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Ca-OXALATE FILMS AND MICROBIOLOGICAL INVESTIGATIONS OF THE INFLUENCE OF ANCIENT PIGMENTS ON THE GROWTH OF LICHENS
Thermogravimetric/Thermomicroscopic analyses

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Abstract

Simultaneous thermogravimetry and thermomicroscopy were performed on Ca-oxalates which play an important role in the appearance of oxalate films connected with lichens superficially growing on human artefacts. Ca-oxalate exists in two well-described modifications: as the more stable monoclinic monohydrate whewellite and the less stable tetragonal dihydrate weddelite. Weddelite serves for lichens as a water absorbing and accumulating substrate which transforms to whewellite when humidity drops. To follow these morphological changes optically and the water loss gravimetrically at the same time, a combination of thermomicroscopy and thermogravimetric analysis was constructed. The reflection light microscope was connected to a video camera and computer so that the morphological changes and the thermogravimetric curves could be shown simultaneously on the screen as function of time and temperature.

Lichens – double organisms composed of algae and fungi – form surface layers not only on natural organic and inorganic material but also on human artefacts. In calcareous artefacts such as the famous Chinese terracotta soldiers or Egyptian epigraphs they lead to a destruction of the surface by forming Ca-oxalate layers and thus to a deterioration of the historian work of art. But in places where the surface is covered by some blue colours (Egyptian and Chinese Blue, Chinese Purple) the growth of lichens is inhibited and the artefacts are well preserved. The copper ion contained in the pigments is responsible for this effect since copper is a strong poison for microorganism.

As lichens exhibit an extremely slow growth under natural and laboratory conditions the two lichen components: algae and fungi were investigated separately. The three mentioned ancient pigments have very low solubility products and thus do not act on the quickly growing fungi. But under special experimental conditions Egyptian Blue formed clear halos on the growth plates of the algae *Chlorella minutissima* and *Trebouxia glomerata*. These halos were compared with those of the easily soluble copper sulphate as a standard.

Keywords: algae, copper, fungi, human artifacts, lichens, oxalate films

Introduction

Modern physical techniques and special combinations of different methods allow for new approaches to questions of archaeology and preservation. This is of ut-

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most importance since ancient human artifacts of irreplaceable value not only suffer under the influence of inanimate factors like air pollution or acid rain but to a similar degree under the deteriorating effects of living organisms, especially of lichens.

During the investigation of colour layers of the bust of Nefertete it was seen that blue surfaces were firmly attached to the artefact while the surface stability was strongly reduced in other areas of the bust. In these parts a significant contamination with lichens was observed while the blue zones were free of them. Similar observations with the Chinese Terracotta Soldiers, the Papyrus Berolina and the Papyrus Ebers (both Berlin) led to the conclusion that copper containing blue pigments are responsible for these protecting effects [1]. This idea stimulated an intensive research into ancient pigments on the one hand side and lichens and their components – fungi and algae – on the other.

Three copper containing pigments were chosen for the investigations: Egyptian Blue, $CaCuSi_4O_{10}$, Chinese Blue, $BaCuSi_4O_{10}$, and Chinese Purple, $BaCuSi_2O_6$, as it was supposed that copper was the essential element in their protecting action. Copper is intensively applied as protectant in vini- and agriculture. It acts as a potent cell poison which changes the structure of proteins, blocks the oxydative phosphorylation or alters the energy metabolism and growth of microorganisms. The cited ancient pigments possess an extremely low solubility in water with a strongly retarded poisoning action (Egyptian Blue: $3\cdot10^{-7}$ mol l^{-1}; Chinese Blue:$<4\cdot10^{-8}$ mol l^{-1}; Chinese Purple:$<6\cdot10^{-8}$ mol l^{-1}). Therefore, Blue Vitriol, $CuSO_4\cdot5H_2O$, was included in the investigation as a sort of easily soluble "standard" (1.27 mol l^{-1}).

Lichens are the main cause of biological deteriorating effects on human artifacts due to formation of calcium oxalate thallus/substrate interfaces [2]. They show a very slow growth and are difficult and tedious laboratory objects, nearly never kept in culture. They are double organisms composed in a symbiotic manner of an algal and a fungal component. These components can be cultivated as pure strains, which grow by far faster under usual experimental conditions. Therefore, we decided to investigate some typical represents of the lichen partners separately. For this end, four algae (*Chlorella saccharophila ellipsoidea, C. minutissima, Trebouxia glomerata, T. gigantea*) and four fungi (*Aspergillus niger, Exophiala jeanselmei, Penicillium oxalicum, P. purpurogenum*) were included in the copper investigations. Three lichens (*Aspicilia sp., Cetraria pinastri, Xanthoria elegans*) grown on different rocks were directly analysed by X-ray diffraction, combined thermogravimetry/mass spectrometry, combined thermogravimetry/thermomicroscopy and optical and scanning microscopy.

Methods and materials

Based on the phenomena observed at the mentioned ancient artifacts a detailed study of the processes initiated by lichens was undertaken. At first, thermogravimetric/mass spectroscopic (Fig. 1) as well as simultaneous thermogravimetric/microscopic studies (Fig. 2) were performed on Ca-oxalate crystals which are frequently found in nature: in kidney stones, plant cells, different animals and as

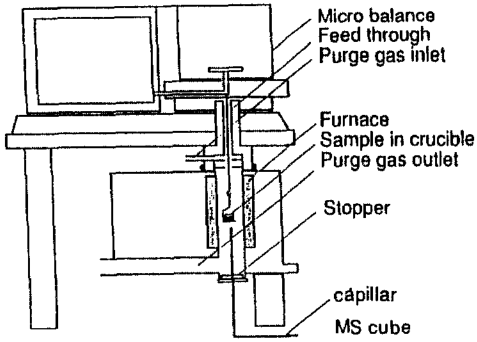

Fig. 1 Thermobalance with capillary connection to a mass spectrometer cube (Balzers)

oxalate films connected with lichens superficially growing on human artifacts. Such oxalate films could be detected by thermogravimetric surface analysis of historical artifacts such as the bust of Nefertete, the Terracotta Soldiers from China, ancient Egyptian papyri or Chinese Magao Grottoes Freschi [3].The oxalate films exist in two well-described modifications: as the more stable monoclinic monohydrate whewellite ($Ca(C_2O_4)\cdot H_2O$) and the less stable tetragonal dihydrate weddelite ($Ca(C_2O_4)\cdot 2H_2O$). In nature both oxalates can be transformed into each other by hydration and dehydration indicating the prevailing humidity conditions. Weddelite serves in lichens as a water absorbing and accumulating substrate which changes to whewellite when humidity drops. Under high humidity the dihydrate forms and in a second step the dissolution leads to the liberation of oxalate ions. The deterioration of artifacts is decisively influenced by these reactions as the binding layer of calcium carbonate ($CaCO_3$) is dissolved and the pigment surface easily falls off the work of art.

To follow these morphological changes and the corresponding water loss optically and gravimetrically at the same time, a microscopic balance was developed of highest sensitivity [3]. Its sample holder in the hot stage is attached to the balance by an aluminium oxide capillary (Fig. 2). The sample is observed during heating or cooling procedures by means of a reflected light microscope connected to a stand-

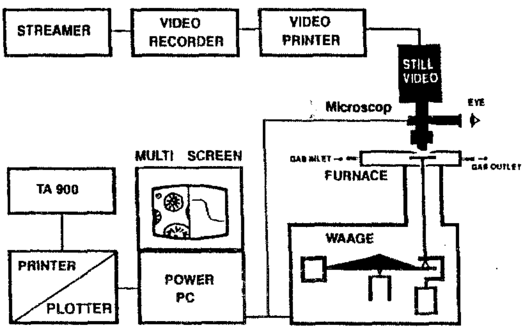

Fig. 2 Scheme of instrumental setup of hot stage microscopy with simultaneous thermogravimetry

ard camera or still video camera plus computer for the documentation of the changes in the sample. The thermogravimetric curves can be superimposed on the morphological picture and shown simultaneously on the screen as a function of time and temperature. Various gas environments at flow rates up to 5 cm^3 min^{-1} could be used in this setup with heating rates of 0.1 to 10°C min^{-1} in a temperature range between 20 and 370°C and sample masses between 0.1 and 20 mg. The same peripheral equipment as for the simultaneous thermomicroscopy/DSC is used for processing, storage and documentation of the data as described earlier [4].

Fungi and algae were cultivated on recommended standard media (plain or standard soil medium for fungi, soil or MBB medium for algae [5]) in petri dishes at temperatures between 4 and 24°C. After inoculation with cells from precultures the four copper compounds were strewn as a powder in small spots on the solid growth medium. Fungi cultures could be kept in the dark while petri dishes with algae were illuminated with 0.5 to 10 klux (10 to 200 Micro-Einstein m^{-2} s^{-1}). As sign of growth inhibition due to copper we looked for clear (cell free) halos around the powder spots.

Results

The thermogravimetric approach to detect oxalate films on artifacts with its extreme high sensitivity of 10^{-8} g was even there successful where light microscopy failed to trace oxalate layers. The fact that no such layers could be found on nearby surfaces painted with copper containing pigments stimulated the present investigations on the influence of these pigments on lichens, fungi and algae.

Thermogravimetric curves of the red lichen *Xanthoria elegans* (Fig. 3) rendered four discrete peaks in the temperature range from ambient to 800°C partly connected with decomposition of oxalate: loss of bound water from the plant (around 120°C), dramatic weight loss due to the destruction of the lichen (around 310°C), loss of CO from oxalate (around 450°C) and loss of carbon dioxide from carbonate (around 650°C). These peaks could also be observed clearly by the simultaneous mass spectrometry [3].

Although many plants and among them especially lichens are known to accumulate and tolerate high concentrations of heavy metals [6], lichens are used in their normal habitat to monitor environmental air pollution in a simple and effective way since more than hundred years. Growth retardation or inhibition are clearly seen in places with high contaminations by sulphur dioxide or heavy metals [7].

In all cases, the algal component in the lichen is the sensitive part [8]. As expected from such microbiological reasoning the algal cultures were by far more sensitive towards copper than the fungi. This is because copper in the algal cell acts on chlorophyll molecules which are lacking in the fungi. The latter are highly resistant against various poisons and thus play a minor role in the inhibition of lichen's growth and metabolism. Because of this the main investigations concentrated on the four chosen algae strains.

Nevertheless, all four fungi were tested and reacted with suppression of growth in a distinct halo around the Blue Vitriol spot (Fig. 4). Chinese Blue could show

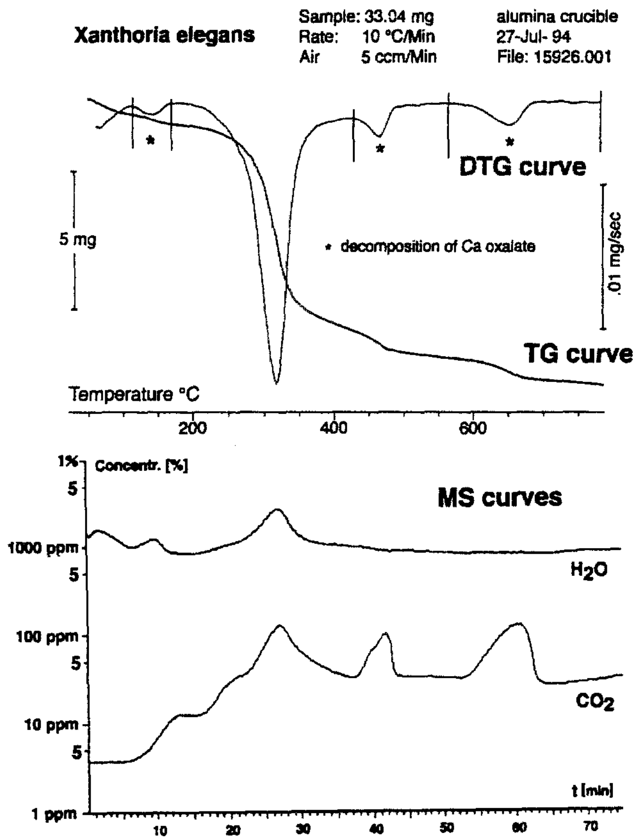

Fig. 3 Thermogravimetric (TG), derivative thermogravimetric (DTG) and mass spectrometric (MS) curves of Ca-oxalate containing lichen *Xanthoria elegans*

some faint influences, if fungal growth was retarded by lower experimental temperatures. Chinese Purple and Egyptian Blue had nearly no effects on the four fungi. After a few days all strains had over-grown the powder spots. These results may be explained by the very low solubilities and thus small diffusion coefficients of the ancient pigments and the high fungal growth rates.

Algal growth is much slower under the chosen experimental conditions so that copper has a better chance to act on the metabolism. *Chlorella minutissima* showed distinct halos around Egyptian Blue and also – but less obvious – with the other pigments. *Trebouxia gigantea* and *Trebouxia glomerata* rendered similar results (Fig. 5). In all cases these halos were compared with those of the easily soluble copper sulfate as a standard.

One way to monitor the physiological state of lichens and especially that of the algal component in them is (micro)fluorometry [8]. As seen under the microscope or even with the naked eye lichens show a strong dark mid-red fluorescence under blue or UV excitation light and change this colour over brown to white when de-

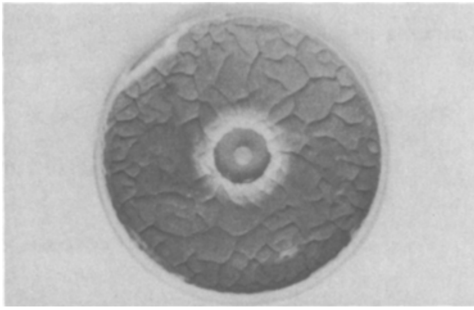

Fig. 4 Clear zone without fungal growth around a (light grey) circular piece of filter paper soaked with Blue Vitriol on a petri dish with a lawn of *Penicillium purpurogenum*

stroyed by exogeneous effects [8, 9]. We applied this method for the algal cultures and observed the described dark red fluorescence for the healthy regions on a petri dish and a brown to black spot where the pigments were active. Excitation wavelength was 366 nm in most cases, sometimes together with 254 nm. Thus, fluorescence offers a simple and quick method to monitor poisoning effects by copper even in cases where no distinct halos are detectable.

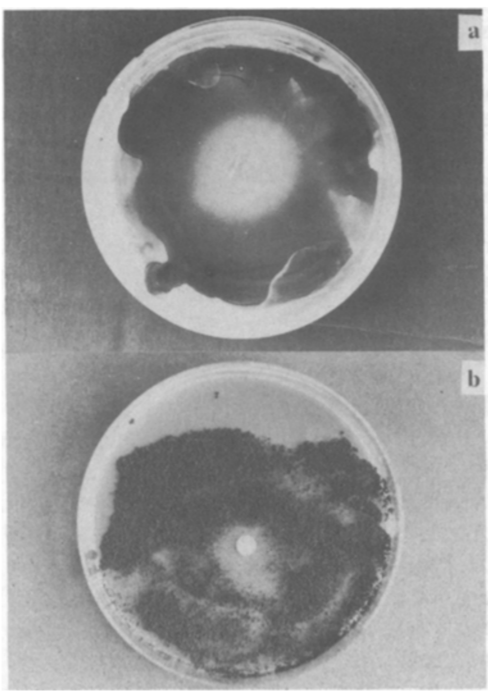

Fig. 5 Clear zone without algal growth around a) a diffuse spot of Egyptian Blue with a culture of *Chlorella minutissima*, b) around a (white) circular piece of filter paper soaked with Blue Vitriol on a petri dish with a culture of *Trebouxia glomerata*

The preliminary results shown above point in the supposed direction. When organisms are growing at very slow rates like lichens in common or algae and fungi under adverse conditions they are exposed to higher copper concentrations near the three chosen colours. Significant antimicrobial effects and thus a protection of the underlying substrate can be expected even when the solubility products are as low as those of the three ancient pigments.

Further investigations with other fungi and algae are now on the way. Special attention will be paid to copper compounds of different solubility products to connect the halo sizes with the change in solubility of such compounds.

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