture)	Scraping
EU187	ISG1	Flake	60′	40,7	60,98	17,2	34	48,07	Hide and S4A pigment (paint mixture)	Scraping
EU188	ISG1	Flake	60′	39,3	27,08	6,86	58	36,05	Dry hide and S9 pigment (paint mixture)	Scraping
EU189	ISG1	Flake	60′	48,3	22,14	6,81	25	33,85	Dry hide and organic black pigment (paint mixture)	Scraping

video-microscopy system. A scanning electron microscope (SEM) Quanta 200 model it was also used. This microscope has also an energy dispersive X-ray spectrometer (EDAX, SDD Apollo 40) that can obtain compositional information quickly and effectively. This low vacuum model SEM avoids, in turn, the materials metallization to be observed (with gold or coal), which will allow complementary studies with other methodologies.

During SEM observations a series of control points were chosen (comparing used edges with fresh ones), allowing to document the gradual stages of edge modifications during use as well as use-wear formation processes.

In this first instance, the residues were observed in situ, that is, photographed, identified and located on the tools through these series of points that also served as reference for documented different kinds of use-wear (gloss, micro-polish, micro-scars, breakages, striations and rounding). All the samples has at least five control points, the EDX measure were on the micropolish, on the cutting edge without residue, on the identified residues and in two areas away from the worked edge without residues and micropolish. The variables included were: shape, colour, size, orientation in relation to the used edges, location in relation to other use-wear traces and observable features of its microstructure at this analysis level (Lombard, 2007; Lombard and Wadley, 2009; Langejans and Lombard, 2015).

3. Results

3.1.1. Experimental organic residues: bone working

Preys processing activities, bone tools and bones decorated by engraving and incision technique were mainly recorded in Cueva Maripe site and commonly associated with middle Holocene occupations (from ca. 7500 to 3000 years BP). It is believed that they would be good raw materials for working hide or fibers (Miotti and Marchionni, 2013). The presence of these materials and the previous identification of bone micro-polishes on lithic tools (Lynch, 2016) have generated the need to consider this material in the reference collection.

Therefore, on the fresh bone work unretouched artefacts were used and the periosteum removal was also included on this activity (Table 1).

The micro-traces developed are similar to those previously described for this type of material worked (Mansur-Franchomme, 1983; Álvarez, 2003; Lynch, 2016). In the first minutes of the activity abundant micro-scars were observed on the edge worked. The micro-polish developed was diagnostic of the bone work; it was generated at high areas of the microtopography, bright, thick and with craquelé. It was also observed transverse and longitudinal striations that allowed identifying the motions used (Fig. 1a).

The residues observed were homogeneously recorded along the edges, extending to the striking platform, on use-wear and on the area adjacent to use-wear (5 mm). They presented a greasy, amorphous appearance and white/yellowish coloration; adhered on high and low surfaces on the artefacts microtopography and in some cases, the motions directionality were recognized (Fig. 1b). In some areas, remnants of the periosteum were visible in reddish tonalities.

Under the SEM (500 × and 2000 ×) the main features of bone micro-polish and bone residues were better observed with light tonalities. In the unused area, constituent elements as Si (43%), Al and K (6% in each case) were identified (Fig. 1c–d). In the residues, higher concentrations of other elements such as C (14%), Ca (22%) and P (12%) were registered by the EDX spectrograms, while Si, Al and K concentration decreased significantly (3%).

In the micro-polish area, there were few residues deposits and C, Ca and P concentrations were $< 5\%$, while Si (37%), Al (8.55%) and K (9.3%) increased (Fig. 1e).

3.1.2. Wood working

In Cueva Maripe site the stone tools related to the wood working are significant and in some cases the use of different shafts made of wood has been inferred through the micro-wear analysis (Lynch and Hermo, 2015; Lynch and Miotti, 2016).

Therefore, the work on semi-hard wood from Patagonian steppe environment (*Condalia microphylla*) was included in the experimental series. This material was used in the same way as bone, with unretouched artefacts in cutting and scraping motions (Table 1).

The use traces identified had abundant striations that allowed inferring the motions employed and also a well-developed micro-polish of the material used.

In scraping motions, the micro-polish was found on a marginal extension in the used edge due to a right work angle; while in cutting motions was developed inside the edge and in dorsal and ventral surface (Fig. 2a).

At low magnification (45 × to 70 ×) some residues on the used edge were identified (Fig. 2b–c); these were translucent-greenish coloration and in some cases, part of the processed plant tissue and fibers could be identified. The fibers usually present white colour and their distribution was mainly on the area adjacent to use-wear and especially inside fractures and micro-scars.

Under SEM and particularly on the unused areas of the edge, the elements identified by the EDX spectrograms mainly correspond to high concentration of Si (40%) and lower percentages of Al and K (7% in each case).

On the micro-polish area, although high percentages of these elements were identified, there was a slight increase in the organic elements concentrations such as C (24%) and O (36%); as well as less concentrations of Na, Mg, Ca and Fe, which did not exceed 4% in each case.

On the residues higher concentrations of organic material, C (56%) and O (32%), and smaller proportions of other elements such as Na, Mg, K, Ca and Fe ($< 2\%$ in each case) were identified by the EDX spectra (Fig. 2e).

3.1.3. Skin working

In Patagonia sites the presence of stone tools used in the leathers processing is considerable and in some cases natural conditions of deposit have allowed its preservation in the archaeological record (Marchione and Bellelli, 2013). This allowed identifying that their treatment included different colouring substances not only due to a decorative function but also for a better drying and conservation (Musters, 1871; Cooper, 1946; Casamiquela, 1981; De La Fuente et al., 2013; Mansur et al., 2009). Nowadays several studies have been focused on the different ochre sources used for the paint production, on the production techniques and the use for which they were intended (Rowe, 2001 and López et al., 2012).Considering these observations, in the experimental series the dry skin was worked naturally and with different additives in its treatment (natural pigments and pigment mixtures). In this sense the first step was scraping the hides alone for degreasing the skin and then tanning the skin to make it more durable and less prone to decomposition (Mansur et al., 2009).

At a microscopic level (45 to 200 ×) a well-developed hide micro-polish was identified on the worked tools, with an homogeneous distribution in the microtopography, a matte gloss, strong rounding of the edge, deep parallel striations and extended development inside the edge due to an acute working angle (between 45° and 70°) (Fig. 3a). The micro-scars were scarce and specially identified in acute edges. At higher magnification (5000 ×) white organic fibers were found all around the used edges mainly recognize on the area adjacent to use-wear and outside this area (Fig. 3b–f).

The EDX spectrograms results in the skin residues allowed determining the highest concentrations of C (77%) in the whole experimental series and lower proportions of Na (0.04%) and Ca (0.55%) (Fig. 3g).

3.1.4. Experimental inorganic residues: natural pigments used

Natural pigments were widely use in South of Patagonia and it is a

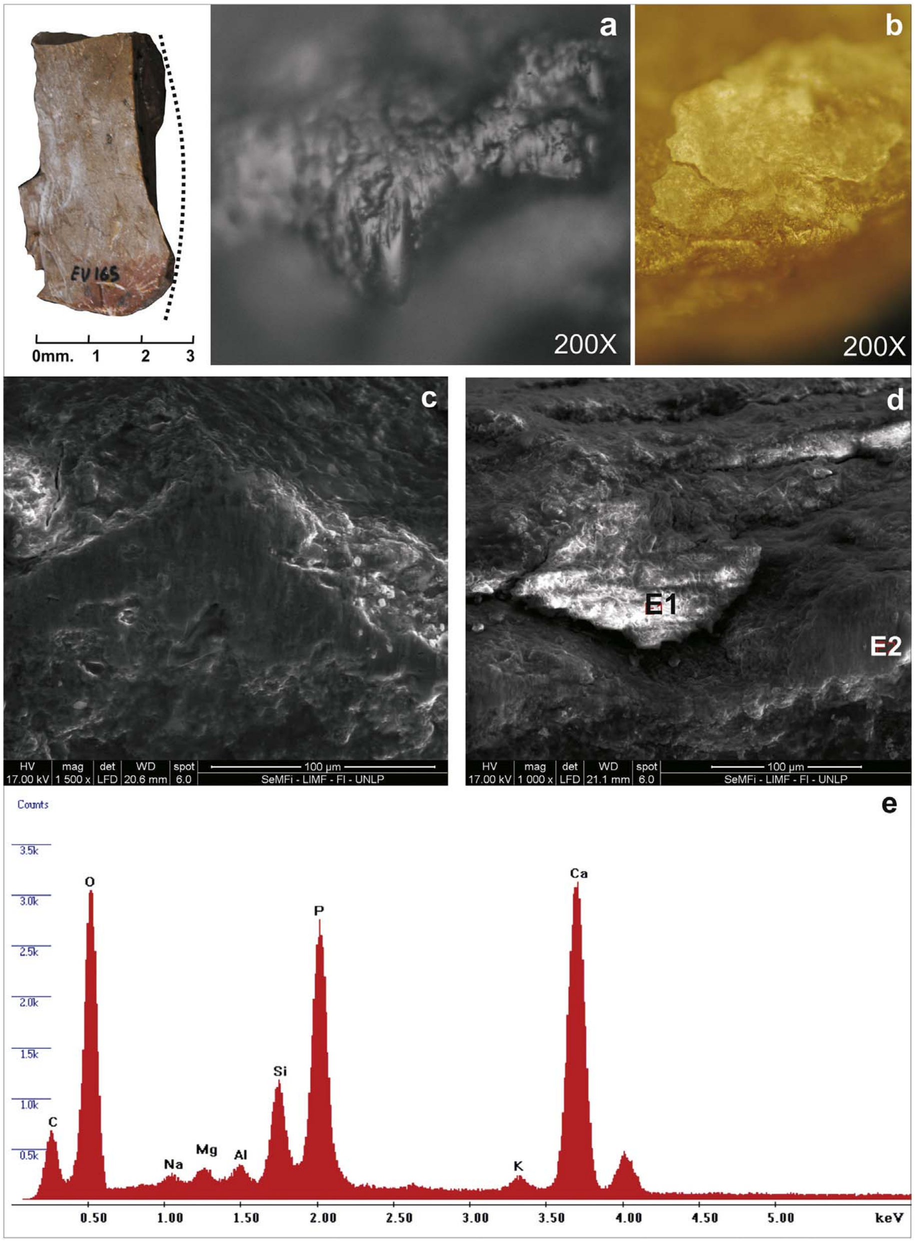


Fig. 1. a. Stone tool used on bone working and bone micro-polish on transverse motions (200 ×). b. Amorphous appearance and white/yellowish bone micro-residues (200 ×). c–d. Bone micro-polish and residues at high magnifications (1000–1500 ×). e. E1: EDX spectra on bone residue (qualitative analysis). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

variable record due to is presented in cave paintings, on different artefacts (pottery, lithic and bone tools), human burials, clothing and awnings (Casamiquela, 1981; Yacobaccio et al., 2008; De La Fuente et al., 2013; Carden et al., 2014). In Cueva Maripe site, pigments in a stratigraphic position, as well as ochres on the surface in short and long distance of the site were found (Carden et al., 2014).

Therefore, the analyses carried out in this paper are related to the identification and characterization of the inorganic fractions of the colouring matter. For this reason, pigment mixtures were conducted using three different colours (red, ochre and black) and taking into account previous analysis about painted caves identified on the study area (Carden et al., 2014) (Table 2). The mixture included 5 g of pigment milled by different pebbles, 1 g of animal fat (*Puma concolor*) as a binder and 30 drops of water, leaving to rest approximately 20 min.

3.1.5. Red paint (S9 pigment use)

After the skin working with red paint, abundant residues on the used edge were recorded. Under a low power microscope (200 ×), edge rounding and perpendicular striation were identified as also a well-development micro-polish of the worked material (matt gloss, homogeneous, with deep striations and of medium extension due to a working angle between 45° and 70°), however in this case the striations with different lengths increased. Abundant residues of the paint used as well as of the dry skin worked were also observed along the contact and handling part as outside of the use-wear area (Fig. 4A).

Under SEM on the residues, high concentrations of C (50%) and between 8% and 5% of Si, Al, Ca and K were detected by the EDX spectrograms; while Ti (0.80%) and Fe (0.3%) were identified in lower proportions (Fig. 4a). In turn, the use of this same pigment in powder, recorded variations with respect to the concentrations of the red paint elements. In this sense, a decrease of C (5.9%) and the absence of Mg, Ca and Ti were record. While an increase of Fe (2.59%) and the presence of S (0.53%) was identified.

3.1.6. Ochre paint (S4A pigment use)

The colouring matter used for this paint has smaller sizes clasts than the red one (Table 2). After their use, abundant residues on the tool's

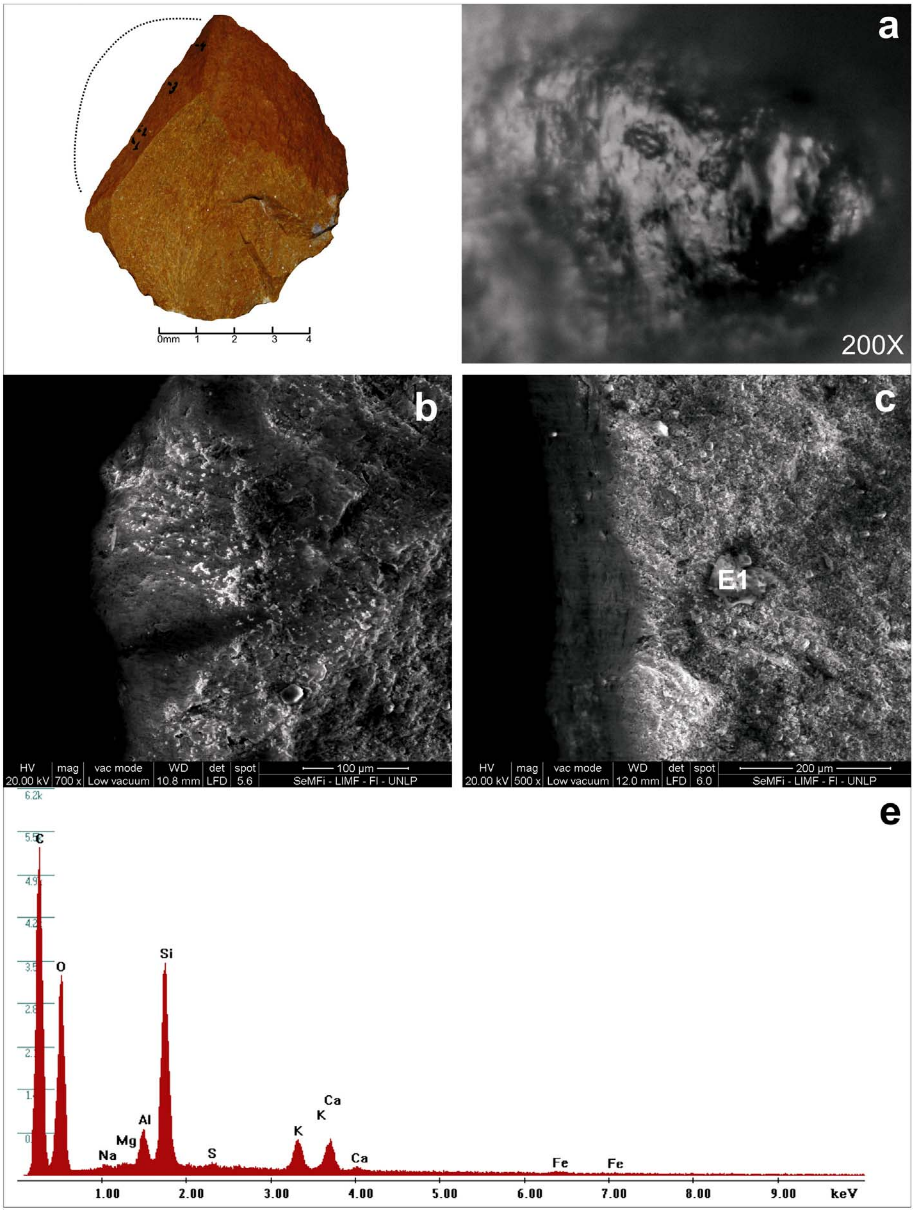


Fig. 2. a. Stone tool used on wood working and wood micro-polish on transverse motions (200 ×). b–c. Wood micro-polish and residues identified under SEM at high magnifications (500 ×–700 ×). e. E1: EDX spectra on wood residue (qualitative analysis).

edges were record extending towards more than half of the technological axis, mainly outside of the use-wear area. The residues identified were granular deposits mainly concentrated in micro-scars and fractures of the rock (Fig. 4b).

At low magnifications, abundant micro-scars were identified due to an extremely sharp angle of the edge ($< 35°$). However, after 30 min of activity a certain degree of edge rounding and hide micro-polish was recognized.

Under SEM the chemical elements identified on the residues showed a decrease of Si (20.13%), Al (4.51%) and K (1.58%) concentrations and an increase in C (33.70%), Na (0.40%), Ca (3.35%) and Fe (4.74%) (Fig. 4b).

On the other hand, the use of S4A pigment in powder, recorded considerable concentrations of Na (1.45%) and Fe (10.2%) and other elements in low concentrations such as Cl (3.41%) and S (0.6%), absent in the pigment mixture (Fig. 5).

3.1.7. Inorganic black paint (commercial ferrite)

The black paint was used in the Deseado massif rock art and in some cases related to early occupations of the area (Carden et al., 2014). In La Primavera canyon (Santa Cruz province, Argentina), negative hands painted in black were recorded in different caves and it is believed that its production would have been associated with the use of charcoal or some mineral agent such as manganese oxide (Carden et al., 2014). However, an effective source has not been identified so far and nor have specific studies been made related to its components. Therefore, this paper evaluates two different ways of production for the application on dry skin. A first mixture was made from ferrite. This proved to be more homogeneous due to the colouring matter granulometric and mineralogical composition.

Under binocular loupe a strong rounding of the edge as well as abundant residues were identified on micro-scars and fractures of the rock. At higher magnification (200 ×), transversely striations and a well-developed hide micro-polish were registered. The residues were mainly identified on the use-wear and adjacent area (Fig. 4c).

The results obtained by SEM-EDX on the residues recorded considerable concentrations of O (39.01%), C (14.64%), Mg (13.45%) and Ca (23.73%). It should be noted that this high magnesium concentration occurred only in the use of inorganic black paint. While the other elements identified (Si, Al and K) were also recorded in non-used areas of the edge but in higher proportions (Fig. 6).

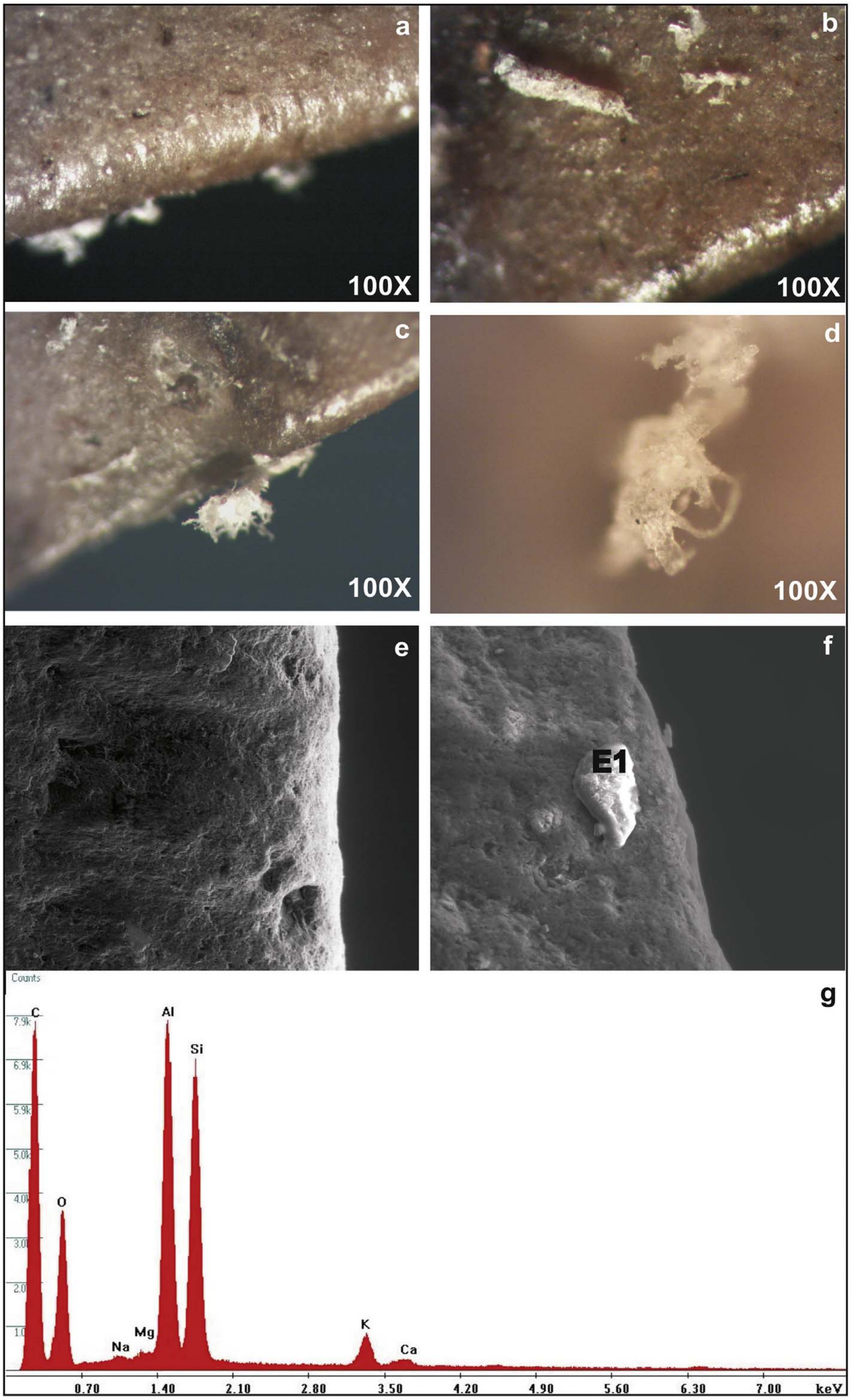


Fig. 3. a. Skin micro-polish on scraping motions. b–d. Fibers and skin residues identified on the used edge. e–f. Micro-polish and residues observed at higher magnifications under SEM. g. E1: EDX spectra and qualitative analysis on the residues.

Table 2
The main features of pigments used and archaeological sources.

Pigment used	Locality	Geological formation	Colour code	Shape	Distance from Cueva Maripe
S9	La Primavera	Chön Aike	2.5 Y 7/8 yellow	Pebbles	< 1 km
S4A	La Primavera	Chön Aike	10 R 3/6 dark red	Pebbles	3,3 km

3.1.8. Organic black paint

It was made from charcoal and ground calcined bones, which resulted in a heterogeneous mixture unlike inorganic black paint. Even so, it allowed covering and penetrating effectively the worked material. The differences observed in this paint production were not significant with respect to its distribution on the edge used and to the residues morphology registered at low magnifications. However, characteristic and exclusive chemical elements of its production were determined. In this sense, the EDX spectrograms detected high concentrations of C (66.44%), P (0.3%) and Ca (0.94%). These last two elements were not

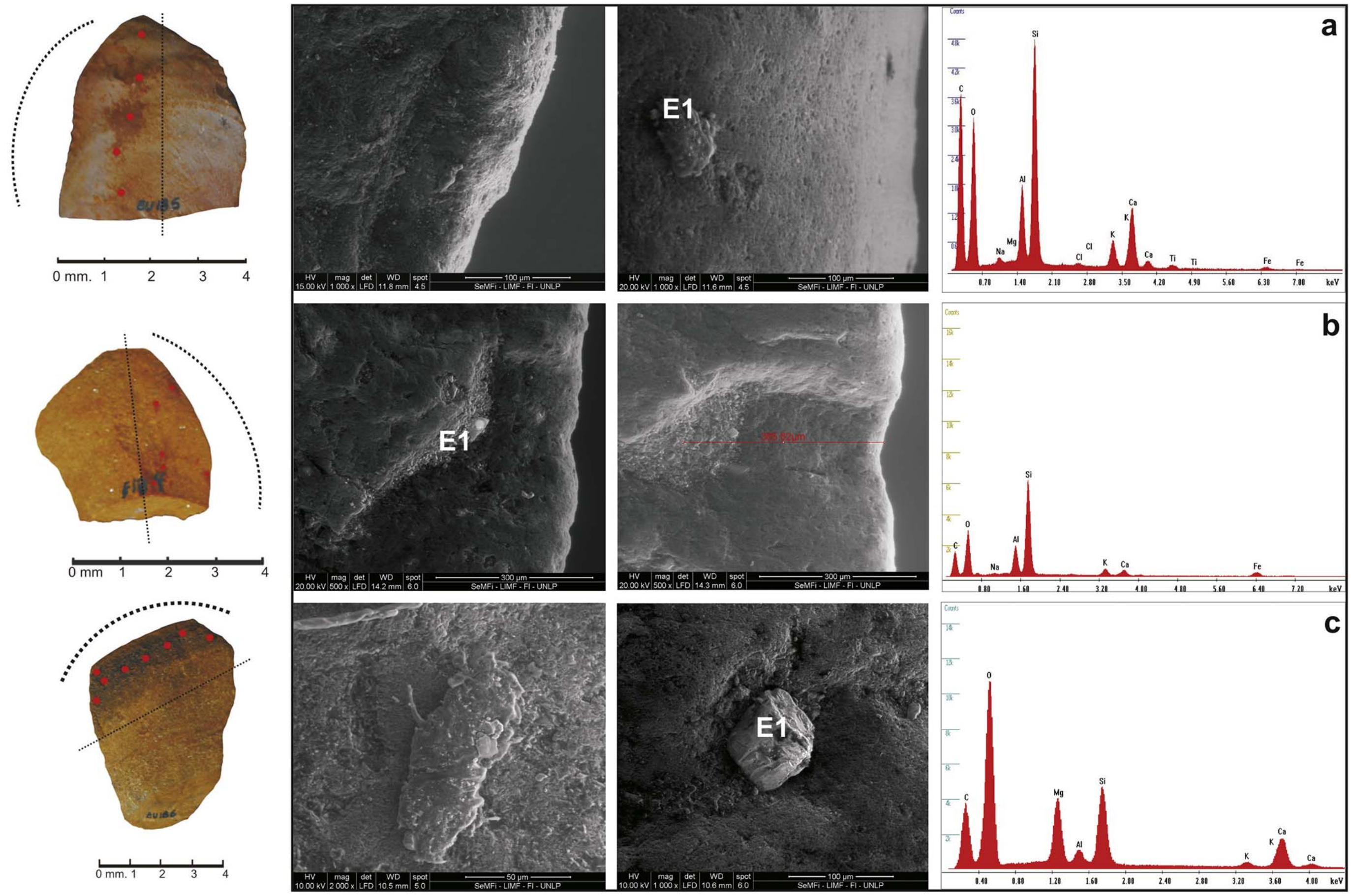


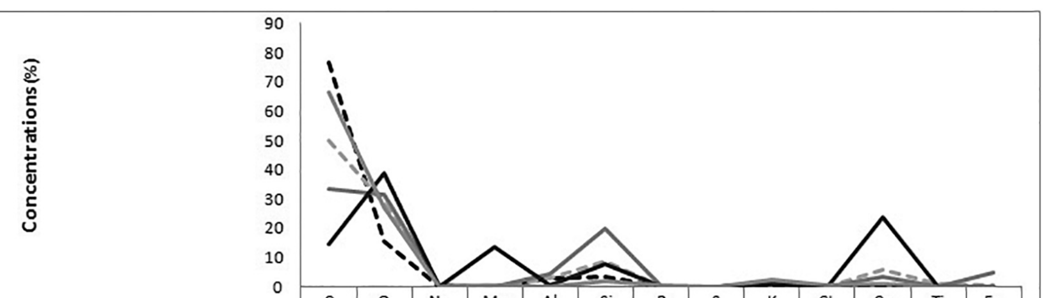
Fig. 4. a. Skin working with red paint (S9 pigment), skin micro-polish and residues. E1: EDX spectra and qualitative analysis on residues identified. b. Skin working with ochre paint (S4A pigment), skin micro-polish and residues. E1: EDX spectra and qualitative analysis on residues identified. c. Skin working with black inorganic paint. E1: EDX spectra and qualitative analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

registered in the inorganic mixture and therefore would be diagnostic to determine its production process (Fig. 6).

4. Archaeological preliminary results

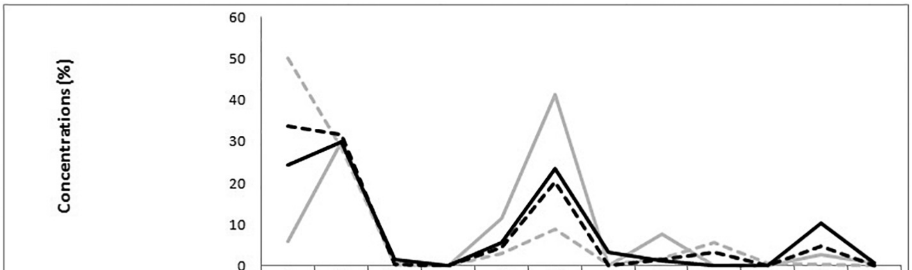
The archaeological material analysis is complementary to the creation of an experimental reference collection. This means that use-wear found on archaeological implements can influence the development of the experimental activity as well as induce to change some variables at the experimental protocol.

As we said before on this paper the use-wear analysis at Cueva Maripe archaeological record was already done but no residues analyses following this protocol have been performed yet. The first reason of this is that part of the archaeological material was observed without



	C	O	Na	Mg	Al	Si	P	S	K	CL	Ca	Ti	Fe
Skin	76,69	15,6	0,04	0	2,7	3,59	0	0	0,7	0	0,55	0	0
Skin and red paint	50,02	28,59	0,47	0,09	3,03	8,9	0	0	1,8	0,23	5,77	0,78	0,32
Skin and ochre paint	33,7	31,59	0,4	0	4,51	20,13	0	0	1,58	0	3,35	0	4,74
Skin and black inorganic paint	14,64	39,01	0	13,45	0,58	7,68	0	0	0,9	0	23,73	0	0
Skin and black organic paint	66,44	27,24	0	0,32	0	1,89	0,3	0,07	2,32	0,47	0,94	0	0

Fig. 5. Quantitative and qualitative EDX analysis of skin worked with different paints.



	C	O	Na	Mg	Al	Si	Cl	K	Ca	Ti	Fe	S
Red paint (S9)	50,02	28,59	0,47	0,09	3,03	8,9	0,23	1,8	5,77	0,78	0,32	0
Pigment in powder (S9)	5,9	29,95	0,31	0	11,55	41,42	0	7,65	0	0	2,59	0,63
Ochre paint (S4A)	33,7	31,59	0,4	0	4,51	20,13	0	1,58	3,35	0	4,74	0
Pigment in powder (S4A)	24,44	29,93	1,45	0	5,73	23,54	3,41	1,31	0	0	10,2	0,6

Fig. 6. Quantitative and qualitative EDX analysis of skin worked with different paints and pigments in powder.

taking in to account some methodological precautions and specially connected to modern contaminants. However, new archaeological materials recovered can contribute to a suitable microscopic residues analysis.

Anyway, preliminary results are promising, as use-traces have been documented as also some inorganic remains (pigments) and in this sense additional experiment and more archaeological data are necessary before making any kind of interpretation.

5. Discussion and conclusions

The residues analysis is a complementary methodology of the micro-wear analysis, especially when diagnostic micro-polishes have not been developed, either because of the short time of use, states of the worked materials or the raw material used in tools production. In this paper the analyses developed allowed generating a first approach of the micro-residues identification on stone tools.

A controlled experimental program allowed isolating indicators for a better understanding of technical possibilities of the morphological residues studies and the SEM-EDX analysis provided a greater support for their chemical identification.

In this regard, animal tissue (i.e. blood, bone, muscles, lipids, collagens, etc.) has particular cellular structures but is not readily visible without proper biochemical analysis.

Nevertheless, the artefacts used in the bone working at the experimental series, recorded inorganic diagnostic elements that allow its identification. The high proportions of calcium and phosphorus identified on the residues during the bone working would be related to calcium carbonate and calcium phosphate, major components of hydroxyapatite or bone mineral.

The mature compact bone is constituted by 70% of this mineral and 30% of collagen type 1 (Lombard and Wadley, 2007). In turn, bone residues also often present periosteum, irregular connective tissue similar to the dermal and mainly composed of an organic component (protein fibers = collagen). These elements can be recognized by SEM-EDX from the high percentages of carbon and oxygen identified in the spectrum (Fig. 1e).

Moreover, the presence of calcium in the micro-polish area could be indicating that part of the worked material has been trapped in this area. However, the penetration depth of the electron beam into the rock does not exceeded 1 μm, which implies only a surface material analysis.

In the wood working, the residues identify were mainly concentrated on the use-wear and adjacent areas, this could be due to the low moisture degree of the plants processed. Other experimental studies identified that residues are more widely spread on the experimental tools used to work plants with a high moisture degree, suggesting that the water content of the contact material is a major factor that influence the residues distributions on lithic tools (Xhauflair et al., 2017).

On the other hand, the low concentrations of Na, Mg, K, Ca, Fe (< 2%) were identified and would be related to the secondary components and minerals of this material expressed in ash. In these sense, Watson and Dadswell (1962) argue that variations in the wood's mineral substances, such as calcium, phosphorus and iron, could be due to undetected moisture and to the soil fertility where the plant is grown.

In turn, the large reabsorption of phosphorus (P) and potassium (K) when the sapwood is transformed into heartwood was studied by Bamber (1976) in *E. grandis*, determining a phosphorus content of 0.0103% and 0.0007% and potassium between 0.1087% and 0.0238%. However, when heartwood is formed in E. pelularis, phosphorus can range from 0.0043% to 0.0005% and potassium from 0.494% to 0.007%.

In addition, it should be noted that plant tissues and fibers are composed of cellulose fibers embedded in hemicellulose, lignin and other polysaccharides and proteins (Blanchette, 2000). Although these plant tissues and fibers can be recognized by double wall cells, long vacuoles and organelles with chloroplasts (Devlin and Witham, 1983; Mauseth, 1988), Langejans (2010) mentions that their preservation and identification in the archaeological record at morphological level is often difficult. This is due to the micro-residues lose their characteristic coloration, taking shades of the sediment that was in contact and to the maceration process during the activity developed that makes impossible to observe microstructures (Langejans and Lombard, 2015). Therefore the SEM-EDX studies would give greater support to the results obtained at a morphological level of this micro-residues type.

On the other hand, the SEM-EDX analysis in the pigmentary elements, allowed obtaining a topographic image of the pigment grains; as well as chemical contrast images with different shades of the elements present and a chemical spectrum of these elements in the samples.

In the hide working with red paint (S9 pigment) the considerable percentages of carbon and calcium (50% each one) on the residues identified would be related to the worked material as well as the organic elements used in the production process (animal fat). However, the low concentrations of titanium (0.80%) and iron (0.3%) would correspond to other elements associated with the mineral characteristic of the pigment used.

The hematite is a mineral composed of ferric oxide that possesses traces of titanium (Ti), aluminium (Al) and manganese (Mn). Therefore, the iron presence (0.32%), would indicate that the chromatophore element would be iron oxide, which together with titanium (0.78%), would explain the darker coloration of the pigment used. In contrast, the use of the S4A pigment, of a lighter coloration than the previous one, only recorded iron in higher concentrations (4, 74%) as a chromatophore element (Fig. 6).

In turn, the high proportions of Mg, Ca and P identified in residues generated from the use of black paint were strong indicators of the organic and inorganic nature of this paint, as well as other elements identified (carbon and calcium) that would be related to the animal origin binder used.

It is important to note that although the use of SEM-EDX provides a greater support to the morphological residues studies, it also limits greater inferences and comparisons with respect to them, since we only have qualitative and elementary analysis, hindering the mineralogical characterization or quantification of the samples components. In spite of this, being a non-destructive method since the samples are not metallized for low vacuum, they can be reused and analyzed by other physical-chemical techniques that allow a better organic elements identification (FTIR, Raman, LC-MS) (Christensen, 1997; Fiore et al., 2005; Yacobaccio et al., 2008).

Finally, the problem of possible contamination on archaeological and experimental materials cannot be overlooked. In the first case, the sediment extraction in contact with the recovered archaeological material should be considered at the future (this control sample will be analyzed to verify if the residue is related to contamination with other materials), while in an experimental level, specific laboratory conditions and special handling procedures will reduce the potential contamination. However, we believe it is of fundamental importance to continue with specific studies related to this issue.

Therefore, to the results obtained in this first approach, is very important to carry out specific complementary analysis (FTIR and Raman spectroscopy, residues extraction, etc.), generating a large reference collection to apply these studies in the Patagonian archaeological record.

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