The Effects of Plant Dyes, Watercolours and Acrylic Paints on the Physical, Chemical and Biological Stability of Japanese Tissue Paper Used in Paper Conservation

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Abstract

Despite substantial literature on the dyeing of textiles, there is a lack of research about dyeing and colouring Japanese mending papers for paper conservation purposes. In this study, a range of scientific techniques have been applied to improve understanding of the physical, chemical and biological properties of Japanese mending papers after treatment with various dyes and pigments. A variety of toning materials including plant dyes, watercolours, acrylic paints, inks, pastels, gouaches, and colour pencils are commonly used by conservators for paper toning purposes. Plant dyes are often used in artisanal practices including painting, handicrafts, textiles and paper dyeing; however, the chemistry of such colourants and their interaction with Japanese mending papers used in paper conservation has not been studied. The basic premise of this study is that a conservation treatment should not contribute to the physical, chemical or biological degradation of mending papers used for paper conservation purposes and should, ideally, prevent such degradation.

In this study, two Japanese tissue papers (Yukyu-shi and Sekishu Mare) were treated with selected plant dyes, watercolours and acrylic paints. Paper specimens were subject to both moist-heat artificial ageing and accelerated photoageing and colour changes were measured using spectrophotometry and microfading tests (MFTs). Physical experiments (folding endurance, tear resistance) and chemical tests (pH) were used to investigate the paper degradation mechanisms to achieve a better understanding of how paper deteriorates as a result of artificial ageing. The results show that, in general, the papers treated with plant dyes are more acidic than those treated with watercolours and acrylic paints. Almost all of the plant dyes tested in this work showed some degree of fading as measured by spectrophotometry, compared to untreated controls and those samples treated with watercolours and acrylic paints. By contrast, synthetic artists’ pigments were relatively stable to colour change. Acrylic paints and watercolours are the most widespread colourants used by paper conservators and their continued use over plant dyes is justified by this study. While their use is undergoing a revival and they are seen to have heritage value as a
traditional product, plant dyes may not be suitable for colour-matching the retouched parts of ancient books and documents because of their propensity for colour change over time.

Dyed papers also displayed less folding and tear resistance after ageing and there was a difference in these properties between Yukyu-shi and Sekishu papers. The untreated Sekishu papers and the Sekishu papers treated with watercolours and acrylic paints exhibited greater tear resistance than the Yukyu-shi papers. The Sekishu and Yukyu-shi papers in untreated form and when treated with acrylic paints, as well as the Yukyu-shi papers treated with plant dyes, demonstrate effective folding endurance after ageing.

A further aim of this thesis was to quantify the growth of *Aspegillus niger* and *Penicillium rubrum* fungal species on Japanese tissue papers with the aid of real time polymerase chain reactions (PCR). This technique amplifies deoxyribonucleic acid (DNA) from the target species which is a proxy for species abundance. Universal PCR primers amplified DNA from both *A. niger* and *P. rubrum* and these species were found to grow preferentially on Yukyu-shi paper, regardless of the treatment. Sekishu papers treated with most plant dyes and chemical colourants were more resistant to fungal growth than similarly treated Yukyu-shi papers.

In summary, this study suggests that for the best long term preservation outcomes for paper materials in archives, libraries, galleries and museums, acrylic artist paints generally perform better in conservation terms than plant dyes and watercolours. This must be balanced against the fact that traditional paper conservation practices may have particular cultural values in some circumstances. Important new insights and opportunities to improve conservation outcomes, and safeguard unique cultural heritage, can be based on the innovative use of an array of scientific techniques that question established cannons.
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# Table of Contents

ABSTRACT ...................................................................................................................... I

ACKNOWLEDGMENTS .................................................................................................. III

CONTRIBUTIONS ........................................................................................................ VII

TABLE OF CONTENTS .................................................................................................... VIII

LIST OF FIGURES ........................................................................................................ XIII

LIST OF TABLES ............................................................................................................ XVIII

APPENDICES ................................................................................................................ XIX

ABBREVIATIONS ........................................................................................................... XX

1. INTRODUCTION........................................................................................................ 1

1.1 Outline of the thesis ................................................................................................. 3

1.2 Original contribution ............................................................................................... 4

2. LITERATURE REVIEW .............................................................................................. 7

2.1 Introduction .............................................................................................................. 7

2.2 Paper materials ........................................................................................................ 8

  2.2.1 Machine-made paper production techniques ...................................................... 9

  2.2.2 Japanese tissue papers ....................................................................................... 10

2.3 Dyes and pigments .................................................................................................. 12

  2.3.1 The history of dyes and pigments ................................................................... 13

  2.3.2 Dyes ................................................................................................................ 15

    Anthraquinone dyes.................................................................................................. 16

    Naphthoquinone dyes ............................................................................................. 16

    Henna ...................................................................................................................... 17

    Flavonoid dyes ........................................................................................................ 19

    Black tea ................................................................................................................ 19
Table of Contents (continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus leaves</td>
<td>22</td>
</tr>
<tr>
<td>2.3.3 Pigments</td>
<td>24</td>
</tr>
<tr>
<td>Watercolours</td>
<td>27</td>
</tr>
<tr>
<td>Acrylic paints</td>
<td>28</td>
</tr>
<tr>
<td>2.4 Paper conservation ethics</td>
<td>29</td>
</tr>
<tr>
<td>2.5 Physical, chemical and biological properties of paper materials</td>
<td>34</td>
</tr>
<tr>
<td>2.5.1 Ageing</td>
<td>34</td>
</tr>
<tr>
<td>2.5.2 Folding endurance</td>
<td>39</td>
</tr>
<tr>
<td>2.5.3 Tear resistance</td>
<td>41</td>
</tr>
<tr>
<td>2.5.4 Colour change</td>
<td>43</td>
</tr>
<tr>
<td>2.5.5 Micro fading</td>
<td>45</td>
</tr>
<tr>
<td>2.5.6 The effect of acidity on paper</td>
<td>49</td>
</tr>
<tr>
<td>2.5.7 Biological properties of paper materials</td>
<td>51</td>
</tr>
<tr>
<td>Biological properties of dyes and pigments</td>
<td>54</td>
</tr>
<tr>
<td>Molecular techniques in heritage conservation</td>
<td>55</td>
</tr>
<tr>
<td>2.6 Objectives</td>
<td>59</td>
</tr>
<tr>
<td>2.7 Conclusion of literature review</td>
<td>61</td>
</tr>
<tr>
<td>3. SURVEY OF PAPER CONSERVATION PRACTITIONERS</td>
<td>63</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>63</td>
</tr>
<tr>
<td>3.2 Survey participants</td>
<td>64</td>
</tr>
<tr>
<td>3.3 Use of Japanese tissue papers in paper conservation</td>
<td>65</td>
</tr>
<tr>
<td>3.4 Characteristics of Japanese mending papers</td>
<td>66</td>
</tr>
<tr>
<td>3.5 Colourants used for toning Japanese tissue papers</td>
<td>68</td>
</tr>
<tr>
<td>3.5.1 Colourants used for toning Japanese tissue papers in Australia, European countries and the United States</td>
<td>69</td>
</tr>
<tr>
<td>3.6 Types of plant dyes for toning Japanese tissue papers</td>
<td>70</td>
</tr>
<tr>
<td>3.7 Popular brands of toning materials used in paper conservation</td>
<td>72</td>
</tr>
<tr>
<td>3.8 Reasons for choosing toning materials</td>
<td>73</td>
</tr>
<tr>
<td>3.9 Studies on the effects of dyes and pigments on Japanese tissue papers</td>
<td>74</td>
</tr>
</tbody>
</table>
3.10 Long term effects of toned Japanese tissue papers ........................................ 75
3.11 Conclusions .................................................................................................................. 77

4. METHODOLOGY ................................................................................................................. 80
4.1 Sample preparation ........................................................................................................... 80
  4.1.1 Collection and processing of samples ................................................................. 80
  4.1.2 Extraction and dyeing conditions ........................................................................ 83
  4.1.3 Dyeing of Japanese tissue papers ......................................................................... 84

4.2 Experimental testing of properties ................................................................................ 85
  4.2.1 Artificial ageing ....................................................................................................... 85
  4.2.2 Folding endurance ................................................................................................. 88
  4.2.3 Tear resistance ........................................................................................................ 90
  4.2.4 Colour change ......................................................................................................... 91
  4.2.5 Micro fading test .................................................................................................... 94
  4.2.6 pH .......................................................................................................................... 96
  4.2.7 Fungal growth test ................................................................................................. 97
    Preparation of the samples ............................................................................................ 97
    Fungal strains and growth conditions ........................................................................... 99
    Spore quantification using hemocytometer .................................................................. 101
    Inoculation of paper samples ....................................................................................... 102
    DNA extractions ........................................................................................................... 103
      Organic extraction ........................................................................................................ 104
      QIAamp® mini kit DNA extraction ............................................................................ 106
    DNA quantification using Qubit® 2.0 fluorometer .................................................... 107
    Selection of primers ..................................................................................................... 108
    Polymerase chain reaction (PCR) ............................................................................... 111
  4.2.8 Statistical analysis .................................................................................................... 117

5. RESULTS ................................................................................................................................. 118
5.1 Results of folding endurance test ................................................................................... 118
  5.1.1 Results of folding endurance test for the untreated papers and the papers treated
       with plant dyes ............................................................................................................ 118
  5.1.2 Results of folding endurance test for the untreated papers and the papers treated
       with watercolours ....................................................................................................... 119
  5.1.3 Results of folding endurance test for the untreated papers and the papers treated
       with acrylic paints ...................................................................................................... 121

5.2 Results of tear resistance test .......................................................................................... 123
5.2.1 Results of tear resistance test for the untreated papers and the papers treated with plant dyes .................................................................................................................. 123
5.2.2 Results of tear resistance test for the untreated papers and the papers treated with watercolours ........................................................................................................ 124
5.2.3 Results of tear resistance test for the untreated papers and the papers treated with acrylic paints ...................................................................................................... 125

5.3 Results of colour change .................................................................................. 127
5.3.1 Results of colour change on the untreated papers and the papers treated with plant dyes .................................................................................................................. 127
5.3.2 Results of colour change on the untreated papers and the papers treated with watercolours ........................................................................................................ 129
5.3.3 Results of colour change on the untreated papers and the papers treated with acrylic paints ...................................................................................................... 130

5.4 Results of microfading tests .......................................................................... 131
5.4.1 Microfading tests on the untreated papers .................................................. 132
5.4.2 Microfading tests on the treated papers .................................................... 134

5.5 Results of pH test .......................................................................................... 137
5.5.1 Results of pH test on the untreated papers and the papers treated with plant dyes .................................................................................................................. 137
5.5.2 Results of pH test on the untreated papers and the papers treated with watercolours ........................................................................................................ 138
5.5.3 Results of pH test on the untreated papers and the papers treated with acrylic paints ...................................................................................................... 139

5.6 Results of fungal growth tests ....................................................................... 140
5.6.1 Results of fungal growth on the untreated papers (PCR was performed with the UN1 universal primer pair) ........................................................................ 140
5.6.2 Results of fungal growth on the untreated papers and the papers treated with plant dyes (PCR was performed with the UN1 universal primer pair) .......... 142
5.6.3 Results of fungal growth on the untreated papers and the papers treated with watercolours (PCR was performed with the UN1 universal primer pair) .......... 144
5.6.4 Results of fungal growth on the untreated papers and the papers treated with acrylic paints (PCR performed with the UN1 universal primer pair) .......... 145
5.6.5 Results of fungal growth on the untreated papers (PCR performed with the UN2 universal primer pair) ........................................................................ 147
5.6.6 Results of fungal growth on the untreated papers and the papers treated with plant dyes (PCR was performed with the UN2 universal primer pair) .......... 148
5.6.7 Results of fungal growth on the untreated papers and the papers treated with watercolours (PCR performed with the UN2 universal primer pair) .......... 150
5.6.8 Results of fungal growth on the untreated papers and the papers treated with acrylic paints (PCR performed with the UN2 universal primer pair) .......................... 151

6. DISCUSSION .......................................................................................................................... 154

6.1 Introduction .......................................................................................................................... 154

6.2 The effects of acidity on paper ............................................................................................ 155

6.3 Folding endurance ............................................................................................................... 157

6.4 Tear resistance ..................................................................................................................... 161

6.5 Colour change after artificial ageing .................................................................................. 163

6.6 Colour change after photoageing ........................................................................................ 167

6.7 Fungal growth ....................................................................................................................... 170

7. CONCLUSIONS AND FURTHER WORK .......................................................................... 177

REFERENCES ............................................................................................................................ 184
List of Figures

Chapter 2:

Figure 2-1: Chemical structure of an anthraquinone-----------------------------16
Figure 2-2: Chemical structure of 1, 4-naphthaquinone-------------------------17
Figure 2-3: Chemical structure of lawson --------------------------------------17
Figure 2-4: Chemical structure of catechin (C)-------------------------------19
Figure 2-5: Chemical structure of theaflavins ---------------------------------20
Figure 2-6: Chemical structure of thearubigin -------------------------------20
Figure 2-7: Chemical structure of epicatechin (EC) ----------------------------21
Figure 2-8: Chemical structure of gallocatechin (GC) -------------------------21
Figure 2-9: Chemical structure of catechin gallate (CG) ----------------------21
Figure 2-10: Chemical structure of epicatechin gallate (ECG) -----------------21
Figure 2-11: Chemical structure of epigallocatechin (EGC) ---------------------22
Figure 2-12: Chemical structure of epigallocatechin gallate (EGCG) ---------22
Figure 2-13: Chemical structure of quercetin -------------------------------23
Figure 2-14: Chemical structure of rutin --------------------------------------23
Figure 2-15: Chemical structure of alizarin crimson ------------------------26
Figure 2-16: Japanese tissue paper used as support for Persian document conservation ---33
Figure 2-17: Papers affected by fungi due to previous inappropriate storage conditions --54

Chapter 3:

Figure 3-1: Frequencies of the survey sample demographics ------------------65
Figure 3-2: Use of Japanese tissue papers by paper conservators--------------66
Figure 3-3: Use of toning materials for colouring Japanese tissue papers for paper conservation. -----------------------------------------------69
Figure 3-4: Use of toning materials in Australia, the European countries and the United States of America. --------------------------------------70
Figure 3-5: Use of plant dyes as toning materials in paper conservation -------71
Figure 3-6: Most popular brands of watercolours and acrylic paints used by paper conservators. --------------------------------------------72
Figure 3-7: Reasons of choosing toning materials by paper conservators-------74
Figure 3-8: Awareness of longevity of toned papers over time ----------------76

Chapter 4:

Figure 4-1: Filtration process for henna --------------------------------------84
Figure 4-2: Yukyu-shi paper after treatment with alizarin crimson acrylic paint ---85
Figure 4-3: Untreated and treated paper samples were artificially aged at 70 °C and 65% relative humidity (RH) for 12 days.

Figure 4-4: Left to right: untreated Sekishu (S) and Yukyu-shi (Y) papers and papers treated with henna, black tea, dried and fresh eucalyptus leaves respectively. The figure shows the paper specimens both before and after moist-heat artificial ageing.

Figure 4-5: MIT folding endurance tester was used to study the folding endurance of treated and untreated papers before and after ageing.

Figure 4-6: Tear tester machine - Yukyu-shi papers treated with alizarin crimson watercolour are located between the machine jaws.

Figure 4-7: Three dimensional representation of the CIE Lab colour space

Figure 4-8: The spectrophotometer used for measuring colour shifts of treated and untreated paper specimens before and after ageing.

Figure 4-9: Microfading tester used for identifying fading resistance of untreated papers and papers treated with the selected dyes and pigments.

Figure 4-10: Whatman® Cellulose Filter Paper control used for fungal growth test (left) and treated paper specimens stored individually in petri dishes (right).

Figure 4-11: Paper specimens for DNA extractions were prepared in a biosafety cabinet.

Figure 4-12: Aspergillus niger (left) and Penicillium rubrum (right) after 6 days growth at room temperature.

Figure 4-13: Fungal stocks in saline solution (0.85% NaCl) which were stored at room temperature.

Figure 4-14: Haemocytometer used for counting the spores

Figure 4-15: Preparation of the spore suspension by gently scraping the surface of the agar plate with the aid of a plastic swab.

Figure 4-16: Paper specimens were placed in individual eppendorf tubes after incubation and placed in the freezer at -20 °C for at least 12 hours before DNA extractions.

Figure 4-17: Qubit® 2.0 fluorometer used to measure the DNA concentrations of the paper samples.

Figure 4-18: Standard curve generated with UN2 primer set and Aspergillus niger. The lowest DNA concentration was 4.16 ng/µL.

Figure 4-19: Standard curve generated with UN1 primer sets and Aspergillus niger. The lowest DNA concentration was 4.16 ng/µL.

Figure 4-20: Standard curve generated with UN2 primer sets and Penicillium rubrum. The lowest DNA concentration was 2.38 ng/µL.

Figure 4-21: Standard curve generated with UN1 primer sets and Penicillium rubrum. The lowest DNA concentration was 2.38 ng/µL.

Chapter 5:

Figure 5-1: Folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes before and after artificial ageing (outliers are indicated in the
Figure 5-2: Folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours before and after artificial ageing (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.

Figure 5-3: Folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints before and after artificial ageing (outliers are indicated in the form of circles and asterisks in the graph). Error bars represent confidence intervals at 95% level.

Figure 5-4: Tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes before and after artificial ageing. Error bars represent confidence intervals at 95% level.

Figure 5-5: Tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours before and after artificial ageing. Error bars represent confidence intervals at 95% level.

Figure 5-6: Tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints before and after artificial ageing. Error bars represent confidence intervals at 95% level.

Figure 5-7: Colour change shifts on untreated papers (Sekishu and Yukyu-shi) and papers treated with plant dyes (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.

Figure 5-8: Colour change shifts on untreated papers (Sekishu and Yukyu-shi) and papers treated with watercolours (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.

Figure 5-9: Colour change shifts on untreated papers (Sekishu and Yukyu-shi) and papers treated with acrylic paints (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.

Figure 5-10: Microfading curves obtained for BW1, BW2 and BW3 as well as untreated Yukyu-shi and Sekishu papers after 10 minutes exposure to an intensive light source.

Figure 5-11: Microfading curves obtained for BW1, BW2 and BW3 as well as the Yukyu-shi and Sekishu papers treated with black tea and henna after 10 minutes exposure to an intensive light source. There is a missing data point for the Yukyu-shi paper treated with henna at 3 minutes.

Figure 5-12: Microfading curves obtained for BW1, BW2 and BW3 as well as the Yukyu-shi and Sekishu papers treated with fresh and dried Eucalyptus leaves after 10 minutes exposure to an intensive light source.

Figure 5-13: Microfading curves obtained for BW1, BW2 and BW3 as well as the Yukyu-shi and Sekishu papers treated with watercolour and acrylic paint of alizarin crimson after 10 minutes exposure to an intensive light source.
Figure 5-14: pH of untreated Sekishu and Yukyu-shi papers and papers treated with plant dyes before and after ageing. .................................................. 138

Figure 5-15: pH of untreated Sekishu and Yukyu-shi papers and papers treated with watercolours before and after ageing. ............................. 139

Figure 5-16: pH of untreated Sekishu and Yukyu-shi papers and papers treated with acrylic paints before and after ageing. ................................. 140

Figure 5-17: Concentrations of DNA recovered from untreated papers (Yukyu-shi, Whatman and Sekishu) at the time of inoculation with Aspergillus niger and Penicillium rubrum (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). ............................................................. 142

Figure 5-18: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes at the time of inoculation with Aspergillus niger and Penicillium rubrum (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). ............................................................. 143

Figure 5-19: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours at the time of inoculation with Aspergillus niger and Penicillium rubrum (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). ............................................................. 145

Figure 5-20: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints at the time of inoculation with Aspergillus niger and Penicillium rubrum (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). ............................................................. 147

Figure 5-21: Concentrations of DNA recovered from untreated papers (Yukyu-shi, Whatman and Sekishu) at the time of inoculation with Aspergillus niger and Penicillium rubrum (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). ............................................................. 148

Figure 5-22: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes at the time of inoculation with Aspergillus niger and Penicillium rubrum (day 0) and after 10 days of incubation at 27 °C and 80% RH. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). ............................................................. 150
Figure 5-23: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). -------------------------------------------------- 151

Figure 5-24: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level). -------------------------------------------------- 152

Chapter 6:

Figure 6-1: pH versus folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints before and after artificial ageing. -------------------------------------------------- 161

Figure 6-2: pH versus tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints before and after artificial ageing. -------------------------------------------------- 163

Figure 6-3: pH versus colour change of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints after artificial ageing. -------------------------------------------------- 164

Figure 6-4: Chemical structure of flavone--------------------------------------------------------------- 165

Figure 6-5: Chemical structure of flavonol----------------------------------------------------------- 166

Figure 6-6: pH versus colour change of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, a watercolour and an acrylic paint of alizarin crimson after photo ageing. -------------------------------------------------- 170

Figure 6-7: pH versus DNA concentration for untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair. -------------------------------------------------- 172

Figure 6-8: pH versus DNA concentration for untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair. -------------------------------------------------- 173
List of Tables

Chapter 4:

Table 4-1: List of commercial watercolours and acrylic paints with properties as declared by the manufacturers. .........................................................82
Table 4-2: Primers used to target fungal species ........................................110
Table 4-3: Components of the qPCR master mix used to quantify fungal DNA ..........112
Table 4-4: Cycling conditions for the PCR reactions. Annealing temperature is primer set specific. .................................................................113

Chapter 5:

Table 5-1: Treatments resulting in significant differences between folding endurance before and after ageing after a two-way ANOVA test (p ≤ 0.05). The folding endurances for these treatments were either significantly reduced (↓) or increased (↑) after ageing. .........................................................122
Table 5-2: Treatments resulting in significant differences between tear resistance before and after ageing after a two-way ANOVA test (p ≤ 0.05). The tear resistance for these treatments were either significantly reduced (↓) or increased (↑) after ageing. ------ 126
Table 5-3: Colour change summary (total exposing over 10 minute fading run is approximately 1mlx hour). .................................................................132
Table 5-4: Summary of the two-way ANOVA significant differences (treatments resulting in significantly higher (↑) or lower (↓) concentrations of DNA than other treatments after 10 days of incubation at 27 °C and 80% RH). (p < 0.05). ..............................153

Chapter 7:

Table 7-1: Summary of results obtained from folding endurance, tear resistance, colour change, pH and fungal growth for the papers. A tick (✓) indicates a desirable conservation outcome and a cross (✗) represents a poor, or less desirable, conservation outcome. .................................................................................................178
Appendices

**Appendix 1: Ethics Approval** 212

**Appendix 2: Survey questions (colouring Japanese tissue paper)** 214

**Appendix 3: Shifts in $L^*$, $a^*$, $b^*$ coordinates of the untreated Yuku-yu-shi and Sekishu papers and the papers treated with plant dyes, watercolours and acrylic paints before and after artificial ageing followed by the total mean of $\Delta L^*$, $\Delta a^*$, $\Delta b^*$, and $\Delta E$.** 216
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>A. niger</td>
<td><em>Aspergillus niger</em></td>
</tr>
<tr>
<td>APP</td>
<td><em>Aspergillus</em> and <em>Penicillium</em> primers</td>
</tr>
<tr>
<td>AP</td>
<td>Acrylic paints</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>ASP1-ASP3</td>
<td><em>Aspergillus</em> primers</td>
</tr>
<tr>
<td>ASPn</td>
<td><em>Aspergillus niger</em> primers</td>
</tr>
<tr>
<td>a*</td>
<td>redness-greenness</td>
</tr>
<tr>
<td>b*</td>
<td>yellowness-blueness</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>BS</td>
<td>British Standard</td>
</tr>
<tr>
<td>BWS</td>
<td>Blue Wool Standard</td>
</tr>
<tr>
<td>BWE</td>
<td>Blue Wool Equivalent</td>
</tr>
<tr>
<td>BWFSs</td>
<td>Blue wool fading standards</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>ºC</td>
<td>degree centigrade</td>
</tr>
<tr>
<td>C</td>
<td>catechin</td>
</tr>
<tr>
<td>CG</td>
<td>catechin gallate</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CIE</td>
<td>International Commission on Illumination</td>
</tr>
<tr>
<td>Ct</td>
<td>cycle threshold</td>
</tr>
<tr>
<td>ΔE</td>
<td>colour change</td>
</tr>
<tr>
<td>ΔL*</td>
<td>lightness change</td>
</tr>
<tr>
<td>Δa*</td>
<td>redness-greenness change</td>
</tr>
<tr>
<td>Δb*</td>
<td>yellowness-blueness change</td>
</tr>
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<td>ΔC</td>
<td>chromaticity change</td>
</tr>
<tr>
<td>Δh</td>
<td>hue change</td>
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<td>deoxyribonucleic acid</td>
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<td>dinucleotide triphosphate</td>
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<td>degree of polymerisation</td>
</tr>
<tr>
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<td>double stranded deoxyribonucleic acid</td>
</tr>
<tr>
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<td>dithiothreitol</td>
</tr>
<tr>
<td>dH2O</td>
<td>deionised water</td>
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<td>epigallocatechin</td>
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<td>epigallocatechin gallate</td>
</tr>
<tr>
<td>E. leaves</td>
<td>Eucalyptus leaves</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>G</td>
<td>Guanine</td>
</tr>
<tr>
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<td>gravity</td>
</tr>
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<td>GC</td>
<td>gallocatechin</td>
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<tr>
<td>g/L</td>
<td>grams per litre</td>
</tr>
<tr>
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<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>g/m²</td>
<td>grams per square metre</td>
</tr>
<tr>
<td>IBM</td>
<td>International Business Machines</td>
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<tr>
<td>IDT</td>
<td>Integrated DNA Technology</td>
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<tr>
<td>ISO</td>
<td>International Standards Organisation</td>
</tr>
<tr>
<td>ITS</td>
<td>Internal Transcribed Spacer</td>
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<tr>
<td>K</td>
<td>Kelvin</td>
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<tr>
<td>Kg</td>
<td>kilograms</td>
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<td>Kilopascal</td>
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<td>L*</td>
<td>lightness-darkness</td>
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<tr>
<td>Lux</td>
<td>lumens per square metre</td>
</tr>
<tr>
<td>Lysis buffer</td>
<td>10mM Tris base, 1mM EDTA, 100mM sodium chloride and 2% Tween 20 with pH 8.0</td>
</tr>
<tr>
<td>MΩ.cm</td>
<td>megohm centimetre</td>
</tr>
<tr>
<td>MFT</td>
<td>Microfaing test</td>
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<td>Mlx</td>
<td>millilux</td>
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<td>Mean difference</td>
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<td>millinewton</td>
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<td>micromolar</td>
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<tr>
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<td>micrometre</td>
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<tr>
<td>u/µL</td>
<td>units per microlitre</td>
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<td>microlitre</td>
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<tr>
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<tr>
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<td>nanometre</td>
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<td>NaCl</td>
<td>sodium chloride</td>
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<td>PD</td>
<td>Plant dyes</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PEN</td>
<td><em>Penicillium</em> primers</td>
</tr>
<tr>
<td>pH</td>
<td>Power of Hydrogen</td>
</tr>
<tr>
<td><em>P. rubrum</em></td>
<td><em>Penicillium rubrum</em></td>
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<tr>
<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>R</td>
<td>Registered</td>
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<tr>
<td>RFU</td>
<td>relative fluorescence unit</td>
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<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>Rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SCI</td>
<td>Specular component included</td>
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<tr>
<td>Sekishu Mare</td>
<td>Sekishu Mare</td>
</tr>
<tr>
<td>T</td>
<td>Thymine</td>
</tr>
<tr>
<td>TAPPI</td>
<td>Technical Association of Pulp and Paper Industry</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>TE buffer</td>
<td>10 mM Tris, 0.1 mM EDTA, pH 8.0</td>
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<tr>
<td>TM</td>
<td>Trademark</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>Tris</td>
<td>2-Amino-2-hydroxymethyl-propane-1,3-diol</td>
</tr>
<tr>
<td>UN1</td>
<td>Universal primers</td>
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<td>UN2</td>
<td>Universal primers</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>W</td>
<td>watt</td>
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<tr>
<td>WC</td>
<td>Watercolours</td>
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Chapter 1: Introduction

1. INTRODUCTION

Libraries and archives aim not only to preserve books and documents of cultural value for future generations but also to make them accessible to researchers and to the communities who value them. However, the physical, chemical and biological properties of paper materials can be adversely affected by ageing (Zervos 2010). The stabilisation of compromised paper documents and art-works is a crucial practice in paper conservation and Japanese tissue papers have long been used for paper repair. Their long, strong and flexible fibres make them stable against ageing (Kurosawa & Hashino 2012; Doming 2005). However, the colour of these papers needs to be visually adjusted to be in keeping with the tonality of the original document. Conservators sometimes have to use different colourants to tone Japanese tissue papers to not only match the various tonalities of old manuscripts, but also to maintain the aesthetic qualities of the original document. Today, toning materials including black tea, coffee, yasha (Alnus japonica), watercolours, acrylic paints, inks, gouaches, colour pencils and pastels are commonly used in paper conservation practice (Ormsby & Learner 2010; Poulsson 2008; Winter 2008; Owen, Ploeger & Murray 2004; Gyles & Maver 2002; Grantham & Webber 2002; McAusland 2002; Norton 2002; Townshend 2002). A toning process (defined as inpainting, retouching or colouring) is a subjective issue in paper conservation and there is no standard rule in paper conservation practice to cover it (Poulsson 2008). The toning process should ideally be performed with colourfast colourants. Fugitive colourants are, however, subject to fading and then the colour of the repaired paper becomes noticeably different to that of the original paper.

Although natural colourants have been used by artisans and craftsmen from ancient times, the application of such eco-friendly colourants is of relatively recent interest in textile
dyeing and there is substantial literature about the application of plant dyes in the textile industry (Degano et al. 2009; Samanta & Agarwal 2009; Bechtold, Mahmud-Ali & Mussak 2007; Flint 2007; Bechtold et al. 2003). Dyeing the fibres used in textiles constitutes an important and time-honoured aspect of Iranian art and industry (Afshar 2001). In the 16th and 17th centuries (Safavid and Qajar periods), craftsmen used plant dyes in Iranian miniature paintings (Purinton & Watters 1991). More specifically, Sadiqi Bek—a royal painter in the Safavid period—discussed the techniques of manufacturing artists' supplies and the application of colourants in his treatise, Ghanon al-Mosavar. Many toning materials have been used by Iranian artisans drawing on past experience. These were studied by Barkeshli and Ataie (2002) who found that saffron can act as a buffer against the corrosive reactions of some natural colourants including verdigris (copper acetate) in ancient Iranian manuscripts and miniature paintings. The scientific study of traditional art and crafts can be a reliable way in the preservation of such tangible heritage. Further, the revival of traditional art and crafts can be beneficial to artists, conservators and museum professionals, increasing their understanding of the materials and methods used in the past.

An artificial ageing test is used in this study to determine the long-term effects of toning materials used for paper conservation purposes. This thesis sets out to identify the physical stability of the Yukyu-shi and Sekishu Mare (Sekishu) paper samples treated with selected plant dyes, watercolours and acrylic paints—particularly those which may occur in paper conservation practice—using a number of physical, chemical, optical and molecular techniques. The findings of this study will compare the effects of toning materials on the mending papers to determine which papers and treatments have the most effective physical and chemical properties in long term storage conditions in museums, galleries, archives
and libraries. The results provide a comparative assessment of the fungal bioreceptivity — defined as the ability of a material to be colonised by fungi — of the untreated papers and those that were treated with the selected dyes and pigments, and identifies the encouragement or inhibition properties of the toning materials for the paper samples.

1.1 Outline of the thesis

This dissertation is organised into seven chapters with several sub-sections within each chapter. The introductory chapter covers the application of toning materials including plant dyes and chemical colourants for toning Japanese tissue papers in paper conservation.

- **Chapter 2** is a comprehensive literature review. It starts with an introduction to paper materials, followed by a short history of paper production, differences between machine-made papers and Japanese hand-made papers, and the chemical structures and fungal bioreceptivity properties of the selected plant dyes as well as the characteristics of watercolours and acrylic paints used for toning the Japanese mending papers examined in this thesis. The chapter will also review the literature on the ethics of paper conservation. Finally, a review of the physical, chemical and microbial tests used in this study will lead into a statement of the objectives of the thesis.

- **Chapter 3** discusses the results of a survey of paper conservation practitioners, which reviews the toning materials and mending papers used by the participants who filled in the survey. This provides an overview of current paper conservation practice and reinforces the objectives of the research.

- **Chapter 4** introduces the methodology for preparation and extraction of plant dyes and chemical colourants followed by the toning process of the Japanese tissue
Chapter 1: Introduction

papers. It also sets out the methodology for the physical experiments (folding endurance, tear resistance, colour change and microfading), chemical test (pH) and also the fungal growth tests on the untreated papers and those papers treated with the selected dyes and pigments.

- In Chapter 5, the results and findings of the physical, chemical and the fungal growth tests on the papers are detailed.

- Chapter 6 is a more detailed discussion and analysis of the results set out in Chapters 3 and 5.

- To conclude, Chapter 7 draws together the findings of the previous chapters and describes further work that might be needed in the field of paper conservation.

1.2 Original contribution

This thesis has made some substantial and novel contributions to the field of heritage conservation – especially paper, textile and painting conservation. The original contributions are highlighted below.

- Paper conservation evolved out of traditional practices such as bookbinding and repair of manuscripts. Many conservators are trained like artisans or apprentices, using the techniques passed down from the masters in the past. The old apprenticeship system focused on the aesthetic aspects of paper objects. This study aims to question some of these traditional techniques and to analyse scientifically the properties and performance of the materials used. In this way, this research will help to develop new scientifically based conservation knowledge around past practice.
This thesis aims to investigate the long term effects of toning materials used in Japanese tissue papers for paper conservation purposes. Hence, it was decided to design an on-line survey, which mostly targeted paper conservators, to identify the different repairing papers and toning materials commonly used for paper conservation purposes around the world.

This study represents the examination of both the physical and biological stability of Japanese tissue papers treated with plant dyes such as henna, black tea, fresh and dried leaves of *Eucalyptus cinerea*, and selected watercolours and artists’ acrylic paints. To understand the colourfastness (defined as the resistance of a material to changes in its colour characteristics or the extent of transfer of its colourants to adjacent white materials) of the treated Japanese tissue papers, traditional colourfastness techniques are generally applied (Samanta & Agarwal 2009; Christie, Mather & Wardman 2000). Analytical techniques such as spectrophotometer and microfading tests (MFTs) are considered to permit colour change identification in a non-destructive manner. This study employs such existing colour measurement methods on both untreated and treated Japanese mending papers. This technique provides new opportunities for paper, textile and painting conservators to determine which papers and colourants have the best colourfastness properties when used as toning materials in conservation treatments.

A further original contribution of this thesis is the identification of the fungal bioreceptivity of a wide range of pigments and dyes, particularly those which are mostly used for toning Japanese tissue papers, using a number of DNA and polymerase chain reactions (PCRs) techniques. While DNA extractions and PCRs are able to identify many species of fungi in forensic laboratories, this work has
been constrained by the limited number of existing databases of destructive fungi relevant to paper conservation research. Molecular biology techniques can be readily applied to study the destructive fungal attack of paper. Thus, this thesis sets out to examine a range of DNA extraction techniques, which will be useful for paper and painting conservators. It brings innovative methods into the realm of conservation, through collaboration between conservation and microbiology. The findings of this thesis indicate how successfully fungi are able to colonise different papers treated with different colourants, thereby indicating which papers and colourants have the most effective antifungal properties.
2. LITERATURE REVIEW

2.1 Introduction

The aesthetically pleasing rehabilitation of paper objects has been of paramount importance in paper conservation practice. As an art-work consists of aesthetic and physical aspects, objective, scientific and subjective criteria will impact the conservation decision (Poulsson 2008). Thus, the toning process used in paper conservation needs to balance both ethical and aesthetic requirements and will reflect the changing attitudes and cultural contexts of conservators. The application of Japanese tissue papers for repair, lining and filling in the lost parts of an aged paper has increased from the early 1980s with the growing awareness of the quality of these papers (Webber 2006; Wills 2002). In paper conservation practice, criteria for assessing the qualities of the colouring materials are also worthy of study. A suitable pigment or dye should be stable on re-wetting and avoid the transfer of colour to the original object (Wills 2002). Additionally, the dyes and pigments used for toning purposes should be lightfast and be resistant to colour change over time. This study will compare both the physical and chemical stability of the selected paper samples to reveal which dyes or pigments raise or lower the papers’ resistance after artificial ageing. Further, DNA extractions and PCR techniques were used to assess the fungal bioreceptivity of the selected dyes and pigments on the paper samples. This will help to understand which dyes or pigments have antifungal properties once applied to Japanese tissue papers for paper conservation purposes.
2.2 Paper materials

Paper is a multi-component material, which is made of wood derived fibres composed of cellulose, hemicellulose, lignin and additives such as starch, gelatine, minerals, natural dyes or synthetic pigments (Cappitelli et al. 2010; Cappitelli & Sorlini 2010; Pasquariello et al. 2008). It was introduced as a writing material by Cai Lun in China in 105 AD and its production remained as a secret among Chinese craftsmen for centuries (Stuart 2007; Cappitelli & Sorlini 2005). In 751 AD, the Chinese paper-making technique was transferred to Arab countries and at the same time to China’s neighbours including modern day Japan, Korea and India which also profited from this invention. Paper-making knowledge reached European countries such as Spain and Italy several centuries after the Arabs began making paper (Strlič, Kolar & Scholten 2005).

Paper production is divided into two historical periods: the first before the 19th century which includes mostly hand-made papers and the second after the 19th century with the introduction of machine-made papers (Cappitelli et al. 2010). Hand-made paper is manufactured from cotton, linen and hemp rags (Cappitelli et al. 2010; Pasquariello et al. 2008). These materials are composed of pure cellulose fibres and sizing compounds including starch and gelatin. The sizing materials are used for glazing paper and make it ready for writing. Hand-made paper involves a high degree of polymerization (DP) for the production of higher quality paper. Older papers lack even colour due to the absence of a bleaching process, so the colour ranges from a creamy to a light brown tone.

The demand for paper increased after the invention of the printing press in the 16th century by Gutenberg (Strlič, Kolar & Scholten 2005). When the Hollander beater (a machine for
beating rags to shorten the fibres) was developed in 1680 AD, the quality of the papers produced becomes a concern for paper conservators (Holik 2006). Further, during the 19th century, machine-made papers were produced from wood pulp sources in which the earlier sizing materials are replaced by alum (potassium-aluminum sulfate), which increases acidity and accelerates the paper ageing over time (Ravikumar, Rao & Karigar 2012; Abdel-Maksoud 2011; Afsharpour, Rad & Malekian 2011; Area & Cheradame 2011; Jablonsky et al. 2011; Cappitelli & Sorlini 2010). The adverse effects of bleaching materials including chlorine and hypochlorite, which accelerate the deterioration process of the papers, have been studied for many years (Cappitelli & Sorlini 2010; Havermans 2008).

2.2.1 Machine-made paper production techniques

Wood pulping paper production methods can be divided into different groups: mechanical or grinding, semi-chemical and chemical pulp processes (Sequeira, Cabrita & Macedo 2012; Cappitelli & Sorlini 2010). Mechanical or grinding actions remove the bark from the wood without the use of chemical reagents. This type of paper contains a high percentage of lignin (85-95%) and is used for papers with low stability against mechanical resistance such as newspapers. The removal of lignin during paper-making procedures raises the physical quality of the final paper. Resistance to microorganisms; on the other hand, might be decreased due to the absence of the protective action of lignin against biological degradation (Cappitelli & Sorlini 2010). Semi-chemical pulping is a combination of mechanical process and a light chemical treatment resulting in a medium quality of final paper. The most commonly used chemicals for chemical pulping are sodium sulfate and calcium sulfate. Chemical pulping removes lignin and the paper manufactured through this
process is of higher quality than the mechanical and semi-chemical papers (Pasquariello et al. 2008).

**2.2.2 Japanese tissue papers**

Japanese hand-made papers were used extensively until 1874 AD, the year of the invention of the first machine-made paper in Japan (Kurosawa & Hashino 2012). The microscopic structure of the fibres of Japanese tissue papers are described by Gettens and Stout (1966, p. 241): “They are nearly transparent under the microscope and show transverse jointing as well as longitudinal striae. The central canal generally shows as a well-defined line and the ends are sometimes blunt and rounded, sometimes fringed”. Japanese tissue papers are composed of a large amount of hemicellulose, cellulose with low lignin content, plant mucilage and cooking alkaline (Barrett 1983). The hemicellulose facilitates water absorption and acts as a glue-like substance to improve the inter-fibre bonding during the drying process. It also improves the plastic properties of the fibres during cooking and beating.

Japanese tissue papers are made from various plants including kozo (*Broussonetia kazinoki*), gampi (*Wikstroemia canescens*), and mitsumata (*Edgeworthia papyrifera*) (Yum 2011; Taylor 2007; Barrett 1983; Hunter 1978). The kozo is a mulberry tree which grows up to two metres in height with stems up to two centimetres in diameter. The kozo fibre (twelve millimetres average) is longer than mitsumata (three millimetres) and gampi (four millimetres) (Barrett 1983). The length of the fibres is an important element in final paper quality: the longer the fibres, the greater the quality of the paper (Kurosawa & Hashino 2012; Uyeda et al. 1999; Murphy & Rempel 1985). Today, the kozo bark is used for
making approximately 90% of ‘washi’ (Japanese traditional paper). It is believed that the kozo paper is appropriate for almost any type of paper conservation uses because of its long, heavy and coarse fibres (Cunha & Cunha 1971). It is used for hinging, attaching covers to books, repairing page tears and backing very heavy maps or documents. The long, strong and flexible fibres of the Japanese tissue papers make them stable against ageing and improve their colour uptake (Kurosawa & Hashino 2012; Doming 2005).

In a study by Kurosawa and Hashino (2012), the historical background of Japanese papers from hand-made to machine-made is described. There are three differences between Japanese traditional papers and modern Western papers. First, the bast fibre (inner white bark) of the kozo, the mitsumata or the gampi plant is used for making washi paper. Second, Japanese paper-making employs the discharge paper-making technique, which produces thin paper with long fibres. In this technique, neri, the mucilage of the tororo-aoi plant (*Abelmoschus manihot*) is employed for dispersing the wood pulp and producing stronger papers. Neri facilitates fibre dispersion in water, prevents clumping in fibres, and controls the final product uniformity during the paper-making process (Yum 2011; Barrett 1983). Finally, soymilk or the extract of funori (a kind of seaweed) is sometimes added to the pulp to reduce streaking and also improve the quality of the paper sheets (Ginsberg 2007).

The quality of washi papers depends on different factors including: plant species, plant habitat, farming, harvesting, fibre cleaning and drying techniques. The preparation of the fibres involves different stages: cooking, rinsing, cleaning, and beating (Barrett 1983). During the cooking process, some chemical residuals might remain in the pulp and they can affect the final tonalities of the paper. The inner bark of the plant is separated by
boiling in mild alkaline solutions such as wood ash (calcium carbonate) and soda ash (sodium carbonate) to dissolve lignin, pectin, waxes and gums. For making papers with natural tonality and strong physical properties, mild alkalis are used by paper craftsmen (Ginsberg 2007; Inaba et al. 2002; Uyeda et al. 1999; Murphy & Rempel 1985). However, the stronger alkalis such as caustic soda (sodium hydroxide) and lime (calcium hydroxide) are used for producing papers with brighter tonality. These strong alkaline chemicals might affect the paper degradation over time (Inaba et al. 2002; Uyeda et al. 1999). Stream water or snow has traditionally been used for bleaching to make light coloured papers. The washing and cooking processes influence the final quality of the paper. For instance, extended washing after the cooking process yields lighter paper compared with shorter washing which yields darker papers. Drying can be performed on a wooden or metal board. The wooden board dryer imparts some advantages compared to the steam heated metal dryer, which has been common in Japan since World War II. Those papers that were bleached under the sun and dried on the wooden board have higher quality than the paper samples dried on the steam heated metal dryer (Barrett 1983).

2.3 Dyes and pigments

Colourants can be divided into two groups: dyes and pigments. Dyes are generally defined as compounds that undergo chemical interaction with substrates such as paper and textiles (Siva 2007; Aspland 1998; Lewis 1998). A dye molecule has two principal chemical groups: a chromophore and an auxochrome. The former usually incorporates an aromatic ring, which is associated with the colouring property, and the latter helps the dye molecule to combine with the substrate. Pigments are composed of solid and insoluble granules, which are suspended in solvents. They are responsible for maintaining the optical

### 2.3.1 The history of dyes and pigments

The application of natural dyes and dyeing goes back into prehistory when humans left handprints or wall paintings in palaeolithic caves. Humans also used pigments to colour their skin, using black, white, yellow and red natural inorganic pigments during festivals and wars (Siva 2007). It is imagined that humans believed that colour would give them magical powers and protect them in battle. The earliest examples of the use of plant dyes are from China and India in the third millennium BC (Siva 2007). Natural dyestuffs have been applied to different substrates and for different purposes such as paintings, textiles, wood artefacts and manuscripts. Natural dyes are derived from plants (e.g., black tea and henna), insects (e.g., cochineal and lac), animals (e.g., some species of molluscs and shellfish) and minerals (e.g., iron oxide and ochre), without chemical processing (Kadolph 2008). Plant dyes are not only used for colouring textiles, paper, leather and wood, but they have also been used in the cosmetic, pharmaceutical, and food industries.

Natural dyes have some advantages. They are less toxic, polluting and hazardous to health, because they are non-carcinogenic and non-poisonous (Shahid, Shahid ul & Mohammad 2013; Sharma et al. 2012; Baliarsingh et al. 2012; Tiwari et al. 2010; Gaur 2008; Türkmen et al. 2004). Additionally, they are environmentally friendly and can be recycled after use. Although natural dyes have several advantages, there are some limitations as well. These include the difficulties involved in the collection of plants and the lack of availability of precise technical knowledge of extracting and dyeing procedures (Siva 2007). During the
20\textsuperscript{th} century, natural dyes were replaced by synthetic ones because of advances in organic chemistry which led to the mass production of chemical dyes. These dyes are cheaper to produce and require less time to extract colour compared with the procurement of the materials for natural dyes, which are more complicated and time consuming.

The Egyptians began to use synthetic pigments in 4000 BC (Barnett, Miller & Pearce 2006). Egyptian blue (calcium copper silicate) was one of the first stable pigments produced in ancient Egypt. This stable pigment is made by mixing a calcium salt (carbonate, sulphate or hydroxide), a copper compound (oxide or malachite) and silica sand. Vermilion, a red pigment, was developed by the Chinese around 2000 years ago, “made by mixing mercury with molten sulphur and heating the mixture to produce the compound” (Barnett, Miller & Pearce 2006, p. 447). The Greeks developed white and red lead pigments that remained the most popular white and red pigments until the 19\textsuperscript{th} century (Barnett, Miller & Pearce 2006). Tyrian purple was possibly first used by the ancient Phoenicians in the first millennium BC (McGovern & Michel 1985). Archaeologists have long known from ancient texts such as Pliny that purple dyes were produced by extraction from the molluscs \textit{Murex trunculus} and \textit{Purpura haemastoma} which were found in the Mediterranean Sea (Siva 2007; Barnett, Miller & Pearce 2006). The dye continued to be produced in later periods and it became a royal colour in ancient Rome (Siva 2007; McGovern & Michel 1985).

In the European renaissance, oil painting became popular as artists changed from paintings on walls (fresco and egg tempera painting techniques) to painting on portable substrates (canvas and wood mediums) (Jones et al. 2005). Pigments such as raw umber, burnt umber, raw sienna and burnt sienna were invented in the late 15\textsuperscript{th} century. The first
chemically synthesised pigment, a blue pigment which was known as Prussian blue, was
made by Diesbach in Germany in 1704 (Barnett, Miller & Pearce 2006). Since William
Henry Perkin invented the first synthetic dye (mauve) in 1856, the use of natural dyes has
deprecated (Siva 2007; Grierson 1989). Thus, the application of chemical dyes has increased
in the textile industry because of its higher lightfastness properties compared to that of
natural dyes (Samanta & Agarwal 2009; Cristea & Vilarem 2006).

2.3.2 Dyes

There is a large volume of published studies describing the definition of dyes and pigments
and their application as colouring materials (Sharma et al. 2012; Mongkholrattanasit et al.
2011; Naz & Bhatti 2011; Moiz et al. 2010; Ali, Hussain & Nawaz 2009; Degano et al.
Norton 2002; Deo & Desai 1999). During the colouring process glycoside bonds in
cellulose chains are broken and new bonds are formed between the fibre and the dye
molecule and the final product is a water insoluble colour (Sequin-Frey 1981).

Natural dyes are divided into two groups according to their application: additive and
substantive. Additive dyes are fugitive. Metallic salts (mordants) including aluminium
potassium sulphate and copper sulphate can raise the colourant’s resistance against ageing
(Ahmed 2009; Melo 2009; Kadolph 2008; Cardon 2007). Conversely, the substantive dyes
bond to the fibres without requiring any chemical assistance.

For the purposes of this study, three different plant dyes (black tea, henna and fresh and
dried Eucalyptus cinerea leaves) have been used for toning the selected Japanese tissue
papers. There is limited literature on the use of plant dyes and chemical pigments for
colouring Japanese mending papers in paper conservation. The following section reviews the literature on the most significant chemical groups of natural dyes: anthraquinones, naphthaquinones, and flavonoids. Knowledge of the chemistry of dyes provides a better understanding of their behaviour at the molecular level. In this thesis, the molecular structures of the dyeing compounds (anthraquinone, naphthaquinone, and flavonoid) have been depicted graphically to assist in this understanding.

**Anthraquinone dyes**

Anthraquinones are organic compounds with structures involving carbonyl (C = O), which are found in a number of naturally occurring plant dyes (Christie 2001). Anthraquinones are mainly red and are derived from plants such as madder (*Rubia tinctorium*) or insects including kermes (*Kermes vermilio*) and cochineal (*Coccideae*) (Degano et al. 2009). Figure 2.1 shows the molecular structure of an anthraquinone.

![Figure 2.1: Chemical structure of an anthraquinone](image)

**Naphthoquinone dyes**

Naphthoquinones are yellow crystalline substances, which include a two-ring naphthalene base in their structure. Henna (*Lawsonia inermis*) and green walnut shell (*Juglans*) are categorised in the naphthoquinone family (Yusuf et al. 2012; Ashnagar & Shiri 2011; Habbal et al. 2011; Ali, Hussain & Nawaz 2009; Dev et al. 2009; Abdulmoneim Saadabi...
Figure 2.2 shows the molecular structure of 1, 4-naphthaquinone. The following section reviews the literature on the dyeing and antimicrobial properties of henna.

![Molecular structure of 1, 4-naphthaquinone](image)

**Figure 2-2: Chemical structure of 1, 4-naphthaquinone**

**Henna**

Henna belongs to the *Lythraceae* family of flowering plants, which is cultivated in countries including India, Iran, Pakistan, Egypt, Yemen, and other parts of Africa as well as Australia (Yusuf et al. 2012; Ali, Hussain & Nawaz 2009; Bechtold 2009; Cardon 2007). The dyeing element of henna leaves is lawson or 2-hydroxy-1, 4-naphthaquinone (Figure 2.3) (Yusuf et al. 2012; Abdulmoneim Saadabi 2007). It produces an orange colour due to the presence of the auxochrome group in its structure (Cardon 2007). Henna is also composed of flavonoid, various tannins, gallic acid, phenolic acids, xanthones and resin (Yusuf et al. 2012; Ashnagar & Shiri 2011; Chengaiah et al. 2010; Cannon & Cannon 1994).

![Chemical structure of lawson](image)

**Figure 2-3: Chemical structure of lawson**

In the last decade, the use of plant dyes including henna has increased in the cosmetic, pharmaceutical and textile dyeing industries due to their dyeing, antifungal and
antibacterial properties (Baliarsingh et al. 2012; Selvam, Singh & Kalirajan 2012; Habbal et al. 2011; Chengaiah et al. 2010; Prusty et al. 2010; Sathianarayanan et al. 2010; Babu & Subhasree 2009; Samanta & Agarwal 2009; Abdulmoneim Saadabi 2007; Siva 2007; Singh et al. 2005; Gupta, Khare & Laha 2004; Malekzadeh & Shabestari 1989). Several studies were conducted to study the dyeing and antimicrobial characteristics of henna when used for dyeing textile fibres (cotton, wool, polyester, chitosan and linen) (Ali, Hussain & Nawaz 2009; Dev et al. 2009; Bechtold et al. 2003). The antimicrobial properties of henna leaves on woollen yarn substrates have also been studied (Yusuf et al. 2012). The aqueous extract of powdered leaves of henna was used to dye wool yarn to achieve orange-brown to light yellow colour. Henna inhibits microbial growth on fabrics including woollen yarn substrates and the chitosan treated wool fabrics. Chitosan is a natural biopolymer used on textile substrates to increase the dye uptake of fabrics and antimicrobial properties (Yusuf et al. 2012; Dev et al. 2009). Henna extract in concentrations higher than 10% was found to inhibit the growth of the fungus Aspergillus flavus (Barkeshli, Ataie & Ali-Mohammadi 2008). In a study by Habbal et al. (2011), the antibacterial activity of henna leaf was examined. Although both fresh and dried leaves of henna show antibacterial activities against Pseudomonas aeruginosa, the fresh leaves of henna had the highest anti-\textit{P. aeruginosa} activity. The antimicrobial properties of most plant dyes, including pomegranate (\textit{Punica granatum}), henna, green walnut shell and black tea, are due to the presence of anthraquinones, flavonoids, tannins, naphthoquinones, terpenoids, alkaloids, polypeptide, and polyacetylenes in their structure.
Chapter 2: Literature review

Flavonoid dyes

Flavonoid dyes constitute a large group of plant dyes including black tea and *Eucalyptus* leaves (Séquin-Frey 1981). “Almost 50% of all natural dyes used to colour textiles are flavonoid compounds” (Crews 1987, p. 65). They mainly yield yellow to orange-yellow colours (Bechtold et al. 2009). The following section reviews the literature on dyeing and the antimicrobial properties of black tea and Eucalyptus leaves.

*Black tea*

The first consumption of tea as a beverage was in China over 3000 years ago (Peterson et al. 2004). Today the consumption of tea has increased and it is categorised as the second most widely consumed beverage in the world (Wu et al. 2007). Black tea is used for dyeing purposes in the textile industry (Moiz et al. 2010; Bechtold et al. 2003). The tea plant, *Camellia sinensis*, prefers acidic mountain soils and a wet climate. During the processing of black tea for dye, the green tea is macerated to start fermentation in which the polyphenol enzyme oxidases and acts as a catalyser in the oxidation of catechins (Figure 2.4) into quinone. New chemical compounds (theaflavins and thearubigins) are formed during the oxidation processes. Theaflavins (Figure 2.5) are orange-red, while thearubigins (Figure 2.6) are brownish-red (Obanda, Owuor & Oka 2001).

![Chemical structure of catechin (C)](image-url)

Figure 2-4: Chemical structure of catechin (C)
Among polyphenols, catechins (C) are the most common compounds. The most important catechins include epicatechin (EC) (Figure 2.7), gallocatechin (GC) (Figure 2.8), catechin gallate (CG) (Figure 2.9), epicatechin gallate (ECG) (Figure 2.10), epigallocatechin (EGC) (Figure 2.11), and epigallocatechin gallate (EGCG) (Figure 2.12) (Obanda, Owuor & Oka 2001). ECG, C and EGCG are reported to act as antimicrobial elements for black tea (Friedman 2007; Hamilton-Miller 1995). In a recent study by Bansal et al. (2013), EGCG - the most abundant catechin of green tea (50-80% of the total catechin content) has been noted for its effective antimicrobial action (Obanda, Owuor & Oka 2001).
Figure 2-7: Chemical structure of epicatechin (EC)

Figure 2-8: Chemical structure of gallic acid (GC)

Figure 2-9: Chemical structure of catechin gallate (CG)

Figure 2-10: Chemical structure of epicatechin gallate (ECG)
Chapter 2: Literature review

Figure 2-11: Chemical structure of epigallocatechin (EGC)

Figure 2-12: Chemical structure of epigallocatechin gallate (EGCG)

**Eucalyptus leaves**

The *Eucalyptus* plant is in the *Myrtaceae* family, which includes 133 genera (Ashnagar & Shiri 2011; Silva et al. 2011; Elaissi et al. 2011; Marzoug et al. 2011; Gilles et al. 2010). It is most often an evergreen tree with a large number of species. Although *Eucalyptus* is today being grown in many different countries, it is a native genus to Australia (Elaissi et al. 2011; Silva et al. 2011; Marzoug et al. 2011; Gilles et al. 2010). Many species of *Eucalyptus* can be used for dyeing including *E. cinerea*, *E. crenulata*, *E. nicholii*, *E. globulus*, *E. bicostata* and *E. cordata* (Cannon & Cannon 1994). The chemical compounds of *Eucalyptus* leaves include up to 11% tannin (gallic acid, ellagic acid, benzopyrano), flavonoids (quercetin) (Figure 2.13) and rutin (Figure 2.14) (Mongkholrattanasit et al.)
Various species of *Eucalyptus* leaves have been used to tint textile fibres to achieve different tonalities (Flint 2007; Afshar 2001). Fresh and dried *Eucalyptus* leaves produce green and brown shades respectively (Flint 2007).

![Figure 2-13: Chemical structure of quercetin](image)

![Figure 2-14: Chemical structure of rutin](image)

*Eucalyptus cinerea* is one of the species of *Eucalyptus* with dyeing properties. The main chemical compound of *E. cinerea* is 1, 8- cineole (Silva et al. 2011). *Eucalyptus* oils have been used in the pharmaceutical industry as an antiseptic and also in the cosmetic industry because of their antimicrobial and antifungal properties. Different *Eucalyptus* species were used by indigenous Australians to treat fungal infections (Ashour 2008). Several studies have been undertaken to examine the antimicrobial properties of different species of *Eucalyptus* leaves including *E. oleosa, E. cinerea, E. camaldulensis, E. olida, E. staigeriana* and *E. dives* (Siramon, Ohtani & Ichiura 2013; Elaissi et al. 2011; Marzoug et
a recent study, the antibacterial properties of the essential oils from 20 *Eucalyptus* species leaves including *E. cinerea* were studied by Elaissi et al. (2011). *Eucalyptus cinerea* showed antibacterial inhibition ability against the Gram negative bacterium *P. aeruginosa.* The potential antimicrobial activities of *Eucalyptus* leaves might be due to the presence of terpenoid and phenolic compounds especially 1, 8-cineole (Elaissi et al. 2012; Silva et al. 2011; Marzoug et al. 2011; Naz & Bhatti 2011; Gilles et al. 2010).

### 2.3.3 Pigments

Since pigments do not form chemical bonds with the substrate, they have to be mixed with a binder such as gum Arabic in watercolour or acrylic polymer in acrylic paint to be in dispersion in the relevant medium. Pigments provide colour to paint and usually consist of insoluble particles with size varying from 0.2 µm to 20 µm diameter (Charkoudian et al. 2010; Learner 2004; Szczepanowska & Cavaliere 2003). The dry pigments are composed of primary particles, which aggregate through some intermolecular forces including Van der Waals and ionic. The latter is the potential intermolecular force for most inorganic pigments (Christie, Mather & Wardman 2000; Lewis 1998).

The high insolubility property of many pigments can be explained by intermolecular hydrogen bonding in the crystal lattice of pigments (Learner 2004). This strong intermolecular hydrogen bonding in many pigments raises colourfastness for the colourants. Pigment particles need a physical reaction during wetting to react with the substrate (Learner 2001). Accordingly, the vehicle or solvent needs to displace the air on the surface of the pigment particles to form a heterogenic compound. This process is often
easier with organic solvents such as acrylic and polyester than water because pigments are originally hydrophobic materials (they lack affinity with water based systems).

Pigments are divided in three categories: natural inorganic pigments, such as ochre, sienna and umber; synthetic inorganic pigments including iron oxide, cadmium and chromium oxide and synthetic organic pigments, such as azo and phthalocyanine (Winter 2008; Auhorn 2006). The oldest pigments had natural compositions and were derived from inorganic materials, but today most pigments are produced through synthetic processes. Metal oxides such as iron, copper, manganese, cobalt, nickel, tin, zinc and gold are the most common substances used to produce pigments in industry. Lakes, which are a subgroup of pigments, are formed by the precipitation of an organic dye in metallic salts such as aluminum sulfate (Varella 2012). Natural lakes include saffron (Crocus sativus) and Persian berries, while alizarin crimson is an example of synthetic lake.

Both watercolours and acrylic paints of raw sienna, burnt sienna, raw umber, burnt umber, cobalt blue and alizarin crimson were compared in this study. The following section reviews the literature on the pigments used in this study.

- **Alizarin crimson** is a lake pigment containing colourant alizarin, 1, 2-dihydroxyanthraquinone (Figure 2.15) (Crews 1987). Alizarin crimson is the principal colourant in madder lake which is one of the oldest plant dyes used for textile dyeing since ancient times (2000 BC) (Türkmen et al. 2004). The synthetic alizarin crimson has higher lightfastness than natural madder (Afsharpour, Rad & Malekian 2011; Korenberg 2008; Grim & Allison 2003; Crews 1987; Gettens & Stout 1966; Padfield & Landi 1966).
- **Burnt sienna** is prepared by calcining raw sienna. During the process, raw sienna undergoes a considerable change in hue and depth of colour (Gettens & Stout 1966). Burnt sienna is composed of fine particles with higher density (g/cm³) compared with raw sienna. These factors raise its hiding power, relative to raw sienna (Patton 1973). The following reaction occurs during the calcination process to convert raw sienna to burnt sienna:

\[
\text{Fe}_2\text{O}_3 \cdot \text{XH}_2\text{O} \text{ (yellow)} + \text{heat} \rightarrow \text{Fe}_2\text{O}_3 \text{ (red)} + \text{XH}_2\text{O}
\]

- **Burnt umber** contains iron oxides (FeO⋅XH₂O). The lightfastness properties of all iron oxide compound pigments are excellent (Winter 2008; Nassau 1998). Burnt umber is coarser than raw umber with similar hiding power properties. The density of the former; however, is higher than the latter (Patton 1973).

- **Cobalt blue** was isolated in the first half of the eighteenth century, but not developed as a pigment until 1802 when it replaced the similar colour, smalt (Barnett, Miller & Pearce 2006). This blue colourant contains oxides of cobalt and aluminium (CoO⋅Al₂O₃) (Harley 1982). The pigment includes excellent lightfastness properties (Nassau 1998). Cobalt blue may cause various diseases such as lung diseases, skin allergies or even cancer (Christensen & Poulsen 1994). There are some indications that the cobalt complex present
in cobalt blue has antimicrobial activity against various fungi including *Aspergillus* sp. and *Cladosporium* sp. (Belaïd et al. 2008; Chohan et al. 2006; Chohan et al. 2004).

- **Raw Sienna** is hydrated iron oxide (Fe$_2$O$_3$.H$_2$O) with alumina and silica. The colour of raw sienna can be compared with yellow ochre. The former is more transparent than the latter (Gettens & Stout 1966). Raw sienna pigment is yellow in colour and might include possible antifungal properties due to high concentration of iron compounds in its structure (Dauda et al. 2012; Wadley, Williamson & Lombard 2003).

- **Raw umber** is composed of iron oxides with hydrated manganese oxides and aluminium silicate. The high proportion of manganese dioxide gives the pigment brown shades (Wehlte 1975). Raw umber has lightfastness properties and catalytic effects on drying oils in oil paintings due to the high concentration of manganese in its structure. The pigment has greenish brown colour due to the effects of the yellow iron oxide, the brownish manganese dioxide and the black carbon deposits (Patton 1973).

**Watercolours**

Watercolour is composed of very tiny pigment particles, providing the paint colour, and a binder such as gum Arabic, which causes the pigment particles to stick together (Kokla, Psarrou & Konstantinou 2010). Gum Arabic, which is the most common binder used in watercolours, is produced by several species of *Acacia* growing in Senegal, Pakistan, Iran and India (Riedo, Scalarone & Chiantore 2010). Gum Arabic not only decreases the surface tension, but also prevents the flow of paint as a result of the abrupton of hydrogen bonding capability (Brasuel, McCarter & Bower 2009; Ormsby et al. 2005). Water is added to the pigment in the watercolour technique to dissolve or suspend all ingredients on
The role of pigments in the drying process is important; they affect the oxidation process and breakdown of hydro peroxides (Weerd, Loon & Boon 2005). Honey and various forms of sugar were added to watercolours to increase the strength and transparency of the colour (Ormsby et al. 2005). The pigments used in watercolours give rise to several problematic issues over time. They may undergo chemical and physical alterations, which cause discolouration of the paint. Further, the pigments have the ability to create a water absorbable surface which provides susceptible conditions for paper based materials to swell and deteriorate.

**Acrylic paints**

The physical, chemical and biological properties of synthetic resins used in acrylic paints may be affected by ageing, environmental conditions and conservation treatments. Acrylic, alkyd, and polyvinyl acetate (PVA) resins are the most important mediums in modern paints (Learner 2004). Acrylic emulsion is known to ‘latex’ due to the dispersion of acrylic polymer in water. Acrylics have been widely used since the Second World War because of their excellent affinity with water (Pintus, Wei & Schreiner 2012; Learner 2004). The additives, including calcium carbonate, clay or fibres can modify the strength of the polymer (Stuart 2007). The flexibility of the polymer can be improved by adding plasticisers. Stabilisers are also added to polymers to improve their lightfastness properties. The widespread use of numerous synthetic resins challenges conservators in selecting proper treatments for art-works because of the variety of conservation issues encountered.

An acrylic paint is composed of a pigment and high molecular weight acrylic polymers that are based on the esters of acrylic acid and methacrylic acid (Jones 2004; Learner 2004; Learner 2001). For example, the ester formed between ethanol and acrylic acid is known
as ethyl acrylate (EA). Acrylic colours are polymerised before the paint is manufactured, so there is no chemical reaction to form an acrylic film. Although acrylic paints can be chemically deteriorated, the destruction process takes more than a hundred years in optimal environmental conditions (Jones 2004). The chemical alteration of polymers breaks down the intermolecular chains and leads to the production of smaller molecular chains and also cross linking chains (Jones 2004; Learner 2001).

Acrylic resins can be divided into two different categories: thermoplastic and thermosetting. The thermoplastic types include longer chain lengths compared with the thermosetting type. The thermoplastic acrylics are available in two distinct forms: acrylic solution and acrylic emulsion. The former consists of the acrylic polymer dissolved in a defined organic solvent such as mineral spirit while the latter includes two-phase systems in which acrylic polymer is dispersed in an aqueous phase. The acrylic emulsion involves molecules with a hydrophobic portion including a long hydrocarbon chain that is chemically attached to a hydrophilic end which will be able to interact physically with both the acrylic and water phases. Most artists’ acrylic paints are constituted in accordance with this formulation (Learner 2004).

2.4 Paper conservation ethics

Paper conservators hold that Japanese tissue papers need to not only strengthen the original paper, but also create an aesthetically pleasing result when used as mending papers. Although it is impossible to completely hide repairs in paper conservation, conservation practice generally requires that the repair should not be visually obtrusive (Gyles & Maver 2002). A toning process is a restoration technique to infill missing areas of media which
can be performed directly onto the original paper or on overlay papers. The former is an interventive toning method, which is considered to be a kind of restoration, while the latter is a non-interventive method. When undertaking the interventive toning approach, accepted conservation ethics require that issues such as reversibility and authenticity need to be considered (Poulsson 2008).

Heritage conservation has developed a distinctive vocabulary of specifically defined terms, including ‘conservation’ which is defined as the means by which the ‘true nature’ of an object is preserved (Caple 2000) or, in the terms of the Australian Burra Charter, all the means via which cultural significance is maintained (Australia ICOMOS 1999). The true nature of an object is defined as evidence of its origin, construction, composition and manufacturing technology. Today, digitisation techniques are also seen as an effective preventive conservation strategy (aiming to retain the object in ideal conditions for the prevention of further damage and decay) for protecting old books, as the digitised copies can be provided for researchers instead of the unstable original books.

By the mid-twentieth century the concept of reversibility was understood as the capability of science and technology to create desirable materials, which remain stable and removable once applied to cultural and historical objects. In this period, the concept of reversibility evolved in response to the observation of artefacts that were perceived to have been adversely affected by earlier conservation treatments, thus compromising their authenticity (Caple 2000). However, since paper is a fibrous and absorbent material, it is generally not possible for toning to be reversed (Poulsson 2008). Since paper conservators understand that many of these toning techniques are not reversible, in practice they make a compromise between the ethics of reversibility and the visual aesthetic qualities achieved by integrating the colour of the repaired papers with those of the original object. Further,
paper conservators are also looking for the best long-term solution when repairing the original artefacts to minimise the need to repair them in the future. Thus, they may often choose a permanent treatment which they understand is non-reversible.

Towards the end of the nineteenth century, authenticity came to be considered as a crucial determinant of cultural heritage value. In heritage conservation, authenticity correlates with the notion of the ‘original’ and the ‘genuine’. ‘An authentic historical object or building is thus one that is true to its origins in terms of its date, material, form, authorship, workmanship and, in many cases, its primary context and use’ (Jones 2010, p. 184). Conservation of cultural heritage in all its forms and historical periods is rooted in this notion of the authentic and in the values attributed to this quality in the present (Jones 2009; Stovel 2008; Jokilehto 2006). According to the principles of the Charter of Venice (1964 as cited in Jokilehto 1985, p. 5), ‘the intention in conserving and restoring monuments is to safeguard them no less as works of art than as historical evidence’. ‘Authenticity is the key word of the great majority of documents enunciating either a theory or criteria of choice with respect to safeguarding cultural heritage… it is no exaggeration to say that this concept lies at the base of all modern doctrine on the conservation and restoration of historical monuments’ (Lemaire as cited in Starn 2002, p. 15). Authenticity is thus the underpinning concept in all scientific studies of cultural heritage and its conservation and restoration (Stovel 2008). Thereby, conservation treatment plans for cultural heritage objects aim to maintain authenticity by maximising the retention of original or historical material and by being in harmony with the original object (Jokilehto 1993 as quoted in Stovel 2008, p. 13). However, the Nara Charter developed in 1994 demonstrates how the concept of authenticity has evolved in global conservation practices, allowing that authenticity is not a universal constant but culturally
contingent. In paper conservation it would thus be difficult to understand the authenticity of documents and manuscripts if their original form has not been maintained and they are allowed to become fragmentary. Thus the imperative to maintain the original appearance and form of these objects tends to prevail over considerations of non-intervention and reversibility, and the introduction of new materials is thereby justified. A thorough understanding of the physical and chemical properties of toning materials is important to help conservators to understand the implications of decisions that they make for aesthetic and other cultural reasons. Since it is difficult to prevent the influence of the toning materials on the adjacent areas of the original paper, the toning process needs to be performed before repair. Repair adhesives are usually water-based, which can also cause toning materials to spread to the original part when toning materials are applied directly on the paper.

Early methods of paper conservation were not well documented. Until the seventeenth century, paper conservation knowledge was transferred traditionally and the first paper restoration methods followed the methods of textile conservation (Poulsson 2008). Early conservators aimed to carefully imitate the original artist’s work, making their repairs and treatments aesthetically pleasing in that they recreated the completeness and integrity of the original artefact.

A paper object is a complex artefact that conveys past intellectual traditions, cultural and historical information, spiritual beliefs and also aspects of the lifestyles of previous generations. The ultimate aim of conservation and restoration of manuscripts is the retention of these values and spiritual meanings (Schädler-Saub 2011). The application of any new mending papers and toning materials to the original paper object for repairing
missing parts should be carefully assessed beforehand. Conservation professionals are responsible for selecting appropriate materials and treatments, which should protect the original object from any adverse effects in the future. Before undertaking any toning process, conservators need to match the repair paper as nearly as possible to the original paper—in terms of the paper’s weight, tonality, wave direction, fibre orientation and thickness (McAusland 2002).

In summary, the conflict between a paper object’s aesthetic qualities and its perceived authenticity remain a subjective issue in paper conservation. Aesthetic considerations may be secondary and the practice of toning may be used to preserve the legibility and composition of the paper object (Poulsson 2008; Gyles & Maver 2002). Conservators should select appropriate toning materials according to the objectives of the repair and after consideration of the specific cultural values attributed to the object. The aim of this research is to provide paper conservators with a greater understanding of the long term performance of materials that may be chosen for diverse cultural reasons.

Figure 2-16: Japanese tissue paper used as support for Persian document conservation
2.5 Physical, chemical and biological properties of paper materials

Organic materials such as paper and textile are susceptible to physical, chemical and biological deterioration. The type and quality of the raw material is crucial for the longevity of paper materials (Kronkright 1990). Paper objects can deteriorate through physical strains and stresses that make them swell or condense respectively. A paper with a heterogeneous matrix responds differently to physical forces depending on the properties of the individual fibres and also the interfibre bondings in its cellulose structure (Karademir, Imamoğlu & Çetin 2004). Of all the degradation vectors on paper, chemical deterioration is probably the most difficult to control (Strlič, Kolar & Scholten 2005). The following section reviews the literature on ageing, folding endurance, tear resistance, colour change, photoageing, pH and fungal growth tests.

2.5.1 Ageing

Paper materials age naturally during their lifespan. Artificial ageing speeds the natural ageing process of paper by subjecting it to extreme conditions for certain periods of time, and is used to determine the lifespan or permanence of paper or predict the long term effects of conservation treatments (Strlič, Kolar & Scholten 2005). High temperature and humidity inside the artificial ageing chamber accelerate the oxidative and hydrolytic reactions and promote paper deterioration (Łojewski et al. 2010; Barański 2002). The continued reactions lead to interruption in cellulose bonds and formation of crosslinking products (HavlÍnová et al. 2009). The final products will be shorter polymer chains which lack stability against external forces including folding, tearing and tensile stress (Zervos 2010).
Paper samples need to be preconditioned prior to ageing and subjecting to physical tests due to the hysteresis phenomenon in which the moisture content of the paper is much more important than the relative humidity (RH) of the environment (Zervos 2010). Water degrades the cellulose polymer of paper through chemical, physical and photochemical reactions. The chemical degradation occurs through hydrolysis or oxidative reactions which cause abruption of cellulose bonds, scission of inter-fibre bonds, and formation of smaller molecules. The physical reactions cause the loss of hydrogen bonds between the substrate, pigments and vehicle. The photochemical reactions accelerate the degradation of the cellulose fibres through the hydroxyl radicals (Feller 1994).

The effects of moisture and heat inside the artificial ageing chamber could affect the fading rate of colourants; the more water content in the chamber, the faster the colourant will fade (HavlÍnová et al. 2005). Additionally, the fading rate of the colourants relates to the level of acidity and alkalinity of the paper samples during manufacture. The acidity may originate from the ageing products of the deteriorated papers, atmospheric pollution, migration of acidic substances from neighbouring materials, and the acidic remains of microorganisms in the paper matrix. The acidic hydrolysis may also be derived from metal ions such as Al\textsuperscript{3+} derived from alum during paper-making procedures or Fe\textsuperscript{3+} produced by oxidation of Fe\textsuperscript{2+} from iron gall ink – the popular ink from the 6\textsuperscript{th} to 20\textsuperscript{th} centuries (Zervos 2010). The destructive effects of acidic compounds in paper cause autocatalysis reactions. Many books and documents from the 19\textsuperscript{th} and 20\textsuperscript{th} centuries are vulnerable to this form of reaction (Strlič & Kolar 2002).

Artificial ageing process may also affect the physical properties of paper materials (Lee & Inaba 2013). Some studies indicate that suspension methods or closed ageing system (an
ageing system in which paper specimens are placed inside glass tubes, and then the tubes are placed in a chamber with controlled humidity and temperature) involve more advantages than the open ageing system (an ageing system in which paper specimens hang freely on the climatic chamber racks) (Lee & Inaba 2013; Area & Cheradame 2011; Porck & Teygeler 2000). The closed ageing system is a breakthrough in understanding the paper ageing process because it allows the degradation products of the papers to be retained for further analysis. Area and Cheradame (2011) indicate that no statistically significant difference can be observed between the degree of polymerisation and tensile strength of the measured data in both closed and open systems of ageing. However, the pH and whiteness index values of the papers had meaningful differences in the two mentioned ageing systems. Choosing the appropriate ageing methods and conditions in paper conservation science relates to the final objectives of the research. To examine the volatile organic compounds that are emitted from paper during ageing process, the suspension method can also be worthwhile (Lee & Inaba 2013; Lattuati-Derieux, Bonnassies-Termes & Lavédrine 2006).

Several studies have been conducted to explain the production of acidic materials in artificially aged papers (Lee & Inaba 2013; Jablonsky et al. 2011; Lattuati-Derieux, Bonnassies-Termes & Lavédrine 2006; Porck & Teygeler 2000; Shahani, Hengemihle & Weberg 1989). The effects of fluctuations in RH on the ageing rate of single sheets and within a bound book are different (Lee & Inaba 2013). The ageing process of the bound book in an artificial ageing environment (high humidity and temperature) is faster than the ageing for single sheets (Winter 2008). The trapped adverse acidic materials inside the book structure accelerate its ageing process, relative to the single sheets. Further, the
acidity toward the centre of a paper stack is greater than the regions located near the outsides of the sheets.

Artificial ageing conditions can be produced by different conditions including dry oven, oxygen and nitrogen atmospheres, and moist and dry climatic chambers (Porck 2000). Several studies infer that the findings of either natural or artificial ageing are similar (Bansa 2002; Zou, Uesaka & Gurnagul 1996). The oxidation, hydrolysis and crosslinking reactions happen during both natural and artificial ageing. Formic, lactic, acetic, succinic and oxalic acids are the final products of aged papers. The formation of these acids causes auto-catalysis and degradation of the macro molecule chains of the cellulose (Jablonsky et al. 2011). The final degradation products of artificial ageing depend on the temperature and RH of the oven or climatic chamber.

The effect of fluctuations in RH on the degradation of paper-based materials under artificial ageing is studied to gain a better understanding of the optimal environmental conditions for long term storage of papers and documents (Zou, Uesaka & Gurnagul 1996). There are obvious differences between degradation products of paper aged under moist heat and dry heat conditions. The results of ageing of Whatman® Cellulose Filter Paper under both moist heat and dry heat ageing conditions, performed by Erhardt in 1987, were explained in a study by Shahani (1995). Shahani indicates that glucose and xylose are the products of the hydrolytic degradation of cellulose in the moist heat conditions compared with the very little amount of glucose produced in the dry heat conditions, demonstrating that hydrolysis of cellulose was not the actual reaction during dry heat ageing process. Additionally, the effects of dry heat ageing diminish the mechanical
strength of the papers, especially folding endurance, compared with moist heat ageing (Karlovits & Gregor-Svetec 2012).

Natural ageing of paper can be affected by many elements including chemical reactions (acid hydrolysis, oxidative reactions, alkaline degradation and enzyme attacks), mechanical failures (loss in folding endurance, tear resistance and tensile strength), thermal deterioration (high temperature or the fluctuation in temperature), radiation energy (ultra violet or visible radiation spectrums) and environmental conditions (high heat and humidity, aerosol gases) (Area & Cheradame 2011).

In a study by Zou, Uesaka and Gurnagul (1996), moist heat and dry heat artificial ageing tests were used to compare the results of the artificial ageing conditions with naturally aged lignin free papers (aged for 22 years). The results of the study confirm a good relationship between the natural and artificial ageing. According to the Technical Association of Pulp and Paper Industry (TAPPI) Standard Test Method T 544 cm-08 (2008), moist heat artificial ageing of cellulose is much more sensitive than the dry heat artificial ageing, and that dry heat artificial ageing lacks the ability to rank paper samples according to their stability as accurately as moist heat. Porck (2000) shows that artificial ageing tests are often used to determine the permanence of paper materials and the rate of paper degradation. Nonetheless, it can predict the long-term effects of conservation treatments on the paper materials. At this time, no standards exist for parameters in artificial ageing with moist heat conditions; however, the conditions of 70–80 °C and 65% RH are endorsed by many conservation scientists (Bansa 2002; Feller 1994). For the experiments described in this thesis, the untreated paper controls and those that were
treated with different dyes and pigments, are subjected to a moist heat artificial ageing inside an open ageing system.

2.5.2 **Folding endurance**

Folding endurance can be interpreted as a fatigue strength property to determine the stability of paper. It is a modified tensile strength test of paper where failure results in breakage of the paper. Of all the physical properties used for assessing the strength of paper, folding endurance is the most sensitive test of the deterioration of paper by ageing and it is widely used in the paper and board industry as well as in paper conservation (Lee & Inaba 2013; Zervos 2013; Karlovits & Gregor-Svetec 2012; Area & Cheradame 2011; Zervos 2010; Zervos & Moropoulou 2006; Karademir, Imamoğlu & Çetin 2004; Bansa & Ishii 1997; Caulfield & Gunderson 1988).

Folding endurance testing imitates the repeated action of opening and closing a book or the number of repeated forward and backward folds that a paper can withstand under tension before it breaks (Sothornvit & Sampoompuang 2012). The results of folding endurance tests are more useful for papers used in book preservation than for flat paper artefacts. The results of folding endurance tests are highly dependent on the conditions of the artificial ageing as well as the physical and chemical properties of the paper samples (Caulfield & Gunderson 1988).

The mechanical properties of paper objects are absolutely dependent on the individual characteristics of cellulose fibres, the intrinsic structure of cellulose networks as well as the chemical compounds added to papers during the making process (HavlÍnová et al. 2009). The folding endurance of paper is dependent on both the elastic and viscoelastic
properties of the paper (Caulfield & Gunderson 1988). The entanglement and flexibility of the fibres impart two significant factors in reduction or accession in folding endurance for paper materials (Karlovits & Gregor-Svetec 2012). Thus, the more flexible the fibres, the greater the folding endurance exhibited by the paper. The degree of polymerisation, length of fibres, adequate fibre bondings, inter-fibrile bondings and the formation of hydrogen bonds between the cellulose fibres can affect the folding endurance properties of papers (Caulfield & Gunderson 1988).

In a study by Havlínová et al. (2009), the folding stability of the selected alkaline and acidic papers was measured after exposing to dry heat and moist heat artificial ageing methods. Alkaline paper specimens exhibited higher resistance to folding endurance compared to the acidic papers. The acidic papers showed loss of folding endurance after exposure to a high temperature and humidity chamber. The authors confirm that alkaline reserves in paper slow the ageing rate induced by different artificial ageing methods.

Folding endurance is a sensitive physical property of paper that could be affected by ageing (Zervos 2010). Both folding endurance and tear resistance tests before and after ageing are recommended by Shahani (1995) to evaluate paper permanence. These are the most useful tests for detecting alterations caused by ageing on papers (Shahani 1995; Caulfield & Gunderson 1988). In a recent attempt by Karlovits and Gregor-Svetec (2012), cellulose papers and synthetic papers were exposed to dry heat, moist heat and xenon lamp ageing methods. Different ageing approaches diminish the folding endurance compared with tensile strength for the papers. Additionally, the papers exposed to dry heat ageing indicate higher loss in folding endurance compared with the other ageing methods.
Physical stabilities of a number of hand-sheets made from unbeaten and unbleached paper samples were examined to identify correlations between the properties of paper fibres with physical tensions including folding endurance, tear resistance and tensile strength (Karademir, Imamoğlu & Çetin 2004). Those papers which are made of undamaged and flexible fibres such as kraft paper are mechanically more resistant than those made of damaged and short fibres including refiner mechanical pulp paper. Jablonský et al. (2011) study the effects of acetic acid and formic acid formation induced by artificial ageing of newspapers samples. The decrease in folding endurance during artificial ageing aligns with the brittleness index of paper. The findings of the study indicate that the folding endurance test is less sensitive for brittle and weak papers. Moreover, the embrittlement of the paper fibres correlates with the increase in carboxylic, acetic and formic acid concentrations. The results of folding endurance, used in this study, can help paper conservators to select the most stable Japanese tissue papers for the long-term preservation of restored paper materials with a greater resistance to folding.

2.5.3 Tear resistance

A tear resistance test reflects the internal tearing strength of paper samples and it is widely used in the paper industry and to evaluate the effectiveness of various conservation treatments in paper conservation. The tearing test is performed in the paper-making industry and in conservation practice to measure the mean internal tear resistance of papers to the propagation of a deliberately initiated tear (Zervos 2010). The physical stability of paper materials can predict the impact of conservation treatments in terms of the durability of documents and books in long term storage conditions in archives and libraries. The tear resistance test can simulate the kind of tearing occurring to a nicked book page or
document (Reyden 1992). Several studies have measured the tear resistance of cotton and polyester textiles (Triki et al. 2012; Triki, Dolez & Vu-Khanh 2011). They conclude that the tearing properties of the fabrics relate to the type of weaves, density and characteristics of the yarns.

Physical properties can show independent effects on the durability of the cellulose polymer of paper. The tear resistance of paper depends on the flexibility of fibres, strength of intermolecular bondings, strength of individual fibres, type of fibres, length and thickness of fibres, imperfections in fibres, pattern of fibre lattice, number of inter-molecular bonds, strength of individual bonds, weight of paper, density of paper and moisture content in paper (Chaiarreki et al. 2011; Karademir, Imamoğlu & Çetin 2004; Reyden 1992; Caulfield & Gunderson 1988).

The beating process during paper-making increases the entanglement of fibres and decreases the tear resistance properties for the papers. Water acts as a viscoelastic or plasticiser element in the fibres of paper and increases the ability of the fibrils of the cellulose fibres to stretch rather than break (Reyden 1992). In high humidity conditions, the tear resistance of paper might be diminished because of the disruption of interfibre lattice bonds and deterioration in the polysaccharide chains as well as disruption of hydrogen bonds.

For the first time, Barrow and Sproull (1959) declared that the loss in mechanical properties of papers, including folding endurance and tear resistance, correlate with the degradation of papers. There is also a good correlation between the acidity of papers and loss of mechanical strength (Lee & Inaba 2013; Barrow & Sproull 1959). The tearing
resistance of rice straw made paper depends on the number of inter fibre bondings in the cellulose structure of paper samples (Sothornvit & Sampoompuang 2012). The physico-chemical properties of naturally aged papers were studied after exposing them to a moist heat artificial ageing (Lee & Inaba 2013). The tear resistance and fail indexes of the papers were measured after artificial ageing to compare their physical stabilities. The findings of this study confirm a good correlation between the number of cellulose chain breaks and the loss of tear index. The tear resistance of commercial papers commonly used in archives and libraries was also examined to optimise gamma irradiation conditions to eliminate insects and fungi (Area et al. 2014). The findings of this study show that there is a minimum lowering in the tear resistance and brightness properties of papers after exposure to gamma irradiation.

2.5.4 Colour change

Several methods can describe colour change properties of paper including colourimeters, spectrophotometers (Reyden 1992) and microfading testers (Ford 2011; Whitmore & Tao 2011; Connors-Rowe, Morris & Whitmore 2005; Whitmore, Bailie & Connors 2000; Whitmore, Pan & Bailie 1999). The evaluation of colour measurements for observing the effects of conservation treatments is invaluable. Colour measurement is a useful tool for monitoring paper degradation after artificial ageing (Zervos & Moropoulou 2006). An increase in lightness and decrease in yellowness index are desirable since such alterations raise the contrast between text and paper. However, the opposite effect, that is a decrease in lightness and increase in yellowness manifest the degrading effects of a range of chemical reactions, such as those that result in acidity. In a study by Cocca, D’Arienzo and D’Orazio (2011), the natural ageing of Whatman® Cellulose Filter Paper was simulated.
using different artificial ageing methods. The colour changes of paper specimens were analysed using the spectrophotometric techniques and the findings of the examination confirm that artificial ageing causes colour change on the paper samples.

Colour measurements of several commercial acrylic paints have been made using spectrophotometric analysis after artificial UV ageing. The experimentally determined lightfastness (the chemical stability of the pigment or dye after exposure to light) was not always similar to what was claimed by the manufacturers (Pintus et al. 2005). The spectrophotometric method was also used to evaluate the appearance and colourfastness properties of fluorescent watercolours. The concentration of the paint could affect the appearance of fluorescent watercolours and increase their colourfastness properties (Connors-Rowe, Morris & Whitmore 2005).

Spectrophotometry has been used to evaluate the colour change properties of textile fibres including jute, cotton and wool treated with black tea (Moiz et al. 2010; Deo & Desai 1999). The findings of spectrophotometric analysis confirm that plant dyes including madder and green walnut shell promoted colour change for the textile fibres (wool yarn and linen fabric) (Bechtold et al. 2003). The results of colour change measurements showed that post-mordanting procedures resulted in superior colourfastness. In another study involving spectrophotometric colour change analysis, wool fabrics dyed with Eucalyptus camaldulensis were found to have superior colourfastness relative to cotton fibres (Mongkholrattanasit et al. 2011). Extraction of henna in alkaline solution (sodium hydroxide) was found to cause less colour change when dyeing cotton fibres than extraction in distilled water (Ali, Hussain & Nawaz 2009). However, few studies have been performed on the colourfastness of dyed Japanese conservation papers (Eriko, Inaba
& Masahiro 2010; Gordon 2008; Yoshida et al. 2001; Inaba 1991; Cass et al. 1989). This study provides information about the potential changes in colour that treated papers may experience during exhibition or storage.

2.5.5 Micro fading

The optical properties of paper are defined as colourimetric, brightness, whiteness, yellowness, reflectance, gloss, opacity and transparency (Reyden 1992). The absorption of light energy in a molecule promotes electrons from a low energy state to an excited state. This process is called electronic transition. The energy difference (\( \Delta E^* \)) is between the electrons in a low energy state and the electrons in an excited state according to Planck’s relationship (Christie, Mather & Wardman 2000):

\[ \Delta E^* = hv \]

where \( h \) is Planck’s constant and \( v \) is the light frequency.

Feller (1994) indicates that the photo-chemical reactions of light accelerate chemical reactions in an object after exposure to an intensive light source. The results of these primary reactions are a series of secondary chemical reactions, which are generally influenced by heat and moisture. According to the reciprocity principle, the total photochemical reactions are equal to the times of exposing to an intensive irradiation.

Colour perception depends on different factors such as illumination, the observer and the surroundings of the object (Kubik 2006). Fading of dye molecules is a process of photochemical reduction in cellulose and an oxidation reaction in protein substrates (Feller 1994). A dye molecule in an excited state can extract hydrogen from a substrate or water molecule; which subsequently results in the production of free radicals in either dyes or
substrates. The lightfastness property of dyestuffs depends on the kind of substrate, the chemical affinity between the substrate and dyes, the degree of absorption and the dispersion or aggregation of dyes (del Hoyo-Meléndez & Mecklenburg 2011).

Archival materials and library collections are subject to visible light when they are on display in exhibitions and when they are being used for research in study rooms. However, no studies have been performed on the colourfastness properties of Japanese mending papers treated with various dyes and pigments for paper conservation purposes. Various groups of colourants are used in paper conservation and they may perform differently in response to environmental exposure in both museum exhibitions and storage environments.

Some pigments, including those that are composed of natural or synthetic iron oxides, impart excellent lightfastness properties (Winter 2008; Nassau 1998). Conversely, the lightfastness properties of plant dyes depend on the chemical structure of the dyes and the host fibres (Padfield & Landi 1966). Materials including paper, parchment, leather, glue, ink, dyes and pigments may be damaged by photochemical reactions with visible light (Vavrova et al. 2011). The destructive effects of light include photo oxidation and photo reduction, leading to darkening in organic and inorganic pigments (Nassau 1998). A photo degradation process might occur in the forms of oxidation or reduction in cellulose and proteins based fibres respectively (Cristea & Vilarem 2006). The photo degradation is initiated by the absorption of an energetic photon by either organic or inorganic colourants, which in turn causes the formation of an excited state in the colourant molecule (T. Padfield 2012; Pers. Comm., 21 November). Since the energetic photon has the ability to interact with oxygen and moisture in the atmosphere, this process is called photo oxidation.
or photo bleaching. The result of this interaction is disruption of the conjugated system, loss of colour, darkening or lightening.

The light bleaching behaviour of the coloured papers depends on the compositions of the colourants. The destructive part of the light spectrum including UV needs to be removed in both exhibition and storage areas. The UV light has a higher energy (shorter wavelength) than visible light, which could break down the carbon-hydrogen (C-H) bonds in the cellulose structure of paper. The carbon radicals react with the oxygen molecule in the environment to form free radicals. Additionally, the autoxidation of cellulose polymer may be started with the production of carbonyl, hydroxyl and carboxyl acidic groups (Kronkright 1990).

There are inconsistencies about the lightfastness properties of plant dyes and chemical paints in the reviewed literature. A large and growing body of literature has investigated the application of both plant dyes and chemical paints for textiles (Yurdun & Dolen 2012; Prusty et al. 2010; Degano et al. 2009; Samanta & Agarwal 2009; Kadolph 2008; Bechtold, Mahmud-Ali & Mussak 2007; Flint 2007; Cristea, & Vilarem 2006; Bechtold et al. 2003; Ye, Salmon & Cass 2000). Several studies explain the lightfastness properties of plant dyes (Kadolph 2008; Cristea & Vilarem 2006; Crews 1987; Padfield & Landi 1966). Some authors mention that plant dyes remain unfaded over a long period of time, whereas chemical paints begin to discolour and fade away some years after the application on artworks (Kadolph 2007; Türkmen et al. 2004; Sensei 1999).

Conversely, Padfield and Landi (1966) report that natural dyes have poor lightfastness properties when applied on cellulose based materials and stored in a museum environment.
There is no doubt that dyes affect their substrate and vice versa. Indigo is much more light resistant on wool than on cotton, as opposed to madder which fades faster on wool (Winter 2008). Indigoid dyes may create a photo-excited state in their fibre host, transferring energy to the surrounding areas. The molecular vibration can open the fibre to chemical reactions, or even affect the interstitial air molecules (Cristea & Vilarem 2006).

The lightfastness properties of some selected natural dyes (madder, weld and woad) on antioxidants and UV absorber treated fabrics show that the application of those treatments improved the lightfastness properties of dyed fabrics (Cristea & Vilarem 2006). Most stable red dyes have anthraquinone in their structure while yellow dyes are less resistant to fading (Melo 2009). Microfading just reveals the photo-bleaching of paper under the visible light conditions. Concurrent thermal reactions and other reactions initiated by light lead to yellowing and might be continued during subsequent dark storage (post-actinic processes) (Whitmore, Bailie & Connors 2000). The post-irradiation effects depend on different factors including the spectrum of the light source, the light dose, type of light source, the structure of paper (the compounds of paper including lignin content), and finally temperature and RH in the storage environment (Vavrova et al. 2011).

The lightfastness properties of inorganic and organic pigments are different when they are exposed to illumination. Organic pigments are less stable to light than inorganic pigments in painting materials (Ravikumar, Rao & Karigar 2012). The former are brighter, more transparent and they involve strong tinting as well as good gloss properties. Conversely, inorganic pigments impart excellent hiding power, heat and photochemical stability with poor tinctorial strength and brilliance (Wilker, Ohleier & Winter 2004; Christie 2001; Zollinger 1991).
2.5.6 The effect of acidity on paper

Paper materials are affected by chemical reactions during their life time. The weakly alkaline environment is more favourable for archives and libraries due to its less destructive effects on papers (Lee & Inaba 2013; Strlič et al. 2007; Lattuati-Derieux, Bonnassies-Termes & Lavédrine 2006). Although acidity increases the hydrolysis of cellulose fibres, the deterioration of cellulose fibres does not cease in high alkaline conditions, it just modifies from acidic hydrolysis to autoxidation (Area & Cheradame 2011; Zervos 2010; Strlic et al. 2004). These oxidised groups that form through alkaline degradation or reduction reactions of cellulose have the ability to degrade paper fibres. Additionally, the oxidation of cellulose may be accelerated in alkaline environments by free radical elements. Deacidification treatments (calcium carbonate and magnesium bicarbonate) impart stabilising effects for paper while metal residuals including copper and iron can act as catalysts to degrade paper materials through oxidation processes (Stephens et al. 2008; Daniels 2002). The pH of paper after deacidification is frequently higher than 7 (neutral pH). The deposited carbonate in paper results in an alkaline reserve, which subsequently prevents paper materials from future damage due to acid neutralisation (Strlič, Kolar & Scholten 2005).

In a study by Malesic, Kolar and Strlic (2002), three paper samples (bleached chemical pulp, rag made papers and purified cotton made fibres) were deacidified with magnesium bicarbonate. The deacidification procedure decreased the pH of the bleached pulp papers after the artificial ageing tests compared with the rag made papers and purified cotton made papers. In a study by Stephens et al. (2008), an inverse correlation between pH and
yellowness index was indicated. Paper samples with lower pH manifest high yellowness shift while the yellowness change is slow in alkaline paper specimens.

Chemical damage can be caused by the oxidation of cellulose chains with the subsequent production of free carboxylic groups, which promote yellowness and brittleness for the paper structure. Several studies suggest that the degradation of cellulose causes the generation of several acids including formic, acetic, lactic, and oxalic (Lee & Inaba 2013; Jablonsky et al. 2011; Princi et al. 2008; Stephens et al. 2008; Kronkright 1990). These acids continue to accumulate within paper, attaching themselves through strong intermolecular bonds. With continuing oxidation or acid hydrolysis processes, the physical resistance of paper is noticeably reduced. A pH test was performed on paper samples naturally aged for 27 years and the results were compared with those papers after exposing to an artificial dry ageing (Batterham & Rai 2008). The findings of the study indicate that papers with lower pH are often degraded faster than alkaline papers.

The effects of wood ash, soda ash, lime and caustic soda on acidity and alkalinity of Japanese mending paper samples have also been studied (Inaba et al. 2002). The findings of the pH test indicate that stronger alkalis such as caustic soda and lime increase acidity for paper samples compared with milder alkali such as soda ash and wood ash. It can be extrapolated from Zou’s study (1996) that there is a good correlation between lower pH of papers and their faster degradation. Volatile acids can be absorbed by water in cellulose networks and accelerate the paper degradation while other acids remain in paper objects with no influence on the paper degradation (Barański 2002).
The influence of pH on the formation of fungi on paper substrates has been studied previously (Weitz et al. 2001; Nittérus 2000). These studies show that acidic conditions (low pH index) cause more fungal growth than an alkaline environment. However, Rakotonirainy, Heraud and Lavedrine (2003) examine the relation between pH and fungal growth on untreated, acidified and alkalinised papers. They indicate that there is not a good correlation between the fungal growth and the pH index of the papers.

Few studies have examined the pH of Japanese conservation papers and correlated this with their physical properties and bioreceptivity (Inaba & Sugisita 1990; Inaba & Sugisita 1988). For the aims of the present study, a pH test is performed on both untreated papers samples and those that were treated with plant dyes, watercolours and acrylic paints to evaluate the acidity and alkalinity level of papers after colouring and also after exposure to an artificial ageing environment. The pH test can help to understand the effects of acidity and alkalinity on the physical stabilities (folding endurance and tear resistance), appearance (colour change) and encouragement or inhibition of fungal growth on Japanese mending papers.

**2.5.7 Biological properties of paper materials**

Organic materials are susceptible to biodeterioration especially in humid and warm climates. Fungi and bacteria are the most harmful microorganisms which attack not only cellulosic materials such as books, textiles, paintings and wooden objects, but also protein based materials including parchment, leathers and vellum (Valentin 2003). A paper with a hygroscopic structure is a good source of microbial nourishment which makes it vulnerable to biodeterioration. Biodeterioration is defined here as “any undesirable change
in the properties of a material caused by the vital activities of organisms” (Allsopp, Seal & Gaylarde 2004, p. 1). Microorganisms target paper materials in high RH and temperature; therefore, the control of environmental conditions in the store areas of archives and libraries is essential (Cappitelli et al. 2009). Fungi can adjust themselves to a wide range of environmental conditions and live with a low water content (Manente et al. 2012; Cappitelli et al. 2010; Cappitelli et al. 2009). Many Aspergillus and Penicillium species are active in cellulose based materials with moisture content of 7-8% (Winter 2008; Nugari & Salvadori 2003; Valentin 2003). Aspergillus species prefer warm climates while Penicillium species predominate in moderate climates (Florian 2003).

The cellulose fibres of paper can be dissolved by most of the filamentous fungi through the action of cellulolytic enzymes, with the subsequent production of organic acids that cause deterioration of the structure of historical and cultural paper based materials (Pinzari et al. 2011; Michaelsen et al. 2009; Szczepanowska & Cavaliere 2003). Those fungi that feed on cellulose based substrates including papers are extremely dangerous for them and need to be separated and isolated from the remaining documents and books to prevent transferring spores to other documents (Area et al. 2014).

Penicillium spinulosum, Penicillium steckii, Penicillium restrictum, Penicillium turbatum (Zotti, Ferroni & Calvini 2008), and Penicillium rubrum (Sterflinger 2010; Michaelsen et al. 2006; Zyska 1997).

Textile materials are susceptible to fungal growth since their surface can easily absorb moisture and subsequently provide suitable environmental conditions for fungal growth (Prusty et al. 2010; Szostak-Kotowa 2004). Paper is susceptible to fungal growth because it has a heterogeneous, organic surface that can easily absorb moisture. The role of water in dyeing Japanese mending papers might make papers more susceptible to biological attacks. The interference of mordants in textile dyeing processes is well-known –these metallic salts may produce toxic effects against microorganisms (Baliarsingh et al. 2012; Prusty et al. 2010).

There is increasing literature about the harmful effects of fungi in museum and archival environments (Clark et al. 2011; Cappitelli et al. 2010; Strlič et al. 2009; Zotti, Ferroni & Calvini 2008; Florian 2003; Rakotonirainy, Heraud & Lavédrine 2003). Fungi cause irreversible deterioration in historical and cultural paper objects. The microbial agents appear on the paper materials in different shapes, sizes and pigmented colours (purple, red, yellow, black and brown) (Manente et al. 2012; Pasquariello et al. 2008; Cappitelli & Sorlini 2005).

Microorganisms deteriorate paper components through cellulase, protease and amylase enzymes. Proteases and amylase facilitate fungal attack on the documents with paper used as a nutrient for fungal growth (Rojas et al. 2009). The compositions of papers including additives, colourants, varnishes, and sizing might help microorganisms to degrade paper.
materials. The paper-making process can also provide a wet, warm and nutritious environment for most microorganisms (Cappitelli & Sorlini 2010; Szczepanowska & Cavaliere 2003). Jerusik (2010) studies the role of raw materials such as wet pulp in fungi infestation of papers. The fungi infection might occur during manufacturing of paper products or after the production of papers. The following section reviews literature on the biological properties of dyes and pigments followed by molecular techniques used in heritage conservation especially paper conservation practice.

![Image](image_url)

**Figure 2-17: Papers affected by fungi due to previous inappropriate storage conditions**

**Biological properties of dyes and pigments**

A number of studies have found that some plant dyes have antimicrobial properties, which make them suitable to use in the textile industry (Yusuf et al. 2012; Ali, Hussain & Nawaz 2009). Henna is one of such plant dyes that is well-known for its antimicrobial properties (Selvam, Singh & Kalirajan 2012; Yusuf et al. 2012; Habbal et al. 2011; Prusty et al. 2010; Babu & Subhasree 2009; Dev et al. 2009; Barkeshli, Ataie & Ali-Mohammadi 2008; Abdulmoneim Saadabi 2007; Singh et al. 2005; Gupta, Khare & Laha 2004). The
antimicrobial properties of plant dyes such as black tea (Bansal 2013; Friedman 2007; Wu et al. 2007; Łuczaj & Skrzydlewska 2005; Hamilton-Miller 1995) and *Eucalyptus* leaves (Siramon, Ohtani & Ichiura 2013; Elaissi et al. 2012; Elaissi et al. 2011; Marzoug 2011; Silva et al. 2011; Gilles et al. 2010; Ashour 2006) have already been studied.

Watercolour pigments contain a small amount of organic binder (gum Arabic), which makes them susceptible to microbial deterioration (Abdel-Kareem 2010; Szczepanowska & Cavaliere 2003). Most fungi attacking watercolour pigments are *Penicillium*, *Aspergillus*, *Trichoderina* and *Phoma pigmentovora*. It is generally believed that synthetic paints are less susceptible to biological degradation. However, many synthetic resins and polymers used for conservation treatments can also be attacked by fungi (Cappitelli, Zanardini & Sorlini 2004). The contamination can occur during the manufacturing process or it might relate to the additives or impurities added to the polymer resin. Cappitelli and Sorlini (2008) tested three synthetic resins (acrylics, polyvinyl acetate and alkyds) to identify their stability against microbial growth. They indicated that acrylics are the most resistant product against biodeterioration compared with the other resins.

**Molecular techniques in heritage conservation**

Deoxyribonucleic acid (DNA), which is comprised of long chains of repeating units (nucleotides), imparts the genetic material of all living cells. “Each nucleotide comprises of a carbon and nitrogen containing base attached to a sugar (deoxyribose) that is linked through a phosphate group to the sugar of the next nucleotide” (Bower et al. 2010, p. 1). The information within the DNA is carried out by the sequence of the four nitrogenous bases: adenine, guanine, thymine and cytosine (Goodwin, Linacre & Hadi 2007).
In recent years, genetic identification has been introduced to the science of cultural heritage conservation; however, the application of molecular approaches in paper conservation are still in their infancy (Michaelsen et al. 2013; Pinzari et al. 2011; Cappitelli et al. 2010; Michaelsen, Piñar & Pinzari 2010; Michaelsen et al. 2009; Michaelsen et al. 2006; Di Bonaventura et al. 2003). Species such as *Aspergillus* and *Penicillium* are the most widespread and destructive fungi found in heritage materials (Michaelsen et al. 2006; Bonaventura et al. 2003; Gonzalez 2003). Molecular techniques can identify microorganisms from their DNA, ribonucleic acid (RNA) and proteins (Gonzalez 2003). Polymerase chain reaction primers (PCRs) can be designed to target the desired species. PCRs have become an effective tool for the amplification of DNA molecules, and the reaction can be performed on a small amount of the sample material (Michaelsen et al. 2013; Kraková et al. 2012; Sterflinger & Pinzari 2012; Cappitelli et al. 2010; Michaelsen et al. 2009; Rakotonirainy, Heude & Lavédrine 2007; Michaelsen et al. 2006; Di Bonaventura et al. 2003; Florian 2003; Makimura, Murayama & Yamaguchi 1994).

Traditional culturing methods, while considered the “gold standard”, are slower and less able to differentiate precisely between species and sub-species. However, the traditional culturing methods have still been used for fungal identification in paper conservation. Perhaps the most serious disadvantage of traditional culturing methods is the inconsistency of the results compared with molecular approaches (Gonzalez 2003). Most of the microorganisms are in dormant stages and cannot be grown on the provided culture medium in traditional microbial culturing techniques (Pinzari et al. 2010). In an optimum condition, just 1% of the microorganisms present in the asset can be cultured.
Additionally, the recognition of the species of fungi should be performed by paying attention to the physiological characteristics of the species affecting the paper materials.

Conversely, the molecular techniques need procedures for the separation of the DNA and identifying fungi that make them somewhat complicated (Zotti, Ferroni & Calvini 2008). Further, the molecular methods need to define a DNA isolation protocol to be followed. A considerable amount of literature has been published on the destructive effects of fungi on paper materials especially the well-known foxing spots on old books and documents (Michaelsen 2010; Rakotonirainy, Heude & Lavédrine 2007; Gallo 1992). Molecular techniques were used in a study by Di Bonaventura et al. (2003) in conjunction with a traditional culturing medium to identify fungal species from the contaminated paper samples. The results of the study indicated that although traditional culturing methods may identify fungal species, those techniques cannot properly differentiate the fungal species.

The introduction of PCR by Kary Mullis in 1985 resulted in DNA amplification of museum samples such as parchment, animal skins, leather and paper (Eklund 2012). At present, a mix of synthetic DNA polymerases is used to obtain the best results during PCR reactions. RNA or DNA extraction is the first step in all molecular methods; however, the extraction of nucleic acids from cultural and historical paper materials is somewhat challenging. In the first instance, most DNA analysis of precious paper materials requires the sacrifice of a small amount of the original paper. Further, it has recently been found that the effect of the leather tanning process has inhibited PCR reactions (Eklund 2012). Hence, the efficiency and accuracy of PCR products rely on the optimisation of the reaction conditions, pipetting accuracy, elimination of contamination and use of sterile materials.
A considerable amount of literature has been published on ribosomal sequences used as phylogenetic identification (Pinzari et al. 2010; Zhao et al. 2001; Larena et al. 1999). Ribosomes are found in all living cells and they are comprised of two major subunits, which, in fungi, are designated the small (40S) and large (60S) subunit. Each subunit is comprised of RNA and proteins. The small subunit contains 18S ribosomal RNA (18S rRNA) which is highly conserved between species (Jaeger et al. 2000; Glass & Donaldson 1995). The use of PCR primers targeting the 18S rRNA gene is a common method employed to identify fungi. This approach takes advantage of the conserved regions when designing the primer binding sites as well as the variability in DNA sequence between the primer binding sites which can be used to distinguish and identify different fungal species (Anderson, Campbell & Prosser 2003). Another target commonly used in identification is the variable internally transcribed spacer regions (ITS) which, like 18S rRNA, are sufficient to recognise species from different genera from less than a nanogram of DNA (Lord et al. 2002).

Traditional culturing methods in conjunction with molecular techniques were used to identify fungal colonisers in the restoration of a 16th century Italian book (Michaelsen et al. 2009). DNA extracts from manuscript paper dating from the 13th century were also amplified by PCR targeting the ITS regions of fungi and the 16S rRNA gene of bacteria (Michaelsen, Piñar & Pinzari 2010). The effects of different antifungal treatments were studied through both classical culturing and molecular techniques to compare efficacy of the genetic identification versus traditional culturing (Michaelsen et al. 2013). The ribosomal sequencing method was also used to survey the yellow and grey fungal colonisation on the walls of Altamira Cave in Spain (Sterflinger 2010). The 16S rRNA gene was applied to describe the differences between various structures of the stained
pages of Leonardo da Vinci’s Atlantic Codex (Principi et al. 2011). ITS regions have also been used to identify fungal strains in a range of studies on culturally significant historic paper objects (Kraková et al. 2012; Michaelsen, Piñar & Pinzari 2010; Mesquita et al. 2009; Michaelsen et al. 2009; Rakotonirainy, Heude & Lavédrine 2007; Michaelsen et al. 2006; Di Bonaventura et al. 2003).

In the present study, real time PCR has been used to target polymorphic DNA sequences with the aid of an intercalating fluorescent dye which binds to amplified double stranded DNA. The results obtained from DNA extractions and PCR reactions show the fungal bioreceptivity of a wide range of pigments and dyes used for colouring Japanese tissue papers for paper conservation purposes. DNA is isolated from paper samples treated with three different groups of colourants: plant dyes, watercolours and acrylic paints and fungal growth is inferred from changes in DNA concentration in extracts. The concentration of DNA is determined in paper samples inoculated with fungal spores directly after inoculation and then after 10 days incubation in an artificial ageing environment.

### 2.6 Objectives

Despite a substantial literature on dyeing textiles, there is a lack of comprehensive studies about dyeing Japanese mending papers for paper conservation purposes. Thus, this study aims to compare the physical stabilities of dyed paper samples with the selected dyes and pigments both pre and post artificial ageing. It will explore which colourants raise physical resistance for papers samples and make them suitable for long-life storage and exhibition conditions.
This study aims to provide a better understanding of toning materials and their colourfastness properties so that paper conservators and museum professionals can make better decisions about the colourants chosen for conservation treatments and are also informed about the potential impacts on colour stability of the treated papers when used for exhibition and research. Many toning materials including plant dyes, watercolours, acrylic paints, inks, pastels, gouaches, and colour pencils are commonly used for paper toning purposes. Most plant dyes have poor lightfastness properties compared with synthetic pigments; hence, the colours of plant-dyed textiles in museums are often different from their original colours (Duff et al. 1977). Analysing the colour change properties of natural dyes helps to infer the original colour of ancient textiles and also the effects of conservation treatments on such textiles. Anecdotally, plant dyes are used in artisanal practices including painting, handicraft, textile and paper dyeing; however, the chemistry of such colourants and their interaction with Japanese mending papers has not been studied. Thus, there is a need to fill this knowledge gap in paper conservation practice. Spectrophotometric methods and microfading tests are employed in this study to measure the colour change properties of dyes and pigments used for dyeing paper substrates. These methods can assist in exploring which dyes and pigments cause less colour changes on the papers over time. MFTs are employed to determine which dyes or pigments have superior lightfastness properties for the same papers.

This study aims to examine the fungal bioreceptivity of a wide range of pigments and dyes, used for colouring Japanese tissue papers for paper conservation purposes. The aim of this thesis is to select the most efficient primer pairs for the rapid quantification of A. niger and P. rubrum fungal species in pure culture mediums with the aid of real time PCR reactions. The real time PCR is used to target polymorphic DNA sequences with the aid of
an intercalating fluorescent dye which binds to amplified double stranded DNA. This technique is used to measure the concentration of DNA in paper samples inoculated with the fungal spores at the time of inoculation and then after 10 days of incubation in an artificial ageing environment. This indicates how successfully fungi are able to colonise different papers treated with different colourants, thereby indicating which papers and colourants have the most effective antifungal properties. This new knowledge will assist paper conservators in making better choices to ensure the long term preservation of restored paper materials, with a greater resistance to fungal attack.

2.7 Conclusion of literature review

Paper is one of the most important carriers of information about culture, science, business, politics, the arts and history which needs to be transferred among and between generations. There is a great need for applied and fundamental research on paper ageing and test methods for determining the stability of papers over time. In this study particular consideration is given to the mechanical and optical properties of the treated papers samples. The changes in the colour and mechanical stability (folding endurance and tear resistance) of paper are measured before and after artificial ageing. The mechanical properties of paper should be the principal focus of all stability studies of paper materials. Identification of pH is also the most widespread chemical test in studies of paper permanence. This test determines a paper acidity/alkalinity as a crucial regulating mechanism of degradation. Visual perception is a significant aspect in conservation especially of paper, textile and graphic art objects. Colour change imposed by conservation treatment is an unwanted issue, which can be measured by colour measurement techniques
using spectrophotometer and MFT methods. Thermal ageing should be conducted to
demonstrate the vulnerability to degradation of objects under visible light.
An area of study which needs further work is the biological degradation of paper based
materials. The application of innovative methods such as DNA extractions and PCR
reactions that are frequently used in environmental and forensic studies can help to better
understand the biodeterioration processes of the paper materials compared with traditional
culturing techniques.
3. SURVEY OF PAPER CONSERVATION

PRACTITIONERS

3.1 Introduction

In order to provide information about day to day practice in conservation laboratories that is not available from published literature, an on-line survey was undertaken to provide further insights into paper conservation practice in different parts of the world, and how paper conservators make decisions about the materials and methods they use. The aims of the survey included learning more about which types of Japanese tissue papers, dyes and pigments are being used for paper conservation purposes and why they are being used, as well as seeking participants’ opinions about the need for more research on the performance of these materials in conservation. As discussed earlier, there is generally a lack of research on the different types of mending papers and toning materials frequently used in paper conservation practice; hence, this on-line survey was designed to assist in filling this knowledge gap. While this thesis will focus on the scientific and theoretical aspects of paper conservation problems, this chapter shifts the focus to how and why decisions are made in day to day practice. It is hoped that as well as strengthening the findings of this thesis the survey will also provide a useful resource for further research by archives, libraries, museums, conservation centres and other cultural institutions with paper-based collections. The questions posed in the survey included:

- What kind of mending papers do you mostly use for repairing old papers and books?
- What kind of dyes or pigments do you often use for colouring mending papers to achieve the same tonality of original paper objects?
Are you familiar with the effects of the dyes and pigments on mending papers over time?

Is there a need for more research on the long-term effects of toning materials on mending papers over time?

The survey was designed using the Survey Monkey on-line research tool <URL: http://www.surveymonkey.com/s/ZYWJZPT> and one hundred and forty five responses were received within three months (April-June 2013). All of the respondents were practicing conservators with most being paper conservators. The survey was distributed via major conservation organisations, including the Conservation DistList (an interdisciplinary forum for those involved in preservation and conservation of cultural property) (http://cool.conservation-us.org/byform/mailing-lists/cdl/2013/0360.html), the Australian Institute for the Conservation of Cultural Materials (AICCM) (<http://www.aiccm.org.au/>) and the American Institute for Conservation, Book and Paper Group (AIC-BPG) (<http://cool.conservation-us.org/coolaic/sg/bpg/>) as well as Network Temático MEEP Forum(<http://orlandinivaleria.blogspot.com/>). All responses were de-identified such that they were anonymous, in accordance with ethics approval from the University of Canberra Committee for Ethics in Human Research (Project number 13-32) (Appendix 1). Appendix 2 contains the questions in the survey.

3.2 Survey participants

The majority of respondents (59%) were from the USA, followed by European countries (including England, France, Greece, Italy, Austria, Germany, the Netherlands, Spain, and Portugal) at 21% and Australia, at 15%. A smaller number of respondents (5%) were from a wide range of other countries such as India, Iran, Malaysia, Japan, Mexico, Argentina,
Chapter 3: Survey of paper conservation practitioners

Colombia, New Zealand, Guatemala, Canada, Trinidad and Tobago, and Brazil. Figure 3.1 shows the responses received by country of origin.

Figure 3-1: Frequencies of the survey sample demographics

3.3 Use of Japanese tissue papers in paper conservation

The survey asked for a yes/no response to the question ‘do you often use Japanese tissue paper to repair old documents and books’? As would be expected, 143 out of 145 respondents agreed that they use Japanese tissue papers for paper conservation purposes. As shown in Figure 3.2, ninety eight per cent of respondents claimed that they mostly use hand-made Japanese tissue papers while 73% suggested they mostly use machine-made Japanese tissue papers, indicating that most respondents frequently make use of both hand-made and machine-made Japanese tissue papers in paper conservation practice. Twelve per cent of respondents expanded on these answers in a free text section noting the use of a range of other mending papers including: Chinese (2.6%), Thai (0.65%), Korean (5.3%),
Manila hemp obtained from abaca (*Musa textilis*) (0.65%) and European papers (e.g., French hand-made papers) (2.6%).

![Figure 3-2: Use of Japanese tissue papers by paper conservators](image)

### 3.4 Characteristics of Japanese mending papers

The survey asked for a yes/no response to the question ‘are you familiar with the physical and chemical properties of the Japanese tissue papers that you use in paper conservation processes’? Ninety seven per cent claimed they are aware of the characteristics of Japanese tissue papers compared with 3%, who stated that they are not aware of the properties of these papers. Thirty respondents added further comments in relation to this question, including the following (direct quotes from the survey):

- ‘In a general sense, they are long fibred lignin free, that hold their strength as they age and do not discolor’
• ‘To a certain extent -some suppliers like Hiromi, Talas and the University of Iowa Centre for the Book are better about providing detailed manufacturing information’

• ‘I feel familiar enough to use them with confidence but do not know all the scientific details’

• ‘I feel I am moderately familiar. I do not have the strongest chemistry background and I know there are things I have never understood or forgotten. I am not sure of the importance of various cooking and drying methods. I also sometimes add additional colour to coloured kozo or gampi from one of several conservation paper vendors, especially in matching dark loss colours. I have never thought about the colourant since the time of purchase’

• ‘Sometimes it is hard to know the properties of these papers because companies that sell them do not have that kind of information’

• ‘We rely on the technical information provided by our Japanese paper suppliers, but also test the papers for acidity, for example, especially if they have been stored for a period of time’

Key themes emerging from these comments include:

• Most respondents confirmed that they are aware of the physical properties of Japanese mending papers including their long and strong fibres with sufficient alkaline reserve. Some comments asserted that Japanese tissue papers do not discolour and retain their strength as they age.
The findings of the survey indicate that most respondents use Japanese tissue papers according to their practical experience, relying on the information provided by Japanese paper suppliers, which tends not to include scientific information about the physical and chemical properties of the papers and their performance over time. This suggests that there is a need to study the long-term properties of Japanese tissue papers in a scientific way rather than just relying on practical experience and transferred information from the vendors.

3.5 Colourants used for toning Japanese tissue papers

When asked ‘what types of colours do you use to match the colour of Japanese tissue paper with the tone of the aged paper’, acrylic paints and watercolours were the most frequently used at 79% and 73% respectively (Figure 3.3). Plant dyes and gouaches were used only by a small number of respondents at 16% and 9% respectively. Forty four percent and 37.3% of respondents also mentioned that they use colour pencils and pastels respectively. Forty respondents provided further information about other toning materials used for colouring Japanese tissue papers in a free text section. Free text responses remarked on the application of various toning materials such as: Chinese ink (4%), Japanese ink (12%), leather dyes (4%), dry pigments (16%), textile dyes (24%), tinted charcoal (4%), synthetic dyes (e.g., azo) (12%), pre-dyed Japanese tissue papers (8%), susu (colourant obtained from paper extract) (12%), and caramel colour (E150) (4%). It is also noteworthy that one respondent mentioned that they deliberately aged papers to achieve the same tonalities as the original paper without any consideration of the effects of this process on the long-term stability of the object.
Chapter 3: Survey of paper conservation practitioners

3.5.1 Colourants used for toning Japanese tissue papers in Australia, European countries and the United States

The results of the previous question were filtered to show answers from Australia, the USA, and the European countries which were the largest groups responding to the survey. According to Figure 3.4, 11 out of 77 (14%) of respondents from the USA use plant dyes compared with the five out of 20 in Australia (25%) and 3 out of 31 in the European countries (9.7%). Comments made in the free text section revealed that yasha -the extract of Japanese Alder cones- is used by the American, Australian and European respondents (6 out of 77, 5 out of 20 and 2 out of 31 respectively). American respondents (2 out of 77) also use susu for toning Japanese tissue papers. Susu is made by boiling deteriorated papers as well as old backing board to achieve the same tonality of aged papers; subsequently, they use the boiled liquid solution as a toning material.

The findings of the survey confirm that watercolours and acrylic paints are the most popular colourants used by respondents in the USA, Australia and the European countries.
Colour pencil and pastels are the third most popular colourant used by American respondents. Seven out of 31 (22.9%) of the European respondents preferred to use colour pencils while only one out of 20 (5%) Australian respondents use colour pencils for toning purposes. Nine out of the 77 (11.7%) American respondents use gouaches for toning purposes, compared to 3 out of the 20 (15%) of Australian respondents. Interestingly, there is no use of gouaches among the European respondents (0 out of 31).

Comments made in the free text section revealed that respondents from the Netherlands are also interested in using ethanol-based pigments or toning Japanese tissue papers while azo dyes and anionic direct dyes are popular in Germany and UK.

![Use of toning materials in Australia, the European countries and the United States of America.](image)

**3.6 Types of plant dyes for toning Japanese tissue papers**

When asked ‘If you use plant dyes, what type of dyes do you use’, 56% respondents reported the use of black tea (Figure 3.5). The survey indicates that black tea is the most
popular plant dye used by respondents from the USA, Australia, UK, the Netherlands, France, Malaysia, Mexico, Iran and Lao PDR. Yasha was the second most popular plant dye, used in Australia, the Netherlands, Japan, the USA, UK and France.

The survey also revealed that turmeric and henna are used as colourants by Indian and Iranian paper conservators while coffee is used in New Zealand and the indigo plant was used in Australia and the USA. The use of different plants by paper conservators might be related to the availability of plants, and their traditional use by artisan and craftsmen in that region. For example, the use of henna, green walnut shell, pomegranate rind and black tea by Iranian paper conservators at the National Archive of Iran, National Museum of Iran and the Parliamentary Library was observed by the author whilst working at these institutions in 2010 and 2011. Since these plants are grown in Iran, they are accessible to both artisans and paper conservators who wish to use them.

Figure 3-5: Use of plant dyes as toning materials in paper conservation
3.7 Popular brands of toning materials used in paper conservation

When asked ‘if you use watercolour or acrylic paints, what types of paints do you use’, 76.6% of respondents selected their palette of colourants from the Winsor & Newton™ brand, followed by 56.3% who used the Golden brand (Figure 3.6) indicating that these brands are overwhelmingly the preferred choice of the respondents to this survey. Brands such as Kremer (17.2%) and Derivan® (2.75%) were of lower interest. A number of other brands of watercolours and acrylic paints were also mentioned: Liquitex® (14%), Schmincke (7%), Maimeri (0.7%), Daler-Rowney watercolour (2.75%), Utrecht® Artists’ Watercolour (1.4%), Daniel Smith watercolour (1.4%), Grumbacher® (0.7%), Reeves™ (0.7%), Pèbéo (0.7%), Russian brands (0.7%), Tri-Art (0.7%), Sennelier pastels (2%), Dr. Ph. Martin’s® transparent watercolours (0.7%) and also Faber-Castell colour pencils (0.7%). Winsor & Newton™, Kremer and Liquitex® colourants were the most used by the survey’s participants; however, the Derivan® brand was selected for tests based on its widespread use in Australian practice and the results are relevant and can be extrapolated to the other brands.

Figure 3-6: Most popular brands of watercolours and acrylic paints used by paper conservators.
3.8 Reasons for choosing toning materials

When asked ‘why do you prefer to use your chosen type of colourant’, respondents were asked to choose which of the following properties a good colourant needed to possess: lightfastness properties, physical stability, humidity-heat resistance, environmental friendliness, chemical resistance, compatibility with original paper, antimicrobial properties and aesthetic qualities. As shown in Figure 3.7, the majority of respondents agreed that toning materials are chosen for:

- aesthetic reasons,
- compatibility with original paper,
- physical stability, and
- lightfastness properties.

Humidity and heat resistance, chemical and antimicrobial properties as well as the environmental effects of the colourants were less significant factors in the choice of colourants. The availability of the colourants in the region, the colour range of the palette, ease of use, solubility, repeatability, reversibility, permanence, and quick drying properties are other factors which were cited as affecting the respondents’ selection of toning materials. Once again aesthetic considerations are paramount and physical and biostability do not appear to be significant factors in the choice of colourants (as shown in Figure 3.7)
3.9 Studies on the effects of dyes and pigments on Japanese tissue papers

When asked ‘in your opinion, are there enough studies about the effects of dyes and pigments on Japanese tissue paper over time’, the majority of the respondents (93%) mentioned that there are few studies about this issue compared with 7% of the respondents who answered that there are adequate studies on this issue. When given the opportunity, 40 respondents expanded on this answer in a free text section. Free text responses included comments such as (direct quotes from the survey):

- ‘No, of course, but nevertheless, there is enough to postulate a comfortable durability of chosen treatments’
- ‘with dyes the questions are about the stability of the colourant; with some metallic pigments there might be problems with the pigment sensitising the paper to light, resulting in increased paper degradation; we are mostly making assumptions based on the colouring materials selected by paintings conservators as suitable for inpainting on easel paintings’
‘I’d like to know more about the effects of tannins and other acids used in tinting mending tissues on the object being treated’

‘I believe we make our selections based on certain assumptions and expectations - "we do the best we can with what we’ve got" mentality. I would love to see scientific studies on the effects of these choices over time, and would certainly use this research to make more reliable choices’

‘I would be interested in reading any new information about the long-term stability of various media on Japanese papers, and would utilize this information to inform my decisions on choosing media in the future’

Respondents therefore overwhelmingly supported the need for further research about the long-term effects of toning materials on Japanese tissue papers used in paper conservation practice. In particular, respondents had concerns about a lack of understanding of the effects of certain materials, such as tannins and metallic pigments, and the fact that choices tended to be made without any scientific basis.

3.10 Long term effects of toned Japanese tissue papers

The survey then asked ‘are you aware of the long term effects of dyes and pigments on Japanese tissue paper over time’, and responses were ranked from one (indicating extremely aware) to five (not at all aware). The findings of the survey (Figure 3.8) show that most respondents (41%) were moderately aware of the effects of toning materials on Japanese tissue papers over time. While 11% of respondents were not at all aware of the effects over the long terms, 15% of the respondents suggested that they are very aware of
the effects. Thirty three respondents expanded on this answer in a free text section, raising issues such as (direct quotes from the survey):

- ‘what I use, watercolour is stable, but I am not sure about black tea’

- ‘I am aware that watercolour, natural dyes, and, to a lesser degree, acrylic paints fade when exposed long term to long wave ultraviolet light’

- ‘Honestly, it’s something of which I might make a mental note when a previously repaired item comes into the lab or goes on exhibit, but like many of my colleagues, I rarely have the time to go back and examine past repairs in a comprehensive way. This is one reason that deliberate, publishable research on the topic would be so helpful’

This suggests that respondents are more aware of the physical properties, such as lightfastness, of the treated papers than the long-term effects of dyes and pigments on the stability of Japanese mending papers when stored for a long time, or when they are on display or exhibition.

![Figure 3-8: Awareness of longevity of toned papers over time](image)

Figure 3-8: Awareness of longevity of toned papers over time
3.11 Conclusions

Japanese tissue papers are a very popular choice in paper conservation and they are widely used in different weights and thickness for a range of purposes. The findings presented here suggest that both hand-made and machine-made Japanese tissue papers are favoured for repairing paper objects. In terms of matching the tonality of original papers with the repaired paper, acrylic paints and watercolours are more popular with paper conservators in different countries depending on the availability of the colourants in the region.

The results of the on-line survey showed that 98% of respondents used Japanese hand-made papers for repairing purposes. Further, Japanese machine-made papers, Chinese, Korean, Thai and European papers have also been used by respondents. Survey results indicate that for colourants approximately 79% and 73% of the participants used acrylic paints and watercolours respectively. Survey participants further indicated that gouaches and plant dyes are of lower interest. The survey respondents reported that they use toning materials for colouring Japanese mending papers mostly due to aesthetic reasons, their compatibility with original paper, physical and lightfastness properties. These properties were of much greater importance to conservators than their antimicrobial or chemical properties.

The findings of this survey suggest that there is a need to study the long-term properties of Japanese tissue papers in a scientific way rather than just relying on practical experience and transferred information from the vendors. In this thesis, the physical, chemical and microbial properties of watercolours and acrylic paints are analysed and the results of the on-line survey confirmed that they are the most popular colourants used by survey respondents. This survey also indicates that black tea is the most widely used plant dye by
Chapter 3: Survey of paper conservation practitioners

the survey’s participants and this plant dye is also the subject of analysis in the following chapters. Most respondents confirmed that there is a need for more scientific research on the effects of toning materials on the stability of Japanese mending papers in long-term storage conditions.

This survey has provided some very useful information, but also raised some further questions. In the discussion chapter (Chapter 6), key questions emerging from the findings of the survey will be further examined, questions such as:

- What are the effects of various toning materials on physical stability of Japanese tissue papers?
- What are the effects of different dyes and pigments on the chemical permanence of Japanese tissue papers?
- Is there a difference between different Japanese tissue papers in terms of physical, chemical and biological performance?
- Is there a link between the treated paper’s pH and their physical resistance?
- Is there a link between the treated paper’s pH and their colour fastness properties?
- Is there a link between the treated paper’s pH and their fungal growth or inhibition properties?
- Which groups of colourants (acrylic paints, watercolours, plant dyes) are durable toning materials in terms of physical, chemical and biological performance?

This is one of the first times that the quality of Japanese tissue papers has been studied under different experimental conditions. Identification of the quality of toning materials on Japanese tissue papers will help the conservation community to be better informed about
the selection of appropriate materials in an increasingly confusing market of conservation supplies.

The findings of this survey highlight the issue of paper conservators mostly relying on aesthetics in their choice of toning materials for colouring Japanese tissue papers in all paper treatment circumstances, without being aware of the long-terms properties of these materials. Further work to identify the range of conservation methods that are currently used by conservation institutions in different parts of the world would be beneficial. Conservators from different countries could share their successful methods for retouching in paper conservation for the benefit of a wider audience.


4. METHODOLOGY

4.1 Sample preparation

4.1.1 Collection and processing of samples

A wide range of Japanese tissue papers possess desirable qualities for conservation purposes. Two types of hand made papers were used in this study as representative of the many Japanese tissue papers that are available: Yukyu-shi (16 g/m$^2$) and Sekishu (21.4 g/m$^2$) (Hiromi Paper Inc.). Both of these are 100% composed of the domestic kozo plant. They lack any clay, sizing or additives and are made by artisans using Nagashizuki -the traditional practice of Japanese paper-making. The kozo for Sekishu is harvested around Shekishu (Shimane prefecture) and the Yukyu-shi is harvested around Toyama prefecture in Japan (H. Katayama 2013, pers. comm., 9 October).

To make the Yukyu-shi paper, the kozo plant is steamed to remove the bark while the Sekishu paper is made by keeping the middle bark layer (H. Katayama 2013, pers. comm., 9 October). A mild alkaline (soda ash) is used during the cooking process for both Sekishu and Yukyu-shi papers. The kozo fibres of Sekishu are bleached with mountain spring water and sun while the fibres of Yukyu-shi are bleached on snow and sun. Both Sekishu and Yukyu-shi papers are dried on a wooden board under the sun. For the aims of this thesis, the physical, chemical and microbial properties of the untreated Sekishu and Yukyu-shi papers was compared with those that were treated with selected plant dyes, watercolours and acrylic paints.
Fresh *Eucalyptus cinerea* leaves were hand harvested from natural bushland close to Canberra in September 2012. Commercially available loose black tea (Glenbog Fine Teas) and Indian powdered henna leaves (Ayur Rajasthani) were purchased from providers in September 2012. The watercolours and acrylic paints used in the study consisted of alizarin crimson, cobalt blue, raw sienna, raw umber, burnt sienna and burnt umber. These colourants are proprietary brands (for watercolour, Winsor & Newton™ Cotman; and for acrylic paint, Derivan® Matisse). They were selected because they are widely used by paper conservators to provide a range of tonalities to match those of old papers and books (Gyles & Maver 2002; Norton 2002). The complete list of commercial colourants used in this study is presented in Table 4.1. The lightfastness ratings for the Derivan® acrylic paints follows the American Society for Testing and Materials (ASTM) method 4303 and these are I, II, and III corresponding with excellent, very good, and good lightfastness, respectively. Winsor & Newton™ has its own rating for Cotman watercolours and these are set out in their “permanence” system of AA, A, and B to classify their colourants as extremely permanent, permanent and moderately durable, respectively.
Table 4-1: List of commercial watercolours and acrylic paints with properties as declared by the manufacturers.

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Colour Name</th>
<th>Colour</th>
<th>Pigment Name</th>
<th>Main Chemical Components</th>
<th>Colour Index (CI) Number</th>
<th>Lightfastness</th>
</tr>
</thead>
<tbody>
<tr>
<td>W&amp;N</td>
<td>Blue</td>
<td>Complex sodium aluminosilicate containing sulphur, coprecipitated zinc sulphide/barium sulphate</td>
<td>PB 29, PW 5</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W&amp;N</td>
<td>Red</td>
<td>Quinacridone pyrrolidine, quinacridone</td>
<td>PR 206</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W&amp;N</td>
<td>Brown Raw sienna</td>
<td>Synthetic iron oxides</td>
<td>PR 101, PY 42, PR 101</td>
<td>AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W&amp;N</td>
<td>Brown Burnt sienna</td>
<td>Synthetic iron oxide</td>
<td>PR 101</td>
<td>AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W&amp;N</td>
<td>Brown Raw umber</td>
<td>Natural iron oxide, synthetic iron oxide</td>
<td>PB 7, PY 42</td>
<td>AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W&amp;N</td>
<td>Brown Burnt umber</td>
<td>Calcined natural iron oxide, synthetic iron oxide</td>
<td>PB 7, PY 42</td>
<td>AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Blue</td>
<td>Oxides of cobalt and aluminium</td>
<td>PB 28</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Red</td>
<td>Blend quinacridone and naphthol carbamide</td>
<td>PR 122, PR 170</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Brown Raw sienna</td>
<td>Natural iron oxide</td>
<td>PY 43</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Brown Burnt sienna</td>
<td>Synthetic and natural iron oxide</td>
<td>PB 7, PR 101</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Brown Raw umber</td>
<td>Natural iron oxide containing manganese</td>
<td>PB 7</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Brown Burnt umber</td>
<td>Calcined natural iron oxide</td>
<td>PB 7</td>
<td>I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)W&N = Winsor & Newton, D = Derivan; \(^b\)as defined in the text.
4.1.2 Extraction and dyeing conditions

The ultra-pure water used for extraction and dyeing was treated by both reverse osmosis and deionisation. The resistivity of the purified water was always greater than 18 MΩ.cm. Fresh *Eucalyptus cinerea* leaves were washed under tap water to remove dust particles, and were then dried in sunlight for at least one month. Plant dyestuff extraction was carried out by boiling the plant materials in deionised water for 1 hour with occasional stirring, maintaining a liquor ratio of 1 g plant material to 20 mL of deionised water (Yusuf et al. 2012; Bechtold et al. 2003). Following this ratio, 20 g of both black tea and henna powder were placed into separate beakers and 400 mL deionised water was added so that the plant material was completely covered. The contents were brought to boiling and simmered on a hotplate for one hour. Similarly, 30 g of fresh and dried Eucalyptus leaves were separately added to 600 mL of deionised water. In all cases, the dyeing liquor was allowed to cool and then the insoluble residue was removed by sedimentation and filtration through Whatman® Cellulose Filter Paper Grade 4 (GE Healthcare, Category No. 1004-150). Watercolours and acrylic paints were prepared by adding 1 g to 50 mL of deionised water. Figure 4.1 shows the filtration step for preparation of henna dye after boiling and cooling.
4.1.3 Dyeing of Japanese tissue papers

A Japanese soft wool brush (Talas) was used to tint the paper samples with the prepared plant dyes and chemical paints. Each paper was tinted with a minimum of 25 brush strokes. The treated papers were dried at room temperature for at least 24 hours on a flat surface to ensure even dispersion of the colourants. Hanging the treated papers results in uneven dispersion and concentration of dye at one end. Figure 4.2 shows the Yukyu-shi paper after treatment with alizarin crimson acrylic paint. Dyeing experiments were conducted at the Heritage Conservation Laboratory, Faculty of Arts & Design, University of Canberra.

Figure 4-1: Filtration process for henna
4.2 Experimental testing of properties

Japanese tissue papers toned with plant dyes, watercolours and acrylic paints were then subjected to further tests of their physical, chemical, and biological properties. Untreated paper samples were used as controls.

4.2.1 Artificial ageing

The natural ageing process of paper is too slow to observe changes in paper materials in a manageable time scale (HavlÍnová et al. 2009). In order to simulate long-term deterioration processes over a manageable time scale, a moist heat artificial ageing test was performed on the untreated papers and those that were treated with plant dyes, watercolours and acrylic paints. The paper samples were individually cut to 21 × 29.7 cm (A4) size and then subjected to artificial ageing at 70 °C and 65% RH in a climatic chamber (Angelantoni EKOCHL 700) for 12 days according to the Technical Association
The standard test temperature was altered from 80 °C to 70 °C to accommodate the limitations of the climatic chamber.

The moist heat treatments were applied because the degradation rate of cellulose is very sensitive to the amount of moisture in the paper sample. The paper sheets were hung vertically on the climatic chamber racks which permitted free air-flow around the paper specimens. Stainless steel paper clips were used to clamp the edges of each paper so that the paper did not come into contact with the other papers. Figure 4.3 shows the paper specimens after preparation and inserting into the climate chamber.

Figure 4-3: Untreated and treated paper samples were artificially aged at 70 °C and 65% relative humidity (RH) for 12 days.
In this study, an artificial ageing test in the moist heat climatic chamber was used to compare the effects of various toning materials on physical, chemical and optical properties of the paper specimens before and after ageing. Results from the following tests were measured both before and after artificial ageing: folding endurance, tear resistance, pH and colour change. Pre-conditioning is a pre-requisite before running the physical tests due to the hysteresis phenomenon, in which the moisture content of a material depends somewhat on its previous history (Daniels 2006; Caulfield & Gunderson 1988). In accordance with the TAPPI Standard Test Method T 402 sp-08 (TAPPI 2008), the paper specimens were conditioned at 23 °C and 50% RH in the climatic chamber (Angelantoni EKOCHL 700) for at least 24 hours before artificial ageing. The artificial ageing test was performed in the forensic laboratory of the Faculty of Education, Science, Technology and Mathematics at the University of Canberra. Figure 4.4 illustrates the visual difference of the untreated Yukyu-shi and Sekishu papers and the papers treated with plant dyes before and after artificial ageing.
4.2.2 Folding endurance

Mechanical tests were also conducted on both the untreated and treated Japanese tissue papers before and after artificial ageing. The folding endurance test is performed to measure the stability of a paper in response to a repeated forward and backward folding force. The test was conducted in accordance with TAPPI Standard Test T511 om-02 (TAPPI 2006), using a Folding Endurance Tester (MIT PA-106). Although the folding apparatus is designed to assess the physical resistance of paper samples in industrial
applications, it is also a standard method to measure paper stability against repeated folding, bending and creasing in paper conservation practice (Doming 2005).

The folding endurance test applies more stress than would be expected from normal handling in libraries and archives. Hence, the overall results are indicative of the durability of paper samples over time. The TAPPI Standard recommends using the logarithm (to base 10) of the number of double folds before rupture as an index of folding endurance.

In this study, double folds were applied by the MIT apparatus with the aid of an oscillating jaw-like clamp. The upper jaw that holds the paper is fixed, but the lower jaw swings similar to a pendulum. The rotary oscillating movement of the head is such as to fold the paper through an angle of 135 degrees both to the right and to the left of the position of the unfolded specimen. A counter for registering the number of double folds is also provided. The applied tension in the MIT test machine was fixed at 1 kg. The MIT fold endurance tester folds a 15 mm × 110 mm paper strip, which is held vertically under tension, at a rate of 175 ± 25 double folds per minute until the specimen is ruptured.

The folding standard test samples were subjected to 1000 folds, this level was chosen to represent, in line with the aims of the study, the anticipated level of use (and abuse) in library and archive circumstances (Doming 2005). As folding endurance is very sensitive to the moisture content of the specimen, it is important to observe the requirements for preconditioning before and during the test. The environmental conditions during the test procedure should be around 23 °C and 50% RH. Ten replicates were employed for each paper specimen. The folding test was performed for both untreated and treated Japanese papers before and after artificial ageing. The experiments were performed at the
Conservation Laboratory of the National Archives of Australia. Figure 4.5 shows the apparatus used to study the folding endurance of the paper specimens in this study.

Figure 4-5: MIT folding endurance tester was used to study the folding endurance of treated and untreated papers before and after ageing.

4.2.3 Tear resistance

A tear resistance test was conducted for both untreated and treated papers using the Elmendorf tear test machine (ED30-ED 401. 01) (Figure 4.6). The test measures the internal tearing resistance of paper against a tearing force according to the TAPPI Standard Test Method T 414 om-12 (TAPPI 2012). A pendulum weight of 32,000 mN was used after the instrument was calibrated to measure the average force of a prescribed number of sheets torn simultaneously. A digital encoder immediately converted this to the mean of the tearing force for a single sheet. The air pressure was adjusted to 600 kPa.

Four sheets of each paper specimen were cut into sheets measuring 49.9 mm × 61.0 mm. The four sheets of specimen paper were clamped between the grips. The tear test was repeated four times for both untreated and treated paper specimens before and after ageing.
The index of tear measurement is expressed in mN. The tear resistance tests were performed at the Conservation Laboratory of the National Archives of Australia.

![Tear tester machine with Yukyu-shi papers treated with alizarin crimson watercolour between the machine jaws.](image)

**Figure 4-6:** Tear tester machine -Yukyu-shi papers treated with alizarin crimson watercolour are located between the machine jaws.

### 4.2.4 Colour change

Reflectance spectrophotometry measures colour change in a non-destructive manner. It was applied before and after moist-heat artificial ageing. Reflectance spectra were recorded with a CM 2600d Minolta spectrophotometer (Thermo Fisher) in the wavelength range of 360 nm to 740 nm on several small areas measuring about 8 mm in diameter. Colour data were measured using illuminant daylight 6500 °K (D65), with the standard observer angle of 10 °, the specular component included (SCI) and excluding UV radiation. The instrument was calibrated according to the manufacturer’s instructions.

When examining the colour changes on the paper samples, a template was used to position the spectrophotometer at the same spot on the surface of the paper sample each time a measurement was taken. Accordingly, a transparent film of Mylar® (Archival Survival) was fitted to the surface and fixed to the border of the paper with adhesive tape.
Subsequently, five areas to be investigated were selected and indicated with a marker-pen. A thick black board was located under the paper specimen and a Mylar® sheet in order to prevent intervention from the passage of light from the surface of the paper sample. Circular holes were cut from the Mylar® film corresponding with the selected areas. For each paper specimen, equivalent coloured areas were tested both pre- and post-ageing to compare the colour change. A total of five areas were measured five times. The average of the five measurements was used for the colour calculations.

The colourimetric data were calculated from the acquired data following the International Commission on Illumination (CIE) colourimetry system which mathematically simulates the perception of colour by providing a standard process for measuring and quantifying colour perception (Bacci et al. 2003). Figure 4.7 illustrates a schematic diagram of the CIE colour space system. It is defined according to the three coordinators: $L^*$, $a^*$ and $b^*$ (Luo 2002), where:

- $L^*$ = Lightness (0 = black, 100 = white)
- $a^*$ = Redness-greenness ($a^+ =$ redness, $a^- =$ greenness)
- $b^*$ = Yellowness-blueness ($b^+ =$ yellowness, $b^- =$ blueness)

The differences between $L^*$, $a^*$ and $b^*$ measurements before and after ageing were calculated and expressed as $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ respectively (Yusuf et al. 2012; del Hoyo-Meléndez & Mecklenburg 2011; Cocca, D’Arienzo & D’Orazio 2011; Bacci et al. 2003; Bechtold et al. 2003; Suzuki & Koestler 2003). The overall colour change was calculated using the CIE 1976 (CIE76) (Luo 2002).

$$\Delta E^* = [ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 ]^{1/2}$$
The spectrophotometer (Figure 4.8) has the ability to measure the absolute reflectance of electromagnetic wavelength. The collected data can be converted into ‘tristimulus’ values and expressed as colour change (Mangum & Heginbotham 2002). The three significant values of colour are hue (wavelength), saturation (purity) and luminosity (intensity of reflected light) (Kubik, 2006). Human eyes can differentiate colour change ranges as minute as 0.3 or as large as 1.5-2 tristimulus values (Bacci et al. 2003).

A $\Delta E^*$ value of 5 was considered large enough to be detected by most observers and was used as an initial threshold value in this study. Colour change values greater than 5 indicate extreme colour difference (Yurdun & Dolen 2012; Hoyo-Meíendez & Mecklenburg 2011; Mangum & Heginbotham 2002). The spectrophotometer experiments were conducted at the Heritage Conservation Laboratory, Faculty of Arts & Design, University of Canberra.
4.2.5 Micro fading test

Micro fading tests provide a rapid way to measure the relative lightfastness properties of an object, relative to the traditional accelerated light ageing methods (Ford 2011; del Hoyo-Meléndez & Mecklenburg 2011). MFT was conducted on both untreated Japanese tissue papers and those that were treated with plant dyes, a watercolour and an acrylic paint of alizarin crimson. The instrument used was an Oriel 80190 MFT (Newport®) (Figure 4.9) developed at the Art Conservation Research Center of Carnegie Mellon University in Pittsburgh (Whitmore 2002; Whitmore et al. 1999).
Figure 4-9: Microfading tester used for identifying fading resistance of untreated papers and papers treated with the selected dyes and pigments.

The instrument is comprised of a 75 W xenon light source which was restricted to 400-700 nm by filtering UV light and included a water filter and a hot mirror. An optical fiber and lens assembly projects a 300 µm area of light on to the surface of the paper at a photometric intensity of approximately 6 Mlux (Ford 2011). A second lens assembly collects the diffusely reflected light at 45 ° from the source and directs it to a spectrophotometer via a second optical fiber. Spectral change during the course of the test (approximately 10 minutes) was converted to colour change using the CIE 2000 (CIE00) equation (Luo 2001). The ISO Blue Wool Fading Standards (BWFSs) were employed as internal standards to ensure reproducibility and also to provide an approximate scale against which to rate the lightfastness of the colourants under test using Michalski's estimates of the lightfastness of BWFSs and British Standard (BS) 1006 (Michalski 1987).
Colour change rates were reported as Blue Wool Equivalents (BWE), calculated from the CIE_00. Each standard represents increasing lightfastness by a factor of two compared with the previous one (del Hoyo-Meléndez & Mecklenburg 2010). Paper samples were positioned approximately 1cm from the confocal point of the light beam and the collection lens (Ford 2011). After the final focus, the reflectance spectrum was maximised and the data collection was initiated. Spectra were recorded using a resolution of 1 nm at 60 second intervals for 10-15 minutes and then averaged.

The fading rates of BW1 and BW2 increase moderately when the Blue Wool is exposed to the light source while the fading rate of BW3 and BW4 are slower and closer to those commonly encountered (Whitmore & Tao 2011). All textiles, coloured works on paper, sensitive pigments and natural fibres are usually found to be in the range BW1-4 (Ford 2011; Auhorn 2006; Herbst et al. 2004). Conversely, acrylic paints and durable pigments such as those with iron oxides in their structure are usually categorised in the range BW5-7.

4.2.6 pH

To identify the acidity of both untreated paper specimens and those that were treated with plant dyes, watercolours and acrylic paints, a pH test was performed according to the TAPPI Standard Test Method T509 om-11 (TAPPI 2011); using hydrogen ion concentration (pH) of the paper extracts (cold extraction method). The test compared the pH of the untreated papers with those that were treated with the selected dyes and pigments before and after artificial ageing. The TAPPI method involves soaking equal amounts of the paper sample with deionised water for one hour. A small piece (2.5 × 2.5 cm) of each paper was prepared and cut into 0.5 cm squares with the aid of a scalpel and
then placed in a beaker with 12 mL deionized water so that the paper sample was completely covered. The solution was stirred and covered with a watch glass for at least one hour. Subsequently, the mixture was filtered through Whatman® Cellulose Filter Paper Grade 4 (GE Healthcare, Category No. 1004-150). A pH meter (Thermo Fisher) was used to measure the pH of the solution at 25°C after preparation, according to the TAPPI Standard Test Method. The temperature was controlled by automatic temperature compensation. Calibration of the instrument was performed according to the manufacturer’s instructions using pH 4 and 7 calibration buffers (Sigma). The pH test was performed in the Forensic Laboratory of the Faculty of Education, Science, Technology and Mathematics at the University of Canberra.

4.2.7 Fungal growth test

Preparation of the samples

The untreated Yukyu-shi and Sekishu papers and the papers treated with plant dyes, watercolours and acrylic paints were compared to identify their antifungal properties against Aspergillus niger (A. niger) and Penicillium rubrum (P. rubrum). Whatman® Cellulose Filter Paper Grade 4 (GE Healthcare, Category No. 1004-150) was used as a control paper. Positive controls were consisted of the three untreated papers (Yukyu-shi, Sekishu and Whatman) with three replicates for each paper. DNA concentration was assessed at day 0 (time of inoculation) and day 10 (10 days after inoculation and incubation in an artificial growing environment). Hence, the experimental samples consisted of:
Chapter 4: Methodology

- 3 untreated papers (Whatman, Yukyu-shi & Sekishu) × 3 replicates × 2 fungal species (A. niger and P. rubrum) × 2 time points (day 0 and day 10) = 36 untreated control samples
- 32 treated papers × 1 replicate × 1 time point (day 0) = 32 uninoculated, treated samples
- 32 treated papers × 3 replicates × 2 fungal species (A. niger and P. rubrum) × 1 time point (day 10) = 192 inoculated, treated samples

Paper strips (treated and untreated) measuring 2.5 cm × 2.5 cm were wrapped separately in aluminum foils and placed in a desiccator with silica gel for at least four weeks to minimise the development of any existing fungal growth. In a study by Michaelsen et al. (2006), UV radiation was used for removing existing fungal growth from the paper samples. In this study, the desiccation method was used due to the destructive effects of UV radiations on the dyes and pigments used for treating paper samples. Figure 4.10 presents the Whatman Filter Paper after preparation and the paper specimens stored in separate petri dishes before inoculation with fungal spores.

Figure 4-10: Whatman® Cellulose Filter Paper control used for fungal growth test (left) and treated paper specimens stored individually in petri dishes (right).
The DNA extractions were performed in a Class II laminar flow (biosafety) cabinet within an isolated DNA extraction area (Figure 4.11). The biosafety cabinet was prepared by 70% ethanol (aq). The cabinet and its contents (including pipettes) were irradiated with UV light for 20 minutes before and after DNA extractions. The micro-pipettes and pipette tips were pre-sterilised and contained barrier filters to prevent cross contamination of biological materials. PCRs were prepared in a dedicated PCR cabinet and then performed in a dedicated post-amplification area in the Forensic Laboratory of the Faculty of Education, Science, Technology and Mathematics at the University of Canberra.

Figure 4-11: Paper specimens for DNA extractions were prepared in a biosafety cabinet.

**Fungal strains and growth conditions**

Sabouraud agar was formulated to be selective for *Aspergillus* and *Penicillium* species (Zain et al. 2009). Fungal strains (*A. niger* and *P. rubrum*) were routinely cultured on Sabouraud dextrose agar, pH 5.6, (Oxoid) prepared according to the manufacturer’s instructions. This consisted of mycological peptone (10 g/L), dextrose (40 g/L) and agar.
(15 g/L). The media was sterilised by autoclaving for 20 minutes at 103.4 kPa and 121 °C after which it was distributed into sterile petri dishes and solidified by cooling. The fungal spores were transferred to dedicated plates by using an inoculation loop in a crossing pattern to maximise fungal growth. Subsequently, the agar plates containing both *A. niger* and *P. rubrum* were stored at room temperature for at least 7 days. Figure 4.12 shows *A. niger* and *P. rubrum* after 6 days growth, stored at room temperature.

![Figure 4-12: Aspergillus niger (left) and Penicillium rubrum (right) after 6 days growth at room temperature.](image)

After observing sufficient fungal growth on the plates, the fungi were inoculated onto fresh Sabouraud dextrose agar. This process extended the lag-phase of fungal growth. Old plates were stored at 4 °C to prevent further growth of fungi. After observing sufficient fungal growth on new plates, the previous plates were discarded. For long term storage of fungal stocks, the agar plates with sufficient fungal growth were cut into small blocks and transferred into 50 mL eppendorf tubes with saline solution (Sigma) (0.85% NaCl) (aq) before storage at room temperature (Figure 4.13).
Figure 4-13: Fungal stocks in saline solution (0.85% NaCl) which were stored at room temperature.

**Spore quantification using hemocytometer**

A haemocytometer (Hawksley) was used for cell counting (Figure 4.14). It is composed of a thick crystal slide with nine square subdivision grids. Effective concentrations should be in a range of $10^6$ to $10^8$ cells per mL (Morris & Nicholls 1978). Spore suspensions for *A. niger* and *P. rubrum* were obtained by gently scraping the surface of the agar plates with a plastic swab and transferring to 40 mL of Sabouraud liquid media (Oxoid), containing 20.0 g/L dextrose, 5.0 g/L pancreatic digest of casein, 5.0 g/L peptic digest of animal tissue (pH 5.6). The liquid sample was transferred by pipette to the centre of the cell chamber. The liquid enters the chamber uniformly and is absorbed by capillary action. A Leica DM500 light microscope (Leica) was used to visualise the spores (200 x and 400 x). A dilution series was prepared to estimate the concentration of spores in each sample. Spores were counted using the haemocytometer and concentrations of *A. niger* and *P. rubrum* strains were diluted to 25,000 spores per 10 μL and 32,000 spores per 10 μL of Sabouraud liquid media, respectively.
Inoculation of paper samples

In order to study the antifungal properties of the papers treated with the prepared plant dyes, watercolours and acrylic paints, both untreated and treated paper samples were inoculated with *A. niger* and *P. rubrum* fungal strains. The amount of inoculation solution applied to the papers was determined in a series of experiments in which different concentrations and inoculation volumes were tested. The inoculated papers were incubated individually in petri dishes in an environmental chamber (Angelantoni EKOCHL 700) for 10 days at 27 °C and 80% RH. Figure 4.15 shows the preparation of spore suspension by gently scraping the surface of the agar plate.
Figure 4-15: Preparation of the spore suspension by gently scraping the surface of the agar plate with the aid of a plastic swab.

DNA extractions

Before DNA was extracted, the paper specimens were individually placed in a 1.7 mL eppendorf tube and stored in the freezer at -20 °C for at least 12 hours to avoid possible fungal growth (Figure 4. 16).

Figure 4-16: Paper specimens were placed in individual eppendorf tubes after incubation and placed in the freezer at -20 °C for at least 12 hours before DNA extractions.
In this study, a lysis process was employed in which the cell wall and nuclei are broken down chemically. Fungal species are well-known to include an especially thicker and stronger cell wall which makes DNA extraction more difficult than for bacterial and animal cells (Zhou et al. 2000). Two DNA extraction methods were compared in this study: an organic (phenol-chloroform) extraction with ethanol precipitation and the QIAamp® Mini Kit (Qiagen). In general, the kit has a number of advantages over the organic extraction method. It is easier to perform, takes less time and does not involve the use of toxic chemicals (phenol and chloroform). According to the manufacturer, the QIAamp® mini kit and the method removes PCR inhibitors. The cost of DNA extraction using the kit; however, is more than that required for organic extraction.

**Organic extraction**

The DNA extraction procedure was carried out in a DNA extraction area in a dedicated pre-amplification laboratory, which was physically isolated from the post-amplification laboratory. Throughout the procedure, disposable gloves were worn and disposable pipette tips were used at all times.

The organic method of DNA extraction is simple, inexpensive and can produce a pure final DNA preparation. This method uses a digest buffer which usually contains proteinase K to denature proteins, dithiothreitol (DTT) to reduce disulphide bonds and a metal ion chelator such as ethylenediaminetetraacetic acid (EDTA) to decreases nuclease activity. During incubation, the components of the digest buffer lyse the cell; break down cell walls and degrade proteins, including those involved in the chromosome scaffold. The DNA remains dissolved in the aqueous phase, while denatured cellular proteins are dissociated from the
DNA into the organic phase. The supernatant can then undergo purification and concentration.

Paper strips were placed in individual sterile 1.5 mL micro centrifuge tubes (Axygen Scientific) together with 700 µL of DNA extraction buffer containing 60 µL of 20 mg/µL proteinase K (Sigma Aldrich) per mL of lysis buffer (10mM Tris base (Sigma Aldrich), 1mM EDTA (Sigma), 100mM sodium chloride (Chem Supply) and 2% Tween 20 (Sigma) with pH 8.0) and 6 mg of solid dithiothreitol (Sigma Aldrich) per mL of lysis buffer. After mixing by repeatedly inverting the tubes, the samples were incubated at 56 °C overnight. The next day, 42 µL of 20 mg/mL proteinase K and 26 µL of 6 mg/mL dithiothreitol were added to the samples. Then, they were incubated at 56 °C for at least one hour. To each sample, 350 µL of phenol/chloroform/isoamyl alcohol (25:24:1, pH 8.0, Sigma Aldrich) was added after which they were thoroughly vortexed and then centrifuged at 13800 × g for 15 minutes.

The upper, aqueous layer (200 µL) from each sample was transferred to new sterile 1.7 mL micro centrifuge tube (Scientific Specialities Inc.) to which was added 20 µL of 125 mM EDTA, 20 µL of 3M sodium acetate (Sigma) (pH 5.2) and 500 µL of cold (-20 °C) absolute ethanol(Sigma Aldrich). After inversion, the samples were incubated at -20 °C for 15 minutes and then centrifuged at 2500 × g for 30 minutes. Immediately after centrifuging, the liquid from each tube was decanted after which 70 µL of cold (-20 °C) 70% ethanol was added. The samples were incubated at -20 °C for 15 minutes and then centrifuged at 1700 × g for 15 minutes. The liquid from each tube was decanted after which the DNA pellets were air-dried at room temperature and then resuspended in 50 µL of TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). The samples were stored at 4 °C until
further analysis. For longer term storage, DNA samples were stored in the freezer at -20 °C.

Several experiments were used to reach the optimal conditions for DNA extractions for the aims of the study. The ethanol precipitation was the only purification step during the organic laboratory extraction method. The centrifugal filter units were also examined during the organic extraction method for further purification of the DNA extracts.

The method of filter purification involves repeated centrifugation of the sample with the aid of a Microcon® 100 centrifugal filter (Millipore). The Microcon® was pre-rinsed using TE buffer before applying the sample (2.5 cm × 2.5 cm). The filter was then centrifuged for 10 minutes at 600 × g. After centrifugation, 200 µL of TE buffer was added to the sample and it was centrifuged at 600 × g for 10 minutes. This step was repeated twice. A liquor of 50 µL of TE buffer was then added to the filter which was incubated at room temperature for 5 minutes, inverted into a new tube and centrifuged at 600 × g for 5 minutes. The purified DNA was stored at 4 °C for further use.

**QIAamp® mini kit DNA extraction**

In this study, a modified version of the QIAamp® DNA Mini Kit protocol was employed. The protocol is designed for the isolation of genomic DNA from fungi. Paper strips were placed in individual, sterile 1.7 mL micro centrifuge tubes together with 350 µL of DNA extraction buffer containing 60 µL of proteinase K (Sigma Aldrich) per mL of lysis buffer and 6 mg of solid dithiothreitol (Sigma Aldrich) per mL of lysis buffer. After mixing by repeatedly inverting the tubes, the samples were incubated at 56 °C overnight and then centrifuged at 21200 × g for 10 minutes. The mixture was resuspended in 180 µL of buffer.
AL (Qiagen) and 20 µL of 20 mg/mL proteinase K (Sigma Aldrich), followed by incubation at 55 °C for 15 minutes. After mixing, a further 200 µL buffer AL (Qiagen) was added to the sample which was incubated at 70 °C for 10 minutes. After incubation, 200 µL of cold (-20 °C) absolute ethanol (Sigma Aldrich) was added to the solution and the mixture was transferred to a QIAamp Mini spin column (Qiagen) and centrifuged at 6000 × g for one minute. A series of centrifugation steps with the QIAamp Mini spin column were followed respectively with addition of 500 µL buffer AW1 (Qiagen) and buffer AW2 (Qiagen). Extracted DNA was eluted with 200 µL of buffer AE (Qiagen) in two steps resulting in a total of 400 µL of DNA extract for each sample.

**DNA quantification using Qubit®2.0 fluorometer**

The DNA concentrations of the paper samples were determined using a Qubit®2.0 fluorometer (Life Technologies) (Figure 4.17). The fluorometer utilises an intercalating fluorescent dye to quantify DNA concentrations. The dye only fluoresces when bound to double stranded DNA (dsDNA). The method can be performed within 10 minutes. Instrument calibration was performed according to the manufacturer’s instructions. A dilution of 1 µL in 200 µL of Qubit™ reagent (Life Technologies) in Qubit™ buffer (Life Technologies) was used as a working solution. In a 0.5 mL tube, a volume of 2 µL of the extracted DNA from paper samples was added to 198 µL of working solution. After vortexing to mix, the samples were incubated for 2 minutes at room temperature to allow the intercalating dye (PicoGreen®) to bind to the dsDNA. After incubation, the tube was placed into the tube holder of the Qubit®2.0 fluorometer, which was able to directly calculate the DNA concentrations.
Figure 4-17: Qubit®2.0 fluorometer used to measure the DNA concentrations of the paper samples.

**Selection of primers**

The length of the primer influences the PCR efficiency and specificity. It is believed that a shorter amplification target yields better results because less deoxynucleoside triphosphates are required for the elongation step (Quellhorst & Rulli 2008). However, a short primer may randomly anneal to the template DNA and be less specific. Very long primers, however, might raise their annealing or melting temperature (Tm). A length between 19 and 23 base pairs (bp) is recommended. Melting temperature represents the temperature at which half of the primer concentration is disintegrated from the DNA. The following simplified formula gives the description of the Tm:

\[ Tm = 4(G+C) + 2(A+T), \]

in which the Guanine (G), Cytosine (C), Adenine (A) and Thymine (T) nucleotide bases stand for the associated dNTP.

In a singleplex PCR reaction, only one primer is used; however, it is possible to use multiple primer sets to amplify different fragments at the same time. Application of multiplex PCR is very useful in the context of forensic studies in which fast and reliable
results are required. Further analyses can be applied after PCR reactions because
fluorescence can be yielded by the amplified DNA through one of the primer sets or a
combination of the primer sets. Multiplex PCR is more complicated than normal PCR
since all primer sets used in PCR reactions should include very similar annealing
temperatures. Additionally, achieving optimal concentration of different components in the
PCR mix is crucial and generally has to be developed by a process of experiments. Primer
sets (Table 4.2) used in this study were obtained from the reviewed literature since their
functionality has already been proved.
Table 4-2: Primers used to target fungal species

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Specificity</th>
<th>Primer sequence</th>
<th>Annealing temperature</th>
<th>Amplificon length</th>
<th>Reference</th>
</tr>
</thead>
</table>
| UN1         | All fungi                | Forward: 5’-ATT GGA GGG CAA GTC TGG TG  
Reverse: 5’-CCG ATC CCT AGT CGG CAT AG | 62 °C                  | 500 bp            | (Loeffler et al. 1999) |
| UN2         | All fungi                | Forward: 5’-GAT ACC GTY GTA GTC TTA  
Reverse: 5’-TGT CGG GAC CGT GTG AGT | 55 °C                  | 219 bp            | (Musher et al. 2004) |
| APP         | Aspergillus + Penicillium | Forward: 5’-ACT TTC GAT GGT AGG ATAG  
Reverse: 5’-CAG AAG GAA AGG TCC AGC C | 57 °C                  | 385 bp            | (Makimura, Murayama & Yamaguchi 1994) |
| ASP1        | Aspergillus              | Forward: 5’-CTG TCC GAG CGT CAT TG  
Reverse: 5’-TCC TCC GCT TAT TGA TAT | 55 °C                  |                  | (Schabereiter-Gurtner et al. 2007) |
| ASP2        | Aspergillus              | Forward: 5’-CGG CCC TTA AAT AGC CCG GTC  
Reverse: 5’-ACC CCC CTG AGC CAG TCC G | 62 °C                  | 363 bp            | (Melchers et al. 1994) |
| ASP3        | Aspergillus              | Forward: 5’-GAT AAC GAA CGA GAC CTC GG  
Reverse: 5’-TGC CAA CTC CCC TGA GCC AG | 50°C                   | 384 bp            | (Yamakami et al. 1996) |
| ASPn        | Aspergillus niger        | Forward: 5’-GAT TTC GAC AGC ATT TCT CAG AA  
Reverse: 5’-AAA GTC AAT CAC AAT CCA GCC C | 60°C                   | 245 bp            | (Susca et al. 2007) |
| PEN         | Penicillium              | Forward: 5’-TCC GTA GGT GAA CCT GCG G  
Reverse: 5’-TCC TCC GCT TTA TCG ATA TG | 56 °C                  | 560/600 bp        | (Tiwari, Jadhav & Kumar 2011) |

* UN1 and UN2= Universal primers; APP= Aspergillus and Penicillium primers; ASP1-ASP3= Aspergillus primers; ASPn= Aspergillus niger primers; PEN= Penicillium primers.
Polymerase chain reaction (PCR)

The polymerase chain reaction was used to amplify the extracted DNA. In theory, every cycle duplicates the number of DNA amplicons, such that:

\[
\text{Final number of DNA amplicons} = \text{initial number of DNA amplicons} \times 2^{\text{number of PCR cycles}}
\]

In this study, 40 cycles of the PCR reaction were used, which can detect very low DNA concentrations (Quellhorst & Rulli 2008). Quantification of template DNA is an essential step in the analysis of samples using the polymerase chain reaction, as the efficiency of PCR amplification is dependent on the amount of DNA present in the sample. The amplification of too little genomic DNA may yield only partial results and stochastic effects may be observed. The amplification of too much template may increase the tendency for amplification of spurious products.

The quantitative polymerase chain reaction (qPCR) technique was used for the detection and quantification of DNA from fungal strains on paper samples. The major challenge of this study was identification of appropriate primer sets for PCR. After several experiments with various primer sets described in Table 4-2, the UN1, UN2 and ASP2 primer pairs, used to amplify regions of the 18S rRNA gene, were the only ones to consistently amplify DNA from extracts. The primers obtained from Integrated DNA Technology (IDT®) and desalted. The UN1 and UN2 universal primer pairs resulted in amplification beyond the threshold of 10,000 relative fluorescence unit (RFU) whereas ASP2 primer pair did not; therefore, for the aims of this study the use of the ASP2 primer pair was ignored.

Some preparation or conservation treatments may inhibit PCR amplification. Known inhibitors include phenolic compounds, heavy metals in pigments, fats, pollen, cellulose,
EDTA, tannic acids and some dyes (Eklund 2012; Michaelsen, Piñar & Pinzari 2010). To mitigate against inhibition, bovine serum albumin (BSA) (Sigma) was added to the PCR.

Extracted DNA was added to a PCR master mix (Table 4.3). The PCR for each extracted DNA sample consisted of 1 × reaction buffer, 0.025 units/µL Taq DNA polymerase (MyTaq™HS), 0.2 mM of each dNTP (Bioline), 0.125 µM of each of the forward and reverse primers (IDT®), 1 × Evagreen™ intercalating dye (Biotium), and 0.4 µg/µL Bovine serum albumin (Sigma), 2 µL DNA template and dH₂O to a total volume of 25 µL.

Table 4-3: Components of the qPCR master mix used to quantify fungal DNA

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration</th>
<th>Final concentration</th>
<th>Volume in one reaction (25 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dNTPs</td>
<td>10 mM</td>
<td>0.2 mM</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>Primer forward</td>
<td>25 µM</td>
<td>0.125 µM</td>
<td>0.125 µL</td>
</tr>
<tr>
<td>Primer reverse</td>
<td>25 µM</td>
<td>0.125 µM</td>
<td>0.125 µL</td>
</tr>
<tr>
<td>Taq DNA polymerase</td>
<td>5 u/µL</td>
<td>0.025 u/µL</td>
<td>0.125 µL</td>
</tr>
<tr>
<td>Reaction buffer</td>
<td>5 X</td>
<td>1 X</td>
<td>5 µL</td>
</tr>
<tr>
<td>(changed in trial)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dH₂O</td>
<td>14.875 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evagreen</td>
<td>20 X</td>
<td>1 X</td>
<td>1.25 µL</td>
</tr>
<tr>
<td>BSA</td>
<td>10 µg/µL</td>
<td>0.4 µg/µL</td>
<td>1 µL</td>
</tr>
<tr>
<td>DNA template</td>
<td></td>
<td></td>
<td>2 µL</td>
</tr>
</tbody>
</table>

Negative controls consisted of 2 µL of TE buffer in place of DNA template. Standard concentrations were prepared from a dilution series of DNA extracts from pure cultures of *P. rubrum* (2.38 – 0.0000238 ng/µL) and *A. niger* (4. 16 – 0.0000416 ng/µL). These were used to produce a standard curve for each primer set and fungal species. Once a sample of DNA is placed in the PCR machine, the instrument runs through multiple heating and cooling cycles (Cripps 2006). The PCR reactions consist of three stages: denaturation of the template DNA, annealing the primers and extension of the primers.
All PCRs were performed in a 7500 Real Time PCR instrument (Life Technologies) using PCR Master Mix (Bioline) as described earlier. Thermocycling was performed in the PCR instrument with the following conditions: 10 minutes initialisation at 95 °C, followed by 40 cycles of 1 minute’s denaturation at 95 °C, 1 minute annealing (primer set specific), 1 minute extension at 72 °C; and a 10 minutes final extension at 72 °C. The first step of the PCR procedure is denaturation of the double stranded DNA sample. Hydrogen bonds are disrupted at a temperature around 92 °C for 10 minutes and the double stranded DNA melts forming two single stranded DNA (Smith & Osborn 2009; Cripps 2006). The second step is the annealing of the primers targeting the specific sequences. As each primer has its own optimal temperature, the temperature during annealing stage is primer-specific. Annealing temperatures of 60 °C and 56 °C were used for the UN1 and UN2 primers, respectively. The third stage of the PCR reactions is extension. In this stage, the primer is extended along the single stranded DNA to produce two double stranded DNA. These three stages were repeated 40 times to theoretically achieve one billion copies of the target DNA sequence. Table 4.4 is a summary of conditions for the PCR reactions.

Table 4-4: Cycling conditions for the PCR reactions. Annealing temperature is primer set specific.

<table>
<thead>
<tr>
<th>Name of cycle</th>
<th>Time in minutes</th>
<th>Temperature in °C</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initialisation</td>
<td>10</td>
<td>95</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>1</td>
<td>95</td>
<td>}</td>
</tr>
<tr>
<td>Annealing</td>
<td>1</td>
<td>Primer set dependent</td>
<td>40</td>
</tr>
<tr>
<td>Elongation</td>
<td>1</td>
<td>72</td>
<td>}</td>
</tr>
<tr>
<td>Final elongation</td>
<td>10</td>
<td>72</td>
<td>1</td>
</tr>
</tbody>
</table>

Standard curves (logarithm of concentration versus PCR cycle number at which EvaGreen® (Biotium) fluorescence exceeded a threshold for each primer set) were generated using a fluorescence threshold in the middle of the exponential phase of PCR
amplification for each primer set. Fluorescence passes through a number of phases.
Initially, the fluorescence signal is below the detection limit of the sequence detector.
Then, it enters an exponential phase where PCR amplification is unrestricted. During this
phase, a plot of the logarithm of fluorescence versus cycle number should yield a straight
line and it is here that the signal threshold should be placed.

DNA concentrations for each primer set in each DNA extract were derived from the
relevant standard curve for that species. Microsoft Excel 2010 software was used to
calculate the regression line by calculating the best fit with the quantification standard data
points. The regression line formula has the form of

\[ C_t = m\log Q + b \]

Where \( C_t \) is cycle threshold, \( m \) is the slope, \( b \) is the y-intercept, and \( Q \) is the starting DNA
quantity. Figure 4.18 - 4.21 represent the standard curves of \( C_t \) values versus logarithm of
concentration that were used to quantify \( A. niger \) and \( P. rubrum \) to determine amplification
for the target fungal species.
Chapter 4: Methodology

Figure 4-18: Standard curve generated with UN2 primer set and *Aspergillus niger*. The lowest DNA concentration was 4.16 ng/µL.

Figure 4-19: Standard curve generated with UN1 primer sets and *Aspergillus niger*. The lowest DNA concentration was 4.16 ng/µL.
Chapter 4: Methodology

Figure 4-20: Standard curve generated with UN2 primer sets and *Penicillium rubrum*. The lowest DNA concentration was 2.38 ng/µL.

![Graph showing the standard curve generated with UN2 primer sets and *Penicillium rubrum*.](image)

Figure 4-21: Standard curve generated with UN1 primer sets and *Penicillium rubrum*. The lowest DNA concentration was 2.38 ng/µL.

![Graph showing the standard curve generated with UN1 primer sets and *Penicillium rubrum*.](image)
4.2.8 Statistical analysis

The data were analysed using a two-way analysis of variance (ANOVA) at a 95% confidence level for the quantification of mean concentrations of the fungi identified for the measurements of inhibition in the DNA extraction tests. The test was used to compare means of concentrations of the two fungi strains, which was deemed to be significantly different when the calculated $p$-value was smaller than 0.05. The two-way ANOVA was also used for comparing the results of folding endurance and tear resistance tests before and after artificial ageing. ANOVAs were performed using SPSS Statistics 21 (IBM).
5. RESULTS

5.1 Results of folding endurance test

5.1.1 Results of folding endurance test for the untreated papers and the papers treated with plant dyes

A folding endurance test was performed on both untreated and treated Japanese tissue papers before and after artificial ageing. A two way, between-groups analysis of variance was conducted at a 95% confidence level to determine whether there was a significant difference between folding endurance properties of the paper samples before and after ageing. A $p$-value of less than 0.05 indicates a statistically significant difference at a 95% confidence level. If the mean folding endurance of the paper post-ageing is greater than or equal to the mean folding endurance pre-ageing, the paper has good folding stability over time. If the mean folding endurance of the paper after ageing is less than the mean folding endurance before ageing, the paper is less able to tolerate folding over time.

Figure 5.1 compares the folding endurance of the untreated papers and the papers treated with plant dyes pre and post ageing. The results of the two-way ANOVA indicate that the untreated paper controls did not have any change in folding endurance before and after ageing. Most plant dyes resulted in an increase in folding endurance after ageing for the Yukyu-shi papers, but not for the Sekishu papers. Generally, plant dyes resulted in a dramatic decrease in folding endurance for the Sekishu papers both before and after ageing. There was a statistically significant decrease in folding endurance for the Sekishu paper treated with black tea after ageing ($M_{\text{diff}} = 525, 95\% \ CI [561.4, 36.4], \ p= 0.00$). Conversely, there was a statistically significant increase in folding endurance for the
Yukyu-shi paper treated with black tea ($M_{\text{diff}} = 230$, 95% CI [657, 887], $p = 0.02$). Henna significantly increased folding endurance for the Yukyu-shi paper ($M_{\text{diff}} = 706.3$, 95% CI [115.7, 822], $p = 0.00$). The Sekishu papers treated with henna had poor folding stability both pre and post ageing. The Yukyu-shi papers treated with both fresh and dried *Eucalyptus* leaves indicated a high folding endurance before and after ageing, relative to the effects of fresh and dried *Eucalyptus* leaves on the Sekishu papers before and after ageing.

![Figure 5-1: Folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes before and after artificial ageing (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.](image)

### 5.1.2 Results of folding endurance test for the untreated papers and the papers treated with watercolours

Figure 5.2 compares the folding endurance for the untreated Yukyu-shi and Sekishu papers and those that were treated with watercolours pre and post ageing. After ageing, there was a statistically significant reduction in folding endurance for the Sekishu papers treated with
alizarin crimson ($p = 0.00$), burnt umber ($p = 0.00$), cobalt blue ($p = 0.00$) and raw umber watercolour ($p = 0.01$). Conversely, there was a statistically significant increase in folding endurance for the Sekishu paper treated with raw sienna ($p = 0.00$) after ageing. The Sekishu papers treated with burnt sienna had a stable folding endurance.

There was a statistically significant reduction in folding endurance for the Yukyu-shi papers treated with burnt sienna ($p = 0.00$), raw sienna ($p = 0.00$) and raw umber ($p = 0.00$) after ageing. The Yukyu-shi papers treated with alizarin crimson, burnt umber and cobalt blue had a stable folding endurance. According to the results of the ANOVA test, raw umber watercolour resulted in decreases in folding endurance for both Yukyu-shi and Sekishu papers after ageing. Although treatment with raw sienna raised the folding endurance for the Sekishu paper, it lowered the endurance for the Yukyu-shi paper after ageing. The folding endurance for the untreated papers was higher than those for the papers treated with watercolours both pre and post ageing.
5.1.3 Results of folding endurance test for the untreated papers and the papers treated with acrylic paints

Figure 5.3 compares the folding endurance for the untreated Sekishu and Yukyu-shi papers and the papers treated with acrylic paints before and after ageing. There was a statistically significant increase in folding endurance for the Sekishu papers treated with burnt umber ($p = 0.00$) and raw umber ($p = 0.01$) acrylic paints after ageing. The Sekishu papers treated with alizarin crimson, burnt sienna, cobalt blue and raw sienna acrylic paints had a stable folding endurance. There were no statistically significant differences in folding endurance for any of the acrylic paints on the Yukyu-shi papers after ageing. Hence, the untreated Yukyu-shi papers and those that were treated with acrylic paints had a stable folding endurance.
Figure 5-3: Folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints before and after artificial ageing (outliers are indicated in the form of circles and asterisks in the graph). Error bars represent confidence intervals at 95% level.

Table 5.1 summarises ANOVA test and the statistically significant results in folding endurance for the Yukyu-shi and Sekishu papers treated with plant dyes, watercolours and acrylic paints before and after ageing.

Table 5-1: Treatments resulting in significant differences between folding endurance before and after ageing after a two-way ANOVA test (p ≤ 0.05). The folding endurances for these treatments were either significantly reduced (↓) or increased (↑) after ageing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant dyes</th>
<th>Watercolours</th>
<th>Acrylic paints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sekishu</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black tea (↓)</td>
<td></td>
<td>Alizarin (↓)</td>
<td>Burnt umber (↑)</td>
</tr>
<tr>
<td>Henna (↓)</td>
<td></td>
<td>Burnt umber (↓)</td>
<td>Raw umber (↑)</td>
</tr>
<tr>
<td>Burnt umber (↓)</td>
<td></td>
<td>Cobalt blue (↓)</td>
<td></td>
</tr>
<tr>
<td>Raw sienna (↑)</td>
<td></td>
<td>Raw umb (↓)</td>
<td></td>
</tr>
</tbody>
</table>

| **Yukyu-shi**   |            |              |               |
| No treatment    |            |              |               |
| Black tea (↑)   |            | Burnt sienna (↓) |
| Henna (↑)       |            | Raw sienna (↓) |
|                 |            | Raw umber (↓)  |
5.2 Results of tear resistance test

5.2.1 Results of tear resistance test for the untreated papers and the papers treated with plant dyes

A tear resistance test was performed on both untreated and treated Sekishu and Yukyu-shi papers before and after artificial ageing. A two way, between-groups analysis of variance was conducted at a 95% confidence level to determine whether there was a significant difference between tear resistance properties of the paper samples before and after ageing. A $p$-value of less than 0.05 indicates a statistically significant difference at a 95% confidence level. If the mean tear resistance of the paper post-ageing is greater than or equal to the mean tear resistance pre-ageing, the paper is stable against tearing over time. If the mean tear resistance of the paper after ageing is less than the mean tear resistance before ageing, the paper is not able to tolerate tearing force over time.

Figure 5.4 compares the tear resistance of the untreated papers and the papers treated with plant dyes before and after ageing. There was a statistically significant increase in tear resistance for the Sekishu paper treated with black tea after ageing ($M_{\text{diff}} = 142.85$, 95% CI [886.6, 1029.5], $p = 0.02$). Conversely, there was a statistically significant decrease in tear resistance for the Sekishu paper treated with henna after ageing ($M_{\text{diff}} = 189.4$, 95% CI [660.8, 471.4], $p = 0.01$). The Sekishu papers treated with both fresh and dried Eucalyptus leaves had a stable tear resistance.

There was a statistically significant increase in tear resistance for the untreated Yukyu-shi paper after ageing ($M_{\text{diff}} = 289$, 95% CI [596.1, 885.1], $p = 0.00$) compared with pre-ageing. There was a statistically significant increase in tear resistance for the Yukyu-shi
paper treated with black tea after ageing ($M_{\text{diff}} = 176$, 95% CI $[549.2, 725.3]$, $p = 0.00$).

Conversely, there was a statistically significant decrease in tear resistance for the Yukyu-shi paper treated with henna after ageing ($M_{\text{diff}} = 262.1$, 95% CI $[528.9, 266.7]$, $p = 0.00$).

The Yukyu-shi papers treated with both fresh and dried *Eucalyptus* leaves had a stable tear resistance. The findings of the ANOVA indicate that both Sekishu and Yukyu-shi papers treated with black tea showed an increase in tear resistance after ageing, relative to the papers treated with henna, which indicated a decrease in tear resistance after ageing.

Figure 5-4: Tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes before and after artificial ageing. Error bars represent confidence intervals at 95% level.

### 5.2.2 Results of tear resistance test for the untreated papers and the papers treated with watercolours

Figure 5.5 shows the interaction effects of time and treatment with watercolours on the Yukyu-shi and Sekishu papers. There were no statistically significant differences in tear resistance for any of the watercolours on the Sekishu papers after ageing. There was a
statistically significant increase in tear resistance for the Yukyu-shi paper treated with alizarin crimson watercolour after ageing ($M_{\text{diff}} = 318.9$, 95% CI [466.1, 785], $p = 0.00$). There were no statistically significant differences in tear resistance for the Yukyu-shi papers treated with the other watercolours after ageing.

Figure 5.5: Tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours before and after artificial ageing. Error bars represent confidence intervals at 95% level.

5.2.3 Results of tear resistance test for the untreated papers and the papers treated with acrylic paints

Figure 5.6 shows the interaction effects of time and treatment with acrylic paints on the Yukyu-shi and Sekishu papers. There was a statistically significant increase in tear resistance for the Sekishu paper treated with burnt sienna and alizarin crimson acrylic paints after ageing ($M_{\text{diff}} = 544.8$, 95% CI [994.4, 1539.2], $p = 0.02$) and ($M_{\text{diff}} = 344.8$, 95% CI [1165.05, 1499.85], $p = 0.05$) respectively. There were no statistically significant differences in tear resistance for burnt umber, cobalt blue, raw sienna and raw umber
acrylic paints on the Sekishu papers after ageing. The Yukyu-shi papers treated with all acrylic paints had a stable tear resistance.

![Figure 5-6: Tear resistance of untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints before and after artificial ageing. Error bars represent confidence intervals at 95% level.](image)

Table 5.2 summarises the ANOVA test and the statistically significant results in tear resistance for the Yukyu-shi and Sekishu papers treated with plant dyes, watercolours and acrylic paints before and after ageing.

Table 5.2: Treatments resulting in significant differences between tear resistance before and after ageing after a two-way ANOVA test ($p \leq 0.05$). The tear resistance for these treatments were either significantly reduced (↓) or increased (↑) after ageing.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No treatment</th>
<th>Plant dyes</th>
<th>Watercolours</th>
<th>Acrylic paints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sekishu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (↑)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black tea (↑)</td>
<td></td>
<td></td>
<td></td>
<td>Alizarin (↑)</td>
</tr>
<tr>
<td>Henna (↓)</td>
<td></td>
<td></td>
<td></td>
<td>Burnt sienna (↑)</td>
</tr>
<tr>
<td><strong>Yukyu-shi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (↑)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black tea (↑)</td>
<td></td>
<td></td>
<td></td>
<td>Alizarin (↑)</td>
</tr>
<tr>
<td>Henna (↓)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

126
5.3 Results of colour change

The colour changes of both untreated papers and those papers that were treated with plant dyes, watercolours and acrylic paints were measured using spectrophotometry method. Appendix 3 shows the lightness, redness-greenness, and yellowness-blueness values of the paper samples, demonstrated by \( L^* \), \( a^* \), \( b^* \) respectively before and after ageing. It also provides the total mean in lightness, redness-greenness, yellowness-blueness and colour change values according to CIELAB equation, demonstrated by \( \Delta L^* \), \( \Delta a^* \), \( \Delta b^* \), and \( \Delta E^* \) respectively. A Multivariate ANOVA test was conducted at a 95% confidence level \( (p < 0.05) \) to determine whether there was a significant difference in colour change for the paper samples after subjecting to moist heat artificial ageing.

5.3.1 Results of colour change on the untreated papers and the papers treated with plant dyes

Figure 5.7 shows the colour changes on the untreated Sekishu and Yukyu-shi papers relative to those treated with plant dyes after moist-heat ageing. In general, plant dyes caused higher colour changes on the papers relative to their untreated counterparts. There were significantly larger colour changes on the Sekishu papers treated with black tea \( (p = 0.00) \), fresh eucalyptus leaves \( (p = 0.00) \) and henna \( (p = 0.00) \) compared with the Yukyu-shi papers. Conversely, there was a significantly smaller colour change on the Sekishu paper treated with dried eucalyptus leaves \( (p = 0.03) \), relative to the ones recorded for the Yukyu-shi paper.

Further observations were made by considering the variations in the colourimetric coordinates \( L^* \), \( a^* \) and \( b^* \) as demonstrated in Appendix 3 and show the chromatic direction
of the colour change induced by artificial ageing. All dyed paper specimens showed a reduction in lightness ($\Delta L^* < 0$) after artificial ageing and they became noticeably darker. Colour changes recorded for the Sekishu and Yukyu-shi papers treated with dried and fresh eucalyptus leaves were characterised by increases in redness ($\Delta a^*$) and yellowness ($\Delta b^*$). Both Sekishu and Yukyu-shi papers treated with black tea exhibited a relatively large colour change, which was due to darkening and a reduction in yellowness and increase in redness. Treatment with henna resulted in a smaller colour change on the papers than black tea and dried eucalyptus leaves due to darkening and reduction in both yellowness and redness.

Figure 5-7: Colour change shifts on untreated papers (Sekishu and Yukyu-shi) and papers treated with plant dyes (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.
5.3.2 Results of colour change on the untreated papers and the papers treated with watercolours

Figure 5.8 compares colour changes observed for the untreated Yukyu-shi and Sekishu papers with those treated with watercolours. The papers treated with watercolours showed smaller colour changes relative to those treated with plant dyes. Larger colour changes were observed for the Yukyu-shi papers treated with alizarin crimson \( (p = 0.02) \), burnt sienna \( (p = 0.01) \), burnt umber \( (p = 0.00) \), and raw sienna \( (p = 0.00) \) compared with the Sekishu papers. By considering the variations in the \( L^\ast, a^\ast \) and \( b^\ast \) values in Appendix 3, it can be observed that the larger colour change for the Yukyu-shi papers treated with alizarin crimson was due to a reduction in lightness and increase in redness. The Yukyu-shi papers treated with burnt sienna experienced a reduction in lightness, redness and yellowness and the Yukyu-shi papers treated with burnt umber and raw sienna were darker after artificial ageing.
Figure 5-8: Colour change shifts on untreated papers (Sekishu and Yukyu-shi) and papers treated with watercolours (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.

5.3.3 Results of colour change on the untreated papers and the papers treated with acrylic paints

Figure 5.9 compares the changes experienced by the untreated Sekishu and Yukyu-shi papers with those treated with acrylic paints. Larger colour changes were recorded for the Sekishu papers. The papers treated with both watercolours and acrylic paints showed less colour changes than the samples that were treated with plant dyes. There were significantly greater colour changes on the Sekishu papers treated with burnt sienna \((p = 0.00)\), burnt umber \((p = 0.00)\) and raw umber \((p = 0.02)\) relative to the Yukyu-shi papers. Nevertheless, no significant colour changes were observed after comparing the Yukyu-shi and Sekishu papers treated with alizarin crimson, cobalt blue and raw sienna. The variations in the \(L^*\), \(a^*\) and \(b^*\) values in Appendix 3 show that the larger colour change for the Sekishu papers
treated with burnt umber was due to an increase in lightness and a small reduction in redness and yellowness. The Sekishu papers treated with burnt sienna experienced a reduction in redness and yellowness, whereas the Sekishu papers treated with raw umber had an increase in lightness and yellowness.

Figure 5-9: Colour change shifts on untreated papers (Sekishu and Yukyu-shi) and papers treated with acrylic paints (outliers are indicated in the form of asterisks in the graph). Error bars represent confidence intervals at 95% level.

5.4 Results of microfading tests

Table 5.3 shows a summary of colour change for BW1, BW2, BW3, the untreated Yukyu-shi and Sekishu papers and those papers that were treated with plant dyes, a watercolour and an acrylic paint of alizarin crimson.
Table 5-3: Colour change summary (total exposing over 10 minute fading run is approximately 1mlx hour).

<table>
<thead>
<tr>
<th>Colour</th>
<th>CIELAB(CIE76)</th>
<th>CIE2000</th>
<th>ΔL</th>
<th>Δa</th>
<th>Δb</th>
<th>Δc</th>
<th>Δh</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW1</td>
<td></td>
<td></td>
<td>11.5</td>
<td>4.8</td>
<td>-3.8</td>
<td>10.6</td>
<td>-10</td>
</tr>
<tr>
<td>BW2</td>
<td></td>
<td></td>
<td>5.9</td>
<td>1.7</td>
<td>-1.3</td>
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<td>-5.6</td>
</tr>
<tr>
<td>BW3</td>
<td></td>
<td></td>
<td>2.2</td>
<td>0.5</td>
<td>-1.2</td>
<td>1.8</td>
<td>-2.1</td>
</tr>
<tr>
<td>BW4</td>
<td></td>
<td></td>
<td>0.7</td>
<td>-0.1</td>
<td>0.9</td>
<td>0.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>Yukyu-shi treated with black tea</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sekishu treated with black tea</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.4</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yukyu-shi treated with fresh E. leaves</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.9</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sekishu treated with fresh E. leaves</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.8</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yukyu-shi treated with dried E. leaves</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>4</td>
<td>0.7</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
</tr>
<tr>
<td>Sekishu treated with dried E. leaves</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.9</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yukyu-shi treated with henna</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.5</td>
<td>1.5</td>
<td></td>
<td></td>
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<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yukyu-shi treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3.9</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>4</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>4</td>
<td>0.4</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>3</td>
<td>0.5</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>0.5</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>0.3</td>
<td>0.0</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
</tr>
<tr>
<td>Sekishu treated with alizarin</td>
<td>BW4-BW3</td>
<td>BW3-BW2</td>
<td>0.3</td>
<td>0.0</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
<td>&gt;BW3</td>
</tr>
</tbody>
</table>

5.4.1 Microfading tests on the untreated papers

Figure 5.10 shows microfading test results obtained for BW1, BW2 and BW3 as well as untreated Yukyu-shi and Sekishu paper samples. Colour changes recorded for BW1, BW2 and BW3 after 10 minutes of exposing to a 10 mega lux illuminated spot were 4.8, 1.7 and 0.5 respectively. Equivalent areas of untreated Yukyu-shi and Sekishu papers were also

132
tested to compare the colour change rates. The untreated Yukyu-shi paper specimens showed fading rates between BW2 and BW3 with greater tendency towards the latter. Colour changes recorded for this paper were dominated by reduction in the yellowness parameter and a slight increase in lightness and redness after 10 minutes exposure to an intensive light. The untreated Sekishu paper indicated fading rates between BW2 and BW3 with greater tendency towards the former. The paper experienced an increase in redness and a decrease in yellowness as well as a decrease in lightness, relative to the untreated Yukyu-shi paper, which suggests that the MFT produces a bleaching effect on the Yukyu-shi paper.

![Microfading curves](image)

Figure 5-10: Microfading curves obtained for BW1, BW2 and BW3 as well as untreated Yukyu-shi and Sekishu papers after 10 minutes exposure to an intensive light source.
5.4.2 Microfading tests on the treated papers

Microfading test results for Yukyu-shi and Sekishu papers treated with black tea and henna are presented in Figure 5.11. Both Yukyu-shi and Sekishu papers treated with black tea exhibited a colour change profile over ten minutes of exposure that was similar to BW3. The small colour change was due to some darkening and a slight reduction in yellowness and redness. On the other hand, treatment with henna has resulted in no more or less colour change for both Yukyu-shi and Sekishu papers than for the untreated papers. The Yukyu-shi and Sekishu paper samples treated with henna displayed colour change profiles between BW2 and BW3 caused by a reduction in lightness, yellowness and redness.

![Microfading curves](image)

Figure 5-11: Microfading curves obtained for BW1, BW2 and BW3 as well as the Yukyu-shi and Sekishu papers treated with black tea and henna after 10 minutes exposure to an intensive light source. There is a missing data point for the Yukyu-shi paper treated with henna at 3 minutes.
Figure 5.12 shows microfading test results obtained for the Yukyu-shi and Sekishu papers treated with fresh and dried Eucalyptus leaves. The fading rates of the paper samples treated with fresh eucalyptus leaves were located between BW2 and BW3 with greater tendency towards the latter. Colour changes recorded for the Yukyu-shi and Sekishu papers treated with fresh eucalyptus leaves were dominated by a reduction in lightness and yellowness parameters with no change in redness. The Yukyu-shi paper treated with dried eucalyptus leaves showed a colour change profile similar to BW3. The small colour change was due to a slight reduction in lightness and yellowness. The Sekishu paper treated with dried eucalyptus leaves resulted in slight reduction of yellowness but no redness and lightness changes.

Figure 5-12: Microfading curves obtained for BW1, BW2 and BW3 as well as the Yukyu-shi and Sekishu papers treated with fresh and dried Eucalyptus leaves after 10 minutes exposure to an intensive light source.
Microfading test results for the Yukyu-shi and Sekishu papers treated with alizarin crimson watercolour and acrylic paint are demonstrated in Figure 5.13. The Yukyu-shi and Sekishu papers treated with alizarin crimson watercolour were located between BW2 and BW3 with greater tendency towards the latter. Both Yukyu-shi and Sekishu papers treated with alizarin crimson watercolour showed less redness and more yellowness after a 10-minute exposure. The colour change for the Sekishu and Yukyu-shi papers treated with alizarin crimson acrylic paint were slightly under BW3. The small colour change was due to an increase in yellowness with no change in lightness. The Sekishu paper treated with alizarin crimson acrylic paint had a reduction in redness.

![Figure 5-13: Microfading curves obtained for BW1, BW2 and BW3 as well as the Yukyu-shi and Sekishu papers treated with watercolour and acrylic paint of alizarin crimson after 10 minutes exposure to an intensive light source.](image-url)
5.5 Results of pH test

5.5.1 Results of pH test on the untreated papers and the papers treated with plant dyes

Figure 5.14 compares the pH of the untreated Sekishu and Yukyu-shi papers and the papers treated with plant dyes before and after artificial ageing. The untreated Sekishu and Yukyu-shi papers indicated higher pH than those papers that were treated with plant dyes both before and after ageing. All paper samples treated with plant dyes were slightly below neutral pH before ageing, with the exception of the Yukyu-shi and Sekishu papers treated with fresh Eucalyptus leaves, which had pHs of 7.4, 7.5 respectively. The effect of ageing resulted in an increase in pH for the Sekishu and Yukyu-shi papers treated with plant dyes after ageing, with the exception of the Yukyu-shi and Sekishu papers treated with dried Eucalyptus leaves, which had pHs of 6.7 and 6.8 respectively which show a reduction in pH.
5.5.2 Results of pH test on the untreated papers and the papers treated with watercolours

Figure 5.15 compares the pH of the untreated Yukyu-shi and Sekishu papers and the papers treated with watercolours before and after ageing. The Sekishu and Yukyu-shi papers treated with watercolours were strongly associated with high pH reserve and they had greater pH than the papers treated with plant dyes. Before ageing, the Sekishu and Yukyu-shi papers treated with watercolours had higher pH than the untreated paper controls with a pH range of 7.5-8.7 and 7.2-7.3 respectively. Watercolours make both Sekishu and Yukyu-shi papers alkaline after ageing with a pH range of 8.2-9.
5.5.3 Results of pH test on the untreated papers and the papers treated with acrylic paints

Figure 5.16 presents the pH of the untreated Yukyu-shi and Sekishu papers and the papers treated with acrylic paints before and after ageing. As for the watercolours, the papers treated with acrylic paints showed greater pH than those samples that were treated with plant dyes. Before ageing, the Sekishu and Yukyu-shi papers treated with acrylic paints had greater pH than the untreated paper controls. The Sekishu and Yukyu-shi papers
treated with acrylic paints were alkaline both pre and post ageing with a pH range of 7.6-8.4 and 7.4-8.4 respectively.

5.6 Results of fungal growth tests

5.6.1 Results of fungal growth on the untreated papers (PCR was performed with the UN1 universal primer pair)

DNA was extracted effectively from the papers using the standard phenol/chloroform procedure with ethanol precipitation compared with the QIAamp® Mini Kit, which
resulted in lower amount of DNA extracts. Further, using the additional purification step (centrifugal filter units) caused a lower concentration of DNA. DNA extractions of artificially inoculated paper samples were tested effectively via fungal specific PCR amplification using UN1 and UN2 universal primer pairs with a minimum amplification from representative of non-fungal groups. Due to lower number of negative paper controls, the results of them were ignored in the statistical analyses (one negative paper sample versus three positive paper specimens). A two-way between-groups analysis of variance was used to determine whether there were any significant differences between the mean DNA concentrations of the three groups of treatment on the papers after incubation. A $p$-value of 0.05 was used to indicate significance (with 95% confidence).

Figure 5.17 presents the results of the DNA concentrations with the UN1 primer pair recovered from the two untreated papers (Yukyu-shi and Sekishu) and Whatman® Filter Paper control at the time of inoculation with *A. niger* and *P. rubrum* and then 10 days after incubation in an artificial ageing environment. The findings of the UN1 primers confirm that there was no statistically significant difference between the DNA concentrations on the untreated Sekishu paper and the Whatman® Filter Paper control. Conversely, there was a statistically significant increase in DNA concentrations on the untreated Yukyu-shi papers after inoculation with both *A. niger* and *P. rubrum*, relative to the untreated Sekishu papers [$p = 0.003$ and 0.01 respectively] and Whatman® Filter Paper control [$p = 0.00$ and 0.002 respectively]. The DNA concentrations attributable to *A. niger* and *P. rubrum* were observed on the Yukyu-shi paper at the time of inoculation (day 0) and also with higher concentrations after 10 days of incubation. The Whatman® Filter Paper control did not show a significant change in DNA concentration after 10 days of incubation compared with the time of inoculation.
Figure 5-17: Concentrations of DNA recovered from untreated papers (Yukyu-shi, Whatman and Sekishu) at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).

5.6.2 Results of fungal growth on the untreated papers and the papers treated with plant dyes (PCR was performed with the UN1 universal primer pair)

Figure 5.18 shows the DNA concentrations recovered from the two untreated papers (Yukyu-shi and Sekishu) and the papers treated with plant dyes at the time of inoculation with *A. niger* and *P. rubrum* (day 0) and then after 10 days of incubation in an artificial ageing environment. There was a statistically significant increase in DNA concentrations on the Yukyu-shi papers treated with dried *Eucalyptus* leaves after inoculation and incubation with *A. niger* (*p* = 0.002) compared with the Sekishu papers. The Yukyu-shi papers treated with black tea and henna showed statistically lower DNA concentrations.
after inoculation and incubation with *A. niger* \( p = 0.00 \) and \( p = 0.00 \) respectively] than the untreated Yukyu-shi papers. There were no statistically significant differences in DNA concentrations for the Sekishu papers treated with plant dyes after inoculation and incubation with *P. rubrum*. The Yukyu-shi papers treated with black tea \( p = 0.003 \), henna \( p = 0.002 \) and fresh *Eucalyptus* leaves \( p = 0.007 \) had statistically lower DNA concentrations than the untreated Yukyu-shi papers after inoculation and incubation with *P. rubrum*.

**Figure 5-18:** Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).
5.6.3 Results of fungal growth on the untreated papers and the papers treated with watercolours (PCR was performed with the UN1 universal primer pair)

The DNA concentrations on the untreated Sekishu and Yukyu-shi papers and the papers treated with watercolours after inoculation and incubation with *A. niger* and *P. rubrum* are presented in Figure 5.19. There was a statistically significant increase in DNA concentrations on the Sekishu papers treated with alizarin crimson (*p* = 0.001) after inoculation and incubation with *A. niger* compared with the untreated Sekishu papers and the Sekishu papers treated with the other watercolours. The untreated Yukyu-shi papers had significantly greater DNA concentrations (*p* = 0.00) than the Yukyu-shi papers treated with watercolours after inoculation and incubation with *A. niger*.

There were no statistically significant differences in DNA concentrations on the untreated Sekishu papers compared with the Sekishu papers treated with watercolours after inoculation and incubation with *P. rubrum*. The untreated Yukyu-shi papers had greater DNA concentrations from *P. rubrum* than the Yukyu-shi papers treated with watercolours after incubation.
Figure 5.19: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).

5.6.4 Results of fungal growth on the untreated papers and the papers treated with acrylic paints (*PCR was performed with the UN1 universal primer pair*)

Figure 5.20 compares the DNA concentrations on the untreated Yukyu-shi and Sekishu papers and the papers treated with acrylic paints after inoculation and incubation with *A. niger* and *P. rubrum* at the time of inoculation (day 0) and then after 10 days of incubation in an artificial ageing environment. The untreated Sekishu papers and the Sekishu papers treated with acrylic paints showed lower DNA concentrations from *A. niger* compared with the Yukyu-shi papers. There was a statistically significant decrease in DNA concentrations on the Yukyu-shi papers treated with alizarin crimson (*p* = 0.00), burnt
sienna ($p = 0.00$) and raw sienna ($p = 0.00$) acrylic paints after inoculation and incubation with *A. niger* compared with the untreated Yukyu-shi papers. Conversely, the Yukyu-shi papers treated with burnt umber, cobalt blue and raw umber had greater DNA concentrations from *A. niger* after incubation.

There were no statistically significant differences in the mean of DNA concentrations on the untreated Sekishu papers and the Sekishu papers treated with acrylic paints after inoculation and incubation with *P. rubrum*. There was a statistically significant decrease in DNA concentrations on the Yukyu-shi papers treated with alizarin crimson ($p = 0.00$), burnt sienna ($p = 0.002$), cobalt blue ($p = 0.004$), raw sienna ($p = 0.00$) and raw umber ($p = 0.04$) acrylic paints compared with the untreated Yukyu-shi papers after inoculation and incubation with *P. rubrum*. Conversely, the Yukyu-shi papers treated with burnt umber showed greater concentrations of DNA from *P. rubrum* after incubation.
Figure 5.20: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).

5.6.5 Results of fungal growth on the untreated papers (PCR was performed with the UN2 universal primer pair)

Figure 5.21 compares the DNA concentrations recovered from the two untreated papers (Yukyu-shi and Sekishu) and Whatman® Cellulose Filter Paper control at the time of inoculation with *A. niger* and *P. rubrum* (day 0) and then after 10 days of incubation.

There were no statistically significant differences in DNA concentrations on the untreated Sekishu papers and the Whatman® Filter Paper control. Conversely, there was a statistically significant increase in DNA concentrations on the untreated Yukyu-shi papers after inoculation with both *A. niger* and *P. rubrum*, relative to the untreated Sekishu papers $[p = 0.02$ and 0.02, respectively] and Whatman® Filter Paper control $[p = 0.001$ and 0.001, respectively]. The Whatman® Filter Paper control was a good *P. rubrum* growth inhibitor.
from an initially high DNA concentrations at day 0 compared with the low DNA concentrations at day 10.

Figure 5-21: Concentrations of DNA recovered from untreated papers (Yukyu-shi, Whatman and Sekishu) at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).

**5.6.6 Results of fungal growth on the untreated papers and the papers treated with plant dyes (PCR was performed with the UN2 universal primer pair)**

The mean DNA concentrations on the untreated Yukyu-shi and Sekishu papers and the papers treated with plant dyes at the time of inoculation with *A. niger* and *P. rubrum* and then after 10 days of incubation in an artificial ageing environment are presented in Figure 5.22. There were no statistically significant differences between the mean DNA
concentrations on the Sekishu papers treated with plant dyes after inoculation and incubation with *A. niger*, relative to the untreated Sekishu papers. Both Sekishu and Yukyu-shi papers treated with plant dyes showed lower concentrations of DNA from *P. rubrum* than from *A. niger* after incubation.

There was a statistically significant decrease in DNA concentrations on the Yukyu-shi papers treated with dried *Eucalyptus* leaves (*p* = 0.02) and black tea (*p* = 0.01) after inoculation and incubation with *A. niger*, relative to the untreated Yukyu-shi papers. There were no statistically significant differences between the mean DNA concentrations on the untreated Sekishu papers and the papers treated with plant dyes after inoculation and incubation with *P. rubrum*. Conversely, the Yukyu-shi papers treated with all plant dyes had lower DNA concentrations after inoculation and incubation with *P. rubrum* compared with the untreated Yukyu-shi papers.
Chapter 5: Results

5.6.7 Results of fungal growth on the untreated papers and the papers treated with watercolours (PCR was performed with the UN2 universal primer pair)

Figure 5.23 compares the DNA concentrations on the untreated Yukyu-shi and Sekishu papers and the papers treated with watercolours at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* and then after 10 days of incubation. There were no statistically significant differences between the mean DNA concentrations on the Sekishu papers treated with watercolours and the untreated Sekishu papers after inoculation and incubation with either *A. niger* or *P. rubrum*. Treatment with raw sienna resulted in a lower concentration of DNA from *A. niger* on the Sekishu papers than on the Yukyu-shi papers. The untreated Yukyu-shi papers and the Yukyu-shi papers treated with watercolours resulted in greater
concentrations of DNA from *A. niger* after incubation compared with *P. rubrum*. The untreated Yukyu-shi papers had significantly greater DNA concentrations (*p* = 0.00) than the Yukyu-shi papers treated with watercolours after inoculation and incubation with *P. rubrum*. Treatments with burnt umber resulted in greater concentrations of DNA from *P. rubrum* on the Sekishu papers after incubation when compared to the Yukyu-shi papers.

Figure 5.23: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with watercolours at the time of inoculation with *Aspergillus niger* and *Penicillium rubrum* (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).

### 5.6.8 Results of fungal growth on the untreated papers and the papers treated with acrylic paints (*PCR was performed with the UN2 universal primer pair*)

Figure 5.24 compares the DNA concentrations on the Sekishu and Yukyu-shi papers treated with acrylic paints and inoculated with *A. niger* and *P. rubrum* at the two times of
measurements (day 0 and day 10). The Yukyu-shi papers treated with burnt umber, raw umber and cobalt blue showed greater concentrations of DNA from \textit{A. niger} than for the other treatments. There were no statistically significant differences between the DNA concentrations on the untreated Sekishu papers and the papers treated with acrylic paints after inoculation with both \textit{A. niger} and \textit{P. rubrum}.

PCR amplification with the UN2 primers indicate that the untreated Sekishu and Yukyu-shi papers resulted in lower concentrations of DNA from \textit{P. rubrum}, relative to \textit{A. niger} after incubation. The untreated Yukyu-shi papers had greater DNA concentrations after inoculation and incubation with \textit{P. rubrum} than the Yukyu-shi papers treated with acrylic paints.

![Figure 5-24: Concentrations of DNA recovered from untreated papers (Yukyu-shi and Sekishu) and papers treated with acrylic paints at the time of inoculation with \textit{Aspergillus niger} and \textit{Penicillium rubrum} (day 0) and after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair (outliers are presented in the form of asterisks in the graph and error bars represent confidence intervals at 95% level).]({})
The results of the DNA concentrations from the two universal primer pairs (UN1 and UN2) are summarised in Table 5.4. Treatments include significantly higher (↑) or lower (↓) concentrations of DNA than the other treatments, $p < 0.05$.

Table 5-4: Summary of the two-way ANOVA significant differences (treatments resulting in significantly higher (↑) or lower (↓) concentrations of DNA than other treatments after 10 days of incubation at 27 °C and 80% RH). ($p < 0.05$).

<table>
<thead>
<tr>
<th>Paper</th>
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<th>Primers and fungal species</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td><strong>UN1 primer pair</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>A. niger</strong></td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
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</tr>
<tr>
<td>Plant dyestuff</td>
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</tr>
<tr>
<td>Water colours</td>
<td>Alizarin crimson (↑)</td>
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</tr>
<tr>
<td>Acrylic paints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>Untreated (↑)</td>
<td>Untreated (↑)</td>
</tr>
<tr>
<td>Dried Eucalyptus leaves (↑)</td>
<td>Fresh Eucalyptus leaves (↓)</td>
<td>Dried Eucalyptus leaves (↓)</td>
</tr>
<tr>
<td>Black tea (↓)</td>
<td>Black tea (↓)</td>
<td>Henna (↓)</td>
</tr>
<tr>
<td>Yukyu-shi</td>
<td>Water colours</td>
<td>Alizarin crimson (↓)</td>
</tr>
<tr>
<td>Water colours</td>
<td></td>
<td>Alizarin crimson (↓)</td>
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<tr>
<td>Acrylic paints</td>
<td></td>
<td>Burnt sienna (↓)</td>
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</tbody>
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6. Discussion

6.1 Introduction

Various dyes and pigments are used for colouring Japanese tissue papers in paper conservation, as discussed earlier; however, the physical stability of treated papers and the fungal bioreceptivity of these dyes and pigments is still an underestimated concern. In this study, two Japanese tissue papers (Yukyu-shi and Sekishu) were treated with selected plant dyes, watercolours and acrylic paints. Artificial ageing was used to provide information on whether conservation treatments improve the quality and ageing behaviour of papers over time. Physical experiments (folding endurance, tear resistance, colour change) and chemical tests (pH) were used to investigate the paper degradation mechanisms to achieve a better understanding of how paper deteriorates as a result of artificial ageing. Further, colour changes were measured using accelerated photoageing. The papers treated with plant dyes exhibited pH slightly below neutral than those that were treated with watercolours and acrylic paints. The dyed papers generally were also subject to more colour change than the papers treated with watercolours and acrylic paints. Further, plant dyes result in less folding endurance and tear resistance for the Sekishu papers than watercolours and acrylic paints.

In order to understand more about the fungal bioreceptivity of dyes and pigments, both untreated and treated paper samples were inoculated with A. niger and P. rubrum fungal strains and incubated in an artificial ageing environment. Two universal PCR primers amplified both A. niger and P. rubrum and the Sekishu papers were found to have better antifungal properties than the Yukyu-shi papers.
Almost all of the plant dyed papers tested in this work showed some degree of fading after exposure to an artificial ageing environment. By contrast, synthetic artists’ pigments were relatively stable to colour change. Finally, most plant dyes caused poor folding endurance and tear resistance for the Sekishu and Yukyu-shi papers compared with watercolours and acrylic paints. This might be related to the fact that they make treated papers more acidic.

This chapter will further discuss the findings obtained in Chapter 5 (Results) as well as the findings of the survey of paper conservation practitioners (Chapter 3) to better understand the long-term effects of treating the selected Japanese tissue papers with different dyes and pigments.

### 6.2 The effects of acidity on paper

The pH is the most significant factor in determining the permanence and colour fastness of paper objects (Area & Cheradame 2011). As shown in Figure 5.14, the Yukyu-shi and Sekishu papers treated with plant dyes have lower pH than the untreated paper controls and the papers treated with watercolours and acrylic paints. However, before ageing, the Yukyu-shi and Sekishu papers treated with fresh *Eucalyptus* leaves had pHs of 7.4 and 7.5 respectively and post ageing, they had pHs of 7.9 and 7.7 respectively. A comparison of Figures 5.15 and 5.16 indicates that the paper samples treated with watercolours and acrylic paints were alkaline both pre and post artificial ageing, with pH ranges of 7.2 – 9.

A paper sample with a low pH (high acidity) will show signs of deterioration in its appearance via discolouration after ageing (Stephens et al. 2008; Strlic & Kolar 2004). The acidic structure of plant dyes can promote paper degradation by reducing its degree of
polymerisation (Area & Cheradame 2011). The acidic structure of some plant dyes including henna, dried *Eucalyptus* leaves and black tea may have been the predominant property affecting the long-term stability of the treated Japanese tissue papers in this study. When the pH was alkaline, as for papers treated with watercolours and acrylics, paper specimens only showed a small colour change. However, when the pH was acidic, as occurred after treatment with plant dyes, paper samples exhibited a large colour change. It is suggested that the colour change was probably higher in low pH specimens because the acid present catalyses hydrolysis of the cellulose (Stephens et al. 2008). Colour change is often a sign of the chemical degradation of the cellulose of the paper. This study confirms that colour change values are strongly associated with pH, and that almost all plant-dyed paper specimens were acidic. The relationship between pH and the long-term stability of paper samples found here confirms the findings of Barrow and Sproull (1959) about correlation between the acidity of papers and loss of mechanical strength.

Understanding the mechanisms which affect the long-term stability of paper objects is important in identifying papers that might be at risk of rapid deterioration. This knowledge can therefore assist in the development of appropriate conservation treatments to increase the stability of papers over time. High pH paper specimens appear to be associated with positive physical properties (high folding endurance and high tear resistance) and appearance (low colour change) which favour the stability of papers over time. Low pH paper specimens showed negative signs in conservation terms including greater colour change, as well as physical signs of degradation including low folding endurance and tear resistance. This study indicates that pH may be a major factor affecting the long-term stability of these paper samples. The following sections detail the association of pH with folding endurance, tear resistance, colour change and fungal growth.
6.3 Folding endurance

The arrangements of paper fibres are important in paper-making methods; they affect the reactions that occur as a result of water absorption. Water plays a manifold role in paper deterioration. It is a prerequisite reactant in acidic hydrolysis, expands the cellulose fibres and increases the availability of the paper surface for further reactions (Zervos 2010). Folding endurance is greatly affected by fluctuations in RH (Caulfield & Gunderson 1988). As discussed earlier, the high temperature and humidity inside the artificial ageing chamber would be expected to chemically degrade paper materials through hydrolysis or oxidative reactions which cause abruption of cellulose bonds and scission of inter-fibre bonds (Feller 1994). Further, high humidity inside the ageing chamber can physically react with paper materials and cause the loss of hydrogen bonds between the substrate, pigments and vehicle.

As shown in Figure 5.14, artificial ageing makes the untreated Yukyu-shi and Sekishu paper controls more alkaline after ageing (8.7 and 8.2 respectively) compared to before ageing (7.3 and 7.2 respectively). The folding endurance of the untreated Yukyu-shi and Sekishu papers after ageing was no better than their folding endurance before ageing. This means that it is not pH alone that affects folding endurance as it might be expected that more acidic, pre-aged paper would have reduced folding endurance. However, treatment with some plant dyes increased paper acidity to pH < 7. A general concern with the application of plant dyes in paper conservation is their acidic composition as it has been shown that the acidity and moisture content of paper accelerate paper deterioration (Zou, Uesaka & Gurnagul 1996). The relationship between pH and folding endurance is shown in Figure 6.1. Most plant dyes make both Sekishu and Yukyu-shi papers more acidic than
the untreated paper controls before ageing while watercolours and acrylic paints make the papers more alkaline before ageing. Ageing has the effect of making most papers, treated and untreated, more alkaline. After ageing, the Sekishu papers treated with plant dyes have lower folding endurance than the Sekishu papers treated with watercolours and acrylic paints. The effect of treatment on the Yukyu-shi papers is more complex. According to Figure 5.1, before ageing, henna lowered the folding endurance of these papers, relative to other treatments.

The acidic compounds of plant dyes may also prevent the formation of hydrogen bonds among the cellulose fibres (Reyden 1992). As a result, paper conservators sometimes use alkaline materials such as calcium carbonate to raise the pH of the plant dyes before using them for toning papers (Winter 2008). However, this treatment may affect the final tonality of the treated paper and increase the yellowness index on the paper over time (Area & Cheradame 2011). The significant role of alkaline reserve in paper for slowing the deterioration processes induced by artificial ageing was also confirmed in a study by Havlínová et al. (2009).

The folding endurance for the untreated paper controls was higher than those for the papers treated with watercolours both pre and post ageing as shown in Figure 5.2. After ageing, however, some watercolours (burnt sienna, raw sienna and raw umber) lowered the folding endurance for the Yukyu-shi papers. Before ageing, raw sienna lowered folding endurance for the Sekishu papers and most watercolours (alizarin crimson, burnt umber, cobalt blue and raw umber) also lowered folding endurance for the Sekishu papers after ageing. Therefore, the acidity of plant dyes may explain the lower folding endurance of the Sekishu papers treated with plant dyes before ageing and the Yukyu-shi papers treated
with plant dyes before ageing, but there also seems to be some effect of watercolours on both Sekishu and Yukyu-shi papers after ageing that lowers folding endurance, even though pH in this case is alkaline. In general, the Yukyu-shi paper has greater folding endurance than the Sekishu papers for most treatments after ageing.

Acrylic paints demonstrate the best performance for the Yukyu-shi and Sekishu papers both before and after ageing and the results confirm that treatments with acrylic paints provide long-term stability for the selected Japanese mending papers. The acrylic polymer (the binder in acrylic paints) may cause more production of hydrogen bonds in the treated papers compared with gum Arabic (the binder in watercolours), which is a complex polysaccharide (arabinogalactan) (Cappitelli & Sorlini 2005; Sanchez et al. 2002).

As already mentioned, the entanglement and flexibility of the fibres impart two significant factors in the reduction or accession in folding endurance for paper materials (Karlovits & Gregor-Svetec 2012). During the dyeing process, pigment particles are strongly bound to each other in their solid crystal structure and resist dissolving, while dyes make strong bonds with their substrates (Christie 2001). This very different behaviour of dyes and pigments may affect the physical resistance of the papers. In the case of papers treated with acrylic paints, the aggregation of colourants on the papers may act an elastic layer on the substrate and thereby increase the folding endurance of these papers (Caulfield & Gunderson 1988).

Currently, an enormous effort in archives, libraries and museums is focused on the stabilisation of acidic papers by using deacidification processes and maintaining paper objects in appropriate archival folders (Ipert et al. 2006; Sequeira, Casanova & Cabrita 2006; Bogaard, Morris & Whitmore 2005). The use of archival papers, acid-free papers,
and also acid-free boards and boxes (made either of 100% cotton rag or of highly purified chemical wood pulp by addition of alkaline reserve of calcium or magnesium carbonate) for storing paper objects, is in response to the destructive effects of acidity for paper objects. According to the Canadian Conservation Institute (CCI) Notes 11/1, ‘Making Protective Enclosures for Books and Paper Artifacts’, books and paper objects should be stored either in acid-free products with a neutral pH or alkaline-buffered products to protect them from acidic destructions.

As discussed earlier, the results of folding endurance tests are more relevant to papers used in book preservation than for flat paper artefacts. The results of this study suggest that untreated Sekishu and Yukyu-shi papers, both Yukyu-shi and Sekishu papers treated with acrylic paints and also the Yukyu-shi papers treated with most plant dyes impart effective folding endurance after ageing.
6.4 Tear resistance

Generally, longer fibres improve tear strength because they distribute tearing stress over more bonds compared to short fibres, which concentrate the stress in a smaller area (Caulfield & Gunderson 1988). Figure 6.2 shows the tear resistance of the untreated paper controls, the papers treated with plant dyes, watercolours and acrylic paints and their correlation with pH both before and after artificial ageing. The untreated Yukyu-shi paper controls demonstrated greater tear resistance after ageing compared with the Yukyu-shi papers treated with plant dyes, watercolours and acrylic paints. In general, the Sekishu papers had greater tear resistance than the Yukyu-shi papers, whether treated or untreated, aged or not aged. As discussed in Chapter 2, the tear resistance of paper depends on the type of fibres, length and thickness of fibres, weight of paper, and density of paper.

Figure 6-1: pH versus folding endurance of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints before and after artificial ageing.
The density of the Sekishu paper is greater than the Yukyu-shi paper (21.4 g/m² and 16 g/m² respectively) and this higher density might increase tear resistance. According to Caulfield & Gunderson (1998, p. 35), ‘tearing resistance generally shows a continuous increase with increasing RH over the range of relative humidities’. This increased stretch and viscoelasticity induced by moisture content serves to distribute the stress at the tearing point and promotes fibre elasticity rather than fibre breakage.

As has been discussed, most plant dyes make both Sekishu and Yukyu-shi papers more acidic. The Sekishu papers treated with watercolours and acrylic paints have greater tear resistance than the Sekishu papers treated with plant dyes. Therefore, the acidity of plant dyes may explain the lower tear resistance of the Sekishu papers treated with plant dyes compared with their much smaller effect on the Yukyu-shi papers. Because of the hydrolytic deterioration usually catalysed by acids, the acidic structure of plant dyes could potentially speed up the degradation of fibres in the dyed papers (Zervos 2010).

As shown in Figure 6.2, artificial ageing makes both Sekishu and Yukyu-shi papers more alkaline. Further, watercolours and acrylic paints make both Sekishu and Yukyu-shi more alkaline before and after artificial ageing, relative to the papers treated with most plant dyes. As discussed in Chapter 3, acrylic paints and watercolours are the most widespread colourants used by paper conservators and the results of this thesis confirm that they impart effective tear resistance properties for the Sekishu papers when they are used as toning materials. Therefore, Sekishu papers, both untreated and treated with watercolours and acrylic paints, could help protect repaired papers from tearing when they are used as mending papers for paper objects in archives, libraries and galleries.
6.5 Colour change after artificial ageing

Every object behaves differently in conjunction with light; it may absorb, transmit, scatter, or reflect some parts of the light. The object seems white or black if it respectively reflects or absorbs all wavelengths of the visible spectrum. Coloured objects absorb some part of the light wavelength and reflect the other part (Christie, Mather & Wardman 2000). Figure 6.3 shows the correlation between pH and colour changes of the untreated paper controls and the papers treated with plant dyes, watercolours and acrylic paints after moist-heat artificial ageing. The fading rates of these papers were determined by calculating the colour change ($\Delta E^*$) as discussed in Chapter 4. A $\Delta E^*$ value of 5 was considered large enough to be detected by most observers and was used as an initial threshold value in this study. Colour change values greater than 5 indicate extreme colour difference (Yurdun &
Acrylic paints and watercolours not only make both Sekishu and Yukyu-shi papers more alkaline but also cause much lower colour change on the papers ($\Delta E^* < 5$), relative to the papers treated with plant dyes.

Ideally, the dyed Japanese tissue paper should change colour at the same rate, and in the same direction, as the paper being repaired. There is evidence that high quality hand-made papers, which lack lignin in their structure, do not fade over time (Winter 2008). The findings of this study also confirm that the untreated paper controls did not show noticeable colour changes compared with the dyed papers.

Figure 6-3: pH versus colour change of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints after artificial ageing.
Both Sekishu and Yukyu-shi papers treated with plant dyes were more acidic, and underwent more extreme colour change after artificial ageing, than the untreated paper controls and the papers treated with watercolours and acrylic paints. This suggests that papers repaired with plant dyes are more likely to become a totally different colour to the original document after ageing if matched at the time of repair. As discussed in Chapter 2, the dyeing component of henna leaves is lawson or 2-hydroxy-1, 4-naphthaquinone (Yusuf et al. 2012; Ali, Hussain & Nawaz 2009; Abdulmoneim Saadabi 2007). Quinones consist of aromatic rings with two ketone substitutions in their structure which makes them highly reactive as stable free radicals (Dev et al. 2009). They may react with oxygen and water in a hot, humid environment, resulting in colour change (Winter 2008). Black tea and *Eucalyptus* leaves are flavonoid-containing plants, which are a source of yellow dyes (Mouri et al. 2014; Moiz et al. 2010; Bechtold et al. 2009). The predominant flavonoids in these plants are flavones (Figure 6.4) which are comparatively colourfast (Mouri et al. 2014). Conversely, flavonols (3-hydroxyflavones) (Figure 6.5) are unstable materials. It is possible that heat and humidity result in the formation of flavonols in black tea and *Eucalyptus* leaves, resulting in colour change.

![Figure 6-4: Chemical structure of flavone](image.png)
Colour change is not the only factor influencing visible compatibility. The textures of the original and repaired papers also determine the final colour shifts. Colour differences are more noticeable when the surface of the substrate is smooth and uniform (Lerwill et al. 2008). Apart from the nature of the substrate, the fading rates of coloured papers depend on the size of the pigment particles, the concentration of the colourants, the thickness of the paint layer, chemical affinity between the substrate and dyes, and the binders (e.g., gum Arabic or acrylic polymer) (Connors-Rowe et al. 2004; Whitmore 2002). Fine dispersion of dye molecules can accelerate the fading of the substrate (Suzuki & Koestler 2003; Crews 1987). The dilution and intensity of the pigments in water can also play a role (Lerwill et al. 2008).

Pigments are responsible for maintaining both optical (the gloss of the dried paints) and physical properties of colourants (prevention of cracks in the paint) (Weerd, Loon & Boon 2005). Additionally, pigments are the only ingredients in the paint that can prevent the surface from hygroscopic reaction—a reaction in which the substrate, such as a painted paper, absorbs and retains water molecules from the surrounding environment. Conversely, the mechanical properties of paint layers are maintained by binders, such as gum Arabic in watercolours, linseed oil in oil paintings and acrylic polymer in acrylic paints (Learner 2001).
As mentioned earlier, most plant dyes have poor colourfastness properties compared with synthetic pigments; hence, the colours of plant-dyed textiles in museum collections are often quite different from their original colours (Duff et al. 1977; Padfield & Landi 1966). The results of this study suggest that conservators should consider that colouring Japanese tissue papers, such as Sekishu and Yuku-yshi, with plant dyes may cause noticeable colour change, when compared to untreated paper and paper treated with watercolours and acrylic paints.

**6.6 Colour change after photoageing**

In paper conservation science, the purpose of microfading assays is to identify the possible fading rate of dyes and pigments on paper substrates as a result of exposure to light. More sensitive colourants tend to fade rapidly and fading deterioration can be observed in the forms of cracking and shrinkage of the paint layers (Bowen, Mangum & Montague 2002; Perkinson 2002; Whitmore, Pan & Bailie 1999). While the overall chemical structure of a dye molecule seems to determine its general lightfastness properties, the substituted groups, including hydroxyls, can alter these properties (Crews 1987). Dyes are generally defined as compounds that undergo chemical interaction with substrates such as paper and textiles (Siva 2007). A dye molecule has two principal chemical groups: a chromophore and an auxochrome. The former usually incorporates an aromatic ring, which is associated with the colouring property, and the latter helps the dye molecule to combine with the substrate. Since dye molecules are not chemically stable in their photo-excited form, they can absorb light and decompose. These unstable excited molecules might react with other compounds contained in paper materials (Beek & Heertjes 1966).
A comparison of Figures 5.11 and 5.12 shows that black tea and Eucalyptus leaves are more lightfast than henna after photoageing, suggesting that henna is more subject to photo-oxidation than those papers that were treated with black tea and Eucalyptus leaves. Black tea and Eucalyptus leaves are categorised as flavonoid dyes, and these dyes aggregate into large particles in the paper substrate and form dye-metal complexes (Cristea & Vilarem 2006). The metal ions may quench the excited states and their presence in the dyed substrate thus increasing lightfastness properties (Beek & Heertjes 1966).

The lightfastness properties of dyes also depend on the mordant and the substrate. The presence of mordants can improve the lightfastness properties of natural dyes (Mikropoulou et al 2009). The influences of different mordants were found to play an effective role in the fading of 18 natural yellow dyes. The use of chrome, iron, or copper mordant resulted in effective colourfastness properties, relative to tin and alum mordants (Crews 1981). However, the application of mordants in paper conservation is generally not considered to be acceptable because many metals can affect the physical stability of paper (McCready 1996). The application of mordants, including blue and green copper pigments, in illuminated manuscripts was found to cause corrosion and in most cases irreversible degradation (McCready 1996; Purinton & Watters 1991). Alum resin mordant was also found to accelerate deterioration in many Western papers (Winter 2008). According to the results of the survey of paper conservation practitioners, yasha is the second most popular plant dye used by the respondents who participated in the survey. Mordants such as calcium carbonate are added to the yasha dye bath to improve lightfastness properties; however, paper treated with yasha needs to be thoroughly rinsed before using as a lining for an original paper object (Winter 2008).
Pigments are composed of solid and insoluble granules, which are suspended in solvents (Grim & Allison 2003). They are responsible for maintaining the optical properties of the paint. The strong intermolecular hydrogen bonding in many pigments provides colourfastness (Learner 2001). The results of MFT in this study, as shown in Figure 5.13, confirm that alizarin crimson acrylic paint applied to both the Sekishu and Yukyu-shi paper specimens resulted in the greatest lightfastness (fading less than BW3). The lightfastness properties of alizarin crimson lake pigment can be explained by an absorption spectrum shift caused by the dye-metal complex formation (Giles 1965). An acrylic paint is composed of a pigment and the high molecular weight acrylic polymers that are based on the esters of acrylic acid and meth-acrylic acid. Most artists’ acrylic paints are composed of light-fast pigments (Learner 2001). Inorganic pigments such as iron based pigments (raw sienna, raw umber, burnt sienna and burnt umber) are also considered stable in their painting components and they are identified as lightfast pigments (Winter 2008). Alizarin crimson acrylic paint is a blend of Quinacridone (PR122) and Naphthol carbamide (PR170) in an acrylic polymer. The fading of both papers treated with alizarin crimson watercolour and black tea was equivalent to that for BW3. A comparison of Figures 5.11 and 5.12 shows that dried Eucalyptus leaves conveyed greater light fastness for Yukyu-shi (= BW3) than for Sekishu (> BW3) while the fading of both papers treated with fresh Eucalyptus leaves and henna was similar to that for untreated papers.

Figure 6.6 shows the correlation between pH and colour changes after photoageing of the untreated paper controls, the papers treated with plant dyes, and with alizarin crimson in both watercolour and acrylic paint forms. The untreated Sekishu paper and the Sekishu paper treated with henna have greater colour change (1.2 and 1.1 respectively) after photoageing than the untreated Yukyu-shi paper and the papers treated with other dyes and
pigments. Treatment with alizarin crimson acrylic paint makes both Sekishu and Yukyu-shi papers alkaline and also imparts good lightfastness properties. Otherwise, there is not an obvious correlation between pH and colour change after photo-ageing. The Yukyu-shi and Sekishu papers treated with black tea and dried Eucalyptus leaves are light-fast, even though they are acidic.

![Graph showing pH versus colour change](image)

Figure 6-6: pH versus colour change of untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, a watercolour and an acrylic paint of alizarin crimson after photo ageing.

### 6.7 Fungal growth

Paper objects provide an environment that is suitable for the growth and multiplication of fungi that can result in their physical degradation. Additionally, mycotoxins can pose health hazards, possibly leading to life-threatening infections including inhalation diseases (Sterflinger & Pinzari 2012; Valentin 2010; Suanthie, Cousin & Woloshuk 2009; Sugita et al. 2004; Baldwin 2003). Fungal colonisation of papers can also result in unpleasant odours. Genetic detection of fungal growth in this study shows both *A. niger* and *P.*
rubrum grow preferentially on the Yukyu-shi papers, regardless of the treatment. The Whatman® Filter Paper control was an effective inhibitor of both A. niger and P. rubrum growth.

This study demonstrates the potential of molecular techniques in paper conservation practice. Retrieval of fungal specific DNA from inoculated papers after artificial ageing was successfully employed and a reliable molecular strategy was established to investigate fungal growth or inhibition on both untreated paper controls and those that were treated with the selected dyes and pigments. The major challenge for this study was to identify specific PCR primer pairs for amplification of extracted DNAs. Two PCR primers (UN1 and UN2) successfully amplified regions of the 18S rRNA gene. Two different DNA extraction protocols (organic extraction and Qiagen DNA extraction kits) were employed. The organic (phenol-chloroform) extraction with ethanol precipitation resulted in the greatest DNA recoveries from paper samples.

There are different opinions about the influence of pH on the growth of fungi. It is claimed by several authors that a low pH environment is an ideal environment to stimulate fungal growth (Manete et al. 2013; Weitz et al. 2001; Nittérus 2000) while bacteria prefer alkaline conditions to flourish (Cappitelli & Sorlini 2010; Shafique, Bajwa & Shafique 2009; Gallo 1992). However, Rakotonirainy, Heraud and Lavedrine (2003) claim that the pH of papers does not often influence the growth or inhibition of fungi.

There was no obvious correlation between pH and fungal growth in this study. Figures 6.7 and 6.8 show the pH of treated and untreated papers together with the concentrations of DNA recovered from untreated papers after 10 days of incubation at 27 °C and 80% RH (PCR was performed with the UN1 and UN2 universal primer pairs respectively). These
figures show that most treatments offer greater resistance to fungal growth than the untreated controls for Yukyu-shi papers. In general, the Yukyu-shi papers exhibited more fungal growth than the Sekishu papers for most treatments. Most watercolours (alizarin crimson and burnt umber) seem to encourage fungal growth on the Sekishu papers, relative to other treatments. Most plant dyes and acrylic paints offer better protection against fungal growth for the Sekishu papers. Further, the UN1 universal primers seem more sensitive than the UN2 universal primers, suggesting better binding efficiency and therefore more efficient PCR.

Figure 6-7: pH versus DNA concentration for untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN1 universal primer pair.
Figure 6-8: pH versus DNA concentration for untreated papers (Yukyu-shi and Sekishu) and papers treated with plant dyes, watercolours and acrylic paints after 10 days of incubation at 27 °C and 80% relative humidity. PCR was performed with the UN2 universal primer pair.

The antifungal properties of plant dyes including black tea, henna and Eucalyptus leaves have already been studied (Elaissi et al. 2012; Yusuf et al. 2012; Elaissi et al. 2011; Dev et al. 2009; Łuczaj & Skrzydlewska 2005). The different behaviour of these plant dyes for paper and textile substrates might be related to the different dyeing methods used.

Extraction in aqueous alkaline medium including sodium hydroxide is generally used for textile dyeing (Ali, Hussain & Nawaz 2009). A direct dyeing method without any chemical processing is mostly used for toning papers.

External additives, including dyes and pigments, will have an effect on the biological stability of paper materials over time. Most plant dyes impart intrinsic antimicrobial properties, which make them suitable for using in the textile industry (Yusuf et al. 2012; Ali, Hussain & Nawaz 2009). Henna is one such plant dye that is known for its
antimicrobial properties, and it is used to treat certain mycotic infections (Sharma et al. 2011; Abdulmoneim Saadabi 2007). As already discussed, henna belongs to the anthraquinone chemical group, which is highly reactive. It not only generates stable free radicals, but connects itself strongly with the amino acids of proteins in substrates including wool fabrics (Dev et al. 2009). This inactivation of proteins may cause fungal growth inhibition. Anthraquinones may also prevent microorganisms from accessing protein substrates thus inhibiting fungal growth. Habbal et al. (2011) studied the antibacterial activity of both fresh and dried henna leaves. The fresh henna leaves had the highest anti-

*Pseudomonas aeruginosa* activity. The results of this study confirm that the Sekishu and Yukyu-shi papers treated with henna were better inhibitors of both *A. niger* and *P. rubrum* than the untreated paper controls after incubation.

The survey of paper conservation practitioners (Section 3.6) indicated that black tea is frequently used for toning Japanese tissue papers as it yields various tonalities of yellowish-brown to dark brown (the tonality needed for most old documents). Fungal DNA recovered by the UN2 universal primer pair (shown in Figure 5.22) indicates that black tea inhibited the growth of *A. niger* on the Sekishu paper. Further, the Yukyu-shi papers treated with black tea show significantly lower DNA concentrations than the untreated Yukyu-shi papers after inoculation and incubation with *A. niger*. The potential antifungal activity of black tea may be due to the presence of flavonols (Bansal et al. 2013; Wu et al. 2007).

Artists’ paints such as watercolours are composed of pigments and a small amount of organic binder (gum Arabic) that binds to pigments and holds dry pigments in suspension in the medium. Biocides (chemical compounds used to kill living cells) are incorporated
into the Winsor & Newton™ Cotman watercolours (Winsor & Newton™ -Regulatory Affairs Department 2013, pers. comm., 23 October). The presence of plasticisers, which migrate toward the surface of the paint and exposes the paint layer to biological attacks, could also encourage fungal growth (Cappitelli et al. 2005; Cappitelli & Sorlini 2005). As shown in Figures 6-7 and 6-8, the Yukyu-shi papers treated with watercolours were better inhibited fungal growth than untreated paper controls. Further, raw sienna watercolour inhibited the growth of A. niger more effectively on the Sekishu papers than on the Yukyu-shi papers.

The acrylic paints used in this study include bactericides in their composition to improve the biological stability of the paint (E. McInnes 2013, pers. comm., 10 October). There is little literature about the biological stability of acrylic paints (Cappitelli & Sorlini 2008; Cappitelli, Principi & Sorlini 2006). As was mentioned earlier, acrylic resins were found to have more resistance to biodeterioration than polyvinyl acetate and alkyds (Cappitelli & Sorlini 2008). The findings of this study also confirm that treatments with acrylic paints caused greater fungal growth inhibition on the Sekishu papers, than the Yukyu-shi papers. The widespread use of acrylic resins in cultural heritage conservation is due to conservators’ perception of their long term stability and of the antimicrobial properties of synthetic resins –because of the inclusion of biocides in these products (Cappitelli, Zanardini & Sorlini 2004). The findings of this study also confirm that acrylic artists’ paints have better antifungal properties when used for treating the Sekishu papers compared with the Yukyu-shi papers.

The results of this thesis show that the Yukyu-shi papers treated with plant dyes, watercolours and acrylic paints encouraged the growth of both A. niger and P. rubrum,
relative to the Sekishu papers. The two papers used in this study were made in two different geographical prefectures in Japan. Several factors may influence fungal attack of paper materials treated with colourants including the raw materials, sizing compounds, additives, contaminants (e.g., heavy metals), and environmental conditions (e.g., humidity and temperature) (Yoshinao 2011; Zotti, Ferroni & Calvini 2008). Further, non-cellulosic materials such as pectin and hemicellulose can promote microbiological attack in paper based materials (Winter 2008). The origin of the raw materials, the season of harvesting of the plant and the conditions of the soil may also affect the susceptibility of papers to fungal attacks.
7. CONCLUSIONS AND FURTHER WORK

Due to the susceptibility of organic materials to physical, chemical and biological deterioration, valued paper objects will degrade over their life time. As discussed in Chapter 3, a variety of toning materials including plant dyes, watercolours, acrylic paints, gouaches, colour pencils, and pastels have long been used for toning mending papers (e.g., Japanese tissue papers) in paper conservation practice. This thesis presents a detailed scientific investigation of the use of toning materials for colouring Japanese tissue papers in paper conservation. The Yukyu-shi and Sekishu papers were chosen as examples of the many Japanese tissue papers that may be used in paper conservation and their physical (folding endurance, tear resistance, colour change), chemical (pH) and biological properties, when treated with the selected plant dyes and artists’ paints, were measured. A number of colourants have thus been identified as more effective materials for use in paper conservation.

Artificial ageing was employed to simulate the changes in the physical, chemical and biological properties of paper over time. Recognition of the age-related effects of toning materials on Japanese tissue papers is critically important in identifying which papers may be at risk of rapid degradation. The results of this study confirm that although pH is indeed an important variable in the stability of papers against ageing, it is clear that other variables (paper and colourant) also play an important role. Papers treated with plant dyes were more acidic than those that were treated with watercolours and acrylics paints and, in general, dyed papers displayed less folding and tear resistance and greater colour change after ageing. The results obtained from physical tests (folding endurance, tear resistance,
and colour change), chemical test (pH) and fungal growth tests are summarised in Table 7.1.

Table 7-1: Summary of results obtained from folding endurance, tear resistance, colour change, pH and fungal growth for the papers. A tick (✓) indicates a desirable conservation outcome and a cross (×) represents a poor, or less desirable, conservation outcome.

<table>
<thead>
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According to Table 7.1, the Sekishu papers performed better on a larger range of criteria than the Yukyu-shi papers. Interestingly, there was a difference in the physical properties of the Yukyu-shi and Sekishu papers. The untreated Sekishu papers and the Sekishu papers treated with watercolours and acrylic paints exhibited greater tear resistance than the Sekishu papers treated with plant dyes. The results of this study suggest that Sekishu and Yukyu-shi papers in untreated form and when treated with acrylic paints, as well as the Yukyu-shi papers treated with plant dyes, demonstrate effective folding endurance after ageing. However, paper treated with watercolour paints resulted in less folding endurance after ageing. The Sekishu papers treated with plant dyes exhibited a significant loss of folding endurance, relative to the Yukyu-shi papers. Thus, for the best long term preservation of paper objects, especially for the preservation of books where frequent usage is desirable, plant dyes are not considered to be a good conservation choice for
colouring Sekishu mending papers, as this type of paper lacks folding endurance. Acrylic paints were found to contribute to effective folding endurance and tear resistance when used for colouring the Sekishu and Yukyu-shi papers, perhaps because they make these papers alkaline.

Most of the plant dyes tested in this study showed some degree of fading after exposure to the moist heat artificial ageing environment. By contrast, the Yukyu-shi and Sekishu papers treated with watercolours and acrylic paints were alkaline and they exhibit much lower colour change on the papers, relative to the papers treated with plant dyes. There is not an obvious correlation between pH and colour change after photo-ageing. After photoageing, the papers treated with black tea and Eucalyptus leaves were found to be more light-fast (even though they are acidic) than those papers that were treated with henna and the untreated paper controls. Colouring with alizarin crimson acrylic paint makes both Yukyu-shi and Sekishu papers alkaline and this was found to be an effective light-fast treatment, relative to the untreated papers.

The results of this study have broader application than the field of paper conservation. For instance these findings should also be useful for understanding the stability and colourfastness properties of archaeological artefacts, including textiles that were dyed with one of the dyes or pigments used in this study. Hence, analysing the colour change properties of natural dyes helps to infer the original colour of ancient textiles and also the effects of conservation treatments on such textiles.

Colour measurement techniques such as spectrophotometry and MFT can help paper conservators to understand the long term performance of toning materials on repaired papers. While not perfect indicators of long term colour changes, these methods provide an objective indication of potential colour change as a result of storage in libraries, galleries,
museums and under exhibition lighting, and are to be preferred over visual assessments conducted in short periods of time (e.g., the period of a temporary exhibition) which is also more time consuming and labor intensive. Thereby, understanding the sensitivity of treated Japanese tissue papers to light will allow museums to set limits for the display of significant individual items with confidence. Further work will be needed to investigate the colourfastness properties of other types of papers when treated with different extracts of the plant dyes used in this study and other colourants, including gouaches, pastels, colour pencils and inks.

As discussed in Chapter 3, acrylic paints and watercolours are the most widespread colourants used by paper conservators and their continued use over plant dyes is supported by the findings of this study. While their use is undergoing a revival and they are seen to have heritage value as a traditional product, plant dyes are not suitable for colour-matching the retouched parts of ancient books and documents where some form of ageing is expected. Specifically, the spectrophotometry colour change investigations suggest that those papers treated with plant dyes should not be stored in environments where high temperature and humidity can be expected.

Although traditional microbial culturing methods are still used in the conservation field, they are time-consuming and limited by a lack of morphological distinction between some species (Cappitelli et al. 2010). The results of this study confirm that molecular techniques are useful to conservators and may complement or even replace classical approaches in biodeterioration assessment and heritage preservation since classical culturing methods are slower and less able to differentiate precisely between species and sub-species (Pinzari et al. 2010; Gonzalez 2003).
Of the two Japanese tissue papers examined in this study, Sekishu paper, when used untreated, or when it treated with plant dyes and acrylic paints, displayed the greatest resistance to fungal growth. Also, most colourants do not encourage any more fungal growth than untreated controls for the Yukyu-shi papers when they are used as toning materials. The results of this study suggests that for the best long term preservation outcomes for paper materials in archives, libraries, galleries and museums, artists’ acrylic paints perform better in conservation terms than plant dyes and watercolours.

The findings of this study confirm that the pH of papers does not often influence the growth or inhibition of fungi. However, there was a difference in fungal growth or inhibition properties between Yukyu-shi and Sekishu papers treated with plant dyes and artists’ paints. Most plant dyes offer better protection against fungal growth for the Sekishu papers, despite their acidic structure, relative to the Yukyu-shi papers.

The application of pure fungal strains used in this study represents a starting point for further comparative analyses of conservation practices for paper objects of historic and cultural value. The role of bacterial flora in the degradation of paper materials used in paper conservation is also worthy of further study. Generally, any conservation treatments that are applied to prevent biological deterioration of historical documents and books have to be considered prior to restoration procedures. Further collaboration between microbiologists and conservators is needed to develop effective strategies to prevent fungal degradation of paper materials. Also, further work is needed to test more fungal species and to investigate the possibility of imparting antifungal properties to other types of papers by treatment with different extracts of the plant dyes used in this study.
The Japanese tissue papers examined in this study were found to be generally of good quality, based on pH readings and physical performance; however, toning these papers with various dyes and pigments does affect the quality of these papers. This research is one of the first times that the quality of Japanese tissue papers has been studied under different experimental conditions. Better understanding of the properties and performance of Japanese tissue papers in the conservation community can assist in building consumer demand for providers to produce unvarying, high quality mending papers. Understanding the quality of these papers will also help simplify the choice of mending papers in the increasingly diverse market of conservation supplies.

As discussed in Chapter 2, when performing a retouch or repair, conservators often make an instinctive decision on how best to colour Japanese tissue papers, based on traditions and the conventional practices established in conservation laboratories. Further, these decisions tend to consider the visual aesthetic of the integrated colour as of paramount importance, rather than an in-depth scientific knowledge of the chemical and physical properties of the materials chosen. According to the results of the survey of paper conservation practitioners (Chapter 3), conservators are often more concerned with adjusting the tonality of repairs, than with the long-term stability of the chosen colourants over time; for instance, paper conservators use black tea and yasha to achieve brown tonalities without substantial knowledge about their acidic effects on the repaired parts of the object in the future. Thus, it may not be appropriate to continue to justify the application of these plant dyes on the fact that tea and yasha have been used by conservators in the past to tone paper. Further, paper conservators should be encouraged to base their practice on documented research, and not to simply accept the established use of
particular types of paper or colourants in all paper treatment circumstances unless they are aware of the long-terms properties of these materials.

Generally, any treatment that is applied to Japanese mending papers and subsequently used in conjunction with old papers and books should be carefully assessed beforehand. Further, economic factors including cost of re-treatment, the quantity of paper objects that need to be repaired, and their historical and cultural value also need to be considered before making conservation decisions. The results of the survey of paper conservation practitioners discussed in Chapter 3 demonstrates that further work is needed to develop the toning techniques used by paper conservators around the world. Conservators from different countries also need to share their successful methods of retouching and their thoughts and experience on less advisable toning materials, for the benefit of a wider audience. While traditional paper conservation practices may have particular cultural values in some circumstances, this study shows that important new insights and opportunities to improve conservation outcomes, and safeguard unique cultural heritage, can be based on the innovative use of an array of scientific techniques that question established cannons. It is hoped that the findings of this study open the way for further such research to benefit cultural heritage and the work of the paper conservation community.
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References


References


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References


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References


3 April 2013

Mrs Somayeh Soleymani
Faculty of Arts & Design
University of Canberra
Canberra ACT 2601

Dear Somayeh,

The Human Research Ethics Committee has considered your application to conduct research with human subjects for the project The effects of plant dyes, watercolour and acrylic paints on the physical and biological stability of Japanese tissue paper used in paper conservation.

Approval is granted until 30 June 2013, the anticipated completion date stated in the application.

The following general conditions apply to your approval.

These requirements are determined by University policy and the National Statement on Ethical Conduct in Human Research (National Health and Medical Research Council, 2007).

| Monitoring: | You, in conjunction with your supervisor, must assist the Committee to monitor the conduct of approved research by completing and promptly returning project review forms, which will be sent to you at the end of your project and, in the case of extended research, at least annually during the approval period. |
| Discontinuation of research: | You, in conjunction with your supervisor, must inform the Committee, giving reasons, if the research is not conducted or is discontinued before the expected date of completion. |
| Extension of approval: | If your project will not be complete by the expiry date stated above, you must apply in writing for extension of approval. Application should be made before current approval expires; should specify a new completion date; should include reasons for your request. |
| Retention and storage of data: | University policy states that all research data must be stored securely, on University premises, for a minimum of five years. You must ensure that all records are transferred to the University when the project is complete. |
| Contact details and notification of changes: | All email contact should use the UC email address. You should advise the Committee of any change of address during or soon after the approval period including, if appropriate, email address(es). |

Please add the Contact Complaints form (attached) for distribution with your project.

Yours sincerely
Human Research Ethics Committee

Hendryk Flaegel
Ethics & Compliance Officer
Research Services Office
T (02) 6201 5220 F (02) 6201 5486
E hendryk flaegel@canberra.edu.au

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Postal Address:
University of Canberra ACT 2601 Australia
Location:
University Drive Bruce ACT
Australian Government Higher Education Registered
Provider Number CRICOS: 00213K

212
PROJECT INFORMATION

The following study has been reviewed and approved by the University of Canberra’s Human Research Ethics Committee.

**Project title:**

*The effects of plant dyes, watercolour and acrylic paints on the physical and biological stability of Japanese tissue paper used in paper conservation*

**Project number:**

<table>
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<th>Project number:</th>
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</tr>
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<td>13-32</td>
<td>Mrs Somayeh Soleymani</td>
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INDEPENDENT COMPLAINTS PROCEDURE

1. As a participant or potential participant in research, you will have received written information about the research project. If you have questions or problems which are not answered in the information you have been given, you should consult the researcher or (if the researcher is a student) the research supervisor. For this project, the appropriate person is:

   **Name:** Dr Tracy Ireland  
   **Contact details:** University of Canberra  
   Phone: 02 6201 2957  
   Email: tracy.ireland@canberra.edu.au

2. If you wish to discuss with an independent person a complaint relating to:

   - conduct of the project, or  
   - your rights as a participant, or  
   - University policy on research involving human participants,

   **Please Contact:**

   Mr Hendryk Flaegel  
   Ethics and Compliance Officer  
   University of Canberra  
   Phone: 02 6201 5220  
   Email: hendryk.flaege@canberra.edu.au

   Ethics Committee **Human Ethics Manual**.
Appendix 2: Survey questions (colouring Japanese tissue paper)

Thank you for taking the time to fill out this survey. The survey is a part of the PhD research in Cultural Heritage Conservation being undertaken by Mona Soleymani at the University of Canberra. The aim of this survey is to seek conservators' opinions about the paper conservation processes and the common dyeing materials used in paper conservation centres. Thanks for your help.

Mona Soleymani, PhD candidate, Heritage Conservation, University of Canberra. Mona.soleymani@canberra.edu.au

1. Which country are you working in?
2. Do you often use Japanese tissue paper to repair old documents and books?
   - Yes
   - No
      If no, please specify what kind of paper do you use?
3. What type of Japanese tissue paper do use for paper restoration processes?
   - Hand-made Japanese tissue paper
   - Machine-made Japanese tissue paper
   - Other (please specify)
4. Are you familiar with the physical and chemical properties of Japanese tissue papers that you use in paper conservation processes?
   - Yes
   - No
      Please add comments.
5. What type of colours do you use to match the colour of Japanese tissue paper with the tone of the aged paper?
   - Plant dyes
   - Watercolours
   - Acrylic paints
   - Gouaches
   - Colour pencils
   - Pastels
   - Other (please specify)
6. If you use plant dyes, what type of dyes do you use?
   - Black tea
   - Henna
   - Pomegranate rind
   - Green walnut shell
   - Eucalyptus leaves
   - Indigo
   - Saffron
7. If you use watercolours or acrylic paints, what type of paints do you use?
   - Cotman
   - Winsor and Newton
   - Derivan
   - Kremer
   - Golden
   - Other (please specify)

8. Why do you prefer to use your chosen type of colourants?
   - Light resistance
   - Physical stability
   - Humidity-heat resistance
   - Environmentally friendly paints
   - Chemical resistance
   - Suitable to use with Japanese tissue paper
   - Antimicrobial resistance
   - Aesthetic reasons
   - Other (please add comments)

9. In your opinion, are there enough studies about the effects of dyes and pigments on Japanese tissue paper over time?
   - Yes
   - No
   - Please add comments.

10. Are you aware of the long term effects of dyes and pigments on Japanese tissue paper over time?
    - Extremely aware
    - Very aware
    - Moderately aware
    - Slightly aware
    - Not at all aware
    - Please add comments.

Thank you for completing the survey.
I would be happy for any further comments and experiences about inpainting Japanese tissue paper in paper conservation processes.
Mona Soleymani, PhD candidate, Cultural Heritage Conservation, Faculty of Arts and Design, University of Canberra.
Mona.soleymani@canberra.edu.au
Appendix 3: Shifts in \(L', a^*, b^*\) coordinates of the untreated Yukyu-shi and Sekishu papers and the papers treated with plant dyes, watercolours and acrylic paints before and after artificial ageing followed by the total mean of \(\Delta L', \Delta a^*, \Delta b^*,\) and \(\Delta E^*\).

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