

DATING OF BIODEPOSITS OF OXALATES AT THE *ARC DE BERÀ* IN TARRAGONA, SPAIN

J Girbal¹ • J L Prada² • R Rocabayera¹ • M Argemí³

ABSTRACT. This research stems from an earlier study of the lichen covering with oxalate deposits at the *Arc de Berà* monument. The initial objective of dating these biodeposits opened up other questions concerning the structure of these deposits and how they were formed. Some dating results gave an absolute age greater than the monument itself, which posed various hypotheses on the possible ageing mechanisms.

INTRODUCTION

The origin and age of oxalate-rich surface deposits were studied and subjected to paleoenvironmental dating (Watchman 1991). These deposits form multiple layers on quartz-rich rocks. Watchman used radiocarbon dating to argue that crusts run back from the present to 8000 years ago. This shows that the processes of oxalate formation have been continuous throughout history. This dating of oxalates enabled Watchman to set up a reference system, which could establish the chronology of different styles of prehistoric painting. Later, Russ et al. (1999) dated oxalates, which they thought were of lichen origin.

The *Arc de Berà* is a Roman monument at Roda de Berà (Tarragona) built at the end of the first century BC (Dupré 1994). In recent centuries it has been restored on various occasions: in 1788, 1840, 1936 and, most recently, in 1994–1998. Documentation and research into the construction material and the weathering of the monument can be found in Amorós et al. (1994), Prada (1995), and Argemí et al. (1995). The arrangement of lichens on various parts of the monument enabled distinguishing the chronology of the previous restorations. Because of this, Watchman's research led us to posit the dating of the biodeposits of certain ashlar, which are part of the *Arc de Berà* and are thought to have different ages.

To complement the biodeposit sequence, samples were also taken from the Roman aqueduct at Tarragona, which has similar coverings.

DESCRIPTIVE BACKGROUND

Biological Materials and Covering

The material consists of carbonated rocks (Miocene-lower Serravallian) with sand-sized grains (biocalcarene) (see Amorós 1994; Prada 1995; Argemí in press). On the *Arc de Berà* there are chromatic films that correspond to pictorial coverings from different historical epochs. After the *Arc de Berà*'s construction in the Roman epoch (at the end of the first century BC), a pictorial covering was first applied with a crude wall-painting technique. Later, in 1840, the Arch was extensively remodelled and there is documented evidence that another pictorial layer was applied.

The biological alterations found on the *Arc de Berà* were examined during the restoration work in order to propose adequate treatments for them. The lichen covering is different on each facade, and less extensive on the facades facing south. The crust coverings are formed of various types of lichen

¹Botànica. Fac. Ciències, Universitat Autònoma de Barcelona. 08193 Bellaterra, Spain. Email: josep.girbal@cc.uab.es.

²ESCRBCC. Aiguablava 109-113, 08033 Barcelona, Spain

³Balmes 67, 08225 Terrassa, Spain

(Argemí in press). The most common genus is *Dirina*, which has a very thick grey-whitish thallus and chalky appearance. Due to its metabolism oxalates are formed on a calcareous substrate.

Oxalates can accumulate in the thallus and increase its thickness to 40 or 50% of its weight, which makes the observation of the hyphae difficult. When lichen dies, it leaves a floury-porous looking surface that can be re-colonized (Seaward and Giacobini 1989). *D. massiliensis* is more closely related to coastal climates than *D. stenhammari*, but both are found on vertical walls with little water provision (Nimis 1992). The two species of *Dirina* reached 70% coverage on the northwest and northeast faces of the monument, with a fairly thick layer of oxalates.

Composition and Structure of Biodeposits

Biodeposits were studied through observation of thin layers with a petrographic microscope, X-ray diffraction, HPLC, and the lichen structure were observed using scanning electron microscopy (SEM) and confocal laser microscopy. The crystal texture was also micro-analyzed by X-ray spectrometry with electron probe in SEM with analytic capacity through an energy-dispersive system (XEDS). This was completed with “Z-type Mapping” composition images of different elements and backscattered chemical contrast images (BSE).

The layer of oxalate biodeposits seen through petrographic optic microscopy with crossed Nicols has a dark grey or almost black appearance that is characteristic of isotropic minerals. With a quartz delay wedge, this microscopy shows colors of very live second-order tones, typical of minerals with very low birefringence. In some samples, at the lichen-stone contact, low birefringence and darkening are more marked. These optical characteristics correspond to weddellite, a dehydrated calcium oxalate mineral identified by XRD and the main component of the biodeposits. Because of its crystallographic structure, weddellite has very low birefringence and behaves optically almost like an isotropic material that darkens or is extinguished in crossed polars. This optical behavior of the biodeposit could be accentuated by the presence of organic non-crystalline components (see HPLC analysis).

The only mineral detected by X-ray diffraction as oxalate was weddellite (Figure 1). This is the dehydrated variety and is the main mineral and crystal component. The other minerals detected were calcite, quartz, and gypsum.

The results of the liquid chromatography (HPLC) revealed that the *Arc de Berà* had (in weight percentage) 32.36% oxalic acid content, and the Roman aqueduct 30.09%. Since X-ray diffraction told us that the mineral component was weddellite, it could be easily deduced by stoichiometric calculation that weddellite content in the *Arc de Berà* is 46%, and in the Roman aqueduct 42.78%. This indicates that, as well as the oxalates and a small amount of other inorganic minerals, there are a lot of organic compounds originating in the lichen structure and its metabolism.

To determine the amount of oxalates in the dated biodeposit and distinguish it from other organic ingredients, a total carbon and organic carbon analysis was run (see Argemí in press). The results are presented in Table 1. Since this analysis was insufficient to show the majority presence of other organic compounds, first, the organic carbon belonging to the oxalates was worked out. Then, this amount was taken away from the amount of organic carbon detected analytically in order to find the carbon that originated in other compounds, which are organic and not oxalate. The organic carbon was also taken away from the total carbon to obtain the inorganic carbon of mineral origins corresponding to minerals from the carbonates. The results show more carbon originates in other organic materials than in oxalates (Table 1). This suggests that 40 to almost 50% of this type of biodeposit are oxalates and the rest is mainly composed of other organic compounds. The carbon percentage, which was the basis for the ^{14}C dating, can also be found in these results.

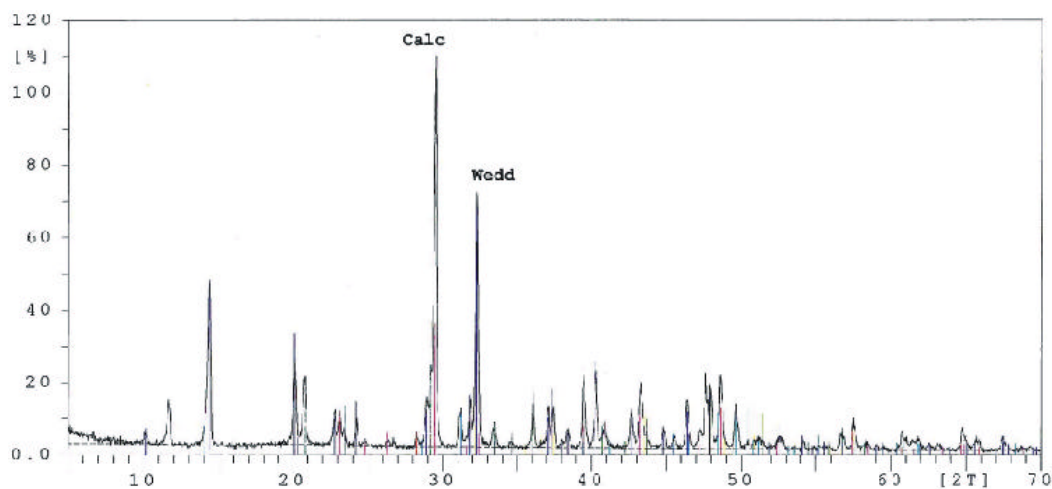


Figure 1 X-ray diffraction pattern of biodeposits

Table 1 Results of calculations to deduce the organic matter: * stoichiometrically deduced data, # analytical data, % weight percentage

Sample	Oxalic acid #	Percentage weddellite*	Total carbon #	Organic carbon #	Inorganic carbon *	Oxalate carbon *	Organic non-oxalate carbon *
Arc Berà 062107	32.36%	46%	22.42%	19.81%	2.61%	6.72%	13.09%
Aqueduct AQ-11	30.09%	42.78%	21.01%	16.35%	4.6%	6.25%	10.1%

Oxalate carbon = (percentage of carbon mass in Weddellite × weight percentage of weddellite)/ 100

Organic carbon - oxalate carbon = organic non-oxalate carbon

Total carbon - organic carbon = inorganic carbon (carbonates)

The samples studied under the electron microscope showed the structure of a crustose lichen to be a relatively thin upper cortex composed of overlapping hyphae. Following this is a somewhat thicker algal area, in which the algae are sometimes surrounded by haustoria. The medulla is formed by hyphae, some of which are smooth and most of which are full of oxalates (see Argemí, in press). The texture of the biodeposit is heterogeneous, without any defined horizontal stratification or concentric structure. Three types of areas can be seen: one without oxalates and with bare hyphae in irregular vertical bands, another area with hyphae completely covered with crystals, and still other with massive deposits, in which hyphae could not be seen (Figure 2). There are also texture differences in the size of the grain, their morphology, and crystalline habit. These differences did not match distribution distinguished according to the biodeposit closeness to the substrate or to the lichen cortex, but rather were spread in patches. Other samples were more mineralized, with denser and more compact deposits. What is mainly seen are huge areas where mineralization is very intense and there is an irregular granular aggregate with remains of hyphae totally covered (Argemí in press).

The stone-oxalate contact has an irregular, winding, and penetrating profile. Some mineral grains from the carbonated substrate look dissolved or corroded (Figure 3). The hyphae penetrating into the substrate were observed individually: some of them were filled with oxalates and some were clean.

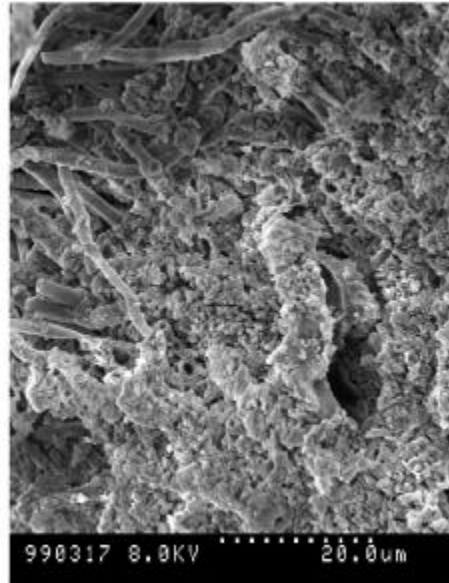


Figure 2 Massive deposits of oxalate crystals and bare hyphae

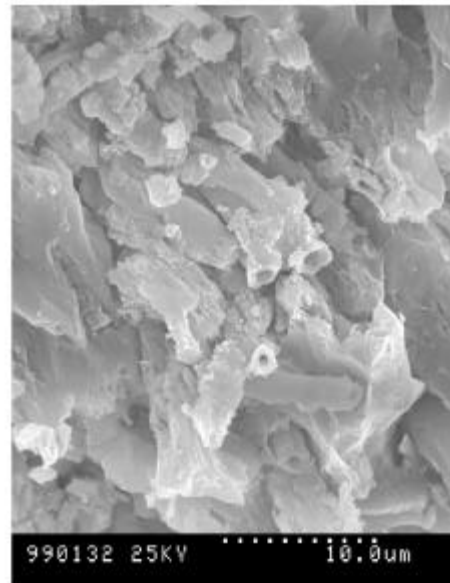


Figure 3 Stone-oxalate contact with substrate corroded

RESULTS

It was uncertain whether the position of the ashlar with the Roman inscription on the NE face of the monument was original, i.e. from the Roman era, or had been changed during the 1840 restoration. These ashlar had a lichen covering linked to the oxalate deposits. To resolve the position and restate certain ashlar after the 1840 restoration, a total of seven oxalate samples were dated. Each sample was collected from a different ashlar. Samples 062093, 062094, and 062095 were taken from the outer faces of ashlar. The other four samples, 062096, 062097, 062098, and 062099 (corresponding to the inscription) were taken from the lower horizontal faces at a distance of about 3 cm from the

edge of the ashlar to exclude any possibility that they were handled during recent restorations. According to the archaeological information, ashlar I12C (062095) is undoubtedly Roman. Ashlar C32B (062093) and I121 (062094) were undoubtedly created or reworked during the 1840 restoration. The task with the remaining four (those of the Roman inscription) was to check whether they were in their original position or if they were adjusted by having their lower face reduced or chipped.

At the time the samples were collected the outer part of the lichen had already been removed by the restoration cleaning process (1994–1998). Therefore, the oxalate samples came from the medulla or an indeterminate area of oxalate accumulation. The samples were extracted with care in order to avoid sampling the carbonated rock from the substrate or pointing mortar. Extraction from the cortex minimized the possibility of choosing air-polluted particles.

The samples were sent for ^{14}C dating at the Research Laboratory for Archaeology and the History of Art in Oxford. Chemical pretreatment (personal communication with R Hedges of this laboratory) was as follows: 1) the samples were treated with 0.5 M NaOH to remove soluble organic material such as humic acids, 2) the insoluble residue was treated with excess 1M nitric acid, first, to destroy carbonates, and secondly, to dissolve calcium oxalate, 3) the filtrate was then brought back to neutrality by adding calcium hydroxide, precipitating calcium oxalate, and 4) the precipitate was washed in alkali, then rapidly in dilute hydrochloric acid and was combusted in a continuous-flow CHN analyzer while still sufficiently acidified to avoid contamination by absorption of atmospheric carbon dioxide (Hedges 1998).

The results of ^{14}C dating gave four samples belonging to the modern period and three to ancient ones with different absolute ages. Samples taken from two ashlar belonging to the 1840 restoration (C32B-062093, I121-062094) gave the ^{14}C activity that corresponds to the present time, while the sample taken from the ashlar of Roman origin (I12C-062095) gave an age of 3505 years. Two of the four ashlar of the inscription gave a modern age; of the other two, one is 365 years old, the other is 3880 years old (see Table 2).

Table 2 The dates are expressed as uncalibrated ^{14}C years BP (before present-AD 1950) and based on the half life of 5568 years. The $\delta^{13}\text{C}$ dates in ‰ relate to PDB standard

Sample	Ashlar	$\delta^{13}\text{C}$ (‰ PDB)	Dating	Supposed age
062093	C32B	-23.9	119% modern	1840
062094	I121	-25.9	108% modern	1840
062095	I12C	-18.2	3505 ± 70	1st century
062096	C32D	-29.0	112% modern	1st century?
062097	C32C	-23.1	110% modern	1st century?
062098	C32F	-28.3	365 ± 70	1st century?
062099	C32E	-28.6	3880 ± 70	1st century?

The $\delta^{13}\text{C}$ of the samples had values between -18.2 and -29.0‰ vs. PDB standard (see Table 2). These figures are consistent with biological origin (Gupta and Polach 1985) and also consistent with the oxalate values measured by Watchman (personal communication) of $\delta^{13}\text{C}$ between -21 and -23‰.

It can be seen that the results of dating show unexpected divergences from the ages estimated in the archaeological and architectural studies. ^{14}C results show that the ages of some biodeposits are more

modern than the date of the last restoration and the ages of other biodeposits are older than the monument itself.

DISCUSSION

The type of activity and growth of the lichens explains why, for various reasons, some samples give modern dates:

In the case of late growths, lichens have grown on ashlar at any period in the monument's history. As such, the process of oxalate formation does not have to coincide with the age of the block—modern deposits can occur. The activity and growth mechanisms of the lichens mentioned explain the more modern dating of samples 062093, 062094, 062096, and 062097.

In addition, recolonization can occur, causing mixtures of deposits of different ages. Unlike the oxalate deposits described by other authors (Watchman 1991), these biodeposits are not laminar but heterogeneous. Recolonization can also take place when spaces left by the loosening of old biodeposits fill up. Dating results for sample 062098 can be explained with either a mixture of deposits of different ages or lichen growth that is the same as the ashlar date found.

The excessive ageing of samples 062095 and 062099 cannot be due to contamination with geological calcium carbonate during the process of sample extraction because a 20%-contamination by this carbonate would be needed to substantially age the sample in order to match dating results (R Hedges, personal communication). Chemical pretreatment, however, eliminated the calcium carbonate, and the $\delta^{13}\text{C}$ data also discounted possible substrate pollution at the collection of the sample since calcium carbonate has a $\delta^{13}\text{C} \geq 0$.

Other sources of ancient carbon, such as old construction materials, conservation treatments, and particles of external origin, were considered. However, the chemical treatments certainly eliminated all these, and so they could not have modified the dating.

After discounting possible sources of ageing, we thought that a biochemical process characteristic of lichen was more likely. Lichens incorporate a certain amount of dead carbon in the form of CO_2 and HCO_3^- through photosynthesis from the algal ingredient. Incorporated into the lichen metabolism, it generates calcium oxalate as its end product. Since this carbon does not contain active ^{14}C , the proportion of ^{14}C in the sample's oxalate is reduced. The incorporation of these compounds can be established through the study of aquatic bryophytes and algae (Peñuelas 1985; Mackereth 1978).

Dead carbon assimilated by lichens may originate from (Figure 4):

a) Formation of oxalates through carbon contributions from air pollution caused by burning fossil fuels (automobile hydrocarbons)—the lichens take in atmospheric pollutants through particles, aerosols, and CO_2 (Saiz-Jiménez 1995).

b) Acid dissolution of the calcareous substrate. This chemical process can occur in two ways: 1) through formation of oxalates from carbon originating in the substrate that is chemically altered by the lichen. The lichen attack causes mechanisms of dissolution (calcium carbonate) and new formation (oxalate) through a complex process of biogeochemical division (Gorgoni 1992; Dutton 1996). Oxalic acid attacks the carbonates of the stone chemically, which leads to the release of CO_2 that is absorbed by lichen in a process similar to that mentioned above. The carbon of the oxalate produced comes from an ancient geological source and does not contain ^{14}C . In addition, the ^{14}C content in the sample oxalate would diminish, 2) another mechanism of calcareous substrate acid dissolution is the

action of carbonic acid coming from rainwater and runoff that also releases CO_2 from the substrate (Caner et al. 1979; Livingstone 1992).

Following the criteria of Dutton et al. (1996) on the metabolism of oxalic acid in fungal cells, we think that the formation of new oxalates by reuse of earlier biodeposits is not very likely.

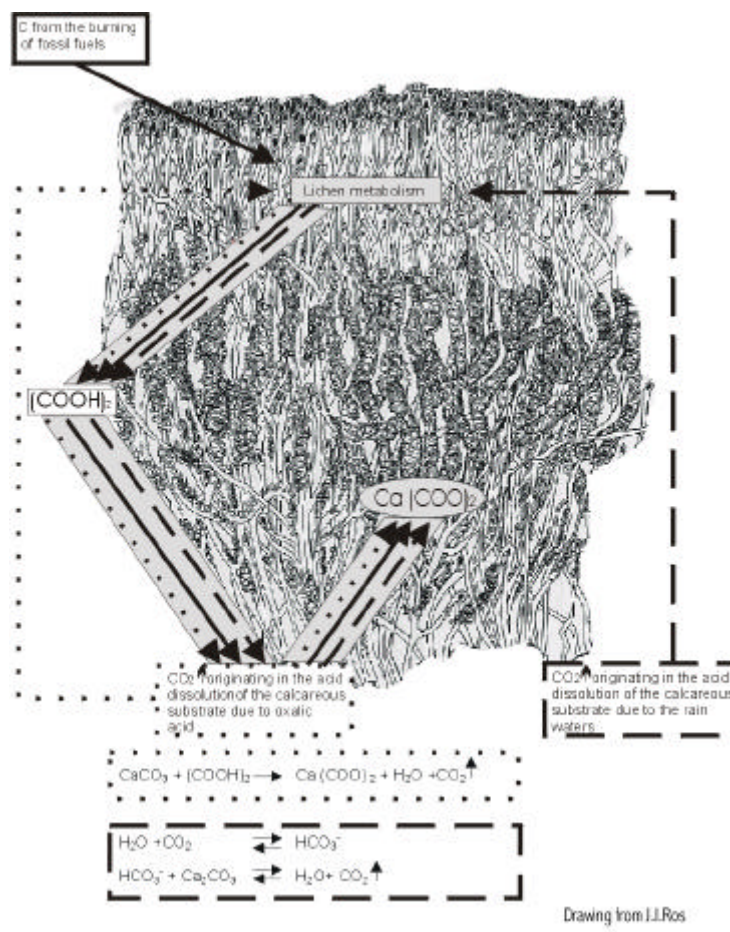


Figure 4 Possible origins of dead carbon

In conclusion, the use of oxalate biodeposits of lichen origin on carbonated substrate as indicators of ^{14}C age is complex. They may be used for comparative dating, but not for absolute dating. It is clear that the question is difficult when carbonated substrates (limestone, dolomites, or sandstone with carbonated cement) are examined, since the assimilation of dead carbon from the substrate causes ageing of the sample. This ageing is not easy to quantify, either to discount it or to correct an apparent age. Nevertheless, it may indicate the deterioration of the ashlar through chemical changes caused by lichen. An alteration coefficient for the substrate could possibly be obtained from this ageing. This assimilation of carbon from the substrate also happens on whitewashed and roughcast surfaces or lime mortars, which makes it even more complicated to establish the result, although the lime of the mortar takes in CO_2 from the air during its hardening process.

ACKNOWLEDGMENTS

The authors are grateful to Alan Watchman and Bogomil Obelic for helpful discussions and criticisms. We thank the Ministry of Public Works, Transport, and the Environment, and its team in charge of restoring the *Arc de Berà*: Jaume Costa i Pallejà, an architect, and Xavier Dupré i Raventós, an archaeologist.

REFERENCES

- Amarós M, Dupré X, Escarré V, Prada JL, Rocabayera R, Valenciano A. 1994. Degradation forms and weathering mechanisms in the Berà Arch (Tarragona, Spain) III *International Symposium on the Conservation of Monuments in the Mediterranean Basin*:673–79.
- Argemi M, Girbal J, Prada JL, Rocabayera R. (In press). Biodeposits of oxalates on the Arc de Berà (Tarragona): their morphology, composition and dating. *5th International Symposium on the Conservation of Monuments in the Mediterranean Basin*. Sevilla, 2000.
- Argemi M, Girbal J, Prada JL, Rocabayera R, Vilaseca L. SAXUM. 1995 *Estudi de l'estat de conservació i proposta de tractament de l'arc de Berà*. Technical report.
- Caner EN, Seeley NJ. 1979. Dissolution and precipitation of limestone. *3rd International Congress on Deterioration and Conservation of Stone*. Venezia:106–29.
- Dupré X. 1994. *L'arc romà de Berà (Hispania Citerior)*. Barcelona Institut d'Estudis Catalans i CSIC: 321p.
- Dutton MV, Evans CS. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Canadian Journal of Microbiology* 42: 881–95.
- Füredi-Milhofer H, Tunik L, Filiponvic-Vincekovic N, Skrtic D, Bavic-Ivancic V, Garti N. 1995. Induction of crystallization of calcium oxalate dihydrate in micellar solutions of anionic surfactants. *Scanning Microscopy* 9(4):1061–9.
- Gorgoni C, Lazzarini L, Salvadori O. 1992. Minerogeochemical transformations induced by lichens in the biocalcarene of the selinuntine monuments. *7th International Congress on Deterioration and Conservation of Stone*, Lisboa:531–9.
- Gupta SK, Polach HA. 1985. Radiocarbon dating practices at AUN. Handbook. Canberra: Radiocarbon Laboratory-AUN.
- Hedges REM, Bronk Ramsey C, van Klinken GJ, Pettitt PB, Nielsen-Marsh C, Etchegoyen A, Niello J, Boschin MT, Llamazares AM. 1998. *Radiocarbon* 40(1):35–44.
- Livingstone RA. 1992. Graphical methods for examining the effects of acid rain and sulfur dioxide on carbonate stone. *7th International Congress on Deterioration and Conservation of Stone*. Lisboa:375–86.
- Mackereth FJH, Heron J, Talling JF. 1978. Water analysis: some revised methods for limnologists. *Freshwater Biological Association scientific publication* 36: 34–53.
- Nimis PL, Pinna D, Salvadori O. 1992. *Lichene e conservazione dei monumenti*. Bologna: CLUEB. 165 p.
- Peñuelas J. 1985. HCO₃⁻ as an exogenous carbon source for aquatic bryophytes *Fontinalis antipyretica* and *Fissidens grandifrons*. *Journal of Experimental Botany* 36(164):441–8.
- Prada JL. 1995. *Caracterización de formas y procesos de alteración, observadas en piedra de construcción de edad miocénica del área monumental de Tarragona*. Doctoral thesis. Facultad de Geología, University of Barcelona.
- Quaessima R, Di Giuseppe E. 1996. Analysis of oxalate films by means of HPLC. *II International Symposium. The oxalate films in the conservation of works of art*. Milan:391–406.
- Russ J, Kaluarachchi WD, Drummond L, Edwards HGM. 1999. The nature of whewellite-rich rock crust associated with pictographs in southwestern Texas. *Studies in Conservation* 44:91–103.
- Saiz-Jimenez C. 1995. Deposition of anthropogenic compounds on monuments and their effect on airborne microorganisms. *Aereobiologia* 11:161–175.
- Seaward MRD, Giacobini C. 1989. Oxalate encrustation by the lichen *Dirina massiliensis forma soredata* and its role in the deterioration of works of art. *La pellicola ad ossalati origine e significato nella conservazione delle opere d'arte*:215–19.
- Watchman AL. 1991. Age and composition of oxalate-rich crust in the Northern Territory, Australia. *Studies in Conservation* 36:24–31.
- Wilson MJ. 1995. Interactions between lichens and rocks; a review. *Cryptogamic Botany* 5:299–305.

APPENDIX

Apparatus:

Philips X'Pert fitted with Cu tube and graphite secondary monochromator. K_{α} radiation of the Cu: 15419Å. Diffraction service, Universitat Autònoma de Barcelona.

Hitachi S-570 scanning electron microscope. Electronic microscopy service, Universitat Autònoma de Barcelona.

Jeol JSM-6300 scanning electron microscope equipped with an energy dispersive spectrometer XEDS Linkis-300 with 138 eV resolution (boro-uranium). Electronic microscopy service, Universitat Autònoma de Barcelona.

Leica TCS 4D Laser Arg-Kr confocal laser optical microscope. Electronic microscopy service, Universitat Autònoma de Barcelona.

AMS 2.5 Tandem. The Oxford Radiocarbon Accelerator Unit (ORAU), General Iomex Corporation.

The chromatographic analysis used an HPLC instrument consisting of a Pharmacia 2148 pump (Amersham Pharmacia, Uppsala, Sweden), a Rheodyne 7125 injector (Cotati, CA, USA) and a Kontron 720-1c detector (Bio-tek Kontron, Milan, Italy). It was fitted with an Aminex HPX87H column (Bio-Rad). 4mM sulfuric acid was used as elution. Work conditions: isocratic, at room temperature. Injection: 100 µL. Detector: at 214 nm, 2aufs. Data acquisition: Merck-Hitachi d-2520 integrator. Standard: oxalic acid ACS (Sigma), dried in the phosphorous void for 24 hr. Calibration straight at 6 points between 15 and 150 ppm. *Serveis Científic-Tècnics* of the University of Barcelona.