Effectiveness of Fumigation using Carbon dioxide on the ancient Egyptian pigments applied on ancient wooden artifacts

فاعلية تكنيك التبخير بثاني أكسيد الكربون على الأصباغ والمواد المونة المصرية القديمة المطبقة على المشغولات الخشبية الأثرية

[1] Ahmed Khairy, [2] Elsayda-Nafesa El-Shamy

[1] Conservator at the Egyptian Ministry of Antiquities (ahmedkhgh@gmail.com)
[2] Conservation Department, Faculty of Archaeology and Tourism Guidance, Misr University of Science and Technology, Egypt (alsaydanafesa@hotmail.com)

Abstract

The sterilization of organic objects using Carbon dioxide is one of the safest methods which used in conservation of organic materials which eliminates the presence of insects and other aerobic organisms. The fumigation process with Carbon dioxide takes two to three weeks and requires a high levels of carbon dioxide “more than 60% (no matter how high the rate of the oxygen).

This study explains this sterilization method and evaluates the effectiveness of the method on the pigments and colors of an ancient Egyptian painted wooden ushabti-box, from the collection of the Egyptian museum of Tahrir to insure if it is safe and has no negative effect. Different analyses techniques have been used such Microscopic Examination for Identification of ancient wooden box, X-ray florescence (XRF) for Identification of Egyptian pigments, Change of color by spectrophotometer, in addition Visual Assessment, Documentation Process by High Resolution Camera, Scanner in Multispectral Imaging (MSI), Multi Spectral Imaging (VIS-UV-IR) and X-Ray Radiography have been applied for documentation procedures, also Isolation and identification of micro- organisms.

The results showed that the wooden Ushabti box depicts on the top a falcon squatting on a cartouche Dates back to Late Period dynasties which highly carved and decorated. It has a layer of Pigments: Yellow, Green, Blue, Red and Black. Microscopic Examination revealed that the wood was sycamore. X-Ray Florescence Spectrometry (XRF) showed that the black pigment has the element Carbon; the Yellow pigment has the elements calcium, iron, and arsenic. Change of color by spectrophotometer indicates that CO_2 has no effect on the pigments. This study proved from the recorded values that the sterilization process by using carbon dioxide does not affect or change the degrees of colors in which indicate that CO_2 has no effect on the pigments on the archaeological wooden box**.**

Keywords: Fumigation; Carbon Dioxide; Egyptian Pigments; wood; color change; sterilization; Ushabti

الملخص

يعد تعقيم المواد العضوية باستخدام ثاني أكسيد الكربون من أكثر الطرق أمانًا التي تستخدم في الحفاظ على المواد العضوية والتي تقضي على وجود الحشرات والكائنات الهوائية الأخرى. تستغرق عملية التبخير بثاني أكسيد الكربون من أسبوعين إلى ثالثة أسابيع وتتطلب مستويات عالية من ثاني أكسيد الكربون "أكثر من 60٪ (بغض النظر عن مدى ارتفاع معدل الأكسجين). تتناول الدراسة شرح طريقة التعقيم هذه وتقييم فعاليتها ومدى تأثيرها على الأصباغ والألوان المصرية القديمة المتواجدة على بصندوق أوشابتي خشبي مطلي، من مجموعة المتحف المصري بالتحرير بهدف التأكد من أنها آمنة وليس لها أي تأثير سلبي.

تم استخدام تقنيات التحليل المختلفة مثل الفحص المجهري، وتفلور الأشعة السينية (XRF)، والتغير اللوني، بالإضافة إلى التقييم البصري، وطرق التوثيق المختلفة بواسطة كاميرا عالية الدقة، والماسح الضوئي متعدد الأطياف، ايضا التصوير متعدد الأطياف بالأشعة تحت الحمراء وفوق البنفسجية، وكذلك تم عزل وتعريف الكائنات الحية الدقيقة. أظهرت النتائج أن بصندوق الأوشابتي الخشبي والمصور في اعلاه بصقر جالسا على خرطوش، يعود تاريخه إلى عصر الدولة المتأخرة والذي تم نحته وتزيينه بشكل عالي الدقة وعليها طبقة من المواد الملونة: أصفر، أخضر، أزرق ، أحمر ، أسود.

وقد كشف الفحص المجهري أن نوع الخشب المستخدم هو جميز. كما اظهرت قياسات تفلور الأشعة السينية (XRF)أن المادة الملونة سوداء اللون تحتوي على عنصر الكربون؛ بينما تحتوي المادة اللونية الصفراء على عناصر الكالسيوم والحديد والزرنيخ. كما اشارت قياسات التغيير اللونى إلى أن ثاني أكسيد الكربون ليس له أي تأثير سلبي على الأصباغ والمواد الملونة. ومن هنا فقد أثبتت هذه الدراسة من القيم المسجلة خلال التحاليل الى أن عملية التعقيم باستخدام ثاني أكسيد الكربون ال تؤثر وال تغير درجات الألوان مما يشير إلى عدم تأثير استخدام التعقيم بغاز ثاني أكسيد الكربون على الأصباغ والمواد الملونة الموجودة على الصندوق الخشبي الأثري.

الكلمات الدالة:التبخير، ثاني اكسيد الكربون، المواد الملونة المصرية، الأخشاب، التغير اللوني، التعقيم، الأوشابتي

1. INTRODUCTION

Carbon dioxide, which is non-toxic in low concentrations and non-flammable, is regarded as one of the inert and inexpensive gases that can be widely used. To ensure the long-term survival of culturally significant materials, conservation professionals employ a number of different preventative and intervention techniques. In museums, preventative techniques are typically used to stabilize a range of dry and fragile materials. CO_2 is one of the most commonly used. CO_2 is non-toxic at low concentrations, non-flammable, inexpensive, inert, and recyclable resource[1]

Dry wood exposed to insect activity and environments of fluctuating humidity is at the highest risk of degradation. In galleries or museums maintaining and controlling a stable environment is a key factor in the preservation of wooden items. Therefore conservators often place wood in an oxygen purged environment, impeding the colonisation of aerobic organisms[2]

[1] Montanari, L., Fantozzi, P., Snyder, J. M. & King, J. W. (1999). Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol. The Journal of supercritical fluids, 14, 87-93

[2] Blanchette, R. A. (2000). A review of microbial deterioration found in archaeological wood

Controlling a stable environment is critical for preserving dry wood and protecting it from the high risk of degradation caused by fluctuating humidity and temperature. This is why conservators decided to use a free oxygen environment to kill and stop the growth of aerobic organisms in some cases. Determining the most secure method of preserving cultural holdings was by many methods and techniques have been developed by conservators. Because of its many advantages, they tended to sterilise it using inert gases[3]

A fumigant is a chemical that, at the proper temperature and pressure, can exist as a vapor or gas that, when released, penetrates objects or enclosed areas in lethal concentrations to pest organisms. This allows them to penetrate the fumigated material and diffuse away afterwards.

Fumigation techniques are extremely adaptable in pest control. They can be used to control wood-destroying insects in structures and furniture where liquid or dust formulations are ineffective or cause damage. Fumigants can be used in some situations to control burrowing rodents that other methods cannot reach. Localized infestations, as the complexity of the infestation grows, it becomes impossible to get the chemical to the pest unless fumigation is used. Carbon dioxide works in a way that it obliterates the breathing cells in insects and prevents oxygen from reaching them even if the oxygen is present at higher levels than normal, so it kills insects by suffocation, its method is different from nitrogen or other inert gases. The suffocation here is because the gas blocks and prevents oxygen from reaching the breathing cells, and not because of the lack of oxygen in the tent or the sterilization environment as in the case of nitrogen[4]

The standard methods of fumigation used to be used are steam, gamma irradiation, ethylene oxide, and hydrogen peroxide sterilization. As previously stated, each method has drawbacks in specific applications. Because of its low cost and effectiveness, steam sterilization is the most commonly used technique.

Steam sterilization, on the other hand, operates at 121 ˚C, causing heat-sensitive materials to be damaged or destroyed. Furthermore, steam sterilization may deposit an oxide layer on metallic devices, reducing the biocompatibility of the treated implants. Irradiation and ethylene oxide sterilization, unlike steam sterilization, can be used on heat-labile materials. On the other hand, irradiation may cause changes in the shear and tensile strength, elastic modulus, and transparency of medical polymers. Ethylene oxide is a flammable and toxic substance that is also a known carcinogen and can cause hemolysis. Ethylene oxide sterilization can also change the chemical properties of materials.

Spilimbergo and Bertucco have summarized the biocidal effects of high-pressure carbon dioxide (CO_2) on various species of bacteria[5]. There are several advantages to using CO_2 as a

from different environments. International Biodeterioration & Biodegradation, 46, 189-204.

[3] Montanari, Opcit. , 92

[3] Montanari, Opcit. , 92

[4] Scott D. Cichowlaz, Fumigation, Volume 5, 2005, pp.7

[5] Spilimbergo, S., A. Bertucco, Non-thermal bacteria inactivation with dense CO_2, Biotechnol. Bioeng, 84, (2003) 627

sterilizer. First, CO_2 is not flammable or toxic; asphyxiation is the leading risk associated with its use. CO_2, unlike ethylene oxide, does not require special handling or ventilation and emits no toxic byproducts. Second, in most cases, CO_2 is inert[66]. CO_2 has a low viscosity (3–7 105 Nsm2) and zero surface tension, allowing it to quickly penetrate complex structures and porous materials. Because CO_2 is cheap and readily available, switching to CO_2-based sterilization is economically feasible. CO_2 is, in a nutshell, inexpensive, non-toxic, non-flammable, physiologically safe, and has a low critical temperature. As a result, high-pressure CO_2 sterilization may be an option for heat-sensitive and porous material sterilization. Compared to pesticides or other environmental pollutants, the percentages used have little effect. Because the CO_2 used can be recycled from other existing industrial processes, it will not negatively impact global warming or ozone layer depletion[7]

Spores are highly resistant to heat, chemicals, and radiation. Extreme temperatures (121 ˚C steam), UV radiation, or highly oxidative chemicals, such as ethylene oxide, are used for sterilization. The standard assay for testing sterilization equipment is spore survivability[8]

Bacterial cells and spores are intricate chemical systems of organic and inorganic components. When the variety of growth media is considered, high-phase CO_2 processing becomes very complex. Temperature, depressurization rate, pressure cycling, treatment time, cell concentration, cell growth phase, and agitation have all been studied. Temperature and pressure are the most critical factors influencing microorganism growth. Each microorganism has a maximum temperature that is unique to its species. Proteins denature at that temperature, cytoplasmic membranes collapse, and cell lyses are inactivated[9]

Carbon dioxide (CO_2) fumigation was investigated to control bed bugs, Cimex lectularius L. The effect of bed bug developmental stage, temperature, and CO_2 concentration on the minimum time required to kill 100% of bed bugs was studied. With a 24-hour exposure time at 25˚C, the minimum CO_2 concentration lethal to all bed bug stages was approximately 30%.

---

[6] McHugh, M., V. Krukonis, Supercritical Fluid Extraction, Butterworth–Heinemann, USA, 1993; Dillow, A.K., F. Dehghani, J.S. Hrkach, N.R. Foster, R. Langer, Bacterial inactivation by using near- and supercritical carbon dioxide, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 10344; Lausmaa, J., B. Kasemo, S. Hansson, Accelerated oxide growth on titanium implants during autoclaving caused by fluorine contamination, Biomaterials 6 (1985) 23; Spilimbergo, S., A. Bertucco, Non-thermal bacteria inactivation with dense CO_2, Biotechnol. Bioeng. 84 (2003) 627.

[7] McHugh, M., V. Krukonis, Supercritical Fluid Extraction, Butterworth–Heinemann, USA, 1993; Matthews, M.A., L.S. Warner, H. Kaiser, Exploring the feasibility of using dense-phase carbon dioxide for sterilization, Med. Device Diagn. Ind. 5 (2001) 140; Cooper, A.I., Recent developments in materials synthesis and processing using supercritical CO2, Adv. Mater. 13 (2001) 1111.

[8] Tortora,] G.J. , B.R. Funke, C.L. Case, Microbiology, An Introduction, Pearson Education, USA, 2002

[9] Madigan, M.T., J.M. Martinko, J. Parker, Brock Biology of Microorganisms, Prentice Hall, USA, 2002.

The minimum fumigation time required to kill 100% of eggs using 100% CO_2 at 20, 25, and 30 degrees Celsius was 3, 7, and 8 hours, respectively; the minimum fumigation time required to kill 100% of adult males/nymphs was 8, 13, and 14 hours, respectively. The minimum time required to kill 100% of adult males/nymphs using 50% and 70% CO_2 at 25 degrees C was 18 and 16 hours, respectively[10]. Temperatures ranging from 0 ˚C to 100 ˚C have been used for high-pressure CO_2 treatment[11]

It is believed that higher temperature enhances deactivation by (a) increasing the fluidity of cell membranes, making them easier to penetrate, and (b) increasing the diffusivity of CO_2.

However, higher temperatures may reduce the ability of CO_2 to extract low-volatility materials and decrease CO_2 solubility in aqueous media. The CO_2-high-pressure method is an environmental oriented disinfection-method and can be considered as a replacement for CH_3Br and other pesticide-chemicals for disinfection. Within our demonstration project, CO_2 being used as an inert gas, under high pressure, in an airtight pressure chamber, to expel the oxygen: vital to harmful vermin/insects. The maximum pressure used during disinfection will be 30 bars. The CO_2-gas will penetrate into the insects, germs, vermin and their eggs, which will kill them. The final pressure-release will disinfect the total product finally including eggs. This guarantees 100% mortality on all insects, germs and vermin. The CO_2-highpressure method is considered by TNO as the most reliable replacement for CH_3Br[12]

Carbon dioxide is commonly used in museums to successfully eradicate insects as an alternative to methyl bromide and toxic insecticide. Insect inhibition and control efficacy for treatment is dependent on insect species, oxygen concentration, temperature, relative humidity, and gas. Within one month, biological deterioration in a museum can be kept below 0.03 percent oxygen concentration or 60 ~ 75 percent Co_2 in temperature and relative humidity. 40-60% of the museum environment is used, and various systems such as bag, tent, bubble, and chamber are used depending on the size and quantity of objects[13]

Historically, painted wooden materials include a wide range of pigments, and binders which

[10] CHANGLU WANG, LIHUA LU¨ , AND MING XU, Carbon Dioxide Fumigation for Controlling Bed Bugs, VECTOR CONTROL, PEST MANAGEMENT, RESISTANCE, REPELLENTS, 2017.

[11] Nakamura, K., A. Enomoto, H. Fukushima, K. Nagai, M. Hakoda, Disruption of microbial-cells by the flash discharge of high-pressure carbondioxide, Biosci. Biotechnol. Biochem. 58 (1994) 1297; Roskey, C.T. , A. Sikes, Effect of hyperbaric carbon dioxide on spores and vegetative cells of Bacillus stearothermophilus, NATICK/TR-94/019 (1994); Sikes, A., C. Martin, Control of thermophilic spore activity with pressurized CO_2 and egg- white lysozyme, Report, NATICK/TR-95/020 (1995).

[12] Lin, H.M. , Z.Y. Yang, L.F. Chen, Inactivation of Leuconostoc dextranicum with carbon dioxide under pressure, Chem. Eng. J. 52 (1993) B29; Hong, S.I., W.S. Park, Y.R. Pyun, Inactivation of Lactobacillus sp. from kimchi by high pressure carbon dioxide, Food Sci. Technol.-Lebensm.- Wiss. Technol. 30 (1997) 681

[13] Ahmed Khairy "The Effectiveness of fumigation by Carbon dioxide and Nitrogen on the ancient Egyptian painted wooden objects" Applied on a selected one, Master's Thesis, Cairo University, 2021

represent in different environmental conditions highly nutritional material for microorganisms and insects. So the authors use carbon dioxide on the ancient Egyptian pigments applied on ancient wooden artifacts to evaluate the effectiveness of this technique of fumigation. Also Emphasis, after practical experience, that sterilization by carbon dioxide is safe on the colors found on most of the various archaeological collections, and not only that it plays a role in terms of sterilization in killing insects and microorganisms, because there are multiple previous studies in that because the sterilization process is applied in many places in the world, but here the proposed research has not been studied before.

Therefore, the research tests the extent of the effect of carbon dioxide gas in the world using standard ratios. Of course it is naturally present in the environment at a rate of 0.03%, but the sterilization process reaches a rate of 75-60%, so it was proved that this percentage is safe for the colors applied to the old wooden antiquities, and we discovered with experimental results that it is safe by 100% on the colors, and from here it can be applied to any other archaeological wooden objects colored in with any of the colors studied in the research.

2. MATERIALS AND METHODS

2.1. Visual Assessment, Documentation Process

Visual Assessment, Documentation Process by High Resolution Camera, Scanner in Multispectral Imaging (MSI), Multi Spectral Imaging (VIS-UV-IR) and X-Ray Radiography. Small wooden box contains unidentified solids. The box is housed at the Egyptian Museum in Cairo (second floor, hall number 22, inventory number J 28846(. That placed as one of the official burial rituals in ancient Egypt.

Canon 600D, 18-55 lens was used as High Resolution Camera for documentation process.

Multispectral Imaging (MSI) is used to capture images through narrow band filters on consecutive wavebands of radiation. It is a valuable time saving tool that allows for preliminary assessment to observe an object, by selecting wavelength ranges on the electromagnetic spectrum. Multispectral Imaging is a helpful technique that uses frequencies across the electromagnetic spectrum to differentiate between and occasionally identifying materials. X-ray radiography is a non-destructive fast and flexible method technique that is widely used in archeological studies. It is able to reveal the internal structure of objects due to the unique induction of X-rays as they pass through the story.

2.2. Microscopic Examination

The authors used microscopic examination for identification of ancient wooden box light optical used to separate different cell types and their arrangements by using the right techniques, such as SEM microscopes are used which can be efficient and accurate in identifying wood.

Investigation of the surface morphology was examined using scanning electron microscope (SEM) using a JEOL-JXA-840A electron probe micro analyzer- Japan. Samples were tested at National Research Center, Dokki, Giza, Egypt.

2.3. X-Ray Florescence (XRF)

XRF is a non-destructive method of analysis and is used for identifying predominant elements in a pigment sample. It is a surface technique and will only detect elements presented on the surface (GOFFER, 2007). For the identification of Egyptian pigments by X-ray florescence (XRF) the authors used X-ray Fluorescence analysis conducted using a portable EDXRF spectrometer (Elio Spectrometer, XGlab srl, Milan, Italy). The instrument can detect elements from Na to U, with a field of analysis extending between 1 and 50 keV. X-ray radiation is generated using a Rh tube, with an electron accelerating voltage from 10 to 50 kV and a filament current from 5 μA to 200 μA.

Fig.1. Showing identification of Egyptian pigments by X-ray florescence (XRF)

2.4.Microbiology investigation

Microbiological investigations were carried out in the Microbiological Laboratories of the American University in Cairo (AUC). The isolation process carried out using swab technique, and then saved in closed plastic bags to culture them over a nutrient media. A chamber frame associated with the UV lamp was used for all purification process.

Fungi Growth Medium For the isolation and purification of fungal spores, yeast extract media (Sigma Aldrich) was obtained 20g/L of yeast extract, 150 g/L of Sucrose, and 20 g/L of agar in distilled H_2O. Autoclave used for sterilizing the media at 121°C for 15minutes, under 1.5 Pa.[14]

To isolate and purify fungal spores, yeast extract media was obtained and then examined

[14] SCOTT, P. M. , J. W. LAWRENCE, AND W. VAN WALBEEK, Detection of Mycotoxins by Thin-Layer Chromatography: Application to Screening of Fungal Extracts, APPLIED MICROBIOLOGY, Nov. 1970, p. 839-842 Vol. 20, No. 5 American Society for Microbiology, Printed in U.S.A.; Lenka Jeszeová1,Andrea Puškárová1,Mária Bučková,Lucia Kraková1, Tomáš Grivalský, Martin Danko, Katarína Mosnáčková, Štefan Chmela, Domenico Pangallo, Microbial communities responsible for the degradation of poly(lactic acid)/poly(3-hydroxybutyrate) blend mulches in soil burial respirometric tests, World Journal of Microbiology and Biotechnology (2018) 34:101 https://doi.org/10.1007/s11274-018-2483

under a light microscope using "the Johnson slide culture method of cultivation" according to their morphology and spores structures[15]. But recently all fungi identification can be done only through DNA analysis.

Fig.2. Showing swapping process and isolation of micro-organisms from different parts of the archaeological objects.

2.5. Fumigation using Carbon dioxide

The sterilization of organic objects using Carbon dioxide is one of the safest methods which used in conservation of organic materials which eliminates the presence of insects and other aerobic organisms. The dimensions of tent after building the frame,
including the wooden box was 100x80x140cm^3. Carbon dioxide has a suffocation effect on pests and micro- organisms infecting the organic objects, the main idea of this methodology is to increase the CO_2 percentage from its volume in the air 0.03% to reach higher than 60% in the controlled environment. The authors used Carbon Dioxide CO_2 Gas cylinders (99.9 % of dry Carbon Dioxide CO_2 in 250 bars cylinders containing about 15Kg of gas at atmospheric pressure from El-Nasr co for intermediate chemicals, Giza, Egypt, Supplied with flow meter) and Carbon Dioxide CO_2 Gas regulator (FCR-50N Crown YUTAKA two stage carbon dioxide regulator and gauges with heater and a built-in carbon dioxide flow meter).
ESCAL film (Polyethylene sheets) was surrounding the framed box and sealed (Sealer Impulse Heat Sealer, HAWO HPL ISZ 300, 450, 630mm, Valdamark Direct Supplies Impulse heat sealer machines that are perfect for welding air proof polyethylene packaging, PVC, polyethylene and polyamide). In addition a few meters of plastic tubes and some valves used to make gas connections with foam strips (any kind of solid foam) is used to build and shape the tent.
Three plastic jars (3 liters, for humidification process of the dry gas comes from the gas cylinders) comprehends the first one has 60% of water. The last jar which contains a thermal hygrometer is connecting with the bubble (120x90x90 cm^2 bubble box) for taking in the humidified gas. The gas will be blown into the tent till filling it, then using the vacuum to take the air from inside. Measurement of the Co_2 level is the main process should be repeated

[15] Harrigan W.F. and M.E. Mc Cance, Laboratory Methods in Microbiology, Academic Press (1966)

till the Co_2 rate reaches the target level.

Two holes were made, one on the side, to allow gas to enter the 'tent', and another on the top to allow air to escape.

Also authors used Data logger (Electronic Thermometer hygrometer Model HTC -1)

- Temperature measurement range: -50 °C ~ 70 °C (14 °F ~ 122 °F)
- Temperature measurement accuracy: ± 1 °C (1.8 °F)
- Temperature resolution: 0.1 °C (0.1 °F)
- Humidity measurement range: 10% RH ~ 99%
- RH Humidity measurement accuracy: ± 2% Dimension: 10 x 10.8 x 2 cm. white.

It is faster and easier to replace 60-80% of the air with carbon dioxide than with oxygen. It is preferable not to use carbon dioxide gas with small-sized containers, many museums use large containers with carbon dioxide in a way that is easy to reuse again, as well as the cost of carbon dioxide being low. The temperature and humidity must remain in the safe range for the organic materials under sterilization, during the 15 days of the process duration. Authors measured CO_2 concentration measurements results by COSMOS XP-3140 CO_2 meter.

On the first day, the 'tent' was sealed and contained the object, data logger, and ambient air. Co_2 saturation inside the 'tent' was 0.03% as the normal percentage in the air, after the start of injection of Co_2, then; the museum vacuum was used to extract

the air inside the tent. The Co_2 level increased to 8 %. This process was repeated, and measurements were taken. The 'tent' was monitored, and measurements were taken daily over two weeks' recording the readings of temperature: Max 23 Min 18, RH: Max 53% Min 48%.

Co_2: Max 76% Min 73 %.

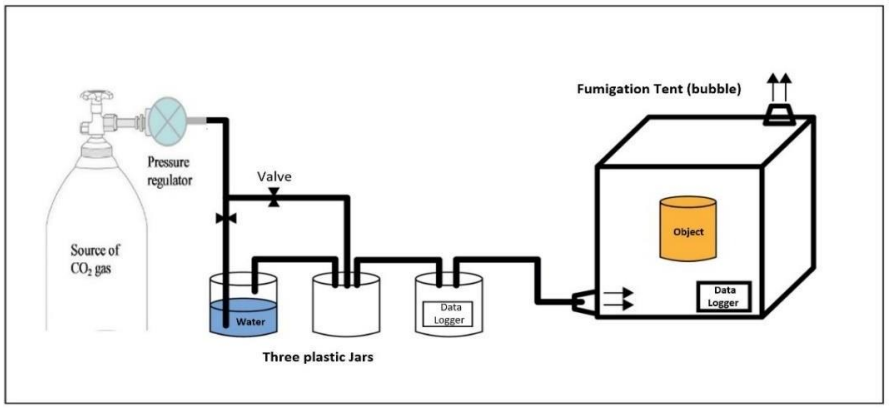


Fig.3. Showing a schematic diagram of the built tent

2.6.Change of color by spectrophotometer

The color change of the pigments studied was measured by a spectrophotometer, precise color reader – WR-10QC model, from the FRU Company. The total color difference ΔE* between two color stimuli $\Delta E = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]^{1/2}$ where the colors are given in CIE Lab coordinates, L* corresponding to the brightness (100

= white, 0 = black), a* to the red-green coordinate (positive sign = red, negative sign

=green), and b* to the yellow-blue coordinate (positive sign = yellow, negative sign = blue)[16]

[16] Wyszecki, G., Stiles, W. S., 2000, Color Science Concepts and Methods. Quantitative Data

RESULTS AND DISCUSSION

The techniques used in this study demonstrated the effect on the evaluated pigments. The majority of sterilization methods eliminated insects and other aerobic organisms. The experimental study proved that there is no clear change happening to the pigments during its exposure in the fumigation processes. The results revealed that there are no changes in color after sterilization which indicates that CO_2 has no effect on the pigments on the archaeological wooden box**.** With the recorded values, it is clear that the sterilization process using carbon dioxide does not affect or change the degrees of colors or the colored materials found on the old wooden antiquities. CO_2 is safe to apply even on the sensitive components and artifacts, specifically the ancient pigments.

2.7. Visual Assessment, Documentation Process by High Resolution Camera, Scanner in Multispectral Imaging (MSI), Multi Spectral Imaging (VIS-UV-IR) and X-Ray Radiography:

The wooden Ushabti box depicts on the top a falcon squatting on a cartouche. On the other four sides is the name of the person, Ba-sou- her as Sahar. Its dimensions are
47 x 25 x 25 cm and Dates back to Late Period dynasties which discovered from Luxor excavations and preserved in the Egyptian museum in Tahrir under the num. of 9096/J28849 (Fig.4). It is believed to contain preserved wrapped unidentified human organs. It is highly carved and decorated. It has a layer of Pigments: Yellow, Green, Blue, Red and Black.

Fig.4. Showing visual assessment, documentation process by high resolution camera of the wooden box

The images obtained in ultraviolet fluorescence (Ultraviolet wave fluorescence between 320-380) can reveal pigments that appear identical to the naked eye and converts them into colours and different shades.

and Formulae, Second Edition, JohnWiley & Sons, Inc. New York.

Visible Light Induced Luminescence (VIL-IR) photos reveal Egyptian blue. Blue pigment one of the studied colors, in the study show broad band emission in the near infrared range (NIR). The inorganic Egyptian blue Pigment belongs to the group of layer silicates, where their solid blue body color is derived from Cu^{2+}.

Radiograph comparisons are determined by the radiation intensity and chemical composition, and size of the object examined. It is very important in the evaluation of cultural objects.

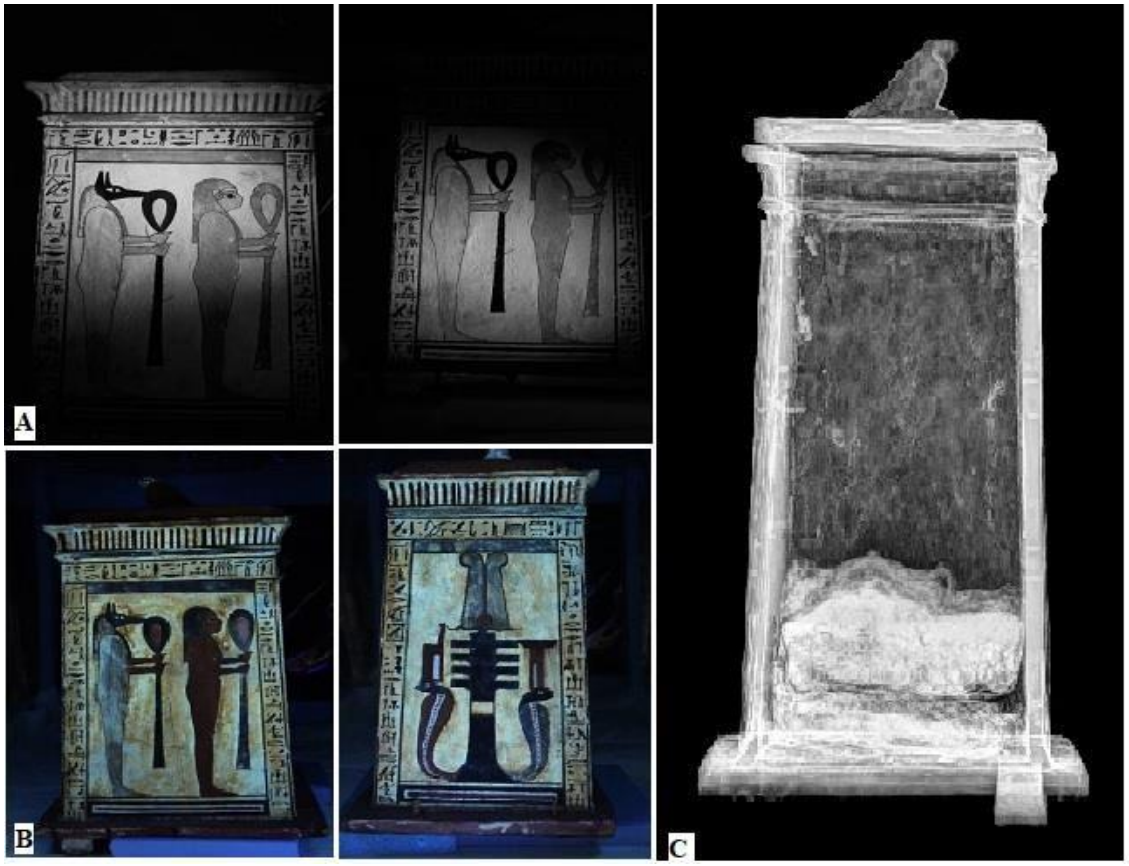


Fig.5. A: Showing Scanner in Multispectral Imaging (MSI); B: Multi Spectral Imaging (VIS-UV-IR); and C: X-Ray Radiography of the wooden box.

2.8. Microscopic Examination for Identification of ancient wooden box

Each type of wood has a unique anatomical structure that creates a difference in wooden structures and ultimately determines the suitability of a particular use. The results revealed that the type of wood of the archaeological object which examined was sycamore figs.6, 7.

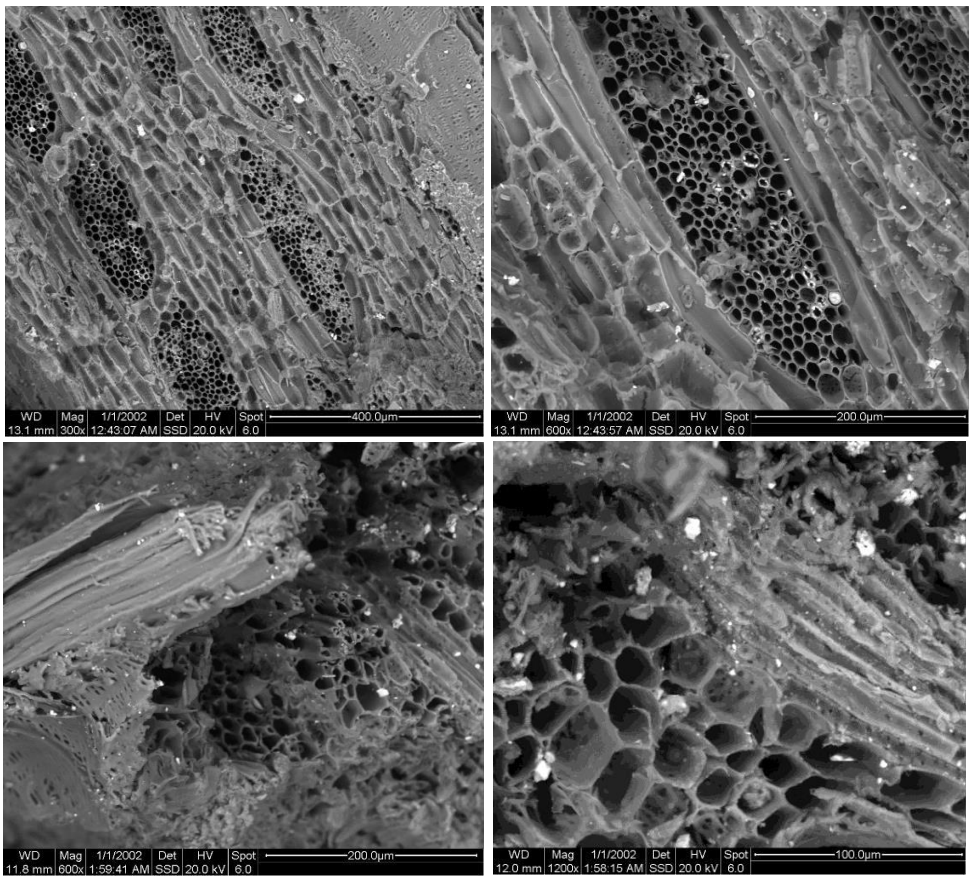


Fig.6. showing Microscopic Examination for Identification of ancient wooden box by SEM microscope

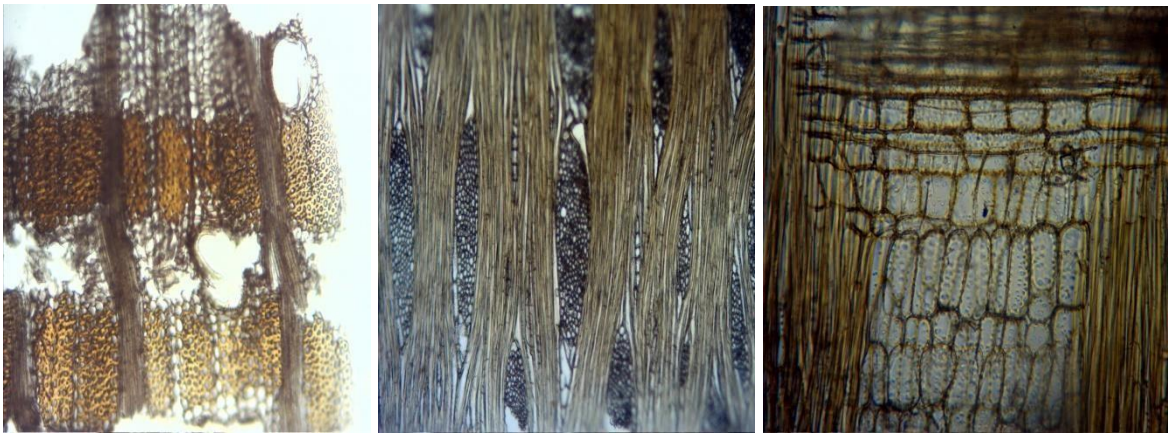


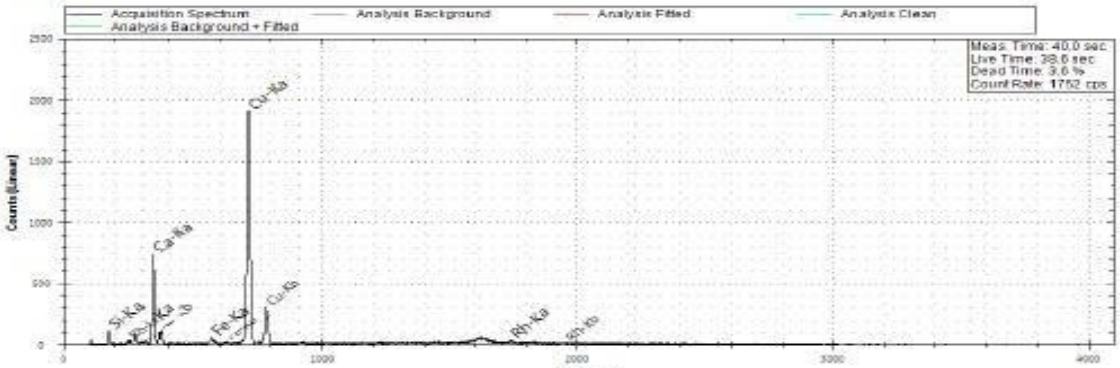
Fig.7. showing Microscopic Examination for Identification of ancient wooden box by light optical microscope

2.9. Identification of Egyptian pigments by X-ray florescence (XRF)

X-Ray Florescence Spectrometry (XRF) used to characterize pigment components fig.8 The results showed that the black pigment has the element Carbon; the Yellow pigment has the elements calcium, iron, and arsenic; the Green pigment has the element copper mixed with calcium; the Red pigment has the elements iron and calcium; the Blue pigment based on copper, which produces the color known as Egyptian blue Shown in table (1)

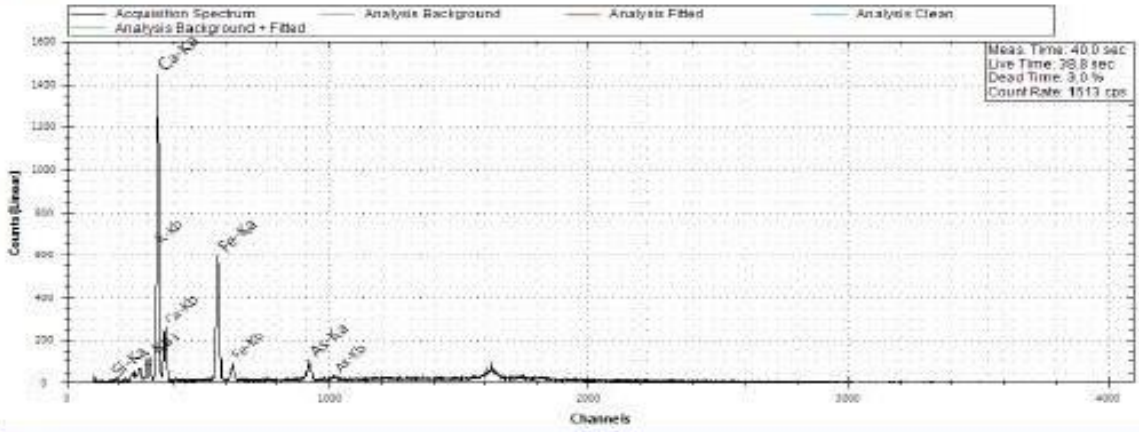
Measurement Time: 40,0 s
Tube Voltage: 40 kV
Tube Current: 20 µA
Tube Target Material: Rh
Elio Device: SN177
Device Mode: Head
Acquisition Mode: Manual
Acquisition Channels: 4096
Sample to Detector Material: Air

Spectrum:



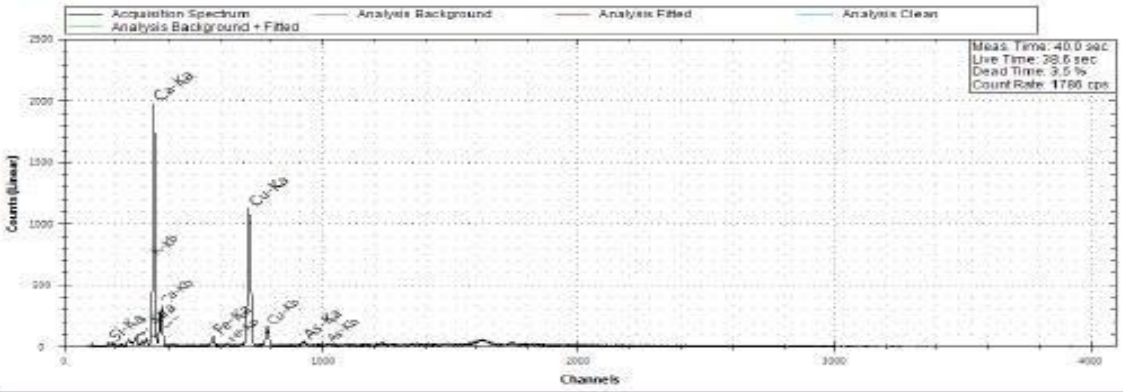
Measurement Time: 40,0 s
Tube Voltage: 40 kV
Tube Current: 20 µA
Tube Target Material: Rh
Elio Device: SN177
Device Mode: Head
Acquisition Mode: Manual
Acquisition Channels: 4096
Sample to Detector Material: Air

Spectrum:

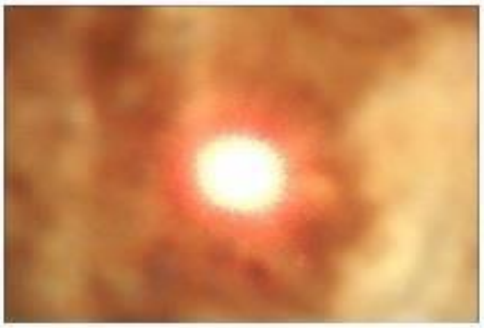


Measurement Time: 40,0 s
Tube Voltage: 40 kV
Tube Current: 20 µA
Tube Target Material: Rh
Elio Device: SN177
Device Mode: Head
Acquisition Mode: Manual
Acquisition Channels: 4096
Sample to Detector Material: Air

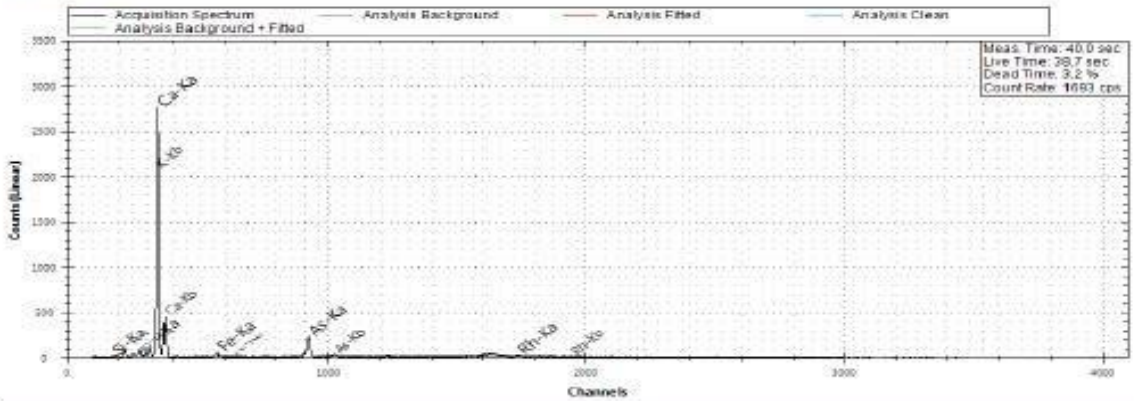
Spectrum:



Measurement Time: 40,0 s
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



Spectrum:



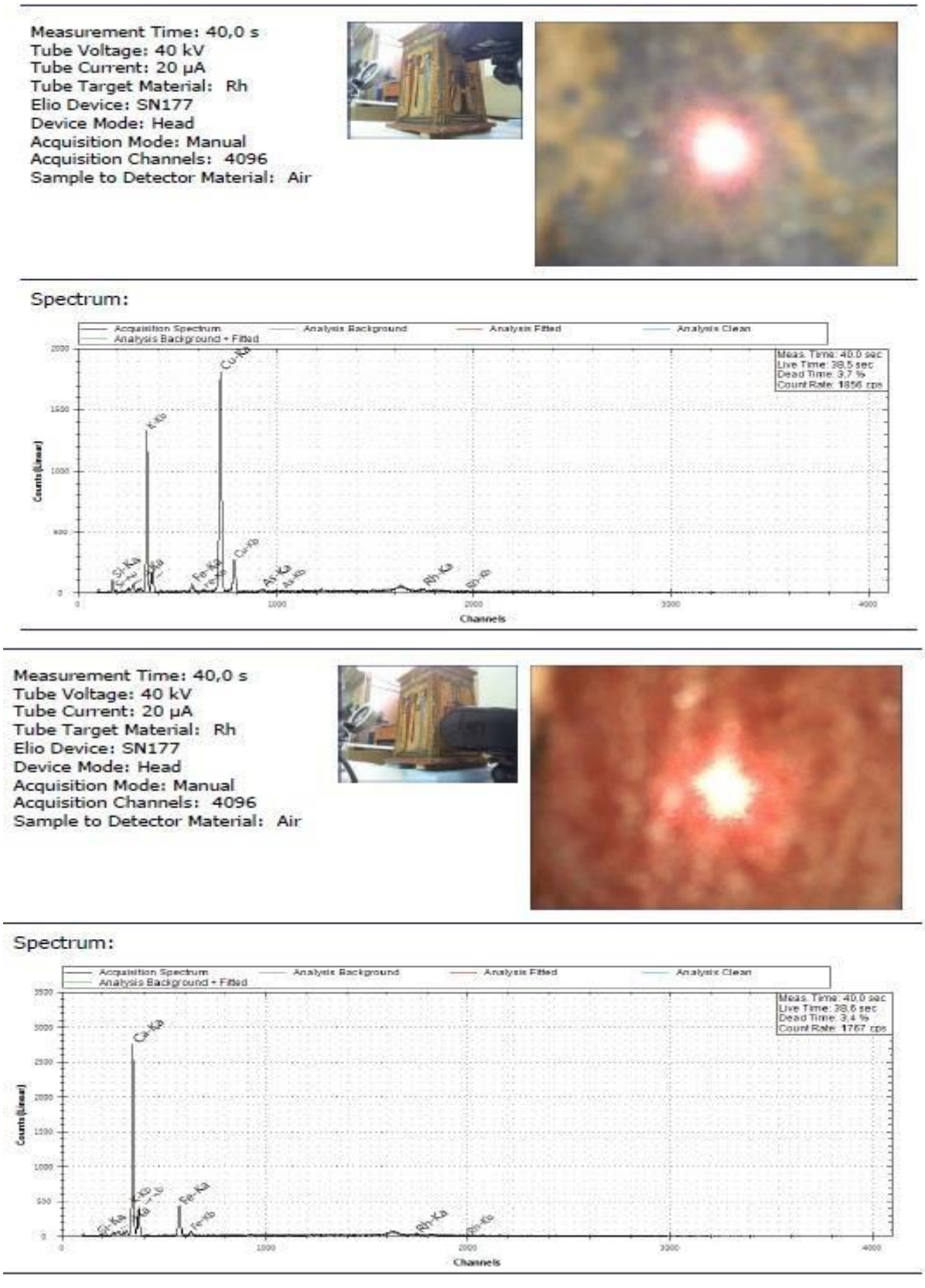


Fig.8. showing Identification of Egyptian pigments by X-ray florescence (XRF)

Table (1) explained Identification of Egyptian pigments by X-ray florescence (XRF)

Color	Primary pigment	XRF results of elements
Black	Carbon black	**Ca – Fe – As** (the ancient Egyptians' source of the black color is iron oxide)
Yellow	Orpiment	Ca – Fe – As
Green	Egyptian green	Si – Ca – Cu - As
Red	Red ochre	Si – Ca – Fe
Blue	Egyptian blue	Ca – Cu - As

2.10. Isolation and identification of micro-organisms

Fungi were identified based on their morphology and spore structures. The process showed that *Aspergillus niger* is the dominant microorganism founded on samples.

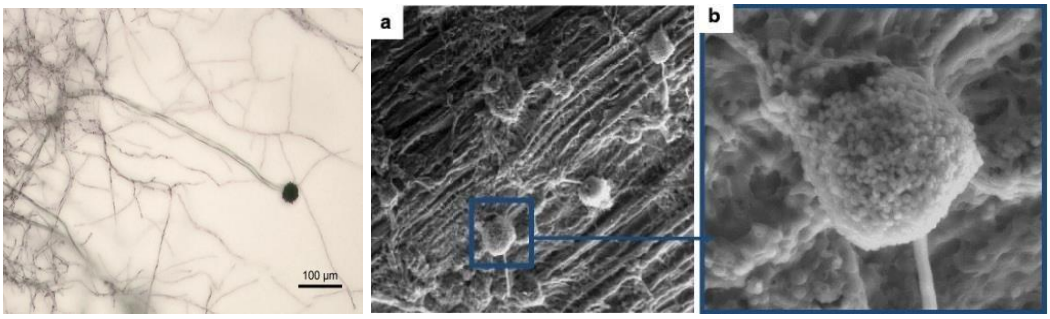


Fig.9. showing *Aspergillus niger* spores adhered onto sycamore wood sample (Soumya, *et al*., 2010).

2.11. Fumigation using Carbon dioxide

The data logger was reset to take daily measurements and ensure there was no leakage Table (2). Carbon dioxide percentage inside was 0.03%. The humidified pure Co_2 gas started to be injected into the tent till it was swollen, after a short wait to enable the air inside to be mixed the museum vacuum started the suction of the air inside. The carbon dioxide level was measured at a percentage of 8%. The process was repeated and measurements were taken Table (3). If a leak is discovered, the injecting process should be redone once or twice (depending on the leakage percentage) to get back to the 75% of Co_2 and preserve the Co_2 rate over 70%.

Fig.10. Showing Fumigation process using Carbon dioxide with tent

Table (2) shows the 1st 8 hours of injecting gas**.**

Hour	Temperature (˚C)	Relative humidity (%)	Carbon dioxide Co_2 (%)
1	**22**	**53**	**0**
2	**22**	**53**	**8**
3	**22**	**52**	**21**
4	**21**	**51**	**25**
5	**20**	**50**	**41**
6	**19**	**50**	**51**
7	**21**	**50**	**63**
8	**20**	**49**	**76**

Table (3) the process of measuring carbon dioxide level which was repeated in 15 days.

Day	Temperature (˚C)	Relative humidity (%)	Carbon dioxide CO_2 (%)
1	**21**	**52**	**76**
2	**21**	**51**	**73**
3	**20**	**51**	**74**
4	**20**	**50**	**75**
5	**19**	**50**	**74**
6	It is Friday (museum vacation)		
7	**18**	**49**	**75**
8	**18**	**48**	**74**
9	**20**	**50**	**75**
10	**19**	**49**	**75**
11	**21**	**49**	**75**
12	**20**	**48**	**75**
13	It is Friday (museum vacation)		
14	**21**	**50**	**74**
15	**21**	**50**	**74**

2.12. Change of color by spectrophotometer

As revealed by the results in Table (4), there are no changes to Lightness (L). Therefore, there are no changes in color before or after sterilization. L, a and b didn't change before and after sterilization with large range. This indicates that CO_2 has no effect on the pigments. The slight change in ΔE is due to the area measurement of 1×1 cm^2. Colorimetric results of CO_2 fumigation are probably caused by the shift of the reading spot location.

Table (4) colorimetric results of CO_2 fumigation

Color	Sample	Before			After						ΔE (±3)
		L	a	B	L	ΔL	A	Δa	b	Δb	
black	1	21.17	1.27	3.97	20.34	-0.83	1.44	0.17	4.71	0.74	1.1249
yellow	2	53.8	13.39	35.5	52.89	-0.91	14.23	0.84	35.3	-0.2	1.254472
yellow	3	60.23	9.68	33.17	60.16	-0.07	9.73	0.05	33.94	0.77	0.77479
blue	4	44.35	-3.65	5.31	44.79	0.44	-3.86	-0.21	4.83	-0.48	0.684178
red	5	34.64	19.68	15.29	33.25	-1.39	19.14	-0.54	15.14	-0.15	1.498733
black	6	23.92	0.97	4.18	23.72	-0.2	0.83	-0.14	4.7	0.52	0.574456
blue	7	33.49	1.88	12.94	33.25	-0.24	1.71	-0.17	13.2	0.26	0.392556
white	8	68.98	3.41	18.19	69.21	0.23	3.17	-0.24	17.93	-0.37	0.422019
blue	9	42.87	-5.05	-0.31	42.56	-0.31	-5.2	-0.15	-0.68	0.42	0.50547
right side above	10	32.94	16.36	13.19	33.18	0.24	16.34	-0.02	13.61	0.57	0.484149
green	11	38.72	-0.65	12.92	38.87	0.15	-0.47	0.18	13.49	0.19	0.616279
white	12	63.74	4.48	11.08	65.13	1.39	4.08	-0.4	11.27	1.25	1.458835
the crown	13	54.25	-2.76	7.3	54.35	0.1	-2.48	0.28	8.55	-0.07	1.284874
light blue	14	54.17	-1.25	12.43	54.21	0.04	-1.37	-0.12	12.36	0.05	0.144568
Dark blue	15	33.63	-3.88	3.98	34.15	0.52	-3.62	0.26	4.03	0.28	0.583524
red	16	32.92	16.71	12.33	32.95	-0.27	16.9	0.19	12.61	0.69	0.339706
The head of the stick of the standing person	17	52.47	14.21	15.49	52.2	-0.27	14.66	0.45	16.18		0.866891

3. CONCLUSION

The techniques used in this study demonstrated the effect on the evaluated pigments. The majority of sterilization methods eliminated insects and other aerobic organisms. The experimental study proved that there is no clear change happening to the pigments during its exposure in the fumigation processes. The results tell that fumigation using CO_2 is safe to apply even on the sensitive components and artifacts, specifically the ancient pigments.

4. Acknowledgements

We would like to give our thanks and appreciation to the management of the Egyptian Museum in Tahrir and all the team there. Also a thank you to Mr. Moamen Othman the Head of the museums sector at the Ministry of Tourism and Antiquities Egypt, and all his team. Including a thank you to all the different libraries, whether outside or inside Egypt, which helped us to collect all the scientific data material for this study.

BIBLIOGRAPHY

- Ahmed Khairy "The Effectiveness of fumigation by Carbon dioxide and Nitrogen on the ancient Egyptian painted wooden objects" Applied on a selected one, Master's Thesis, Cairo University, 2021
- Blanchette, R. A. (2000). A review of microbial deterioration found in archaeological wood from different environments. International Biodeterioration & Biodegradation, 46, 189-204.
- Cooper, A.I., Recent developments in materials synthesis and processing using supercritical CO2, Adv. Mater. 13 (2001) 1111.
- CHANGLU WANG, LIHUA LU¨ , AND MING XU, Carbon Dioxide Fumigation for Controlling Bed Bugs, VECTOR CONTROL, PEST MANAGEMENT, RESISTANCE, REPELLENTS, 2017.
- Dillow, A.K., F. Dehghani, J.S. Hrkach, N.R. Foster, R. Langer, Bacterial inactivation by using near- and supercritical carbon dioxide, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 10344. GOFFER, Z. , *Archaeological Chemistry. Second Edition*, (Chemical Analysis. A Series of Monographs on Analytical Chemistry and Its Applications), New York 2007.
- Harrigan W.F. and M.E. Mc Cance, Laboratory Methods in Microbiology, Academic Press (1966)
- Hong, S.I., W.S. Park, Y.R. Pyun, Inactivation of Lactobacillus sp. from kimchi by high pressure carbon dioxide, Food Sci. Technol.-Lebensm.- Wiss. Technol. 30 (1997) 681
- Lausmaa, J., B. Kasemo, S. Hansson, Accelerated oxide growth on titanium implants during autoclaving caused by fluorine contamination, Biomaterials 6 (1985) 23.
- Lenka Jeszeová1,Andrea Puškárová1,Mária Bučková,Lucia Kraková1, Tomáš Grivalský, Martin Danko, Katarína Mosnáčková, Štefan Chmela, Domenico Pangallo, Microbial communities responsible for the degradation of poly(lactic acid)/poly(3-hydroxybutyrate) blend mulches in soil burial respirometric tests, World Journal of Microbiology and Biotechnology (2018) 34:101 https://doi.org/10.1007/s11274-018-2483-y
- Lin, H.M. , Z.Y. Yang, L.F. Chen, Inactivation of Leuconostoc dextranicum with carbon dioxide under pressure, Chem. Eng. J. 52 (1993) B29.
- Madigan, M.T., J.M. Martinko, J. Parker, Brock Biology of Microorganisms, Prentice Hall, USA, 2002.
- Matthews, M.A., L.S. Warner, H. Kaiser, Exploring the feasibility of using dense-phase carbon dioxide for sterilization, Med. Device Diagn. Ind. 5 (2001) 140
- McHugh, M., V. Krukonis, Supercritical Fluid Extraction, Butterworth–Heinemann, USA, 1993.
- Montanari, L., Fantozzi, P., Snyder, J. M. & King, J. W. (1999). Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol. The Journal of supercritical fluids, 14, 87-93.
- Nakamura, K., A. Enomoto, H. Fukushima, K. Nagai, M. Hakoda, Disruption

of microbial-cells by the flash discharge of high-pressure carbondioxide, Biosci. Biotechnol. Biochem. 58 (1994) 1297.

- Roskey, C.T. , A. Sikes, Effect of hyperbaric carbon dioxide on spores and vegetative cells of Bacillus stearothermophilus, NATICK/TR-94/019 (1994).
- Scott D. Cichowlaz, Fumigation, Volume V, 2005, pp.7-14
- Soumya El Abed & Fatima Hamadi & Hassan Latrache & Houssaini Mohamed Iraqui & Koraichi Saad Ibnsouda, Adhesion of *Aspergillus niger and Penicillium expansum* spores on Fez cedar wood substrata, Ann Microbiol (2010) 60:377–382 DOI 10.1007/s13213-010-0045-0
- SCOTT, P. M. , J. W. LAWRENCE, AND W. VAN WALBEEK, Detection of Mycotoxins by Thin-Layer Chromatography: Application to Screening of Fungal Extracts, APPLIED MICROBIOLOGY, Nov. 1970, p. 839-842 Vol. 20, No. 5
- Sikes, A., C. Martin, Control of thermophilic spore activity with pressurized CO_2 and egg-white lysozyme, Report, NATICK/TR-95/020 (1995).
- Spilimbergo, S., A. Bertucco, Non-thermal bacteria inactivation with dense CO2, Biotechnol. Bioeng. 84 (2003) 627.
- Tortora,] G.J. , B.R. Funke, C.L. Case, Microbiology, An Introduction, Pearson Education, USA, 2002
- Wyszecki, G., Stiles, W. S., 2000, Color Science Concepts and Methods. Quantitative Data and Formulae, Second Edition, JohnWiley & Sons, Inc. New York.