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CHROMATIC ALTERATION OF EGYPTIAN BLUE AND EGYPTIAN GREEN PIGMENTS IN PHARAONIC LATE PERIOD TEMPERA MURALS

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ABSTRACT

This study provides a new reason for the colour alteration of the first man-made pigments (Egyptian blue, EB, "cuprorivaite" and Egyptian green, EG, "wollastonite"). The study focuses on the identification of the most efficient bacterial and fungal species that can grow upon copper-based pigment materials (Egyptian blue and Egyptian green); and their role in the discolouring phenomenon of these pigments. For this purpose, pigment materials from a limestone stela at the Egyptian Museum in Cairo (TR 4.1.21.1, SR 5/10603) were studied using light optical microscope (LOM), scanning electron microscopy-with energy dispersive spectroscopy (SEM-EDS), and x-ray fluorescence (XRF) to identify their composition. Pigment materials were also investigated biologically to determine the role played by microorganisms in their decay, and a spectrophotometer at 542 nm wavelength was used for detecting the chromatic alteration. The study proved that the black pigment is magnetite, red pigment is hematite, yellow pigment is orpiment, blue pigment is Egyptian blue and green pigment is Egyptian green. The most effective fungal species on both E.B and E.G are *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus Flavus*, while the most effective bacterial genus on both E.B and E.G are *Pseudomonas taetrolens*, *Bacillus subtilis* and *Serratia rubidaea*.

KEYWORDS: Stelae; XRF analysis; SEM-EDX analyses; pigment materials; copper-based pigments; Egyptian blue; Egyptian green; Bacterial growth

1. INTRODUCTION

An unusual limestone stela from the Egyptian Museum at Cairo (TR 4.1.21.1, SR 5/10603), which has been dated to the 26th Dynasty was the case study of this research, the stela shows the lady Ta-waher adoring Harmakhis on right and Atum on left; the stela is 37cm in height and 27cm in width (SACP, 2016). According to Peter Munro's classification of stelae (1973), it was placed in the "Edfu I" group and it was dated back to 620-570 B.C. This classification was also based on the Egyptian Museum database which relies the stylistic and textual comparison with other limestone and wooden stelae (Zakareya, 2015). Moreover; through literature research in the department of Ancient Egypt and Sudan at the British museum, and especially through the information obtained from the inscriptions on a coffin kept in New York (MMA 86.1.30), it was concluded that the stela was made at Thebes (modern Luxor) in the 26th Dynasty (664-525 BC), (Pischikova et al., 2014). Pharaonic mural paintings (support, mortar, plaster, pigments and paint media) are susceptible to many degradation agents especially in situ. The moisture and heat are the major factors affecting the durability of these substrates; moisture initiates chemical, physical and biological degradation processes either by itself or by transporting the substances that take part in degradation mechanisms (Addleson et al., 1991, Moussa 2019; Helmi et al., 2016). The lower the temperature, the better, because the rates of chemical reactions and biological activity decrease as the temperature decreases (Stenseth et al., 2005), increased heat can lead to the breakdown of the paint medium which binds the pigment to the mural surface, while UV may lead to colour fading (Benavente et al., 2007). Bio-deterioration is strongly linked to moisture and heat, fungi and bacteria might discolour the painting layer through their own pigments, they can also use the organic compounds from the paint layer as growth substrates (Rolleke et al., 1996). Artificial copper based pigments like Egyptian blue (E.B) $CaCu(Si_4O_{10})$ and Chinese blue $BaCu(Si_4O_{10})$, cannot withstand against bacteria and fungi which cover them with oxalate layer *in situ* but in controlled conditions (museums) these pigments can resist (Sorlini et al., 1987; Wiedemann et al., 1996, 2002). Unlike these findings, the current study aims essentially to identify the chromatic alteration of copper-based pigments due to the growth of bacterial and fungal species inside the high controlled environments (museums). Thus, a new methodology was devised, which concerns the introduction of Egyptian blue (E.B) (widely used in ancient Egypt, see also Abdelmoniem et al., 2020 for wooden coffin) and Egyptian green (E.G) which act as a source of copper for microorganisms, and then as dominant and unique nutrient in a mineral salt medium. The study proves that certain species of fungi and bacteria are responsible for the discolouring of E.B and E.G.

2. SAMPLING AND METHODOLOGY

Blue and green pigments in addition to the other pigment materials of the studied stela; have been sampled and laboratory analysed to determine their chemical composition by mean of scanning electron microscope, Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 KV, magnification 14x up to 100,0000 and resolution for Gun.1n). Tilts (0.00); take-off: (36.47); ampt (35.0); detector type (SUTW-sapphire); resolution (129.87), FEI Company, Netherlands. Samples were investigated using digital USB light optical microscope (Dino-Lite Digital Microscope), with magnification power (500x). XRF analysis was achieved using a BRUKER instrument with Measurement Time: 40.0s, Tube Voltage: 40 kV, Tube Current: 20 µA, Tube Target Material: Rh, Elio Device: SN177, Device Mode: Head, Acquisition Mode: Manual, Acquisition Channels: 4096, Sample to Detector Material: Air. Biological samples were taken from the stela for Mycological and Bacteriological analyses, concerning bacterial isolation; swabbing cotton sterile technique was applied. The swabbed stela samples were collected and suspended in 10ml distilled sterilized water then shacked on mechanical shaker for 15 minutes. Water bath processed at 70 °C for 15 minutes to eliminate vegetative bacterial cells and isolate spore-forming bacteria (dominant Bacillus); samples were then inoculated on nutrient agar medium. All inoculated petri dishes were incubated at 27 °C for 48hrs. The growing colonies were selected and purified by more streaked on the same medium. Purification was insured by Gram and spore stains then microscopically examined. Isolates were identified on the basis of: 1. Gram-stain reaction of smears. 2. Shape: The shapes of bacterial cells were microscopically examined at a magnification of 1500x. 3. Motility: this was examined by the hanging drop technique using broth cultures old 18 – 20 hrs. 4. Sporulation: the presence, shape and position of spores' stained smears were noticed using brilliant green pigmentation. Biochemical tests were also performed by a standard procedure based on Bergey's manual (2008). Chromatic alteration was detected using a Spectrophotometer model: jenway 6305 UV/Vis. Fig.1 shows the places of swabs taken from the studied stela.

Figure 1. The sampling spots of the fungal and bacterial isolates taken from the studied stela

3. RESULTS AND DISCUSSION

3.1. Investigation

Based on the data obtained from the light optical microscope; pigment materials in the studied stela are showing different degradation symptoms amongst: powdering of the paint layers; detachment of the paint layer "flaking"; loss of the paint layers; chromatic alterations; natural deposits (dust, insects' broods and blood; efflorescence; black shadows on the white washing looks like fungal colonies); blistering; spalling of pigments' surfaces; black spots; discoloured area without any pictorial layer under them; addition structures: brown and thick mycelia; addition structure: very thin mycelia; mycelial threads; gaps as a result of losing pictorial layer because of humidity, salt efflorescence or bio-deterioration; detachment of the pictorial layer. Colour change appears clearly in Egyptian blue and Egyptian green in the studied stela, colour alteration is a common phenomenon in these two synthetic pigments; the presence of chlorides catalyses the decay process and causes what is known as "bronze disease", this becomes clear when the samples contain a certain amount of chlorine (Cl) as it was detected by SEM-EDX analysis. The chloride ion replaces the hydroxide ion and forms a soluble metal chloride which has a hygroscopic nature. This is very dangerous in the presence of the Egyptian blue "Cuprorivaite, $CaCu(Si_4O_{10})$" as a pigment material. Schiegl, 1991, 1992 and Schiegl et al., 1989 ascribed the discolouring phenomenon of the Egyptian blue into green colour "copper chloride cancer" to that reason, noting that in the presence of chloride salts it acts with Cu which been added to the pigment's alloy forming green copper chlorides "green atacamite $[Cu_2Cl(OH)_3]$ and paratacamite", and as a result of the migration of Cu and Cl ions in the NaCl solution it precipitates atacamite which tends to change after losing the water to a powder of paratacamite, or to a new pigment material known as wollastonite green $(CaCu)_3(Si_3O_9)$. The discolouring here is due to the instability of the Egyptian blue pigment in the glassy phase which results from the increasing of the silicate amount in the alloy. Figs 2-4 make it clear how the original pigments were discoloured in the studies stela.

Figure 2. The colour alteration of the blue pigment in the studied stela

Figure 3. Detachment of the paint layer and colour change of the blue pigment in the studied stela

Figure 4. Powdering of the paint layer, colour alteration of the green pigment and black shadows look like fungal colonies

Basic copper paratacamite or atacamite, is found in analyses of green pigment samples from surfaces as late as the end of 12th dynasty, but recent investigations led to the conclusion that atacamite may be a degradation product of artificial copper pigments, and that only a few examples of green earlier than the new kingdom were applied as green (Lee and Quirke, 2001). Chemically, salts in reaction with the pigment components play a catalyst role in the discolouring phenomenon (Moussa and Ali, 2013).

Concerning other pigment materials; it has been observed that the red pigment is suffering serious problems related to its fragility and stability, the pigment is cracked and has lost its cohesion and consistency (Fig.5), in other places; red pigment is lost due to cracks in the limestone support itself (Fig.6).

Figure 5. Fragility and cracking of the red paint layer in the studied stela

Figure 6. The cracking of the limestone support itself and its effect on the red pigment in the studied stela

Man-made deterioration was also observed by the LOM investigation; a previous non-accurate consolidation process was detected, an excessive amount of paraloid B72 was added into the surface of the limestone support in a high concentration led to the formation of a bright thick non-porous film upon it (Fig. 7), while salt efflorescence was observed upon many pigmented places on the studied stela (Fig. 8).

Figure 7. False consolidation treatment of the limestone support and its effect on the studied stela

Figure 8. Salt efflorescence and its effect on the pigment materials in the studied stela

Figure 9. SEM micrograph of the blue pigment sample from the studied stela

Figure 10. SEM micrograph of the green pigment sample from the studied stela

The SEM investigation which was carried out upon the blue and green pigment has detected the same fragility and loss of cohesion in the two pigments (Fig. 9 and 10).

3.2. Analyses

In case of such objects; non-destructive methods should be applied for analysing the components. Thus; portable XRF was applied abreast with SEM-EDX in order to point out the chemical composition of the pigment materials. Based on the data obtained from the XRF analysis; it has been concluded that the pigments were not applied directly into the surface of the limestone, but a thin layer of gypsum ($CaSO_4.2H_2O$) whitewash was applied first (Fig. 11).

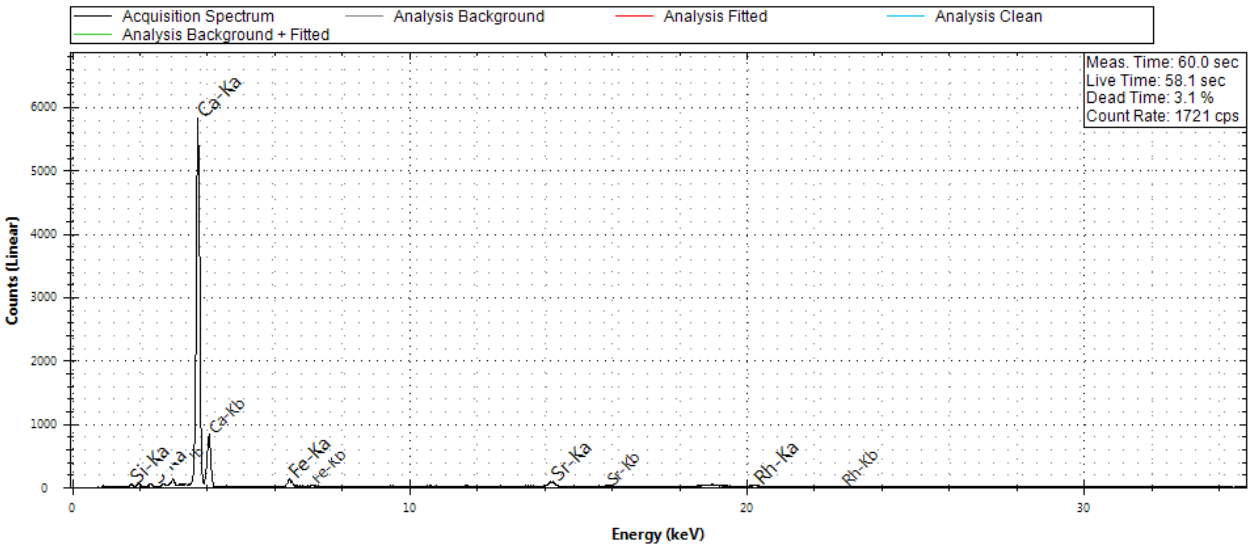

Figure 11. XRF spectrum of the gypsum white wash layer applied on the surface of the studied stela

As it is known for Egyptian mortars; the dominant component used in this culture is gypsum, in conclusion the ability to manufacture lime mortar by a calcination process was known by the Egyptians as well, although natural resources were scarce and the culture developed the use of an alternative, abundant material, gypsum which is more suitable for the arid Egyptian climate (Lucas 1962; Casagrande et al., 1997). Magnetite (Fe_3O_4) was found to be the black pigment used in the studied stela; XRF emphasized that (Fig. 12).

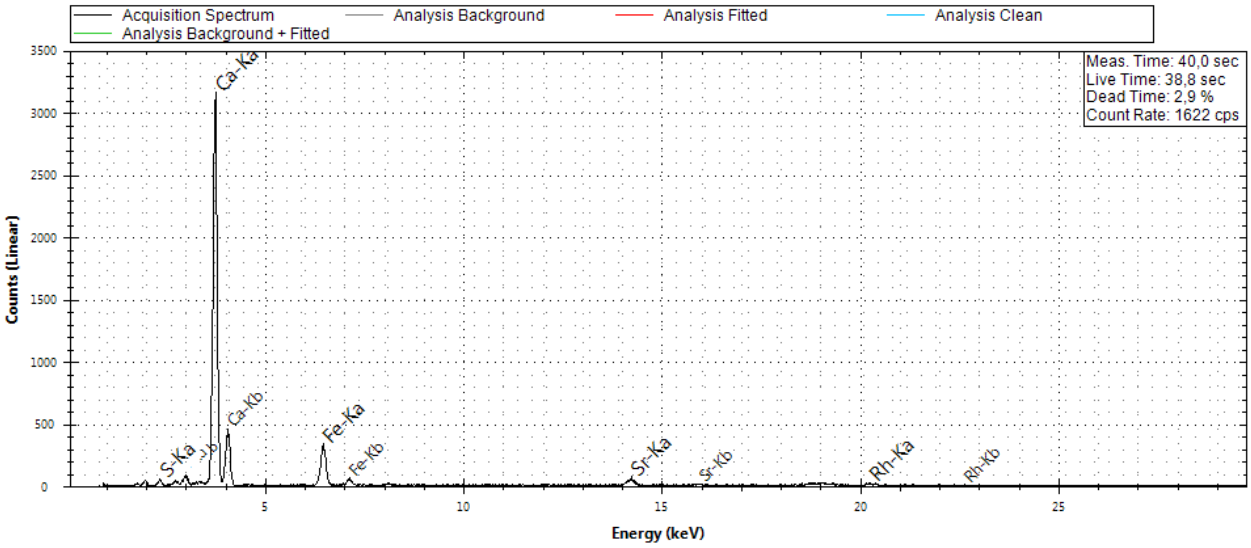

Figure 12. XRF spectrum of the black pigment material from the studied stela

Small grains of magnetite occur in almost all igneous rocks and metamorphic rocks. Magnetite also occurs in many sedimentary rocks, including banded iron formations. Magnetite is sometimes found in large quantities in beach sand. Magnetite reacts with oxygen to produce hematite (Pearce et al., 2006); magnetite was used as a black pigment in ancient Egypt after it was well grinded and mixed with paint medium. Yellow pigment in the studied stela (Fig. 13) is orpiment (As_2S_3).

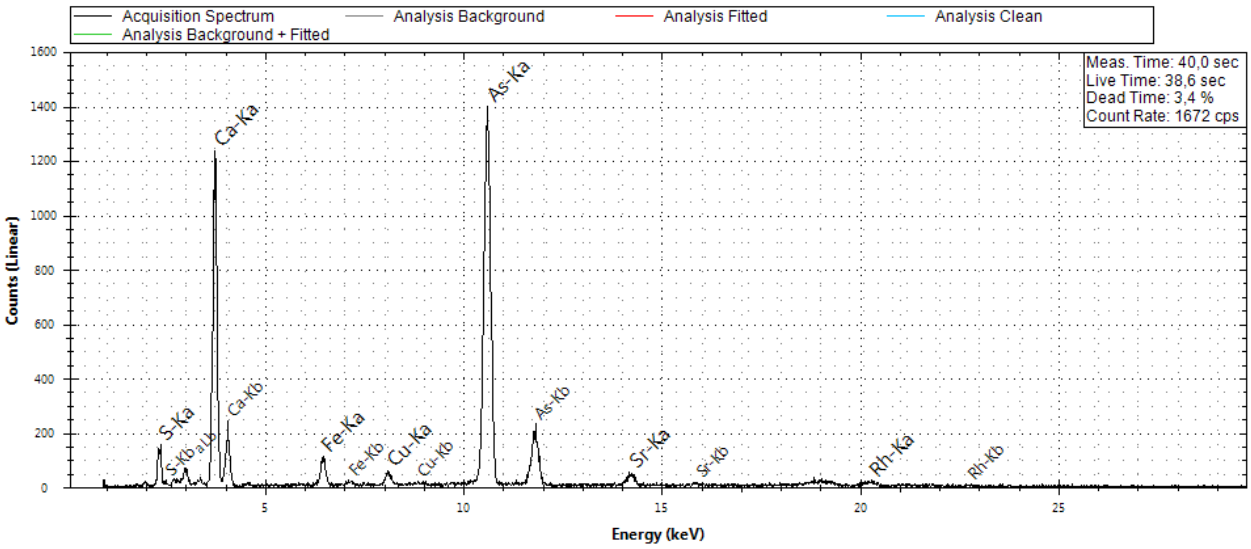

Figure 13. XRF spectrum of the yellow pigment material from the studied stela

This pigment has been used for centuries until the 19th century. The use of orpiment as a pigment ended almost entirely with the advent of the cadmium yellows, Orpiment is also known as "King's Yellow", "Chinese Yellow" and "Yellow Orpiment", in ancient Egypt; orpiment has been mixed with goethite for a bright yellow color (Noll 1978). The red pigment (Fig. 14) of the studied stela is hematite (Fe_2O_3). In ancient Egypt; this pigment has been used in a thick layer upon the gypsum white wash to have a good hiding power because the metal is very hard to be well grinded or to be good mixed with paint medium (Dang et al., 1998, Rozenberg et al., 2002 and Cornell et al., 2003).

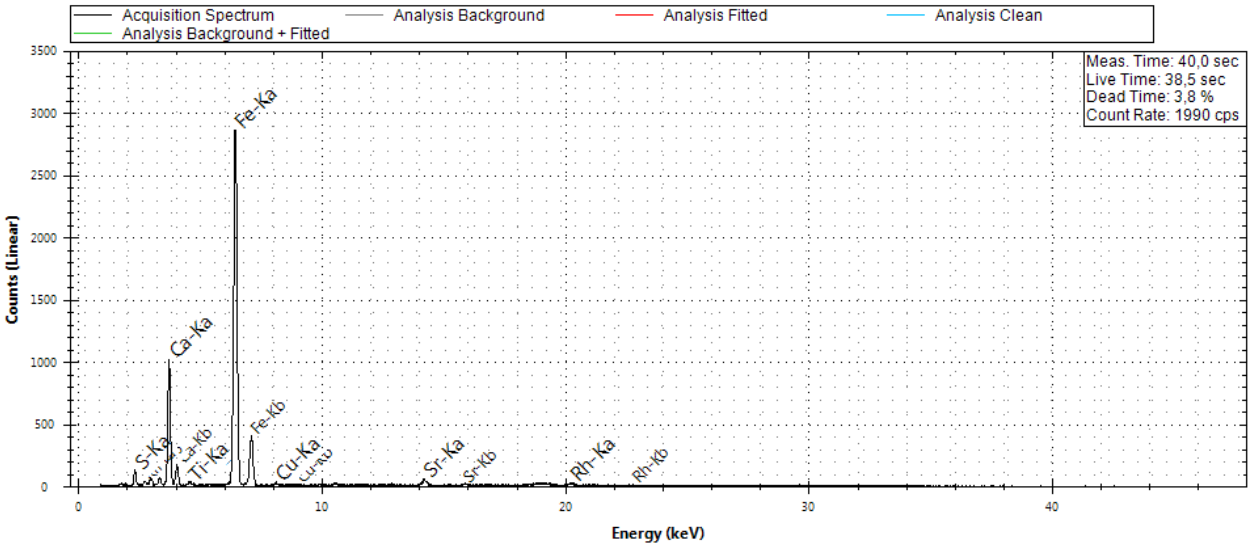

Figure 14. XRF spectrum of the red pigment material from the studied stela

Blue pigment from the studied stela was analysed by mean of XRF, the test proved that it is Egyptian blue "Cuprorivaite CaCuSi4O10" (Fig. 15), which is a synthetic pigment composed of various phases containing silica, copper and calcium, made by heating together silica, copper alloy filings or a copper such as malachite, lime (calcium oxide), and an alkali such as potash or natron which is found naturally occurring in Egypt in Wadi El Natrun (Moussa, 2007).

The green pigment in the studied stela is Egyptian green "Wollastonite CaCuSi3O9" according to the XRF analysis (Fig. 16).

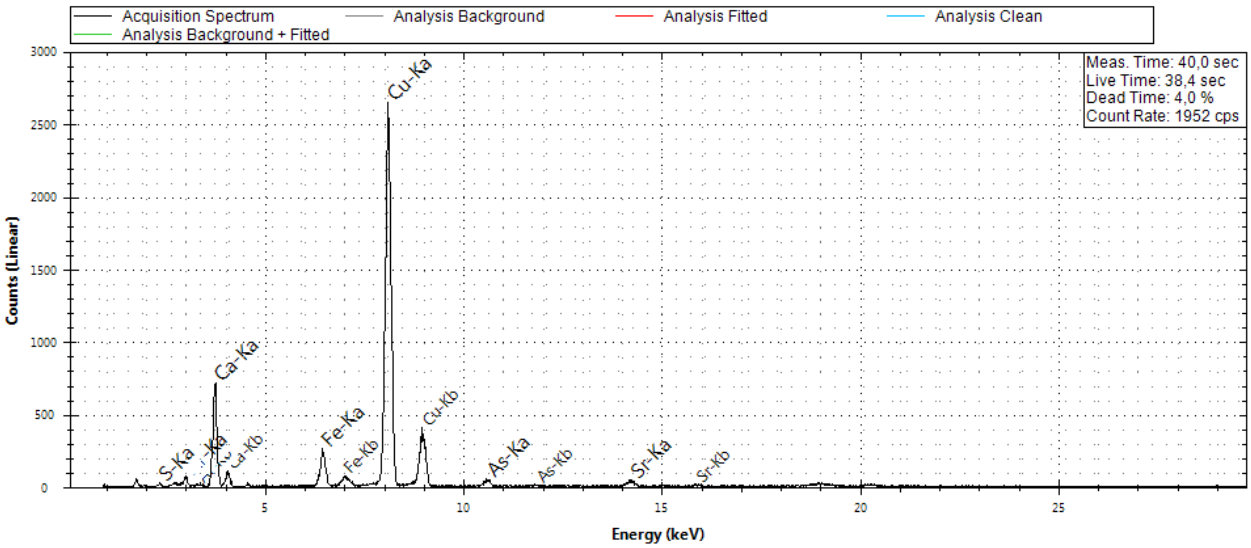

Figure 15. XRF spectrum of the blue pigment material from the studied stela

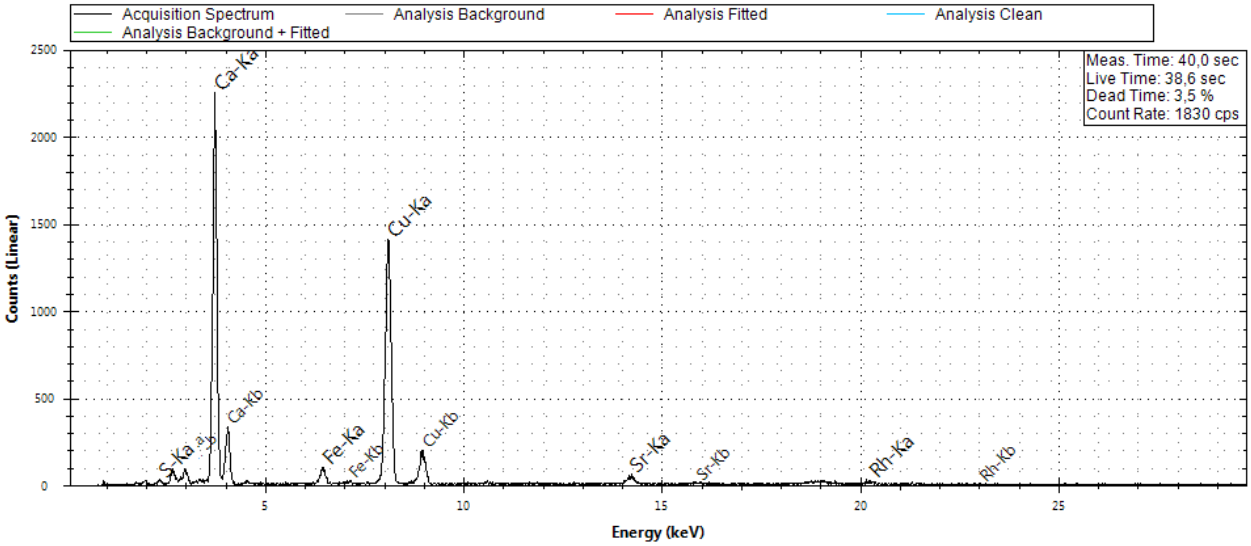

Figure 16. XRF spectrum of the green pigment material from the studied stela

The ancient Egyptians employed for the green color a synthetic material which is often termed "green frit". The major phases of this material are copper, sodium and potassium chlorides. It is made in reducing conditions by mixing similar ingredients as for Egyptian blue, but with higher lime, and lower copper content (Moussa et al., 2009). The results of the blue and green pigments were confirmed by mean of SEM-EDX, the two materials are Egyptian blue (Fig. 17) and Egyptian green (Fig. 18) respectively.

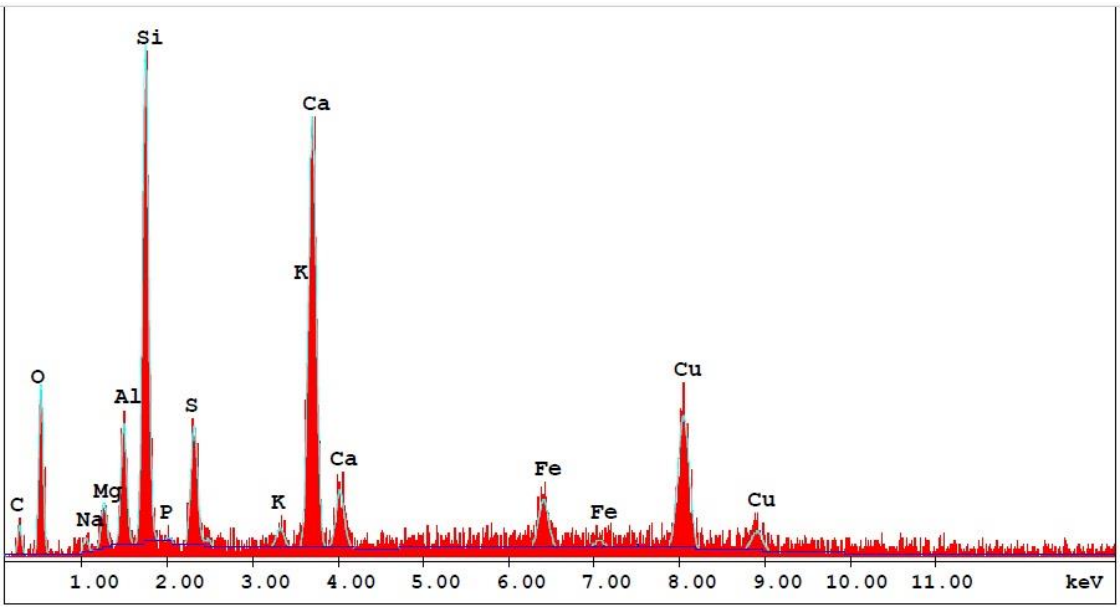

Figure 17. SEM-EDX spectrum of the blue pigment material from the studied stela

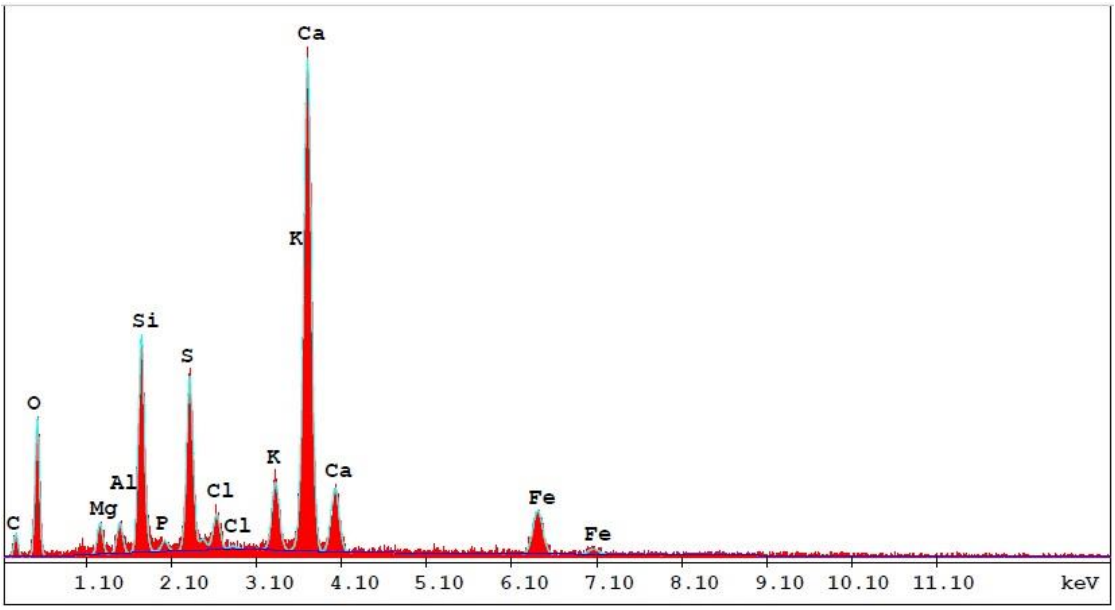

Figure 18. SEM-EDX spectrum of the green pigment material from the studied stela

3.3. Biological investigation

Limestone consists of a dense calcareous matrix allowing mainly a superficial microbial contamination, it may also contain a significant amounts of weathering-prone minerals (i.e., feldspars, clays and ferruginous minerals, >5% w/v) are particularly susceptible to the development of microorganisms. Even digenetic organic residues in sedimentary stones can be considered as possible nutrient sources for the stone-inhabiting microflora. Soluble nylon coatings which widely used in conservation and restoration, despite their name, are insoluble compounds not easily removed by physical and chemical treatments. Furthermore, it must be noted that bacteria were detected on the wall paintings treated with this product (Urzi 1999). Even polymers are potential substrates for heterotrophic microorganisms including bacteria and fungi (Gu 2003; Szaraz and Beczner, 2003). Concerning pigment materials; all previous authors studied the ability of micro species to grow upon them, but no attention was payed to their role in the discolouring phenomenon. Fungal and bacterial species which were swabbed from the surface of the stela were grown on a soil-yeast extract media which is suitable for the growth of most microbes; then they were incubated at 28-30 °C for 48 hrs. Bacteria were purified using streak plate method and grown on a nutrient agar (NA) medium. Fungal isolates need a medium characterized by its high concentration of sugary substances and carbohydrates, the best one is the potato

dextrose agar medium (PDA), fungi were purified by the growth upon the aforementioned medium abreast with the incubation at 27 °C for 72 hrs (Hemraj et al., 2013).

The final results showed that 7 fungal isolates and 9 bacterial isolates were obtained. To reveal the effect of these isolates (bacteria and fungi) on the Egyptian blue and the Egyptian green pigments, these species were grown on a mineral salt medium (Pitt 1979); it composes of: $CuSO_4$, KH_2PO_4, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, $FeSO_4$, the test depends basically on replacing ($CuSO_4$) as a source of copper with the two pigments (E.B and E.G), the medium was filtered until nothing left but the pigment and bacterial and fungal secretions only. An RPM (5000 c/m) was used for 10 minutes, with the aim to increase the precipitation value of the microbial secretions (acids and enzymes), the results of colour alteration were measured after one week, two weeks and three weeks (7, 14 and 21 days); using a spectrophotometer at 542 nm wavelength, the instrument was used to measure the colour spectrum of the medium and measuring the colour spectrum of the pigment residues as well as measuring the value of bacterial and fungal consumption of the pigment as a unique source of carbon and energy.

3.3.1. Egyptian blue

Concerning Egyptian blue; the results proved that the most effective bacterial species on its colour alteration are the isolate number (B4, B6 an d B7) as shown in Table 1 and Fig.19.

Table 1: The bacterial effect on the Egyptian blue pigment according to the spectrophotometer measurements

Isolates	1 Day	1week	2 weeks	3 weeks
Control	0.911	0.892	0.890	0.812
B1	0.908	0.537	0.352	0.322
B2	0.905	0.919	0.857	0.753
B3	0.912	0.781	0.704	0.692
B4	0.909	0.132	0.101	0.088
B5	0.913	0.739	0.553	0.479
B6	0.908	0.325	0.154	0.135
B7	0.911	0.355	0.268	0.120
B8	0.911	0.628	0.420	0.325
B9	0.904	0.669	0.593	0.578

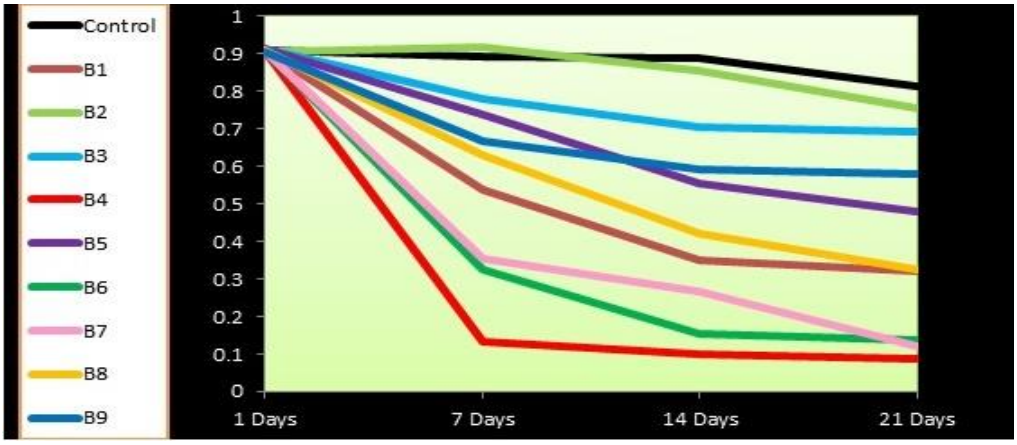

Figure 19. The bacterial effect on the Egyptian blue pigment according to the spectrophotometer measurements

Table 2: The fungal effect on the Egyptian blue pigment according to the spectrophotometer measurements

Isol.	1 Day	1 week	2 weeks	3 weeks
Control	1.116	1.106	0.838	0.838
F1	1.118	0.749	0.749	0.557
F2	1.100	1.149	1.149	0.998
F3	1.103	0.637	0.637	0.378
F4	1.098	0.768	0.768	0.638
F5	1.105	0.468	0.468	0.163
F6	1.116	0.942	0.942	0.766
F7	1.112	0.682	0.682	0.321

As for the fungal species and their effect on the Egyptian blue pigment; the results obtained from the spectrophotometer proved that the most effective isolates are those carrying number F3, F5 and F7; as shown in Table 2 and Fig.20.

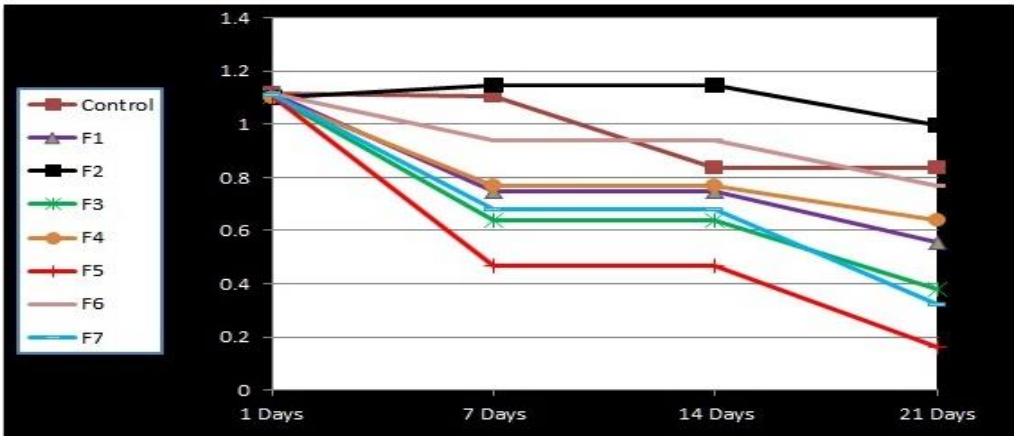

Figure 20. The fungal effect versus time on the Egyptian blue pigment according to the spectrophotometer measurements

Table 3: The bacterial effect on the Egyptian green pigment according to the spectrophotometer measurements

Isol.	1 Day	1 Week	2 Weeks	3 Weeks
Control	1.19	1.17	1.16	1.15
B1	1.13	1.01	0.79	0.69
B2	1.22	1.08	0.97	0.90
B3	1.13	1.03	0.80	0.54
B4	1.18	1.06	0.72	0.52
B5	1.20	1.07	0.87	0.72
B6	1.14	1.04	0.79	0.66
B7	1.13	1.01	0.74	0.69
B8	1.13	1.05	0.85	0.77
B9	1.18	1.08	0.98	0.87

3.3.2. Egyptian green

Concerning Egyptian green; the same test was applied and the results were almost the same, it has been proved that the most effective bacterial species on the Egyptian green colour alteration are the isolate number (B4, B6 and B7) as shown in Table 3 and Fig. 21.

As for the fungal species and their effect on the Egyptian green pigments; the results obtained from the spectrophotometer proved that the most effective isolates are those carrying number F3, F5 and F7; as shown in Table 4 and Fig. 22.

Table 4: The fungal effect on the Egyptian green pigment according to the spectrophotometer measurements

Isol.	**1 Days**	**7 Days**	**14 Days**	**21 Days**
Control	1.29	1.09	1.07	1.03
F1	1.22	1.05	0.76	0.65
F2	1.19	1.02	0.82	0.73
F3	1.23	1.04	0.66	0.44
F4	1.26	1.01	0.85	0.70
F5	1.11	1.00	0.67	0.42
F6	1.25	1.01	0.74	0.62
F7	1.15	1.03	0.65	0.42

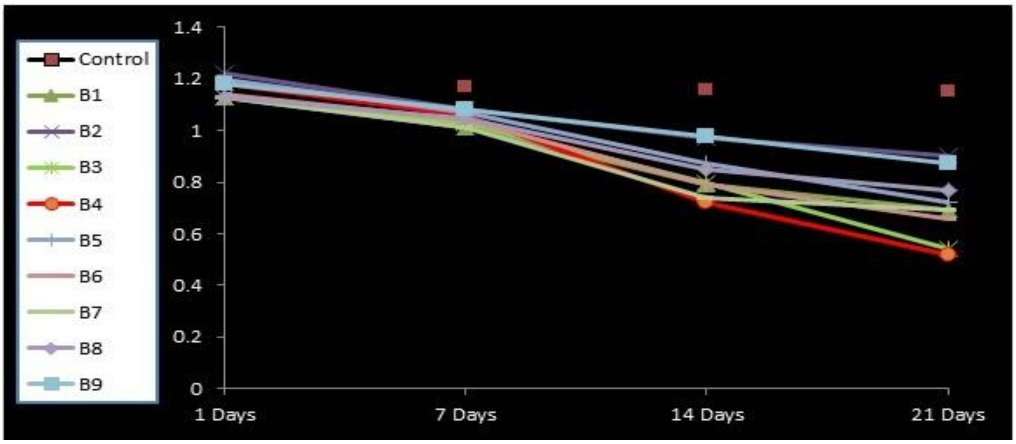

Figure 21. The bacterial effect versus time on the Egyptian green pigment according to the spectrophotometer measurements

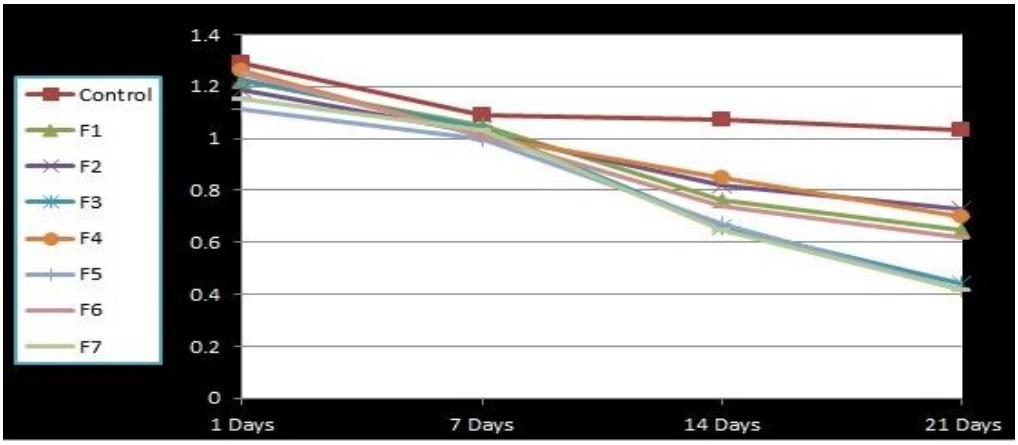

Figure 22. The fungal effect on the Egyptian green pigment according to the spectrophotometer measurements

The results obtained from the biological study and the spectrophotometer instrument proved that three fungal species are the most effective upon Egyptian blue and Egyptian green pigments among the isolated seven species, these fungi are F3, F5 and F7; while (F) refers to (Fungi) and the number refers to the isolate number, according to the identification process of these species; it was found that they belong to *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus Flavus* respectively (identification of fungi was achieved according to Ellis et al., 2007), while there were also three bacterial genus more effective on the Egyptian blue and the Egyptian green pigments than the isolated nine genus, these genus are B4, B6 and B7; while (B) refers to (Bacteria) and the number refers to the isolate number, the most effective bacterial isolates were streaked on nutrient agar plates and incubated at 28±2 °C for 48 hrs. After the incubation period, the colony of each isolate was examined for the general morphological characteristics namely, form of colony, elevation, margin, colour, surface, edge and transparency of colony, according to the identification process of these genus; it was found that they belong to *Pseudomonas taetrolens*, *Bacillus subtilis* and *Serratia rubidaea* respectively (identification of bacteria was carried out according to Bergey's Manual 1994 and Hemraj et al., 2013). The laboratory experimental work assured that the optimum biocide for both fungi and bacteria is marjoram oil (*Origanum vulgare* L. "Lamiaceae"), this oil possesses strong antibacterial and antifungal activities (Fikry et al., 2019), it has been used as solution composes of (2 cm^3 marjoram oil, 2 cm^3 acetone and 100 cm^3 distilled water), the biocide was applied using the spray technique, the biocide has no side effects related to aesthetic or chemical composition of the pigment materials in the studied stela.

4. CONCLUSIONS

This study proves that pigment materials kept in high controlled environments such as museums are also susceptible to the effect of bio-deterioration; especially the effect of fungal and bacterial species. Unlike the previous studies; this study concludes that there is a new reason for colour alteration of the Egyptian blue and the Egyptian green pigments that differs from the widely discussed by the previous authors; fungal and bacterial species uses the copper based-pigments as a nutrient and as a main source for carbon and energy leading to the chromatic alteration of these pigments.

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