IRON-GALL INK STUDIES ON ACID AND ALKALINE PAPERS AND THEIR RELATION TO CELLULOSE MICROBIOLOGICAL DEGRADATION

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Abstract

This paper describes the effect of various iron-gall inks on acid and alkaline papers and their relation to biodeterioration. Different types of papers were used in the present study, where several tests were performed: spot-tests for lignin and starch detection, chemical digestion to quantify total iron content, desorption studies to remove iron from the papers, followed by accelerated artificial aging, scanning electron microscopic studies and biodeterioration tests. Results indicated that lignin was only detected on acid papers, while starch was detected both on acid and alkaline papers. Treatment with calcium phytate proved to be an efficient process to remove excess iron from the inks. Scanning electron microscopic images showed distinct surface spreading of inks on the papers, depending on the iron content. Finally, in the absence of any other carbon source, papers strips containing iron-gall inks proved to be amenable to biodeterioration, indicating that chemical and biochemical deterioration simultaneously occur.

Keywords: Acid papers; Alkaline papers; Scanning electron microscopy; Biodeterioration; Iron content

Introduction

Paper was the most important support for the storage of historical and cultural information during centuries [1]. In the final years of the 19th century, with the increasing demand for paper and the scarce availability of cellulose fibers from linen and cotton, wood became the main source of this compound. Consequently, cellulose, hemicellulose and lignin, both from wood pulp, became the main constituents of paper [2]. It is evident that the evolution in the manufacture of paper through centuries clearly shows a marked modification in its structure, both in relation to the origin of cellulose fibers, as well as in relation to the types of additives used. In this context, the process of sizing plays a key role, directly affecting the mechanism of water and ink absorption by paper fibers [3].

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From the fifteenth century until the first decades of the nineteenth century, as iron-galls were widely used by Western civilization, and in Brazil its application continued until the beginning of the 20th century, being considered one of the most important writing materials in history. Basically, iron-gall ink has four main ingredients: galic acid, ferrous sulphate, gum Arabic and water. However, although it sounds like a simple recipe, there was a wide variability of formulations available. Beyond this, the ink can be formed by a wide variety of natural constituents, usually contaminated with high levels of impurities [4, 5]. This contributes to different mechanisms of degradation, darkening and loss of mechanical properties of the paper, in a process named iron-gall ink corrosion [6, 7]. Unfortunately, the loss of mechanical properties in degraded manuscripts, which is an irreversible process, makes the manipulation of paper more and more difficult to handle [8].

Beyond iron ions, copper ions as impurities can catalyze oxidation processes in cellulose, probably as centers able to form free radicals through a sequence of Fenton reactions [9].

An efficient treatment to stabilize corrosion due to the presence of iron-gall ink requires the addition of alkali substances to neutralize acid hydrolysis of paper, and also the addition of antioxidant agents to inhibit oxidation [10, 11]. Some possibilities are being investigated [12-14], but the treatment with calcium phytate remains as the most widely used chemical [15, 16].

The characterization of paper components plays a key role in this field and archaeometric techniques are being widely used to identify additives and chemical composition of inks in historical documents. This way, the use of scanning electron microscopy is very important to point the morphology of different papers, particularly effects of ink interactions in cellulose matrix and the mechanism of ink impregnation in fibers [17-20].

In the middle ages the paper was manufactured more selectively and had a better quality. Due to the dissemination of wood pulp at the 19th century in substitution to other cellulose fibers, a marked decrease in the quality of paper was observed, due to physical and chemical processes during manufacturing. Because of this, newer papers are naturally more susceptible to attack by microorganisms such as bacteria, fungi and actinomycetes; being the most common fungi, due to their greater tolerance to environmental conditions and because they can live in environments with low humidity [21, 22].

The main objective of this study was to evaluate the effect of incorporation of iron-gall inks with different iron contents on different types of acid and alkaline papers. It is expected to obtain accurate information regarding the incorporation of the ink and the microscopic effects of the type of sizing on the papers, in order to understand the process of accelerated degradation of certain papers by the oxidation of iron gall inks, with regard to the conservation of objects of cultural heritage. Additionally information about biodeterioration in the absence and presence of the ink was investigated.

Materials and Methods

Iron-gall inks

For the preparation of iron-gall inks ferrous sulphate, tannic acid, gum Arabic and ethanol (Vetec, Brazil) were used, according to Table 1.

Ink 1 was formulated with stoichiometric amounts of iron and tannic acid, and is considered the reference ink. Ink 2 presents ten times the stoichiometric amount of iron and was prepared to simulate inks with excess of iron in their composition and is mainly responsible for the degradation of cellulosic substrates by the action of free oxidized iron. Iron-gall inks were prepared at ambient pressure and temperature and exposed to ambient air for 24 hours, to help
in strengthening the color after the formation of the gallotanic complex (gallic acid and ferrous sulphate) and in the fixation of components in the paper strips. After this step, inks were kept in sealed amber jars protected from light, at room temperature.

Table 1. Composition of the iron-gall inks synthesized for the current study

<table>
<thead>
<tr>
<th>Ink</th>
<th>Ferrous sulphate (g)</th>
<th>Tannic acid (g)</th>
<th>Gum Arabic (g)</th>
<th>Ethanol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>5.5</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>5.5</td>
<td>4.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Amounts to 50mL of ink.

Acid and alkaline papers

Eight papers with different characteristics, provided by the Museum of Astronomy and Related Sciences, Rio de Janeiro, Brazil, were used to evaluate some characteristics of paper, such as the presence of lignin, the os sizing through spot-tests, and mainly to evaluate the impregnation of inks 1 and 2 and the efficiency of treatment with calcium phytate solution. Paper samples were coded as A, B, C, D, E, F, G and H and a brief description of its characteristics are shown in Table 2, with their respective specifications and image. All the papers/documents used corresponding to duplicate documents, to be discharged. By date, it was possible to assign the acid paper value for samples A, B, C, D and E, alkaline paper for samples F, G and H respectively.

To conduct the spot-tests, paper samples were cut into a standard size of 2.0x7.0cm, with one side of paper strips immersed in a solution of phloroglucinol, for lignin detection and immersed in lugol's iodine (potassium iodide + iodine), for the detection of starch.

For lignin detection, tests on the samples were done by using the method proposed by D.A. Johansen [23]. The quantity of 0.1g of phloroglucinol was dissolved in 5mL of methanol, 5mL of concentrated hydrochloric acid and 5mL of distilled water. The positive result for lignin is confirmed by the reddish color on the samples.

Tests for the detection of starch in paper samples were conducted according to the methodology proposed by H.P. Schramm and B. Hering [24]. A solution of 0.17g of potassium iodide and 0.25g of iodine in 10mL of distilled water was prepared. The positive result for starch is detected if the paper sample presented a dark blue color after addition of this solution.

Impregnation of papers with stoichiometric and concentrated iron-gall inks

Initially, 10.0mL of each ink were added to a 50mL beaker and then paper strips (6.0x0.5cm) were immersed for 5 minutes. The immersion was performed vertically until the ink impregnated in the paper reached the height of 2.0cm. After that, papers were dried at room temperature for 24 hours and then subjected to different treatments:

- Chemical digestion to quantify total iron concentration in the samples by atomic absorption spectrometry;
- Desorption of iron with calcium phytate solution, to remove excess iron on the surface of paper strips, followed by chemical digestion of the papers;
- Treatment with calcium phytate, accelerated artificial aging [25, 26] followed by a second washing with calcium phytate in order to verify the necessity of application of a second treatment after artificial aging, followed by chemical digestion;
- Evaluation of paper samples through scanning electron microscopy; and,
- Biodeterioration tests.

All the tests were performed in paper strips containing inks 1 and 2.
Table 2. Papers/Documents used in the tests, historical information and images

<table>
<thead>
<tr>
<th>Paper no / image</th>
<th>Specifications</th>
<th>Paper no / image</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Authentic duplicate document</td>
<td>B</td>
<td>Authentic duplicate document</td>
</tr>
<tr>
<td></td>
<td>Dated 1940</td>
<td></td>
<td>Dated 1936</td>
</tr>
<tr>
<td></td>
<td>Brazilian Ministry of Finance</td>
<td></td>
<td>Brazilian Ministry of Agriculture</td>
</tr>
<tr>
<td></td>
<td>Small thickness and transparent</td>
<td></td>
<td>Presents resin, oil or wax</td>
</tr>
<tr>
<td></td>
<td>Apparently presents wood fibers</td>
<td></td>
<td>Apparently presents wood fibers</td>
</tr>
<tr>
<td></td>
<td>Printed watermark</td>
<td></td>
<td>Printed watermark (America Bond)</td>
</tr>
<tr>
<td></td>
<td>Some information written with iron-gall ink</td>
<td></td>
<td>Some information written with iron-gall ink</td>
</tr>
<tr>
<td>C</td>
<td>Authentic duplicate document</td>
<td>D</td>
<td>Cover of a document</td>
</tr>
<tr>
<td></td>
<td>Dated 1949</td>
<td></td>
<td>No information about date</td>
</tr>
<tr>
<td></td>
<td>Brazilian Ministry of Agriculture</td>
<td></td>
<td>Brazilian Ministry of Work, Industry and Commerce</td>
</tr>
<tr>
<td></td>
<td>Apparently presents wood fibers with partial removal of lignin</td>
<td></td>
<td>Yellowish aspect</td>
</tr>
<tr>
<td></td>
<td>Printed watermark (Bond)</td>
<td></td>
<td>Apparently presents wood fibers and thick sizing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Some information written with iron-gall ink</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acid paper, no treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Contains lignin</td>
</tr>
<tr>
<td>E</td>
<td>Acid rough paper</td>
<td>F</td>
<td>Treated paper with removal of lignin</td>
</tr>
<tr>
<td></td>
<td>No information about date</td>
<td></td>
<td>No information about date</td>
</tr>
<tr>
<td></td>
<td>Yellowish aspect</td>
<td></td>
<td>Presence of synthetic resin</td>
</tr>
<tr>
<td></td>
<td>Apparently presents wood fibers</td>
<td></td>
<td>Alkaline paper</td>
</tr>
<tr>
<td></td>
<td>Contains lignin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Paper brand Arches (France)</td>
<td>H</td>
<td>Paper brand Montval</td>
</tr>
<tr>
<td></td>
<td>No information about date</td>
<td></td>
<td>No information about date</td>
</tr>
<tr>
<td></td>
<td>Apparently contains gelatine sizing</td>
<td></td>
<td>Apparently contains gelatine sizing</td>
</tr>
<tr>
<td></td>
<td>Presence of cotton fibers</td>
<td></td>
<td>Presence of wood fibers</td>
</tr>
<tr>
<td></td>
<td>Alkaline paper</td>
<td></td>
<td>Alkaline paper</td>
</tr>
</tbody>
</table>

Treatment of iron-gall ink with calcium phytate followed by chemical digestion

For the removal of excess iron from paper strips impregnated with inks 1 and 2, a treatment was run with calcium phytate solution using 0.8mL of an aqueous solution of phytic acid 50% (Aldrich) and 0.22 g of calcium carbonate (Vetec) according to the methodology.
proposed by da A.C.A. de Costa et al. [27]. This solution was dissolved in about 100mL of distilled water and the final volume was adjusted to 1.0L, in volumetric flask, under constant stirring; the pH was adjusted to 5.5-6.0.

For each paper sample, and for both inks, two groups of samples were prepared. For both groups, 5.0mL of the phytate solution were added in glass tubes and inserted in the tubes, so that the area filled by the ink was completely immersed in the liquid for a period of 30 minutes; this is the time generally used in this type of treatment [27].

In a first group, paper strips impregnated with the inks were washed with phytate solution, dried for 24 hours at room temperature and subjected to acid digestion. In a second group, paper strips impregnated with the inks were washed with phytate solution, artificially aged at 100°C for five days in glass tubes, in accordance to the procedure previously adopted [26]. After that, strips were washed again with phytate solution for 15 minutes and subjected to acid digestion. Digestion of paper strips was carried out by an acid solution containing a mixture of 6mL of nitric acid and 2mL of hydrogen peroxide, followed by addition of 4mL of sulphuric acid until complete digestion of the strips.

**Atomic absorption spectroscopy for quantification of iron in papers**

The chemical digestion of strips of paper was performed in order to quantify the total iron present in each strip, under the following conditions:

a) Paper strips impregnated with inks 1 and 2, separately, without washing with calcium phytate.

b) Paper strips impregnated with inks 1 and 2, separately, washed with calcium phytate.

c) Paper strips impregnated with inks 1 and 2, separately, washed with calcium phytate, followed by accelerated artificial aging and a second washing with calcium phytate.

All measurements were performed by an atomic absorption spectrometer, with a continuous source of high resolution, ContrAA 300 (Analytik Jena AG, Jena, Germany) equipped with flame air/acetylene, responsible for atomization of the metal of interest. To measure the element concentration, aspiration of the solution was fixed at 10mL/min, and all measurements were performed in triplicate.

**Scanning electron microscopy of acid and alkalyne papers in the presence of iron-gall inks**

Samples impregnated with inks 1 and 2, separately, without any prior treatment, were used in the analysis the morphology of the aggregate ink on the surface of the paper strips observed by scanning electron microscopy (FEI-Inspect S50, Czech Republic).

Several images were obtained for each sample in distributed and representative areas for each sample. Samples were prepared with gold lining in cathodic pulverization equipment (Emitech K550X) during 3min at 20mA. Images were taken under high vacuum at 15kV and 400 times magnification.

**Air biological contaminants in acid and alkaline papers in the presence and absence of iron-gall inks**

In order to evaluate the growth of microbes in each type of paper, a culture medium was prepared containing paper strips as the sole carbon source. The composition of the culture medium is shown in Table 3.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄</td>
<td>0.5</td>
</tr>
<tr>
<td>KCl</td>
<td>0.5</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>3.0</td>
</tr>
<tr>
<td>FeSO₄.7H₂O</td>
<td>0.01</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

* Paper strips, as previously described, were introduced as carbon source

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The culture medium was prepared at room temperature, and the pH adjusted to 5.0 measured by a digital pH meter (Phtek, PHS-3B, Japan) with HCl 1.0M solution. After this step, the solution was sterilized in a vertical autoclave (Phoenix, AV 75, Brazil) at 1.0atm pressure and 120°C for 20 minutes. After complete sterilization of the medium, it was transferred to sterile Petri dishes. Each paper strip was inserted in each Petri dish, and exposed to ambient air for a period of 2 hours and then incubated at 25°C (Fanem, 347-CDG, Brazil) for 12 days.

Experimental Results

Spot tests of the papers and treatment of iron-gall ink with calcium phytate followed by chemical digestion

Results of spot-tests are shown in Figure 1. It can be seen that tests were positive for lignin for samples D and E, confirmed by the color developed on these papers, as reported in Table 2. These papers, due to their lignin content, not undergone any treatment, are probably better to absorb the ink, as their surfaces are most vulnerable and permeable, allowing a deeper penetration of the ink inside the fibers. This characteristic, however, cannot be considered as a positive point, when a good quality of writing is needed, because, according to Y.H. Guo et al. [28], since the ink can easily penetrate the paper fibers, after drying, may be blurred. Thus, it is important that the paper is semi-permeable to prevent ink from sticking. Additionally, the presence of lignin is a factor which can also accelerate the degradation of cellulose.

![Fig. 1. Spot-tests for acid and alkaline papers. On the left of each sample, lignin detection; on the right, starch detection](image)

With respect to the detection of starch used as sizing agent, the most evident results were seen in samples B, C, F, G and H. The bluish aspect, differentiated for each sample, indicate the presence of starch as a sizing agent in different proportions. Figure 1 indicates that papers B and C appear to have a small amount of starch in comparison with others, where the blue staining was much more intense.

It is emphasized that tests for detection of gelatin were not conclusive, since the presence of starch markedly interfere with the detection of this compound.

The results of chemical digestion of paper strips to quantify the iron contained in each sample from each experimental condition are shown in Table 4. Paper strips impregnated with ink that had no treatment with calcium phytate showed higher iron concentration in the
digested solution when compared to the other two groups treated, especially those containing ink 2. Inks from the same group presented similar concentration values. This is an indication that a single washing with calcium phytate would be enough to prevent the document damaged by excess iron oxidation. This is in accordance to the study performed by da A.C.A. de Costa et al. [27] where authors concluded that there was no need for a second washing after aging of a document with iron-gall ink initially treated with calcium phytate.

<table>
<thead>
<tr>
<th>Paper sample</th>
<th>Fe Ink 1 (mg/L)</th>
<th>Fe Ink 2 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.0</td>
<td>1.6</td>
</tr>
<tr>
<td>B</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>C</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>D</td>
<td>4.1</td>
<td>4.0</td>
</tr>
<tr>
<td>E</td>
<td>4.3</td>
<td>0.7</td>
</tr>
<tr>
<td>F</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>G</td>
<td>2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>H</td>
<td>3.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

a) Paper strips impregnated with inks 1 and 2, separately, without washing with calcium phytate.
b) Paper strips impregnated with inks 1 and 2, separately, washed with calcium phytate.
c) Paper strips impregnated with inks 1 and 2, separately, washed with calcium phytate, followed by accelerated artificial aging and a second washing with calcium phytate.

S. Sequeira et al. [29] obtained results for inhibition of cellulose depolymerization using aqueous and non-aqueous treatment. Thus they concluded that long-term aqueous treatment, due to higher ion mobility, will be one the factors that mostly prevents the degradation of cellulose, even causing the change in their original color dramatically.

From the results from Table 4 interesting information can be extracted about the incorporation of inks in the paper as a function of acidic or alkaline nature of the paper, as well as due to the type of sizing confirmed in spot-tests.

It can be observed that iron concentrations in the paper strips impregnated with ink 1 were equivalent in concentration, with a higher concentration of iron in papers D and E (4.1 and 4.3 mg/L respectively), precisely those where there was no prior treatment to remove lignin, confirmed by the spot-tests of Figure 1. The remaining papers (B, C, F, G and H) presented lower levels of iron after chemical digestion due to the presence of starch that permits the deposition of ink, but without internal fibers absorption due to sizing process. In relation to paper A, negative for the presence of starch and lignin, probably was subjected to a process of sizing with gelatin, presenting an iron content after digestion equivalent to that received by sizing using starch.

Additional results (ink 1), after washing the strips with phytate solution, it can be observed a decrease in the concentration of iron present in the strips due to the partial removal of the non-reactive iron. Except for paper D, all other papers showed a lower iron concentration, corroborating that even stoichiometric inks are not homogeneously absorbed by the paper.

After absorption, washing, aging and a new washing with phytate, the iron content did not change significantly, showing the ineffectiveness of two washing procedures after non-reactive iron removal. Analogous results were found for the ink 2, considering however, that the standard of ink distribution on the paper surface cannot be understood by the same explanation.
obtained from ink 1. Being ink 2 ten times more concentrated in iron and with the double of gum Arabic than ink 1, the standard distribution of this ink on paper is based absorption and scattering in the contact area with the papers and the formation of a thick upper layer due to the viscosity and density characteristics of ink 2. This distribution standard forms a rind over the paper. Thus, treatment with phytate will remove non-reactive iron surplus; in addition, it gradually removes this upper rind that is not closely linked to the structure of the paper.

These observations are confirmed by the results of Table 4 (ink 2, column a) where the concentrations of iron are substantially higher than the ones from ink 1. However, after the first washing with phytate, there was a reduction of over 90% in iron concentration present in the paper strips, confirming the effectiveness of treatment with phytate on removal of the non-reactive iron (ink 2, column b). It is noteworthy that the results of the post-phytate digestion of the samples with ink 2 produced a concentration equivalent to the ink 1 (column a), confirming that phytate removes everything that was not reactive and incorporated by paper fibers. A second wash with phytate, followed by aging and further washing did not produce significant differences in iron concentrations of those of column 2/b, thus confirming the need for a single wash step.

U. Henniges et al. [30] investigated naturally occurring degradation pathways in historic papers sample, with and without ink, doing the identification a typical pattern of the molecular weight distribution in naturally aged papers. They concluded that although the historic samples had been more severely oxidized than model papers, the inhibition of further oxidation and hydrolysis by the calcium phytate/hydrogen carbonate treatment was evident and could be proven for the first time on the molecular level.

Conclusions about the type of sizing or charging (gelatin, starch, lignin without treatment, etc.) could not be obtained for ink 2, since the standard of absorption of inks by paper was severely compromised due to viscosimetric characteristics of these concentrated iron-gall inks.

In papers with the probable presence of gelatin sizing (A), which has many metal coordination sites due to the presence of active groups in its structure, including amino groups (-NH₂) and thiomethyl groups (-SCH₃), Fe³⁺ ions are coordinated with gelatin macromolecules to form metal-polymer complex [31, 32]. Because that complex (iron-gelatin) is the external layer of the paper, washing with calcium phytate can easily remove the metal. In contrast, paper E presented a significant reduction in the concentration of iron, reaffirming what has been observed in SEM images: a flooding of this ink on the surface.

J. Kolar et al. [33] pointed out in their work that the homogeneous original documents inks used can be stabilized by calcium phytate quite differently in each paper, although with a high efficiency. Their results demonstrated the successful stabilization of two historic documents with iron-gall ink using this treatment. However, while the degradation of the treated samples was 10 times slower than in the case of unstabilized samples, the stabilization factor was almost 20 times in some cases.

**Scanning electron microscopy of acid and alkaline papers in the presence of iron-gall inks**

Observations by SEM allowed to study at high magnification the morphology and adhesion of the film formed by iron-gall ink on each sample of paper. From Figure 2 it can be seen that papers A, B, C, F, G and H even when using iron-gall ink in stoichiometric proportions (ink 1), presented a thick layer of ink throughout the surface area of the paper where it was impregnated, indicating that although the fibers have absorbed part of ink, intrinsic and unique characteristics of each paper, for example the type of sizing, can lead to the formation of a film on the surface, favoring the accelerated degradation processes of these supports if not properly stored. With respect to ink 2 the amount of ferrous sulphate added to produce the ink is ten times higher than the stoichiometric, and has twice the concentration of gum Arabic, making it more viscous than ink 1 and hindering penetration through the fibers.
Fig. 2. Images SEM - From top to bottom: samples A, B, C, D, E, F, G and H. From left to right: without ink/with ink 1/with ink 2
With regard to the papers G and H, the excess ink on the paper surface when applied to ink 1 was lower if compared to papers A, B, C and F. This fact may be related, again, to the type of sizing of these two papers: gelatin. Gelatin contributes to the protection of fibers in relation to ink, however the ink can be washed, increasing its permeability. Furthermore, according to M.V. Callol [34], although gelatin is a good adhesive, it favors microbial attack and the yellowing of paper. Corroborating this statement, particularly on paper G, it was found in SEM images (in the strip of paper without ink soaking) microorganisms (mainly diatomaceous algae) that may have been manifested due to the presence of raw materials (Figure 3). It was also observed that the excess ink on the paper surface when ink 2 was used, the formation of sub-layers on the same surface.

Fig. 3. Sample G without impregnating ink: the presence of microorganisms

Paper D images revealed that there was a good impregnation of ink 1 into the paper fibers, i.e., the stoichiometric ink. However, this was not observed for ink 2, which is understandable in view of what has been discussed above on the concentrations of the ingredients of these inks. Thus, it is expected that paper D containing ink with this composition undergoes a faster oxidation process, if not properly treated.

For paper E it was possible to observe a good impregnation of ink 1 in paper fibers, although it is still possible to observe a drenching of this ink on the surface. However, ink 2 was not well absorbed by the fibers, forming huge crusts on the surface of the paper strip.

The observations of the SEM images of eight different papers impregnated with ink 1 showed that papers D and E were the most suitable for writing with iron-gall ink, since the others, in varying degrees, presented a larger amount of ink deposited on the surface. All eight different types of paper impregnated with ink 2 had excess ink on their surfaces.

These observations corroborate the results obtained in spot-tests and the results of atomic absorption for samples D and E, confirming that these papers are the best for the use of iron-gall ink, due to its high permeability and that the iron-gall ink is fixed in the internal fibers of the paper to prevent or, at least, to retard the degradation process of the cellulose of important documents by oxidation or by acid hydrolysis.

According to J. Kolar et al. [35], the most important factors that influence the degradation of historical documents are the width of ink line, which is correlated with the amount of ink applied to the paper, the pH of the area containing the ink and the paper weight.

A. Gambaro et al. [36], studying some original documents dating from the 19th century observed in one of the samples that the ink was completely absorbed by the paper fibers. They attributed this to the use of aqueous solvents for the production of paint. In contrast, it was observed, in another sample the distribution of a discontinuous film, and also cracked and flaked parts. Based on these observations, researchers have assumed that, in this case, the ink is
distributed only on the paper without penetrating into the fibers. In addition, they stressed the importance of this fact in the acceleration of the aging processes of the paper.

T.A. Salah [17] inspected small sample pages (1 mm²) of a heavily corroded manuscript using SEM. They found that brittle morphology of these parts of the manuscript was mostly exposed to corrosion. That is, although both investigated micro samples were taken from the same manuscript, a different state of corrosive attack was clearly seen between the written portion and the blank portion. Thus, they concluded that the influence of iron-gall ink on the macroscopic structure of the fiber is limited only to the area covered by it.

In the work of E. Princi et al. [20] it was discussed the consequences of natural and artificial aging of paper, and the effects on the decomposition of cellulose. According to them, as the oxygen does not penetrate the cellulose matrix, aging paper in closed and dark environments can only lead to superficial oxidation of cellulose. Therefore, if the paper is kept in darkness for an unlimited time, the cellulose matrix will be unchanged. In contrast to oxygen, which only attacks the surface of the cellulose, UV rays can penetrate deep into the cellulosic fibers. Considerations regarding the effect of artificial aging on the treatment and conservation of long-term papers were also discussed by L. Hajji et al. [37].

T. Ersoy et al. [38] found in his work that even with sizing, historical documents and works of art made of paper can have various types of surface contaminants, including degraded glue, ink stains, suspended particles and biological agents. The sizing treatment in old papers was carried out mostly from large molecules capable of forming a film only on the surface of the fibrous network, thereby contributing to the strength of the paper. Currently, this treatment is carried out especially with smaller molecules, which penetrate inside the fibers, thereby increasing the internal strength of the paper [39]. The iron-gall ink requires a strong interaction with the internal fibers of the paper, where ambient air is unable to oxidize the free iron present in the composition of this ink. Therefore, it is only favorable for paper without treatment, because the sizing acts as a problem for iron-gall ink, protecting the paper against ink blurs, preventing a suitable permeation of the ink into the paper fibers [28, 40, 41]. Therefore, it is natural to form an ink film on the surface of the paper. Thus, it is expected that the vast majority of the cultural heritage made of paper from different parts of the Western world written with iron-gall ink during any period of history is threatened, requiring categorically preventive conservation or, in extreme cases, a corrective restoration.

Air biological contaminants in acid and alkaline papers in the presence and absence of iron-gall inks

The results of the microbiological testing are shown in Figure 4. In these figures it can be seen that even in the absence of carbon source in addition to the strip of paper (cellulose), there was growth of micro-organisms in all cases, particularly in the region where the paper strips were placed on the plate, and in their surroundings. This shows that the selected culture medium supplied with the necessary nutrients for cosmopolitan fungi, supplemented with a source of cellulosic carbon was adequate.

In Figure 4 it can be seen that the microorganisms grew more abundantly in paper containing starch, since this polysaccharide is a common carbon source for fungi, confirming the cellulosytic nature of the contaminating species. Therefore, the growth of fungi in sample E was not substantial, probably due to the absence of such polysaccharide, corroborating the results of spot-tests.

In the remaining Petri dishes, independently of the ink (1 or 2) impregnated into paper strips and inoculated into culture medium, it was found that fungal species grew both in the area of the paper without the ink and in the area with ink, indicating that the presence of the ink, although it is a facilitating of chemical degradation of paper, does not prevent fungal proliferation. It was also observed that the higher the quantity of starch (determined by the more pronounced coloration in the spot-tests) on the sample surface, the greater the growth of fungi (samples F and H). Furthermore, note that the presence of alkali charge in papers F, G and H, which did not prevent the samples were contaminated with fungus.
Fig. 2. Plates with culture medium containing paper as single carbon source: unpainted samples (1st Column), with the ink 1 (2nd Column) and with the ink 2 (3rd Column).
All fungi produce organic acids, which reduce the pH of paper, conditioning the dynamics of its growth in secondary attacks and accelerating corrosion of the metal component of the iron-gall ink and subsequently causing the biodeterioration of the paper [7, 21, 42, 43]. Thus, the presence of micro-organisms on paper strips proves that those papers, primarily due to the type of sizing, are quite vulnerable to biological attack, especially by fungi. According to M. Drdácký et al. [21], a particular kind of alteration due to fungi is the discoloration of inks due to tannase enzyme, produced by some strains, that catalyses the hydrolysis of gallotannate. Besides that, the corrosive nature of iron-gall ink becomes also responsible for opening the way for microbial deterioration.

This statement can be proven by the results observed in the samples of the paper is impregnated with inks 1 and 2, in which can be seen the marked growth of fungi in the exactly dyed area, these remaining absent in the non-impregnated areas of the paper strips.

M. Manso et al. [44] studied three historical manuscripts dated 1589–1592 that were strongly attacked by fungi, mainly around the written text. They used portable energy-dispersive X-ray spectroscopy to analyze the supports and identified characteristic elements (Fe, Cu, Zn, and Mn) from iron gall inks. It was also observed a high amount of Zn in the non-degraded ink regions. In this way, in vitro tests were carried out in the presence of zinc sulfate, revealing inhibition capacity for the majority of fungi sampled from these manuscripts.

Conclusions

The papers interacted with the iron-gall ink 1 and 2 in different ways, depending directly on its specific characteristics, such as type of fiber, lignin content, type of sizing, charge, etc.

Treatment with aqueous solution of calcium phytate significantly reduced iron concentration in excess of the original samples. The group of samples which received a second treatment with calcium phytate showed no significant iron concentration values, indicating that a second wash is unnecessary.

Papers D and E absorbed the stoichiometric iron-gall ink (ink 1) in its fibers better than the other samples. All attempts to use ink 2 formed a thick layer on the support surface.

The non-destructive technique of scanning electron microscopy proved to be adequate to validate the results obtained by atomic absorption spectroscopy, to corroborate the efficient treatment with calcium phytate to remove excess iron.

The chemical corrosion of the support, caused by the use of iron-gall ink, favored microbiological contamination by fungi in all types of paper, irrespective to the presence or absence or iron in paper strips both in acidic and alkaline papers.

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