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BIOCODICOLOGY: UNVEILING THE BIOLOGICAL AND MATERIAL COMPOSITION OF ANCIENT MANUSCRIPTS

Mansour Mohamed Sabri[1*]

International Institute of Islamic Thought and Civilization (ISTAC), International Islamic University Malaysia

E-mail: Mansour.sabri@hotmail.com

ABSTRACT

Biocodicology is an emerging interdisciplinary field that integrates biology, archaeology, and information science to study ancient manuscripts. This paper explores how biological analysis, including DNA and protein analysis, combined with advanced imaging techniques such as Reflectance Transformation Imaging (RTI), Multispectral Imaging (MSI), X-ray Fluorescence (XRF), and Fourier Transform Infrared (FTIR) Spectroscopy, offers new insights into the origins, production, and preservation of historical manuscripts. DNA extraction and protein analysis reveal the biological origins of the materials, while non-invasive physicochemical methods uncover the chemical composition of inks and pigments. The integration of bioinformatics and machine learning tools provides a holistic understanding of manuscript production techniques and environmental conditions. These methodologies contribute significantly to the study of historical manuscripts and inform strategies for their conservation and preservation.

Keywords: *Biocodicology, DNA analysis, protein analysis, Reflectance Transformation Imaging (RTI), Multispectral Imaging (MSI), X-ray Fluorescence (XRF), Fourier Transform Infrared (FTIR) Spectroscopy, manuscript preservation, bioinformatics, cultural heritage conservation.*

ABSTRAK

Biokodikologi adalah bidang interdisipliner yang sedang berkembang yang mengintegrasikan biologi, arkeologi, dan ilmu informasi untuk mempelajari manuskrip kuno. Artikel ini mengeksplorasi bagaimana analisis biologis, termasuk analisis DNA dan protein, yang dikombinasikan dengan teknik pencitraan canggih seperti Reflectance Transformation Imaging (RTI), Multispectral Imaging (MSI), X-ray Fluorescence (XRF), dan Fourier Transform Infrared (FTIR) Spectroscopy, menawarkan wawasan baru tentang asal usul, produksi, dan pelestarian manuskrip sejarah. Ekstraksi DNA dan analisis protein mengungkap asal biologis dari material, sementara metode fisikokimia non-invasif mengungkap komposisi kimia tinta dan pigmen. Integrasi alat bioinformatika dan pembelajaran mesin memberikan pemahaman holistik tentang teknik produksi manuskrip dan kondisi lingkungan. Metodologi ini berkontribusi secara signifikan terhadap studi manuskrip sejarah dan memberikan informasi untuk strategi konservasi dan pelestarian.

Kata kunci: Biokodikologi, analisis DNA, analisis protein, *Reflectance Transformation Imaging* (RTI), *Multispectral Imaging* (MSI), *X-ray Fluorescence* (XRF), *Fourier Transform Infrared* (FTIR) *Spectroscopy*, pelestarian manuskrip, bioinformatika, konservasi warisan budaya.

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I. INTRODUCTION

Biocodicology is an emerging interdisciplinary field that merges biology, archaeology, and information science to study ancient manuscripts and books. This field draws on methods and insights from bioinformatics and bioarchaeology to analyze the biological and historical aspects of these texts. By incorporating biological analysis, such as examining DNA, proteins, and other organic materials in manuscripts, biocodicology provides a novel way to explore the historical, cultural, and environmental contexts of ancient texts.

Like bioinformatics, which integrates biology, computer science, and information technology to analyze biological data (Singh, 2007; Ramos et al., 2018; Ragunath et al., 2014), biocodicology combines biological examination with the study of historical texts. It utilizes technological tools to store, analyze, and interpret biological data derived from manuscripts. The integration of these technologies is critical for understanding the complex biological information encoded within the materials used in manuscripts, such as parchment and ink, and how these materials can shed light on historical societies.

Moreover, the biological analysis in biocodicology closely mirrors the methods used in bioarchaeology, where human remains are studied to gain insights into past societies (Larsen, 2015). In a similar way, biocodicology investigates biological residues, such as DNA and proteins, in ancient manuscripts to reveal important information about their production, use, and preservation. By analyzing these biological markers, researchers can trace the origins of the materials, understand how they were processed, and uncover details about the historical environments in which these manuscripts were created and stored.

Technological integration is another key aspect of biocodicology, where computational tools and databases are essential for interpreting biological data from manuscripts (Singh, 2007; Ramos et al., 2018; Ragunath et al., 2014). This technological support enables researchers to handle large datasets, compare biological materials across different manuscripts, and generate insights about the evolution of writing technologies and preservation techniques over time. This approach aligns with the practices in bioinformatics, where advanced computational methods are used to decipher biological complexity.

Finally, biocodicology not only contributes to our understanding of the historical context of manuscripts but also has practical applications for the preservation of cultural heritage. The knowledge gained from analyzing the biological composition of manuscripts informs better preservation strategies. For example, understanding the degradation processes of parchment or ink can guide conservators in developing effective methods to protect these valuable cultural artifacts from further deterioration (Fiorini, 2018; Collins et al., 2019). This application of biocodicology ensures that ancient texts, which hold immense historical and cultural significance, can be preserved for future generations. While biocodicology is still developing, its interdisciplinary nature, combining biology, archaeology, and technology, mirrors approaches seen in related fields such as bioinformatics and bioarchaeology. These parallels provide a framework for understanding the potential of biocodicology to deepen our knowledge of ancient manuscripts while also playing a vital role in their preservation for the future.

Biocodicology, as an emerging interdisciplinary field, aims to understand the biological and material composition of ancient manuscripts. However, several challenges arise in the analysis and preservation of these historical artifacts. These challenges include:

1. **Biological Degradation:** How can the biological materials found in ancient manuscripts, such as DNA and proteins, be analyzed and preserved despite centuries of degradation?
2. **Technological Limitations:** What are the limitations of current imaging and molecular techniques (such as RTI and DNA analysis) in accurately studying the materials used in manuscript production?
3. **Non-Invasive Methods:** How can non-invasive methods be improved to study and analyze manuscripts without causing further damage to these fragile documents?
4. **Data Integration and Interpretation:** How can the vast amounts of data generated from different techniques (e.g., DNA sequencing, imaging, and molecular analysis) be effectively integrated and interpreted to provide meaningful insights into the historical, cultural, and biological contexts of manuscripts?

II. METHODS

To address these research problems, a multidisciplinary approach will be adopted, involving both molecular and imaging techniques:

2.1 Protein and DNA Analysis

a. DNA Extraction and Sequencing:

This method will be used to study the biological origin of the parchment and ink in manuscripts. Polymerase Chain Reaction (PCR) will be employed to amplify ancient DNA fragments, and advanced sequencing methods will help identify the species of animals or plants used in the production of manuscripts (Fiddyment et al., 2019; Collins et al., 2019).

b. **Protein Analysis:**

Mass spectrometry will be employed to identify proteins, which may provide additional information on the processing methods and the materials used in manuscripts (Collins et al., 2019; Ryder et al., 2020).

2.2 Imaging Techniques

a. **Reflectance Transformation Imaging (RTI):**

RTI will be used to capture surface details of manuscripts, revealing any hidden writings, tool marks, or subtle textures that can offer insights into production methods and historical usage (Bucklow et al., 2018).

b. Multispectral Imaging (MSI):

MSI will be applied to detect invisible texts, chemical compounds, and degradation processes by capturing images across multiple wavelengths (Castro et al., 2022).

2.3 Non-Invasive Physicochemical Analysis

X-ray Fluorescence (XRF) and Fourier Transform Infrared (FTIR) Spectroscopy. These techniques will be employed to analyze the chemical composition of the manuscripts' materials, such as the types of ink and pigments used, without causing any damage to the documents (Fiorini, 2018; Collins et al., 2019).

2.4 Data Integration

The data collected from DNA, protein analysis, and imaging techniques will be integrated using bioinformatics tools to construct a comprehensive understanding of the manuscripts' biological and historical origins. Machine learning algorithms will also be used to analyze patterns in the data, offering deeper insights into the materials and production techniques (Ryder et al., 2020).

III. RESULTS AND DISCUSSION

3.1 DNA and Protein Analysis

3.1.1 DNA Extraction and Sequencing

The analysis of ancient manuscripts using DNA extraction and sequencing has provided significant insights into the biological origin of the materials used in their production. Using Polymerase Chain Reaction (PCR) to amplify ancient DNA fragments, researchers were able to identify the animal species used for parchment production. Most notably, DNA sequencing revealed that medieval parchments were predominantly made from sheep and goat skins, with some regional variation in the types of animals used. For instance, goat skin was more commonly used in Southern Europe, while sheep skin was favored in Northern and Western Europe (Fiddyment et al., 2019; Teasdale et al., 2017). These findings confirm historical trade routes and animal husbandry practices.

The DNA analysis also detected microbial DNA, shedding light on the microbiomes present on the manuscripts. This provides clues about the storage environments, handling, and preservation conditions of these ancient documents over the centuries (Piñar et al., 2022). The presence of specific microbial communities further suggests potential degradation processes that may have affected the condition of the manuscripts, offering insights into how different environmental factors have influenced the aging process.

3. 1.2 Protein Analysis

Mass spectrometry, particularly through peptide mass fingerprinting, was used to identify the specific proteins present in the parchment. The proteins, primarily collagen, indicated the type of animal skins used and provided additional details on the processing techniques applied during the parchment-making process. The analysis revealed that collagen from sheep and goats was most prevalent, confirming the results from DNA analysis. In some cases, the analysis also uncovered the presence of bovine collagen, suggesting that cow skins were occasionally used, particularly in regions where other animals were scarce (Collins et al., 2019).

Further, the protein analysis allowed researchers to examine how the parchment was processed. Differences in the type of collagen present indicated variations in processing techniques, such as the use of lime for

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dehairing. This process had a direct impact on the preservation quality of the manuscripts, as certain treatments improved the longevity of the parchment, while others contributed to its degradation over time (Ryder et al., 2020).

a. Microscopical and Physicochemical Analyses

The application of X-ray fluorescence (XRF) and Fourier-transform infrared (FTIR) spectroscopy provided additional information about the elemental composition and chemical properties of the manuscripts. XRF identified elements in the ink and pigments used, allowing researchers to determine the composition of the materials and trace their geographical origins (Fiorini, 2018). FTIR was instrumental in detecting changes in the chemical composition of the parchment, offering further insights into the degradation processes influenced by storage environments and handling practices.

These physicochemical analyses were particularly useful in understanding the condition of the manuscripts without causing any damage to the delicate materials. The non-invasive nature of these techniques makes them ideal for ongoing conservation efforts, ensuring that the manuscripts are preserved while still enabling detailed scientific analysis (Castro et al., 2022).

b. Bioimaging

Advanced bioimaging techniques, such as electron microscopy and X-ray tomography, provided detailed visualizations of the structural composition of the manuscripts. These methods revealed hidden features of the manuscripts, such as faint inscriptions or scribal corrections, that were not visible through traditional imaging techniques. Magnetic resonance imaging (MRI) allowed for non-invasive visualization of the internal structure of manuscripts, offering insights into the materials used and their state of preservation (Bucklow et al., 2018). These bioimaging techniques proved essential for revealing the microstructural details of the parchment and ink, facilitating a deeper understanding of the manuscripts' history and composition.

c. Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) was employed to separate and identify various compounds within the manuscript materials. The versatility of CE, combined with detection systems such as mass spectrometry and fluorescence, allowed researchers to isolate and analyze specific molecules within the manuscripts, including ink and pigment components (Collins et al., 2019). This technique provided valuable information on the chemical makeup of the manuscripts, contributing to the broader understanding of the materials used in their production.

The integration of DNA, protein, and imaging analyses has significantly advanced our understanding of ancient manuscripts. By combining data from these techniques, researchers have gained a comprehensive view of the materials, processes, and environmental conditions involved in manuscript production. For example, by analyzing both the biological and chemical composition of the parchment, it has been possible to trace trade routes and understand the availability of different animal skins in various regions (Fiddyment et al., 2019; Piñar et al., 2022).

Additionally, bioimaging techniques have allowed researchers to identify hidden details in manuscripts that were previously undetectable. The combination of these techniques with non-invasive analyses like XRF and FTIR ensures that manuscripts are preserved while undergoing detailed examination (Fiorini, 2018).

Bioinformatics tools played a critical role in integrating the diverse datasets generated by these analyses. By combining the results from DNA, protein, and imaging techniques, researchers were able to create comprehensive models of the manuscripts' production and historical use (Ryder et al., 2020). Machine learning algorithms were employed to analyze these large datasets, uncovering patterns that provided deeper insights into the regional and historical contexts of the manuscripts.

These tools allowed researchers to identify correlations between the materials used and the geographical origins of the manuscripts. For instance, manuscripts from Southern Europe tended to use goat skins, while those from Northern Europe were predominantly made from sheep skins. This analysis also revealed variations in ink and pigment compositions, indicating regional differences in scribal practices and materials used (Castro et al., 2022; Fiddyment et al., 2019).

The findings from these analyses have important implications for the preservation of ancient manuscripts. Understanding the biological and chemical degradation processes has allowed conservators to develop more effective strategies for preventing further deterioration. For example, the identification of specific environmental factors that contribute to the breakdown of parchment, such as humidity and temperature fluctuations, has led to improved storage and conservation techniques (Fiorini, 2018; Ryder et al., 2020).

Additionally, the ability to non-invasively analyze the chemical composition of manuscripts using XRF and FTIR has provided critical insights for conservators without causing harm to the documents themselves. This approach ensures that future generations will continue to benefit from these historical and cultural treasures (Collins et al., 2019).

3.2 Imaging Techniques

3.2.1 Reflectance Transformation Imaging (RTI)

Reflectance Transformation Imaging (RTI) is a powerful technique used in biocodicology to document and analyze the surface details of cultural heritage artifacts. The key components of RTI include:

a. Fixed Camera Position:

RTI involves capturing multiple images of an object from a static camera position while varying the direction of the light source around the object (Clarke & Christensen, 2016; Newman, 2015; Mytum & Peterson, 2018).

b. Variable Lighting Directions:

The technique requires changing the direction of incident light for each image, which helps in capturing the surface's interaction with light from different angles (Clarke & Christensen, 2016; Newman, 2015; Mytum & Peterson, 2018).

c. Mathematical Modeling:

Several methods are used to model the local reflectance of each pixel, including:

1. **Polynomial Texture Mapping (PTM):** Uses second-order polynomial functions to model reflectance (Castro et al., 2022; Newman, 2015; Shaoliang et al., 2022).
2. **Hemispherical Harmonics (HSH):**

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Models reflectance using spherical harmonics (Castro et al., 2022; Newman, 2015; Shaoliang et al., 2022).

3. **Discrete Modal Decomposition (DMD):** A more recent method for modeling reflectance (Castro et al., 2022; Newman, 2015; Shaoliang et al., 2022).

d. Data Processing and Analysis:

The captured images are processed to create an interactive digital image where the light source position and reflectance properties can be digitally modified. This allows for enhanced visualization of surface details (Mytum & Peterson, 2018; Newman, 2015; Castro et al., 2022).

e. Uniform Light Distribution:

Ideally, a uniform distribution of light positions over the hemisphere is assumed, although this is often challenging to achieve in practice. Non-uniformity can introduce artifacts, which can be corrected using local density estimation and weighted regression techniques (Castro et al., 2022; Newman, 2015; Shaoliang et al., 2022).

f. Applications in Cultural Heritage:

RTI is particularly useful for visualizing fine surface details such as cracks, tool marks, and inscriptions that are not visible to the naked eye, making it invaluable in the study and preservation of cultural heritage artifacts (Mytum & Peterson, 2018; Shaoliang et al., 2022; Clarke & Christensen, 2016).

By integrating these components, RTI provides a comprehensive method for documenting and analyzing the intricate surface details of biocodicological artifacts, enhancing our understanding and preservation of cultural heritage (Clarke & Christensen, 2016; Mytum & Peterson, 2018; Castro et al., 2022; Newman, 2015).

3.2.2 Multispectral imaging (MSI)

Multispectral imaging (MSI) has several applications in biocodicology, particularly in the analysis and preservation of historical manuscripts and cultural heritage objects. Here are some key applications:

a. **Historical Manuscript Analysis**: MSI is used to reveal properties and structures in historical manuscripts that are not visible to the naked eye. This includes the detection of hidden texts, corrections, and annotations that have faded over time. The technology helps in the detailed examination of the materials and techniques used by scribes, which is crucial for understanding historical contexts and preserving these artifacts (Fiorini, 2018).

b. **Paleography**: MSI aids in computerized paleography, which involves the study of ancient writing. Techniques such as multispectral imaging and Raman spectroscopy are employed to enhance the legibility of ancient texts, reconstruct broken character strokes, and evaluate the facsimile of characters. This is particularly useful for analyzing ink-written ostraca from the Hebrew Iron Age, helping to address long-standing epigraphic questions (Faigenbaum-Golovin et al., 2015).

c. **Chemical Analysis**: MSI can identify chemical species on the surface of manuscripts, which is essential for conservation efforts. By using specific highly selective thin film filters, MSI can detect and analyze the chemical composition of inks and pigments, providing insights into the materials used and their degradation processes (Lumeau et al., 2017).

d. **Chromatic Aberration Correction**: In the context of historical manuscript analysis, MSI systems are calibrated to correct chromatic aberrations, ensuring that images are in focus across different wavelengths. This enhances the accuracy and quality of the captured images, making it easier to analyze fine details in the manuscripts (Brenner & Sablatnig, 2019).

These applications demonstrate the versatility and importance of MSI in biocodicology, offering non-invasive methods to uncover and preserve historical and cultural heritage information. Table 1 below summarizes various applications in the field of biocodicology, including historical manuscript analysis, paleography, chemical analysis, and chromatic aberration correction. Each of these applications provides valuable insights into understanding and preserving ancient manuscripts.

Table 1. Summary of Applications in Biocodicology

Application	Description
Historical Manuscript Analysis	Reveals hidden texts and annotations in manuscripts
Paleography	Enhances legibility and reconstructs ancient writing
Chemical Analysis	Identifies chemical species in inks and pigments
Chromatic Aberration Correction	Ensures in-focus images across different wavelengths

These insights highlight the significant role of MSI in the field of biocodicology, providing advanced tools for the study and preservation of historical texts and artifacts (Fiorini, 2018; Faigenbaum-Golovin et al., 2015; Lumeau et al., 2017).

3.3 Non-Invasive Physicochemical Analysis

The application of non-invasive physicochemical analysis techniques, such as X-ray Fluorescence (XRF) and Fourier Transform Infrared (FTIR) Spectroscopy, provided significant insights into the chemical composition of the materials used in the manuscripts.

a. XRF Analysis:

XRF was employed to analyze the elemental composition of the inks and pigments used in various historical manuscripts. The results revealed the presence of elements such as iron, copper, and zinc, commonly found in the composition of traditional inks, such as iron gall ink. The identification of these elements helped trace the geographical and historical origins of the manuscripts, as the composition of the inks varied by region and time period. Additionally, XRF detected traces of other elements that pointed to the use of certain pigments, allowing researchers to identify the types of materials used in the artwork or annotations of the manuscripts. This non-invasive method allowed for a detailed understanding of the materials without causing any physical damage to the manuscripts (Fiorini, 2018; Collins et al., 2019).

b. FTIR Spectroscopy:

FTIR spectroscopy was used to analyze the molecular structure of the parchment and the ink's organic components. This technique provided detailed information about the chemical bonds within the materials, allowing researchers to identify organic compounds such as proteins, cellulose, and carbon-based pigments. The analysis revealed that certain manuscripts used vegetable-based inks, which differed from iron-based inks in terms of chemical composition. This distinction is essential for understanding the different ink production techniques and their geographical distribution. Furthermore, FTIR helped detect chemical degradation patterns in the manuscripts, particularly in the inks and parchment, which can be linked to environmental factors such as humidity and temperature over time (Piñar et al., 2022).

3.4 Data Integration

After the data collection from DNA, protein analysis, and imaging techniques, bioinformatics tools were employed to integrate the results and construct a comprehensive understanding of the manuscripts' biological and historical origins. The integration of multiple datasets allowed researchers to analyze the relationship between the biological materials and the chemical composition of the manuscripts, providing a holistic view of their production techniques.

a. Bioinformatics Tools:

These tools enabled the collation of diverse datasets from XRF, FTIR, DNA, and protein analysis. By combining the elemental and molecular data with biological information, such as DNA traces of the animal skins used for parchment, researchers constructed a more complete picture of the manuscripts' origins. This integration revealed patterns in the usage of different materials across regions and time periods, contributing to the understanding of historical trade routes and material availability (Fiddyment et al., 2019).

b. Machine Learning Algorithms:

Machine learning techniques were applied to analyze patterns within the integrated data. These algorithms helped identify correlations between the types of materials used in the production of manuscripts and specific historical periods or regions. For example, machine learning identified clusters of manuscripts that shared similar chemical compositions of ink and parchment, indicating that they were likely produced in the same geographical area or time period. Additionally, these algorithms were able to predict the likelihood of certain materials being used based on incomplete data, offering deeper insights into manuscript production techniques that were previously unavailable (Ryder et al., 2020).

The use of non-invasive techniques such as XRF and FTIR has proven to be highly effective in the field of biocodicology. These techniques allowed for the detailed analysis of the chemical composition of the materials used in manuscripts without compromising their physical integrity. The identification of elements in the ink and pigments through XRF provided critical information on the origin and production techniques of the manuscripts. Meanwhile, FTIR offered a detailed view of the organic components, shedding light on the molecular structure of the parchment and ink, and revealing patterns of degradation (Fiorini, 2018; Collins et al., 2019).

IV. CONCLUSION

Biocodicology, by merging biology, archaeology, and technology, has revolutionized the study of ancient manuscripts. Through the use of DNA and protein analysis, researchers have uncovered valuable information about the materials used in parchment production and the historical environments where manuscripts were created. Imaging techniques such as RTI and MSI have allowed for the detailed examination of manuscript surfaces, revealing hidden texts and structural features, while non-invasive methods like XRF and FTIR have provided critical insights into the chemical composition of the inks and pigments without causing damage. Data integration using bioinformatics tools and machine learning has further enhanced our understanding of the geographical and historical contexts of manuscript production. The combined application of these methods has not only deepened our knowledge of ancient manuscripts but also provided critical guidance for their preservation, ensuring the longevity of these invaluable cultural treasures for future generations.

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