SUPERCRITICAL DRYING PROCESS IN CONSERVATION OF WATERLOGGED OSTEOLOGICAL REMAINS.

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ABSTRACT

This poster shows a supercritical drying process in conservation of waterlogged osteological remains. The specimens were collected during the 1997-2000 excavation campaigns in a Neolithic village, now submerged, located in the South-Eastern area of the Bracciano Lake in a locality called "La Marmotta" (Anguillara Sabazia, Rome, Italy). The escavation started in 1989 and it is still continuing by the Soprintendenza Speciale al Museo Nazionale Preistorico ed Etnografico "Luigi Pigorini". A first set of experiments were performed on bench-scale equipment (DIONEX 703, 32 ml volume cell) to verify the technical feasibility of the process. Then, the process was performed on bigger scale (800 and 3000 ml volume extractors). The process is divided into two steps. First, a supercritical mixture of CO_2 and methanol flows through the specimens and slowly removes the water, substituting it. Then, treatment with pure supercritical CO_2 removes the methanol residues. During the process, viscosimetric measures were performed to control the water content of the methanol "extract" collected in the separation unit: evaluating the amount of extracted water, it was possible to stop the process when the specimen was allegedly dry. The process was applied to different kind of osteological remains ("hard" and "soft") with good results in terms of water recover and drying of the archaeological artefacts.

INTRODUCTION

The archaeological context

The Neolithic settlement of "La Marmotta", formerly located on the edge of the South Eastern sector of the Bracciano Lake (Northern Latium, Italy), is now at a depth of about 8 meters under the water level and at about 350 meters from the shore [1]. The archaeological levels are sealed by about three meters of silt that preserved them from the erosive action of water and currents. The village, characterized by a series of exceptional ligneous structures, yielded a large quantity of pottery of different shapes, dimensions, and functions. In the different periods of occupation of the village, this pottery was decorated with impressions, painted motives or in the end with incisions. Of great interest are the numerous wooden objects, also composite ones and objects made of vegetal fibers that are usually not preserved in open air sites; from these objects it is possible to draw much information on the daily life and on the technical abilities of the Neolithic population of the village. Tools made of flint and obsidian, as well as ground-stone and bone artifacts are also very abundant. Some pendants and decorative elements made of wild boar canines are also present. Noteworthy is the finding and

the salvage of **two** large monoxylous pirogues, one more than 10 m long, made out of a trunk of oak, with a slightly tapering prow. The recovery of some little clay models of boats, the oldest recovered so far in Europe, could be related to cult practices. On the basis of the absolute dates and of the characteristics of the material culture, the village of La Marmotta can be considered the oldest Neolithic settlement on the shore of a lake in Western Europe. In terms of absolute calibrated chronology, so far it has been possible to recognize three different occupation phases. The oldest phase is referable to 5690 years B.C., the intermediate one is 5550/ 5450 years B.C., and the most recent one is about 5260 years B.C.. Paleobotanical remains indicate that in the village a rich agricultural economy, based on cereal and pulse cultivation, was developed; the collection of wild plants and fruits was also quite important. The faunal remains examined so far indicate that the exploitation of animal resources was based on the husbandry of four main species: cattle, sheep, goat, and pig; furthermore, hunting of medium-large and small mammals as well as of aquatic birds was constantly practiced. Occasional fishing activity is documented by some fish remains.

The conservation of the faunal remains.

The presence of a large, and still growing, sample of waterlogged faunal osteological remains, raised the problem of their conservation and preservation. The characteristic of these specimens is that they are completely waterlogged and therefore in case of rapid water loss there is, almost in all cases, a complete deformation, exfoliation, and fragmentation of the specimen, with evident problems for the subsequent scientific analysis. A literature review evidenced that the techniques employed so far on organic materials from wet environments do not seem to be satisfying as regards the speed of the treatments, the steadiness and durability of the results, the conservation of the original shape and dimensions, but mainly because such techniques tend to obliterate the legibility of the surfaces. On the basis of these considerations, the Sezione di Paleontologia del Quaternario ed Archeozoologia of the Soprintendenza Speciale al Museo Nazionale Preistorico Etnografico "L. Pigorini" started experimenting a new conservation technique based on the dehydration by means of repeated and prolonged immersions in pure acetone and subsequent consolidation [2, 3]. This process seems to produce, in most cases, satisfying results both for the structural stabilization of the skeletal elements and for the legibility of the surfaces also by optical microscopy. Only in a small sample of the treated specimens there has been exfoliation of a more or less wide surface; this occurred when before the treatment the surface layer of the bone was soft while the inner part was much harder. In order to deal with these particular cases the supercritical drying process has been tested. In fact, over the last ten years, the supercritical drying technique was studied and developed to the conservation of waterlogged archaeological remains [4,5,6].

MATERIALS AND METHODS

Reagents: methanol used for both viscosimetric calibrations and in SFE pilot plant trials was reagent grade from Baker (Mallinckrodt Baker, Deventer, Holland). Solutions of water in methanol were prepared as follows: in a 25 ml flask the volumetric amount of water required were measured using a pipet. The flask was then filled with methanol. Due to volume reduction effects and bubbling during the mixing of the two solvents, the mixture was left to rest prior the final filling.

Archaeozoological remains:

Thermal dehydration. Two main classes of initial conditions of preservation are recognized in the osteological assemblage from La Marmotta: “hard” and “soft” bones. Some of these osteological remains are chosen to determine the initial water content in different kinds of animal bones (see Table 1)

Specimen	***Species***	Anatomical element	Preservation
19129	*Bos taurus* (domestic cattle)	6th cervical vertebra	“soft”
19799	*Ovis vel Capra* (ovicaprine)	right metacarpus	“soft”
22867	*Bos taurus*	1st phalanx	“soft”
25371	*Sus scrofa dom.* (pig)	left astragalus	“soft”
19393	*Bos taurus*	left metatarsus	“hard”
20110	*Ovis aries*	left metatarsus	“hard”
20111	*Ovis vel Capra*	axis	“hard”
20200	*Sus scrofa dom.*	diaphysis of right femur	“hard”
V. 19948	*Ovis vel Capra*	upper molar	“hard”

Table 1: osteological remains for water content tests

Table 2 shows the specimens treated with supercritical drying process. Furthermore, the process also is tested on a “soft” fragment of stone (nr. 11128).

Specimen	***Species***	Anatomical element	Preservation
19250	*Bos taurus*	right humerus	“hard” and “soft”
7908	*Ovis vel Capra*	right humerus	“hard”
24434	*Small herbivore*	awl on long bone diaphysis	“hard”

Table 2: osteological remains for supercritical drying process

Thermal dehydratation of archaeozoological remains: the archaeozoological remains were taken out of their preservation water, washed superficially with acetone and then gently dried with a cotton cloth, to remove the superficial film of water. Then, they were weighted with a precision of ±0,01 g. The remains were placed in a ventilated oven at 353,15 °K for 20 hours, weighted, then the oven temperature was raised to 393,15 °K, and the remains were left to dry for another 28 hours. During this period, they have been weighted four times after 3, 6, 24 and 28 hours respectively. The trial has been stopped as, after 28 hours, all the remains didn’t lose water any longer.

Viscosimetric measures: viscosimetric measurements were carried out with an Ubbelohde viscosimeter. Flowing times were taken with a Nokia digital cronometer, that was able of measuring time with a precision of ±0,01 s. All measures have been repeated six times for each specimen, to allow a good statistical elaboration of data. Calibration measures have been carried out on water-methanol solutions, prepared as described in “Reagents” section. Measures during the Supercritical Drying Process has been carried out on the spilled

methanol, that was left two minutes to degas prior measurement. The influence of temperature on viscosity wasn't taken into account. All measures have been carried out at room temperature.

Supercritical Drying Process:

To verify the technical feasibility of the process, a first set of tests was performed on the DIONEX SFE-703 (cell volume 32 ml) equipped with a Modifier Unit; afterwards, tests on a bigger scale, were planned. Figure 1 shows the process that can be divided into two steps. The first one is a supercritical drying process and a supercritical mixture of CO_2 and methanol flows through the specimens, allocated into the extraction unit (SS), and slowly removes the water, substituting it. The mixture water/methanol was collected into the separation unit (SEP) and analyzed; the analytical procedure was repeated till the solution collected resulted pure methanol. The second one is a supercritical cleaning process consisting in a treatment with pure supercritical CO_2 with the aim to remove, from the specimens, the methanol residues. The tests were performed both on a Muller plant utilizing a 3000 ml extraction unit and a Luwar plant with a 800 ml extraction unit depending on the specimen's geometry. The operating conditions was: I° STEP – DRYING: Extraction Unit (SS): Pressure: 18 MPa; Temperature: 323 °K; CO_2 Flow rate: 20 Kg/h; Time: 2 h 40 min ; Methanol Flow rate: 1.8 Kg/h (7% weight).
Separation Unit (SEP): Pressure: 5 MPa; Temperature: 301 °K;
II° STEP – CLEANING: Extraction Unit (SS): Pressure: 20 MPa; Temperature: 323 °K; CO_2 Flow rate: 20 Kg/h; Time: 2 h;
Separation Unit (SEP): Pressure: 5 MPa; Temperature: 301 °K;

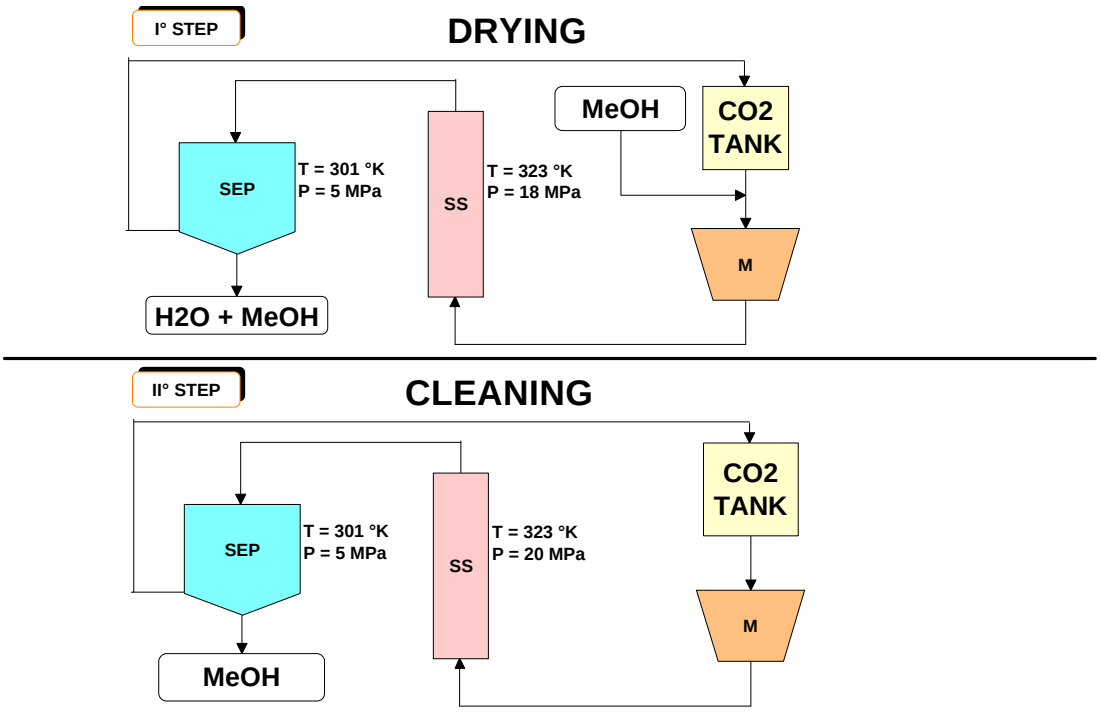

Figure 1 : Supercritical Drying Process

RESULTS AND DISCUSSION

Thermal dehydratation: this step was necessary to assess, the content in water of the osteological remains under examination. Nine different artefacts were chosen, in the hope that their variety could represent the different kinds of artefacts that would have been later dried with supercritical fluid. The weight of the remains was measured after 20, 23, 26, 44, 48 hours tillthe value was constant. From the data, it was possible to obtain the water percentage in each specimen. The results are shown in table 3:

Specimen	Starting mass (g)	Final mass (dried) (g)	Mass water fraction (%)
19129	181,31	81,71	54,93±0,01
19799	14,63	8,59	41,3±0,2
22867	19,01	8,10	57,4±0,1
25371	10,57	5,16	51,2±0,2
19393	160,98	94,78	41,12±0,02
20110	19,56	9,95	49,1±0,1
20111	27,83	13,58	51,2±0,1
20200	17,10	12.27	28,2±0,1
V.19948	3,81	2,77	27,3±0,6

Table 3: water content of osteological remains.

Viscosimetric calibration: the viscosimetric measurement was chosen for the assessment of water content in methanol. The calibration curve (flowing time vs. the content of water in water-methanol mixtures) was built in the whole composition range. The part of the curve that comprises the water content composition range of 0-30 % was approximately linear (and thus univocal). It was then interpolated with least square method, obtaining a good linearity (R=0,99871). From this linear interpolation, we obtained a formula of F_v (percentage volumetric fraction of water in methanol) vs. flowing time:

$F_v(\%)= [t(s)-16,987]/0,589$ %.

The uncertainty in this measure is expressed by the formula:

$\Delta F_v(\%)= \pm \Delta t(s)/0,589$ %.

The uncertainty on the flowing time has been statistically evaluated in every single point. Then, these values have been mediated, and Δt was found to be 0,12 s. As we had to consider both the standardization and the measure uncertainty, the global error on the measure is double that value. Thus, the uncertainty on the measure comes to be:
$\Delta F_v (\%)= \pm 0,4$ %,
and the final result for $F_v(\%)$:

F_v (%)= [(t(s)-16,987)/0,589] ± 0,4%.

Supercritical drying : two drying trials have been conducted with different supercritical plants depending on different geometry of the specimens. During these trials, viscosimetric measurements of the methanol coming out from the plant were carried out. The goal of these measurements was to assess the loss of water, so that it could be decided when to stop the mixture CO_2/Methanol flow, and to start pure CO_2 flow. Methanol was spilled from separation unit and the mixture was analyzed with viscosimeter. The results of the analysis during the two trials are reported in table 4 and 5:

Specimen: 19250 – right humerus of *Bos Taurus*							
Spilling time (min)	Volume (ml)	Water (%)	Water (g)	Total water (g)	Flowing times (sec)	Single error (g)	Total error (g)
30	31	20,18	6,25	6,25	28,89	± 0,12	± 0,12
45	103	7,01	7,22	13,47	21,12	± 0,37	± 0,49
60	153	5,63	8,61	22,08	20,31	± 0,54	± 1,03
75	257	5,45	14,00	36,08	20,20	± 0,94	± 1,97
90	235	4,43	10,41	46,49	19,60	± 0,86	± 2,83
105	660	4,35	28,69	75,18	19,55	± 2,42	± 5,25
105	150	4,48	6,72	81,90	19,63	± 0,55	± 5,80
120	100	2,50	2,50	84,4	18,46	± 0,36	± 6,16
135	370	3,02	11,18	95,58	18,77	± 1,34	± 7,50
150	300	2,91	8,73	104,31	18,61	± 1,09	± 8,59
165	210	2,72	5,70	110,01	18,59	± 0,78	± 9,37
Inf	380	1,58	6,00	116,01	17,92	± 1,39	± 10,76
Inf	245	3,21	7,86	123,87	18,88	± 0,90	± 11,66
Inf	300	4,97	14,92	138,79	19,92	± 1,08	± 12,74
Inf	59	2,82	1,66	140,45	18,65	± 0,22	± 12,96

Table 4: Supercritical drying process data

Specimen: 24434 – awl on long bone diaphysis of *Small herbivore* Specimen: 11128 – fragment of stone Specimen: 7908 – right humerus of *Ovis vel Capra*								
Spilling time (min)	Volume collected (ml)	Flowing times (s)	Water content (%)	Water content (g)	Total water (g)	Single error (g)	Total error (g)	Relat. Error (%)
15	75	25,27	14,06	10,55	10,55	±0,31	±0,31	±2,94
30	135	19,50	4,27	5,76	16,31	±0,54	±0,85	±5,17
45	135	18,47	2,52	3,40	19,71	±0,55	±1,40	±7,06
60	132	18,27	2,18	2,88	22,59	±0,54	±1,94	±8,54
80	156	17,75	1,30	2,02	24,61	±0,63	±2,57	±10,39
100	142	17,89	1,53	2,18	26,79	±0,59	±3,16	±11,74
120	107	17,56	0,973	1,04	27,83	±0,44	±3,60	±12,88
140	156	17,39	0,684	1,07	28,90	±0,64	±4,24	±14,61
160	164	17,22	0,233	NA	NA			
Residue	280	16,99	0	0	NA			

Table 5: Supercritical drying process data

At the end of both trials, dehydrated specimens were weighted to estimate water loss. Water loss estimated during the extraction with viscosimetric measurements is consistent with the weight loss found weighting the specimen after the plant is opened. Figures 2-7 show the results.

CONCLUSION

The SFE dehydration process provided favourable results for the preservation of the macroscopic characteristics of the bone remains (shape and dimension). In particular, on the specimen n. 19250, which originally presented an extremely “soft” external part (superficial film) overlaying a more compact and relatively “hard” internal portion, a good preservation of wide superficial areas , which could have been lost otherwise, has been observed. As regards the preservation of the microscopic characteristics, the comparison between manufacturing and use-wear traces observed with the SEM on replicas of specimen n. 24434 made before and after the SFE dehydration process, evidenced a worsening in the legibility of the traces. In particular it has been possible to observe a considerable loss of detail at the bottom of the traces, the complete disappearance of some of them as well as appearance of new striae probably produced by the friction of the particles suspended in the fluid.

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REFERENCES

[1] FUGAZZOLA DELPINO,M.A., D'EUGENIO,G., PESSINA,A., "La Marmotta" (Anguillara Sabazia, RM). Scavi 1989. Un abitato perilacustre di età neolitica. Bullettino di Paletnologia Italiana, 84 (n.s. II), **1993**, p.181,315.

[2] CERILLI,E., FUGAZZOLA DELPINO,M.A., TAGLIACOZZO,A., (2000) - Restauro e conservazione dei materiali ossei provenienti da scavi subacquei: l'esempio dell'insediamento neolitico di "La Marmotta" di Bracciano (RM), 2° Convegno Nazionale di Archeozoologia, Asti 14-16 novembre 1997, **2000**, p.49,57.

[3] BONDIOLI,L., CERILLI,E., FUGAZZOLA DELPINO,M.A., MACCHIARELLI,R., TAGLIACOZZO,A., Conservation and preservation of bone materials from underwater excavations: the example of the neolithic settlement of "La Marmotta" (Bracciano lake, Rome, Italy). Proceedings 2nd Intern. Congress on *“Science and Technology for the Safeguard of Cultural Heritage in the Mediterranean Basin”*. Parigi, Elsevier, **2000**, p.1169,1174.

[4] KAYE,B.,COLE-HAMILTON,D.J., ‘Hidden Dimensions, the Cultural Significance of Wetland Archaeology’, Ed.K.Bernick (Vancouver,Canada) **1998**, p.329,339

[5] KAYE,B.,COLE-HAMILTON,D.J., ‘A celebration of wood’, Ed.J.A.Spring (York,UK) **1994**, p.25-48

[6] KAYE,B.,COLE-HAMILTON,D.J., International Journal of Nautical Archaeology, Vol. 24.2, **1995**, p.147-158

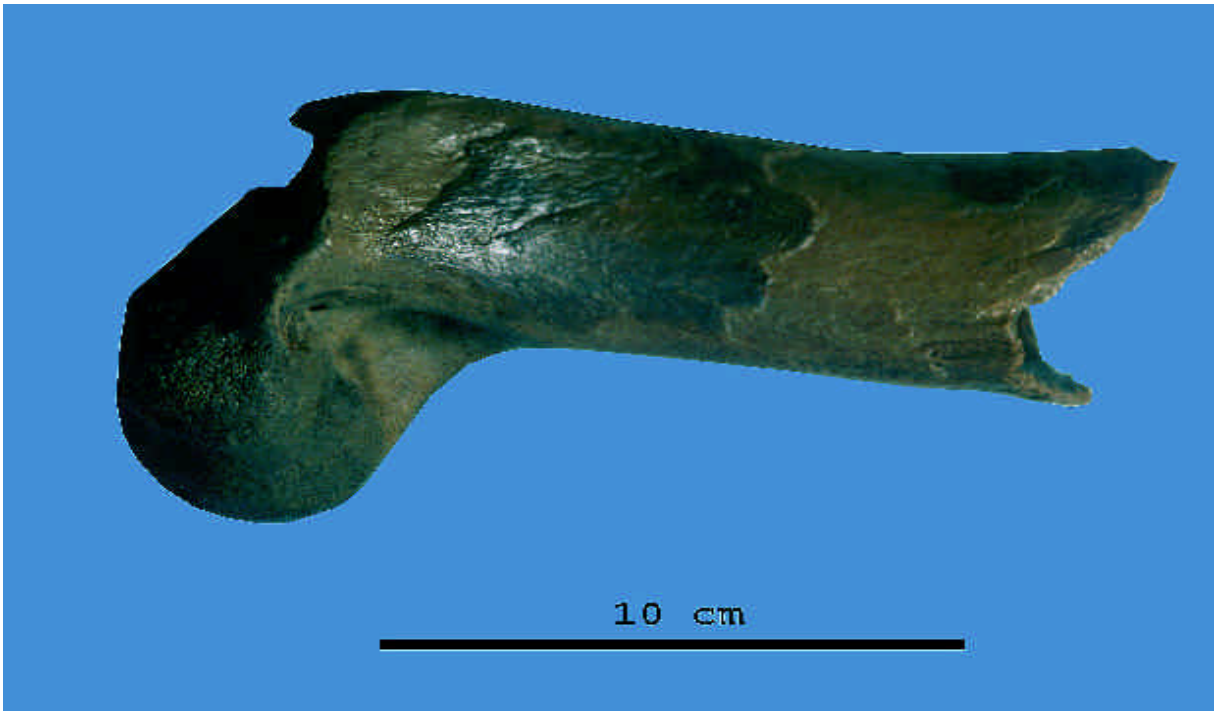

Figure 2 : La Marmotta (Anguillara Sabazia, Rome, Italy) nr. 19250.
Distal epiphysis with diaphyss of right humerus of domestic cattle (*Bos taurus*) before the supercritical fluid process.

Figure 3 : La Marmotta (Anguillara Sabazia, Rome, Italy) nr. 19250.
Distal epiphysis with diaphysis of right humerus of domestic cattle (*Bos taurus*) after the supercritical fluid process.

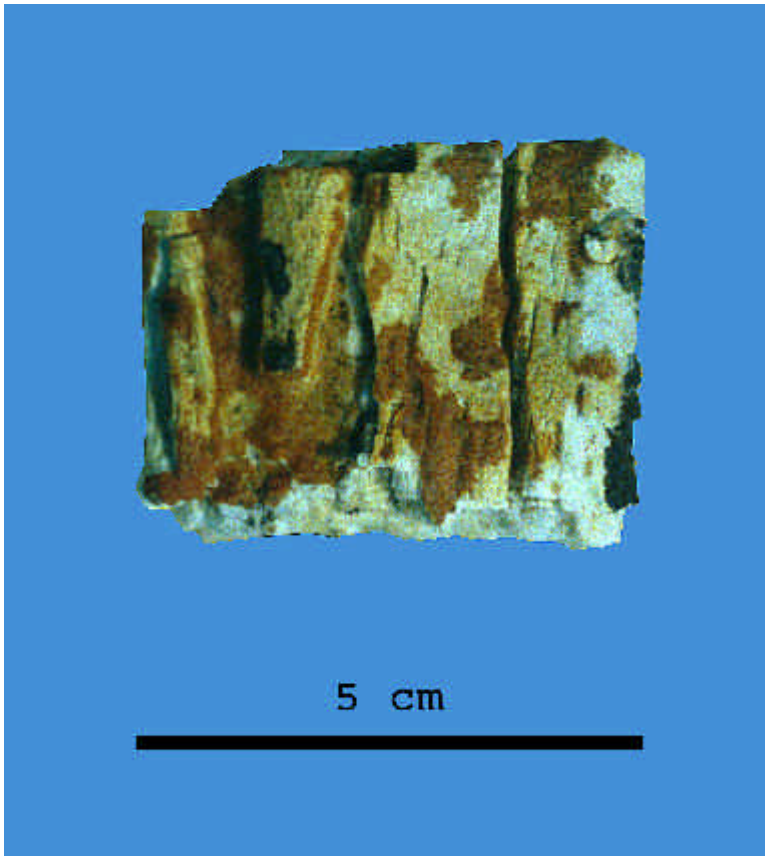

Figure 4 : La Marmotta (Anguillara Sabazia, Rome, Italy) nr. 11128.
Fragment of stone before the supercritical fluid process, extracted sample

Figure 5 : La Marmotta (Anguillara Sabazia, Rome, Italy) nr. 11128.
Fragment of stone after the supercritical fluid process

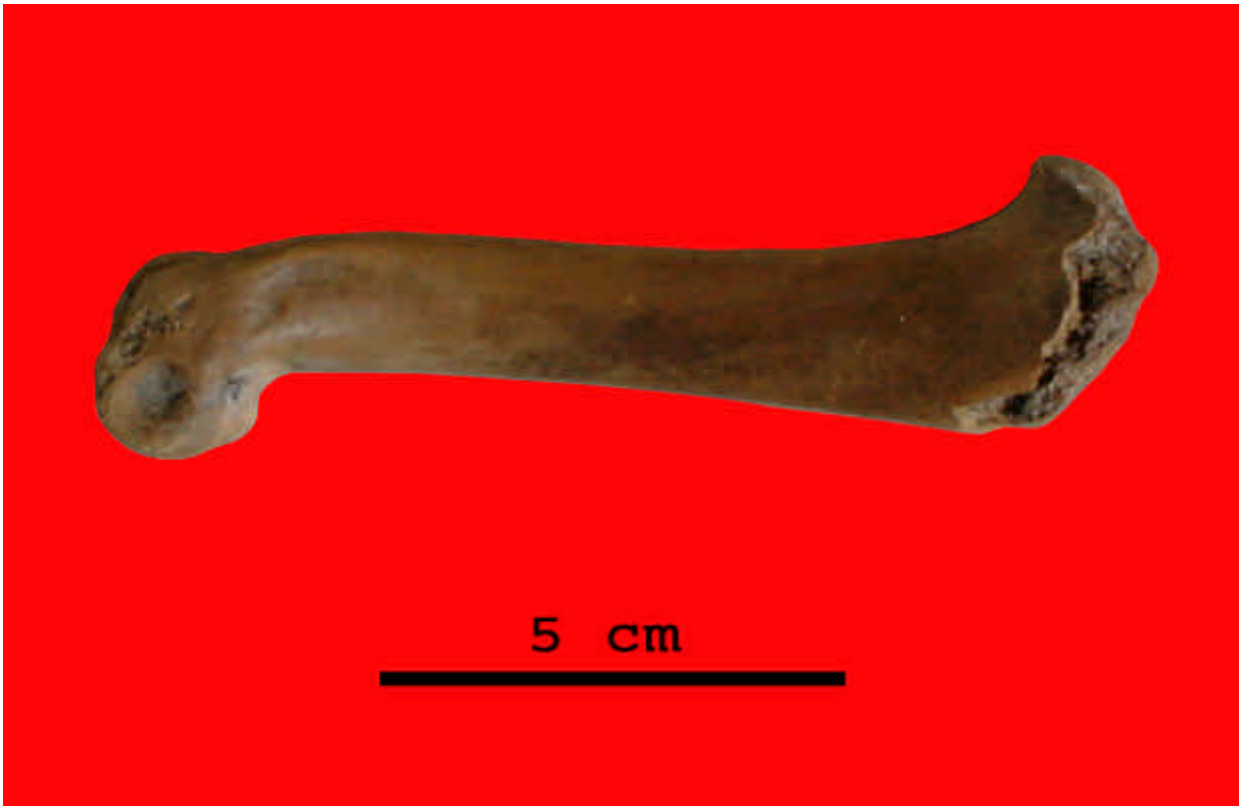

Figure 6 : La Marmotta (Anguillara Sabazia, Rome, Italy) nr. 7908.
Distal epiphysis with diaphysis of right humerus of ovicaprine (*Ovis vel Capra*) after the supercritical fluid process.

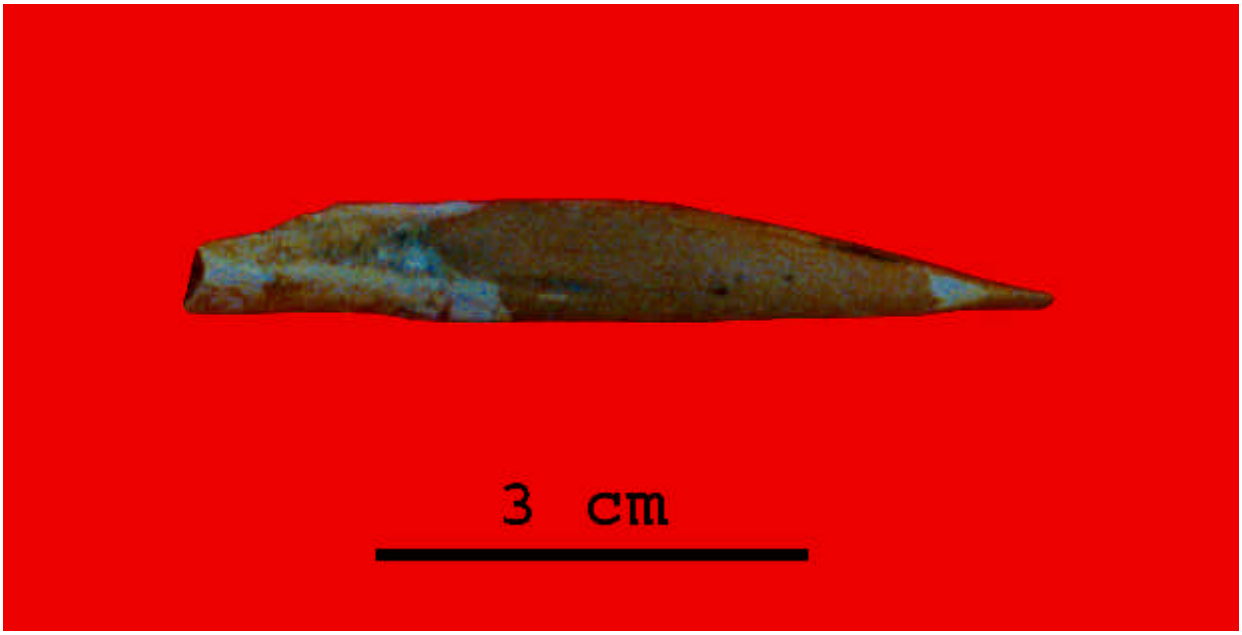

Figure 7 : La Marmotta (Anguillara Sabazia, Rome, Italy) nr. 24434.
Fragment of awl made from a portion of long bone diaphysis of small herbivore after the supercritical fluid process.