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Abstract
The effectiveness and the mechanism of copper stain removal from stones by agar gels was systematically studied using marble laboratory specimens, stained and cleaned in well-controlled and reproducible conditions. The same cleaning procedure was also applied on the marble base of Napoleon's statue by A. Canova. The water release from agar gels to stones was investigated by capillarity absorption and unilateral Nuclear Magnetic Resonance. The cleaning by different agar gel formulations (pure and added with chemicals) was studied both on the stone substrate (optical microscopy and colour measurements) and in the gels (inductively coupled plasma-atomic emission spectrometry, electron paramagnetic resonance spectroscopy). Among the considered cleaning systems, the most effective ones for copper removal were agar gels 3% containing additives,with no significant difference among the used additives. However, agar gelswith additives host copper in different ways: in gels added with ethylenediamine tetraacetic acid (EDTA), all copper centers are coordinated, while copper centers are also dispersed in water within gels added with ammonium citrate tribasic (TAC). The stain cleaning process of stones probably starts with the diffusion of water at the gel-stone interface, but it finds the driving force in the copper coordination.

Keywords: copper, agar gel, stain removal, unilateral NMR, EPR, ICP-AES

1. Introduction

The cleaning of building heritage is a hard task, since high effectiveness should be obtained while avoiding any possible damage. Within this issue, the removal of stains from stone materials is crucial, especially when they are related to the presence of bronze and/or iron elements on building surfaces. In these cases, stains are due to the precipitation of copper salts and/or iron oxides or hydroxides, giving green and/or orange discolorations,

respectively. Just a few studies focused on the removal of copper sulphates from stones [1, 2].

Formation of salts inside building materials causes decay phenomena due to salt crystallization pressure on the material pore walls, such as loss of cohesion among grains, cracking, pulverization and exfoliation [3-8]. In addition, stains are particularly harmful when they stand out on a light coloured substrate, as marble. Accordingly, the reducing of salt content is mainly aimed to a better conservation of the stone material, besides allowing the correct reading of the formal values [9-13].

The most used methods for the removal of salts from building materials are based on a chemical approach. At this purpose, water, organic solvents, chelating agent solutions and ion exchange resins are used both individually and in a complex mixture. However, harmful effects can be observed when aggressive chemicals are added in order to improve the cleaning effectiveness. For example, further salt formation, and consequent enhancement of staining, can be observed when acid or alkaline agents are used [11].

A better extraction effectiveness can be obtained by mixing free liquids with a thickener, such as wood, paper pulp, cellulose paste [14], micronized silica, sepiolite and other clay materials [15]. This approach delays the evaporation of the solvent and favours its penetration within the stone pores, minimizing any mechanical impact on the material surface [10]. Recently, gelling agents were developed, which generate high viscous systems able to control the fluid release to the substrate. For instance, poly(acrylic acid) [16,17], modified cellulose, gellan gum and others have been used [18]. For these systems, the contact time can provide an easy and functional parameter in order to obtain reproducible cleaning operations. The main drawback of these systems is the difficulty to completely remove gels from the substrates, with the consequent need of several washings with swabs soaked in suitable solvents. Therefore, the risk to leave organic residues on the treated surface must be taken into account.

This drawback has been overcome by the development of semi-rigid gels [19] and, in particular, of agar gels [20, 21]. Agar is a polysaccharide derived from red seaweeds of the Gelidiales and Gracilariales orders. It can easily form semi-rigid, thermo-reversible and hydrophilic gels by dispersing the raw powder in water, subsequent heating and final cooling. During the last step, agar chains arrange in an ordered structure, where aggregates of co-axial helices form the junctions of the three-dimensional gel network [22, 23]. This peculiar supramolecular structure provides interesting properties for application as salt bridge in electrochemistry, gelatine substitute in cuisine, growth media for microbiological culture and many others [24]. In the conservation field, agar gels are used as cleaning systems for the removal of several kind of soiling from building materials, due to the peculiar effectiveness combined with other useful requirements: confined release of the solvent at the interface pad/substrate, low impact on the artworks, low cost and “green” approach. In fact, this material can operate on several substrates, in different forms and environmental conditions [21].

Several studies showed that the effectiveness of salt removal from building materials by poultices is governed by stone porosity and by salt-related factors (solubility, concentration and distribution) [10]. Furthermore, the cleaning conditions, such as the agar concentration in the gel and the gel–stone contact conditions (temperature, relative humidity and contact time), play an important role in the salt extraction process carried out by agar gels. For what concerns concentration, best practices suggest to prepare agar gels by dissolving raw powder in water in the range 0.5-5 % w/w. An improvement is also obtained by adding functional chemicals, such as ethylenediaminetetraacetic acid (EDTA), ammonium carbonate (AC) or ammonium citrate tribasic (TAC) [19, 25, 26].

The determination of the most effective agar formulation on a real case material could be misleading, since the staining is not homogeneous throughout the surface and the gel-stone contact degree could be a critical issue due to surface roughness. Therefore, in order to assess the effectiveness of the salt removal by different agar gels formulations, laboratory model specimens should be developed [25], stained and cleaned in well-controlled and reproducible conditions. With the aim of establishing a new analytical protocol for the salt removal, in the present paper marble laboratory specimens were stained, cleaned by different formulations of agar gels both pure and with added chemicals, in the same experimental conditions. The same cleaning procedure was applied on the marble base of the bronze statue of Napoleon (Fig. 1) by Antonio Canova, located in the Brera Gallery courtyard, Milan (Italy).
Raman spectroscopy was used to compare the copper mineralogical phases produced by artificial staining on the models surface with those present on the statue base. Cleaning effectiveness is strictly related to the removal mechanism of copper compounds from the surface and near-surface regions, which is not known in detail. Hence, the water release from agar gels to stone porous systems was investigated: the penetration of water into the substrate and the water molecules mobility in agar gels were studied by capillarity absorption and by unilateral Nuclear Magnetic Resonance (NMR), respectively. With the latter technique, the magnetic field is applied to one side of the object, allowing measurement to be performed in situ without sampling and completely preserving the integrity of the object [27, 28]. The cleaning by different agar gel formulations was studied on substrate by colour measurements and on gels by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The copper coordination in gels was investigated by electron paramagnetic resonance spectroscopy (EPR).
Summarising, this paper has multiple objectives:

1. to study the water release of agar gels into a stone porous structure and to highlight some aspects of the stain cleaning mechanism from porous stone systems;
2. to evaluate the effectiveness of agar gels in removing copper stains from stone substrates;
3. to establish an analytical protocol for copper stains removal.

2. Materials and methods

2.1. Preparation of laboratory specimens

Specimens sized 5 cm × 5 cm × 2 cm of the so-called Sivec dolomitic marble, quarried in Prilep (Macedonia), were prepared. For better homogeneity, one surface (5 cm × 5 cm) of each specimen was polished with Struers RotoPol-11, using SiC paper having 180 μm grain size. The polished 5 cm × 5 cm surface was stained by contact with a 0.1 M solution of $CuSO_4 \cdot 5H_2O$ (Carlo Erba) in Mill-Q water, at room temperature. The contact was assured by keeping the surface of specimens dipped in the solution under-head for few millimetres and it was prolonged for 72 h. This procedure guaranteed staining in the light green palette, very similar to the one observed in real cases, homogeneous on the whole specimen surface and reproducible for all the specimens.

2.2. Agar gel preparation

Six different formulations of agar gel (AgarArt, CTS) were prepared, both as pure polysaccharide and added with disodium EDTA (Merck-Millipore), AC (Bresciani S.r.l) and TAC (Bresciani S.r.l.), as reported in Table 1.

The concentrations 1, 3 and 5% of the agar gels were chosen according to those previously giving good results in cleaning procedures [26].

Table 1 Formulations of agar gels

Agar code	Agar concentration (%, w/w)	Additive (%, w/w)
A	1	/
B	3	/
C	5	/
D	3	EDTA (1)
E	3	EDTA (1) + AC (0.2)
F	3	TAC (1)

It is known that agar powder in water forms a sol at 85 °C and that, by cooling below 38 °C, a gel can be obtained [21]. Accordingly, gels were prepared by dissolving the proper amount of powder in Mill-Q water, then heating the solution in microwave oven at 700 W for 2 min, then cooling in air. During this last step, additives were introduced, according to the desired percentage (Table 1). The pH of the solutions was equal to 5.0 (Agar D), 7.0 (Agar E), 7.0 (Agar F).

2.3. Agar gels application

Agar gels were applied by brush, taking care of obtaining a constant thickness (0.5 cm), then they were left on site for 1 h. In the case of the laboratory specimens, gels were applied only on the stained surface displayed horizontally. On the Napoleon's statue base, they were applied on the front vertical surface measuring 10 cm × 5 cm (Fig. 2).

2.4. Capillarity absorption measurements

Water absorption by capillarity is standardized in the Italian Standard UNI 10895 [29]. Accordingly, 5 specimens of Noto calcarenite (5 cm × 5 cm × 2 cm) were contacted with agar A, B and C. Noto calcarenite was chosen for its high porosity (36.2%), which allows reliable measures of water absorption [33]. Measurements were carried out at the following steps: 5, 10, 20, 30, 60 min; 2, 4, 8, 24, 48, 72 h.

2.5. Unilateral NMR measurements

Measurements of ^{1}H depth profiles and transverse relaxation times (T_2) were performed at 13.62 MHz on a portable NMR instrument (Bruker Biospin), interfaced with a purposely built single-sided sensor by RWTH Aachen University, Aachen, Germany [31].

Transverse relaxation times were measured with a CPMG pulse sequence [32], and 4096 echoes were recorded at a depth of 2 mm inside the sample with an echo time 2τ of 71.2 μs. The experimental data obtained were normalized and fitted using the following equation:

$$Y(t) = \sum_{j=1}^{n} W_i \, e^{-(t/T_{2i})}$$

where n is the number of components of the decay, W_i the weight of the i^{th} component, and T_{2i} the transverse relaxation time of the i^{th} component.

^{1}H depth profiles were obtained as the addition of the first four echoes acquired with a CPMG pulse sequence with an echo time of 86 μs and a nominal resolution of 23 μm. To optimize the duration of measurements, profiles were acquired by repositioning the single-sided sensor in steps of 150 μm to cover the desired spatial range, from the surface of the specimens to a depth of 10 mm. All profiles were obtained measuring both sides of the specimens with a total field view of 20 mm.

2.6. Stone characterization

Magnified images were taken on laboratory specimens with a Leitz Wild M420 stereomicroscope connected with a digital camera Nikon DS-5M/USB. For the Napoleon's statue base, a digital optical Microscope DG-3x with a continuous zoom system able to magnify in the range 25x-200x was used.

Raman spectra were recorded on a Senterra (Bruker) spectrometer, connected with a microscope Olympus BX51 (532 nm laser line).

Colour measurement data were acquired using a Konica Minolta Chromameter CM-700d with D65 source, d/8° analytic geometry in CIE L*a*b* system, measuring a circular area corresponding to a 6 mm diameter. The parameter L* represents the lightness of colors from 0 (black) to 100 (white); a* denotes the red/green values and b* the yellow/blue values, both ranging from +60 to -60. For each specimen, 25 measures were acquired. Given ΔL^*, Δa^*, Δb^*, the total color difference can be stated as a single value known as $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$, representing the distance of two color points on the CIE L*a*b* diagram [30].

2.7. Agar gel characterization

In order to determine the copper amount extracted from substrates, agar gels (5 cm × 5 cm × 0.5 cm) were removed after 1 h contact and dissolved in HNO_3 (70 %, Carlo Erba). Then, the solution was diluted in Mill-Q water and analysed by an ICP-AES spectrometer (38 Sequential, Horiba Jobin Yvon), with copper analyte line at 327.369 nm and argon (99.999 %, Sapio) as carrier gas. Calibration was performed using copper standard solutions (Sigma Aldrich) diluted in Mill-Q water.

For EPR characterization, agar gels were inserted into quartz tubes having an internal diameter of 3 mm and pushed towards the bottom of the tube using a Teflon rod. Then, EPR spectra were recorded at 123 K with a Bruker EMX spectrometer working at the X-band frequency, equipped with a variable temperature BVT 2000 unit, using a microwave power of 5 mW, a modulation amplitude of 3.0 G, and a modulation frequency of 100.0 kHz. The g values were determined by standardization with *α, α'*-diphenyl-*β*-picryl hydrazyl (DPPH) radical. Since it was not possible to compact gels at the bottom of the EPR tubes, any quantitative comparison among different samples was avoided.

3. Results and discussion

In the following, the agar gel codes refer to the formulations reported in Table 1.

3.1. Study of water release from agar gels

3.1.1. Capillarity absorption measurements

The penetration of water into the substrate was studied by capillarity on 5 Noto calcarenite specimens kept in contact with agar gels (Fig. 3). Absorption capillary coefficients (CA),

proportional to the absorption rate, were calculated as suggested in UNI 10859 [29] and are reported in Table 2.
Results show that both the amount of water absorbed by capillarity and the absorption rate decrease when agar gel concentration increases. The asymptotic value of the curve of agar A is very similar to the one obtained for a multiple layer prepared with filter paper [34], used as a reference. This result shows that agar gel 1% performs almost as a "free" water reservoir and suggests that for this gel diffusion of water at the gel-stone interface plays an important role.

Table 2 Absorption capillary coefficients (CA) for Noto calcarenite in contact with different agar gels

Gels	**CA ($mg/cm^2s^{-½}$)**
A	6.1 ± 0.9
B	0.6 ± 0.2
C	0.13 ± 0.01

3.1.2. Unilateral NMR measurements
In order to study the water release from agar gels to stone porous system and the distribution of water inside the stone, ^{1}H depth profiles were measured on Noto calcarenite specimens after water capillary absorption. Pure gels A, B and C were kept in contact with the specimens for 30 min, 1 h and 4 h. The depth profile of dry Noto specimen was also measured and used as a reference (Fig. 4).
The amplitude of ^{1}H depth profile is directly proportional to the amount of absorbed water, and is reported as a function of the depth scanned. Therefore, each experimental point represents the amount of water at the corresponding depth of measurement [35, 36]. Depth profiles showed different trends with both the application time of the gel and the agar concentration (Fig. 4). Specifically, by using agar A, water penetrated the whole thickness of the specimens (20 mm) also at the shortest time of application. Instead, by increasing the gel concentration (B and C), the penetration depth of water decreased: after 1 h it was 10 and 8 mm, respectively. After 4 h of application, water from agar B penetrated the whole specimen, whereas from agar C a penetration depth of 15 mm was achieved. A reduction of the amplitude of the profiles was observed by increasing the gel concentration and reducing the application time, indicating that the amount of absorbed water was reduced. Thus, ^{1}H depth profiles showed that the kinetics of water absorption through the specimens was strongly correlated with the concentration of agar and was the fastest for gel A. These results are in good agreement with those obtained by the curves of capillarity.
In order to evaluate the variation of water in agar gels during water desorption, transverse relaxation times were measured on gels A, B and C after contact with stone for 30, 60 and 240 min (Table 3). It is known that relaxation times depend on temperature, time and some molecular parameters, such as molecular weight, degree of branching, cross-link density, and size of the side groups [36]. Furthermore, relaxation measurements are affected by both rotational and translational molecular motions [37].
In all samples, transverse relaxation times of water in agar gels were found to be bi-exponential. As reported in the literature [37], two proton species must be taken into account: one with a short transverse relaxation time, T_{2A}, related to "bound" water, and another one with a long transverse relaxation time, T_{2B}, related to "free" water. Protons related to "bound"

water are affected by hydrogen bonding interaction and/or chemical exchange with agar macromolecules. In the present study, for all samples the amount of "bound" water was found to be very small (4%-7%, as obtained by the relative weights of T_{2A} and T_{2B}) with respect to "free" water. The shortest transverse relaxation time (T_{2A}), measured after the application on the stone, did not show any net trend with the time of desorption, whereas the longest one (T_{2B}) decreased as the time of desorption increased. Transverse relaxation times decrease as the agar concentration increases.
In order to evaluate the reduction of "free" water mobility at the gel-stone interface as a function of agar concentration, the $R_{T_{2B}}$ parameter was calculated as follows:

$$R^{i}_{T_{2B}} = \frac{T^{0}_{2B} - T^{t}_{2B}}{T^{0}_{2B}} * 100$$

where T^{0}_{2B} is the transverse relaxation time of agar gel before the application on the stone, and the T^{t}_{2B} is the transverse relaxation time at different desorption times.
The mobility of "free" water decreased as the agar concentration increases, like transverse relaxation times. For instance, for agar A and B, after 60 minutes the "free" water mobility reduced to about 30%, and 50%, respectively, whereas in agar C the reduction was about 7%. Thus, the capability of agar gels to release "free" water at the gel-stone interface is a process strongly dependent on the agar concentration. This result can be rationalized considering that agar gels are porous media with flexible cavities [38,39]. The water mobility in agar gels is probably affected by the shrinking of the gel network that occurs during desorption of water. The more the agar chains approach each other, the higher the degree of obstruction on the translational motion of "free" water.

Table 3 Transverse relaxation times (T_{2A} and T_{2B}) and reduction of "free" water mobility $R^{i}_{T_{2B}}$ in agar gels after contact with Noto calcarenite at different times.

Time (min)	**A**			**B**			**C**		
	T_{2A} (ms)	**T_{2B} (ms)**	**$R^{A}_{T_{2B}}$ (%)**	**T_{2A} (ms)**	**T_{2B} (ms)**	**$R^{B}_{T_{2B}}$ (%)**	**T_{2A} (ms)**	**T_{2B} (ms)**	**$R^{C}_{T_{2B}}$ (%)**
0	5.4 ± 0.3	40 ± 2	0	4.7 ± 0.2	31 ± 1	0	4.0 ± 0.2	23 ± 1	0
30	5.2 ± 0.3	31 ± 2	22	4.2 ± 0.2	22 ± 1	28	4.0 ± 0.2	22 ± 1	3
60	3.9 ± 0.2	27 ± 1	32	4.5 ± 0.2	15 ± 1	50	4.0 ± 0.2	22 ± 1	7
240	4.9 ± 0.2	26 ± 1	35	3.4 ± 0.2	14 ± 1	54	4.0 ± 0.2	17 ± 1	25

3.2. Marble specimens cleaning

3.2.1. Specimens characterization
Observations and measures were carried out on the marble surface before staining (at time t_0), after staining (at time t_1) and after cleaning with agar gels for 1 h (at time t_2).

Optical microscope images showed that at t_0 each specimen has a compact surface without morphological flaws (Fig. 5a). This feature allowed the formation of a homogeneous copper staining. The image of the stained surface at t_1 suggests that copper compounds do not remain as external formations, but penetrate to subsurface regions, probably in correspondence of more porous areas (Fig. 5b). The observation at the microscope of specimens at t_2 allowed to highlight a more defined granular texture. It is possible to assume that copper compounds were removed from the intergranular spaces leaving them cleaned, clearer in colour and more visible.

The mineralogical phase formed at t_1 on the marble surface was highlighted by Raman spectroscopy. Chalcanthite, $CuSO_4 \cdot 5H_2O$, was observed on each sample (Fig. 6).

Color variations of specimens were measured after 1 h of agar gel application (Table 4 and Fig. 7). For pure agar gels, small ΔE* values (≈1) were measured, independently on the agar concentrations. After cleaning with additivated gels, ΔE* values are higher and range around 3; moreover Δa* values show a higher decrease of the green component with respect to pure gels. These results show that additivated agar gels are the most effective in removing copper compounds; by a chromatic point of view, they bring back the surface close to the original color features. For each cleaning test, the **ΔE*** values raised up from a contribution approximately equal of the three chromatic parameters (ΔL*, Δa*, Δb*). All the measured **ΔE*** values are at the border line of what is considered the threshold of the of the human sensitivity [30].

Table 4 Chromatic parameters (L*, a*, b*) before (t_1) and after (t_2) cleaning of laboratory specimens

Gels	**t_1**			**t_2**		
	L*	**a***	**b***	**L***	**a***	**b***
A	86.9 ± 0.5	-9.9 ± 0.6	-0.3 ± 0.6	87.6 ± 0.5	-9.2 ± 0.6	-0.2 ± 0.5
B	87.2 ± 0.5	-9.2 ± 0.5	0.3 ± 0.4	87.7 ± 0.3	-8.7 ± 0.4	-0.3 ± 0.2
C	87.1 ± 0.7	-9.7 ± 0.7	-0.1 ± 0.5	87.4 ± 0.8	-9.2 ± 0.6	-0.7 ± 0.3
D	82.1 ± 0.4	-6.3 ± 0.3	1.7 ± 0.3	84.2 ± 0.3	-4.9 ± 0.2	-0.2 ± 0.1
E	80.4 ± 0.7	-6.9 ± 0.4	2.3 ± 0.6	82.8 ± 0.4	-5.3 ± 0.3	0.5 ± 0.2
F	82.0 ± 0.6	-6.6 ± 0.4	1.5 ± 0.4	84.1 ± 0.7	-5.1 ± 0.4	-0.0 ± 0.3

3.2.2. Agar gel characterization

Copper centers in agar gels were studied both before and after 1 h contact with laboratory specimens. The copper content in the gels was quantified by ICP-AES. The symmetry field of copper centres within the gel was investigated by EPR spectroscopy.

ICP-AES data showed that agar gels as prepared contain negligible amounts of copper.

After 1 h contact with specimens, the amount of copper detected in pure gels was rather small, below 15 μg/cm^2 of agar surface area, and reached the lowest value when agar C was used (Fig. 8).

By using agar containing additives (D, E, and F), the removal of copper was about twice more effective, with no significant differences among the used additives. The higher effectiveness displayed by agar gels containing additives is in agreement with both the results obtained in the literature for copper ion removal from wastewaters by agar gel containing EDTA [40] and the color measurements reported above.

For what concerns the EPR characterization, spectra of agar gels as prepared showed the presence of a weak sharp isotropic signal, probably due to impurities present in the agar network. Instead, resonances attributable to Cu(II) centers were never observed.
After 1 h contact with marble specimens, agar gels were removed, inserted into the EPR quartz tubes and frozen in liquid nitrogen.
Pure agar gels A, B, and C displayed weak axial EPR signals (Fig. 9), having values of g and A tensor components (Table 5) consistent with those of Cu(II) centers in a tetragonal symmetry field of oxygen atoms [41,42]. Thus, these signals can be attributed to magnetically diluted Cu(II) centers coordinated by agar gels.
For agar B, a sharp isotropic signal was observed (g = 2.003), besides the Cu(II) signal. This signal is starred in Fig. 9, and is identical to the one observed on gels before contact with marble specimens; thus it can be attributed to impurities present in agar batches.
Gels added with EDTA or co-added with EDTA and AC showed two different axial signals, attributable to two different magnetically diluted Cu(II) centers in a tetragonal symmetry field of oxygen atoms. One is weaker and has the same EPR parameters as those observed for pure agar gels, while the other has higher intensity and tensor components (g// = 2.07 g⊥ = 2.29 A// = 149 G), which can be attributed to copper(II) centers coordinated by the additives.
For agar F, resonances attributable to magnetically diluted Cu(II) centers, identical to those observed in gels D and E, were observed. In addition, an isotropic intense broad band was observed (g ~ 2), attributable to interacting Cu(II) centers in frozen water solution [42, 43].
Thus, EPR spectra show that copper centers are coordinated by agar gels in all the studied samples and by additives, when present. For 3 % gels added with TAC, copper is also dispersed in water. Thus, all agar gels with additives are equally effective for copper stain removal, even if they host copper in different ways.

Table 5 EPR parameters of signals observed on agar gels after cleaning of laboratory specimens

Gels	$\mathbf{g}_{\perp}$	$\mathbf{g}_{\parallel}$	$\boldsymbol{A}_{\parallel}$ **(G)**
A	2.08	2.39	135
B	2.08	~ 2.4	135
C	2.08	2.39	135
D	2.07	2.29	149
	2.08	2.39	135
E	2.07	2.29	149
	2.08	2.39	135
F	2.08	2.39	135

3.3. Napoleon's statue base cleaning

3.3.1. Statue base characterization

In the case of Napoleon's base, copper stains are naturally formed during an aging of several years and it is not possible to get information about the original surface before the natural soiling. Hence, only t_1 and t_2 measurements were possible.
At a microscopic investigation, the Napoleone's base marble seems to be compact and sound with significant copper stains homogeneously distributed on the entire surface (Fig. 2).
The presence of brochantite was detected by Raman spectroscopy (Fig. 10).
Agar gels were applied for 1 h on the front vertical surface. Optical images show that, after 1 h contact, pure gels provoke no tone variation and remove only the incoherent atmospheric particulate deposited on the surface. Instead, at the same application time the additivated gels show good cleaning results (Fig. 11).
Colorimetric measurements reported in Table 6 represent the chromatic values before and after 1 h contact with gels. The high standard deviation values are due to the lack of homogeneity in colour parameters measured on the statue base surface, which underwent natural decay mechanisms.
Agar gel F shows the cleaning with the highest ΔE* value and a considerable variation of a* (Fig. 12). On the other hand, agar gels D and E show comparable ΔE* values to those obtained with pure gels and a feeble decrease of green tone (a*).

Table 6 Chromatic parameters (L*, a*, b*) before (t_1) and after (t_2) cleaning of Napoleon's base

Gels	**t_1**			**t_2**		
	L*	**a***	**b***	**L***	**a***	**b***
A	69 ± 1	-12 ± 2	3.4 ± 0.9	69.0 ± 0.8	-12 ± 2	3.1 ± 0.8
B	67 ± 1	-14 ± 2	3.2 ± 0.3	67 ±1	-13 ± 2	3.4 ± 0.5
C	67 ± 1	-15 ± 1	3.8 ± 0.4	67 ± 1	-15 ± 1	3.6 ± 0.6
D	67 ± 1	-16 ± 2	3.5 ± 0.8	67 ± 1	-16 ± 2	3.7 ± 0.8
E	68 ± 1	-17 ± 1	2 ± 1	67.9 ± 0.9	-16 ± 1	2 ± 1
F	67 ± 1	-15 ± 1	3.8 ± 0.6	68.4 ± 0.8	-13 ± 2	3.6 ± 0.7

3.3.2. Agar gel characterization
After 1 h contact with the statue base, the amount of copper detected in all gels was significantly lower than that extracted from laboratory specimens. However, the same trend was observed: added gels allowed to extract higher amounts of copper with respect to the pure gels, with no significant differences among the used additives (Fig. 13).
For what concerns EPR investigation, agar A, B, C, D and E showed spectra identical to those observed after laboratory specimens cleaning.
Instead, agar F showed a spectrum very different from the one observed after specimen cleaning (Fig. 14). By recording the spectrum at different microwave power values (5, 50 and 100 mW), two signals were observed: one is broad, isotropic and very intense ($g \sim 2$), attributable to interacting copper(II) centers; the other displays some components of axial copper signals attributable to magnetically diluted Cu(II) centers coordinated by agar gels and/or by the additive. The latter attribution is evident by the superimposition of the gel F spectrum recorded at 100 mW with the gel D spectrum recorded at 5 mW (Fig. 15).

The EPR results are in agreement with those obtained for the laboratory specimens and confirm the suitability of marble specimens for studying both the effectiveness and the mechanism of copper stain removal by agar gels on real cases.

4. Conclusions

Marble laboratory specimens were stained and cleaned by agar gels in well-controlled and reproducible conditions. This procedure allowed to compare the cleaning effectiveness and the action mechanism of agar gels in different formulations.

By using agar gels containing additives (EDTA, EDTA + AC or TAC), the removal of copper was about twice more effective than using pure gels, independently on the additive used. These gels bring back the laboratory specimen surface close to the original color features. The same trend of effectiveness in copper stain removal was observed both on the laboratory specimens, stained by calchantite, and on the Napoleon's base, stained by brochantite. Thus, the effectiveness of gels does depend neither on the used additive nor on the water solubility of the staining copper compound. The latter consideration suggests the applicability of the used cleaning protocol in any real case.

For what concerns the mechanism of copper removal, the two following steps are active:

1) diffusion of water at the gel-stone interface, with initial release of water from gels to stone, then re-absorption of water into gel. During this process, copper salts are transported by "free water", without any driving force other than the counter diffusion of ions in the gel phase. This step plays an important role for pure agar gel at 1%, which almost behaves as a reservoir of "free water". The capability of agar gels to release "free" water at the gel-stone interface decreases as the polymer concentration increases;

2) coordination of copper centers, both by agar gels and additives (when present). In gels added with EDTA, all copper centers are coordinated, while copper centers are also dispersed in water within gels added with TAC.

Thus, all agar gels with additives are equally effective for copper stain removal, even if they host copper in different ways. This could suggest that the stain cleaning of stones starts with the diffusion of water at the gel-stone interface, but copper coordination, both by agar gels and additives, is the driving force.

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Figure captions

Fig. 1 Napoleon's statue in the courtyard of Brera Gallery, Milan (Italy)

Fig. 2 Selected areas on Napoleone's base during cleaning procedure

Fig. 3 Water absorption by capillarity on Noto calcarenite in contact with different agar gels (■ agar A 1%, • agar B 3%, ▲ agar C 5%). In the inset, the test setting of capillarity measurements

Fig. 4 ^{1}H depth profiles of Noto calcarenite dry (squares) and after absorbing water from the gel for 30 min (black circles), 1 h (white circles) and 4 h (grey circles). Agar gels: A 1 %; B 3 %; C 5 %

Fig. 5 Optical microscope images of Sivec laboratory specimens before (a) and after (b) the staining process

Fig. 6 Raman spectrum of laboratory specimens after the staining process

Fig. 7 Global colour variation (Δa^*, Δb^*, ΔL^* and ΔE^*) before (t_1) and after (t_2) cleaning of laboratory specimens. Agar gels: A 1 %; B 3 %; C 5 %; D 3 % + EDTA; E 3 % + EDTA-AC; F 3 % + TAC

Fig. 8 Copper content per agar surface area after cleaning of laboratory specimens. Agar gels: A 1 %; B 3 %; C 5 %; D 3 % + EDTA; E 3 % + EDTA-AC; F 3 % + TAC

Fig. 9 EPR spectra of agar gels after cleaning of laboratory specimens. Agar gels: A 1 %; B 3 %; C 5 %; D 3 % + EDTA; E 3 % + EDTA-AC; F 3 % + TAC. Starred signal is due to impurities present in agar batches

Fig. 10 Raman spectrum of Napoleon's base

Fig. 11 Stereomicroscopic images 100x of the base surface before (a) and after (b) cleaning by agar gel E 3 % + EDTA-AC; bar = 500 μm

Fig. 12 Global colour variation (Δa^*, Δb^*, ΔL^* and ΔE^*) before (t_1) and after (t_2) cleaning of Napoleon's base. Agar gels: A 1 %; B 3 %; C 5 %; D 3 % + EDTA; E 3 % + EDTA-AC; F 3 % + TAC

Fig. 13 Copper content per agar surface area after cleaning of Napoleon's base. Agar gels: A 1 %; B 3 %; C 5 %; D 3 % + EDTA; E 3 % + EDTA-AC; F 3 % + TAC

Fig. 14 EPR spectra of agar gel F 3 % + TAC after cleaning of Napoleon's base, recorded at different microwave power values: 5 mW (continuous line), 50 mW (dashed line) and 100 mW (dotted line)

Fig. 15 EPR spectra of agar gels after cleaning of Napoleon's base. Agar gels: F 3 % + TAC recorded at 100 mW (continuous line) and D 3 % + EDTA (dashed-dotted line)

Figure 1

Figure 2

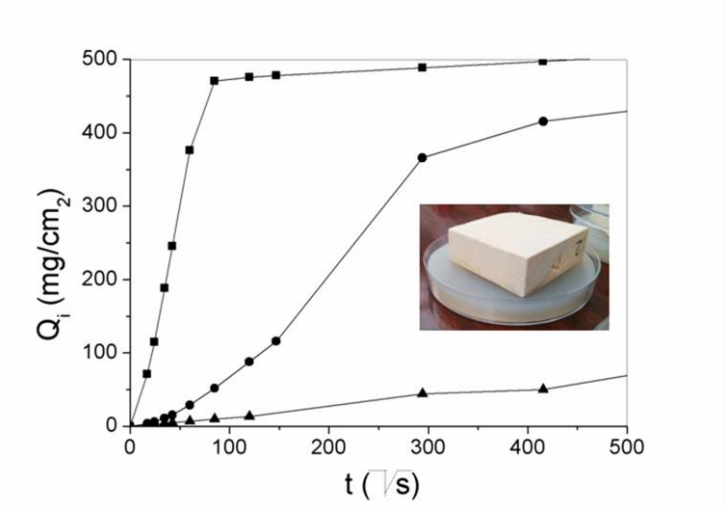

Figure 3

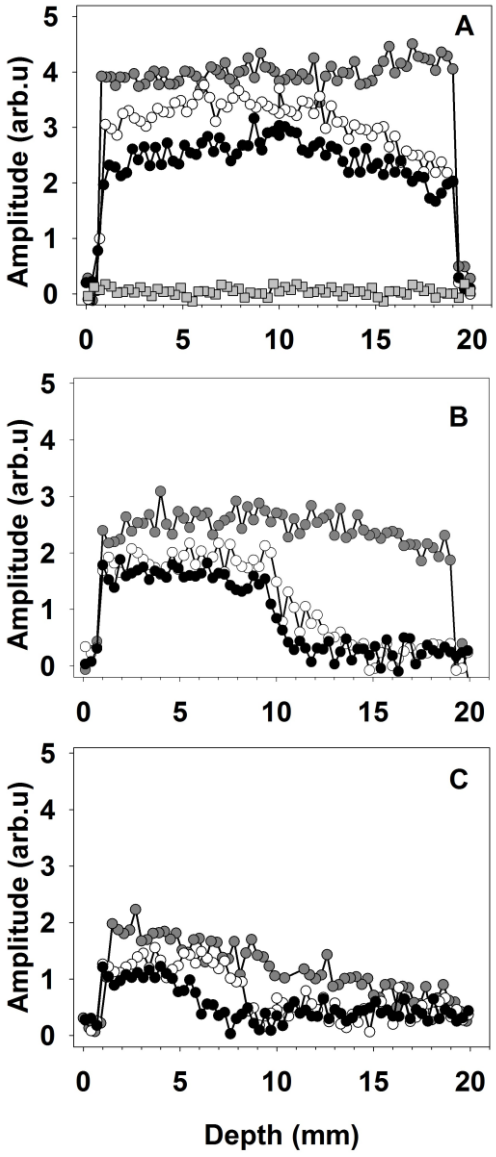

Figure 4

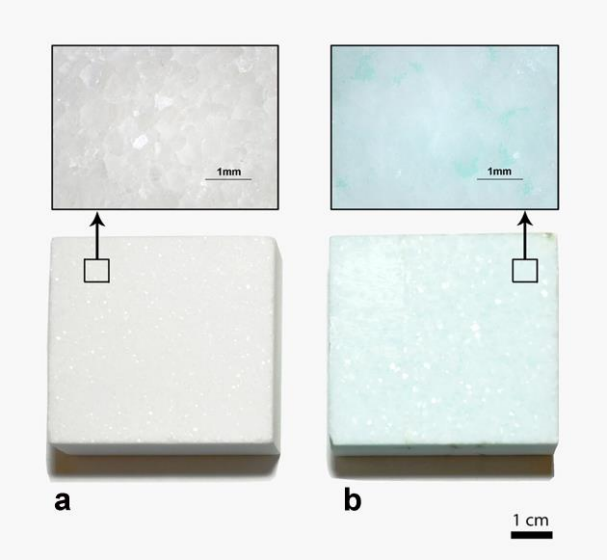

Figure 5

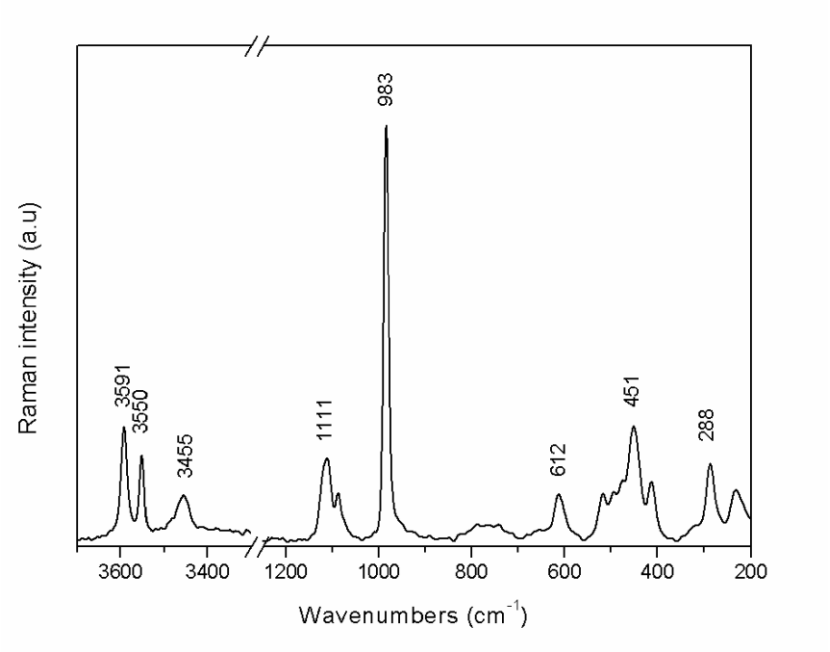
Raman intensity (a.u)
3591
3550
3455
1111
983
612
451
288
3600
3400
1200
1000
800
600
400
200
Wavenumbers (cm-1)

Figure 6

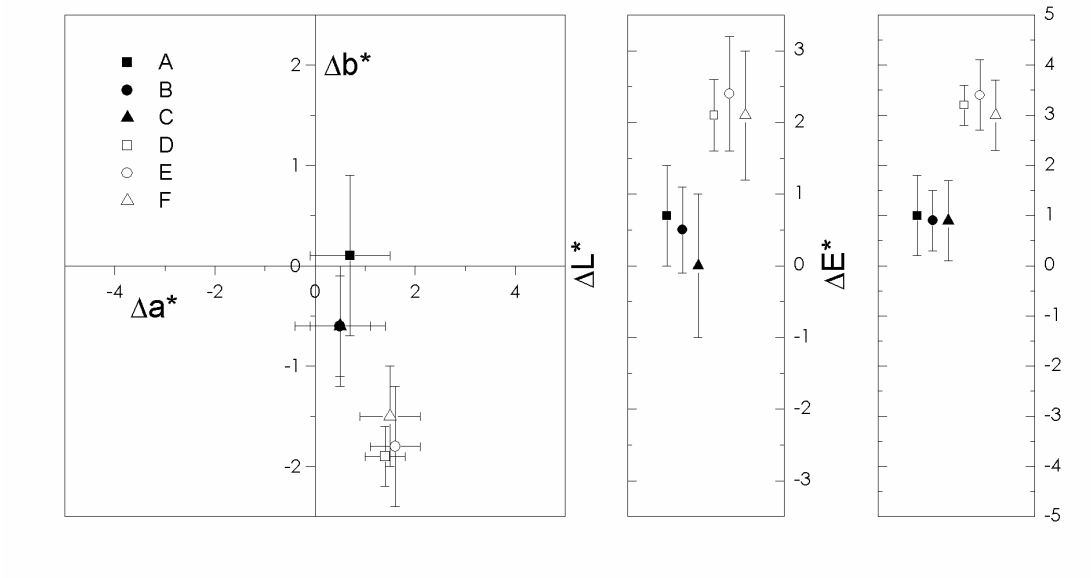

Figure 7

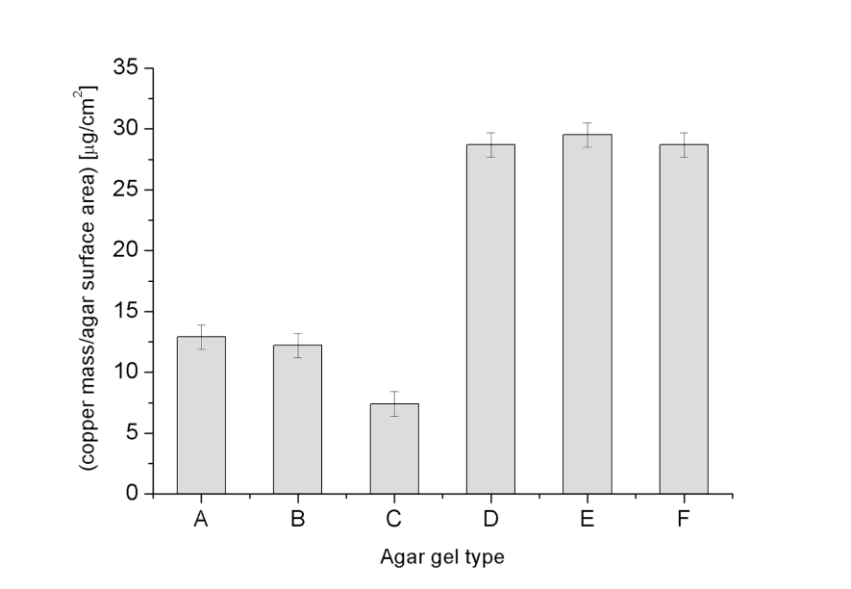
(copper mass/agar surface area) [µg/cm²]
35
30
25
20
15
10
5
0
A
B
C
D
E
F
Agar gel type

Figure 8

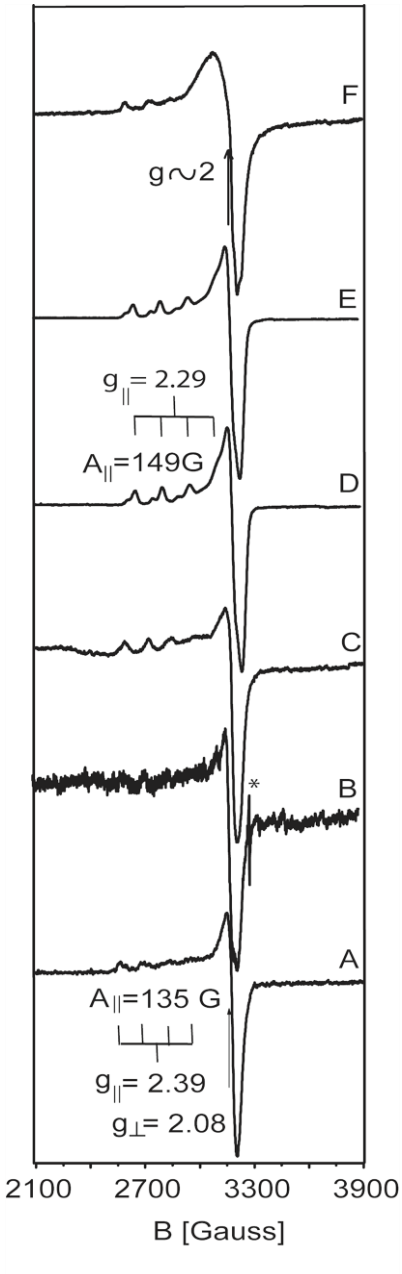
F
g∼2
E
g|| = 2.29
A||=149G
D
C
B
*
A
A||=135 G
g|| = 2.39
g⊥= 2.08
2100
2700
3300
3900
B [Gauss]

Figure 9

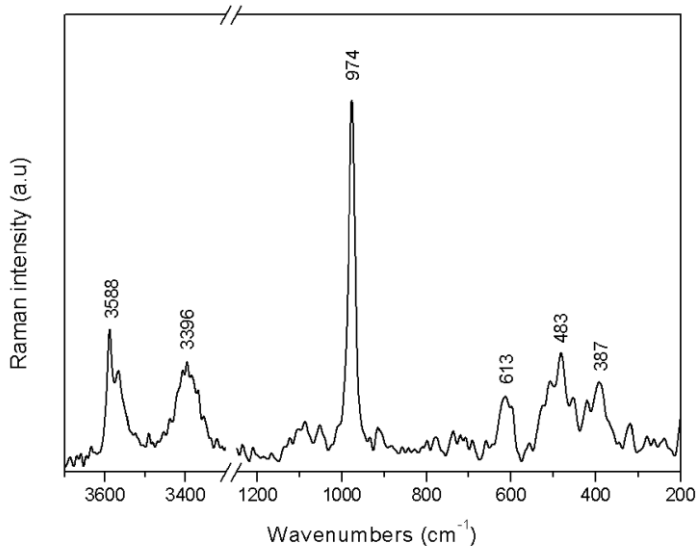
Raman intensity (a.u)
3588
3396
974
613
483
387
3600
3400
1200
1000
800
600
400
200
Wavenumbers (cm-1)

Figure 10

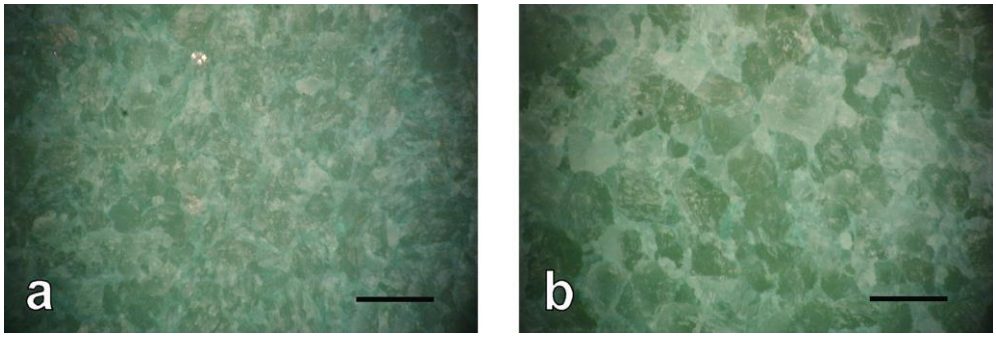

Figure 11

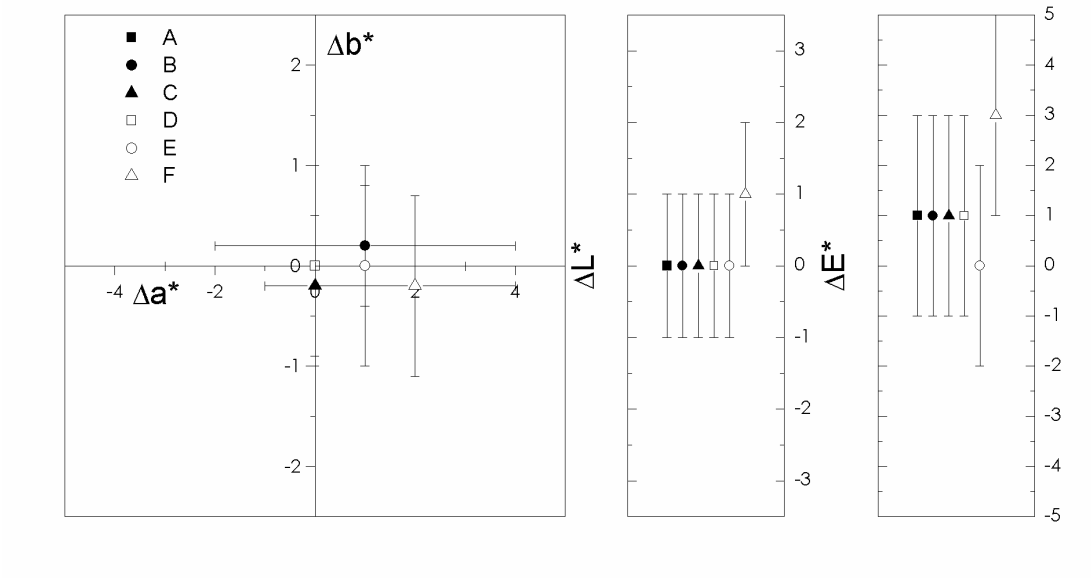

Figure 12

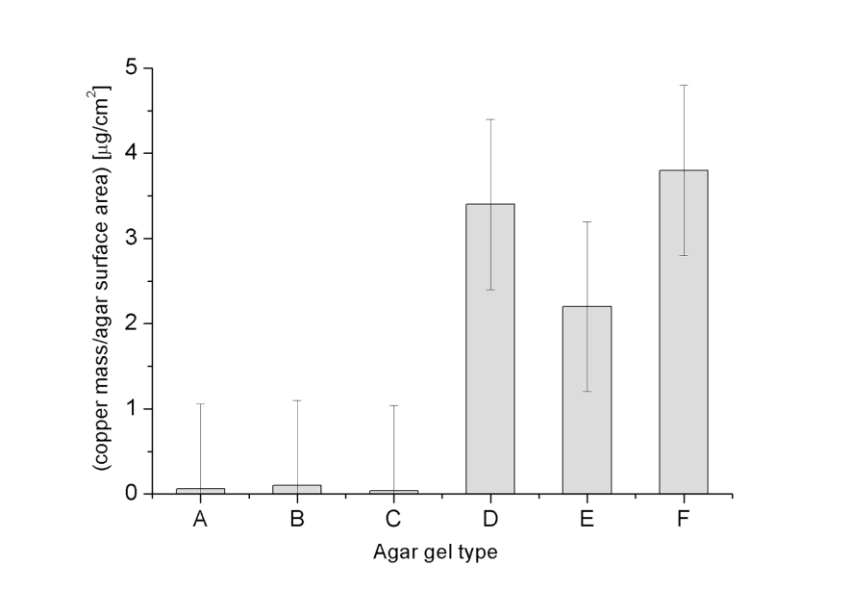
(copper mass/agar surface area) [μg/cm²]
0
1
2
3
4
5
A
B
C
D
E
F
Agar gel type

Figure 13

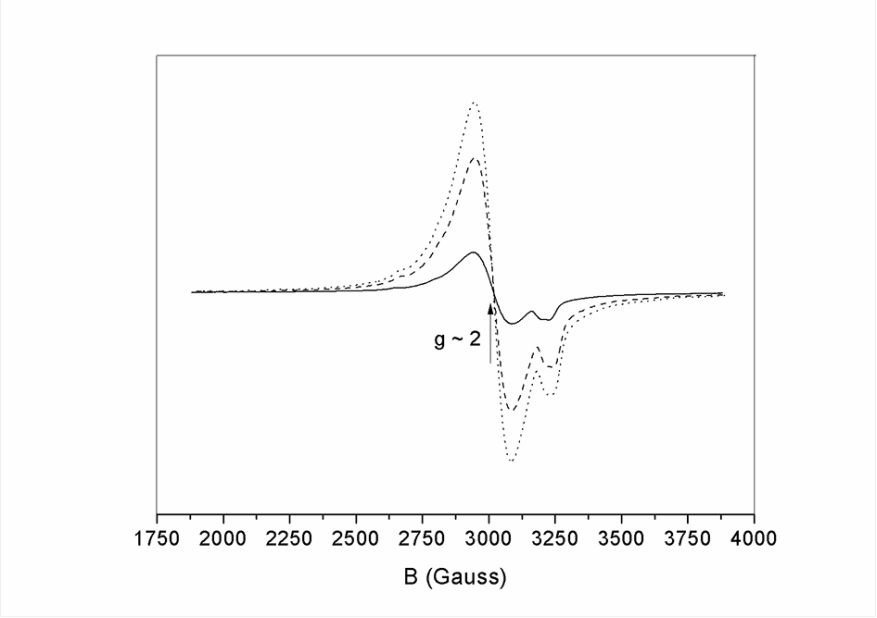

Figure 14

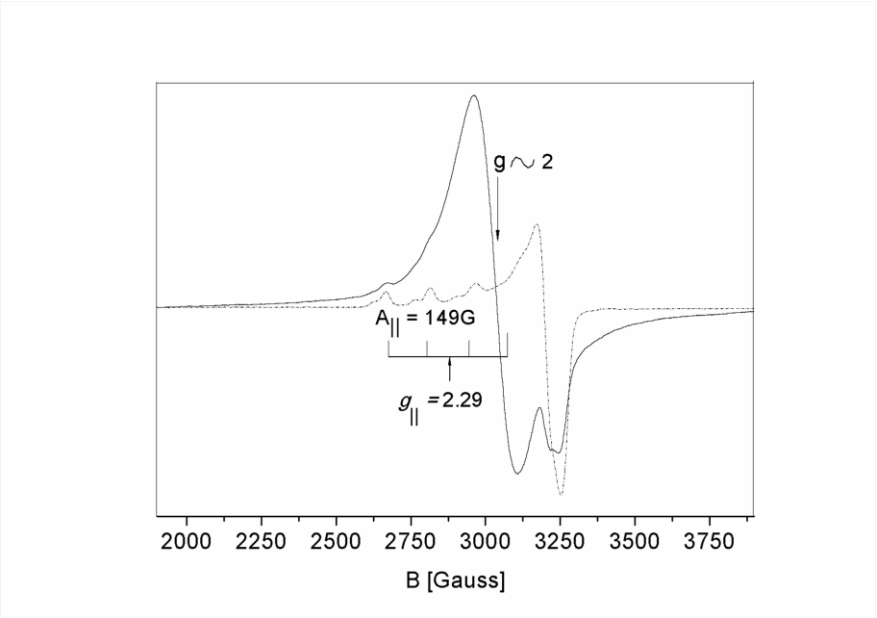

Figure 15