Improving the forensic value of textiles and fibres through the holistic detection and analysis of acquired characteristics due to environmental factors

By

Rachel Morison

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Certification of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis. This research is supported by an Australian Government Research Training Program Scholarship.

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List of Conferences

The research conducted during this project was presented at several international conferences listed below

Year	Conference	Presentation
2016	ANZFSS 23rd International Symposium on the Forensic Sciences, Auckland (New Zealand)	Improving the value of textiles and fibres through the holistic detection and analysis of acquired characteristics due to environmental factors (Oral presentation)
2015	7th European Academy of Forensic Science Conference, Prague (Czech Republic)	Improving the value of textiles and fibres through the holistic detection and analysis of acquired characteristics due to environmental factors (Oral presentation)
2014	ANZFSS 22nd International Symposium on the Forensic Sciences, Adelaide (Australia)	Improving the value of textiles and fibres through the holistic detection and analysis of acquired characteristics due to environmental factors (Oral presentation)

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Abbreviations

ATR	Attenuated Total Reflectance
CE	Capillary Electrophoresis
DRIFTS	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
EFG	European Fibres Group
ENFSI	European Network of Forensic Science Institutes
FT-IR	Fourier Transform Infra-red Spectroscopy
GC	Gas Chromatography
HPLC	High-Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
LA-ICP-MS	Laser Ablation- Inductively Coupled Plasma- Mass Spectrometry
MSP	Microspectrophotometer
nm	Nanometres
PCA	Principal Component Analysis
RMIT	Royal Melbourne Institute of Technology
SEM/EDS	Scanning Electron Microscope/ Energy Dispersive X-ray Spectroscopy
SOP	Standard Operating Procedure
SWGMAT	Scientific Working Group for Materials Analysis
TLC	Thin Layer Chromatography
UV	Ultraviolet
UV-Vis	Ultraviolet -Visible
VSC	Video Spectral Comparator

Abstract

Fibres are a useful form of trace evidence that allows for a suspect, a crime scene or a victim to be linked, based on the comparison of fibres. Currently, analysis of fibres is based on the comparison of manufactured features such as the fibre type, dye and colour. These features are unable to distinguish fibres that have come from a line of mass-produced items, something which has become increasingly common. The use of acquired characteristics to compare fibres is an area of research that has not been substantially explored. This thesis seeks to explore the use of acquired characteristics, specifically laundry detergents, to aid in the comparison of fibres and improve their evidential value.

This study was undertaken by washing textiles in a commercial washing machine, changing variables such as the textile type and colour, detergent brand and number of washes. The analysis of the fibres was split into two categories: fluorescence and composition. Fluorescence analysis included the use of both fluorescence microspectrophotometry and the Video Spectral Comparator to detect optical brighteners in detergent residues on washed samples. Composition analysis was split into chemical (Raman Spectroscopy) and elemental (SEM-EDS and LA-ICP-MS/solution ICP-MS).

Comparison of the fluorescence of washed and control textiles showed that the fluorescence of the sample after washing is highly dependent on the textile type, with cotton showing the most fluorescence, while wool and polyester showed little to no fluorescence. Washed cotton samples produced a consistent fluorescence peak which was common for all detergent. This peak could also be seen in control samples that had been exposed to optical brighteners in the manufacturing process.

Chemical composition analysis of the samples in the form of Raman spectroscopy showed potential to differentiate between different detergents but was unable to detect detergents on the washed samples.

Elemental composition comparison of washed and control samples showed a clear difference in the composition of the two samples, with a consistent trend of elements being removed or added in the washing process.

Blind sample pairs of fibres of the same fibre type were compared using fluorescence microspectrophotometry and solution ICP-MS to determine whether they could be excluded as having come from the same source, based on if they had been washed or not. Fluorescence microspectrophotometry was able to reliably distinguish the majority of the samples, while solution ICP-MS revealed that it had limitations when it came to interpretation due to variation in the elemental concentration of fibres from the same source.

The results in this thesis show that the combination of fluorescence analysis using fluorescence MSP, and elemental composition analysis using solution ICP-MS allows for cotton and wool fibre textiles, but not polyester fibres, to be distinguished based on whether they have or have not been washed before.

Chapter 1: Introduction

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1.1 Forensic examination of fibres

Fibres are a common form of trace evidence that can often be found at crime scenes due to Locard's Exchange Principle. Locard [1] postulated "The truth is that none can act with the intensity induced by criminal activities without leaving multiple traces of his path. [...] The clues I want to speak of here are of two kinds: Sometimes the perpetrator leaves traces at a scene by their actions; sometimes, alternatively, he/she picked up on their clothes or their body traces of their location or presence". This means that fibres may be transferred from the suspect to the victim and crime scene and vice versa. The transfer of fibres can depend on many factors, such as the sheddability of the textile, the force and number of the contacts and the surface area of the contact. The persistence of the fibres decreases the longer the fibres are, the longer the garment is worn, and with increased contact with the area after the original deposition [2].

The forensic examination of fibres seeks to answer two questions. First, can the fibre be linked to a source? This link can be made by comparing the evidence fibre with known fibres from a suspected source. The fibre can then be included or excluded as a source depending on if the fibres can be differentiated or not [3, 4]. The second question is can the fibre be used for the reconstruction of an activity? Fibres can be used to not only link a victim or crime scene to a suspect but also to link a victim or objects to a crime scene. Fibre comparison can be used to identify the crime scene, identify any secondary crime scenes or any modes of transport. This, along with the exact location of the fibres, can be used to recreate a criminal activity. For example, a fibre located on the back of a garment could imply that there was an assault from behind while fibres found on underpants could support sexual activity [2, 5-7].

These fibres need to be recovered from the crime scene and complainant using the most direct and least intrusive method possible [8-10]. The recovery technique chosen depends on the context of the fibres in the crime scene. Small fibres or tufts should be collected by picking (using tweezers) and placed in a secure package. This is a more selective form of collection than the other retrieval techniques. The most common form of fibre

collection is by tape lifting [11]. In this method, an adhesive tape is touched multiple times to a surface to remove loose debris. This tape is then placed on a transparent sheet to prevent further cross-contamination. The benefit of this method is that it allows investigators to determine the number and distribution of fibres on the garment. Other methods of fibre collection include combing (of the hair of victims) and scraping (of buried clothing or a body). When these methods cannot be used, vacuuming of the scene can also be used. This method is not favoured though for fibre collection as it also recovers much extraneous material.

Once the fibres are collected, they must then be examined for the presence of relevant fibres. The selection of which fibres are relevant is usually made on a case by case basis. When both complainant and suspect evidence is being examined, tape lifts will be taken of all the evidence, with complainant and suspect items being examined in separate rooms to avoid contamination. The tape lifts are then examined to determine target fibres (those that should be looked for on other items of evidence). While there may be many evidence textiles available, with many different types of fibres, not all are appropriate to be target fibres. Types of fibres that are not well suited as target fibres include fibres that are very common, textiles that are very smooth and so do not shed a lot of fibres, undyed fibres and fibres from items made with variable reprocessed fibres. There are exceptions to this rule, for example, if a very common fibre is found in an unusual place where they would not be expected to be found, then they have higher evidential value. The best target fibres, suggested by Gaudette [12], are those that can create the best contrast and have the most characteristics for comparison. For example, uncommon fibres that fluoresce, that have a high dye content and that are coarse are better target fibres.

Once the target fibres have been identified, they will be recorded, and the tape lifts of the suspect's garments will be searched in order to find target fibres from the complainant's garments and vice versa. These fibres are called questioned fibres. The aim here is to find evidence of two-way contact.

The target fibres selected can change on a case by case basis depending on what the investigation is looking for. In some cases, there may be no suspect fibres for comparison, and the complainant's garments are searched for fibres that might indicate what surfaces

or textiles the complainant has come in contact with. When no direct link between suspect and complainant can be found, garments may also be examined for a shared target fibre that may indicate contact with a common source. The type of fibre present can also provide information about the type of garment/ source that the fibre came from (e.g. denim cotton, automotive carpet fibres) [10].

1.2 Current methods for analysis of fibres

The forensic examination of fibres follows a hierarchical process that moves from non-destructive to destructive techniques and from general to particular characteristics. Preference is given to techniques that are non-destructive, applicable to the smallest sample sizes, and are highly discriminating. Guidelines for fibre examination were established by working groups such as the European Fibres Group (EFG), which is a part of the European Network of Forensic Science Institutes (ENFSI) [13] and the Scientific Working Group for Materials Analysis (SWGMAT) in the USA [14]. These guidelines are the basis of many published Standard Operating Procedures (SOP) such as those used by the North Carolina State Crime Lab [15] as well as standards created by ASTM [16].

For this reason, the following process is followed [10, 17]:

1. Detection and collection of trace evidence

   The first step in the forensic analysis of fibres is the detection of fibre trace evidence at a crime scene or on a piece of evidence. Techniques for the detection of fibres include general visual searches; visual searches assisted by different types of illumination, such as oblique lighting and alternate light sources (UV, laser, high intensity); and visual searches assisted by magnification [18]. If fibres are detected, they are then collected using one of the collection techniques mentioned above.

2. Identification of fibre type

   The identification of the fibre type is performed initially by microscopy. In the case of man-made fibres, further analysis with FTIR, Raman spectroscopy or pyrolysis gas

chromatography may be needed. If the fibres are unable to be differentiated based on fibre type, the examiner moves to the third step.

3. Colour examination

Colour examination is performed first with the subjective technique of optical examination followed by the more objective technique of UV-vis microspectrophotometry. The colour of the fibre can be caused by the dye used during manufacture as well as any acquired colour change post-manufacture such as staining.

4. Dye examination

If the fibres are found to be indistinguishable by colour, the dye composition of the fibres is then compared. This can be performed by Raman spectroscopy or by extracting the dye from the fibre and performing separation techniques including Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE). The dye type is predominately determined by the manufacturer; however, change in dye composition can also change post-manufacture if items are redyed.

5. Interpretation

The final step of the analysis is the interpretation of the results. This interpretation involves determining whether an evidence fibre and a suspect fibre can be differentiated and, if there is no suspect fibre, identifying the possible source of the fibre based on the fibre and dye type. The interpretation also involves the statistical analysis of the evidence using techniques such as Bayes theorem and taking into account factors such as the frequency of the fibre. Interpretation of the fibre evidence can be improved by considering other aspects that are not routinely used, such as the analysis of residues that can impart some acquired characteristics to the fibres.

The final step, which is the step that is focused on in this project, is the analysis of any residues on the fibres. These residues are acquired after manufacture (acquired characteristics) and can be identified through techniques such as SEM-EDS and other typical methods used for trace evidence analysis.

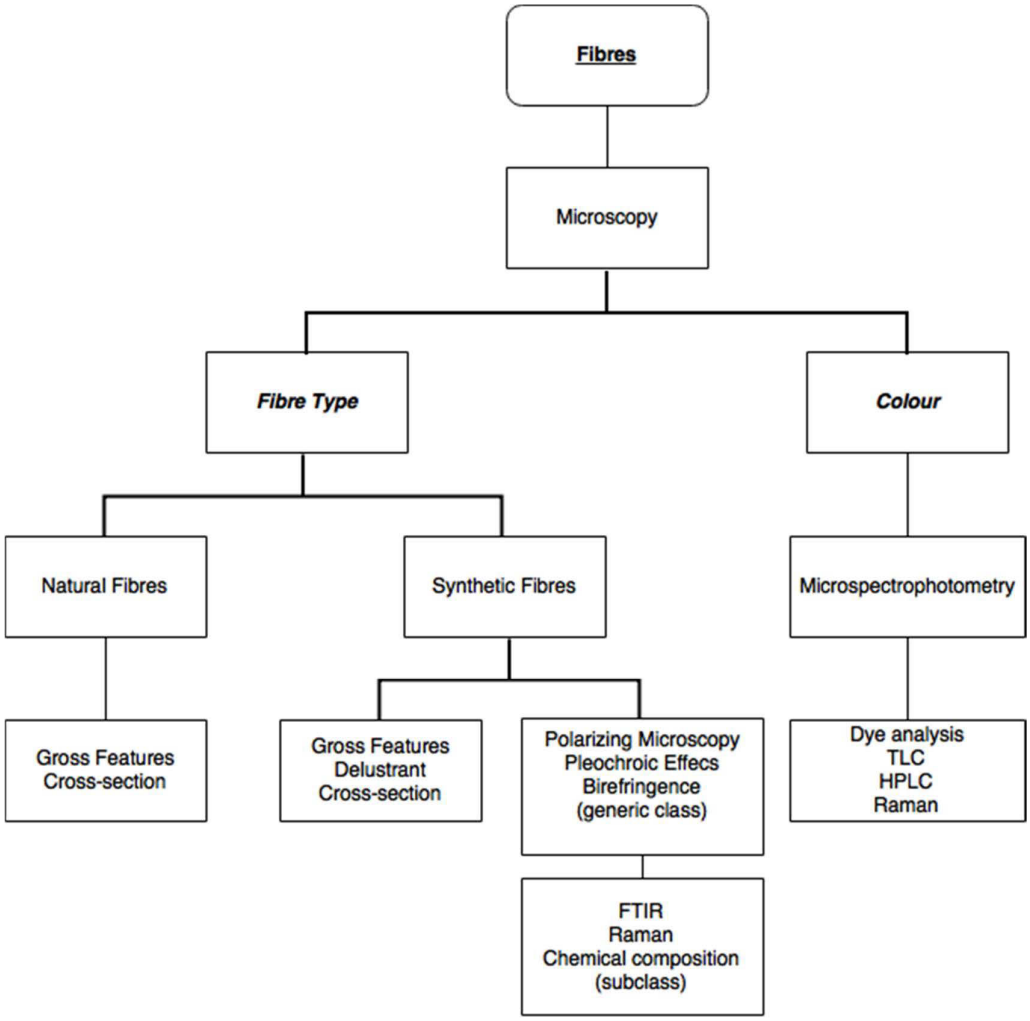


Figure 1-1: Process for the forensic examination of fibres (adapted from [19])

1.2.1 Microscopy

Microscopy techniques [20] are very valuable as they are non-destructive and require little sample preparation, and so are a quick and simple way of discriminating non-similar fibres. The main characteristics used to differentiate fibres are the type of fibre, the cross-section of the fibre and the colour and the weave of the fabric.

There are several types of microscopy used that can provide different types of information about the fibres. Low power stereomicroscopy is usually the first step of fibre examination and is used to aid in the examination of evidence and the recovery of fibres. It is not suitable for the accurate identification of fibres.

High Power Microscopy can be used for the visual comparison of colours and fibre appearance; however, it can also be used under other conditions to provide more aspects for comparison. These conditions include polarised light, interference and fluorescence microscopy. Polarised light microscopy measures the optical characteristics of fibres such as sign of elongation and birefringence. This can lead to a preliminary identification of the type of man-made fibre, though further testing is required to determine the chemical composition and thus the exact type of fibre. Interference microscopy accurately measures the refractive indices/birefringence of fibres and can be used to make distinctions between man-made fibres of one generic class type (e.g. polyesters). Fluorescence microscopy can compare the fluorescence of fibres caused by the materials used in the dyeing and finishing of the textile fibres.

Microspectrophotometry measures the absorption, transmission and fluorescence of the radiation of different wavelengths of light across the ultraviolet and visible (UV–Vis) region creating a spectrum that can be used to compare the colour of very small fibres objectively. The technique is favoured in forensic examination as it is a quick, reproducible and highly discriminating form of analysis [21].

Another form of microscopy is the Scanning Electron Microscope (SEM) coupled with Energy Dispersive Spectrometry (EDS) [22, 23]. The SEM works by scanning an electron beam over the sample, with detectors around the sample detecting the different signals generated by the electron beam to generate an image on the computer screen. SEM allows highly magnified views of individual fibres and, while not regularly used in fibre examination, can be used to investigate fibre fractures and damage[24]. The EDS provides an elemental summary of both the fibre and any particles on the fibre that could provide more information or aid in the linkage of the fibre to a source.

1.2.2 Further Non-destructive techniques

If a specimen cannot be immediately distinguished using microscopy alone, or the results are ambiguous, further analysis is needed. Techniques such as Raman Spectroscopy and Infra-red Spectroscopy are very useful for this purpose as they can provide more detail about the fibre type and, in the case of Raman Spectroscopy, more information about the chemical makeup of the dyes.

Fourier Transform Infra-red (FT-IR) spectroscopy

FT-IR [25-27] is a vibrational spectroscopy technique which is complementary to Raman Spectroscopy. Samples are irradiated with Infra-red light and produce an infra-red spectrum which gives information about the composition of the fibres. Due to the low sensitivity of the Infra-red absorption, the technique is limited to the analysis of fibre type as it is unable to reliably detect the low concentrations of dyes present on fibres. While FT-IR is mainly applied in transmission, the Attenuated Total Reflectance (ATR) method can also be used. In this technique, the specimen is pressed against a crystal (usually diamond or germanium), and the infrared beam interacts with the sample at the interface causing internal reflection of the light which is then detected. The advantages of this technique are that it requires little sample preparation (especially in the case of hard fibres like nylon which are hard to flatten for transmission spectroscopy) and it is a surface analysis technique which can detect surface finishes and pigmentation on the fibre surface. However, as only the first micrometre of the fibre is analysed using ATR, it is recommended to perform transmission spectroscopy as well to gain more spectral information when needed. ATR-FTIR applied with chemometric analysis has been shown to be able to identify pure and mixed fibre textiles, although it was not always able to distinguish between the cellulose-based fibres (cotton, linen and in some cases viscose) and it was only partly possible to distinguish between silk and wool [28]

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) [4, 29] is another IR technique that has been found to be more successful in analysing fibre dyes. As the technique does not follow Beer's law, it is more sensitive using reflectance (rather than transmission) and so can detect smaller concentrations. The technique can discriminate both dye colour and reactive dye state on cotton using chemometric methods such as Principal Component Analysis (PCA) and Soft Independent Modelling of Class Analogies (SIMCA)[30].

FTIR has also been paired with mass spectrometry imaging (MSI) using matrix-assisted laser desorption electrospray ionization (MALDESI) and has been found to be able to provide information on the polymer type and dye from a single fibre analysed in situ on a tape lift [31].

Raman spectroscopy

Raman spectroscopy is a non-destructive technique that is becoming increasingly more common in the forensic laboratory [32]. The use of Raman spectroscopy for the analysis of fibres has been intensively researched since 1986 [33]. Further research [30, 34-39] discovered that the advantages of Raman Spectroscopy were that only small specimen sizes were needed and there was minimal sample preparation. Through the analysis of Raman spectra with multivariate statistics, the technique can be used to identify concentrations of dyes as low as 0.005 % on both natural and synthetic fibres as well as, in some cases, differentiate polymers from different manufacturers [30, 36]. Complications with the analysis of dyes do exist. The Raman response is quite low and thus can generally only identify the major dye component present on the fibre and, in the case of lightly dyed fibres even that might not be detected [40]. These studies also showed that identification of dyes is not an easy task as it requires a complete reference collection of dyes in order to make an identification. As well as this, different dyes may have identical MSP or Raman responses, and so no one single technique will be able to characterise the fibre dye completely.

Research into the optimisation of Raman Spectroscopy is still ongoing as fluorescence of some samples is limiting the ability of the technique. Two collaborative reports by members of the ENFSI (European Network of Forensic Science Institutes) European Fibres Group (EFG) [41, 42] focused on the effect that various excitation wavelengths had on fluorescence.

The first study in 2005 was carried out on three red fibres: two acrylic and one wool. The study used Raman instruments from six different manufacturers as well as nine different laser wavelengths ranging from blue (λ=458 nm) to near infrared-NIR (λ=1064 nm). The results of this study showed that the poorest spectral quality was achieved with red lasers (λ=633 and 685 nm) and average spectra were achieved with blue (458 nm), green (514 nm) and near-infrared lasers (785, 830 and 1064 nm). The best spectra were collected with blue (488 nm) and green lasers (532 nm). However, these results could only be applied to red fibre samples. Another study [43] showed that the optimum laser wavelength for black/grey and blue cotton fibres were the 785 nm and 830 nm lasers and

that, using this wavelength, at least the major dye could be identified. From these two studies, it could be seen that the colour of the fibres has a significant impact on the laser wavelength that should be chosen. This was emphasised by the second collaborative study that was carried out in 2012 on different dyed cotton fabrics[41]. This study reinforced that having a flexible Raman instrument capable of multi-wavelengths is important in the forensic examination of fibres as different wavelengths are needed to detect different dyes in order to get the best detection limit.

1.2.3 Destructive Techniques

When fibres are unable to be discriminated using non-destructive techniques, the dye may need to be extracted from the recovered fibres and the reference fibres for dye comparison using separation techniques such as TLC, HPLC and CE. The extraction of the dyes from the fibres (using methods such as the alkaline hydrolysis of dyed wool fibres and the enzymatic digestion of dyed cotton fibres) destroys the fibre and is, therefore, the main problem when it comes to the analysis of extracted dyes. For this reason, the extraction can only be performed when there is an adequate amount of evidence fibres to allow for retesting or further analysis.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography [44] is one of the oldest methods for the separation of fibre dyes with silica gel being the most commonly used stationary phase. The advantages of this technique are that it is fast, cheap and an easy process to perform.

TLC has been found to have a higher discriminating power than both Raman spectroscopy and MSP for certain colours and textile types [45], however in general MSP has been found to be the more useful technique as it does not require the same lengthy sample preparation as TLC [45, 46]

There are several disadvantages that limit the usefulness of the technique. The first disadvantage is that there is not a universal solvent system for all the classes of dyes and so pre-screening is required before TLC is used, using up even more of the sample. There has been much research into the extraction of dye from fibres and its analysis by TLC and

schemes have been published for dyes on cotton [47, 48], wool [49] and synthetic fibres [50, 51]. The second disadvantage is that it is non-reproducible (due to the variability of the eluent system). This has two effects, the first being that a database cannot be made of R_f values for dyes and so TLC can only be used to compare fibres. The second effect is that it is a less reliable method for casework. The final disadvantage is that the technique is dependent on there being an adequate concentration of dye present in the material being examined, so short fibres or very pale fibres may be unsuitable. In some cases, extraction of dye from the fibre may also be difficult to achieve so TLC cannot be performed.

TLC also has the potential to be used to identify optical brighteners on textiles; however, there are several problems in this process, which are not present in the chromatography of dyes, which makes it an unreliable and complicated process. For example, optical brightening agents that are stilbene or oxazoyl type usually are present in the trans form, but they undergo a partial conversion to the cis form on exposure to UV light [52]. Due to these issues, the European Fibre Group established that they could not recommend this as an acceptable technique for forensic fibre analysis[53].

The problems with the use of TLC on optical brighteners include the fact that optical brightener concentration tends to be much smaller than the amount of fibre present as well as the high probability of interference from other factors such as dyes and other sources of brighteners. TLC can also only be used for the comparison of fibres with known standards of optical brighteners. However the presence of textiles such as cotton can impact the location of optical brightener spots, making comparison difficult [54, 55]

High-Performance Liquid Chromatography (HPLC)

HPLC [4, 23] is a preferred method to TLC as it exhibits better chromatographic resolution, greater sensitivity, and it can be used for quantitation. Before HPLC analysis can be performed, the dye type has to be classified using TLC extraction protocols to determine what chromatographic conditions should be used and what practical considerations should be considered (such as degradation of the dyes).

Reverse-phase HPLC has been employed to separate anionic, cationic, non-ionic, and ionic dyes while basic dyes can be separated using a system based on the ion exchange

properties of the silica stationary phase [4]. HPLC can only characterise dyes based on their retention time, and so, for more information and greater selectivity, it has to be coupled with a suitable detector. For example, a multi-wavelength detector allows for the collection of full UV–visible spectra of each dye in a mixture and allows comparison of those spectra to a database of standard dyes.

Capillary Electrophoresis (CE)

Capillary Electrophoresis [4, 56] is a relatively new technique in the analysis of fibre dyes. Early studies on the use of CE for fibre dye analysis focused on water-soluble dyes and conventional buffer systems [50]. Because of this, the technique was limited by irreproducible migration times, poor sensitivity, and the inability to separate non-ionizable dyes and so it was not commonly used. However, it was discovered that the use of micelles in the electrophoretic buffer (Micellar Electrokinetic Chromatography or MEKC) allowed for the separation of water-insoluble dyes (such as natural dyes like flavonoids and anthraquinones) [51]. When this technique was combined with a related technique, called sample-induced isotachophoresis, the sensitivity was greatly increased, the dye content of both synthetic and natural fibres could be analysed, and the sample size could be reduced to a single fibre. This greatly increased the usefulness of CE in forensic casework [57]. Further research has been performed including the development of a suitable dye extraction process and CE method to extract and analyse acid dyes from wool [58] as well as the development of a separation method for a mixture of dyes that is compatible with the CE-MS configuration and the analysis of single textile fibres [59].

Pyrolysis Gas Chromatography (Pyrolysis GC)

Pyrolysis GC [60], although a chromatographic technique, is different to TLC and HPLC. Instead of separating and analysing dyes extracted from fibres it uses high-temperature fragmentation to break apart larger polymer chains into smaller fragments which are introduced to a Gas Chromatograph (GC) for separation and characterisation. These fragments are characteristic of the larger molecule and can be used to identify and discriminate a wide range of synthetic fibre types (which, due to their similar structures, can be difficult to distinguish using other methods) [61].

The advantages of the technique are that it has good discrimination, involves minimal sample manipulation and has low microgram order of sensitivity. When samples of the same size and composition are heated at the same rate to the same temperature for the same period of time, they form the same pyrolysis products. Therefore, to ensure the reproducibility of the technique, factors such as sample size, final temperature and rate of temperature increase must be kept consistent [62, 63]. A small sample size (in the range of 10 to 150 μg [64]) is also required in pyrolysis GC so that the sample is heated uniformly and to ensure that areas of the sample do not heat further and undergo secondary pyrolysis. This small sample size is advantageous in forensic casework as the technique is not restricted by the size of sample collected at the crime scene. With smaller sample sizes, however, the analysed sample may not be representative of the entire sample and the intra-sample variability may be higher than the inter-sample variability [65]. Due to the sensitivity, high discriminating power and increasing reproducibility of this method, this technique should be revisited in the future for forensic fibre casework, though it is not being explored in this project.

Mass Spectrometry

There are many dyes used in the textile industry, and hundreds of these can appear to be the same colour, have highly similar molecular structures, virtually indistinguishable absorption spectra and identical or highly similar chromatographic retention times or electrophoretic migration times. In these circumstances HPLC, CE and Pyrolysis-GC cannot be used alone and need to be coupled with a highly sensitive and selective detector such as the mass spectrometer. The first study in this area described the identification and quantification of dyes in various matrices using thermospray HPLC-MS (TSP-HPLC-MS) [66]. Since that report, there has been much focus on the analysis of fibre dyes using HPLC coupled to a mass spectrometer through electrospray ionisation (ESI) [67-69]. The coupling of MS to CE allowed the discrimination of four types of textile fibres (acrylic, nylon, cotton and polyester) and the sensitivity of the MS detector allowed fibres as small as 2 mm to be successfully analysed [59]. Recent advances in MS of textile fibres have focused on matrix-assisted laser desorption ionisation (MALDI) time-of-flight (TOF) MS which allows the discrimination of single fibres pre-dyed with acidic and basic dyes by collecting both positive and negative ion mass spectra [58].

Inductively coupled plasma mass spectrometry (ICP-MS) is an analytical technique used to measure the concentration of trace elements [60, 70]. ICP-MS can be used on very small amounts of sample, is extremely sensitive, has very low detections limits (ppt), and is quantitative. Multi-element standards can be used to determine the concentration of several elements in each sample. ICP-MS is not normally used in the forensic analysis of fibres, though a study performed on colourless cotton fibres was able to distinguish between the cotton samples and where the cotton originated from[70]. There are two types of ICP-MS used in this study: solution ICP-MS and Laser Ablation ICP-MS (LA-ICP-MS). In solution ICP-MS the sample must be in liquid form. For fibres, this involves acid digestion of the sample. This sample is nebulised into an aerosol and carried into the plasma using Argon gas (the carrier gas). The advantages of this technique include multi-element capability, isotopic information, high sample throughput, high dynamic range and low detection limits. The disadvantages of using solution ICP-MS is that the technique is destructive, time-consuming, costly and suffers from spectroscopic interferences.

In LA-ICP-MS [70], a laser is used to ablate surface particles of the sample into a carrier gas (usually Helium) that are then transported into the plasma for analysis. As the sample can be analysed in solid form, the need for acid digestion is removed. This reduces the cost and complexity of the technique and means the technique is relatively non-destructive. The high sensitivity of the technique creates difficulty in the interpretation of the results as the variability within a fibre or textiles may be more than that between two fibres from different sources.

1.3 Interpretation of fibre evidence

Once fibres have been analysed, the forensic examiner needs to interpret the results in the context of the case.

In the interpretation and evaluation of forensic evidence, there is a hierarchy of propositions: source, activity and offence [71, 72]. A fibre examiner must determine which proposition they will frame when interpreting their evidence. The source

proposition is the first level in the hierarchy. At this level, comparison of evidence is usually performed to determine the source, and if no meaningful difference can be found between the two, it is said that the fibre could come from the same source. In the case of fibre evidence, a trace fibre found on a crime scene may be compared with a fibre from a suspect's jumper to determine whether the evidence fibre could have come from the jumper. The next proposition level is the activity level. At this level, the evidence is interpreted to find how it ended up in the location it was found and what activities this could be linked to. This involves more than just the comparison of evidence. In the case of fibre evidence, a question may be asked such as "is the type and location of the suspect fibre found consistent with a sexual assault activity" and "is there another reason this fibre may be present in this location?". For this type of analysis, often scenarios are used to investigate the transfer and persistence of fibres in different activities [2, 5-7]. The final proposition level is the offence level. This the level at which a jury would be expected to consider the case, determining whether the suspect is guilty or innocent of the offence [73].

While it can be determined that a fibre could come from a source material, the strength of this connection and the value of the evidence is dependent on many factors. According to Grieve [74], these can be divided into two categories: known and unknown facts

Facts that are known from the outset (or can subsequently be determined):

- the circumstances of the case
- the time that elapsed before collection of the evidence
- the suitability of the fibre types involved for transfer, recovery and comparison
- the extent of the information which can be derived from the evidence submitted
- what evidence might be expected in the light of conflicting hypotheses
- the number of different types of matching fibres
- whether or not there has been an apparent cross-transfer of fibres
- whether there is additional evidence of secondary transfer(s) of environmental background fibres
- the quantity of the matching fibres recovered
- the location of the recovered fibres
- the analytical techniques used to conduct the examinations

Unknown facts:

- the area, duration and pressure involved in contact(s)
- the degree of certainty that specific items were in contact, and to what extent
- the shedding and retaining potential of the textiles involved
- the frequency of occurrence of the matching fibre types.

When it comes to the forensic interpretation of fibre evidence, there are two statistical methods that are commonly used: the frequentist method and the Bayesian approach.

The frequentist method of interpretation only considers evidence from the perspective of the source level and does not consider the probability of the evidence occurring in the context of the activity level[75]. In the case of fibre evidence, it considers only the probability that two fibres can be found to be similar, even though they come from two different sources. If this probability is low, then the hypothesis that the two fibres are from the same source can be accepted. The data used to compute these probabilities come from studies done on the frequency of trace fibres as well as target fibre analysis [76-79]. These studies are conducted by examining various surfaces (such as garments, cinema seats etc.) to reveal information about what fibres can be typically found on these surfaces and their frequency. This reveals how likely it would be to find a similar fibre on a surface if it did not come from a suspect garment.

The second approach used is the Bayesian method[80]. This method puts evidence in the context of two opposing hypotheses, one supporting the prosecution and one supporting the defence. In Equation 1, H_1 is the hypothesis that the suspect is guilty and H_0 is the hypothesis that the suspect is innocent.

$$\frac{\Pr(H_1|E)}{\Pr(H_0|E)} = \frac{\Pr(H_1)}{\Pr(H_0)} \times \frac{\Pr(E|H_1)}{\Pr(E|H_0)}$$

Equation 1: Bayes Theorem [72]

Equation 1can be read as the posterior odds = prior odds × likelihood ratio. The posterior odds is the probability of the evidence (for example a fibre being indistinguishable from a suspects jumper) given that the suspect is guilty over the probability of the evidence given that the suspect is innocent. The prior odds are the probability that the suspect is

guilty over the probability that the suspect is innocent. This is not related to the evidence and is based on background data of the case. The likelihood ratio is what is calculated by forensic examiners and given to the court. The likelihood ratio is determined by finding the probability of the evidence given that the suspect is guilty over the probability of the evidence given that the suspect is innocent. The probability of the evidence given that the suspect is not guilty takes into context the activity put forward by the defence (for example a legitimate reason why the fibre may be present) as well as information including the transfer, persistence and frequency of fibres.

The size of the likelihood ratio will support the strength of the evidence for supporting the hypothesis that the suspect is guilty. Once the forensic fibre examiner has reported the likelihood ratio, the court can take that into account along with the prior odds to formulate the posterior odds of the suspect's guilt. Both the frequentist method and the Bayesian approach can be challenging to apply to fibre evidence due to the lack of information about the population and distribution of fibres.

Unlike DNA evidence, where allelic frequencies used to calculate match probabilities for DNA are known and static, there is very little calculated data for fibre evidence. This is compounded by the fact that the textile industry is frequently changing due to the economy, fashion and many other factors, and so frequency data that is collected is only reliably representative of the time that study was run. Fibre analysts can assign approximate possibilities based on their past experience and data collected using fibre population studies (estimates of the relative frequencies of different fibre types and colour combinations on particular surfaces), fibre studies (estimates of the probability of finding a specific fibre type, morphology and colour combination on a random surface) and colour block studies (the ability of an analysis sequence to discriminate between fibres of a similar fibre type and colour) [81]. The issue when it comes to the use of statistical methods above is that a number (e.g. the likelihood ratio) is been given based on a subjective opinion and an estimation of data that has a risk of being inaccurate due to the changing nature of the textile industry. The lack of databases and the impact it has on the use of statistical evaluation isn't restricted to fibre evidence alone and has been brought up as an issue several times including in the England and Wales Appeal court. [82]

1.4 Challenges in Fibre analysis.

The main challenge in fibre evidence is the mass-produced nature of fibres. Typically, fibres are compared by their shape, size, dye content, chemical composition and microscopic appearances. Each manufacturer uses different types and ratios of chemicals to make one colour dye. Research and development of instrumentation to more accurately identify minor dye constituents is ongoing, improving the ability to discriminate fibres coloured with different dyes and improve their value [4, 83]. However, the biggest limiting factor on the probative value of fibres is that the fibre type and dye are determined by the manufacturer. A manufacturer can mass produce a vast number of products which may be made of the same fibre type and coloured with the same dyes and so are unable to be differentiated using the current forensic methods. Therefore, an evidence fibre linked to a known fibre could be said to come from any number of product lines from the same manufacturer [84, 85]. As discussed, this affects the value of the fibre evidence, as the frequency and population of all fibres of that type and colour need to be considered. The larger the population of the fibres (i.e. the more common it is), the smaller the evidential value.

In forensic casework fibre evidence is predominantly used to support conclusions drawn from other evidence types and there is often not seen any need to differentiate between every fibre. However, the ability to be able to discriminate between fibres from mass produced sources is a matter that goes beyond the use of fibres in a case by case basis. The status of trace evidence is on the decline in forensic laboratories as, on a cost-benefit basis, it is often found lacking as a large amount of instrumentation and expertise is needed to analyse the evidence, resulting in often little evidential value [86, 87]. Therefore, the ability to differentiate between mass produced fibres is beneficial not only on a case by case basis, but for the status of fibre evidence as a whole.

1.5 Differentiating fibres by acquired characteristics

Due to the limitations of the current examination techniques, this project focuses on finding a complementary approach to discriminate fibres to increase their forensic value, in this case by the detection, analysis and comparison of their 'acquired characteristics'. The use of acquired characteristics to compare evidence is already widely accepted in the analysis of impression evidence such as shoe marks, tool marks and bullets where, over an object's lifetime, they acquire unique wear patterns that can aid in comparison. However, despite this, there has not been much focus on acquired characteristics of other forms of evidence. After production, textiles can also obtain acquired characteristics due to their environmental surroundings. These include things like the laundry detergents used, the person's habits (smoking, etc.) and their environment (traces they get exposed to such as metal, paint and dirt). There is currently little to no research into this area of fibre research, so it was decided to focus this project on this gap in knowledge as, to be able to identify these acquired characteristics could allow fibres to be linked to a specific source and greatly increases the probative value of the fibres.

The use of laundry detergents as an acquired characteristic became the focus of this project as there is a wide range of laundry detergents commercially available and most textiles come into contact laundry detergents at some point during their time of use. At the time this project was created, very little research had been undertaken in this area.

There is further scope for future projects to look into other forms of acquired characteristic. Research is being undertaken into the analysis of dust particles on carpet fibres. A study was released in 2013 [88] on the best method of the removal of small particles from carpet fibres. A further study [89] concluded that carpets vary widely in the types and quantities of small particle that are found on their fibres and that, given enough particles, a highly characteristic profile can be created for these particles. It was found that these particles are found on fibres from the same carpet, but are not found on fibres from other carpets. This study focused on environmental contaminants on textiles that are stationary (carpet) and likely will be difficult to apply to other textiles such as clothing which are exposed to a range of different environments.

1.5.1 Laundry detergents

Laundry detergents have been used for thousands of years to aid in the washing of clothing and textiles. Over the years, the composition of these detergents has been altered to offer a lot of different features (front and top loader, powder and liquid, fragrances, etc) and these days supermarket shelves are filled with different brands and types of laundry detergents. Laundry detergents all contain the same groups of chemicals that share the same purpose; the specific chemical chosen from each group varies between detergents. The main groups in detergents are[90, 91]:

- Surfactants
- Builders
- Anti-redeposition agents
- Zeolite
- Alkaline Agents
- Corrosion Inhibitor
- Processing Aids
- Colorants
- Fragrances
- Active oxygen bleaches
- Enzymes
- Optical Brighteners

Surfactants (or surface-active agent) are the main component of detergents and perform three major roles. They aid in the penetration and wetting of the fabric, loosen soils and emulsify them to keep them in the wash solution. Surfactants are long-chain heterogenous molecules which have one polar hydrophilic end and one non-polar hydrophobic end. This allows the surfactant to interact with both polar and non-polar substances. These surfactants come from petrochemicals, vegetable oils and animal fats and can be divided into three main categories: anionic, non-ionic and cationic surfactants

Builders are another main component of detergents and are used to build and enhance the action of the surfactants. They do this by softening the water, aiding the surfactants in

removing soil from fabrics, dispersing the suspended soils and ensuring they do not redeposit on the fabric and increasing the alkalinity of the water to assist with the dissolving of oil-based solids. Calcium and magnesium can affect the hardness of water and can also interfere with surfactants by reacting with them or by precipitating onto fabrics. Builders are chelating agents that form complexes with calcium and magnesium, reducing their effect. There are three types of builders: Sequestrating builders are water soluble and form soluble complexes and include both phosphates (e.g. sodium tripolyphosphate) and non-phosphates, (e.g. citrates, EDTA, etc). Precipitating builders, like sodium carbonate, are water-soluble builders that form insoluble complexes. Ion exchange builders are insoluble and form insoluble complexes and include zeolites and sodium disilicate.

Anti-redeposition agents prevent soil from being redeposited onto fabrics by increasing the negative charge of the surface to repel the negatively charged soil. Examples of these agents include carboxy methyl cellulose (CMC) for cotton and polyvinyl pyrrolidone for wool and synthetic fabrics.

Alkaline agents increase the pH of the water to allow for the breakdown of oily and acidic soil. Sodium carbonate and sodium silicate, among other chemicals, are used to increase the hydroxide concentration in the solution and thus to increase the negative charge of the fabric and soil, allowing more soil to be repelled.

Corrosion inhibitors, like sodium silicate, are used to prevent the corrosion of metallic washing machines, though are becoming less prevalent due to the increase in plastic and stainless-steel machines. These inhibitors work by forming a film over the metal surface, reducing the difference in charge and so preventing a galvanic cell from forming.

Processing aids are a variety of chemicals that are added to detergents to give it the correct physical properties. For example, sodium sulphate is a desiccant used in powder detergent to bind water molecules and create a free-flowing powder. Alcohols are used in liquid detergents as a solvent.

Colourants are added to detergents for mainly aesthetic purposes, allowing the detergents to be individualised and to add to their appeal. Fragrances are another chemical added for

this purpose. Fragrances are added to cover the chemical smell of the detergents as well as to make clothing smell nicer. Fragrances are one of the main features used to distinguish between product ranges in a brand.

Active oxygen bleaches oxidise stains to remove them from the fabrics and make them whiter and brighter. They are preferred to chlorine bleaches, such as sodium hypochlorite, as they can be used on dyed clothing without removing colour. In powder detergent, active oxygen bleaches are present in the form of inorganic peroxygen compounds, such as sodium percarbonate, that convert to the oxidising agent hydrogen peroxide when dissolved in water. Active oxygen bleaches oxidise stains by accepting electrons from the stain. This causes the chemical bonds in the stain to cleave, causing the stain to break down and be removed from the fabric. It can also change the oxidation state of the stain and cause it to discolour.

Enzymes break down large and complex molecules like proteins (which can be found in hard stains like blood and grass), carbohydrates and fats. Once these stains have been broken down, they can either dissolve in the water or become suspended in solution by the surfactants, aiding in their removal

Optical Brighteners (or fluorescent whitening agents) are chemicals that are added to detergents to make clothing appear whiter and brighter. These will be discussed further in 0.

Table 1-1 lists the ingredients that have been identified as being in each of the four detergents as well as their purpose. These ingredients were identified through the product websites, detergent MSDS (Figure A- 1 to Figure A- 4) and through private communication with the companies (Figure A- 5 and Figure A- 6). Of the four detergents used in this project Cold Power and Fab [92] were found to have come from the same parent company (Henkel), Radiant [93] is produced by PZ Cussons, and Omo [94] is produced by Unilever.

It can be seen that while they share some ingredients, the majority of the ingredients are different though can be recognised as coming from the same chemical family.

Table 1-1: Known Ingredients in Cold Power, Fab, Omo and Radiant detergents

Purpose		Cold Power	Fab	Omo	Radiant
Surfactant	***Anionic***	Sodium Dodecyl Benzene Sulfonate	Sodium tridecyl benzene sulphonate (linear)	Sodium dodecylbenzene sulfonate	(Linear) alkylbenzenesulfonic acid, sodium salts
	Non-ionic	C12-15 Pareth-7	n/a	C12-15 Pareth-7	C12-15 Pareth-8
	Cationic	Lauryl dimethyl benzyl ammonium chloride	n/a	n/a	n/a
Builder	***Sequestrating***	n/a	n/a	n/a	Sodium diethylenetriamine pentamethylene phosphonate (DTPMP)
	Precipitating	Sodium carbonate	Sodium carbonate	Sodium carbonate	Sodium carbonate
	Ion exchange	Sodium aluminosilicate	n/a	Zeolite	Zeolite, sodium disilicate

Purpose	Cold Power	Fab	Omo	Radiant
Anti-redeposition	Sodium anionic terpolymer	Sodium anionic terpolymer	Sodium acrylic acid/ma copolymer	Polyvinyl pyridine, Carboxymethyl cellulose (CMC)
Alkaline agent	Sodium silicate	Sodium silicate	Sodium silicate	Sodium silicate
	Sodium carbonate	Sodium carbonate	Sodium carbonate	Sodium carbonate
Corrosion Inhibitor	Sodium silicate	Sodium silicate	Sodium silicate	Sodium silicate
Processing Aids	Sodium sulphate	Sodium sulphate	Sodium sulphate	Sodium sulphate
Colorants	N/a	N/a	CI 74160.	
Active oxygen bleaches	n/a	n/a	Sodium percarbonate	
Optical Brightener	Fluorescent Brightener 71	Fluorescent Brightener 71	Fluorescent Brightener 71	Fluorescent Brightener 351
			Fluorescent Brightener 351	
Anti-foam	Anti-foam compound (not specified)	n/a	Phenylpropyl ethyl methicone	Silicone emulsion

Purpose	Cold Power	Fab	Omo	Radiant
Enzyme	Enzyme	Enzymes	Protease, amylase, mannanase, lipase	Protease, cellulase, lipase, amylase
Other			Tetra acetyl ethylene diamine (TAED) (active oxygen bleach activator	

Optical Brighteners

Optical Brighteners, which are found in most laundry detergents, are also called whitening agents or fluorescent whitening agents due to their use in many materials (detergents, paper coatings, etc.) to make items appear whiter. According to Fink [95], as many polymers absorb light in the blue range they tend to reflect yellow light giving the items a yellowish appearance. Three techniques have been used to try and remedy this effect. The first technique was bleaching of the items; however, this had the disadvantage of causing modification and damage to the fabric. The second technique was to include a blueing pigment to the fabric which causes more blue light to be reflected. However, this also caused the fabric to lose its brightness and appear greyer. The third technique and the focus of my study is the use of optical brighteners.

Optical brighteners provide optical compensation for the yellow colour. This occurs as the optical brighteners absorb light in the ultraviolet range (275-400 nm) and emit blue light (400-500 nm). These blue light waves mix with the yellow light (Figure 1-2) coming from the fabric surface to create white light (additive effect).

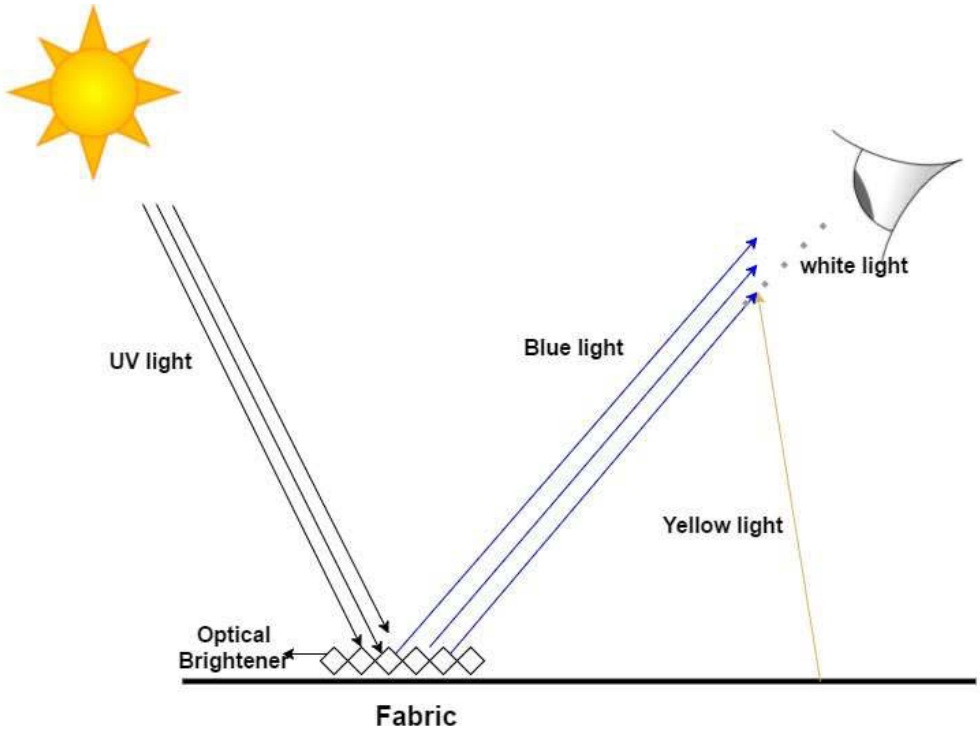


Figure 1-2: How optical brighteners work

Optical brighteners are usually added to textiles in the production process. However, they do degrade after repeated exposure to UV light and so need to be topped up through the washing process. There are said to be about 400 chemically different optical brighteners listed on the Colour Index with close to 4000 active compounds being described in literature such as patents. Nearly all of these brighteners [96] contain groups such as (E)-configured olefins (-CH=CH-, carbonyls (C=O) or (E) -azomethines (-N=CH-). These groups become UV active when they are conjugated to benzene, pyrene, naphthalene or heteroaromatic rings. When these structures are flat and rigid, fluorescence can occur.

There are several different chemical families of optical brightener that have been formulated for different purposes (Table 1-2). Only two families of optical brighteners are suitable for use in detergents: Distyrylbiphenyls and Triazinylaminostilbenes.

Table 1-2: Chemical families of optical brighteners and their purposes

Chemical Group	Subgroup	Purpose
Carbocycle	Distyrylbenzenes	Plastics and synthetic fibres
	Distyrylbiphenyls	Cellulosic fibres Detergents
	Divinylstilbenes	Polyester
Triazinylaminostilbenes	-	Cotton Regenerated cellulosic fibres polyamides detergent paper
Stilbenyl-2H-triazoles	Stilbenyl-2H-naphtho[1,2-d] triazoles	No longer marketed
	Bis(1,2,3-triazol-2-yl) stilbenes	Chlorine and chlorite stable Cottons Polyamides
Benzoxazoles	Stilbenylbenzoxazoles	Polyester Synthetic fibres (polyamide etc) Plastics (polyolefins, polystyrene)
	Bis (benzoxazoles)	
Furans, Benzo[b]furans and Benzimidazoles	Bis(benzo[b]furan-2-yl) biphenyls	Polyamides Cellulosic fibres
	Cationic Benzimidazoles	Chlorine-fast and lightfast Polyacrylonitrile Cellulosic acetate

1,3-diphenyl-2-pyrazolines	-	Polyamides Modified polyacrylonitrile Cellulose acetate
Coumarins	-	Poor lightfastness Wool Cellulose acetate Rayon Polyamide
Naphthalimides	-	Polyester Polyacrylonitrile
1,3,5-Triazin-2-yl Derivatives	-	Polyester

Fluorescent Brightener 71 (CAS 16090-02-1) or Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl) amino] stilbene-2,2-disulphonate is also known as FWA-1 (Figure 1-3). It belongs to the Triazinylaminostilbenes family of optical brighteners and has been identified as the optical brightener present in Cold Power and Fab. It is also listed as an ingredient on the Omo website [94].

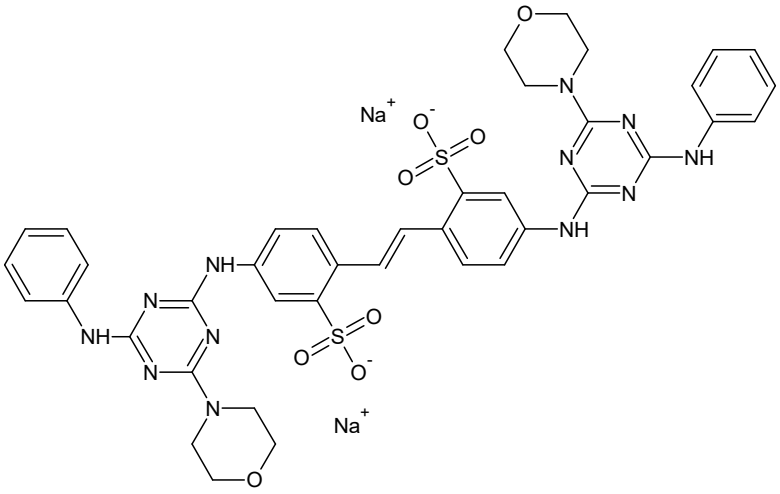


Figure 1-3: Fluorescent Brightener 71

Fluorescent Brightener 351 (CAS 27344-41-8) or Disodium distyrylbiphenyl disulfonate is a member of the distyrylbiphenyl optical brightener family (Figure 1-4). It is listed as an ingredient in both Radiant and Omo detergent.

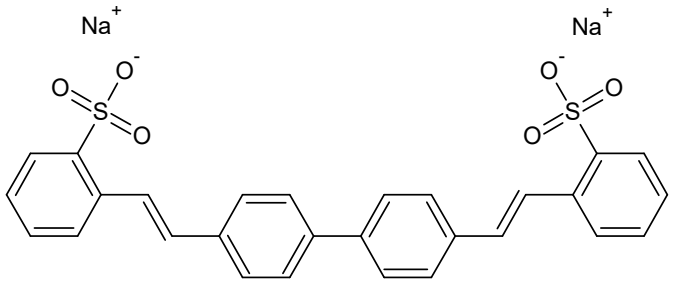


Figure 1-4: Fluorescent Brightener 351

1.5.2 Detecting laundry detergents on fabrics

The use of detergents as an acquired characteristic is something that has not been investigated in forensic science. There is also very little research on detecting detergents on textiles in either forensic science or the broader science community. There are two potential ways detergents could be detected on fibres, firstly, through the detection and chemical and elemental analysis of detergent residues on fibres and secondly, through the fluorescence of optical brightener residues.

There has been no research found to date on the chemical and elemental analysis of detergent residues on fibres. However, there is an increasing amount of research into the fluorescence of optical brighteners.

Due to the fluorescent nature of optical brighteners, the presence and quantity of optical brighteners present on a fibre can be detected using techniques such as fluorescence microscopy and fluorescence spectroscopy. Using these techniques, it may be possible to differentiate fibres based on what sort of detergent was used and how they have been worn. There have been several studies that have looked at the fluorescence of fibres caused by optical brighteners.

The first study, performed by Was-Gubala [97], looked at assessing the colour changes that occur in textiles as a result of the long-term effects of various laundry detergents. The study ran over 14 days and involved submerging blue, red and grey/black cotton, wool,

acrylic and polyester textiles in five detergent solutions. The textiles were removed daily and compared to untreated fibres both visually and using fluorescence microscopy with a UV excitation filter to determine the kinetics of the colour change. This study found that fluorescence of cotton and wool fibres increased over time due to more optical brighteners being deposited on the fibres when exposed to some, but not all, of the detergents. When it came to the two synthetic textiles (polyester and acrylic) no change was seen between treated samples and the controlled samples regardless of what detergent was used. Similar results were seen with the coloured textiles with again polyester and acrylic showing no change. From this study, it could be seen that the fluorescence of fibres due to optical brighteners could be detected, but that the effect of the optical brighteners depended on both the textile and the detergent used. A point was also raised in the article that care must be taken when comparing a fibre collected from a crime scene with one collected from a suspect. If there was enough time between the crime and the suspect's clothing being collected, they might have been washed, changing the fluorescent nature of the fibres. The focus of the study was on the kinetics of the colour change of the fibres during the washing process. The focus of the fluorescence analysis of the fibres was based on visual fluorescence, not spectral, and the comparison was based on the number of washes rather than the detergent used.

The second study was performed by Campiglia and Sigman [29]. This study focused on the use of Room-Temperature Fluorescence–Excitation Emission Matrix (RTF-EEM) Spectroscopy to discriminate fibres based on the fluorescence of the fibre dye, the fibre itself and any impurities that could be present on the fibre. While the main subject of the study was on the fluorescence of the fibre dyes, the fluorescence of acquired environmental contaminants such as PAHs (Polycyclic Aromatic Hydrocarbons) in cigarette smoke and the optical brighteners in laundry detergent was also explored. They investigated the effect of repetitive washing on the intrinsic fluorescence of fibres, the fluorescence characteristics of detergents and fabric softeners and their effects upon repetitive washing of textile fibres. Their results showed that all the detergents and softeners showed strong fluorescence upon excitation in the ultraviolet spectral region. The fluorescence spectra of the Disperse Red 4 fibre extracts showed an increase in the peak at 414 nm which increased over the washes. This peak was found to overlap with the fluorescence peak of the detergent at 431 nm, and so the increasing intensity could be

attributed to residual amounts of detergent on the fibre. As a result, it could be seen that optical brighteners do contribute to the fluorescence of fibres and that quantity of optical brightener residue present increased with the number of washes. This study only explored the fluorescence of one type of washed sample and did differentiate between samples washed in different types of detergents.

More recently research has been undertaken at the University of Central Florida on the identification of detergents for forensic fibre analysis. In one study [98] dyed acrylic, cotton, and nylon fibres were washed in detergents, and the fluorescence of the detergent on single fibres was analysed using a spectrofluorimeter attached to an epifluorescence microscope. From these results, it was found that the maximum fluorescence was reached after five sequential washes though some detergents showed a statistical difference to the unwashed control fibre after only one wash. Principal component cluster analysis was then applied to the data, and it was determined that the spectra of washed fibres were distinct from the spectra of dyed, unwashed cotton or nylon fibres, but not acrylic fibres. The lack of distinct fluorescence on the acrylic fibres was attributed to the likelihood of the optical brightener adsorbing to the fibre. The brighteners, which are derivatized sulfonated stilbenes, have a strong affinity to the cellulose structure of cotton. The polymer of the acrylic fibre, however, may be unable to form hydrogen or ionic bonds with the optical brighteners, thereby decreasing the chance of the brightener adsorbing to the acrylic fibres. It was also found that the type of dye used on the fibres can also affect the amount of fluorescence detected. On cotton fibres where sulfonated DB dye was present in high concentrations, optical brighteners had to compete for bonding sites, decreasing the amount of fluorescence adsorbed to the fibre. As the textiles were washed more and the dye depleted, fluorescence was found to increase at a faster rate.

The second study [99] performed principal component analysis of clusters of detergent pairs on fibres. It was found that eight different detergent pairs could be resolved and identified on nylon fibres while on acrylic fibres, five detergent pairs were resolved and identified. It was also discovered that detergents could still be resolved even when they contained the same optical brightener, indicating that there could be other factors (such as other detergent ingredients) that could be affecting the fluorescence of the fibres. This

study highlighted the ability to discriminate between two types of known detergents but was not shown to differentiate between a number of different detergents.

From these studies, it can be seen that optical brighteners do contribute to the fluorescence of the fibres and that the fluorescence of the fibres increases as the amount of detergent the samples are exposed to increases. Preliminary results have also been found implying that textiles can be discriminated against based on the detergents used to wash them. No studies have been conducted to holistically analyse and differentiate washed textiles based on a number of different analytical techniques, and a limited amount of work has been done on discriminating between a number of different detergents.

There have been many analysis techniques used for the commercial analysis of laundry detergents [100]. The techniques investigated in this study are chemical composition analysis using Raman spectroscopy [101] as well as elemental analysis using SEM-EDS [102] and ICP [103, 104].

1.6 Aims and Objectives of this project.

The aim of this research project was to determine whether acquired characteristics, in the form of laundry detergents, can aid in the comparison of like fibres and thus increase the evidential value of these fibres. To meet this aim, the fluorescence, elemental and chemical composition of textiles washed in laundry detergents were studied. The objectives under each of these sections are presented below.

1.6.1 Fluorescence

There were several objectives to be met when conducting the fluorescence analysis of the samples. The fluorescence analysis of the samples includes both fluorescence imaging and fluorescence spectra.

The first objective was to determine whether the fluorescence of the detergents can be detected using fluorescence microspectrophotometry and a video spectral comparator, two instruments commonly used in forensic examination.

The second objective was to then determine whether fluorescence can be used to detect the presence of detergents on textiles. In order to find out if it is viable to differentiate fibres based on the fluorescence of the optical brighteners, it is very important that it is determined whether that fluorescence can be detected. The differentiation of washed and unwashed textiles can also provide some evidential value.

Following on from this objective, samples were analysed after a successive number of washes to determine the rate at which fluorescence increases, and after how many washes does the fluorescence reaches its maximum.

The fourth objective was to determine whether different brands of detergents would result in different fluorescence imaging and spectra. This was explored to evaluate how useful the fluorescence of optical brighteners is to differentiate fibres. If it can be found that the fibres can be differentiated based on the brand of detergent used, the probative value of fibres can be greatly increased as it would allow many fibres that have the same fibre type and dyeing agent to be distinguished from evidence fibres.

The final objective was to determine whether the fluorescence of samples change after periods of time in storage. This is important to ensure that the comparison technique is used reliably and correctly. In case work, there is often a period of time between the collection of fibre evidence from a crime scene and the acquisition of a suspect garment and in this time the garment and fibre would have been exposed to different conditions which may affect their fluorescence. The usefulness of fluorescence being used for comparison relies on the fluorescence of the fibres not fading quickly or being greatly impacted by storage conditions as this would make it impossible to accurately compare fibres. Knowledge of the degradation of the fluorescence of fibres however can also provide additional information when the two fibres being compared are known to come from the same source.

1.6.2 Composition

The objective here was to investigate if the composition of washed textiles could provide information on the detergents used. Techniques used were SEM/EDS and ICP-MS (Laser Ablation and solution) to analyse the elemental composition of washed textiles while

Raman spectroscopy determined the chemical composition. For each technique, there was a series of objectives.

The first objective was to investigate whether the composition of detergents could be found using analytical methods. Before a technique is used to detect detergent residues on textiles it must first be established whether the detergent can be detected using the technique, and secondly whether any differences can be seen between the detergents.

The second objective was then to determine whether the presence of detergent residues on the textiles could be detected using each technique. This objective establishes whether there were any residues on the textiles to be detected and also whether the techniques were sensitive enough to detect the presence of these residues. The evidential value of the textiles is also increased if washed and unwashed textiles are able to be compared and differentiated.

The final objective when looking at chemical and elemental composition was to determine whether the type of detergent used could be identified based on the composition of any residues on the fibres. The identification of the brand of detergent used to wash a textile would greatly increase the evidential value of the fibres.

1.6.3 Blind Tests

The final aspect of the study is the application of the information collected through fluorescence and composition analysis to unknown samples whose washing history is unknown.

The objective is to determine whether the techniques explored in this study can be reliably used to determine whether an unknown sample has been washed or not.

The first objective is to compare the fluorescence of a blind sample to another sample of the same fibre type and colour to determine whether the blind sample can be discriminated based on its fluorescence, and from that, wherever it can be inferred if the blind sample has been washed or not.

The second objective is to compare the elemental composition of the blind samples pairs which could not be excluded using fluorescence analysis. The concentration of the elements in the two samples will be compared to determine whether the samples can be distinguished. The trend in the concentrations will also be compared to determine whether this is consistent with the sample having been washed.

Chapter 2: Materials, Equipment and Method

Chapter 2: Materials, Equipment and Method

2.1 Experimental Design

2.1.1 General Approach

In order to fulfil the objectives of this project, various textiles were washed multiple times with commercial laundry detergents. These textiles were then analysed using several different methods to determine whether the presence of the detergents could be detected on the textiles, and if the brand of the detergent used can be identified.

The results from each technique were then assessed to determine whether they add value to the forensic analysis of fibres (that is whether the information produced from the analysis is worth the time, cost and potential loss of sample). Finally, it will be decided whether a technique, or the combination of two techniques, will allow for the detection and analysis of laundry detergents as an acquired characteristic that will improve the forensic value of fibres.

2.1.2 Selection of Textiles

Two main types of textiles were chosen, blank textiles and commercial textiles. The blank textiles were textiles that had not undergone any post-manufacturing treatments, such as dying, that most commercial textiles go through. This was desirable as it meant that any fluorescence detected on these textiles came from only the laundry detergents used and not any other treatments that were applied during the textile processing. This then provided an overview of the effect of laundry detergents on textiles.

The limitation with the use of these blank textiles is that effect on laundry detergents on these untreated textiles may not be the same as the effect on commercially available textiles. The post manufacturing treatments can influence the interaction between the detergent and the fibre.

Cotton, wool and polyester were chosen as the commercial textiles as they are commonly used in the clothing industry and would provide a good overview of the effect of

detergents on the types of items that can be found in casework. All the textiles were 100% pure, not mixed blends, so the effect of the fluorescence could be tied to each textile type alone. As wool and polyester exhibited very little fluorescence after washing they provided little information in the fluorescence analysis chapter. As a result, cotton was the textile predominantly used in this study.

White, red and black textiles were originally chosen to again represent the colours seen in the clothing industry. However, the black textiles were then excluded as the colour prohibited the samples from being analysed using fluorescence spectrometry and Raman spectroscopy. Instead, pink, blue and yellow were chosen to reflect other dye colours that are used in the textile industry.

2.1.3 Selection of Detergents

Cold Power, Radiant, Omo and Fab detergents were used for this study as they are some of the most common laundry detergents sold in Australian supermarkets (based on their presence on supermarket shelves). When possible, regular laundry detergents were chosen, without any added fabric softeners or perfumes, to keep the study consistent across the detergents. Powder detergents were chosen over liquid for two reasons. Firstly, powder detergents have been found to be more popular than liquid detergents (sales of powder and powder concentrated detergents predominate in Australia in 2008 with 71.4% of the market)[105]. Secondly, powder detergent was chosen as it was easier to handle and analyse than liquid detergent due to its solid form. All detergents were top loading washing machine detergents to match the washing machine used for the study.

2.1.4 Selection of Analytical Techniques

Analytical instruments were chosen to examine two factors of the samples- their fluorescence and the composition of the samples. The focus was first placed on instruments that are non-destructive (something that is desirable when there is a small sample size) and preferably already found in forensic laboratories. A Video Spectral Comparator (VSC) and Microspectrophotometer (MSP) were chosen to assess the fluorescence of the samples for two main reasons. Firstly, little sample preparation is needed for these techniques and so it is non-destructive, and secondly, they are both

instruments that are already commonly found in forensic laboratories. VSC is commonly used in document examination [106-108] while MSP is already part of the SOP for fibre examination [13-15].

Raman spectroscopy was chosen for the analysis of the chemical composition of the textiles as it is a sensitive technique that is again non-destructive and is also found in forensic laboratories [41, 106, 109].

A Scanning Electron Microscope/ Energy Dispersive X-ray Spectroscopy (SEM/EDS) was first chosen to analyse the elemental composition of the samples as it is again non-destructive, no sample preparation was needed, and it only requires a small sample size. When this technique was found not to be sensitive enough, Laser Ablation-ICP-MS was then tried as it is more sensitive and only mostly non-destructive. Finally, solution ICP-MS was used in order to quantify the results.

2.1.5 Selection of wavelength for fluorescence analysis

The fluorescence analysis of the washed textiles sought to detect the presence of optical brightener residues on the washed textiles. Optical brighteners are chemicals that fluoresce under UV light (sunlight) and so light with a wavelength in the UV range needed to be selected. The wavelength of 365 nm was chosen for both the MSP and the VSC based. Previous research into the fluorescence of optical brighteners supported this selection [29, 97].

2.1.6 Selection of Elements for ICP-MS

The selection of elements for ICP-MS analysis was based on three factors. Firstly elements were selected that have been determined in literature to be detected in commercially available cotton [70]. Other elements were selected that were reported by the CSIRO to be in a high concentration in laundry detergents, or that were found in a large range (which could potentially be used to discriminate between detergents) [110]. Finally, the composition of the detergents used in this study (Table 1-1) was referenced and sodium was selected due to its prominence in all four detergents while phosphorous was chosen as it was only present in Radiant in the form of DTPMP. Phosphorous and

tin were removed from the selection for ICP-MS due to their absence from the standards used, while sodium, strontium and barium were added to the selection. The elements that were selected and their source are listed below in Table 2-1.

Table 2-1: Elements selected for LA-ICP-MS and solution ICP-MS

LA-ICP-MS	Source of element	ICP-MS
^{11}B	Laundry detergent	^{11}B
^{23}Na	Laundry detergent	^{23}Na
^{24}Mg	Cotton	^{24}Mg
^{27}Al	Cotton/ laundry detergent	^{27}Al
^{31}P	Laundry detergent (MSDS)	
^{55}Mn	Cotton	^{55}Mn
^{56}Fe	Cotton/ laundry detergent	^{56}Fe
^{60}Ni	Laundry detergent	^{60}Ni
^{63}Cu	Laundry detergent	^{63}Cu
^{66}Zn	Laundry detergent	^{66}Zn
^{118}Sn	Laundry detergent	
	Laundry detergent (MSDS)	23 Na
	Cotton	88 Sr
	Cotton	137 Ba

2.2 Materials

2.2.1 Textiles

Blank textiles were textiles that had not undergone any treatments or dying. These textiles were sourced from the School of Fashion and Textiles at RMIT.

Blank Textiles:

- 100% cotton
- 100% wool

- 70% viscose, 30% polyester

Dyed 100% cotton, polyester and wool textiles were sourced commercially.

Dyed Textiles:

- White, red, yellow, pink and blue 100% Homespun cotton from Lincraft
- White and Chinese Red 100% Prima Homespun cotton from Spotlight
- Red and white Stella weave polyester from Lincraft
- Red and white 100% merino wool from The Fabric Store

2.2.2 Detergents

All detergents used were commercially available detergents bought in powder form from a supermarket.

- Cold Power Regular Top Loader Laundry Powder
- Radiant No Sort Laundry Powder Front & Top Loader
- Omo Active Clean Laundry Detergent Washing Powder Front & Top Loader
- Fab Sunshine Fresh Front & Top Loader Laundry Powder

Fluffy fabric softener was also tested on some fabrics, though this softener was then excluded from the study due to lack of significant results.

2.3 Method

2.3.1 Washing

The textiles were cut into 5 x 2 cm swatches and labelled with a Dymo Letratag Labelling Machine using Dymo iron on labels. Swatches from each sample batch were washed together in a mesh laundry bag in a Fisher and Paykel top load washing machine (Table 2-2) on the “Regular” and “Cold” settings with a range of other household clothing items. These settings were chosen as a baseline as they were appropriate for all textile types chosen. These clothing items changed each wash in order to more closely represent the

washing environment of case samples and were not analysed. After each wash the swatches were removed from the mesh bag and allowed to air dry. The swatches were washed in the different detergents 2, 4, 8 and 16 times.

Table 2-2: Fisher and Paykel washing machine details [111]

Parameters	Details
Brand	Fisher and Paykel
Machine type	Top Loader
Model	GW612
Product no.	93196-A
Drum material	Stainless steel
Load size (kg)	6.5
Washing Cycle: Regular	
Wash time (minutes)	12
Wash temp	cold
Rinse	Spray rinse + cold deep rinse
Spin speed	Fast
Spin time (minutes)	6
Cycle load	medium
Cycle time (minutes)	42

2.3.2 Storage

The swatches were stored in a plastic clear file folder in a cupboard in the laboratory out of contact with the sunlight. Each sample was stored in a sleeve only with other samples that had been washed in the same detergent the same number of times to prevent cross contamination.

2.3.3 Analytical Techniques

The parameters chosen for each instrument below were chosen based on current standard operating procedures for the techniques used that are used in the university.

Video Spectral Comparator (VSC)

The control and washed samples were analysed using a Foster + Freeman VSC6000/HR. Photos were taken at 4x and 61x magnification using both UV (365 nm) and a white light source. Using the VSC software, fluorescence spectra were collected at 61x magnification from three different points of two samples (that were washed at the same time), and the average spectra were found. Before each sample, a dark reference was collected by the VSC.

Fluorescence Microspectrophotometer (MSP)

Two fibres from different areas of each textile swatch were removed and placed on a microscope slide on a 20/20 PV™ Craic Microspectrophotometer. A Xenon arc lamp with a 365 nm filter was used to measure the fluorescence of the samples in the reflectance mode. Fluorescence spectra were collected from five points along the two fibres (five spectra total) and the average of those spectra were found using the CRAIC software. The exception was in the case of the red cotton fibres where collection of spectra was targeted to only the sections of the fibres that were visually found to fluoresce. The parameters chosen for the microspectrophotometer are shown in Table 2-3. The sampling time was chosen based on auto calibrations performed by the instrument.

Table 2-3: Parameters for the 20/20 PV™ Craic Microspectrophotometer

Parameters	Option selected
Lamp	Xenon Arc
Filter (nm)	365
Limits	300-900
Scans to average	20
Sampling time (ms)	2000
Resolution factor	5

Raman Spectroscopy

A 1x1 cm portion of the sample was placed on a glass microscope slide under a Renishaw inVia Raman microscope (Table 2-4). Each sample was analysed at 100x magnification and data collection was done on the Renishaw Wire 2.0 software. Five Raman spectra were collected from each sample. As the Wire 2.0 software does not allow the average spectra to be collected and all spectra collected were found to be the same for each sample, the clearest spectra were selected. Baseline correction and smoothing were applied to each spectrum. The 785 nm wavelength laser was chosen over the 655 nm laser for analysis as it has been identified in literature to produce less fluorescence (see section 1.2.2)

Table 2-4: Parameters for Renishaw inVia Raman

Parameters	Option Selected
Laser type	Diode
Laser wavelength (nm)	785
Exposure time (s)	10
Accumulations	2

Scanning Electron Microscope/ Energy Dispersive X-ray Spectroscopy (SEM/EDS)

Control and washed samples were analysed using a Zeiss Evo SEM/EDS (Table 2-5). A 1 cm^2 portion of each textile was cut out, adhered to a stub and was then mounted. Images were taken at 200x and 2000x magnification using a variable pressure detector. On some textiles, additional images were taken using a back-scatter detector. Elemental data was then collected for each sample at a 2000 x magnification. No repeats were analysed for this technique as it was determined that it was unable to produce useful information.

Table 2-5: Parameters for Zeiss Evo SEM/EDS

Pressure	Variable pressure
EHT (kv)	10
Gas	air
Detector	VPSE G3
I Probe	6.7 nA
Chamber	110 pA

Laser Ablation- Inductively Coupled Plasma- Mass Spectrometry (LA-ICP-MS)

Agilent 7500Ce ICP-MS with a New Wave Large Format Laser Ablation Cell was used to perform LA-ICP-MS. 1 x 1 cm swatches were cut from the samples and placed on a glass slide on the new wave laser stage. A NIST SRM 612 glass standard was measured at the beginning of each run to tune the machine and allow for sample correction post analysis. The whole of each sample was ablated in order to find an elemental composition representative of that particular swatch. A repeat was performed of the white cotton control sample to determine the repeatability of the technique. Due to the large amount of variation found in this technique, the lack of repeatability and the limited information acquired compared to the time needed for each analysis, further repeat samples were not analysed. The parameters for the laser ablation instrument are shown in Table 2-6 while the parameters for the Agilent 7500Ce ICP-MS are shown in Table 2-7.

Table 2-6: Parameters for the LA New Wave Software

Parameter	Option Selected
Light	Coax
Shot type	Continuous
Laser rate (Hz)	10
Laser spot size (μm)	100
Laser output (%)	30
Passes	1
Scan speed (μm/sec)	300
Washout delay (sec)	20
Laser warmup time (sec)	10
Rep rate (Hz)	20
Depth (μm)	10

Table 2-7: Parameters for the ICP-MS software

Parameter	Options selected
RF power (W)	1350
Acquisition mode	Time Resolved Analysis
Integration time per point	0.1
Carrier gas flow (L/min)	2.0
Carrier gas	Argon
QP Bias (V)	-8
Oct P Bias (V)	-12

Solution Inductively Coupled Plasma- Mass Spectrometry (ICP-MS)

One of each cotton and wool sample were first digested then analysed using an Agilent 7500 series ICP-MS. Repeats of the same digested white cotton control sample were performed to investigate the variability and thus the repeatability of the technique. Two more samples for each textile and detergent type were then freshly washed 16 times to be used as repeats to further investigate repeatability. During that time, the Agilent 7500 series ICP-MS experienced instrumental problems and was unable to be repaired. As a result, the analysis of the repeat samples had to occur on a new instrument, the Thermo iCAP RQ ICP-MS. Validation of the Thermo ICP-MS was conducted by running the same sample on three different dates.

Preparation of samples

0.2 g of each cotton and wool sample was placed in a digestion tube along with 5 mL of 68 % HNO_3 (baseline ultrapure acid from Choice Analytical. LOT number: 1214070) and digested in a CEM Mars 6 Microwave digestion unit using the Nylon One Touch Method (see Table 2-8). The blank mix and polyester textiles were unable to be fully digested and so were excluded from this analysis. 1 mL of the digested sample was then diluted to 10 mL with ultra-pure water.

Table 2-8: Mars 6 Nylon Heating Method

Stage	1
Temperature (°C)	200
Ramp (mm:ss)	15:00
Hold (mm:ss)	15:00
Pressure (psi)	800
Power (W)	900-1050
Stirring	Off

Preparation of standards

The ICP-MS calibration standard used was catalogue no. ICP-MSCS-M from Choice Analytical. This was a multi-element standard containing 10 µg/mL standards in a 2 % HNO_3 + Tr HF solution. 1, 200, 400, 600, 800 and 1000 ppb standards were made up in 6.8% HNO_3 acid (diluted from the 68% HNO_3).

ICP-MS Instrument

An Agilent 7500 series ICP-MS was used to analyse the first round of samples (Table 2-9).

Table 2-9: Agilent 7500 ICP-MS parameters

Parameter	Options selected	
RF power (W)	1550	
Acquisition mode	Spectrum	
Integration time per point	0.10 (0.30 for Ni)	
Carrier gas flow (L/min)	0.90	
Carrier gas	Argon	
QP Bias (V)	-3	
Oct P Bias (V)	-6	
Detector	Auto	
Peak Pattern	Full Quantification	
Repetition	10	
Elements selected	^{11}B ^{23}Na ^{24}Mg ^{27}Al ^{55}Mn ^{56}Fe	^{60}Ni ^{63}Cu ^{66}Zn ^{88}Sr ^{137}Ba

Due to problems with the Agilent ICP-MS, the Thermo iCAP RQ ICP-MS was used to analyse all repeat samples (Table 2-10).

Table 2-10: Thermo iCAP RQ ICP-MS parameters

Parameters	Option selected
Analysis mode	STD
Dwell time (s)	0.01
Uptake (s)	Min: 30, Max:300
Wash (s)	Min: 30, Max:300
CCT entry lens (V)	-54
Deflection Entry Lens (V)	-35
Extraction Lens 1 Polarity (V)	0
Spray Chamber Temperature (°C)	2.7
Peristaltic Pump Speed (rpm)	10
Cool Flow (L/ min)	14
Sampling Depth (mm)	5
RF Power (W)	1550
Auxilliary Flow (min^{-1})	0.8
CCT Bias (V)	-2
Pole Bias (V)	-1
Repetitions	3
Elements selected	^{11}B ^{60}Ni ^{23}Na ^{63}Cu ^{24}Mg ^{66}Zn ^{27}Al ^{88}Sr ^{55}Mn ^{137}Ba ^{56}Fe

2.3.4 Data Analysis and Treatment

Fluorescence

Fluorescence images of washed samples were visually compared to those of the control textiles and those of textiles washed in other detergents to identify differences in the colour, shade, distribution and intensity of the fluorescence detected.

Fluorescence spectra of the samples were compared to each other to find differences in the shape, location and intensity of the fluorescence peaks.

Covariance Principal Component Analysis was performed on the fluorescence spectra (three spectra for each sample) using Minitab 10 statistical software to determine whether there was any separation or clustering of the samples based on whether they had been washed and the detergent used. One limitation with the use of PCA on this data is the small sample size that the analysis is being applied to.

Raman Spectroscopy

Raman spectra were collected for each sample. The spectrum of each washed sample was then compared to the spectrum of the control sample in order to see whether there was any variation in the peaks. This not only includes determining whether there were any new peaks appearing in the sample spectrum, but also whether any peaks were disappearing or changing in size, as well as any change in the appearance of the spectra themselves. PCA analysis was chosen to not be applied to this data as, firstly, there was little to no visual difference in the Raman spectra collected. Secondly, the fluorescence of many of the samples would have resulted in separation of samples based on these fluorescence peaks rather than the sample and its acquired characteristics.

SEM/EDS

Images of the samples taken with the SEM were examined to determine whether visible evidence of the detergent residues could be seen. The elemental composition was

measured using the EDS and the washed samples were compared with the control samples to determine if the elements from the detergents used have been detected.

LA-ICP-MS data

For each ablation line, the average intensity for each element was found. The average and standard deviation of these values were then found across a sample. To scale the intensities collected on each run so they could be compared, the average intensity of each element of the NIST Glass standard in that run was also calculated. The sample average intensities were then divided by the glass intensities collected in the same run, then multiplied by the glass intensities measured on the 15 July 2016.

The corrected intensities of the control and washed samples were then compared in order to identify trends in elemental intensities.

ICP-MS data

Concentrations and standard deviations were calculated for each sample using the ICP-MS software and calibration standards. The undiluted concentration and standard deviation were then found for each sample by dividing by their dilution factor (calculated during sample preparations). Each concentration was then mass-corrected by dividing by the mass of sample digested and multiplying by 0.2 g.

The corrected concentrations of each sample and their standard deviations were then compared. Further analysis involved finding the average of each element concentration in each sample and then subtracting the control sample element concentrations from the washed sample concentrations to determine if elements increased or decreased after washing.

Repeatability of both the Agilent and Thermo ICP-MS were investigated by analysing the same sample aliquot on different runs and calculating the % RSD (Relative standard deviation) for each element.

Principal Component Analysis (PCA) was performed on the solution ICP-MS data set using the Minitab 10 statistical software. Covariance PCA was performed on the majority

of the samples, but correlation PCA was performed on the blank wool and white wool data as it created a better separation of the score plots. This analysis was not performed on the LA-ICP-MS data as the results were not reproducible.

Blind Samples

Eight pairs of samples (around 0.01 g each) were selected from existing washed and control samples (samples analysed throughout the study) by my supervisor. Each pair of samples consisted of fibres that were known to be from the same raw material before washing (i.e. same fibre type, colour and dye).

Each sample was analysed first using fluorescence microspectrophotometry to determine if the fluorescence of the fibres indicated whether they had come into contact with optical brighteners. Five spectra were collected from three different fibres and averaged. All fibres were viewed under UV light to see if their visual fluorescence was consistent. If the blind sample pairs had different fluorescent characteristics, it was concluded that they could be excluded as having come from the same source material.

Blind sample pairs that could not be distinguished were then analysed using ICP-MS. Samples were prepared by dissolving the sample fibres in 2 mL 68 % HNO_3 (baseline ultrapure acid from Choice Analytical) and digested in a CEM Mars 6 Microwave digestion unit using the Nylon One Touch Method as used in (see Table 2-8). The dissolved fibres were then transferred to a 10 mL sample tube and diluted by 1/5. The samples were run on the Thermo iCap RQ ICP-MS using the parameters in Table 2-10. The dilution factors of each sample were used to find the original undiluted concentration for each sample, and the values were mass-corrected to 0.01 g.

The elemental concentrations of each blind sample pair were compared to determine whether there was any significant differences and, if so, whether the trends in these differences indicated whether one fibre had been washed or not and thus whether they could be excluded as having come from the same source.

Chapter 3: Fluorescence Analysis

Chapter 3: Fluorescence Analysis

3.1 Introduction

Video Spectral Comparator (VSC) and a UV Microspectrophotometer (MSP) were used to measure the fluorescence of both control and washed samples to determine whether the fluorescence caused by optical brighteners in detergents could be detected on fibres. Once this was established, the fluorescence of samples washed 16 times in different detergents was compared to determine whether the brand of detergent has an impact on the fluorescence of the sample. Overall the primary objective was to investigate if detergent can impart acquired characteristics that can be detected by fluorescence examination.

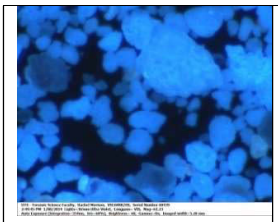
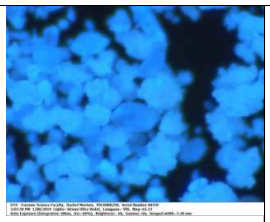
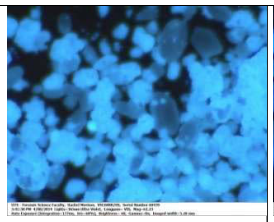
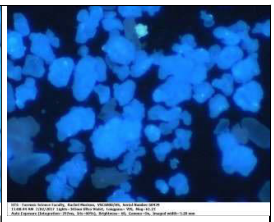
3.2 Video Spectral Comparator (VSC)

3.2.1 Results and Discussion

Fluorescence images

Fluorescence of detergents

Table 3-1: Fluorescence images of Radiant, Cold Power, Fab and Omo powder detergent

			
Radiant	**Cold Power**	**Fab**	**Omo**

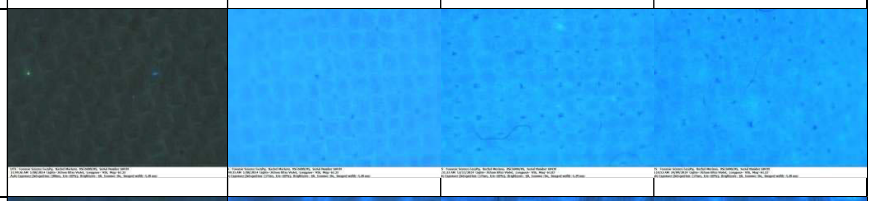
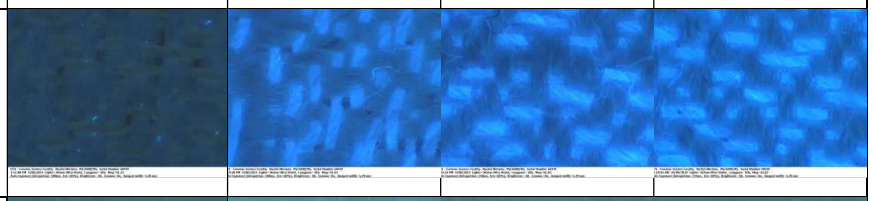
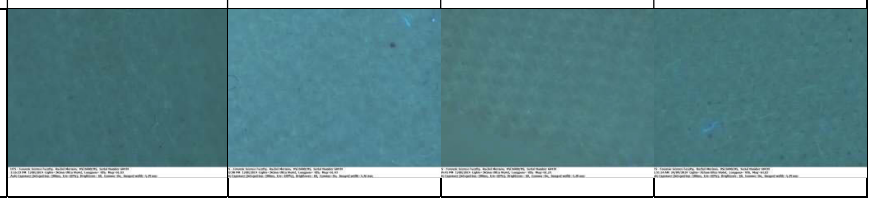
Four powder detergents (Radiant, Cold Power, Fab and Omo) were all viewed under UV light to determine whether the fluorescence from the optical brighteners in the detergent

could be detected. In all four samples (Table 3-1), a significant amount of fluorescence was found, with the fluorescence found to be more concentrated in some granules of the detergent than others. This showed that there is a significant amount of optical brighteners present in the sample, but it is not evenly distributed.

All samples were found to fluoresce the same blue colour and, as a result, the brand of the detergent cannot be identified visually. This also indicates that fluorescence will likely not be able to be used to identify the brand of detergent used to wash the textiles with qualitative visual analysis.

Fluorescence of detergents on blank textiles

Table 3-2: Fluorescence images of blank cotton, viscose/polyester and wool sample controls and washed in Radiant, Cold Power and Fab

Textile	Control	Radiant	Cold Power	Fab
Cotton				
Viscose/ Polyester				
Wool				

Blank samples (ones that had no pre-treatment) were washed 16 times with three different detergents. Examination of these samples under UV (365 nm) light (Table 3-2) showed that, in all samples, little to no fluorescence was detected in the control (unwashed) samples.

The cotton samples all showed an intense blue fluorescence after being washed in each of the three detergents: Radiant, Cold Power and Fab. This fluorescence was consistent across the textile.

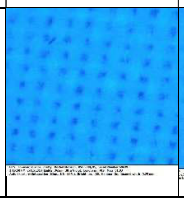
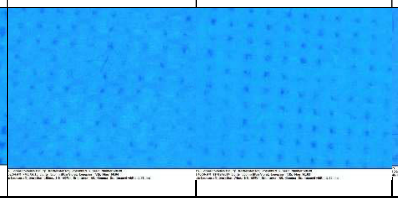
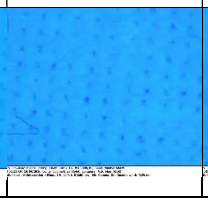
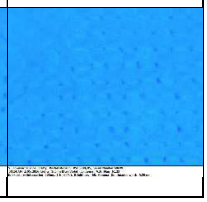
The viscose/polyester blended textile is made up of interlocking viscose and polyester fibres. The images in Table 3-2 show that only the horizontally woven viscose fibres fluoresce, while no fluorescence can be seen on the polyester fibres. This indicates that the type of fibre does affect how optical brighteners attach to the surface of the fibres, supporting the conclusions from other research that showed that optical brighteners have a high affinity to cellulose but may be unlikely to form ionic or hydrogen bonds to acrylic fibres [98].

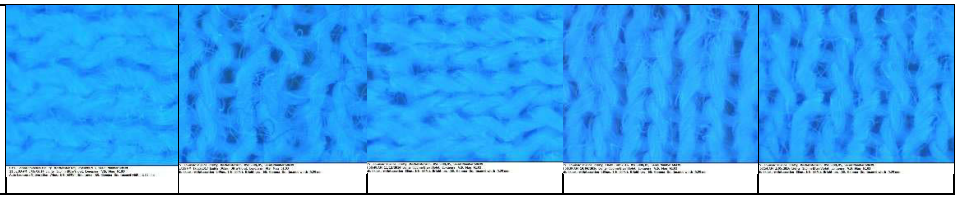
The blank wool sample showed no fluorescence after being washed in any of the three detergents, again indicating that the textile type significantly impacts how optical brighteners interact with the fibres.

From these results, two main observations can be made. The first is that, of the three textiles (cotton/ viscose/polyester and wool) examined, only cotton-based textiles exhibited any visual fluorescence after washing. Secondly, the hue and intensity of the fluorescence appear to be consistent across all three detergents. As a result of this, the identity of the detergent used is unable to be determined through visual examination; it can only be established, in the case of cotton and the polyester/viscose mix, whether the textile has been washed or not.

Fluorescence of detergents on white textiles

Table 3-3: Fluorescence images of white cotton and wool sample controls and washed in Radiant, Omo, Cold Power and Fab

Textile	Control	Radiant	Omo	Cold Power	Fab
Cotton					

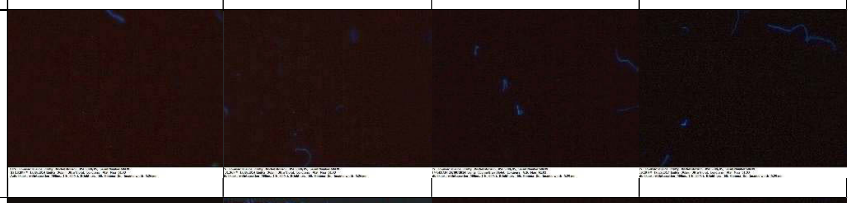
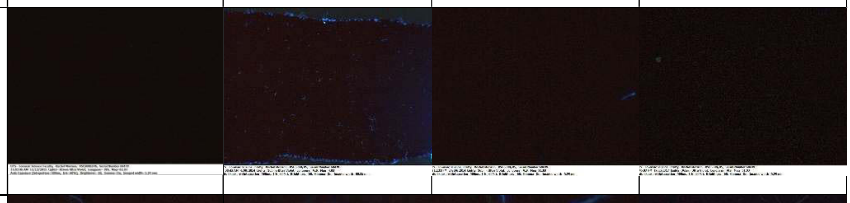
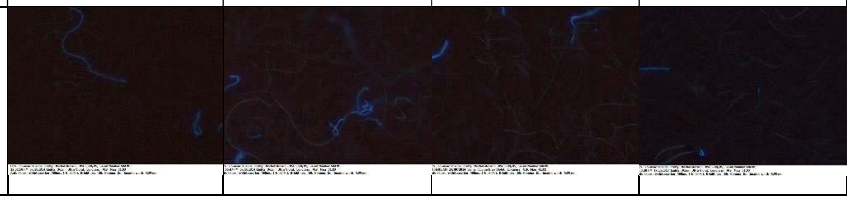
Wool	

When control samples of white cotton and wool were viewed under UV light, both fluoresced a blue colour that was indistinguishable from the fluorescence emitted by the washed blank cotton samples in Table 3-2. There is also no difference in the colour or intensity of the fluorescence of the white samples washed in Radiant, Omo, Cold Power and Fab detergent compared to the fluorescence of the control samples.

This indicates that commercially available white textiles are already pretreated with optical brighteners as part of the treatment process. Due to this, no information about the washing history of white samples can be determined by performing visual fluorescence analysis using a VSC.

Fluorescence of detergents on coloured textiles

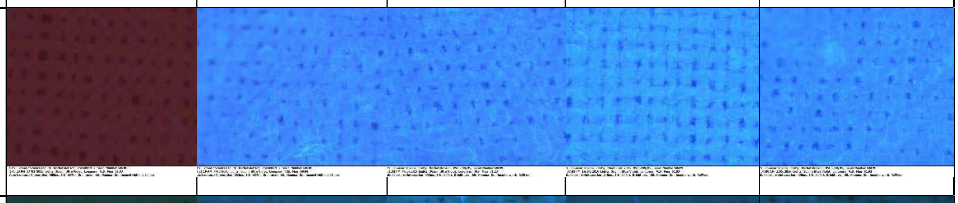
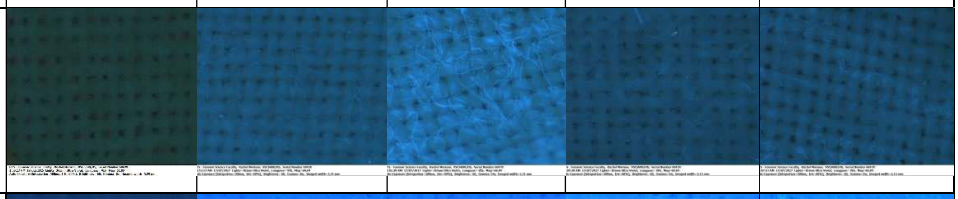
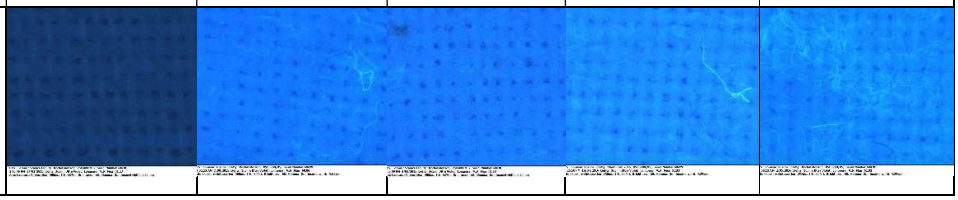
Table 3-4: Fluorescence images of red cotton, polyester and wool sample controls and washed in Cold Power, Fab and Radiant

Textile	Control	Cold Power	Fab	Radiant
Red Cotton				
Red Polyester				
Red Wool				

When red cotton, polyester and wool samples were washed with Fab, Cold Power and Radiant powder detergents, there was no visual change in the appearance of the samples

under UV light at all. In Table 3-4, all the samples appear virtually black under excitation of light at 365 nm. The only fluorescence that is seen is on a few fibres on the surface of the washed textiles, which are likely present due to contact with other textile surfaces throughout the fabrics' lifespan. These results, therefore, show that optical brighteners have no effect on red dyed textiles at the textile level, and so will not be able to be used to differentiate textiles.

Table 3-5: Fluorescence images of pink, yellow and blue cotton sample controls and washed in Radiant, Omo, Cold Power and Fab

	Control	Radiant	Omo	Cold Power	Fab
Pink Cotton					
Yellow Cotton					
Blue Cotton					

After identifying that optical brighteners had no effect on darkly dyed textiles, cotton samples that were dyed in lighter colours (pink, yellow and blue) were then analysed and the images are shown in Table 3-5. The pink cotton control sample showed no signs of fluorescence under UV light. However, both the yellow and blue cotton controls showed a small amount of blue fluorescence.

The pink cotton samples that were washed 16 times in Radiant, Omo, Cold Power and Fab detergent showed a substantial increase in fluorescence with the samples appearing fluorescent blue under UV light with none of the original pink colour remaining. This fluorescence was also consistent across the textile.

The yellow cotton sample also showed an increase in fluorescence after being washed in the various detergents, but not to the same degree as the pink cotton. While a slight fluorescent blue glow could be seen across the textiles, the samples remained predominantly dark.

The blue cotton washed samples, like the pink samples, showed a significant increase in fluorescence compared to the blue cotton control sample. Again, this fluorescence was consistent across the sample.

The four detergents, Radiant, Omo, Cold Power and Fab, visually had the same effect on the three dyed samples and so cannot be differentiated. The difference between the intensity of the fluorescence of the control and the washed samples was significant enough that a control and a washed textile would be able to be differentiated. Care should be taken when comparing the yellow cotton samples, as both the control and the washed samples had low-intensity florescence which is difficult to visually distinguish reliably.

Change in fluorescence of textiles over washes

Table 3-6: Fluorescence of blank cotton, wool and viscose/polyester after being washed in Fab 2, 4, 8 and 16 times

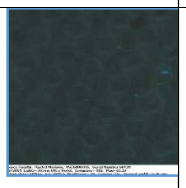
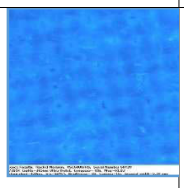
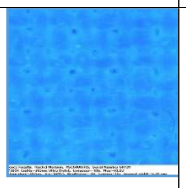
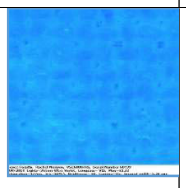
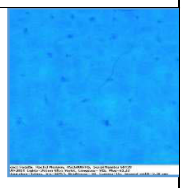
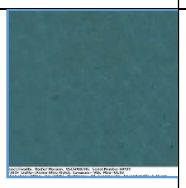
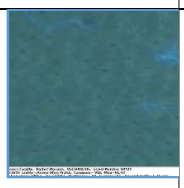
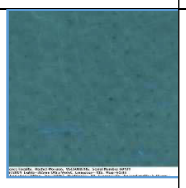
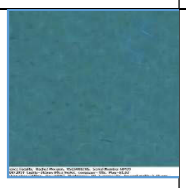
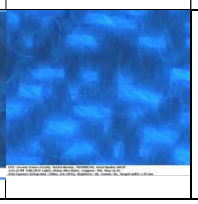
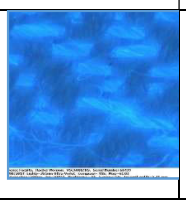
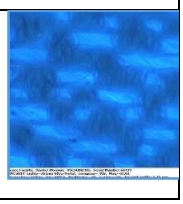
Textile	Control	2x	4x	8x	16x
Blank Cotton					
Blank Wool					
Blank Polyester / Viscose					

Table 3-6 shows fluorescence images of blank samples that were washed 2, 4, 8 and 16 times.

The blank cotton sample showed an increase in fluorescence from the second and fourth wash; however, from 4 washes to 16 washes there was no increase or change in fluorescence observed.

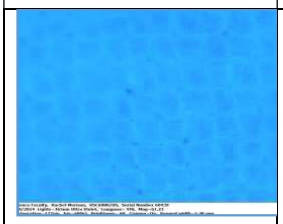
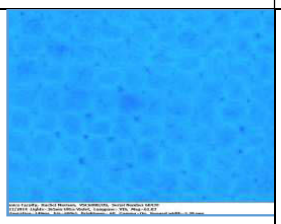
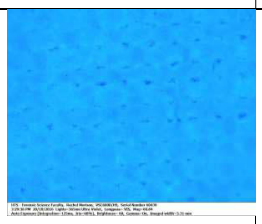
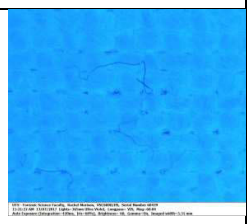
Similar results were seen in the blank polyester/viscose sample, where a small amount of fluorescence was seen on the viscose fibres after two washes, with this fluorescence increasing after four washes, but staying the same from four to 16 washes.

The blank wool samples showed no obvious signs of fluorescence after 16 washes.

These results show that, after two washes, the intensity of the fluorescence is not indicative of the number of times the material has been washed and so is not able to provide information about the materials washing history. However, it indicated that the intensity of fluorescence could be used to discriminate a textile that has been washed less than two times from one that has been washed more than four times. However this would need to be thoroughly tested and validated.

Change in fluorescence over time

Table 3-7: Fluorescence of blank cotton washed in Cold Power after three months and two years in storage

Initial	3 months	2 years	3 years
			

The change in fluorescence of a sample after being kept in storage was observed and recorded in Table 3-7. This comparison was performed to determine whether there was a decrease or degradation in the fluorescence of samples that could interfere with the comparison of textiles that could not be immediately analysed.

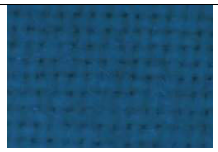
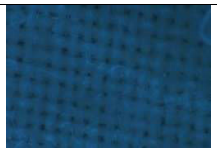
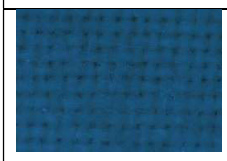
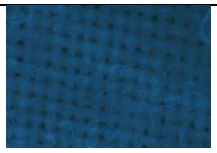
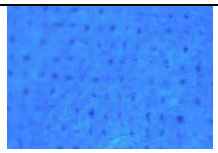
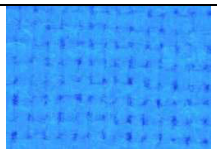
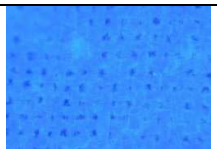
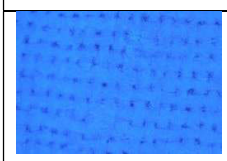
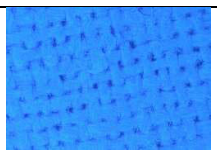
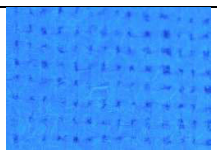
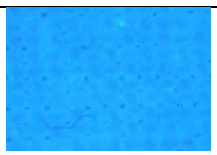
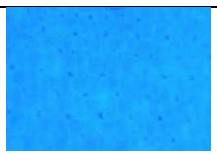
The same blank cotton sample that had been washed 16 times in Cold Power detergent was examined after being kept in storage for three months, two years and three years and these images were compared to the initial images collected.

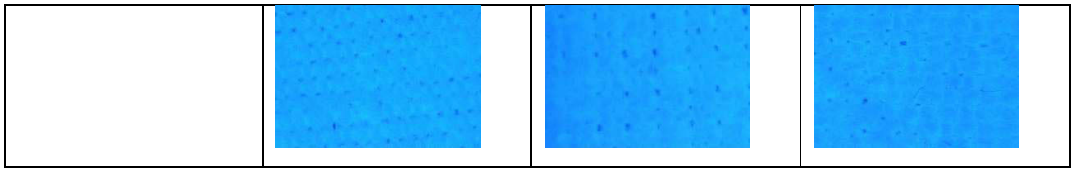
From these results, it can be seen that there was no change in the visible fluorescence observed after three years in storage, in either the intensity or the coverage of the fluorescence.

These results show that samples can be analysed after at least three years in storage in dark conditions without affecting the results.

Repeatability

Table 3-8: Fluorescence images of repeats of yellow, pink and blank cotton

Cotton	Radiant	Cold Power	Fab
Yellow			
			
Pink			
			
Blank			



Visual comparison of the fluorescence images of repeats of yellow, pink and blank cotton was performed to determine the repeatability of this technique. The fluorescence images were taken on different dates and the samples were washed in separate batched. Table 3-8 shows that there can be no difference seen visually in the colour, intensity and distribution of the fluorescence of the samples. As a result it can be determined that the fluorescence images of the washed textile samples can generally be determined to be repeatable.

Fluorescence Spectra

Fluorescence of controls

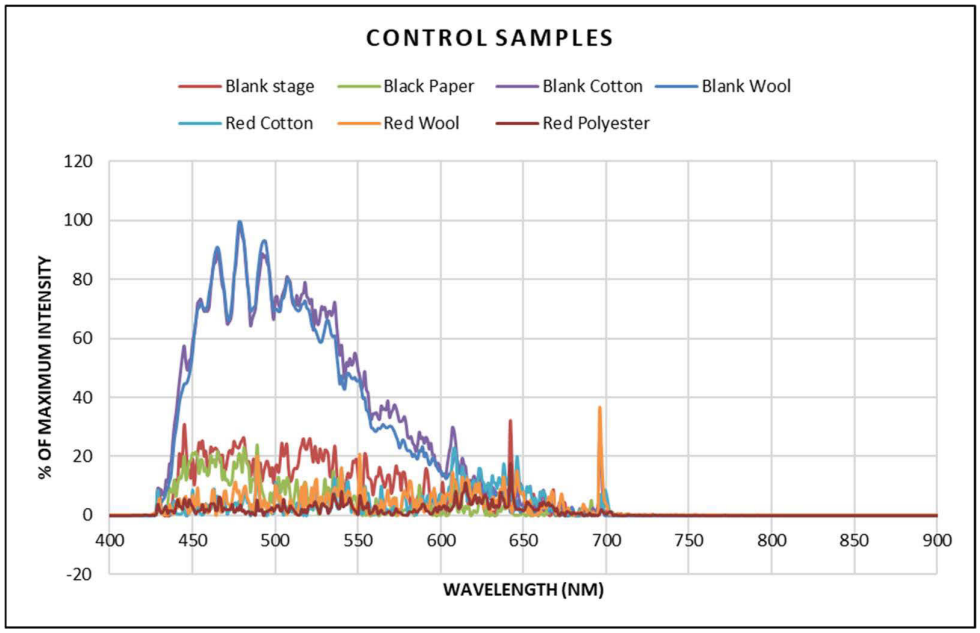


Figure 3-1: Fluorescence spectra of control samples

Fluorescence spectra were collected of various control samples, black paper and of a blank stage to determine what fluorescence peaks are consistent across all spectra and so can be determined to be instrumental artefacts rather than from the samples themselves (Figure 3-1). From the spectra, it can be seen that there are many small peaks present in

all the samples, as well as when nothing is on the VSC stage. The most prominent peaks that can be found in both the nothing spectrum and the blank cotton and blank wool control spectra are 445, 455, 466, 477, 498, 506, 520, 536, 550 and 572 nm. As a result, the presence of these peaks in any sample spectra cannot be attributed to the textile or to any acquired characteristics, but only to the instrument used. It can also be noted that, even when no fluorescence is detected, a noisy spectrum is produced with low intensity peaks. The blank cotton and blank wool samples also produce prominent fluorescence peak between 450 and 650 nm. From this it can be seen that many of the control samples produce fluorescence, even without coming into contact with optical brighteners in detergents. The shape and wavelengths covered by the control fluorescence spectra will therefore need to be compared to any spectra collected from washed samples to recognize if a change has taken place. The red textiles, on the other hand, only produce noise spectra, and therefore the spectra of the washed samples will be examined for an increase in the intensity of the fluorescence peaks.

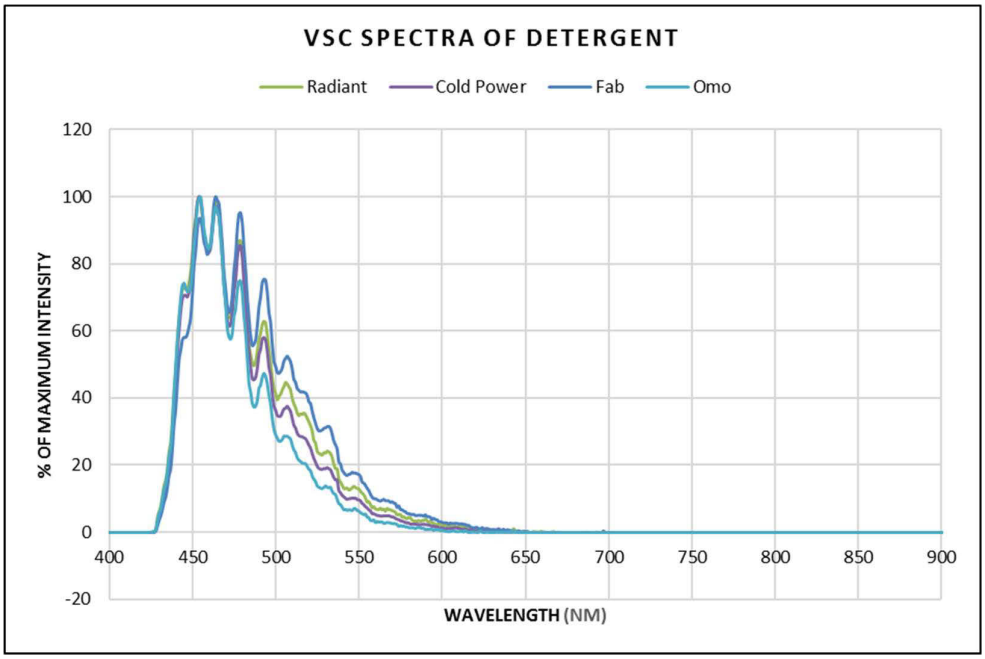


Figure 3-2: VSC fluorescence spectra of Fab, Radiant, Cold Power and Omo detergent powder

Collecting fluorescence spectra of the samples allows the colour and the intensity of the fluorescence of the optical brighteners to be objectively compared to determine whether they fluoresce the same colour and if this can be used to differentiate them.

Figure 3-2 shows the fluorescence spectra collected for Fab, Radiant, Cold Power and Omo detergents and shows that they all exhibit the same fluorescence peak structure. This indicates that all three detergents use optical brighteners that fluoresce the same colour. The wavelength range covered by all four detergent spectra (400-600 nm) is consistent with the detergents fluorescing a blue colour. This coincides with both the visual results in Table 3-1, as well as what is known about how optical brighteners work (section 0).

Four prominent peaks were found at 455, 465, 479 and 494 nm with smaller peaks at 444, 508, 519, 532, 550 and 572 nm. These peaks are consistent with the instrument artefact peaks that were discussed previously and so cannot be used to discriminate between the four detergents.

Fluorescence of detergents on blank textiles

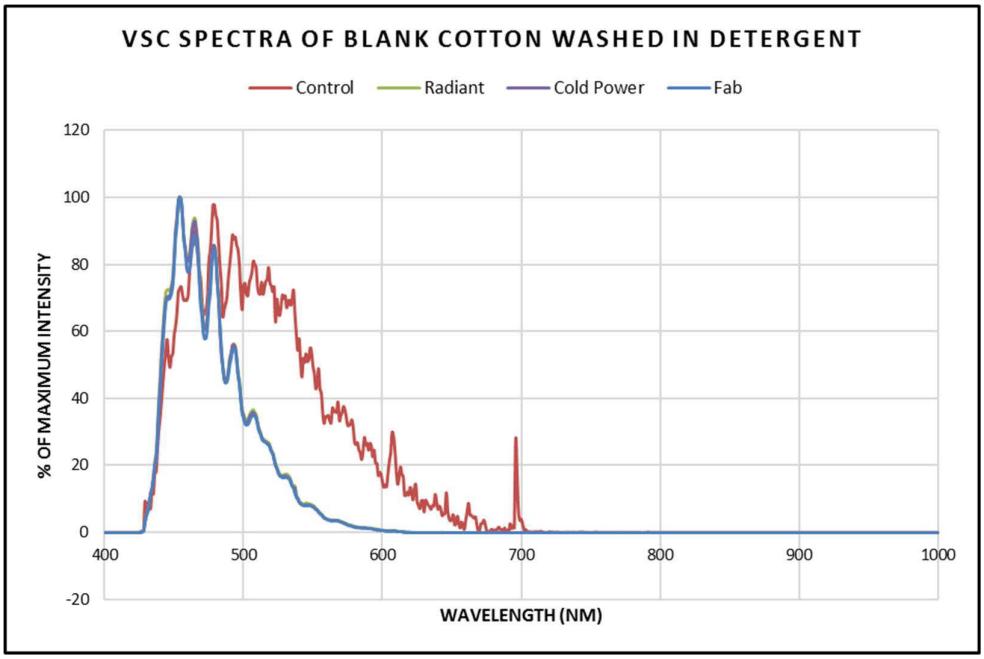


Figure 3-3: VSC fluorescence spectra of blank cotton washed in Radiant, Cold Power and Fab detergent powder

In Figure 3-3 it can be seen that the fluorescence spectrum of the blank cotton control and the washed blank cotton samples are significantly different.

The fluorescence spectrum for the blank cotton control sample shows peaks that range between 430 and 700 nm comprised predominantly of minor peaks. The samples washed

in a detergent exhibit the same fluorescence spectra as each other; these spectra are also identical to the fluorescence spectra for the Fab, Radiant and Cold Power detergents seen in Figure 3-2.

While the blank cotton control spectrum share instrument peaks in common with the washed blank cotton spectra, with peaks at 444, 455, 465, 479, 494 and 508 nm, the rest of the spectrum deviates enough that the fluorescence spectrum of the control and washed samples can be differentiated.

However, as the washed blank cotton samples have the same spectra, it cannot be determined using fluorescence spectra which detergent was used to wash the blank cotton.

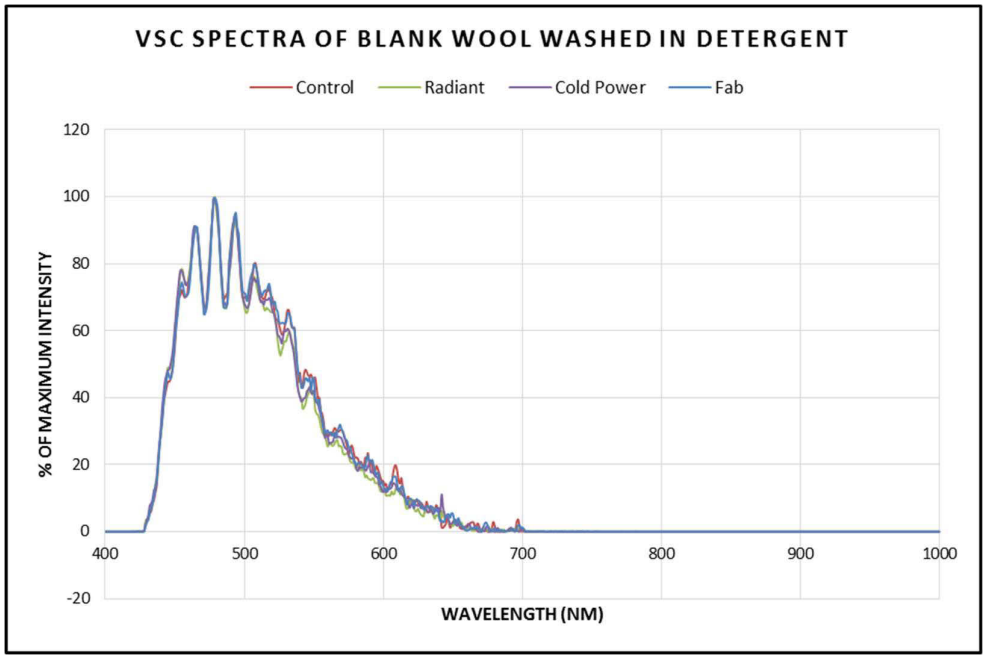


Figure 3-4: VSC fluorescence spectra of blank wool washed in Radiant, Cold Power and Fab detergent powder

Both the washed and control blank wool samples in Figure 3-4 have the same fluorescent peak structure. All four samples show a broad fluorescence peak ranging between 400 and 700 nm. Major peaks at 454, 466, 480, 493, 508 and 533 nm can again be attributed to the VSC instrument. This is consistent with the fluorescence images seen in Table 3-2 that showed that no fluorescence was observed in any of the wool samples.

As no fluorescence can be detected, the fluorescence of optical brighteners cannot be used to discriminate between textiles, or to determine whether a wool textile has been washed or not.

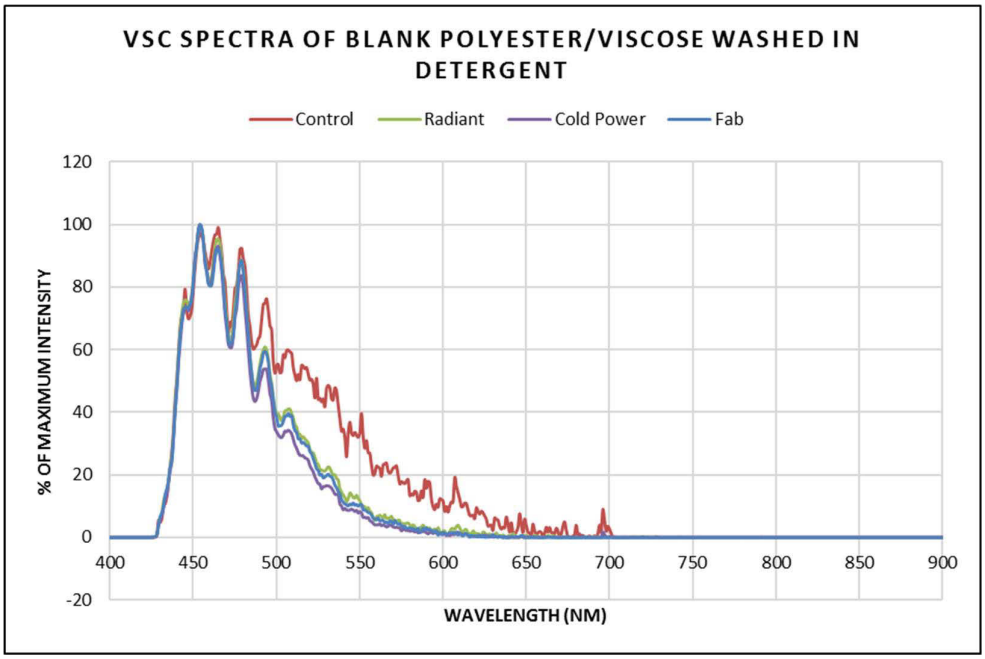


Figure 3-5: VSC fluorescence spectra of blank polyester/viscose washed in Radiant, Cold Power and Fab detergent powder

The fluorescence spectra of the blank polyester/viscose mixed textile shown in Figure 3-5 shows that, like with blank cotton in Figure 3-3, after washing the broad fluorescence peak narrows (from 425-700 nm to 425-600 nm). While the control and washed samples all exhibit the instrument artefact peaks in the same place, the control sample peaks from 525 nm to 700 nm become small and not as prominent in the washed sample.

The washed sample fluorescence spectrum is also found to match those of the detergents in Figure 3-2 and the washed cotton in Figure 3-3 which is indicated to be due to the blue fluorescence emitted by optical brighteners.

Therefore, it can be seen that optical brighteners in laundry detergent were found to affect the fluorescence of the blank polyester/viscose sample. This corresponds to the results in Table 3-2 that showed that, after washing, the viscose fibres in the textile fluoresce a blue colour under UV light.

Again, the limitation with these results is that the type of detergent used is unable to be determined from these results.

Fluorescence of detergents on white textiles

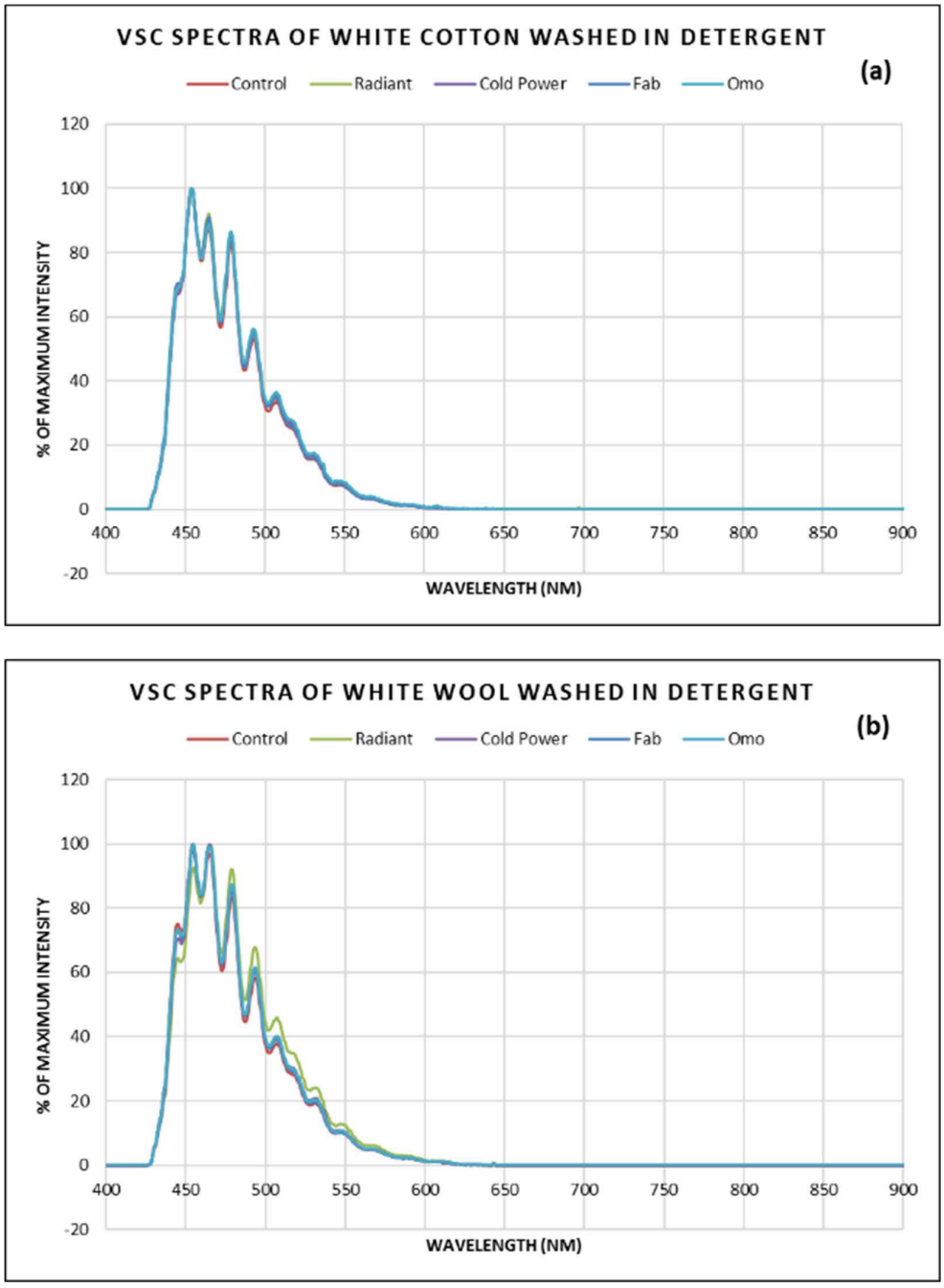


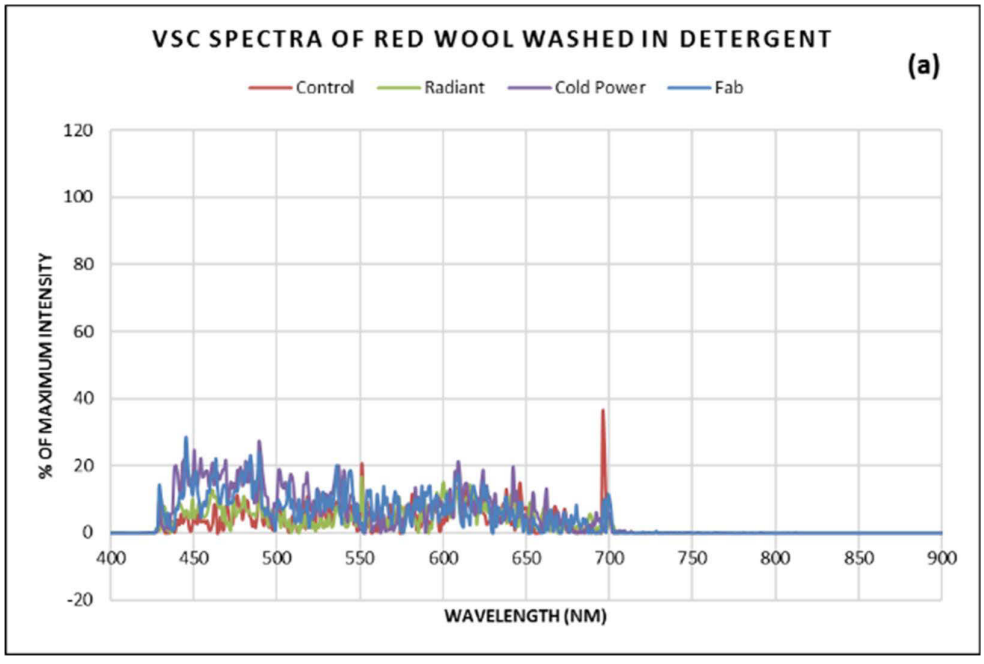
Figure 3-6: VSC fluorescence spectra of (a) white cotton and (b) white wool washed in Radiant, Cold Power, Fab and Omo detergent powder

In Figure 3-6, no difference can be seen between the fluorescence spectra of the white cotton and wool control samples, and those washed in detergents Radiant, Cold Power, Fab and Omo).

The fluorescence peak shape is consistent with that of the blue fluorescence emitted from optical brighteners in detergent. As this same spectrum was produced by both unwashed samples, it indicates that these white textiles are pretreated with optical brighteners in the production process and that these optical brighteners give off the same fluorescence as those seen in detergents. This is something that should be taken into consideration when analysing any white fibres.

As there is no discernible difference between the control and the washed sample spectra, it is unable to be determined whether the fluorescence detected from white textiles is due to the washing process or were already present and so cannot be used to determine if a textile has been washed or not.

Fluorescence of detergents on coloured textiles



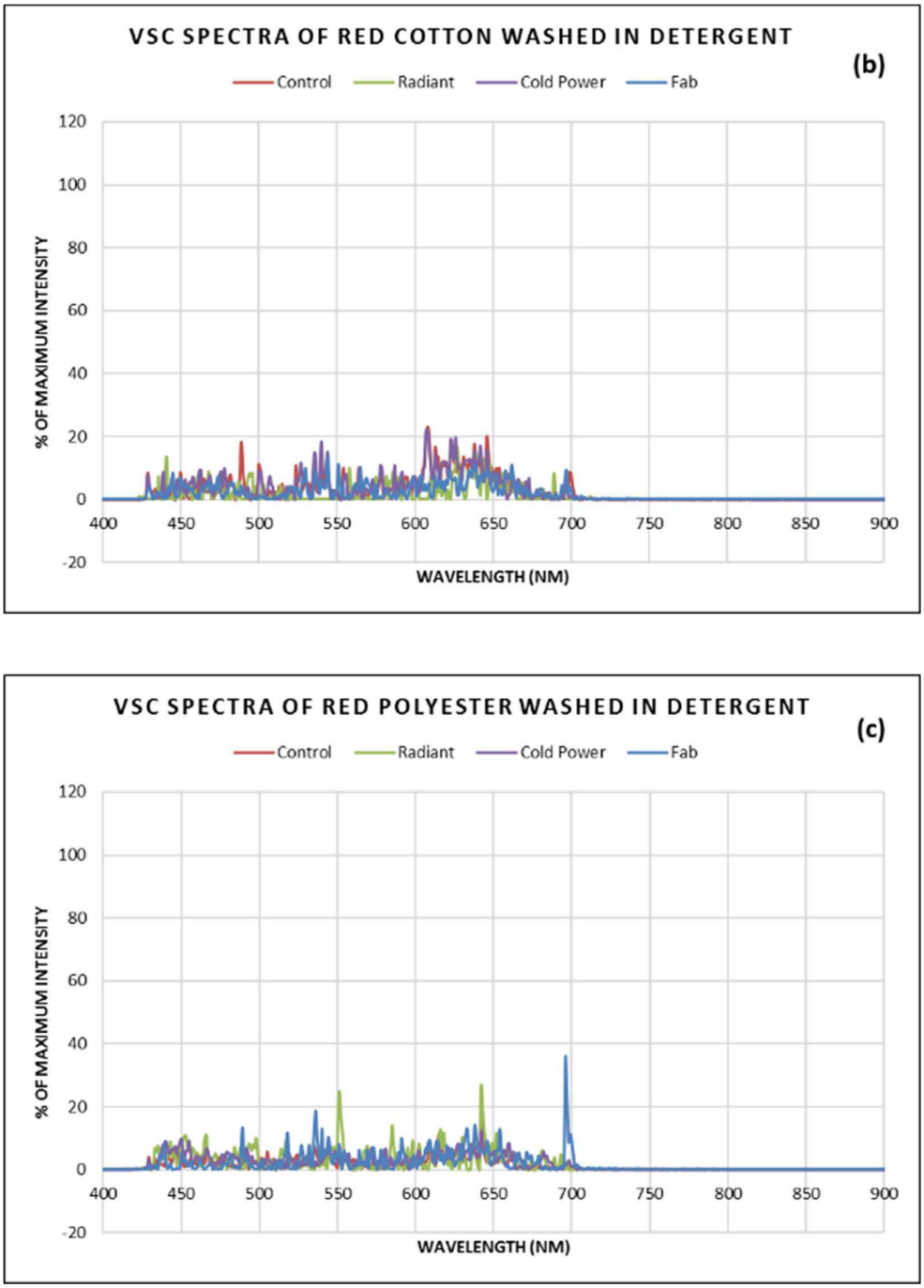


Figure 3-7: VSC fluorescence spectra of red (a) wool, (b) cotton and (c) polyester washed in Radiant, Cold Power and Fab detergent powder

Reflecting what was seen visually in Table 3-4, no fluorescence was detected in either the control or the washed samples of the red cotton and polyester samples (Figure 3-7). A small increase in the intensity of the washed red wool samples peaks is seen between

430 and 500 nm. This could be indicative of a small amount of fluorescence detected, however the large amount of noise in both the control and the washed samples makes this difficult to resolve and, as such, would not be a reliable characteristic to use to discriminate between washed and unwashed samples.

This shows that both the type and colour of textiles can affect how much fluorescence is detected on samples, and hence displays a limitation of this technique for the analysis of fibres.

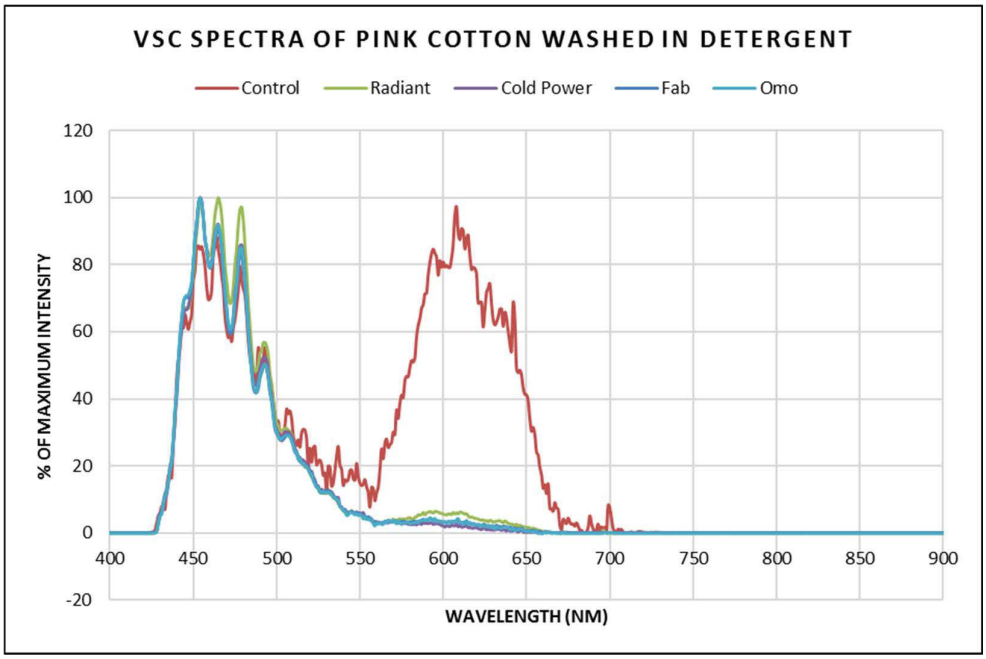


Figure 3-8: VSC fluorescence spectra of pink cotton washed in Radiant, Cold Power, Fab and Omo detergent powder

While no visible fluorescence of the pink cotton control samples was seen in Table 3-5, the fluorescence spectroscopy results in Figure 3-8 shows that fluorescence was detected. The fluorescence spectrum shows two main peak areas, one from 425 to 550 nm, and one from 550 to 675 nm. This fluorescence structure is only detected in the pink cotton sample (out of the samples analysed) and is due to the mixing of blue and red wavelengths to make the pink colour that is emitted by the control sample under UV light. The presence of the blue light in the mixing of the colours explains why the first peak is consistent, in terms of peak shape and placement, to the fluorescence spectra of the optical brighteners used in the detergents. After washing, the red fluorescence is extinguished.

The fluorescence spectra of the washed samples match those of the detergents and the washed samples that are seen in Figure 3-2, Figure 3-3, Figure 3-5 and Figure 3-6. This supports the visual results of the blue fluorescence that was seen in the washed samples and supports the conclusion that most washed textiles will display the same blue fluorescence, despite the detergent used.

The significant difference between the shape of the control and washed pink cotton spectra can be used as a strong indicator whether the sample has been washed or not.

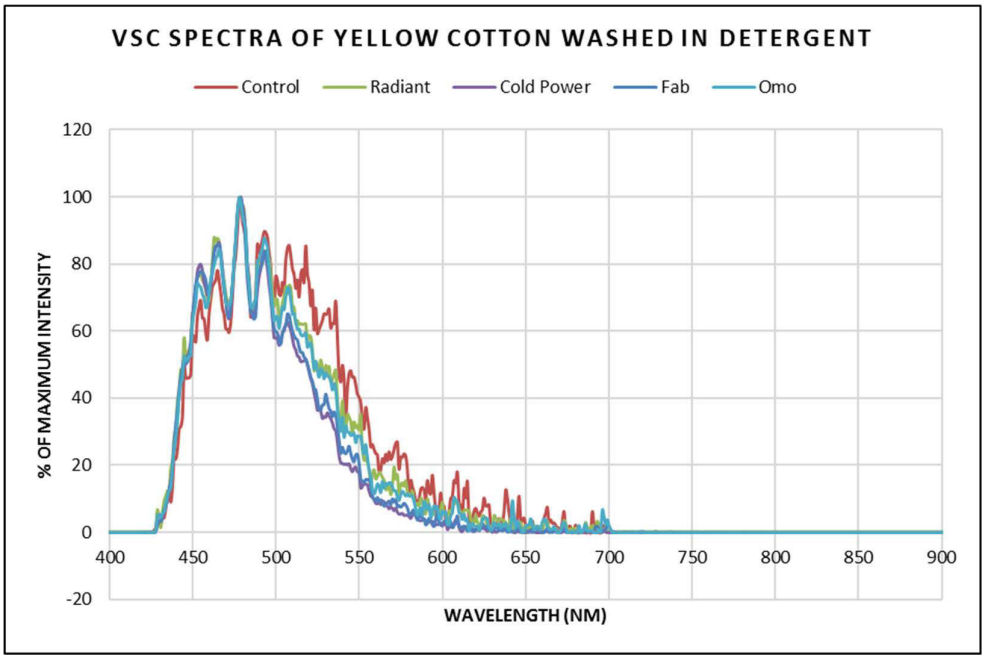


Figure 3-9: VSC fluorescence spectra of yellow cotton washed in Radiant, Cold Power, Fab and Omo detergent powder

The yellow cotton control spectrum in Figure 3-9 showed a fluorescence peak structure quite similar to that of the blank cotton control in Figure 3-3, with the predominant peak area being between 425 and 575 nm. However, unlike the washed blank cotton samples, the washed yellow cotton samples do not show the peak structure that has become characteristic of the blue fluorescence seen emitted from optical brighteners. The primary peak structure of the control and washed samples remain the same, with the main difference being the decrease in the intensity of the peaks between 500 and 550 nm.

These results support the visual results that were seen in Table 3-5 that showed a limited amount of fluorescence detected in the washed samples.

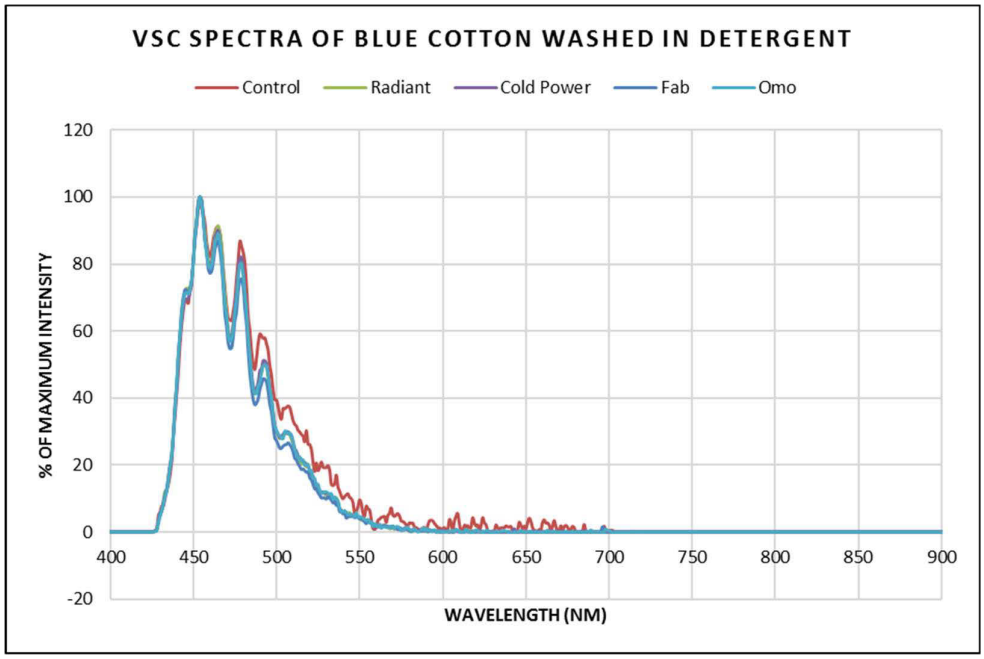
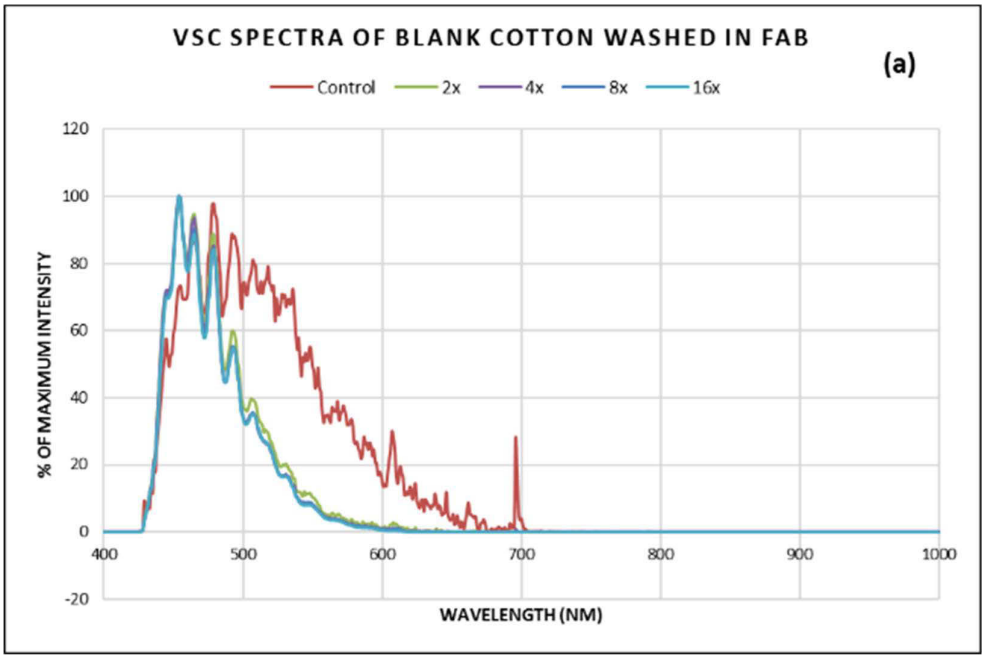


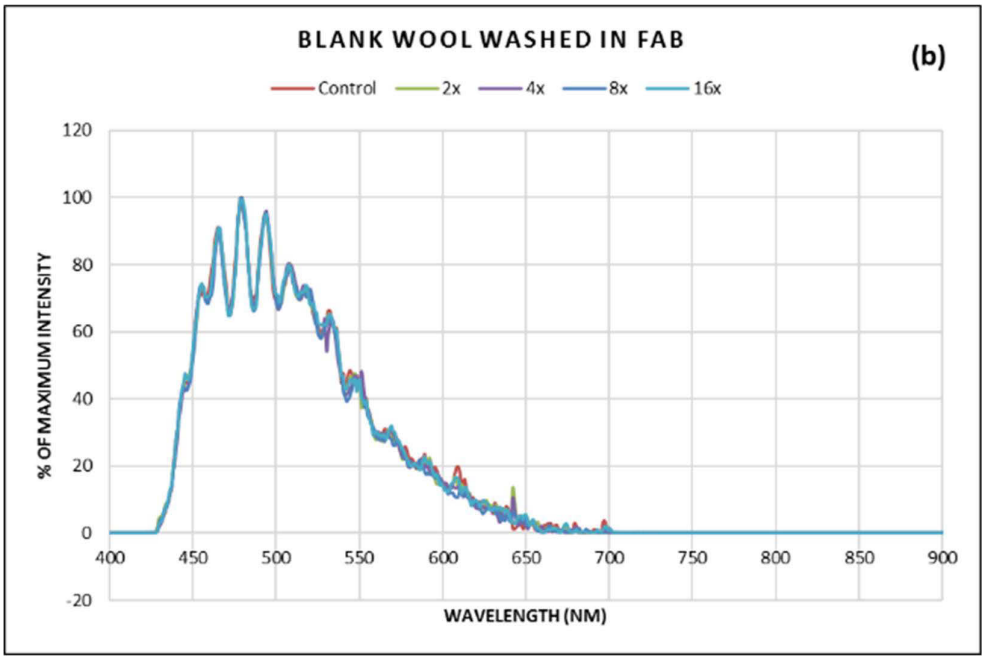
Figure 3-10: VSC fluorescence spectra of blue cotton washed in Radiant, Cold Power, Fab and Omo detergent powder

In Table 3-5, the blue cotton control sample visually already showed fluorescence in the blue range, which was enhanced with washing. These visual results are supported by the fluorescence spectra in Figure 3-10 which show the characteristic blue fluorescence pattern in both the control and washed samples of the blue cotton textile. This is to be expected as the colour of the textile is also blue, contributing to the blue fluorescence emitted. A slight difference can be seen, however, between the control and the washed samples, with a smoothing of the spectra between 500 and 700 nm.

The closeness of the two spectra can create problems when analysing the samples as, without a reference control spectrum, it could be difficult to determine if the spectra is indicative of a washed or unwashed sample.

Change in fluorescence over washes





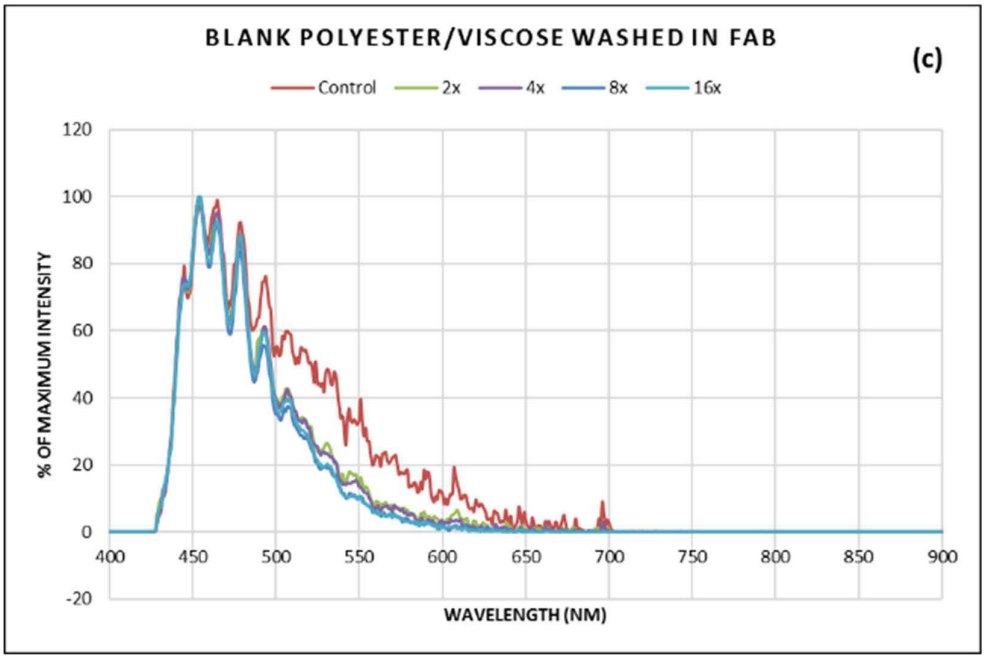


Figure 3-11: Fluorescence spectra showing change in fluorescence of (a) blank cotton, (b) blank wool and (c) blank polyester/ viscose over a number of washes

The fluorescence spectra of blank cotton, polyester/viscose and wool were compared over several washes in Figure 3-11 to determine whether any change could be seen in the spectra.

Unlike the visual change in fluorescence that could be seen in Table 3-6, the only change in the intensity and shape of the fluorescence spectra of blank cotton and polyester/viscose could be seen between the control and the washed samples, with no change over the number of washes.

The fluorescence spectra of the blank wool samples showed no change over the number of washes, which supports the results of the visual fluorescence that showed no signs of fluorescence for any of the washed blank wool samples.

From these results, it can be seen the VSC fluorescence spectra can only provide information about whether a textile has been washed, not about how many times it has been washed.

Change in fluorescence over time

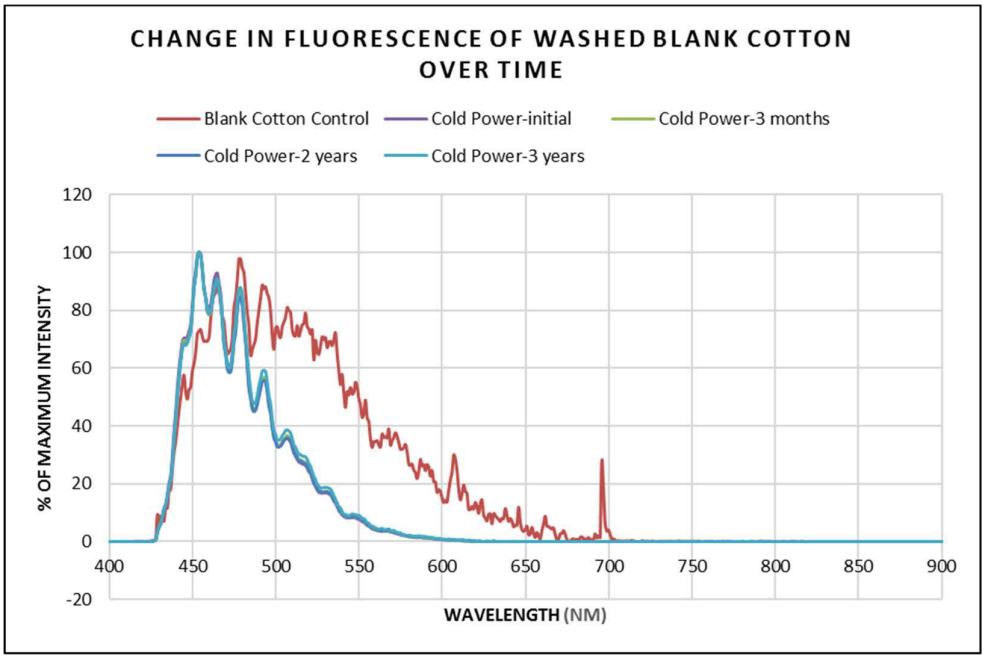


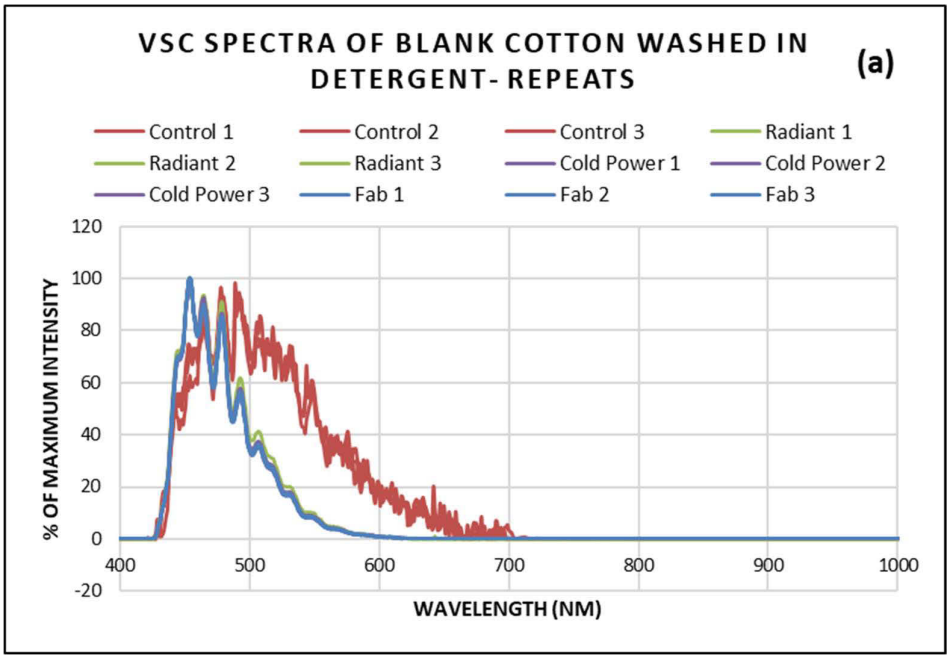
Figure 3-12: Fluorescence spectra of blank cotton washed in Cold Power over time

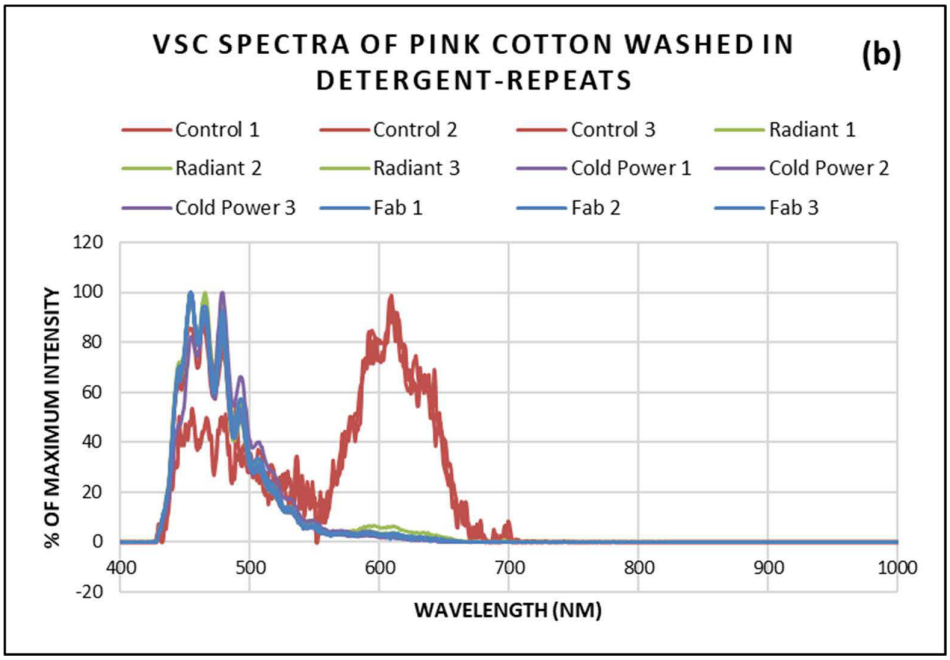
Figure 3-12 shows the fluorescence spectra of blank cotton washed in Cold Power that was analysed 3 months, 2 years and 3 years after the initial analysis. The fluorescence spectra support the visual results in Table 3-7.

The shape and intensity of the peaks in the fluorescence spectra remain the same for washed sample at all time intervals, supporting the conclusion that time in storage in the dark has no impact on the fluorescence of the samples.

This indicates that samples can be reanalysed after three years in storage without any significant impact on the fluorescence. However, it should also be noted that these fibres are kept in the dark throughout their storage. When analysing a stored sample against a new sample it should be recognised that, if the conditions that the new sample were kept in are not known, they cannot be compared reliably as the new sample may have been washed in the interim or exposed to prolonged UV light which is known to degrade optical brighteners [112].

Repeatability





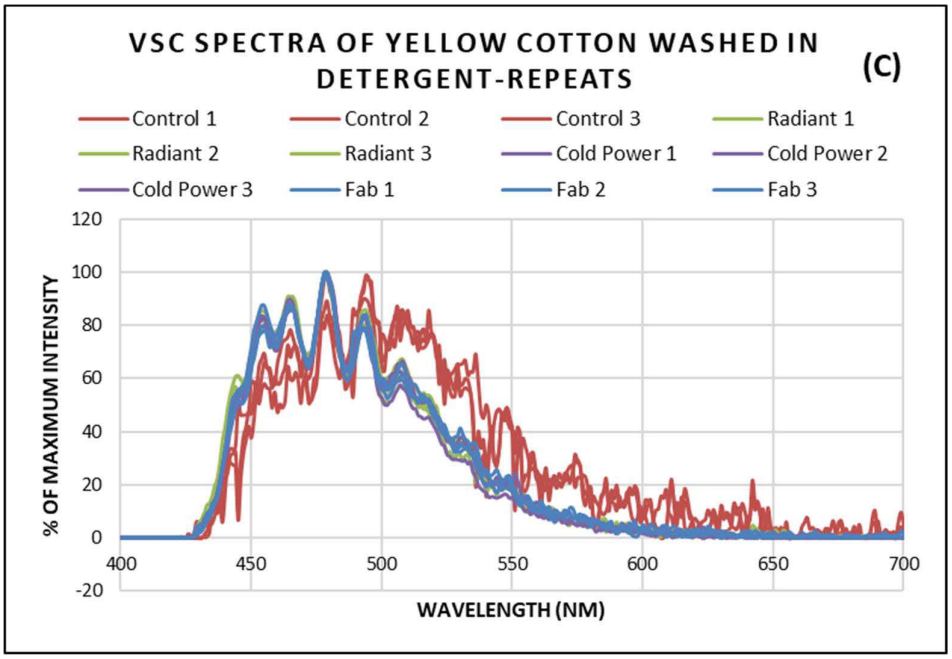


Figure 3-13: Fluorescence spectra of repeats of washed (a) blank, (b) pink and (c) yellow cotton

Figure 3-13 depicts the fluorescence spectra collected of repeat samples of washed blank, pink and yellow cotton. The repeat samples were all washed in separate batches to the original samples.

Visual comparison of the blank, pink and yellow cotton repeats show that the repeat spectra are visually indistinguishable from the original spectra.

The consistency of the fluorescence spectra across the repeats indicates that the technique is repeatable and that the fluorescence spectra collected for these wash spectra is highly indicative of the fluorescence spectra that would usually be collected from these samples.

PCA Analysis

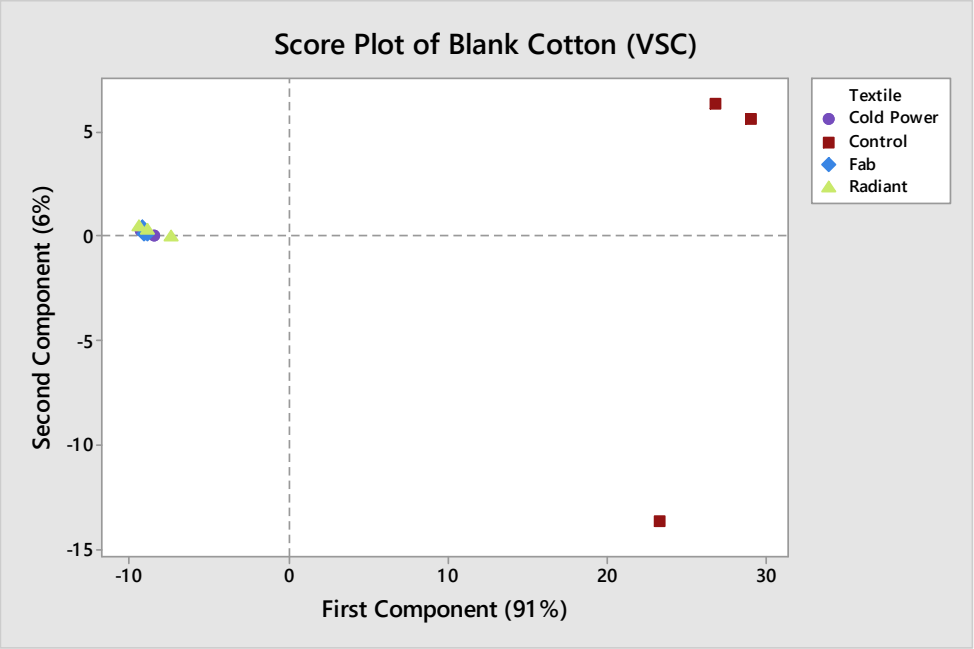


Figure 3-14: Score plot for Blank Cotton (VSC)

The covariant principal component analysis score plot shown in Figure 3-14 shows that there is a clear separation of the control from the washed samples along the first component, which accounts for 91% of the loading of the components. The separation of the samples can be mainly attributed to the presence of high intensity of the fluorescence peak between 500 and 600 nm in the control sample, and the large variation between the peak at 455 nm between the control and the washed samples. No separation is seen along the second principal component with the washed samples clustered at the 0 axes. Instead, a large separation is seen between one control sample and two other control samples. This separation is due to peaks at 434 and 642 nm which have a slight variance due to the noise in the spectra. This PCA score plot supports the visual interpretation of the VSC fluorescence spectra which saw a significant difference in the fluorescence spectra of the washed and control samples.

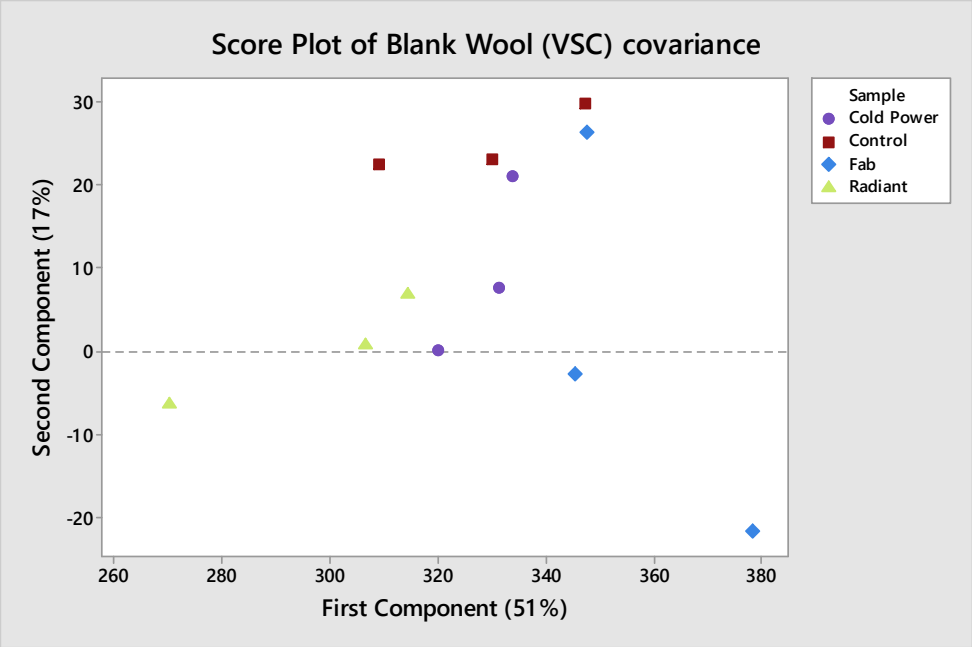


Figure 3-15: Score plot of Blank Wool (VSC)

The covariant PCA score plot of washed and control blank wool samples shows there is little to no clustering of the samples. The separation of the samples is predominantly loaded on the first component (51%). However, this variation can be seen to be predominantly between the washed samples of blank wool where a partial separation can be seen between the three detergents. This separation is due to the difference in intensity of the peaks at 551 and 445 nm, where especially at 551 nm, the Fab sample at the lower right corner of the plot was found to have a high-intensity peak. This variation in the peaks, however, was not consistent across all three repeats and can likely be attributed to instrument variation. A partial separation can also be seen between the control and washed samples along the second component. This separation can be attributed to a slight variation in peak intensity at the peaks 441, 536, 544 and 642 nm. Again, this variation is likely due to instrumental variation, and the separation between the controls and washed samples is not significant enough to discriminate washed from control samples.

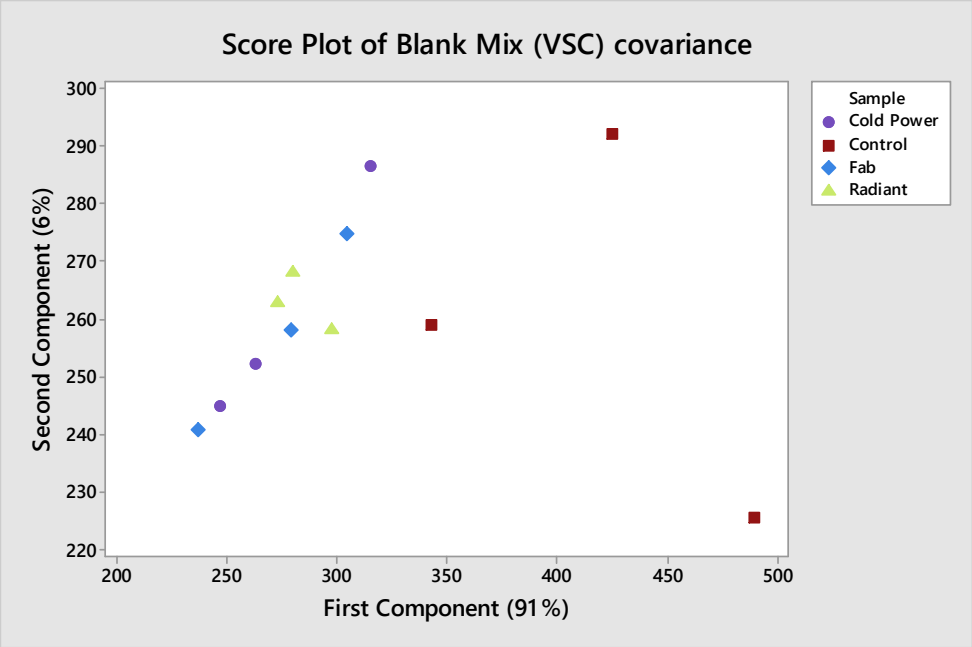


Figure 3-16: Score plot of Blank Mix (VSC)

The score plot of the Blank mix samples in Figure 3-16 shows a clear separation between two of the control samples with the washed samples along the first component which accounts for 91% of the variation. The washed samples can be seen to be clustered along the first component and more spread out along the second component. This separation can be linked to the control samples' higher intensity fluorescent peaks between 500 and 550nm. As a result, the control samples can be clearly discriminated from the washed samples. The spread of the washed samples along the second component is predominantly due to the variation in the peak at 445 nm. As can be seen from the score plot, however, there is no apparent clustering between the detergents showing the intensity of this peak is not related to the type of detergent used. As a result, the samples cannot be separated using PCA based on the detergents they were washed in.

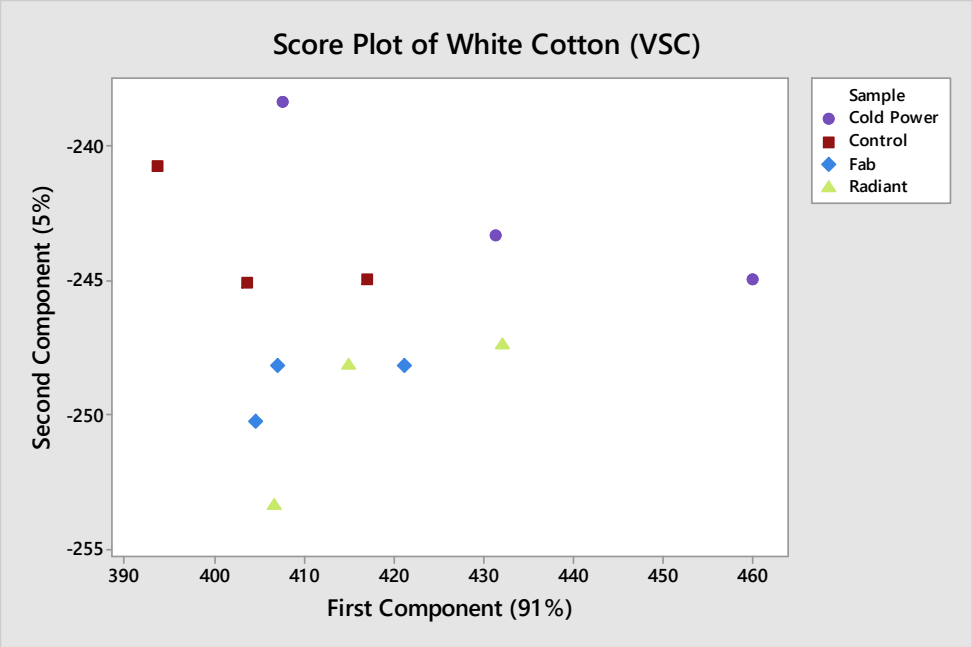


Figure 3-17: Score plot of White Cotton (VSC)

The score plot for white cotton in Figure 3-17 reflects the visual analysis of the spectra in that there is no evident clustering based on whether the samples have been washed or not, and therefore no separation can be achieved. The separation along the first component is mainly due to variation in the intensity of the main peaks between 460 and 600 nm. The second component is predominantly based on the variation of the peak at 443 nm. The difference in the intensity of these peaks across the sample is minor (at most a difference of 10%) and does not appear to be related to the detergent that the white cotton samples were washed in.

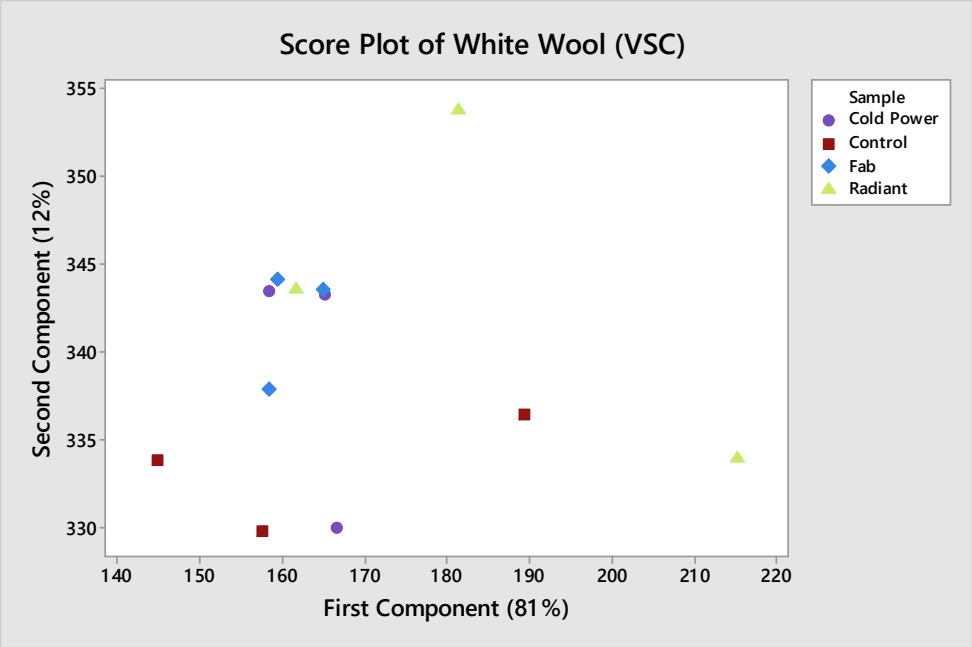


Figure 3-18: Score plot of White Wool (VSC)

As with white cotton, the score plot for white wool shows there is no clear separation between the control and washed samples (Figure 3-18). The variation taken into account by the principal components is predominantly due to a small degree of variation in the peak intensities in the fluorescence spectra. This variation, as discussed previously is due to the instrument and likely small differences in the intensity of the fluorescence detected, not in significant differences in the fluorescence itself.

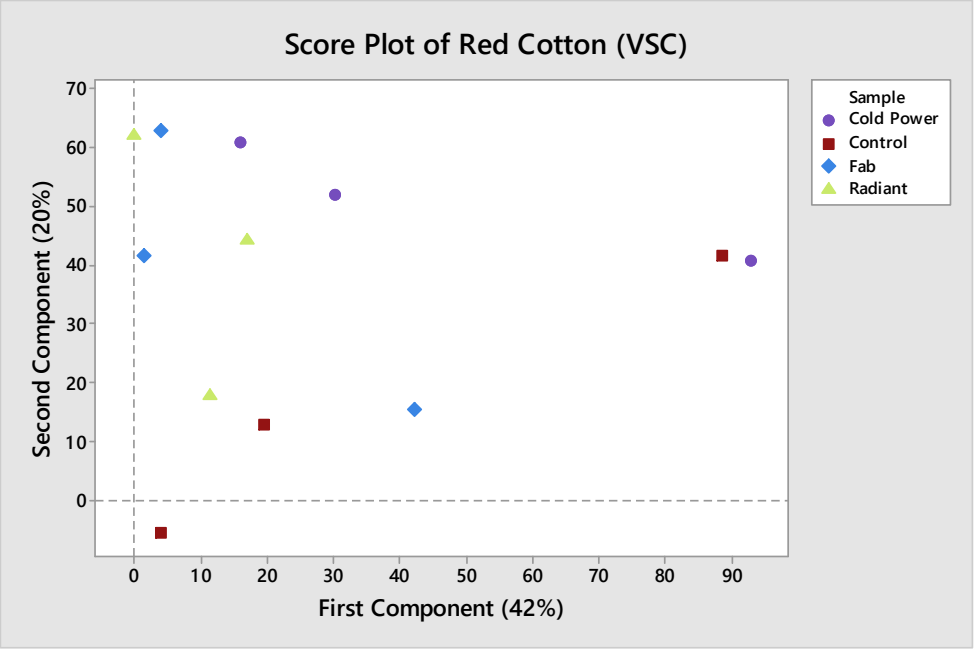


Figure 3-19: Score plot of Red Cotton (VSC)

The score plot for the red cotton samples shows that there is no separation or clustering of the different groups (Figure 3-19). All score points are dispersed over the plot with the variation due to the difference in intensities in several peaks in the fluorescence spectra. As no fluorescence was detected by the VSC, the spectra produced is of background noise. The separation of the samples in the score plot is therefore due to small discrepancies in this noise spectra.

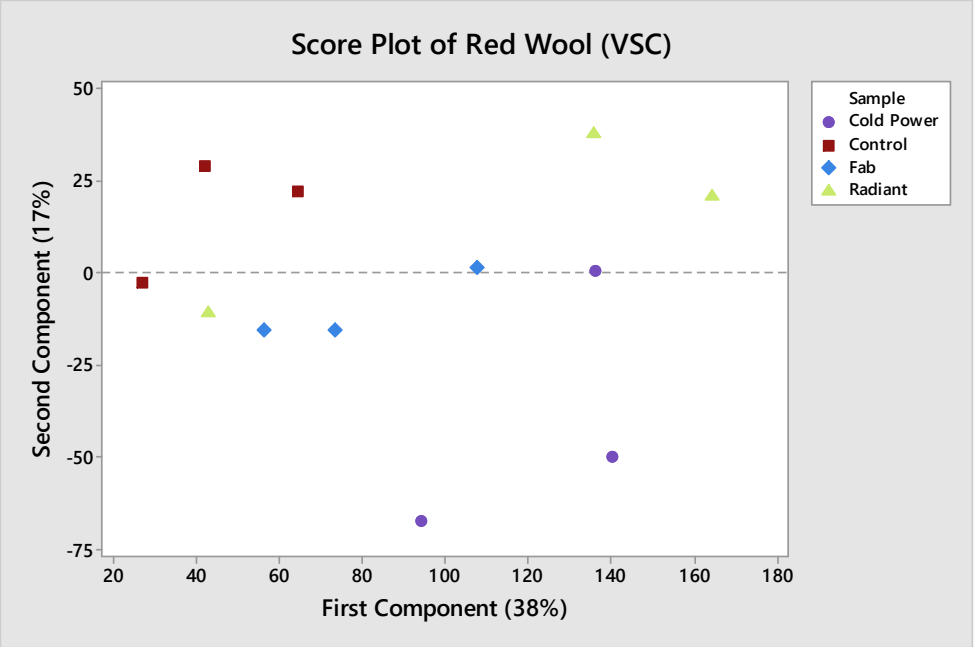


Figure 3-20: Score plot of Red Wool (VSC)

The score plot for red wool (Figure 3-20) shows no separation based on whether a sample had been washed or not. The samples are spread along the first component which is responsible for 38% of the variation and are very spread out with no clustering for any of the group. The separation along this component is predominantly due to the washed red wool samples exhibiting higher intensity peaks between 450-500 nm as well as the peak at 536 nm. However, as with the red cotton fluorescence spectra, there is a significant amount of noise in the spectra, and so, on their own, the difference in these peaks cannot be used to discriminate between washed and unwashed red wool samples reliably.

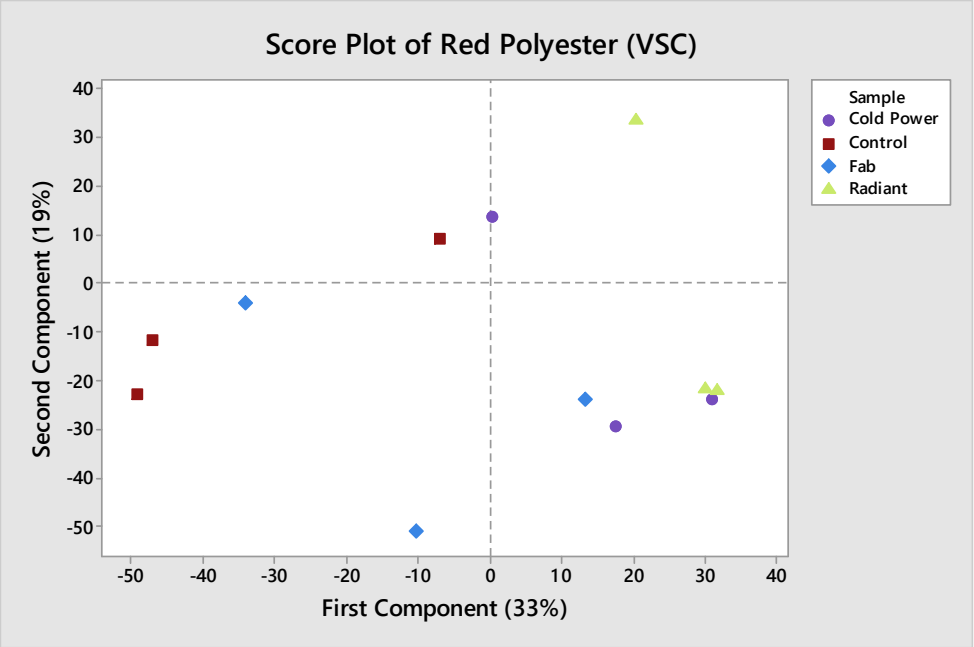


Figure 3-21: Score plot of Red Polyester (VSC)

A partial separation is seen along the first component of the score plot for red polyester (Figure 3-21). This separation is predominantly due to the control samples, and two of the Fab samples having a slightly larger intensity in the peaks at 536, 540 and 544 nm than the other washed samples, while the washed samples have more intense peaks at 551, 552 and 553 nm. The peaks in the spectra are again present due to noise, and fluctuations in the intensity of individual peaks is insignificant.

The PCA results, along with the visual analysis of the fluorescence images and spectra, indicate that optical brighteners have no effect on polyester textile, leading to no fluorescence being emitted. As a result, control and washed polyester samples cannot be distinguished.

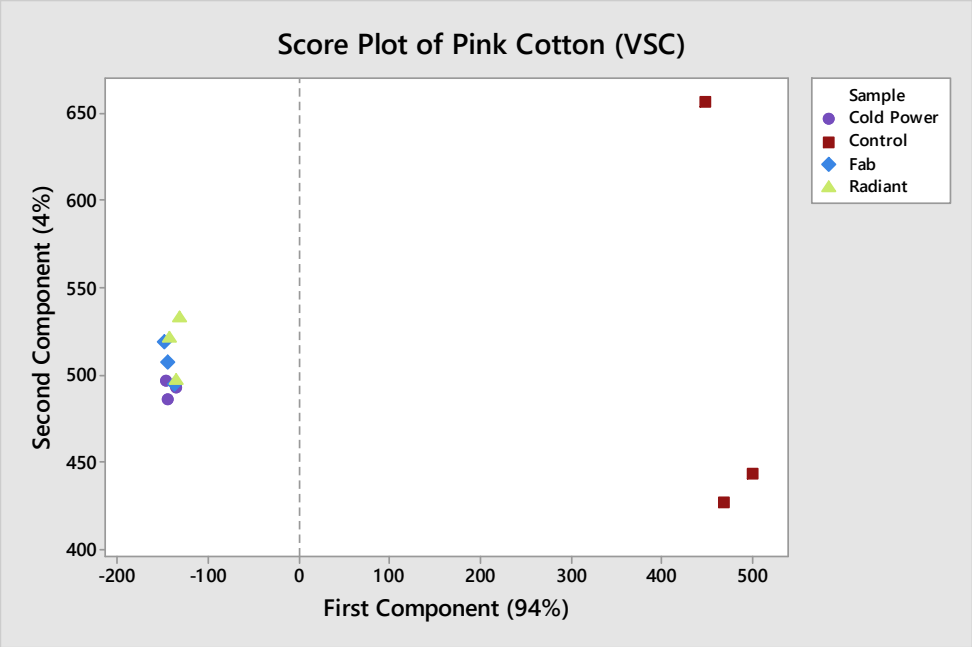


Figure 3-22: Score plot of Pink Cotton (VSC)

The score plot for pink cotton shows a clear and significant separation between the control and the washed samples along the first component (Figure 3-22). The peaks responsible for the variation in the first components are the peaks between 500 and 700 nm. In the fluorescence spectra in Figure 3-8 the control sample has a broad fluorescence peak between 500 and 700 nm which is not present in the washed samples. This separation allows for the control and the wash samples to be clearly discriminated.

No clustering of the different types of detergents can be seen supporting the fact that the pink cotton samples can be separated based on if they have been washed or not, but not what they have been washed in. The distance between two of the control samples and the third control sample along the second component is due to a significant difference in the intensity of the control samples at 464 nm. This peak is part of the main broad fluorescence peak between 420 and 552 nm that both the control and the washed samples have. As this is not in the primary region of peaks used to separate the control and washed samples, this variation has no impact on the use of the score plot to differentiate the samples.

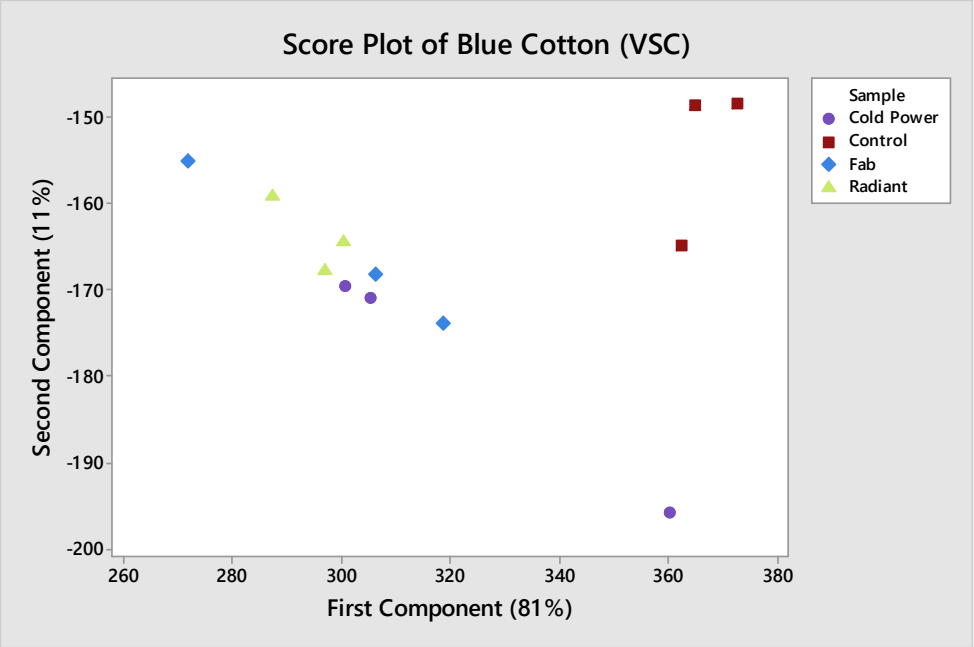


Figure 3-23: Score plot of Blue Cotton (VSC)

The score plot for the blue cotton samples shows a separation between a cluster of washed samples and the control samples along both first and second component (Figure 3-23). This separation is predominantly due to peaks between 480 and 550 nm. As seen in Figure 3-10, the control spectra have a broader fluorescence peak and the peaks are more intense in this region. This indicates that blue cotton control and washed samples can be discriminated.

The presence of the Cold Power sample to the right of the first component, in line with the control cluster, indicates that some washed samples can have spectra that are very similar to the control samples. This similarity can result in false associations between two different samples. As a result, care should be taken when performing this comparison, and it should be used as evidence that two fibres are significantly different to have come from different sources, not as evidence that two fibres came from the same source.

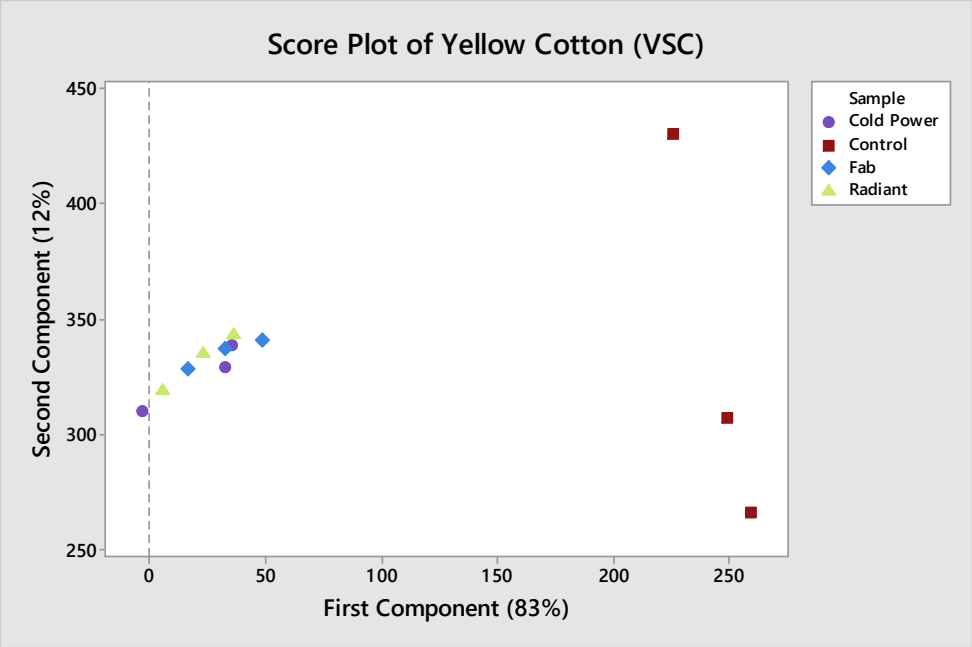


Figure 3-24: Score plot of Yellow Cotton (VSC)

The yellow cotton score plot shows a significant separation between the control score plots and the cluster of the washed sample score plots along the first component (Figure 3-24). The separation is based on two main factors.

The washed samples have a higher intensity peak at 445 nm, with troughs being found at this wavelength in two of the control samples. The second factor is that the control samples have higher intensity peaks between 518 and 640 nm (Figure 3-9). The distance between the control samples on the second component is due to a difference in peak intensities at 536 and 540 nm. No separation can be found between the different detergent groups. As a result, PCA analysis can be used to classify a yellow cotton control sample as being washed or unwashed but cannot determine which detergent was used.

The significant separation achieved in both the blue and yellow cotton score plots highlights the usefulness of PCA analysis as it emphasizes the difference between the control and washed samples. However, as these samples can still be clearly differentiated using the VSC spectra, the extra step of PCA analysis is not pivotal to the analysis.

3.2.2 VSC Conclusion

From the analysis of the results collected it can be seen that the use of fluorescence imaging and spectroscopy using a Video Spectral Comparator (VSC) produced limited information. The amount of fluorescence observed was highly dependent on the type of textile used, with polyester and wool showing little to no fluorescence after washing.

The colour (and pre-treatment) of the textile also had a significant effect. Dark textiles prevented the observation of fluorescence while white and other lightly dyed textiles were found to already contain optical brighteners that fluoresced in the same colour range as the detergent optical brighteners. This meant that no difference could be seen between the control and washed samples.

No difference could also be found between the visual fluorescence and the fluorescence spectra of the detergents tested, which means that while, in some cases, it could be determined that a sample had been washed, the type of detergent could not be identified.

Examination of the change in fluorescence of samples over number washes found that a change in fluorescence could only be seen visually between zero and four washes, but after four washes the fluorescence remained consistent. The fluorescence spectra of these samples showed no change in the fluorescence spectra of the washed samples.

The effect of storage on samples was also examined, where it was found that the intensity of fluorescence remained consistent for at least three years while being kept in storage.

Principal Component Analysis of the fluorescence spectra supported the conclusions drawn from the visual analysis of the spectra. Separation between control and washed groups was present in the plots for the blank mix samples as well as blank, pink, yellow and blue cotton. PCA added value to the analysis of the samples by clarifying small differences in sample spectra that could be open to subjective interpretation. No clusters based on detergent brand were found for any of the textiles analysed.

Therefore, from these results, it can be concluded that, depending on the type of textile used, the fluorescence of a sample can indicate whether a sample has been washed or not,

but not which detergent was used or, after four washes, how many times it had been washed.

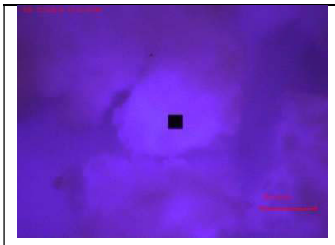
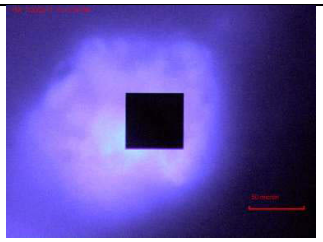
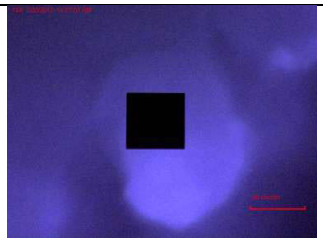
3.3 Fluorescence Microspectrophotometry

3.3.1 Results and Discussion

MSP Fluorescence images

Fluorescence of detergents

Table 3-9: Fluorescence Microspectrophotometry images of Radiant, Cold Power and Fab powder detergents

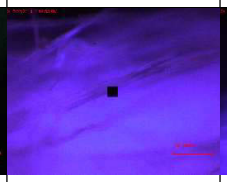
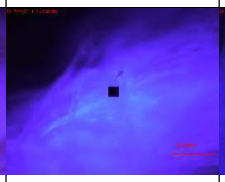
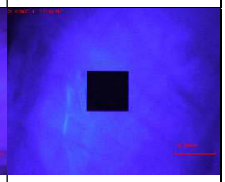
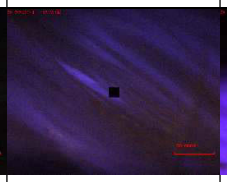
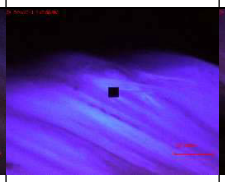
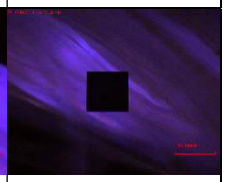
		
Radiant	**Cold Power**	**Fab**

Fluorescence microspectrophotometry images were taken of the powder detergent granules to, firstly, determine whether their fluorescence could be detected and, secondly, to compare with the results obtained using the Video Spectral Comparator. These images are shown in Table 3-9.

All three detergents observed were found to fluoresce a blue colour under UV (365 nm) light which was consistent with the results found using the VSC in Table 3-1, confirming these results. As these samples have the same fluorescence, they are not able to be visually differentiated from each other.

Fluorescence of detergents on blank textiles

Table 3-10: Fluorescence Microspectrophotometry images of blank textiles

Textile	No Detergent	Radiant	Cold Power	Fab
Cotton				
Viscose/ Polyester				
Wool				

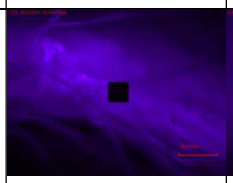
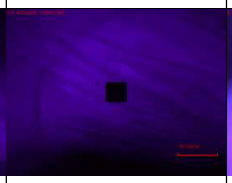
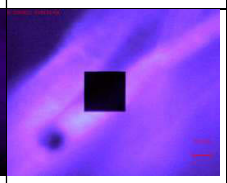
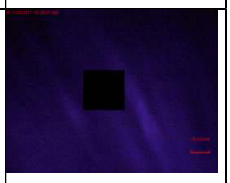
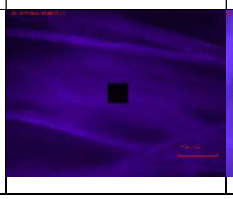
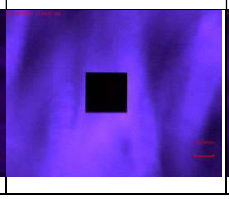
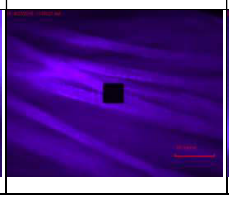
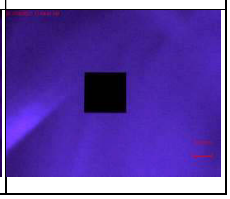
The fluorescence images taken of fibres from blank textiles washed in detergents in Table 3-10 reflects the results found using VSC in Table 3-2.

Blank cotton showed an intense blue fluorescence after washing in all three detergents. Similar results were seen with the viscose fibres from the viscose/polyester mix although the fluorescence was not as intense. Again, no fluorescence was detected on the polyester fibres.

The blank wool fibres showed only a low level of fluorescence both before and after washing. This indicates that the fluorescence is natural fluorescence from the wool itself and that the optical brightener residues are not adhering to the wool fibres.

Fluorescence of detergents on white textiles

Table 3-11: Fluorescence Microspectrophotometry images of white textiles washed in Radiant, Cold Power and Fab

Textile	Control	Radiant	Cold Power	Fab
White Cotton				
White Wool				

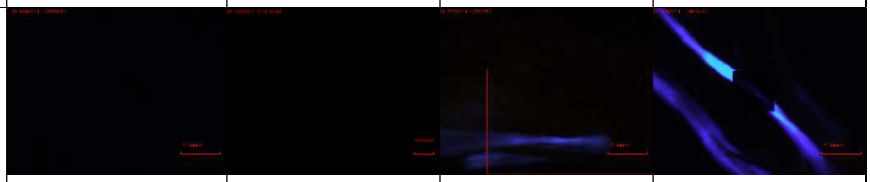
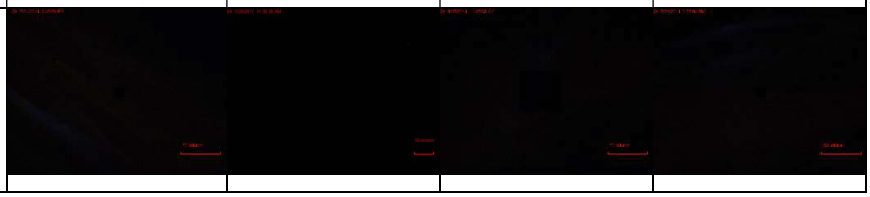
The fluorescence images that were taken of white cotton and wool samples (Table 3-11) supported the VSC results in Table 3-3 which showed that white cotton and wool samples already contain optical brighteners that are obtained through their production process.

No difference can be seen between the control and washed samples visually, and so it cannot be determined whether the fluorescence in the washed samples is due solely to the optical brighteners already contained in the sample, or whether optical brighteners from the detergents also contribute to the fluorescence.

These results again support the conclusion that no information about the washing history of white textiles can be determined using the fluorescence of optical brighteners (see 3.2.1).

Fluorescence of detergents on coloured textiles

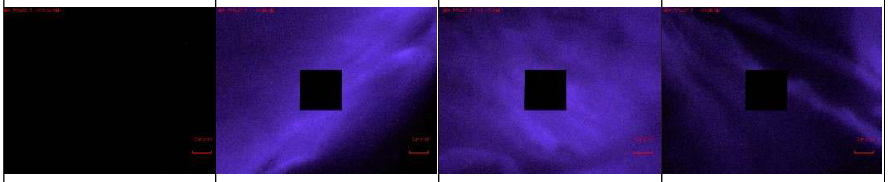
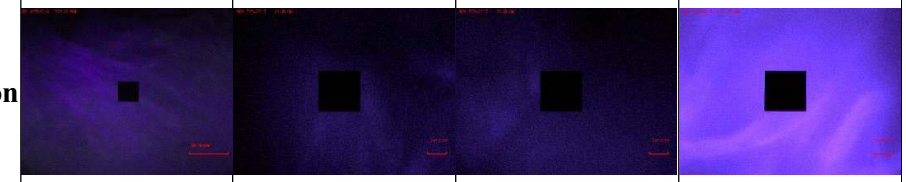
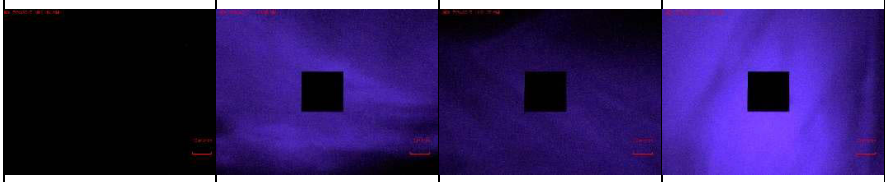
Table 3-12: Fluorescence Microspectrophotometry images of red textiles washed in Radiant, Cold Power and Fab

Textile	No Detergent	Radiant	Cold Power	Fab
Red Cotton				
Red Polyester				
Red Wool				

The fluorescence images taken of red textiles (Table 3-12) were relatively consistent with those taken using VSC in Table 3-4 in that the red polyester and wool emitted no fluorescence either before or after washing.

However, the fluorescence microspectrophotometer was able to detect a small amount of the fluorescence on the washed red cotton fibres which VSC was unable to do. Unlike the washed blank cotton and wool samples that showed consistent fluorescence along the length of the fibre, the washed red cotton samples only contained slight areas of fluorescence that were located randomly along the fibre and were hard to find. The inconsistency of this fluorescence would create problems when it comes to forensic casework, as often only small sample sizes of fibres are available. The small length of the evidence fibres would mean that it would not be likely to detect areas of fluorescence. Due to this, the fluorescence comparison of the red cotton fibres should only be conducted to provide evidence that two fibres are different, or that one of the fibres had been washed. If no difference can be found between the two red cotton fibres, it should not be used as evidence that they came from the same source.

Table 3-13: Fluorescence Microspectrophotometry images of pink, yellow and blue cotton

Textile	Control	Cold Power	Radiant	Fab
Pink Cotton				
Yellow Cotton				
Blue Cotton				

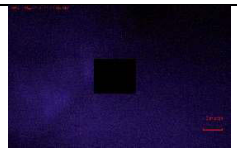
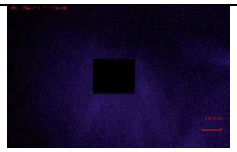
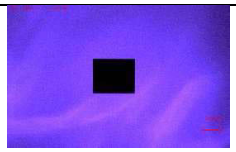
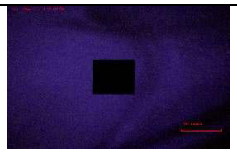
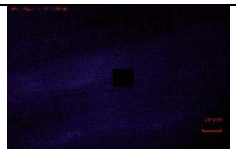
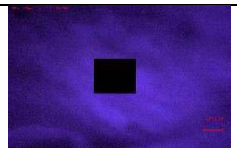
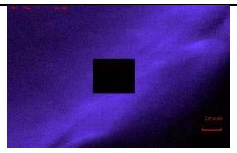
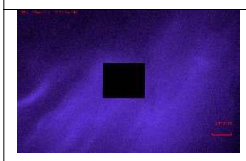
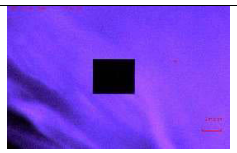
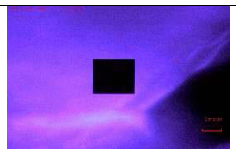
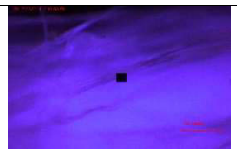
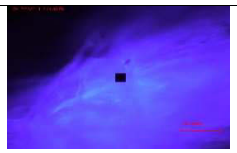
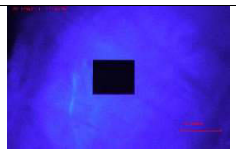
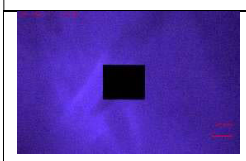
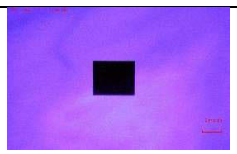
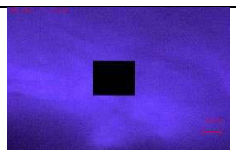
Yellow, pink and blue dyed cotton control and washed samples were analysed using the Fluorescence MSP, and the fluorescence images are shown in Table 3-13. Little to no fluorescence was found on the pink and blue control samples. However, blue fluorescence was seen on the washed samples for all three textiles.

The yellow control sample emitted blue fluorescence that was also detected in all three washed samples. This fluorescence could only be seen to increase in the yellow cotton sample washed in Fab detergent. Due to the similarity of the hue and intensity of the fluorescence, washed and unwashed yellow cotton samples would not be able to be discriminated reliably.

Though the hue of the fluorescence was consistent across all of the washed samples, the intensity of the fluorescence varied. However, this variation could not be linked to being specific to a particular detergent.

Repeatability

Table 3-14: MSP fluorescence images of repeat samples of yellow, pink and blank cotton

Cotton	Radiant	Cold Power	Fab
Yellow			
			
Pink			
			
Blank			
			

Fluorescence images were taken of repeat washed samples of pink, yellow and blank cotton. The repeat samples were washed in a separate batch to the original samples.

Visual comparison of the images in Table 3-14 shows that the hue and intensity of the fluorescence were the same for many, but not all of the samples. Variation of the intensity of the fluorescence can be linked to how many individual fibres were in the fibre bundle in the picture. For example, the image of the original pink cotton washed in Fab contains only a few separated fibres, while the repeat image had many more individual fibres clumped together in the thread, increasing the brightness observed.

Despite this variation, it can still be seen that the fluorescence images of the repeat samples are relatively consistent, indicating that there would not be expected to be a significant discrepancy between two fibres washed in the same detergent.

MSP fluorescence spectra

Fluorescence of detergents

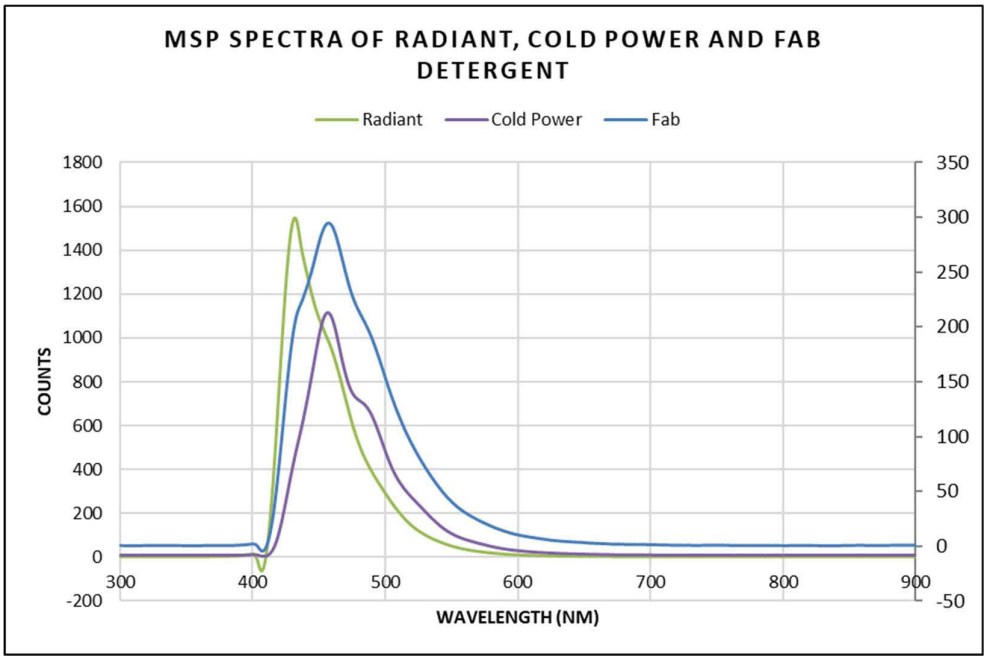


Figure 3-25: Fluorescence MSP spectra of Radiant, Cold Power and Fab powder detergents

The fluorescence spectra collected of Radiant, Cold Power and Fab powder detergent in Figure 3-25 showed that all detergents had a broad peak ranging between 413 and 600 nm, which is consistent with all three detergents fluorescing in the blue range. The Cold Power and Fab detergents both have their maximum peak at 459 nm while Cold Power has a secondary shoulder peak at 487 nm. The Radiant detergent has a fluorescence spectrum that is different to that of Radiant and Cold Power. The width of the Radiant detergent peak is narrower with the maximum fluorescence peak present at 433 nm. The difference in the spectra can be explained by the fact that Cold Power and Fab both contain the same optical brightener (Fluorescent Brightener 71) while Radiant contains a different optical brightener (Fluorescence Brightener 351). The difference in the spectra

indicates that the shape of the fluorescence spectra has the potential to be used to identify the detergent used. The next stage of the analysis then focused on detecting this fluorescence on washed textiles to determine if this difference could also be seen in those samples.

Fluorescence of detergents on blank textiles

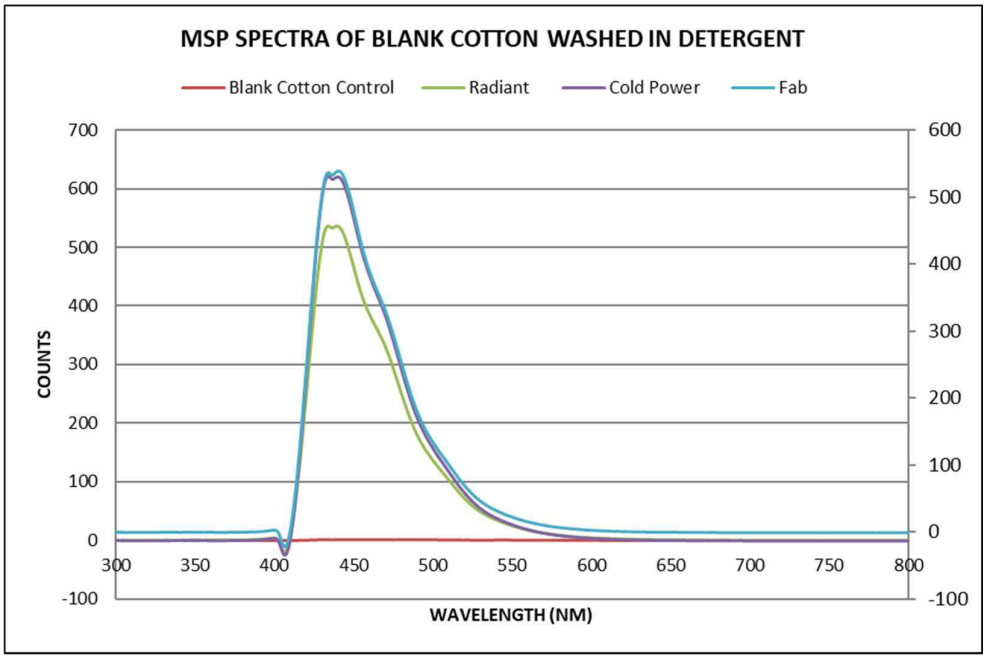


Figure 3-26: MSP Fluorescence spectra of blank cotton washed with Radiant, Cold Power and Fab powder detergent

The fluorescence MSP spectra in Figure 3-26 were collected for the blank cotton control as well as blank cotton washed in Fab, Radiant and Cold Power powder detergents. The spectrum of the control sample shows no fluorescence peaks, proving that no fluorescence is present in the blank sample before it is washed in detergent.

All three washed samples show a broad peak between 410 and 550 nm. The shape of this peak is consistent across all three samples with peaks being formed at 432 and 444 nm. The peaks span the same wavelengths as the detergent fluorescence peaks in Figure 3-25. However, the shape of the peaks and the wavelength of the maximum peak are not in the same location. This is particularly clear in the case of the Radiant samples. While the raw radiant detergent had visually different spectra from the Cold Power and Fab detergents,

the blank cotton washed in radiant cannot be told apart from the blank cotton washed in the other two detergents. This indicates that while the fluorescence of the optical brighteners can be seen on washed textiles, they will not produce the same fluorescence spectra as the raw detergent granules. As the fluorescence spectra collected of washed blank cotton are consistent across all the detergents analysed, it cannot be determined which detergent was used and so they cannot be distinguished from each other.

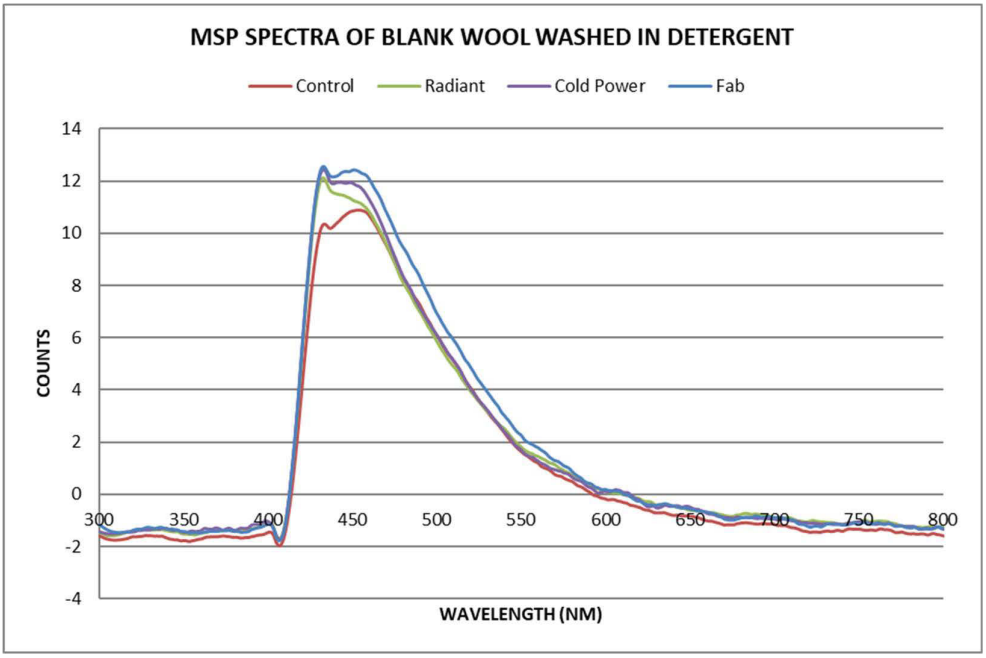


Figure 3-27: MSP Fluorescence spectra of blank wool washed with Fab, Radiant and Cold Power powder detergent

In Table 3-10 it was seen that blank wool samples contained a small amount of fluorescence in both the control and the washed wool samples. It was also seen that there was no significant increase in that fluorescence or change in hue after it was washed. The fluorescence spectra collected of the blank wool samples in Figure 3-27 reflect these results with a low-intensity fluorescence peak ranging from 400 to 600 nm present in both the control and the washed samples. The blank wool control sample was found to have the same intensity spectra as the other washed blank wool samples.

These results are consistent with the VSC results in Figure 3-4 which indicated that the fluorescence spectrum of a blank wool sample does not change after washing and so cannot be used for comparison.

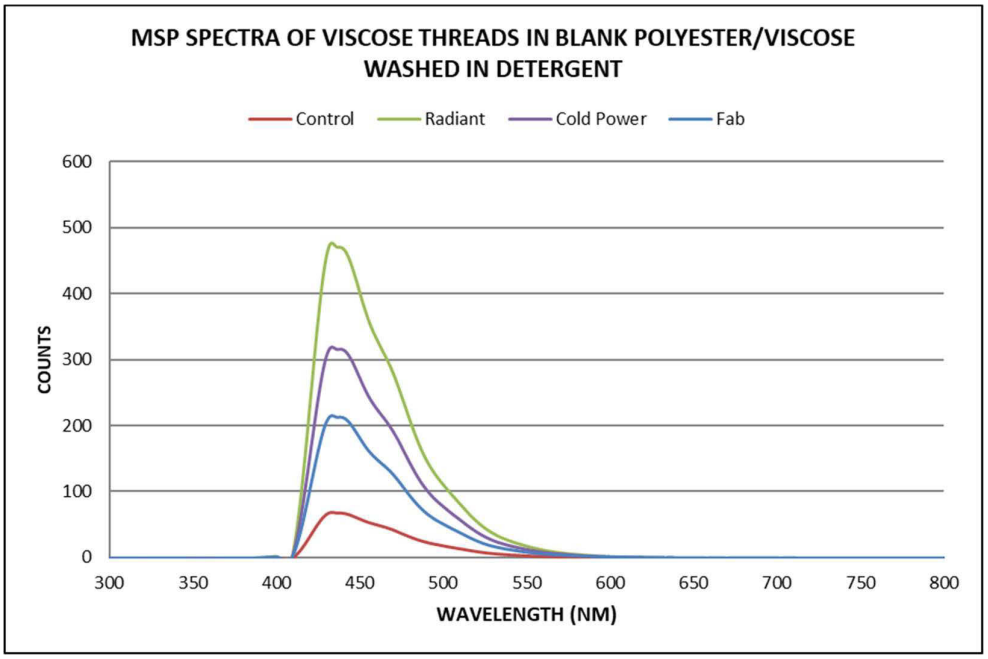


Figure 3-28: MSP Fluorescence spectra of blank polyester washed with Radiant, Cold Power and Fab powder detergent

The blank polyester/viscose sample was made of interlocking polyester and viscose threads. Using the microspectrophotometer, unlike the VSC, a specific fibre could be targeted for analysis. As was concluded using visual fluorescence in Table 3-2 and Table 3-10, the polyester threads emitted no fluorescence both prior to and after being washed in detergents. For this reason, it was decided that fluorescence spectra would be taken of only the viscose threads (the only threads that could be seen fluorescing on the MSP).

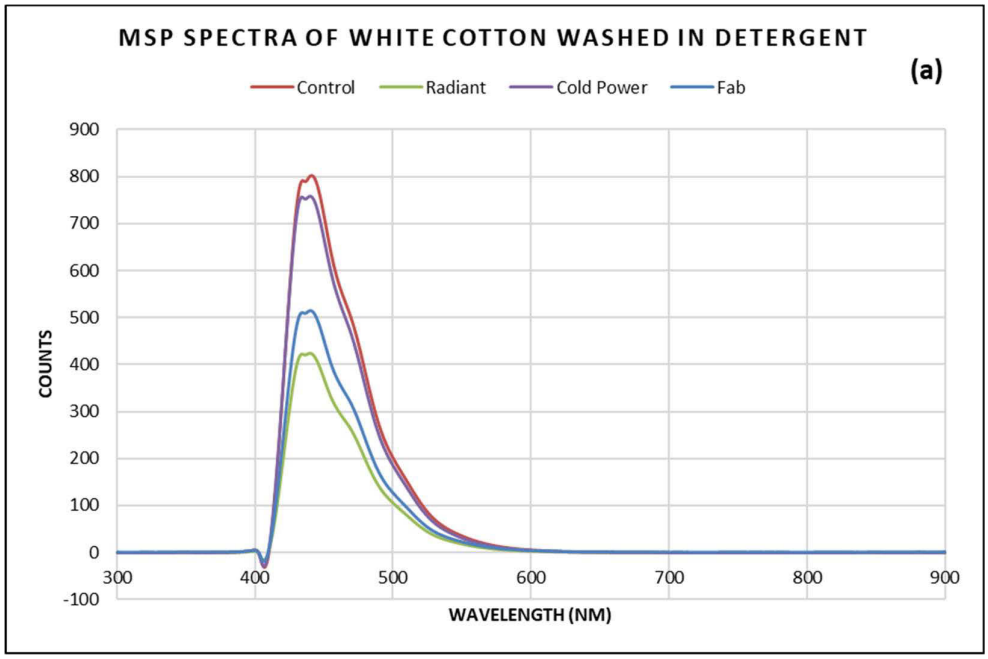
The fluorescence spectra of these viscose threads, shown in Figure 3-28, reveals that a small amount of fluorescence could be detected on the control sample with a low-intensity fluorescent peak between 400 and 600 nm. The same fluorescent peak was also produced by all the washed fibres, though at a higher intensity.

The only difference between the fluorescence spectra of the washed samples is the intensity of the peaks, with Radiant having the highest intensity peaks and Fab having the lowest. However, this does not reflect what was seen visually in Table 3-10 and is most likely due to variation in fluorescence intensity along the length of the fibre. As this

intensity is not characteristic of the detergents themselves, it can not be used to aid in the discrimination of fibres.

Due to the presence of the fluorescence peak in the control sample, the spectra alone cannot be reliably used to determine if a fibre has been washed or not. However, if used in conjunction with the fluorescence images, which in Table 3-10 showed a clear increase in fluorescence intensity after washing, then an indication could be made about the fibres washing history.

Fluorescence of detergents on white textiles



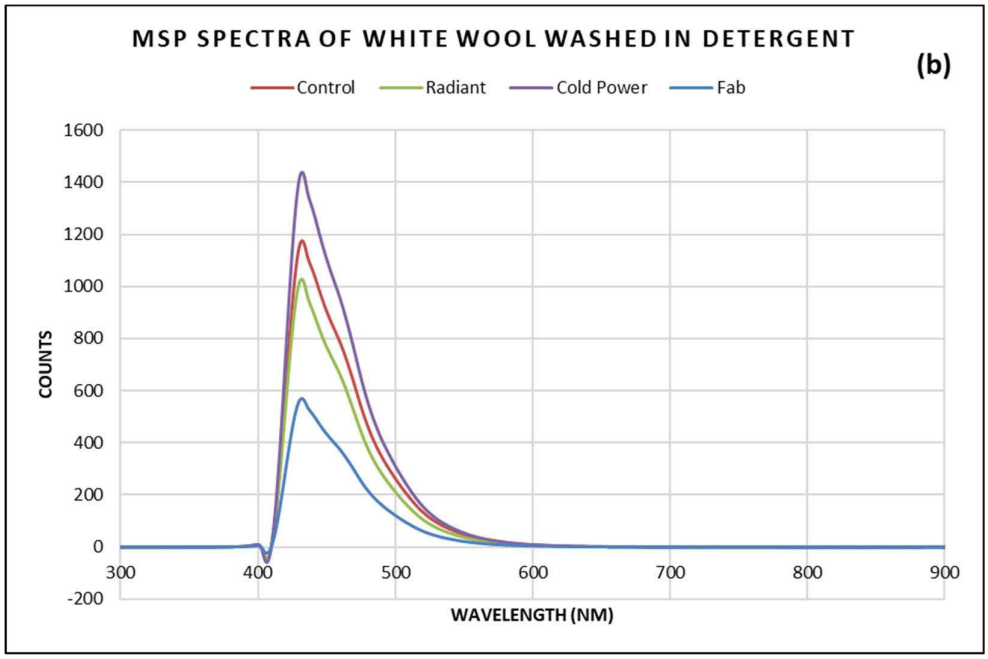


Figure 3-29: MSP fluorescence spectra of (a) white cotton and (b) white wool washed with Radiant, Cold Power and Fab detergent

The MSP spectra collected for the white cotton and wool samples in Figure 3-29 show that there is no difference between the washed and control spectra, reflecting what was seen in Table 3-11. Both the control and the washed white cotton samples share the same fluorescence peak structure with a broad peak between 410 and 550 nm with the predominant peak at 440 nm. These peaks are also consistent with the fluorescence spectra produced by the washed blank cotton samples in Figure 3-26, indicating that the fluorescence of the unwashed white cotton sample is the same as the fluorescence of washed blank cotton.

The white wool samples produced fluorescence spectra in the range of 410 to 550 nm. However, the top of these peaks was found to be at 433 nm and narrower than the fluorescence spectra of the white cotton samples. The spectra indicate that the white wool sample fluoresces the same blue colour as the white cotton samples, but that the wool textile impacts the fluorescence spectra shape. This is unlike the VSC results collected which showed identical fluorescence spectra for both the control and washed white cotton and wool. This highlights the increased sensitivity of Microspectrophotometry as an instrument compared to VSC.

These results again show that the white dyed textiles already contain optical brighteners that fluoresce in the same way as those found in washing detergents, and so they cannot be used to aid in the comparison of white dyed fibres.

Fluorescence of detergents on coloured textiles

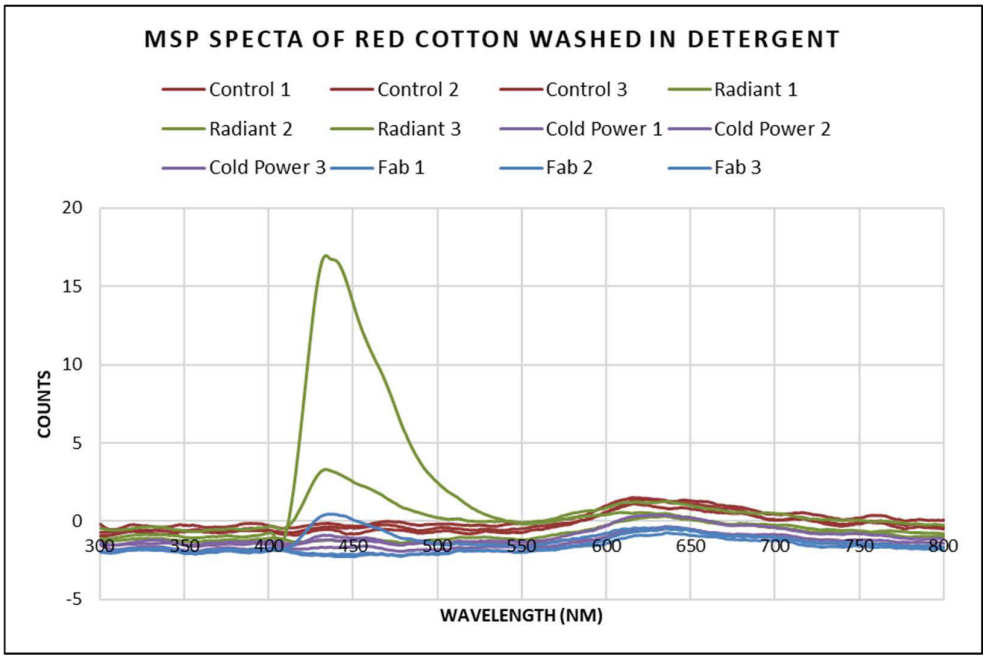


Figure 3-30: MSP Fluorescence spectra of Red Cotton washed in Radiant, Cold Power and Fab detergent

Average fluorescence spectra were collected from three locations along three different fibres for each red cotton sample. Spectra collection was targeted to areas of visual fluorescence when present (as was found in Table 3-12, visual analysis of washed red cotton samples found occasional small areas of fluorescence on some washed fibres). These spectra are shown in Figure 3-30.

The red cotton control sample was found to have no fluorescence peaks which reveals that either the cotton was not treated with optical brighteners, or that they were removed or covered later in the processing. The majority of the washed red cotton samples also showed no signs of fluorescence with the exception of two Radiant samples, and one Fab sample which had a low-intensity broad fluorescence peak between 400 and 550 nm,

consistent with fluorescence spectra of the detergent and other washed samples in this study.

Of the three detergents, red cotton washed in Radiant detergent was found to have, in general, a higher intensity of fluorescence with also more fibres exhibiting fluorescence. This is followed by Fab with one fibre showing a low-intensity fluorescence, followed by the samples washed in Cold Power which had no areas of fluorescence anywhere on the three fibres analysed. The cause of this difference is unknown and would require further investigation. It could be due to the textiles having been washed at different times and handled differently over time, though if this were the case, a similar trend would be expected to be seen in the other washed samples. Another cause of the different fluorescence levels could lie in the detergents themselves, with Radiant detergent adsorbing to red cotton fibres more effectively than Fab or Cold Power detergents.

These results show that, when fluorescence is present, it can be used as an effective indicator that the sample has been washed. However, the inconsistency of the fluorescence fibres also means that the absence of fluorescence in the case of red cotton cannot be used to indicate that the samples have not been washed.

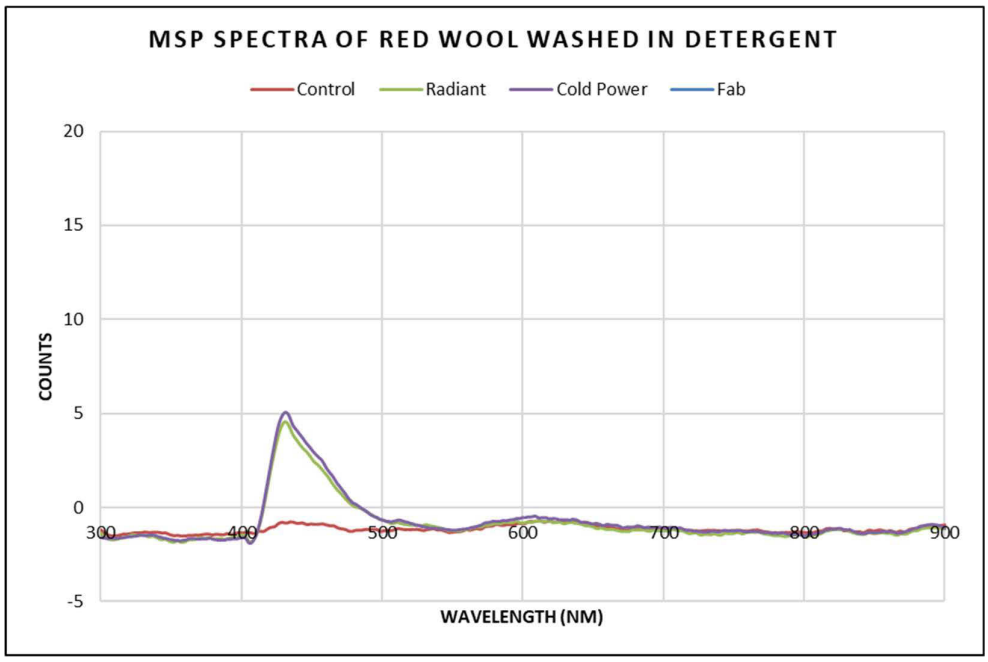


Figure 3-31: MSP Fluorescence spectra of Red Wool washed in Cold Power and Fab detergent

MSP spectra collected of red wool (Figure 3-31) reveal information about the fluorescence of the fibres that could not be seen visually in Table 3-12. The control sample was found to have no fluorescence peaks while all three washed red wool samples had a very small intensity peak between 400 and 500 nm. The low intensity of this peak reflects the low intensity of the fluorescence of the samples and indicates why this fluorescence could not be seen visually.

These results are significant as, using MSP fluorescence spectra, a washed red wool sample is able to be distinguished from an unwashed red wool sample. This separation was not able to be performed using the VSC images and Spectra, or using the MSP images.

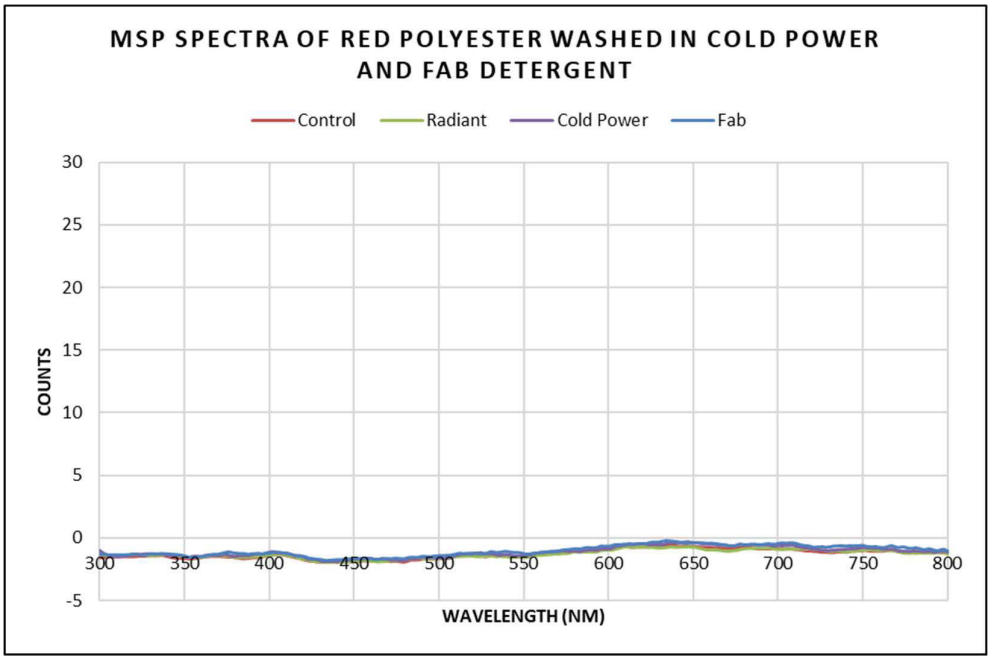


Figure 3-32: MSP Fluorescence spectra of Red Polyester washed in Cold Power and Fab detergent

Both the control and the washed red polyester samples show no visual fluorescence in Table 3-12 which is reflected in the spectra collected in Figure 3-32. This also is consistent with and helps support the results collected using the VSC in section 3.2. This indicates polyester fibres will not undergo any change or increase in fluorescence after washing and therefore fluorescence analysis will be unable to be used to help distinguish them.

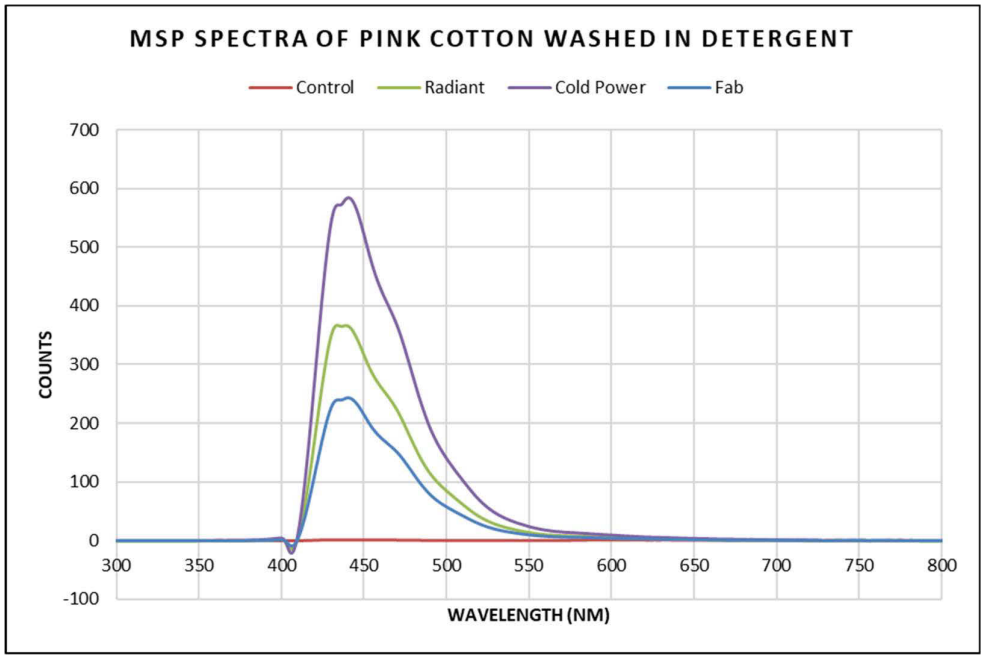


Figure 3-33: MSP fluorescence spectra of pink cotton washed in Radiant, Cold Power and Fab powder detergent

The pink cotton control sample was found to contain no fluorescence peaks in Figure 3-33, supporting the visual fluorescence results in Table 3-13. The pink cotton samples washed in Cold Power, Radiant and Fab, on the other hand, were all found to have a broad peak between 400 and 550 nm, with two small peaks at the top of the maximum peak at 433 and 438 nm. The shape of these peaks is consistent with fluorescence in the blue range and coincides with the fluorescence spectra collected from other washed samples such as blank cotton and viscose/rayon samples.

From these results, it can again be seen that while a pink cotton sample washed in detergent can be differentiated from a pink cotton control sample, there is no difference in the spectra of the different washed pink cotton samples to provide information about which detergent was used.

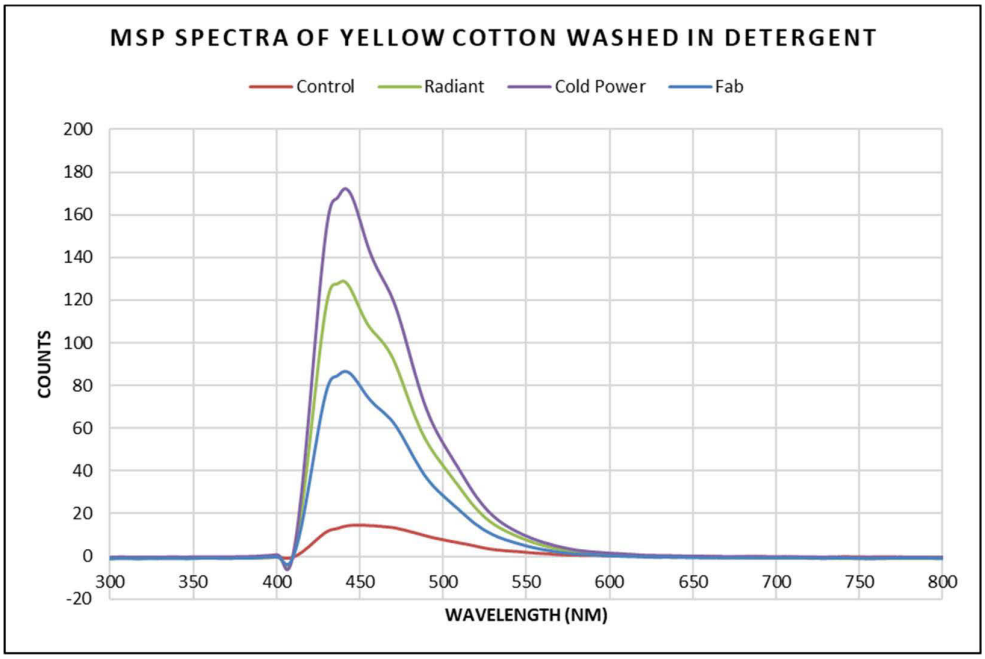


Figure 3-34: MSP fluorescence spectra of yellow cotton washed in Radiant, Cold Power and Fab powder detergent

The yellow cotton control showed visual signs of slight fluorescence in Table 3-13, which is reflected in the fluorescence spectra of the yellow cotton control (Figure 3-34). The control shows a shallow broad peak from 415 to 530 nm indicating that there is a small degree of fluorescence detected from the yellow cotton control sample.

The spectra for the yellow cotton washed in Cold Power, Radiant and Fab also show a large broad peak between 415 to 530 nm. The width and shape of these spectra are consistent with both the fluorescence spectra collected from other washed samples as well as the fluorescence spectra collected of the detergents. The only difference in the spectra of the washed samples is the intensity of the fluorescence peak, with Cold Power having the highest intensity fluorescence and Fab having the lowest.

The value of these results is that, while the washed and control yellow cotton samples were unable to be readily distinguished based on visual comparison of their fluorescence images in Table 3-13, there is a clear difference in their fluorescence spectra.

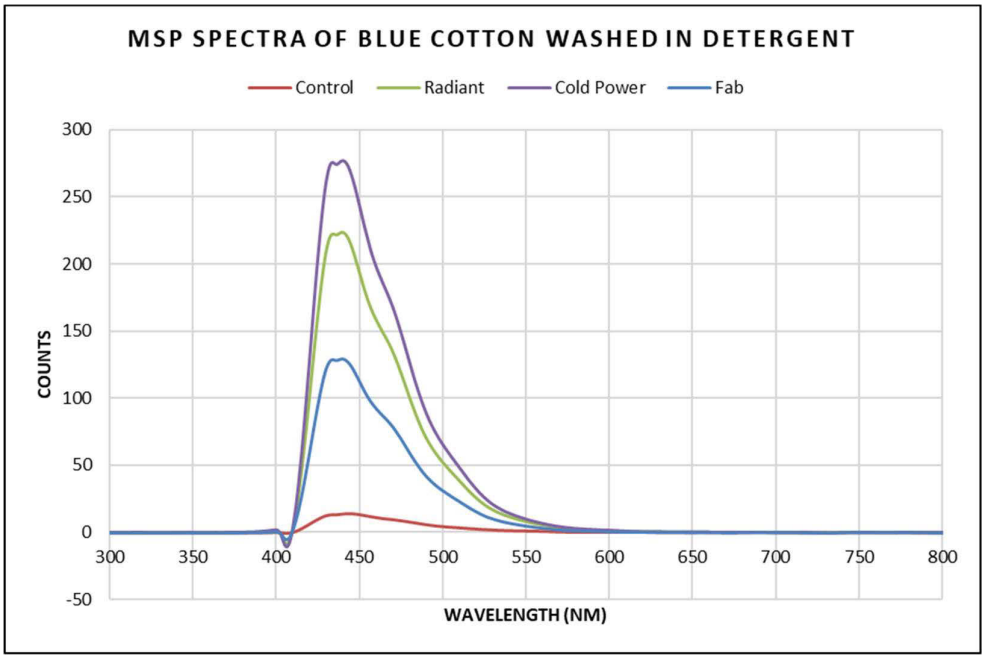


Figure 3-35: MSP Fluorescence spectra of Blue Cotton washed in Cold Power and Fab detergent

The results for the blue cotton samples in Figure 3-35 were found to be similar to that of the yellow cotton samples in Figure 3-34. The blue cotton control, like the yellow cotton control, was found to have a shallow broad peak between 415 and 530 nm.

After washing, the spectra were found to change significantly, with all washed blue cotton samples having a large peak present between 400 and 550 nm, consistent with the blue fluorescence observed in other samples. The only difference between the spectra of the washed samples is the intensity of the peaks, with Cold Power having the highest intensity followed by samples washed in Radiant and Fab. This trend in intensities could also be seen in both the pink and yellow cotton samples.

Repeatability

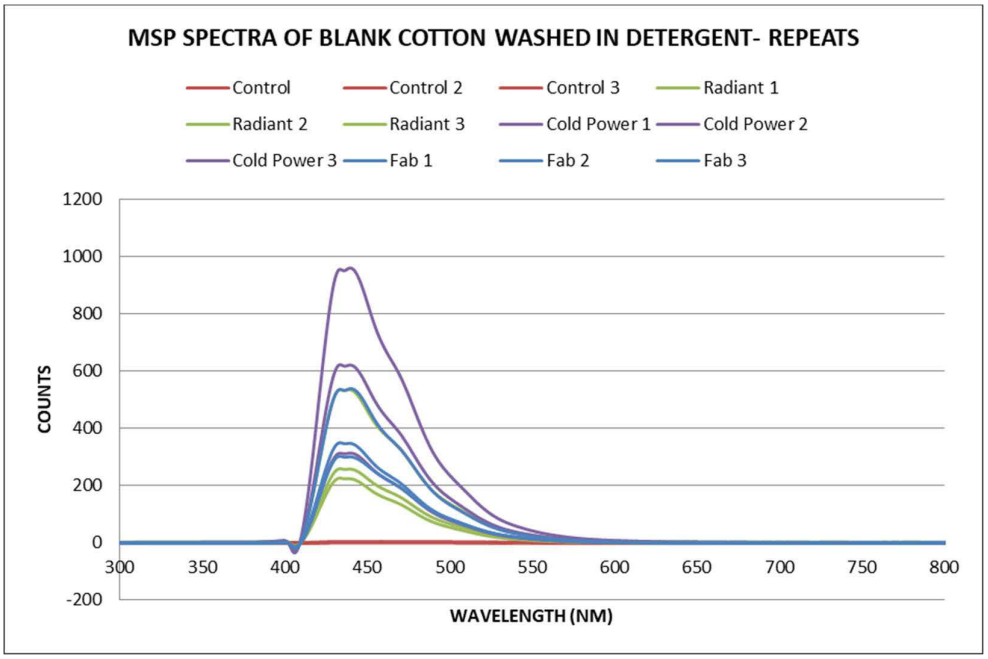


Figure 3-36: MSP Fluorescence spectra of repeats of Blank Cotton samples

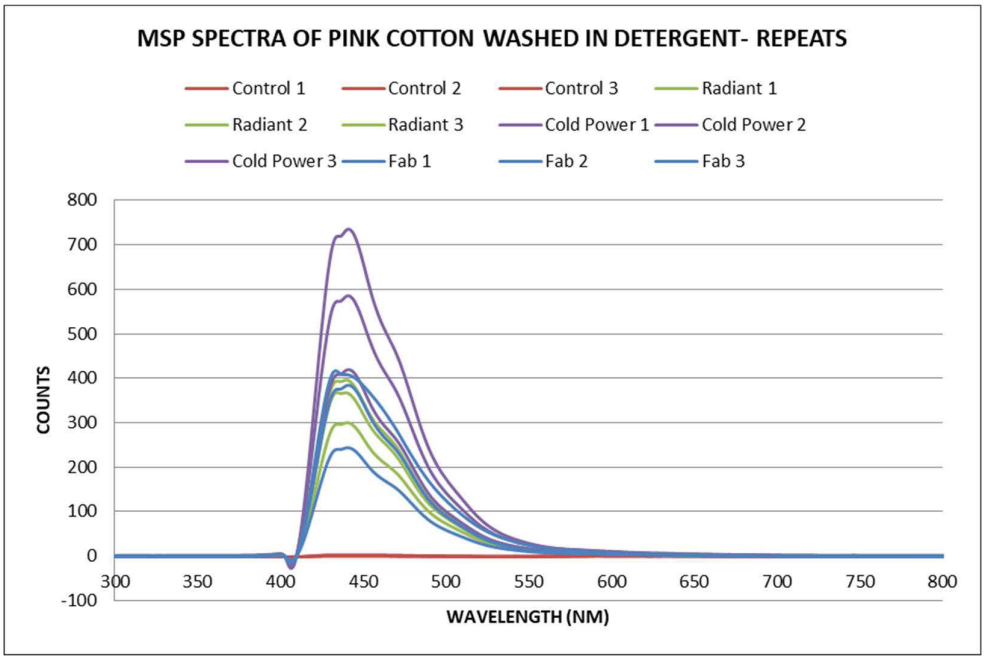


Figure 3-37: MSP Fluorescence spectra of repeats of Pink Cotton samples

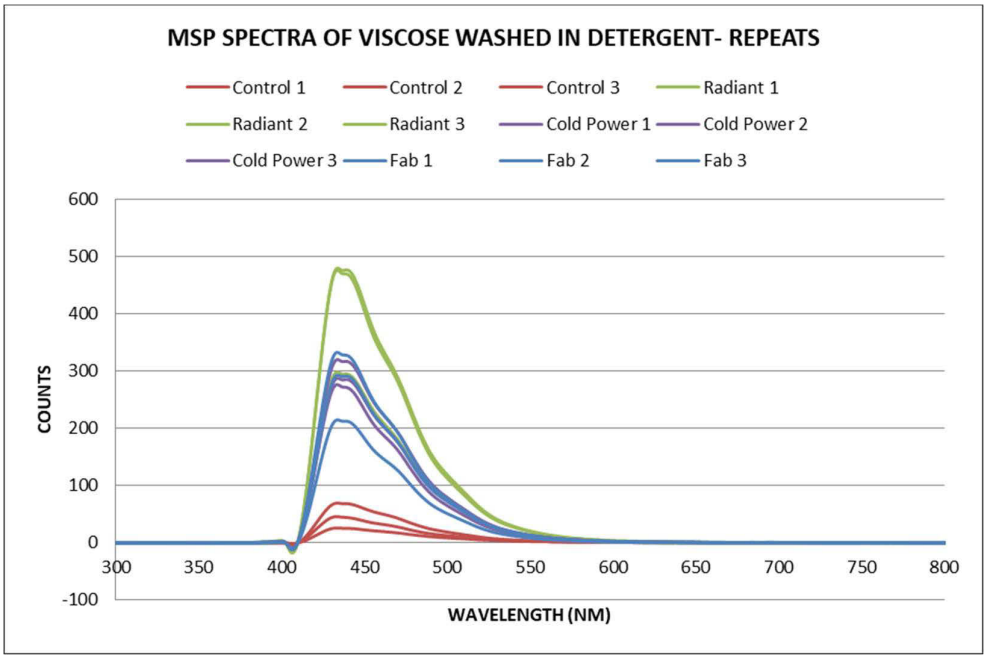


Figure 3-38: MSP Fluorescence spectra of repeats of Viscose samples

Figure 3-36, Figure 3-37 and Figure 3-38 show repeat spectra collected of control and washed samples of blank cotton, pink cotton and blank viscose. Each spectrum is averaged from three spectra collected from three different areas of a fibre. Each fibre comes from a different sample that was washed with the same detergent (16 times) but in a different load. All three figures show that the position and shape of the fluorescence peaks are consistent across all repeats. The intensities of each repeat are not consistent for each sample type, however, although each sample has peaks in a similar range. This indicates that the results from MSP have limited repeatability due to the instrument variation for each sample.

PCA Analysis

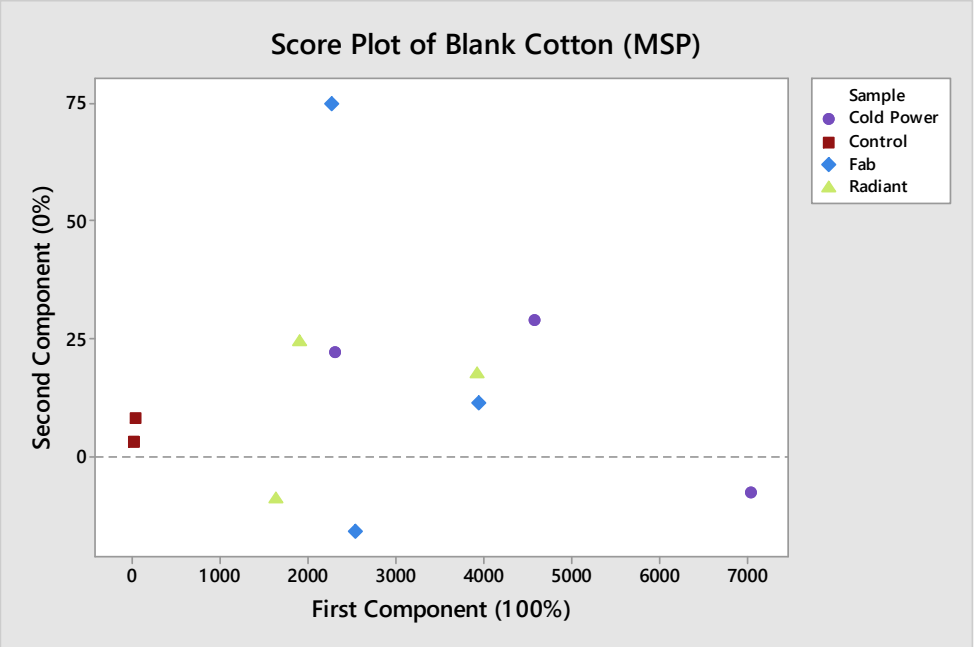


Figure 3-39: Score plot of Blank Cotton (MSP)

The blank cotton MSP score plot in Figure 3-39 shows there is a clear and significant separation between a tight cluster of control sample points and the washed sample points (which are more widely dispersed). This separation is along the first component and is based on the variance in two main peaks. Firstly, at 410.38 nm, the washed sample spectra dip below 0 counts, giving the control samples a higher intensity at this point. The main cause of the variance is the intensity of the peak between 430 and 450 nm. The fluorescence spectra of these samples in Figure 3-26 shows that no fluorescence peaks were detected for the control samples, while a broad fluorescence peak between 400 and 600 nm was detected for the washed samples. The dispersion of the washed sample scores around the score plot was due to each washed sample having different intensities in these peaks. The lack of clustering based on the detergent used shows there is little connection between the detergent and the intensity of the fluorescence.

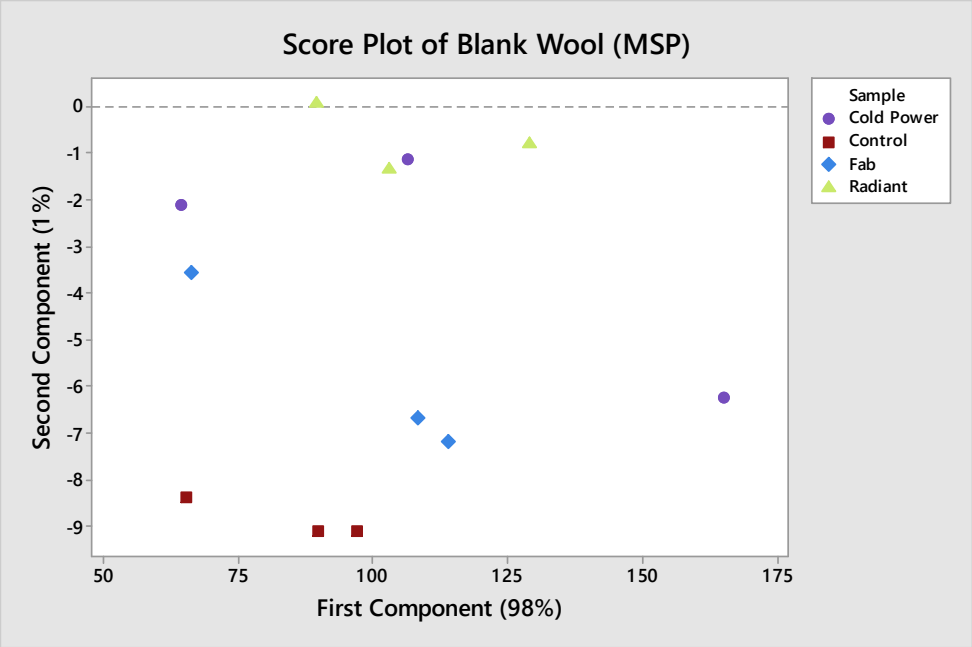


Figure 3-40: Score plot of Blank Wool (MSP)

A partial separation of the control and washed blank wool samples (Figure 3-40) can be found along the second component (which accounts for only 1% of the variation). The separation of the different samples is based on the difference in intensity of the fluorescence spectra between 416 and 435 nm. The fluorescence spectra of the blank wool in Figure 3-27 shows that fluorescence is detected on the control samples. After washing, this fluorescence is found to increase for some of the washed samples (particularly the Radiant samples), while other washed fibres showed the same intensity fluorescence as the control sample. Figure 3-40 shows that, while there is a slight separation between the control samples and some of the washed samples, the difference between these score plots is not large enough to be significant and so therefore cannot be used to indicate that the control and washed sample fibres are different.

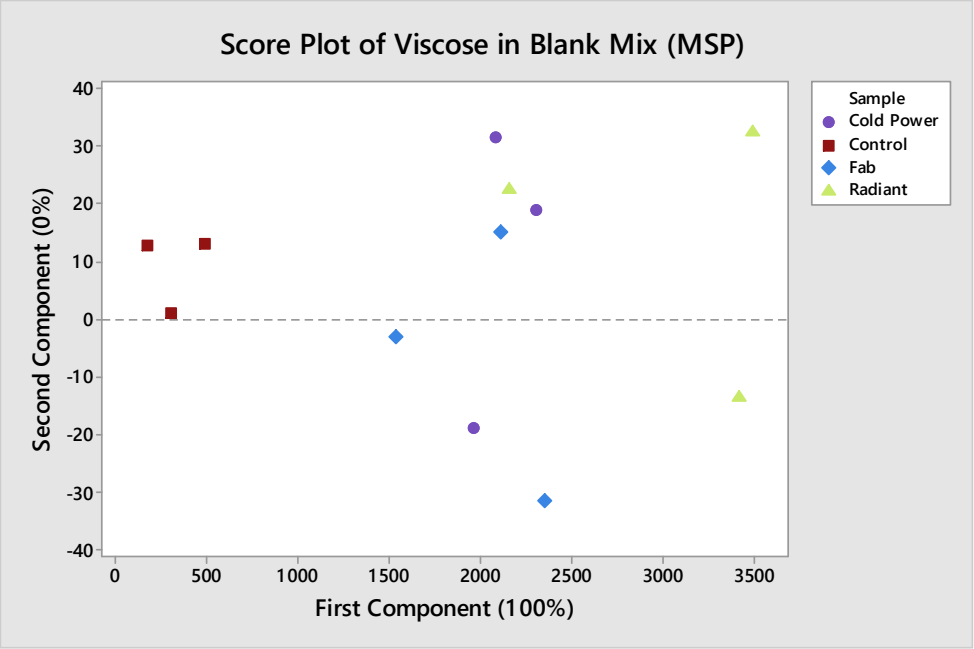


Figure 3-41: Score plot of Viscose in Blank Mix (MSP)

A clear separation is seen between the controls and washed viscose fibres from the blank mix textile in Figure 3-41. This separation occurs along the first component which accounts for 100% of the variation between the samples. The score plot for the viscose samples is very similar to the blank cotton samples in Figure 3-39. This is likely due to the fact that viscose and cotton both contain cellulose. Like the blank cotton samples, the variation along the first component can be predominantly attributed to the difference in fluorescence intensities at 410.38 and between 430 and 450 nm. There is no significant clustering based on the detergent used, and so as a result, the fluorescence and PCA analysis can only be used to determine if the fibres have been washed or not.

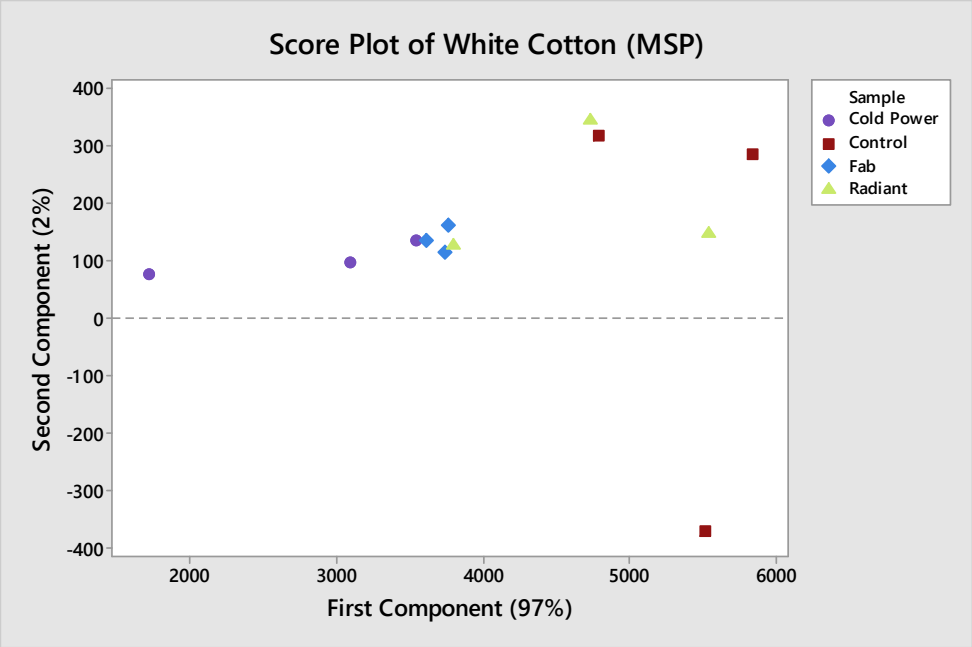


Figure 3-42: Score plot of White Cotton (MSP)

The white cotton score plot in Figure 3-42 shows a partial separation of the samples along the first component, however, there is a significant overlap between the control sample scores and those of the samples washed in Radiant. The variation in the first component is based on a difference in fluorescence intensities at 410.38 nm (left side of plot) where the spectra for all samples dip below 0 counts, and between 430 and 450 nm on the right side of the plot. This region is the part of the main fluorescence peak where the white cotton control samples were found to have more intense fluorescence than many of the washed samples. This indicates that no fluorescence is accumulated after washing and that the washing process may instead be removing some of the fluorescent residues from the white cotton. The lack of clear separation between the control and washed samples, however, shows that fluorescence is not able to reliably discriminate the two groups.

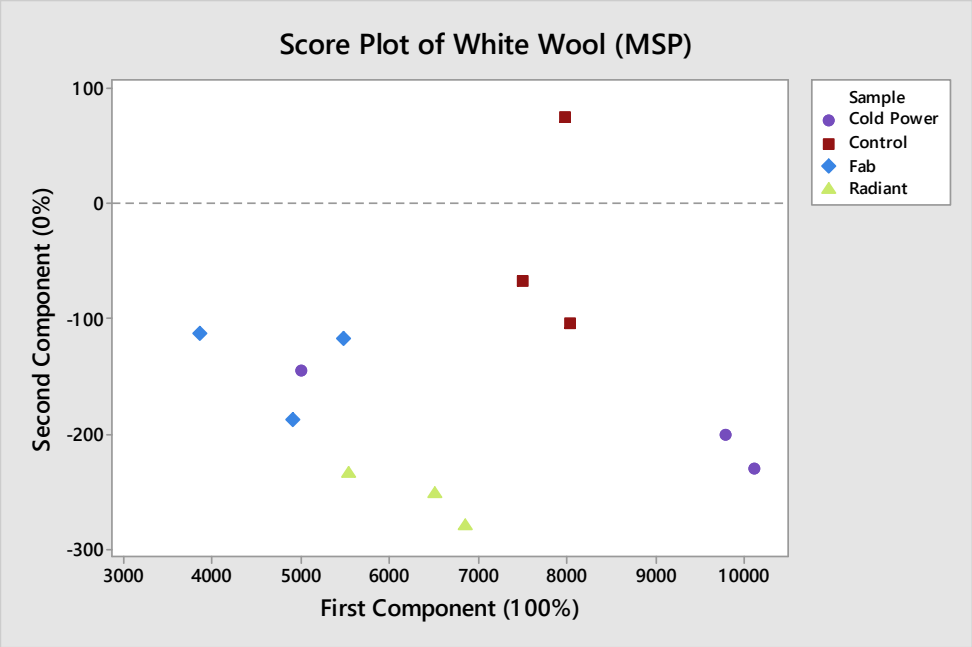


Figure 3-43: Score plot of White Wool (MSP)

Figure 3-43 shows a partial separation of the control and washed samples along the first component (which accounts for 100% of the variation). This variation is due to the difference in the intensity of the fluorescence peak at 432 nm (the highest point of the fluorescence peak). Two of the Cold Power samples, which had the highest fluorescence, are positioned to the right of the score plot while the third Cold Power sample and the Fab samples, which have the lowest intensity fluorescence, are to the left of the component. As with the white cotton samples, the white wool control had a high-intensity fluorescence peak which was less intense than the two Cold Power samples, but more intense than the other washed samples. As there is no clear trend in the accumulation or elimination of fluorescence, limited meaning can be construed from the score plot as it would be impossible to tell whether a sample with an unknown history was a control or a washed sample.

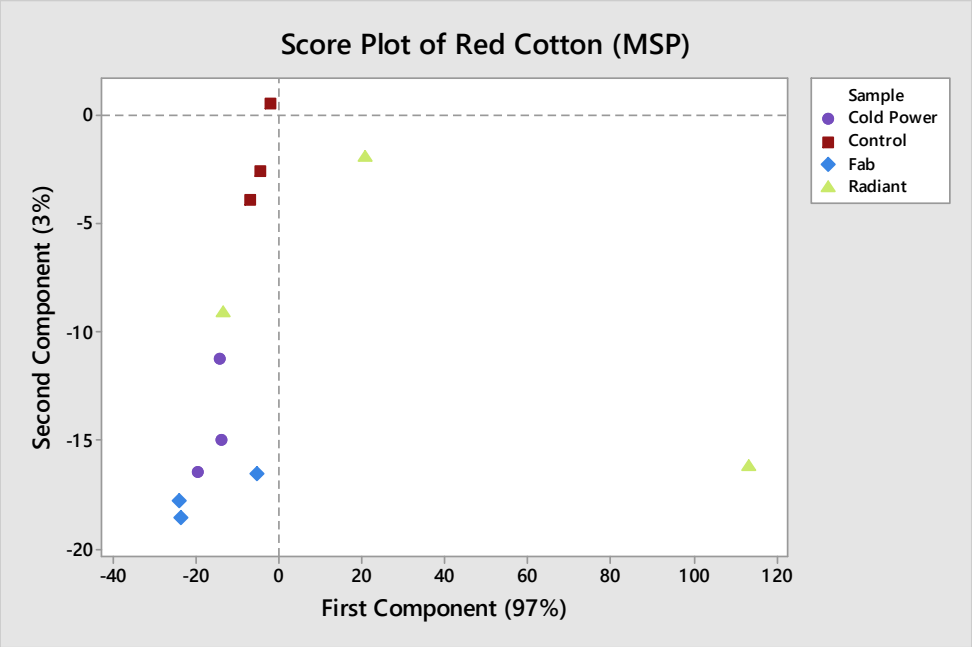


Figure 3-44: Score plot of Red Cotton (MSP)

The score plot of red cotton in Figure 3-44 shows that only two washed samples, both washed in Radiant detergent, can be separated from the control samples. This separation occurs along the first component and is due to the intensity of the fluorescence at 434 nm. In Figure 3-30, it was discussed how most washed red cotton control samples showed no fluorescence, but some, including the two radiant samples, had small areas of fluorescence at various points along the fibre, giving a fluorescence peak between 400 and 550 nm with the top of the peak at 434 nm. Therefore, if fluorescence peak is detected, it is a reliable indicator of the presence of optical brighteners.

Figure 3-45: Score plot of Red Wool (MSP)

There is a partial separation of the samples in Figure 3-45, with all but two of the washed samples scores a significant distance from the group of control samples to the left of the plot. The separation along the first component is based on the intensity of the peak at 430.82 nm. The red wool control samples have a low intensity fluorescence peak between 411 and 480 nm, this fluorescence increased after washing, as seen in Figure 3-31, leading to the separation in the score plot.

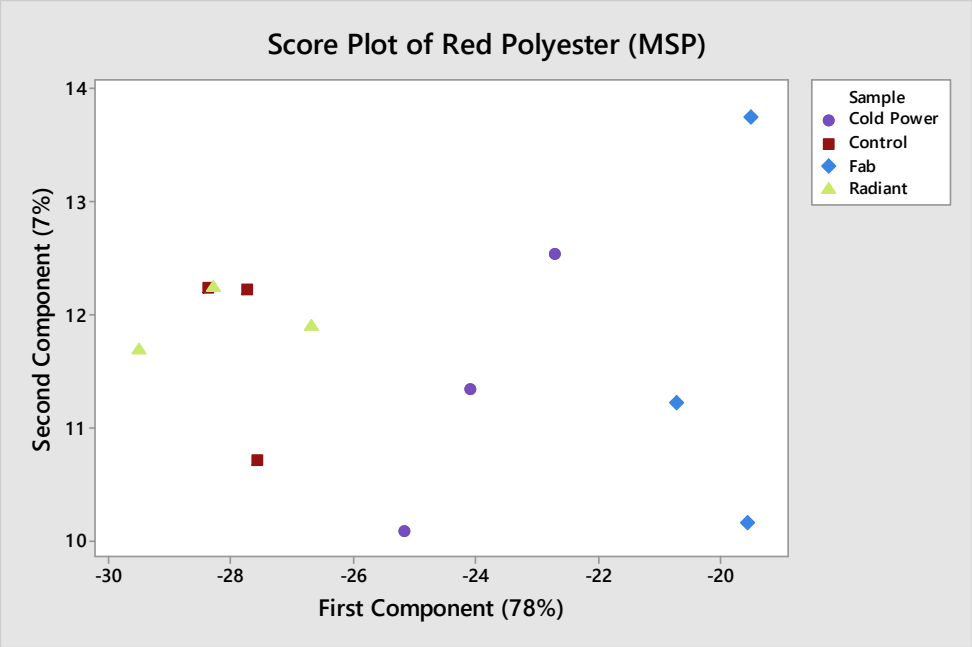


Figure 3-46: Score plot of Red Polyester (MSP)

Neither the red polyester control nor washed samples in Figure 3-32 showed any fluorescence. The only spectra detected was background noise. This is reflected in the score plot in Figure 3-46 which shows no clustering of either the washed or control samples. The variation along the first component is due to divergence of the noise spectra at 300 and 900 nm, at the limits of the wavelengths measured. Therefore, it is not possible to tell whether a red polyester fibre has been washed or not using fluorescence microspectrophotometry.

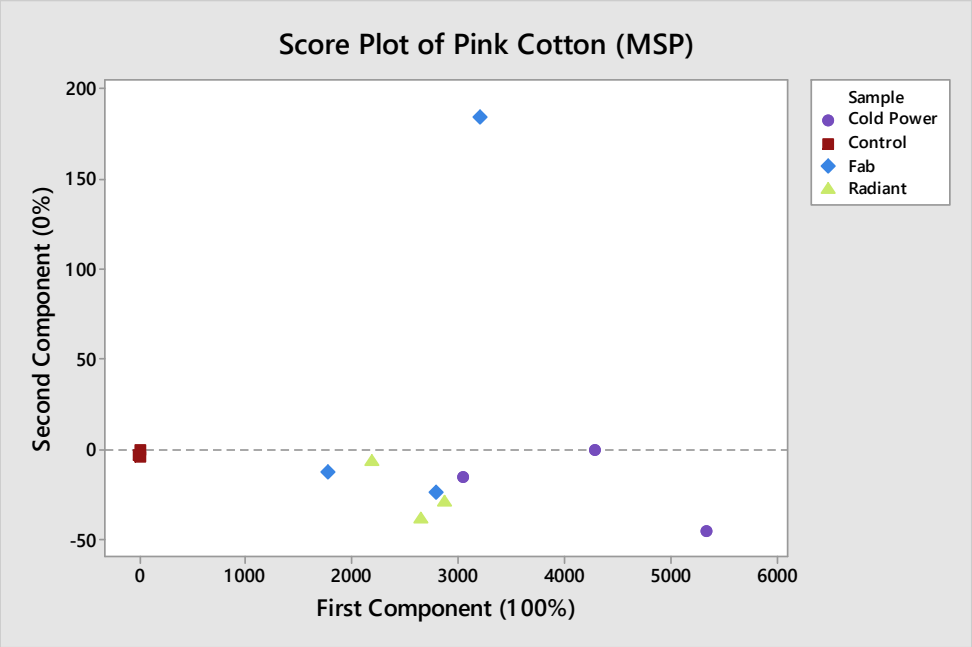


Figure 3-47: Score plot of Pink Cotton (MSP)

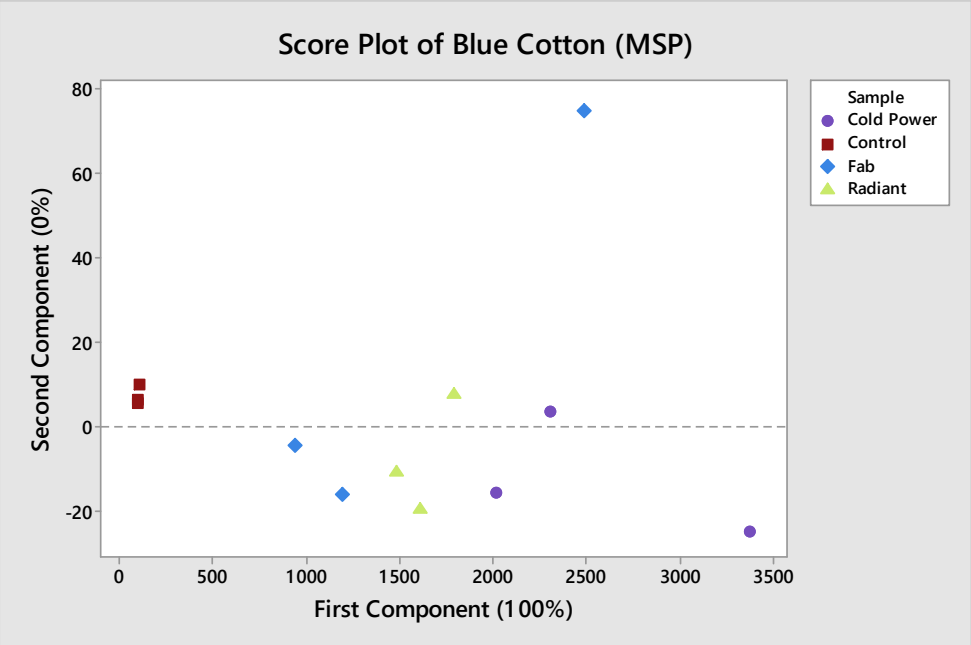


Figure 3-48: Score plot of Blue Cotton (MSP)

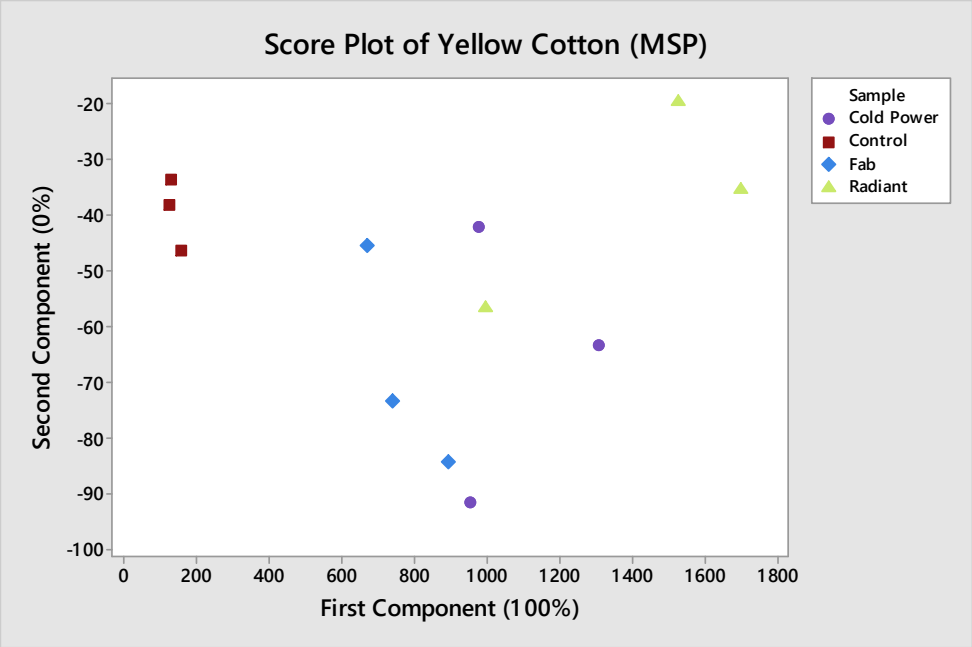


Figure 3-49: Score plot of Yellow Cotton (MSP)

In Figure 3-47, Figure 3-48 and Figure 3-49 there is a clear separation of the control and washed pink, blue and yellow cotton groups along the first component. The variation in the first component is based on the differing intensity of two main sections of the fluorescence spectra. The samples to the left of the score plot, the control samples, have a higher intensity at 410.30 nm, due to there being fluorescence quenching of the washed samples at that point. The samples to the right of the score plot have a higher peak intensity between 430 and 450 nm. This is to be expected based on the spectra in Figure 3-33, which shows that little to no fluorescence was detected from the pink control samples, while the washed samples had a wide fluorescence peak between 400 and 600 nm. The blue and yellow cotton control samples (Figure 3-34 and Figure 3-35) have a low-intensity fluorescence peak, but again there is a large increase in this peak after washing. There is no clustering based on the detergent used, indicating that the intensity of the fluorescence peaks has no connection to the detergent used.

3.3.2 MSP Conclusion

The analysis of the fluorescence of washed samples using microspectrophotometry was found to produce more detailed fluorescence spectra than the Video Spectral Comparator.

The white dyed fabrics were again found to already contain optical brighteners that produced the same fluorescence spectra as the detergents they were washed in. This means that any acquired characteristics cannot be differentiated from the manufactured characteristics.

However, analysis of both blank and coloured (red, pink, yellow and blue) samples identified unique peaks that could be attributed to the detergents used and thus identified as an acquired characteristic. This peak could be used to aid in the differentiation of evidence that have and have not been washed. These peaks were found to be consistent across all the detergents sampled and so cannot be used to identify which specific detergent was used.

3.4 Conclusion

The visual and spectral analysis of the fluorescence of samples, using VSC and MSP, is found to have both positive and negative attributes. The implementation of this form of analysis is advantageous as it is quick, easily incorporated into the Standard Operating Procedures, uses equipment that is already present in most labs and, most importantly, is non-destructive.

Results show that acquired characteristics were found in some samples that could be linked to the presence of detergent. These characteristics could be utilised for the comparison of samples in addition to manufactured characteristics such as their fibre and dye type.

However, the information gathered from these acquired characteristics is limited as it can only reveal if a textile has been washed or not. It does not reveal how many times it has been washed nor is it able to identify which detergent was used. Therefore, the information collected from this type of analysis does not greatly increase the evidential value of the fibres.

Regarding the instruments used for analysis, VSC and MSP, the MSP was found to be a more effective technique. While the VSC was quicker and easier to use, requiring less

analysis time, it was also found to be less sensitive than the MSP. The MSP was able to provide all of the same information as the VSC but was also able to detect fluorescence on samples (red wool) that the VSC was not. The MSP is also able to analyse samples at the fibre level, requiring only a small sample size a few millimetres long. The VSC can only conduct analysis on the textile level and would require a substantially larger sample size, for example, a swatch from a garment rather than just a fibre. As a result of this, if this analysis were to be employed for casework, only the MSP would be required to get the fluorescence results necessary and the VSC need not be used. The PCA results confirmed the visual analysis of both the VSC and MSP samples. As the PCA was not performed on a large enough sample size, and requires extra time and experience from the examiner, this technique is not required for case analysis, however, as it allows for small differences in spectra, that may be missed in visual analysis, to be clearly seen it is a useful tool to use.

Chapter 4:

Chemical Composition

Chapter 4: Chemical Composition

4.1 Raman Spectroscopy

4.1.1 Introduction

Raman Spectroscopy is a quick, non-destructive form of analysis. Control spectra of the four detergents were acquired to determine whether differences could be seen between them. It was then implemented to find whether these detergent residues could be detected on washed textiles by comparing the Raman spectra of the control and washed textiles.

4.1.2 Results and Discussion

Raman analysis of detergents

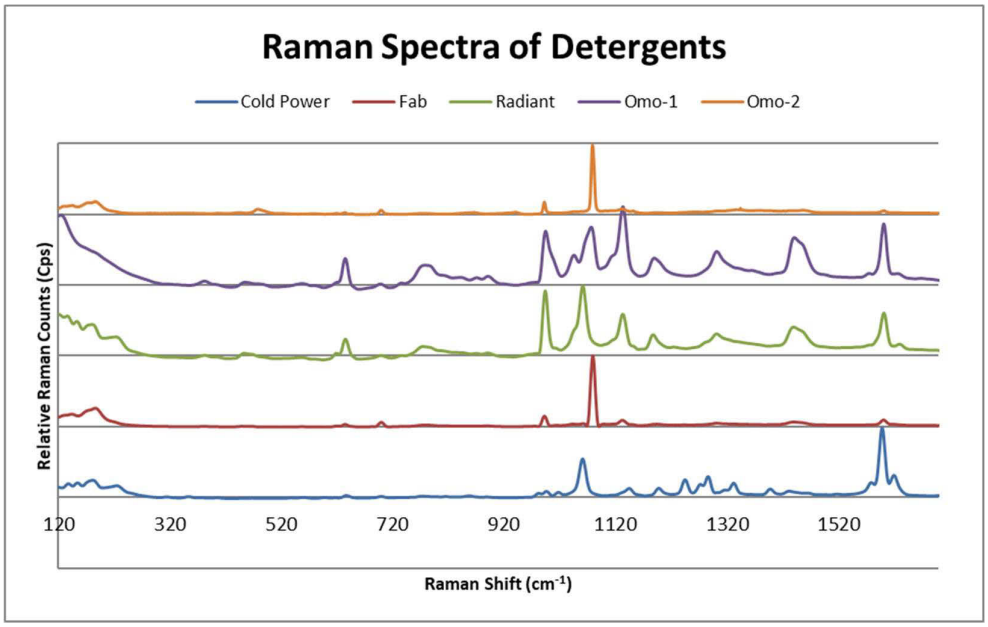


Figure 4-1: Stacked Raman spectra of Radiant, Cold Power, Fab and Omo detergent (120-4000 cm^{-1})

Raman spectra were collected of four powder detergents: Radiant, Cold Power, Fab and Omo (Figure 4-1). The Raman spectra collected of the detergents showed that the Radiant, Cold Power and Fab detergents are homogenous within the brand, with each particle

analysed found to have the same Raman spectra and thus the same chemical makeup. The Omo detergent, on the other hand, was discovered to be heterogeneous (Figure 4-2).

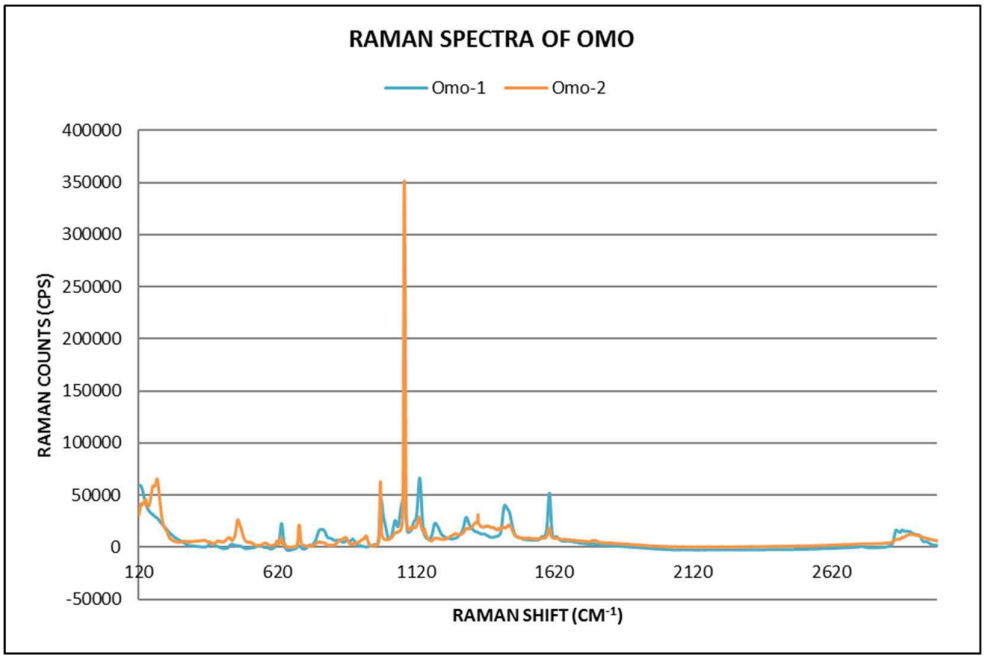


Figure 4-2: Raman spectra of Omo detergent

Of the five Omo particles analysed, two different Raman spectra were identified. Three of the five particles were found to have the Omo-1 spectra, while two had the Omo-2 spectra.

While these two Raman spectra were found to have some peaks in common (125,382, 456, 558, 635, 698, 736, 778, 815, 842, 871, 891, 995, 1045, 1076, 1133, 1190, 1302, 1364, 1441, 1575, 1602, 1626, 2731, 2854, 2875, 2961 and 3065 cm^{-1}), the majority of the peaks were found to be unique to that particular particle.

The difference between the Raman spectra shows that the Omo detergent is a mixture of at least two solids that have different chemical compositions. If a large enough sample size is acquired, this heterogeneous characteristic of Omo detergent can aid in the comparison of Omo to other detergents as there would be two spectra available, both with unique peaks.

Analysis of the Raman spectra collected in Figure 4-1 between 120 and 4000 cm^{-1} reveals that the detergents have many of their peaks clustered around the same Raman shift. This

grouping is because, as discussed in Chapter 1.5.1 in the introduction, laundry detergents are all made up of builders, surfactants, bleaches, enzymes and other chemicals such as optical brighteners and fragrances that aid in the cleaning of the clothes. As a result of this, some of the chemicals used in all four detergents are the same, while others share a similar chemical structure due to their shared purpose.

The peaks that are consistent across all four detergents can be attributed to sodium sulphate and sodium carbonate, the two most abundant chemicals that are present in all the detergents analysed. The peaks at 450 cm^{-1} and 634 cm^{-1} are due to the asymmetric bending of the SO_4^{2-} group, while the peaks in the 990 cm^{-1} range can be attributed to the symmetric stretch of the SO_4^{2-} group and the peaks at 1132 cm^{-1} are due to an asymmetric stretch of the SO_4^{2-} group [113]. The peaks that can be linked to the presence of sodium carbonate are those at 700 cm^{-1} which is due to C-O symmetric in-plane bend, the peak at 1061 cm^{-1} caused by C-O symmetric stretch and the peak at 1430 cm^{-1} which is due to C-O anti-symmetric stretch.

The peak present at 1600 cm^{-1} is due to an aromatic compound in-plane phenyl ring stretching mode. This peak could be due to the presence of surfactants such as sodium tridecyl benzene sulphonate (which is found in Cold Power and Fab), alkyl benzene sulfonic acid sodium salts (Radiant) and Sodium dodecylbenzenesulfonate (Fab). It has also been proposed by the Contact Traces group in the UK that the peak is due to the presence of optical brighteners [114]. Fluorescent Brightener 71 (Disodium 4, 4'-bis [(4-anilino-6-morpholino-1, 3, 5-triazin-2-yl) - amino] stilbene-2, 2'disulphonate) is found in Cold Power and Fab detergents while Fluorescent Brightener 351 (Disodium Distyrylbiphenyl Disulfonate) is in Radiant and Omo detergents.

The final peak that is found in all detergents is at 2854 cm^{-1}. This peak is due to vibrations in the C-H bond which is expected due to the presence of hydrocarbons in the form of surfactants present in all detergents.

However, despite the similarities in peak placement, there is enough variation in the position and intensity of the peaks to allow all four detergents to be differentiated from each other. While the majority of the peaks present can be located in at least one other detergent, there are some peaks that are unique for each detergent.

Cold Power has unique peaks at 315, 354, 598, 857, 978, 1018, 1145, 1271, 1286, 1332, 1465 and 1518 cm^{-1}. The peaks between 1000 and 1400 cm^{-1} are the most significant and are likely due to aliphatic C-C bonds in the long carbon chain of the cationic surfactant Lauryl dimethyl benzyl ammonium chloride which was unique to Cold Power.

The Fab detergent was found to have only one peak unique to it which was located at 1100 cm^{-1}. This is to be expected as, out of the six ingredients reported as being in Fab detergent, only one ingredient, sodium silicoaluminate, was found to be unique to Fab.

Radiant detergent was also found to have only one unique peak present at 1225 cm^{-1}. There were, however, many other peaks that it has in common with either Cold Power or Omo-1 detergent. The similarity in Raman spectra between Cold Power and Radiant is interesting as these two detergents only share two ingredients in common, Sodium Carbonate and Sodium Sulphate, while Radiant has two unique ingredients; alkyl benzene sulfonic acid sodium salts and sodium disilicate.

As discussed previously, the Omo powder detergent was found to consist of two different detergent particles (Omo-1 and Omo-2). Both these two detergent particles exhibited peaks in their Raman spectra which were not found in each other or any of the other detergents. The peaks at 842 and 1364 cm^{-1} were unique to Omo-1, while those at 478, 524, 578, 720, 768, 940, 1344, 1457 and 3198 cm^{-1} was unique to Omo-2. The only peaks that could be found in both Omo-1 and Omo-2, but not in the other detergents, were at 125 cm^{-1}, again showing how different the two detergent particles are.

The Omo-1 detergent was found to have few unique peaks as it shared most of its Raman peak placements with the Radiant detergent with only a few peaks distinguishing them from each other. This similarity in their structure could be due to the fact that Omo and Radiant share the same optical brightener (Fluorescent Brightener 351).

Raman analysis of detergents on blank textiles

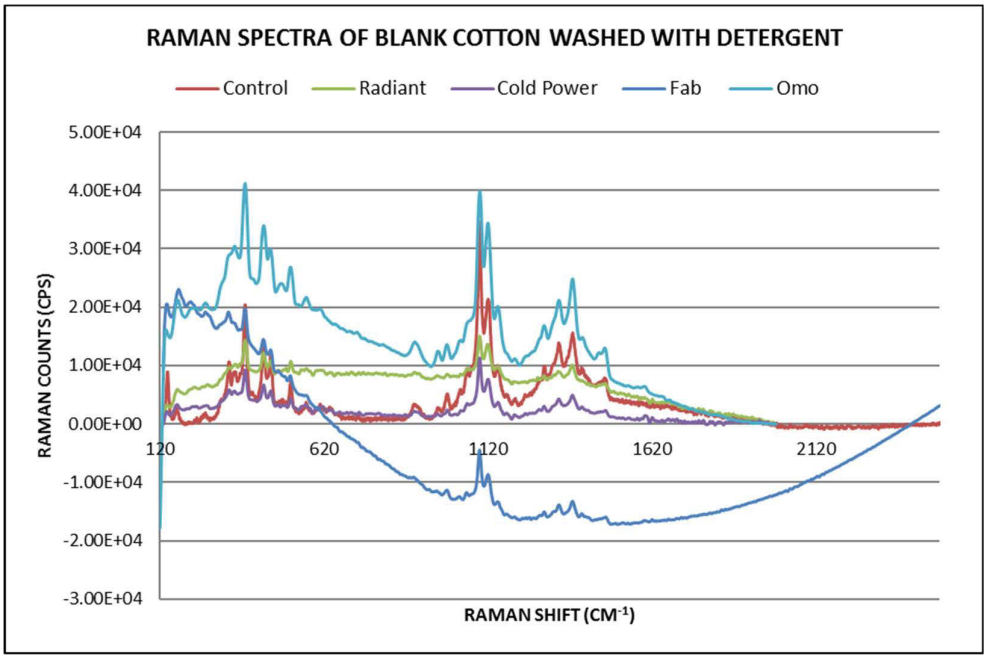


Figure 4-3: Raman spectra of Blank Cotton washed with Radiant, Cold Power, Fab and Omo powder detergents

While Raman spectroscopic analysis of detergent powder particles was able to produce unique Raman spectra that could be used to differentiate and identify each detergent, Raman spectroscopy was unable to detect residual detergent on washed blank cotton. This could be due to two reasons: either the Raman is not sensitive enough to detect residues against the more prominent signal given off by the textile, or there was little to no detergent residue to be detected in the first place.

Analysis of blank cotton samples washed in Radiant, Cold Power, Fab and Omo found that the spectra were all virtually identical to the control with all but one peak being attributed to the chemical profile of cotton. The peaks between 333 and 522 cm^{-1} can be attributed to skeletal C-O-C, C-C-C, O-C-C and O-C-O bends. At 900 cm^{-1} the peak is caused by C-O-C in- plane, symmetric stretch, 1097 cm^{-1} is from C-C and C-O stretch and the 1338 cm^{-1} peak is due to H-C-C, H-C-O, and H-O-C bend. Finally, the peak at 1481 cm^{-1} is present due to H-C-H and H-O-C bend [115].

However, a small peak is located at 1600 cm^{-1} in the washed samples that cannot be found in the blank cotton control sample. As discussed previously, this peak is from an aromatic compound in-plane phenyl ring mode and is thought to be due to the presence of optical brighteners in the detergent. The absence of this peak in the control, and its presence in the washed sample can be explained as the blank cotton used in this experiment has not gone through any dying or post-manufacture processes and so would not have come in contact with optical brighteners before being washed in detergent.

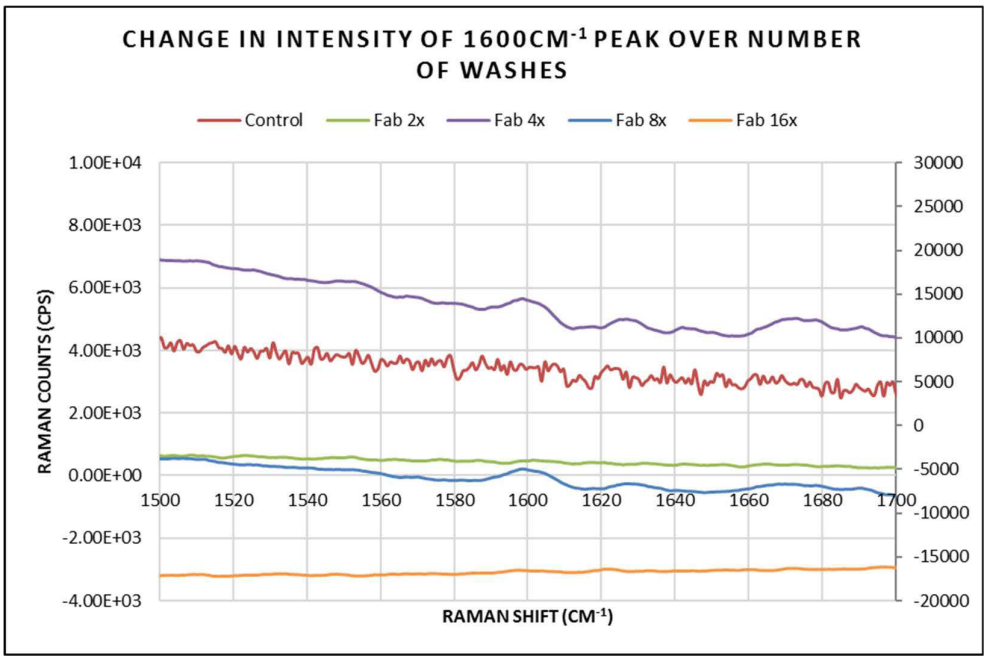


Figure 4-4: 1600cm^{-1} peak in Blank Cotton washed in Fab over a number of washes

Figure 4-4 shows the change in the intensity of the peak found at 1600 cm^{-1} in spectra of blank cotton washed in Fab over a number of washes. No peak could be found in either the blank cotton control sample or in the sample that was washed two times in Fab. A peak can be seen in the 4, 8 and 16 washes implying that signs of a detergent being used can be detected after four or more washes.

The information given by this peak is limited, however, as the size of the peak does not relate to how many times the sample was washed. For example, a larger peak was found in the sample washed four times than in the sample washed 16 times. This variation could reflect that there were fewer residues on the 16x sample, or it could be due to variability when the Raman spectra were collected. This lack of trend in peak size means that, while

a washed sample can be differentiated from an unwashed sample, two samples washed in the same detergent a different number of times are unable to be discriminated.

These spectra also show how very small this peak of interest is. Due to its small size, it does not stand out from the rest of the spectra and has to be specifically looked for. The small size of this peak means that it is not reliable enough to be used in casework.

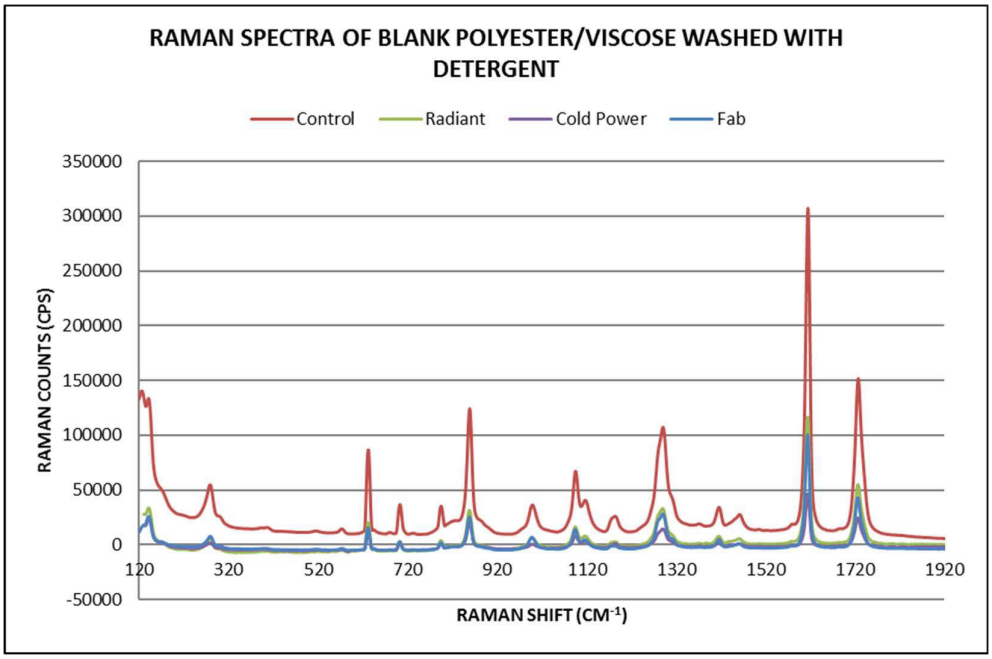


Figure 4-5: Raman spectra of Blank Polyester/Viscose washed with Radiant, Cold Power and Fab detergent

While an acquired characteristic peak was found in the blank cotton spectra in Figure 4-3, analysis of the Raman spectra of Blank Polyester/ Viscose threads in Figure 4-5 showed no signs of any variation between the control and the washed samples. The spectra collected here are for the polyester fibres in the mix, not the viscose fibres, as they were too difficult to isolate. Thus, all these observations relate to the behaviour of polyester fibres when washed.

All the peaks found in the spectra of the washed and control blank mix can be attributed to the textile, not to acquired characteristics. The peaks at 1119 and 1290 cm^{-1} are from the C-O-C ester bond. At 1180 cm^{-1} the peak is due to a para-disubstituted benzene ring while the peak at 1614 cm^{-1} can also be attributed to the presence of a benzene ring.

Finally, the peak at 1726 cm^{-1} is due to a carbonyl bond while the peak at 1450 cm^{-1} is due to CH_2 in the O-CH_2CH_2-O group [116].

Unlike the blank cotton control sample, no acquired peak could be found at 1600 cm^{-1}. The only peak in that range can be found at 1614 cm^{-1} and is attributed to the textile, not to any detergent residues. This reflects what was seen in the fluorescence results in Chapter 3 where no fluorescence was detected on polyester fibres, either in the polyester/viscose mix or the 100% polyester, indicating that the detergent residues used are unable to adhere to the smooth polyester fibres.

As with fluorescence results, the Raman results show that the type of textile significantly affects how many detergent residues are retained and thus how readily these acquired characterises can be detected.

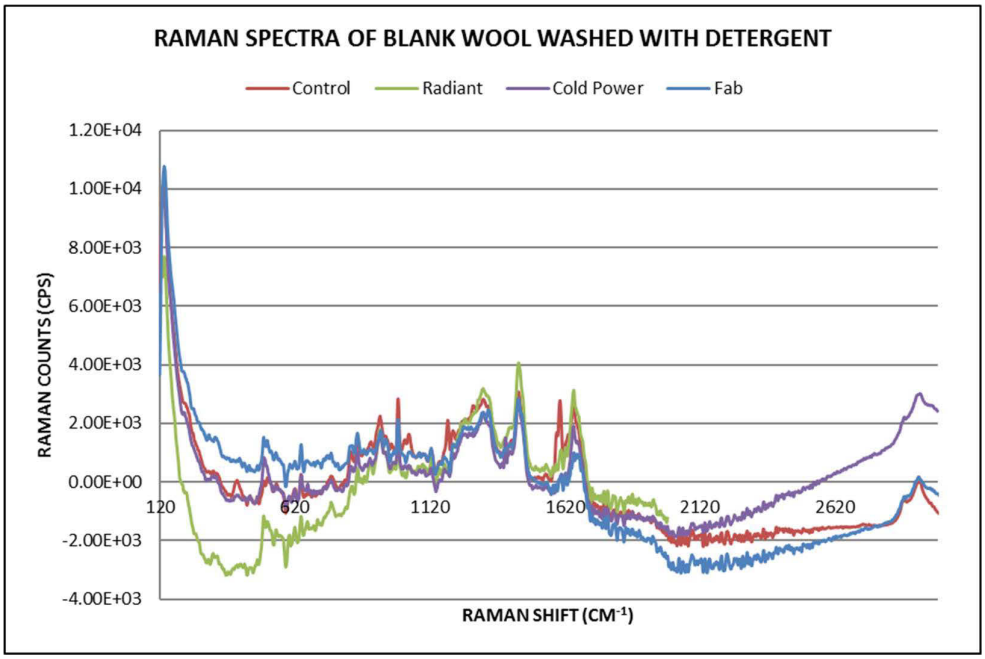


Figure 4-6: Raman spectra of Blank Wool washed with Radiant, Cold Power and Fab powder detergent

As with the polyester fibres in the blank polyester/viscose textile in Figure 4-5, no differences could be detected between the blank wool control spectra and the washed blank wool samples in Figure 4-6. Again all the peaks present can be attributed to the textile itself.

At 518 cm^{-1} there is S-S stretching associated with the disulphide present in wool, with peaks at 623-645 cm^{-1} being connected to C-S bonds. The peaks from 1251 to 1339 cm^{-1} are due to the amide III band while the peaks between 1600 and 1690 cm^{-1} are from the amide I band which is connected with C=O vibrations of carbonyl groups. Finally, at 1451 cm^{-1}, the peak is from CH_2 bend while the peak at 1952 cm^{-1} is from C-H vibrations.[115]

In these spectra, the amide I bands from the wool samples interfere with the optical brightener peak found at 1600 cm^{-1} in the detergents.

The blank wool sample produced resonance fluorescence which created much noise in the Raman spectra resulting in poorly-defined peaks and obscuring smaller peak detail.

Raman analysis of detergents on white textiles

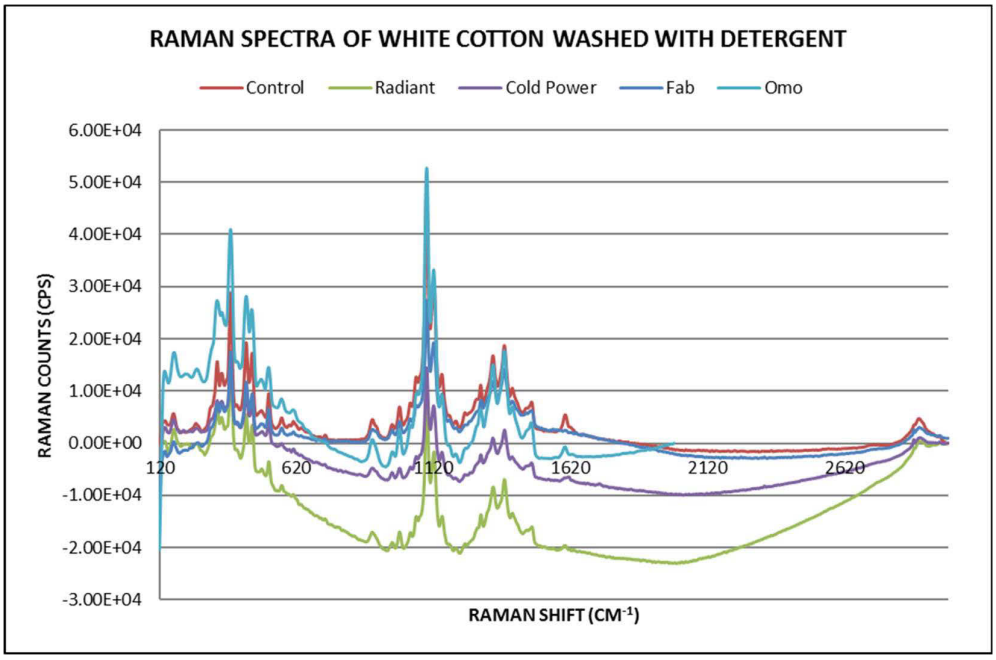


Figure 4-7: Raman spectra of White Cotton washed with Omo, Radiant, Cold Power and Fab powder detergent

The Raman spectra of white cotton samples (Figure 4-7) are almost identical to that of the blank cotton. The only peak of difference is one peak at 1600 cm^{-1}. As discussed previously, this peak was not present in the blank cotton control spectra, and its presence in the washed blank cotton spectra was attributed to optical brightener residues from the detergent on the textile.

The presence of this peak on the control white cotton sample is because, as part of the dying and manufacturing processes, optical brighteners are added to the white textile to enhance its whiteness. The main difference between the acquired characteristic peak of the washed blank cotton sample in Figure 4-3 and the manufactured peak of the control white cotton peak found here is the intensity, the acquired peak only reaching 1000 counts while the white cotton peak reached 5000 counts. This difference in intensity reflects the fact that the white cotton control sample is imbued with a far higher concentration of optical brighteners during manufacturing than the blank textiles acquire in the washing cycle.

The comparison of the washed white cotton with the white cotton control in Figure 4-7 shows that no differences can be found. This is, again, to be expected as the acquired characteristic peak identified on the washed blank cotton samples is already present in the white cotton control samples. As a result of this, while the size of the peak can aid in the comparison, it is unable to be determined for sure whether a peak found at 1600 cm^{-1} in the spectra of an unknown textile is from the manufacturing process or is an acquired characteristic from the washing process.

Most cotton textiles encountered every day will have been through a similar manufacturing process as the white cotton samples, and thus it can be assumed that most textiles examined in casework will contain the peak at 1600 cm^{-1} regardless of whether they have been washed or not.

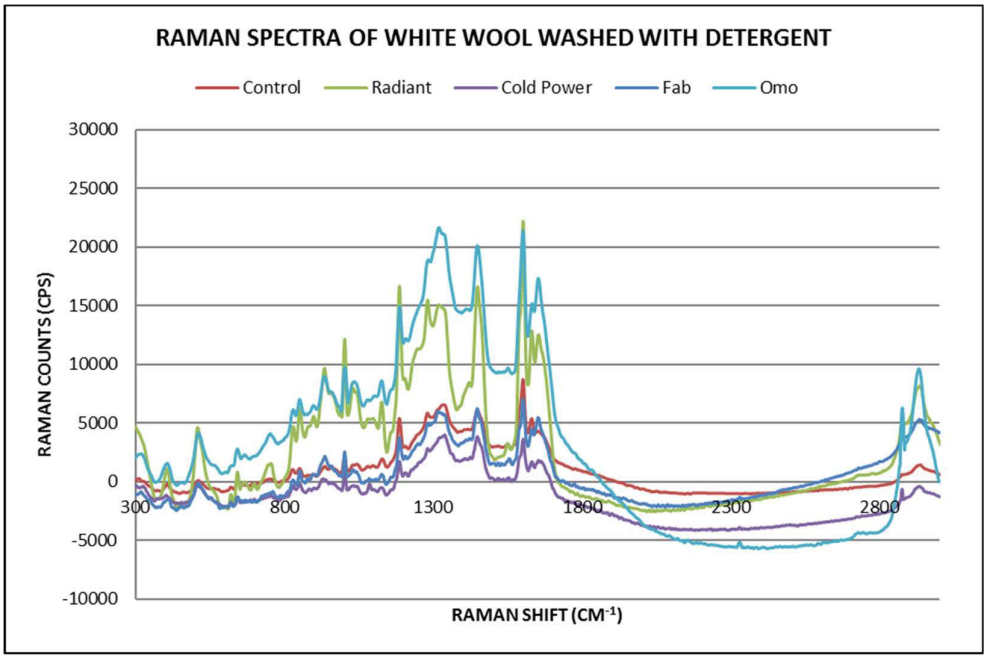


Figure 4-8: Raman spectra of White Wool Washed with Omo, Cold Power, Fab and Radiant powder detergent

As was seen with the analysis of the blank wool Raman spectra in Figure 4-6, the analysis of the white wool spectra in Figure 4-8 found no difference between the control sample and the washed samples.

As discussed in Figure 4-6, blank wool exhibits a Raman peak in the 1600 cm^{-1} range which is where the acquired characteristic peak has been identified as occurring. A peak is found in the same region in the white wool spectra. However, as was seen with the white cotton spectra, the white wool sample was treated with optical brighteners as part of its dying process, and thus the peak at 1600 cm^{-1} is far more prominent than that in the blank wool sample.

The lack of acquired characteristics identified means that the white wool samples are unable to be differentiated by Raman spectroscopy based upon their washing history.

Raman analysis of detergents on coloured textiles

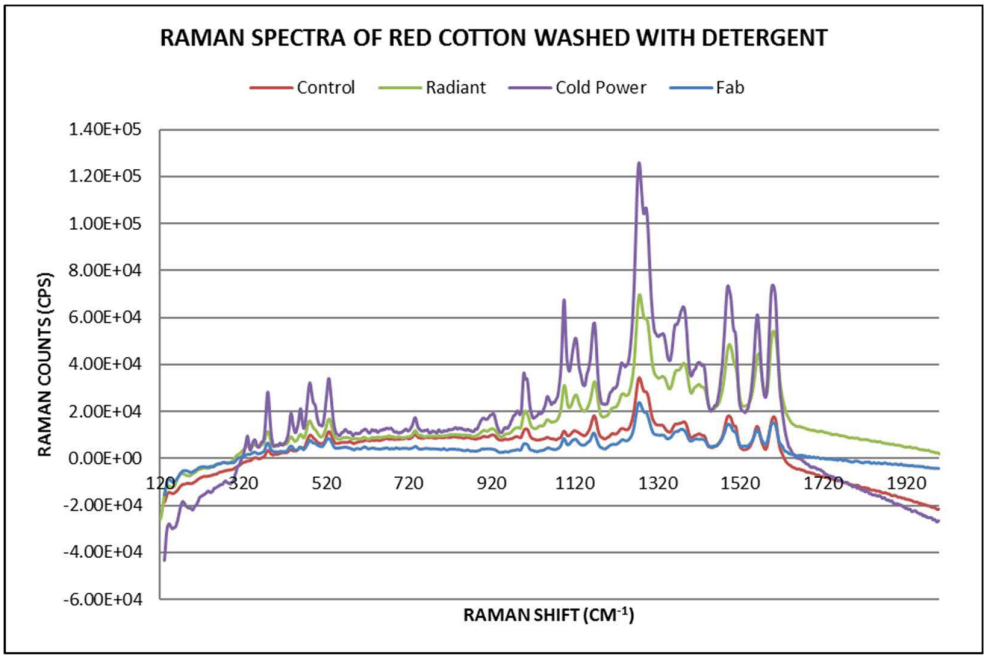


Figure 4-9: Raman spectra of Red Cotton washed with Cold Power, Fab and Radiant powder detergent

Due to the presence of the red dye, the Raman spectra of the red cotton (Figure 4-9) was found to have more Raman fluorescence than the white or blank cotton, and so had less discernible peak detail.

Despite this, it can still be clearly seen that there is no noticeable difference between the control and the washed samples. Peak placement, shape and intensity are consistent across all the samples examined.

It can also be seen that the red dye contributes extra peaks in the spectra than were seen in the blank or white cotton samples, with a far more prominent peak at 1274 cm^{-1} and additional peaks present at 1489, 1564 and 1605 cm^{-1}. These peaks appear in the same region as the acquired characteristic peak at 1600 cm^{-1} however this acquired peak does not appear in these spectra. The absence of this peak shows that no optical brighteners, obtained through either the manufacturing process or through washing, could be detected on the red cotton samples.

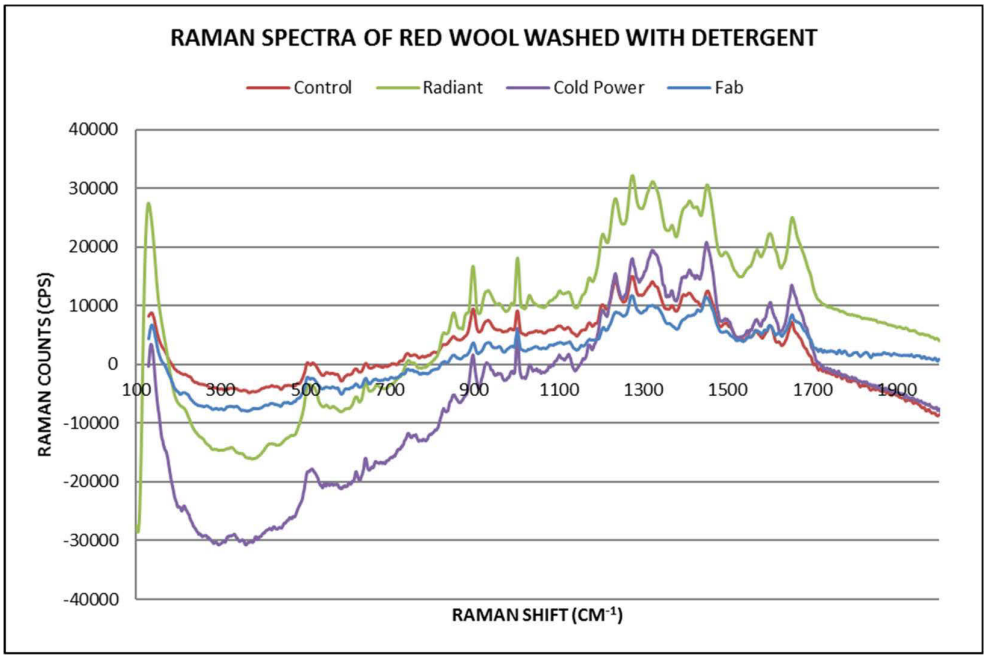


Figure 4-10: Raman spectra of Red Wool washed with Cold Power, Fab and Radiant powder detergent

No differences could be found between the control and the washed spectra of the red wool samples in Figure 4-10. Again a large amount of Raman fluorescence was present which resulted in less definitive peaks, making smaller peak detail harder to see.

What can be seen though is that, as with the blank and white wool samples, the red wool sample has a textile-caused peak at 1600 cm^{-1} which correlates with the position of the acquired characteristic peak. There is also no obvious increase in the intensity of this peak from the control to the washed samples, indicating that the additional presence of optical brighteners is likely not detected in this sample.

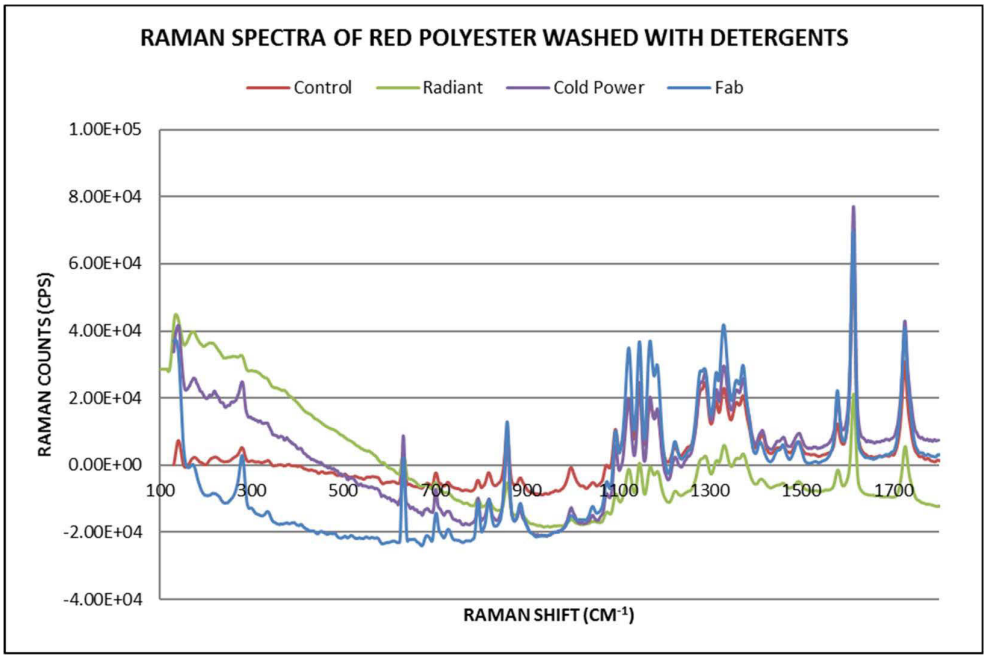


Figure 4-11: Raman spectra of Red Polyester washed with Cold Power, Fab and Radiant powder detergent

Red Polyester in Figure 4-11 is found to have a more complex Raman spectra than that of the polyester fibres in the polyester/viscose mix in Figure 4-5. This is due to the additional chemical profile of the red dye that the fibres have been exposed to. Despite this complexity, it can again be seen that no difference can be found between the control and washed samples and so no evidence of the detergent used can be found.

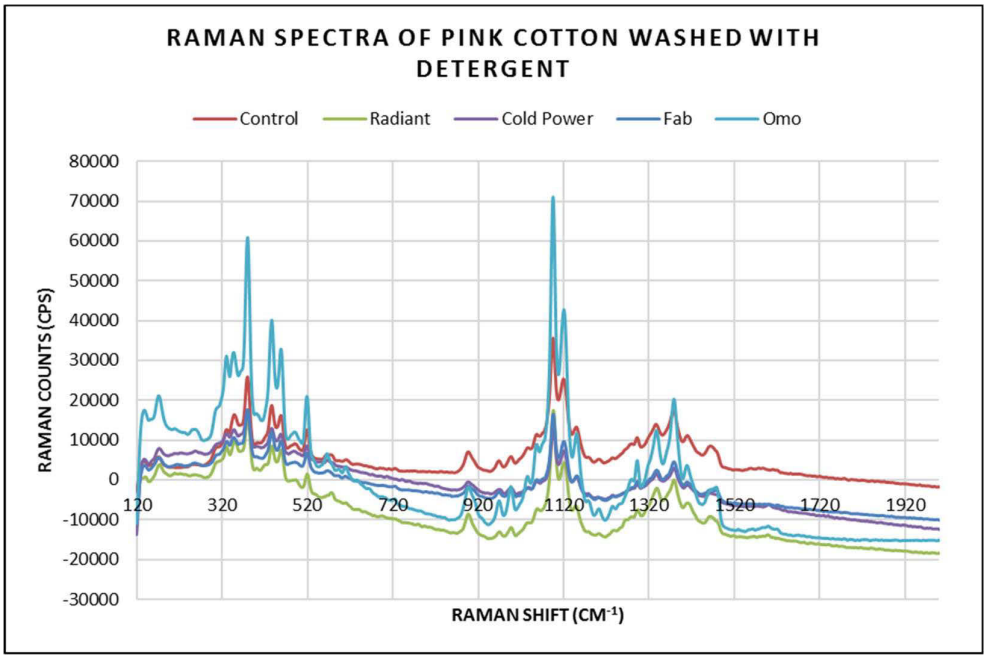


Figure 4-12: Raman spectra of Pink Cotton washed with Omo, Radiant, Cold Power and Fab powder detergent

The Raman spectra for pink cotton (Figure 4-12) is very similar to that of the blank cotton sample, with very little variation in peak number, placement and shape.

As with the other spectra analysed, the main area of the pink cotton spectra that is of interest is the region around 1600 cm^{-1} where the acquired characteristic peak was located in the blank cotton samples. Unlike the blank cotton sample, which had no peak detail in this area, or the white cotton which had a large peak in the control sample, the pink cotton has a wide area of small peak detail from 1525 to 1654 cm^{-1}. The comparison of the washed samples to the control sample shows that the only variation in the spectra can be found in this same area, with a slightly more defined peak being present in the washed samples.

This may indicate that the pink cotton control already contained a type of optical brightener which is acquired in the dying process, while the addition of optical brighteners in the washing process resulted in a more intense band at 1600 cm^{-1}. This is supported by the fluorescence spectroscopy results which showed that, while the pink control samples did fluoresce, its fluorescence was different to that given off by the optical brighteners on

the washed samples. However, due to the lack of intensity of these peaks, it is hard to be definitive with this assessment.

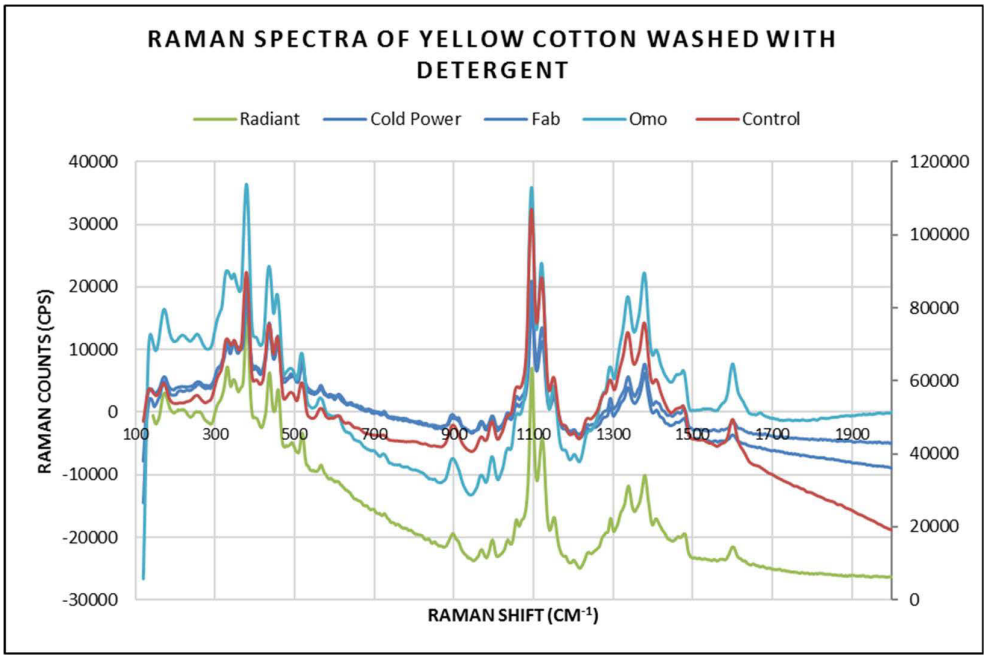


Figure 4-13: Raman spectra of yellow cotton washed in Radiant, Omo, Cold Power and Fab powder detergent

The yellow cotton control samples (Figure 4-13) were also found to have no difference between the Raman spectra of the control and washed sample. It can also be determined that there is virtually no difference between the yellow cotton spectra and the blank cotton spectra except for the presence of an intense peak at 1600 cm^{-1}. This shows that the main component of the sample being detected is the textile, with little evidence of the yellow dye used.

The presence of the intense peak at 1600 cm^{-1} in the control sample again suggests that optical brighteners were applied to the yellow cotton during textile processing to enhance the brightness of the yellow [112, 117]. This not only reflects what was observed with white cotton in Figure 4-7 but also the fluorescence results which showed that the yellow cotton control sample was found to fluoresce under UV light with the same fluorescence spectrum as the detergents used.

As it has been established that optical brighteners were already present on the yellow cotton sample, the presence of an acquired optical brightener peak at 1600 cm^{-1} cannot be

used to differentiate the washed and control sample, and so no further information can be gathered from the Raman spectra.

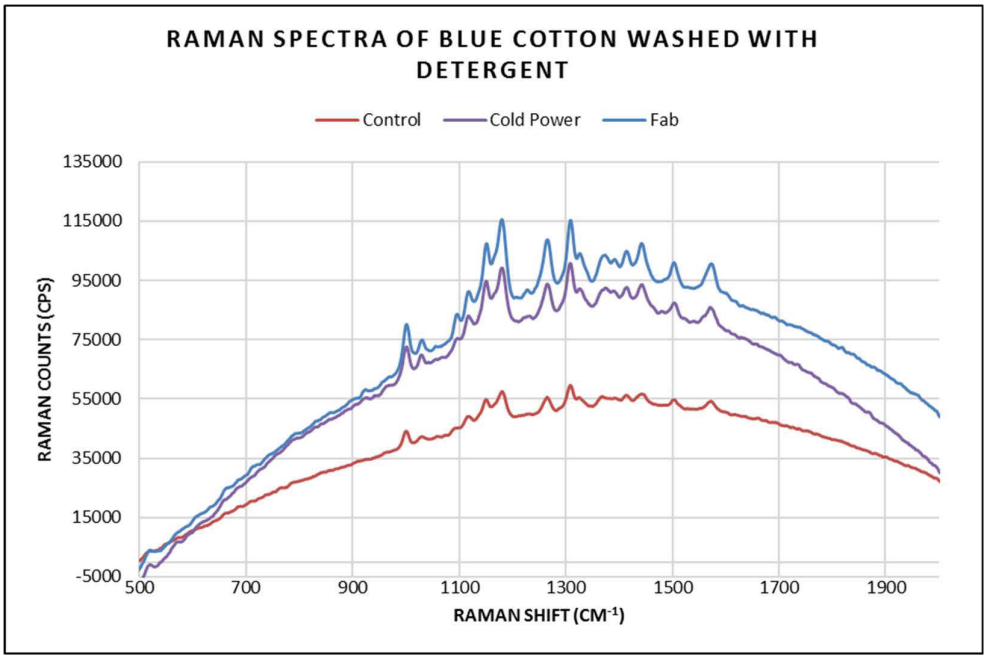


Figure 4-14: Raman spectra of Blue Cotton washed with Cold Power and Fab powder detergent

In Figure 4-14, blue cotton was found to give off a large degree of Raman fluorescence, affecting the quality of the peaks collected.

However, by looking at the peaks that can be clearly seen, it can be determined that there are no differences observed between the Raman spectra of the control and washed samples.

It can also be seen that, while in the fluorescence chapter it was determined that the blue cotton samples fluoresce under UV light, no peak can be observed at 1600 cm^{-1}. A peak can be found at 1575 cm^{-1}, but it is unknown whether its presence is due to the optical brightener or the dye.

4.2 Conclusion

The current Raman Spectroscopic analysis of detergents and the comparison of control textiles to those washed with the detergents yielded limited results, however the fact that the Raman spectroscopy can discriminate between the solid detergent brands means that there is potential for the technique.

Analysis of the detergent identified that each of the four detergents had unique Raman Spectra with different peaks that were characteristic of the particular detergent. It was also discovered that, while Radiant, Cold Power and Fab were all homogenous, the Omo detergent was heterogeneous containing two types of particles with unique chemical composition.

The comparison of the spectra of the control and washed samples identified only one acquired characteristic peak that carried over from the detergents to the washed textile. This peak was present at 1600 cm^{-1} and could be attributed to the optical brighteners present in the detergent. There were, however, many limitations in the use of this peak to discriminate samples. Firstly, this peak could only be detected on cotton samples, not on wool and polyester. It was also found that some cotton samples that were dyed in a light colour, such as white and yellow cotton, already contained optical brighteners that were added in the dying process to brighten the colours. As a result of this, these samples were found to already have a peak at 1600 cm^{-1} in their control spectra.

Finally, in samples where the 1600 cm^{-1} peak was found to be an acquired characteristic, the peak size was very small, and no significant change in this peak could be found when the detergent or number of washes was changed.

As a result of this, it can be seen that the acquired peak at 1600 cm^{-1} can only be used to discriminate between a blank (undyed) cotton sample that has never been washed and one that has been washed four or more times.

Although current analysis is unable to detect the different detergents on the washed fibres, the fact that different Raman spectra were produced for the different raw powder

detergents shows there is potential in this area of research. Future research in this area would be beneficial to explore this potential.

Chapter 5:

Elemental Composition

Chapter 5: Elemental Composition

5.1 Introduction

The elemental composition of each washed sample was collected and compared to the elemental composition of the corresponding control sample to determine whether the composition of the textile changed after washing and whether this could be used to identify the detergent used. Techniques used for elemental analysis were SEM/EDS, LA-ICP-MS and solution ICP-MS.

5.2 Scanning Electron Microscope/ Energy Dispersive X-ray Spectroscopy (SEM/EDS)

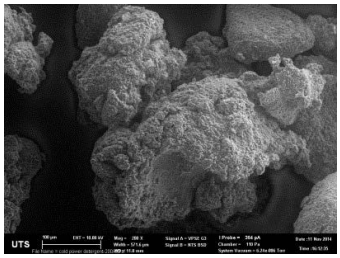
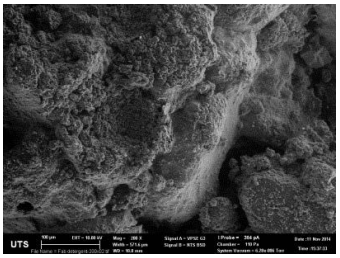
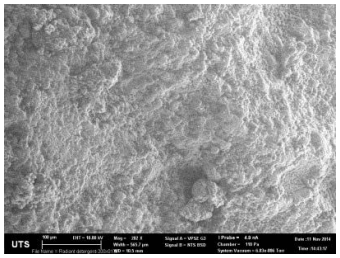
5.2.1 Introduction

A Scanning Electron Microscope was used to determine whether detergent residues could be detected visually on washed textiles. The SEM was coupled with Energy Dispersive X-ray Spectroscopy to see if the elemental composition of these residues could be found. The elemental composition was compared across samples washed in different detergents to determine whether the brand of detergent used had an effect on the elemental composition.

5.2.2 Results and Discussion

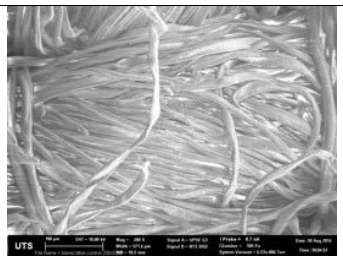
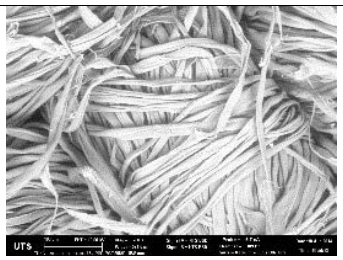
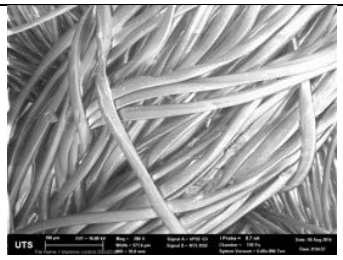
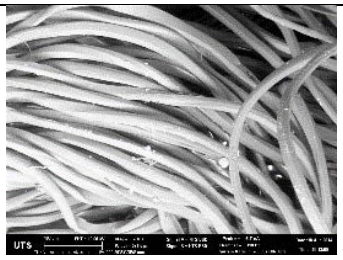
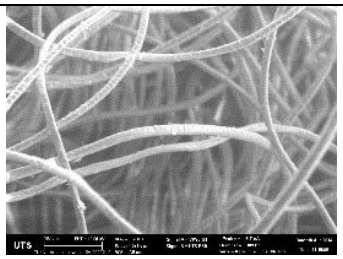
SEM Images

Table 5-1: SEM images of Cold Power, Fab, and Radiant detergent

		
Cold Power	**Fab**	**Radiant**

Images of Cold Power, Fab and Radiant detergent particles taken using the Scanning Electron Microscope (SEM) in Table 5-1 show that the size and texture of the various particles of each detergent do differ from each other. However, as these detergent particles are dissolved in water before they come in contact with textiles, the difference in these particle shapes is unlikely to be carried over to the particular residue that may be found on washed textiles. This can be seen in Table 5-2 where only minute particular residue can be seen on the fibres.

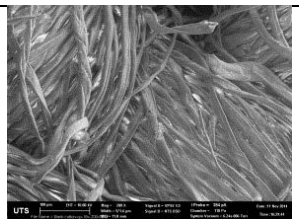
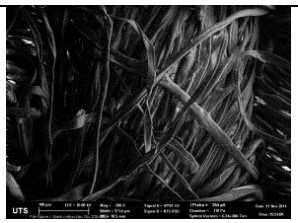
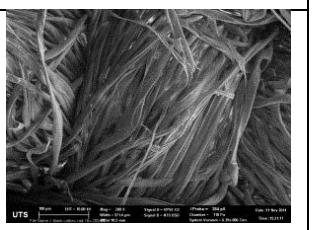
Table 5-2: SEM Images of textiles before and after washing

Textile	No Detergent	Radiant
Blank Cotton		
Blank Viscose/ Polyester		
Blank Wool		

SEM images were taken of both controls (unwashed) samples of material and those washed 16 times using Radiant powder detergent. In these pictures, particular residue can be found on all three washed materials that cannot be found on the control samples. This indicates that it is possible that when the samples are washed, particles of the detergent adhere to the fibres.

The fibres, especially in the wool and cotton samples, are also found to be more disordered after washing, reflecting the mechanical distortions that can occur during the washing cycle.

Table 5-3: SEM images of blank cotton washed in Cold Power, Fab and Radiant

Textile	Cold Power	Fab	Radiant
Blank Cotton			

SEM images of blank cotton washed in three detergents, Cold Power, Fab and Radiant, show particle residue on all three samples (Table 5-3) while no particles could be visibly seen on the control sample in Table 5-2. The particles are very small and fragmented and difficult to see. As a result, it is unclear whether these particles are detergent residue or particles of some other sort of contaminant. The fact that these particles are only seen on the washed fibres supports the proposition that the particles were picked up in the washing cycle.

Elemental analysis of detergents

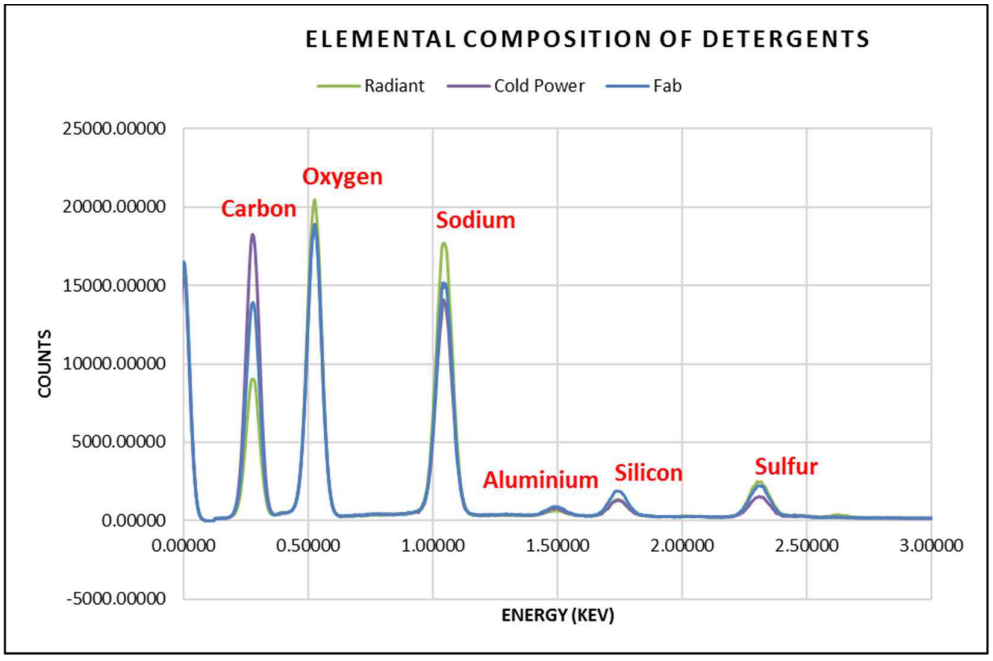


Figure 5-1: Elemental composition of Radiant, Fab and Cold Power detergents

After identifying the presence of possible detergent particles, the next step was to use Energy-Dispersive X-ray Spectroscopy (EDS) to attempt to determine the elemental composition of these residues. First EDS data was taken of three powder detergents, Radiant, Fab and Cold Power. The graph in Figure 5-1 shows that the same six elements were detected at similar intensities in all three samples. Those elements are carbon, oxygen, sodium, aluminium, silicon and sulphur. These results indicate that the basic elemental profile (i.e. based on major elements) of all three detergents is the same and that the samples cannot be differentiated using SEM-EDS.

Elemental Analysis of detergents on blank textiles

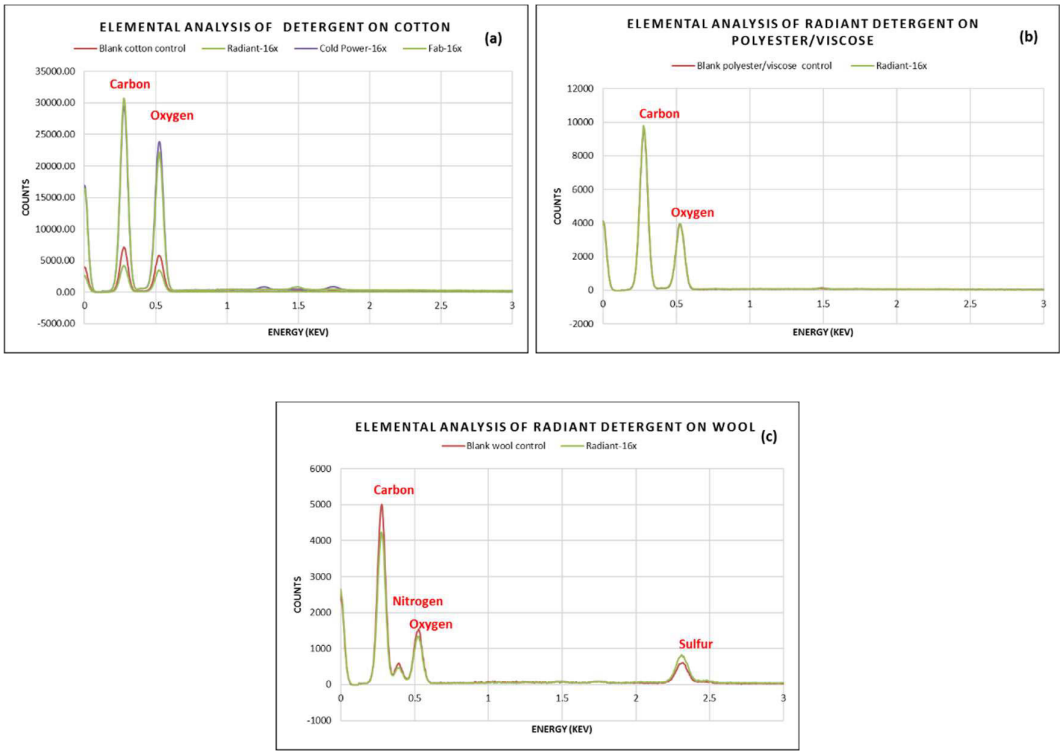


Figure 5-2: Elemental composition of (a) blank cotton washed in Radiant, Cold Power and Fab and (b) blank Polyester/Viscose and (c) blank wool washed in Radiant

Once the elemental composition of the detergents was determined, EDS was then used to attempt to detect and identify residues of these detergents on washed textiles. Figure 5-2 (a) shows the elements detected on a blank cotton sample both before and after washing 16 times with Radiant, Cold Power and Fab. The only elements in all samples were carbon and oxygen. The same elements were also detected on the Blank polyester/ viscose

sample, while carbon, nitrogen, oxygen and sulphur were found in the same intensity on both the wool control and washed wool samples.

Sodium, aluminium, silicon and sulphur, which were identified in the detergent samples, were not detected on any of the control or washed samples while sulphur was only found in the wool sample. The fact that the control and washed samples cannot be differentiated based on their elemental content shows that the EDS was only detecting the elemental composition of the base textile and could not detect any detergent residues on the textile.

5.2.3 Conclusion

These results show that analysis using SEM/EDS, while useful for its nondestructive nature, does not provide information that would aid in the comparison of the fibres.

The pictures taken using SEM did show signs of some particle residues on the washed textiles however the small size and amount of these residues meant that it could not be fully determined whether a sample had been washed or not, and the detergents used could not be identified.

EDS was also found to not be sensitive enough to detect the elemental composition of the residues. This meant that it could not be determined elementally whether a sample had been washed or not, nor would it be able to confirm that the particles seen visually were from detergent rather than some other source.

Elemental analysis was performed at 2000x magnification. There is the potential that elemental analysis could be performed at a higher magnification to allow more focus on the particles, however, there is a risk that at higher magnification, data may be collected which is not representative of the sample itself. This is something, however, which can be investigated in the future.

As this elemental data was not able to be acquired in this project, Inductively Coupled Plasma- Mass Spectrometry, a more sensitive technique, was chosen to analyse the samples using two techniques, LA-ICP-MS and solution ICP-MS.

5.3 Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

5.3.1 Introduction

Laser Ablation Inductively Couples Mass Spectrometry (LA-ICP-MS) was used to analyse fabric in-situ to measure the elemental composition of unwashed and washed samples qualitatively. The elemental profile of the control and washed samples were compared to determine whether a sample could be identified as being washed or unwashed and if the elemental composition of the detergent residues could be used to differentiate between different detergent products.

5.3.2 Results and Discussion

Quantitative analysis of samples using LA-ICP-MS is limited due to the need for a reference standard. There are two types of standards that can be utilised for analysis: internal and external standards. The limitation of external standards in LA-ICP-MS is that the reference standard needs to be composed of the same matrix as the sample since the ionisation of elements is strongly dependent on the matrix [118]. Internal standards need to be incorporated into the sample itself. In the case of textiles, this requires them to be processed and thus destroyed [70]. As a result of this, only qualitative analysis will be used in this study.

Variation in a sample

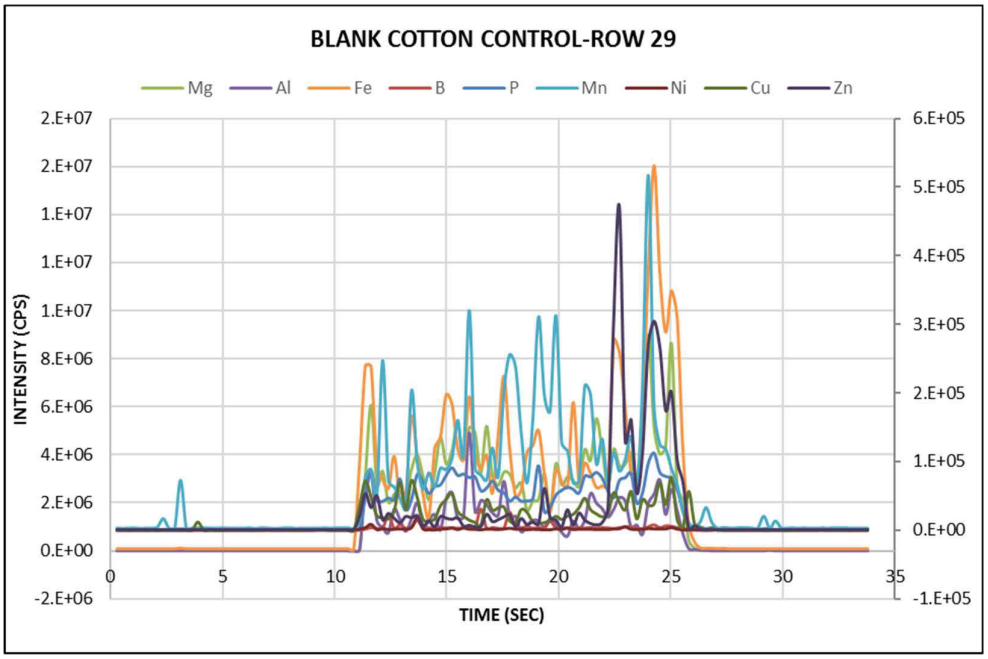


Figure 5-3: Intensity of elements detected across one laser ablation line of Blank Cotton Control

When LA-ICP-MS is used to analyse samples, lines are ablated across the sample using the laser. In Figure 5-3 it can be seen that the intensity of elements detected across the ablated line can vary widely. This variation is due to the lack of homogeneity of the matrix being analysed. As textiles are created by weaving or knitting fibres [119], the surface of the textiles are uneven with spaces between the threads. This results in the peak pattern observed in Figure 5-3 where the intensity of the elements rapidly increases and decreases in a way that is consistent across all the elements, reflecting the woven nature of the cotton textile. This variation in the sample can create a problem when attempting to quantify the average element concentration across the sample. Another factor that must be taken into account, especially when analysing washed fibres, is that deposition of residues is unlikely to be consistent across the fibre, leading to further variation in the intensity of elements detected.

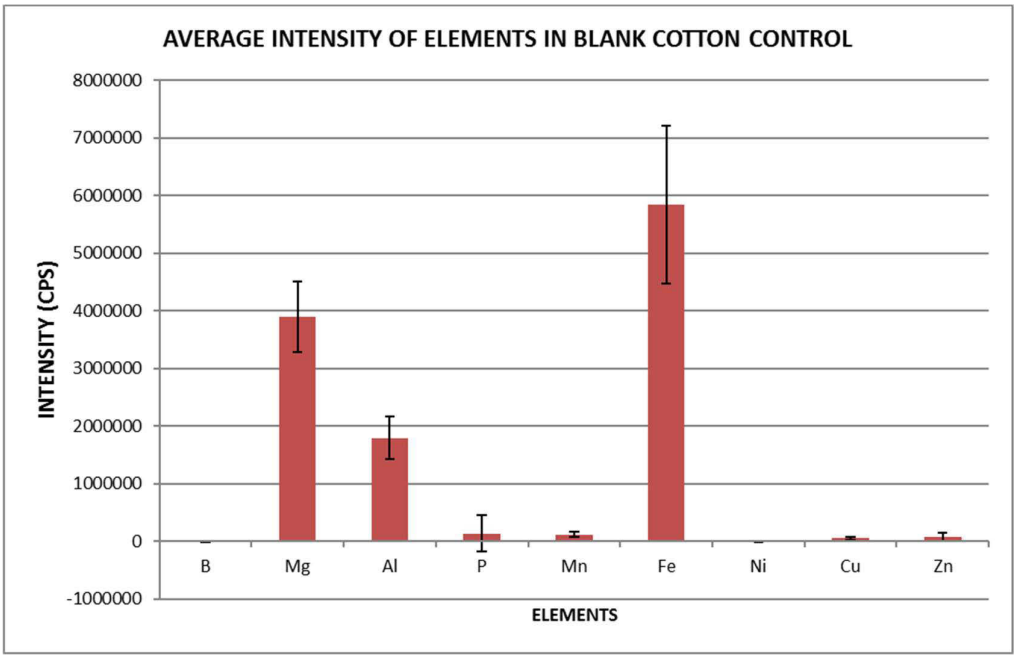


Figure 5-4: Average and standard deviation of the intensity of elements in a 1 cm² Blank Cotton Control sample

Table 5-4: Mean, standard deviation and %RSD of intensities of elements detected in Blank Cotton Control

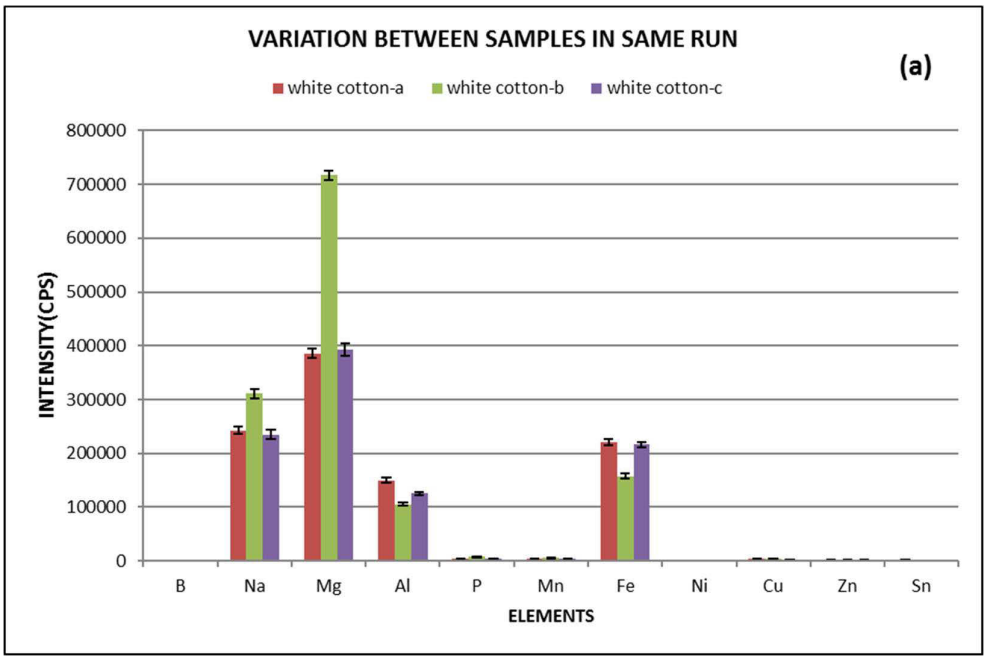
Element	Mean Intensity (Cps)	Standard Deviation	RSD (%)
^{11}B	4291	764	18
^{24}Mg	3898044	615293	16
^{27}Al	1796553	367979	20
^{31}P	136812	310229	227
^{55}Mn	115552	42405	37
^{56}Fe	5843777	1363184	23
^{60}Ni	3121	3877	124
^{63}Cu	48928	20226	41
^{66}Zn	80579	70551	88

The effect that this variation has on the intensity of elements in one sample can be seen in Figure 5-4. The error bars show that there is a significant deviation from the mean

intensity for several elements. Table 5-4 shows this deviation results in a substantial Relative Standard Deviation (%RSD) for all elements in the sample. The smallest variance can be seen in the elements Boron and Magnesium (with %RSD of 18% and 16% respectively) while significantly large variance can be found in Nickel (124%) and Phosphorous (227%).

The variance found within the sample, combined with the qualitative nature of the data, complicates the analysis and comparison of the elemental composition of the samples in the study. Due to the broad range of elemental intensities, the actual intensity of the elements in the sample cannot be accurately determined.

Variation in samples within a run



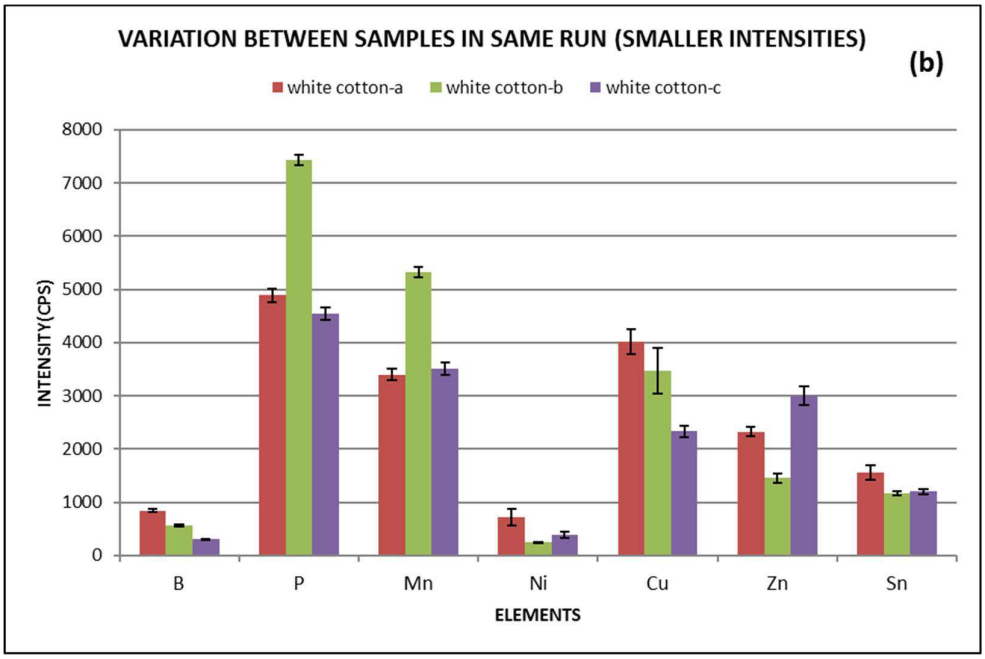


Figure 5-5: Variation in average intensity of (a) all elements, (b) low-intensity elements detected in three white cotton samples in the same run

Three white cotton squares (1 cm^2) from adjacent sections of the same white cotton control textile were analysed in series in the same LA-ICP-MS run. A small variation in the elemental composition of the textile can be expected (due to natural variation in the textile or contact with environmental residues throughout the production, transport and storage of the textile). However, for this technique to be used to reliably differentiate between samples from different textiles, the variance in the elemental composition of the same textiles analysed at the same time should be minimal. Significant variances in composition could lead to comparison textiles being falsely included or excluded when determining if they come from the same sample.

In Figure 5-5, the average intensities and standard error of the elements detected in these three white cotton squares are represented. As discussed earlier, a substantial variation in the intensities of the elements detected in the same sample has led to a significant standard deviation for several of the elements in the sample, especially copper and zinc. Standard error, which takes into account the sample size (55 measurements for each sample), was calculated to determine how accurate the mean intensity of each element is for each sample. These standard errors were then applied as error bars. For it to be concluded that the samples are likely to be from the same textile, it would be expected that there would

be an overlap between the average and standard error of the intensities of each element between the three samples. In Figure 5-5 it can be seen that while white cotton-a and white cotton-c often had very similar average intensities, white cotton-b, which was analysed between the two samples, had reasonably different intensities across most of the elements. Statistical ANOVA single factor and t-tests were applied to the data to determine whether the null hypothesis (that there is no difference between the average intensity of each sample) could be supported (at a 95% confidence interval). The ANOVA test determined whether the three samples could be said to have no difference in mean intensity, while the t-test was applied to pairs of the samples to determine if any of the samples could be found to correlate. The results are recorded in Table 5-5.

Table 5-5: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05

Element	ANOVA (p-value)	t-test (p-value)		
		a + b	b + c	a + c
^{11}B	1.47×10^{-45}	8.33×10^{-12}	2.98×10^{-17}	9.78×10^{-28}
^{23}Na	8.00×10^{-11}	2.19×10^{-07}	9.36×10^{-07}	0.45
^{24}Mg	1.34×10^{-57}	6.46×10^{-30}	1.36×10^{-24}	0.66
^{27}Al	1.59×10^{-14}	8.92×10^{-10}	9.59×10^{-07}	2.24×10^{-4}
^{31}P	9.13×10^{-43}	2.87×10^{-20}	2.49×10^{-24}	0.08
^{55}Mn	3.23×10^{-30}	3.12×10^{-19}	3.24×10^{-16}	0.55
^{56}Fe	7.66×10^{-16}	1.52×10^{-09}	7.81×10^{-12}	0.62
^{60}Ni	3.03×10^{-3}	5.24×10^{-3}	0.02	0.07
^{63}Cu	3.04×10^{-4}	0.26	0.02	1.45×10^{-07}
^{66}Zn	9.07×10^{-15}	4.39×10^{-09}	2.70×10^{-10}	1.50×10^{-3}
^{118}Sn	2.54×10^{-3}	7.96×10^{-3}	0.64	0.02

The p-values obtained for all elements in the ANOVA test were less than the α value of 0.05, and therefore the alternate hypothesis (that the means of the three samples are not

the same) is supported. This reflects the information portrayed in Figure 5-5. However, when the t-test was performed on the three pairs (a and b, b and c and a and c) it was found that there is a correlation between two of the samples. The p-values obtained for the t-test of white cotton-a and white cotton-c were above the α value for half of the elements (sodium, magnesium, phosphorous, manganese, iron and nickel). White cotton-a and white cotton-b were only found to be statistically the same for copper while white cotton b and white cotton-c can be found to have the same average intensity of tin.

From these results, it can be seen that white cotton- a and white cotton-c can be said to correlate to a significant degree and so it could be inferred that they could come from the same source. However, white cotton-b would be falsely reported to be from a different source. The cause of the variation in the second sample could be due to two reasons. Firstly, due to the heterogeneous nature of the textile, there may be different concentrations of elements in that area of the source white cotton textile. This could be caused by natural variation in the cotton fibre or the processing step, or the textile may have come into contact with residues that affected its elemental composition. The second cause could be due to elemental fractionation. Elemental fractionation has been found to be an issue often encountered in LA-ICP-MS, especially when an internal standard is not used. This occurs when the ions detected after m/z separation are not representative of the composition of the original sample. This can happen for many reasons such as the changes in the composition of the ion beam in the mass spectrometer over time, the preference for the ablation of more volatile compounds and the difference in the transport of small and large aerosol particles from the ablation chamber into the ICP. The vaporisation, atomization, and ionisation in the ICP are also found to be less efficient for bigger particles [120-122].

The variability in the samples and the fact that three samples from the same textile analysed at the same time cannot be found to be from the same source increases the risk of false positive or negative identifications when comparing samples. This greatly decreases the accuracy of the technique, and therefore its value for forensic analysis.

To guarantee that the average intensity of elements collected for each sample is representative of the sample itself, a large sample size would be needed which restricts its use in forensic cases where sample sizes are limited.

Variation in samples between runs

Samples from adjacent areas of the same textile were then analysed on different days. This was done for both blank cotton control and white cotton control. The average intensities of each element, along with their standard errors were compared for the two samples to determine whether it could be concluded that they came from the same source textile. ANOVA single factor and t-Test: Paired Two Sample for Means tests were then performed to determine whether this correlation could be proven statistically at a 95% confidence interval.

As discussed previously, the information collected in this study is qualitative as appropriate standards are not available. As a result, the intensities of the elements collected on different days are subject to much variation. To correct this, readings were collected from NIST SRM 612 glass in the LA-ICP-MS on each day of analysis. The intensities of the elements detected in the glass were then used to scale the sample elemental intensities to a common scale to determine if a correlation could be determined between the two samples using this technique. It should be noted that the only elements targeted in this analysis which are present in the glass are boron, copper, nickel and manganese. The elemental intensities of the glass analysed on the 15 July (15/7) were used to correct all the samples.

Another method of comparing samples using normalised data was also investigated (results not shown) however it was not able to identify any relationships or trends in data and it was decided that using the glass method was a more useful method for comparison and just as valid.

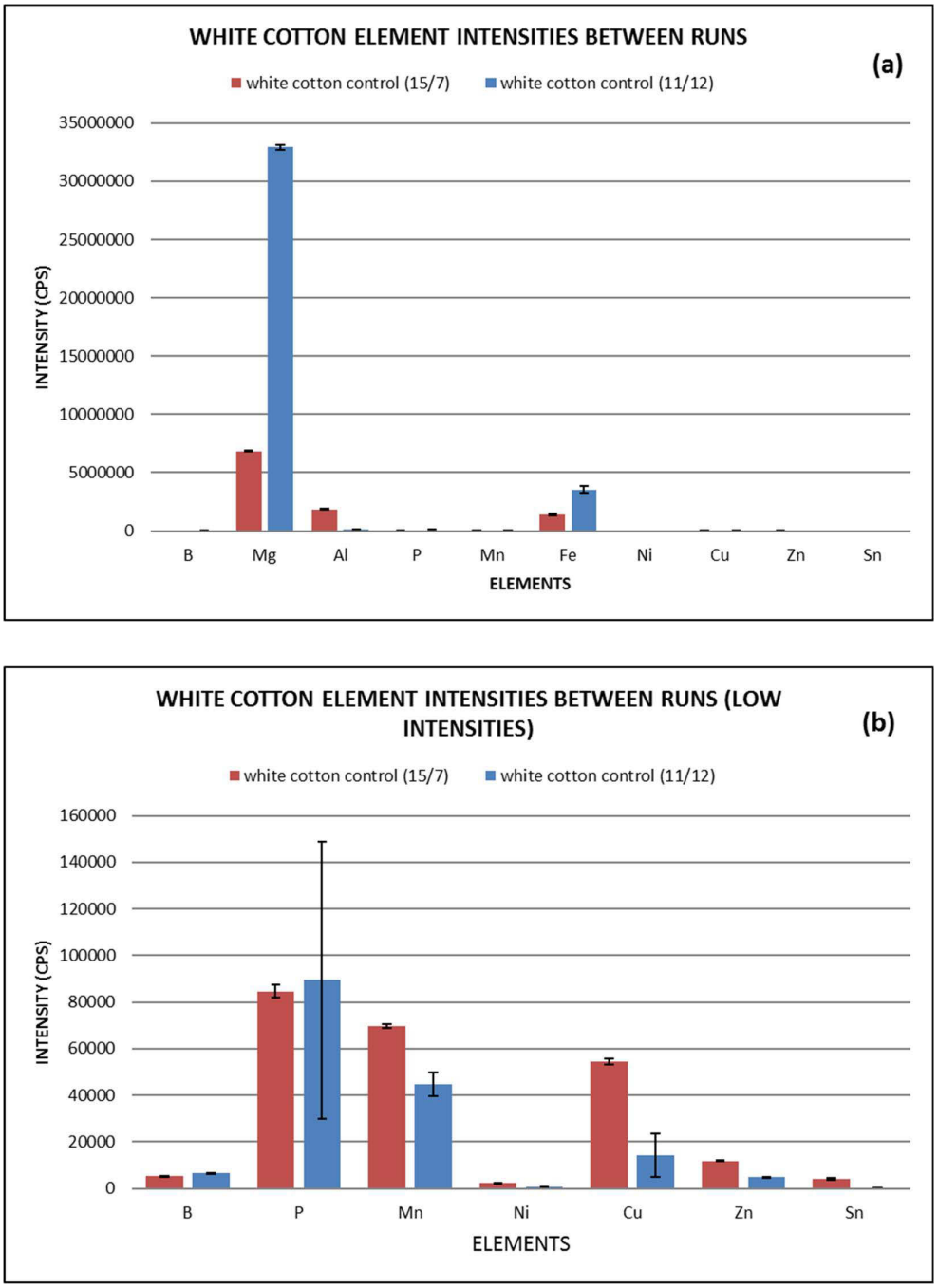
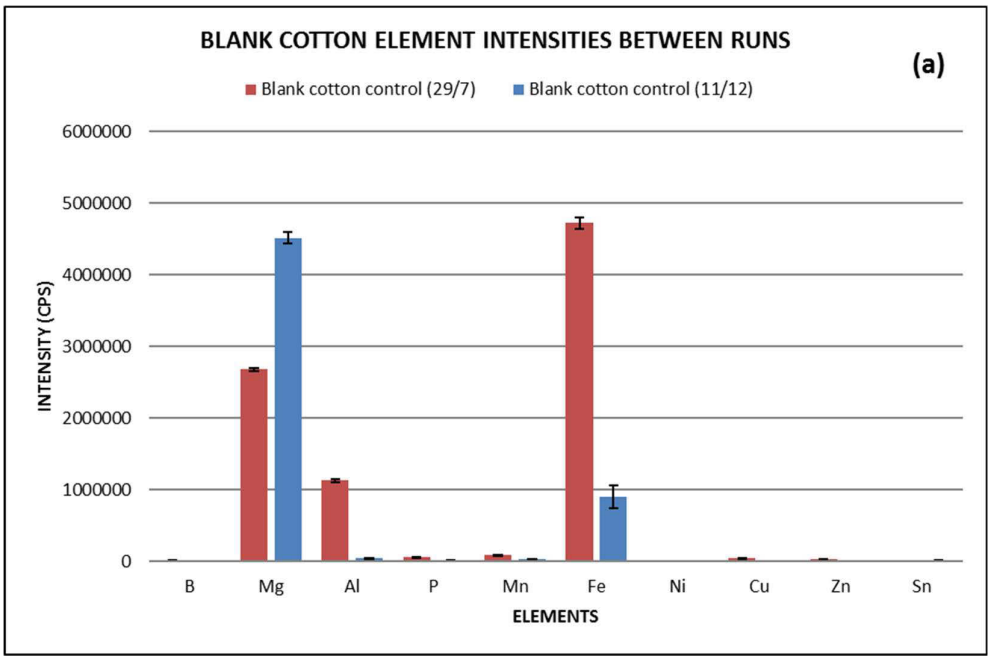


Figure 5-6: Average intensity of(a) all elements, (b) low-intensity elements detected in two white cotton control samples on different days

Comparison of the elemental intensities of the white cotton sample in Figure 5-6 shows that there is little to no connection between the elemental intensities of the two samples. The largest difference was encountered with magnesium where the intensity in white cotton control (11/12) is almost five times the intensity in white cotton control (15/7).

However, this order of magnitude in difference was unique to magnesium. Variation between the intensities of all the other elements, except phosphorous, was encountered, though at a far smaller magnitude. It can also be noted that there is no consistency between the direction or magnitude of the variation, with white cotton control (11/12) having higher intensities of magnesium, iron, boron and phosphorous, but lower intensities of aluminium, manganese, nickel, copper, zinc and tin. The elements with the most similar intensities are boron, phosphorous, nickel, zinc and tin. Phosphorous is the only element, however, to have an overlap between the mean intensity and the standard error. It can also be noted though that the standard error of the phosphorous in white cotton control-11/12 is substantial, indicating a large variability in the concentration of phosphorous detected in the sample itself.



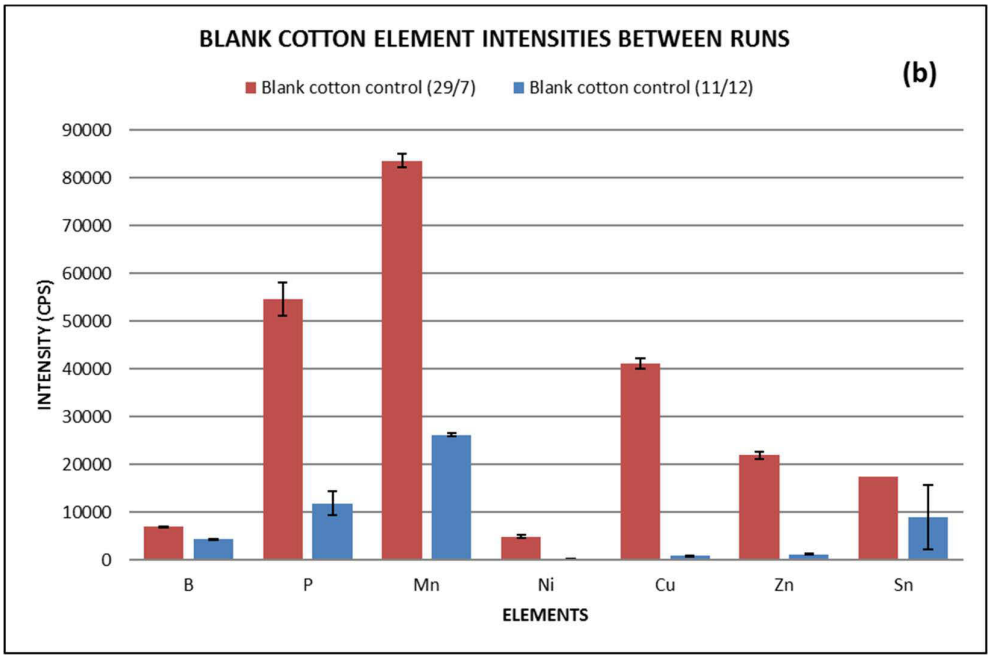


Figure 5-7: Average intensity of(a) all elements, (b) low-intensity elements detected in two blank cotton samples on different days

The comparison of the blank cotton control samples in Figure 5-7 shows a far higher variability than that observed in white cotton control in Figure 5-6. The magnitude of the difference between the intensity of the elements was large for the majority of the elements, with the most significant difference being observed in copper with 45 times the intensity detected in blank cotton control (29/7). The graph also shows that blank cotton control (29/7) consistently had a larger intensity of all elements except magnesium. The only overlap in intensity and standard error was observed in tin. As found in the white cotton sample, this overlap only occurs due to the large standard error calculated for tin in blank cotton control (11/12).

Table 5-6: p- values for t-Test: Paired Two Sample for Means at α=0.05

Element	White Cotton Control	Blank Cotton Control
^{11}B	$1.20\text{x}10^{-27}$	$5.87\text{x}10^{-31}$
^{24}Mg	$4.10\text{x}10^{-101}$	$1.78\text{x}10^{-24}$
^{27}Al	$2.19\text{x}10^{-55}$	$7.81\text{x}10^{-38}$
^{31}P	0.87	$3.18\text{x}10^{-09}$

^{55}Mn	$1.61x10^{-05}$	$9.89x10^{-31}$
^{56}Fe	$6.71x10^{-11}$	$3.50x10^{-28}$
^{60}Ni	$4.23x10^{-08}$	$1.06x10^{-27}$
^{63}Cu	$4.14x10^{-05}$	$1.21x10^{-32}$
^{66}Zn	$6.59x10^{-32}$	$5.97x10^{-30}$
^{118}Sn	$2.95x10^{-17}$	0.37

The p-values obtained in Table 5-6 reflect the observations in Figure 5-6 and Figure 5-7. The p-values for the t-test performed on the white cotton control samples were all below the α value of 0.05 except for phosphorous which had a p-value of 0.87. The only p-value above the α value for the t-tests on blank cotton control was 0.37 for tin. From this statistical analysis, it can be determined that the two white cotton control samples can only be found to correlate with respect to the phosphorous element while the blank cotton control samples only correlate with tin. As discussed previously, this correlation is only due to the considerable variability in the intensity of the phosphorous and tin elements. Because of this, neither of the two pairs of textiles would be able to be determined to have come from the same source.

The NIST glass standard used in this analysis is a standard glass that contains a set concentration of various elements, including boron, manganese, copper and nickel which are targeted in this analysis. Theoretically, the concentration of these elements should remain the same in each run, and therefore the ratio of their intensities to each other should be consistent across each run on each day. Table 5-7 contains the ratio of each elemental intensity to the intensity of nickel in each run.

Table 5-7: Ratio of glass elemental intensities to Ni intensity

Date	**15-Jul**	**29-Jul**	**14-Aug**	**11-Dec**
B:Ni	1.92:1	3.70:1	1.33:1	5.24:1
Mg:Ni	4.60:1	15.11:1	8.99:1	58.23:1
Al:Ni	441:1	1252:1	1411:1	31899:1

P:Ni	0.28:1	0.81:1	0.34:1	2.98:1
Mn:Ni	2.77:1	5.48:1	7.08:1	4.67:1
Fe:Ni	11.05:1	25.69:1	10.60:1	41.88:1
Ni:Ni	1.00:1	1.00:1	1.00:1	1.00:1
Cu:Ni	1.43:1	2.43:1	2.24:1	4.02:1
Zn:Ni	1.46:1	2.05:1	1.75:1	0.93:1
Sn:Ni	0.93:1	1.43:1	2.03:1	1.09:1

The ratios of boron, manganese and copper to nickel are of the most importance in Table 5-7 as the presence, concentration and homogeneity of the other elements are not ensured as they are not listed in the standard information (see Figure A- 8). Examining these ratios, it can be seen that they are not consistent across the dates indicating that either the glass itself is not homogenous or that the concentrations that were detected by the ICP-MS are not consistent. This information is reflected in the variation in the intensity of those target elements in Figure 5-6 and Figure 5-7. From this information, it can be determined that the NIST standard glass is not an appropriate tool to compare samples performed on different days.

To compare samples, an appropriate standard is needed to quantify the results. Without standards, the only information that can be obtained from this analysis is the presence or absence of target elements.

These results also bring to light some limitations of this technique. The variation between runs indicates that all samples analysed using this technique in a casework setting would have to be done so in the same run.

Comparison of samples

As it was found that samples can only be compared to other samples analysed in the same run, only four sample sets will be compared: white cotton control and white cotton-radiant-16x, blank cotton control (29/7) and blank cotton-Omo/fluffy-16x, blank cotton control (11/12) and blank cotton-Radiant-16 and finally pink cotton control with pink cotton-Radiant-4x. However, the variation that was found between the same samples analysed in the same run means that the accuracy of the comparison of the samples cannot

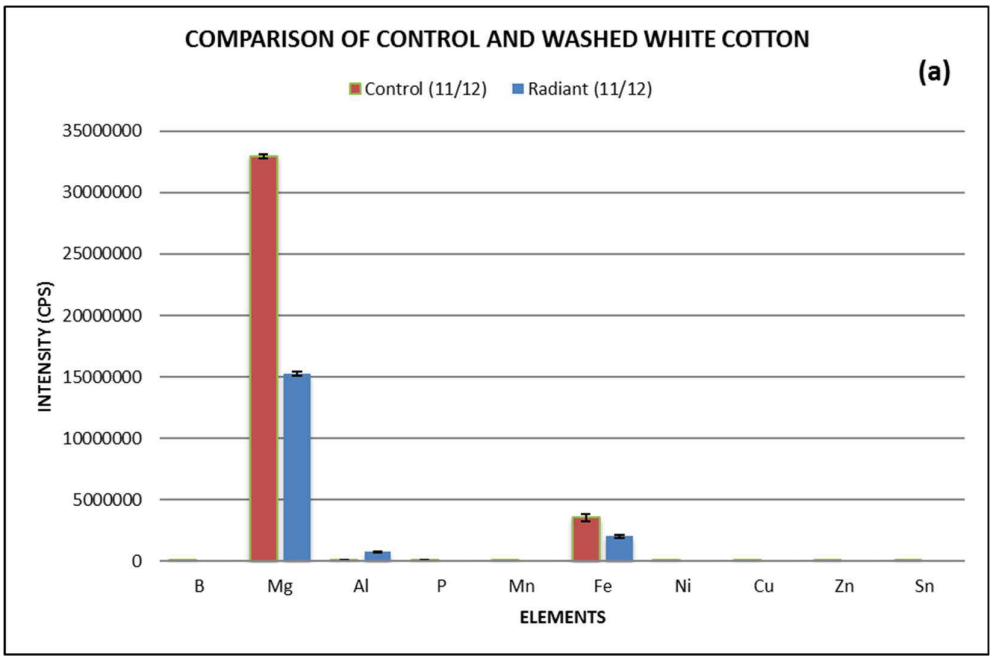
be guaranteed and that, as a result, a difference in the detected elemental intensities may not reflect a difference in the elemental composition of the samples themselves. Due to this, the data was analysed in two ways; firstly a t-test determined whether there is any correlation between the two samples and secondly, general trends in element concetration between the control and washed samples were examined.

Table 5-8: p- values for t-Test: Paired Two Sample for Means at α=0.05

Element	white cotton control/ white cotton-radiant-16x	pink cotton control/pink cotton-radiant-4x	blank cotton control/ blank cotton-radiant-16x	blank cotton control/ blank cotton-omo/fluffy-16x
^{11}B	0.13	$2.58\text{x}10^{-60}$	$1.73\text{x}10^{-43}$	$1.63\text{x}10^{-57}$
^{24}Mg	$1.46\text{x}10^{-66}$	$9.09\text{x}10^{-68}$	$6.03\text{x}10^{-42}$	$1.12\text{x}10^{-46}$
^{27}Al	$8.06\text{x}10^{-57}$	$5.92\text{x}10^{-26}$	$4.00\text{x}10^{-27}$	$2.79\text{x}10^{-32}$
^{31}P	0.40	0.12	0.21	$8.88\text{x}10^{-04}$
^{55}Mn	0.93	0.67	$6.19\text{x}10^{-03}$	$2.72\text{x}10^{-35}$
^{56}Fe	$9.21\text{x}10^{-05}$	$2.91\text{x}10^{-07}$	$9.69\text{x}10^{-06}$	$8.63\text{x}10^{-43}$
^{60}Ni	0.09	0.97	$2.47\text{x}10^{-05}$	0.43
^{63}Cu	0.33	$6.31\text{x}10^{-33}$	$6.69\text{x}10^{-34}$	$1.10\text{x}10^{-04}$
^{66}Zn	$1.12\text{x}10^{-43}$	$9.71\text{x}10^{-05}$	$9.45\text{x}10^{-15}$	$1.40\text{x}10^{-10}$
^{118}Sn	0.10	$6.03\text{x}10^{-04}$	0.23	$1.33\text{x}10^{-04}$

The p-values obtained from the t-test in Table 5-8 show that there is a high amount of correlation between the white cotton control sample and the white cotton sample washed in Radiant. Only four elements (magnesium, aluminium, iron and zinc) were found to not have an equal mean intensity in both samples. These results are consistent with the comparison of two white cotton control samples in Table 5-5, indicating that the white cotton sample washed in Radiant cannot be distinguished from the unwashed white cotton sample. Pink cotton only had matching intensities for three elements (phosphorous,

manganese and nickel). Blank cotton shared two elemental intensities with blank cotton washed in Radiant (phosphorous and tin), and one element with blank cotton washed in Omo and Fluffy (nickel). However, due to the variation that was seen between two of the same samples in Table 5-5, it is not clear whether the discrepancy between the samples is due to the difference in elemental composition of the washed and unwashed samples, or if it is due to instrumental error.



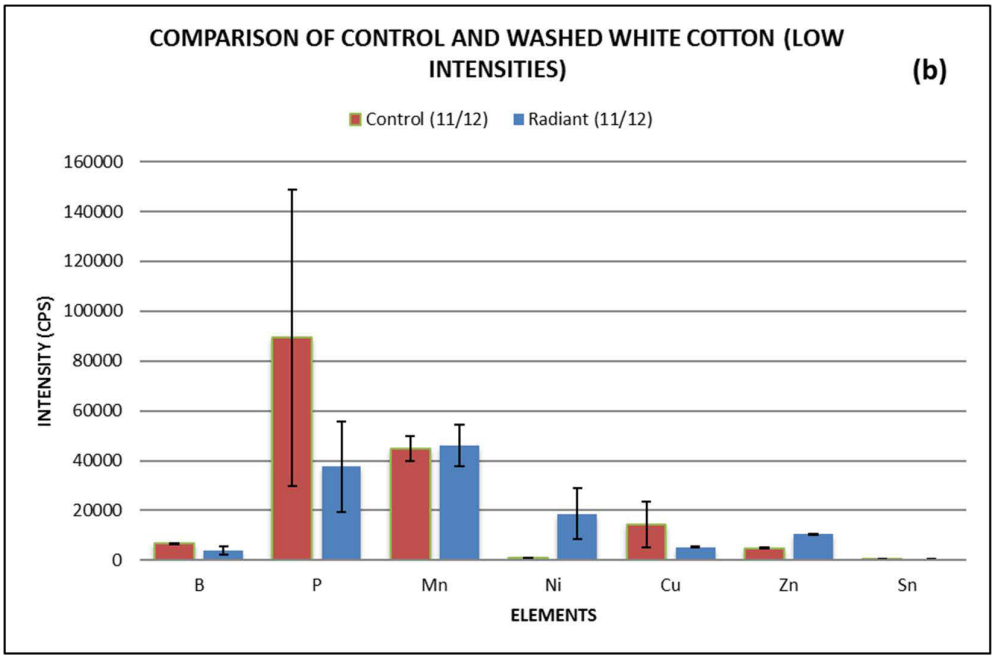


Figure 5-8: Comparison of a) all elements and b) low-intensity elements of white cotton control and white cotton washed in radiant

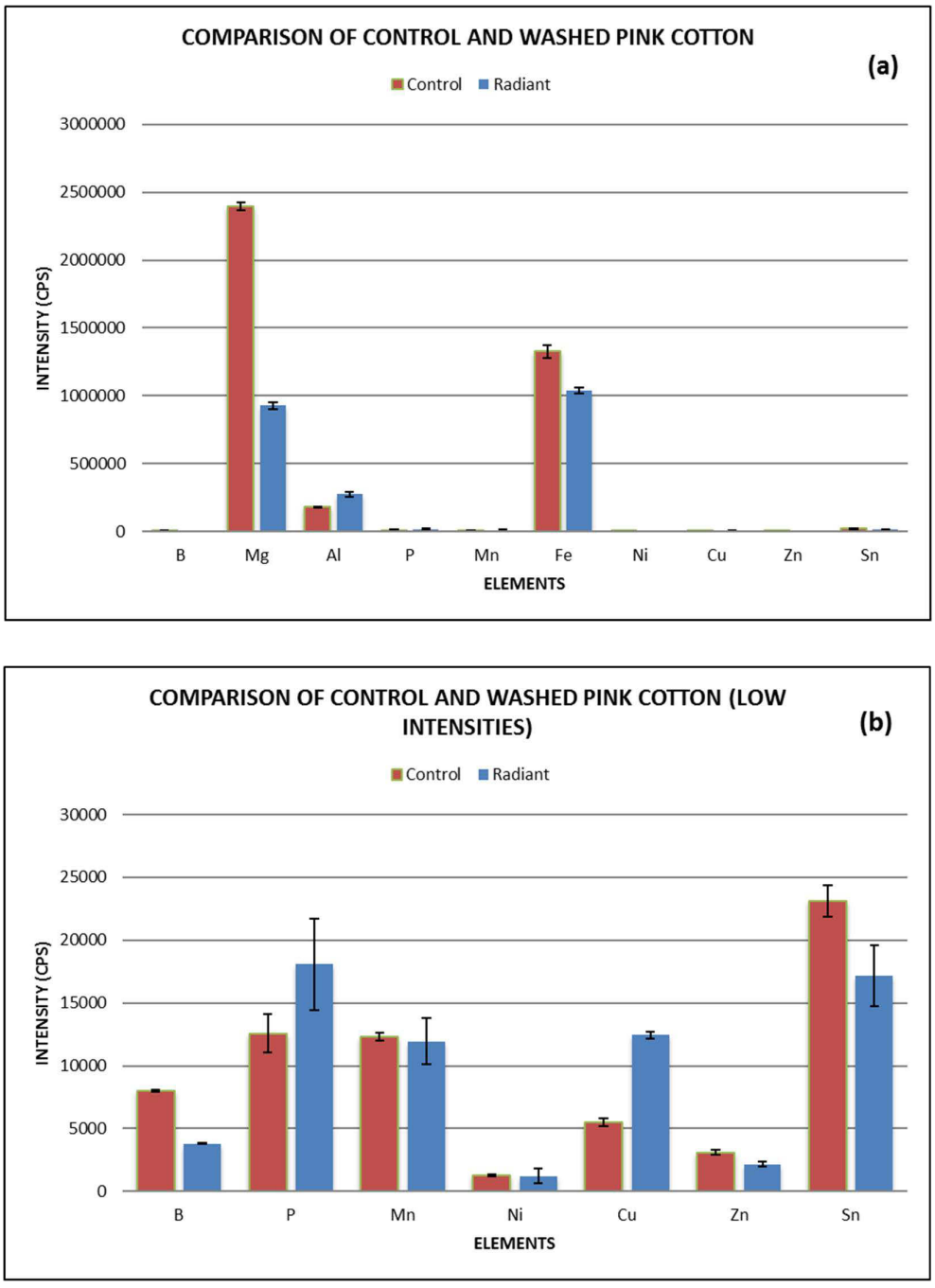


Figure 5-9: Comparison of a) all elements and b) low-intensity elements of pink cotton control and pink cotton washed in radiant

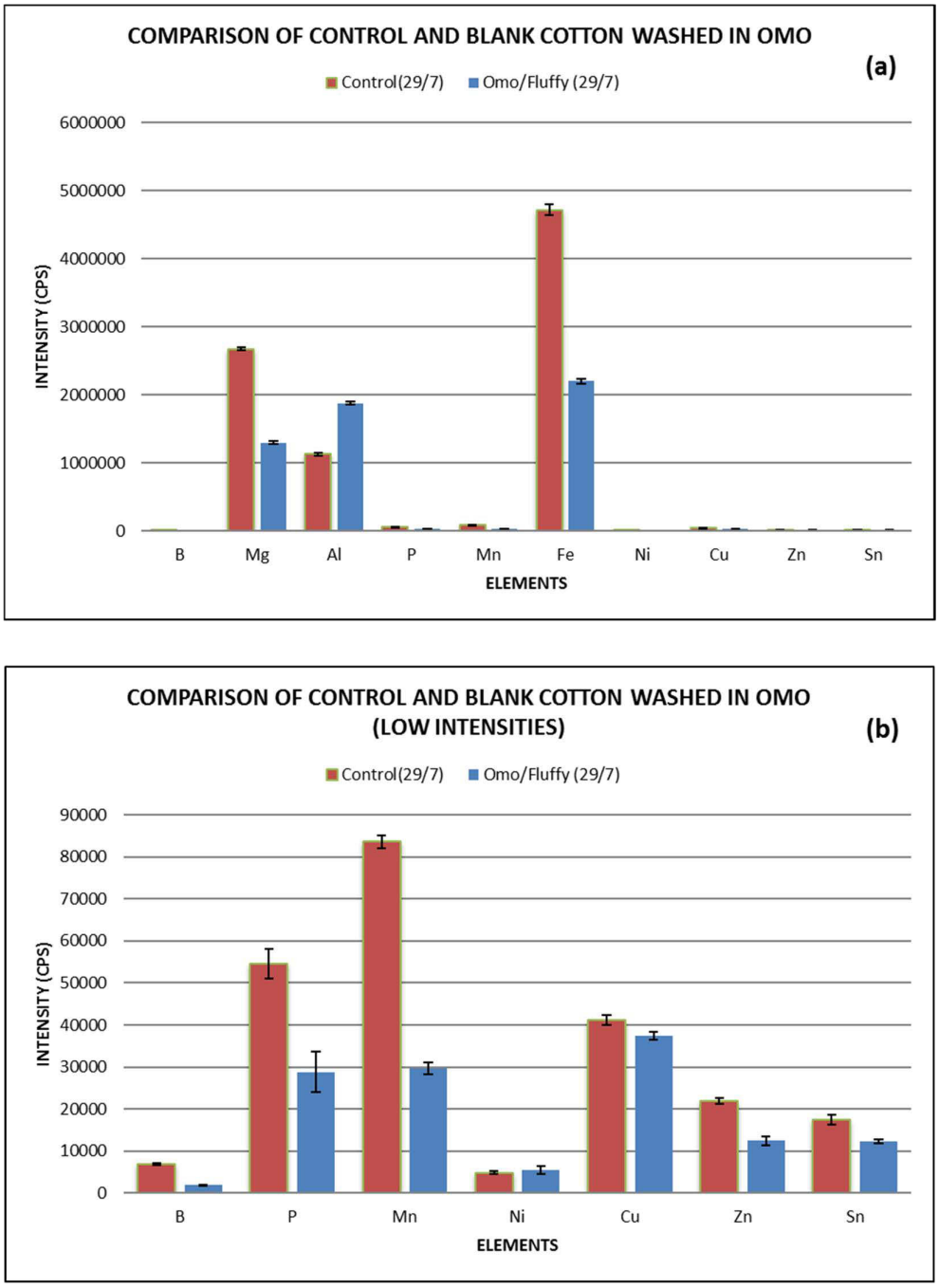


Figure 5-10: Comparison of a) all elements and b) low-intensity elements of blank cotton control and blank cotton washed in Omo

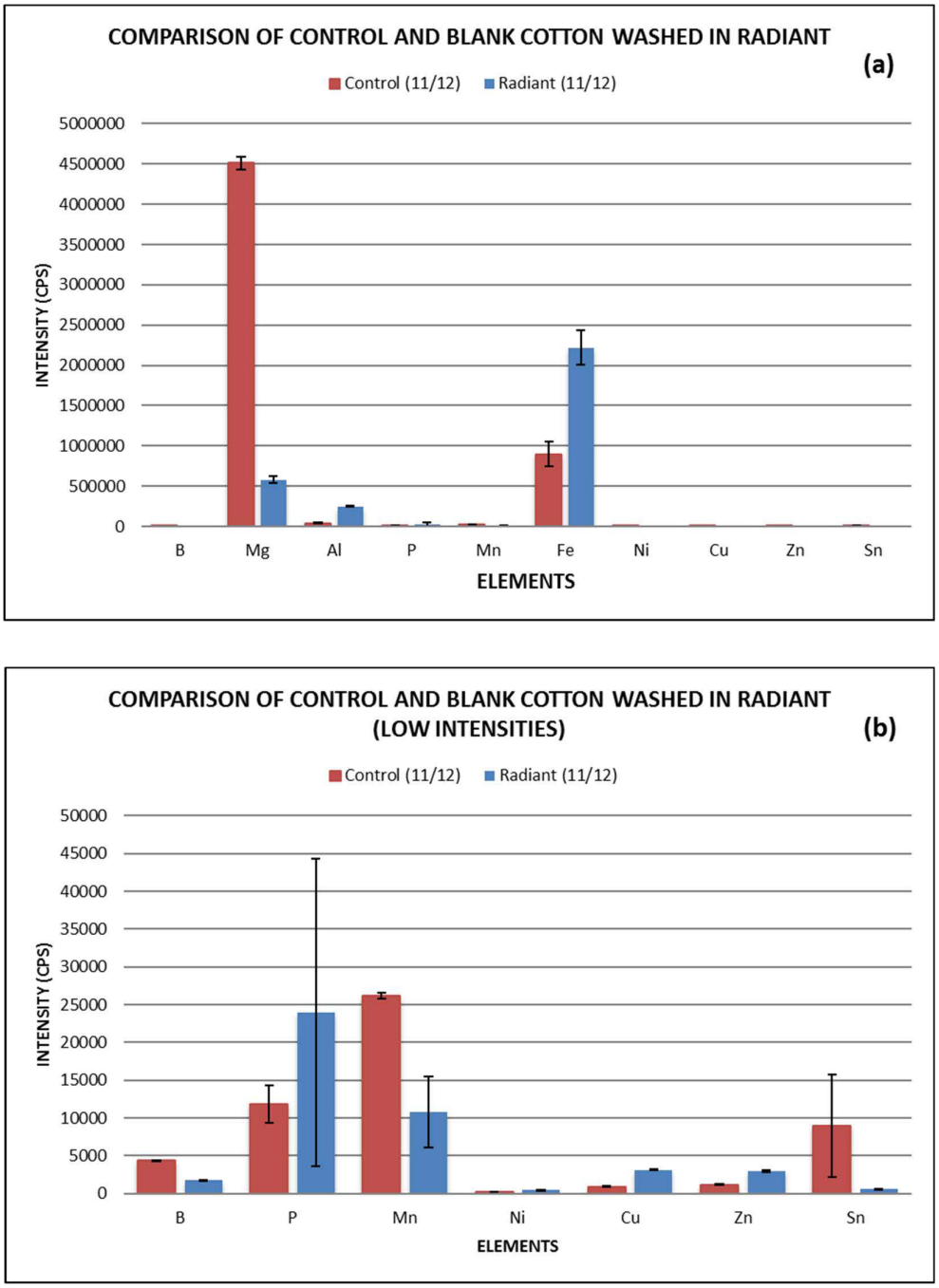


Figure 5-11: Comparison of a) all elements and b) low-intensity elements of blank cotton control and blank cotton washed in radiant

Figure 5-8 − Figure 5-11 show the comparison of the elemental intensities of the control and washed samples of white cotton, pink cotton and blank cotton. It can be seen that for most of the elements there is very little difference in the average intensities. One general trend that can be seen in all samples is that the intensity of the elements seems to decrease

after washing rather than increase. This indicates that the difference observed is due to elements being removed from the control sample in the washing stage, rather than being added as detergent residues. There are two main possible reasons for the removal of these elements. Firstly, it is possible that the textiles were not completely clean prior to washing. The textiles in this project were sources from unwrapped rolls in commercial stores and so could have come in contact with high concentrations of many elements throughout their transport and storage. If this was the case, these contaminant elements could have been removed during the washing process. Secondly, laundry detergents contain builders (see chapter 1.5.1) which chelate with ions such as calcium, magnesium, iron and manganese to reduce the hardness of water. These builders could be responsible for the reduction of some of these elements in Figure 5-8 to Figure 5-11.

One element of note is magnesium which was seen to decrease substantially in all samples. Raw cotton contains insoluble salts of calcium, magnesium and iron on its outer layer of its primary wall. While magnesium ions need to be controlled during the dying process (to reduce water hardness), these ions are necessary in the bleaching step to stop hydrogen peroxide degradation [123], and can also be used in the form of magnesium chloride to increase the crease resistance of cotton textiles. While it is uncertain whether this crease resistance process was applied to the processed cotton textiles (white and pink cotton) there was a high intensity of magnesium in the control samples. It is therefore likely that magnesium was used at some point in the textile processing of these textiles. After washing, the intensity of these elements decreased, indicating that it is likely the magnesium was removed during the washing cycle.

Manganese was an element that also saw a decrease in intensity in the blank cotton samples but not the processed cotton. Manganese is found naturally in raw cotton due to the soils it grows in [124] however the removal of this element is critical in the dying of cotton as it can interfere with several of the processes [123]. It can thus be assumed that excess manganese has already been removed from the dyed cotton samples and so its concentration does not decrease. The blank cotton, which has not been through any dying or processing steps, contains manganese that is removed in the washing cycle, resulting in the decrease in intensity that was seen in Figure 5-10 and Figure 5-11. As all

commercially available textiles have gone through textile processing, it is unlikely that this difference in manganese intensity would be found in casework samples.

5.3.3 Conclusion

From these results, it can be seen that LA-ICP-MS has limited use when it comes to the analysis and comparison of washed and unwashed samples.

Firstly, the lack of internal and external standards means that all results collected are qualitative, not quantitative, which means that samples cannot be accurately compared.

There is also a significant degree of variation in elemental intensities within a sample, due to the heterogeneity of the textile. This is significant in casework when a small sample size can result in a substantial standard error. This, along with factors such as elemental fractionation and sensitivity drift, has an impact on the comparison of samples which can result in the false exclusion of samples from the same source.

The lack of standards, along with changing conditions in the LA-ICP-MS, also results in a large variation between the same samples analysed on different days which cannot be corrected using a NIST external glass standard.

As a result of this persistent variation, samples are unable to be accurately compared either within or between runs. A lack of apparent trends between washed and unwashed samples also emphasises that little information of use can be collected using this technique. Future work could investigate the use of internal standards to determine whether it yields more accurate or useful information that would add value to this technique.

5.4 Solution ICP-MS

5.4.1 Introduction

Through the use of calibration standards, the concentration of target elements in control and washed standards are measured and compared to determine whether elemental composition can be used to compare the samples. The elemental concentrations are then compared before and after washing to investigate whether there are any trends that consistently appear. Finally, the multivariate analysis tool, Principal Component Analysis, will be used to determine whether samples will form clusters on a score plot based on whether they have been washed and which detergent was used.

5.4.2 Results and Discussion

Due to the limited results obtained from LA-ICP-MS, the samples were then analysed using solution ICP-MS. The advantage of this technique is that, through the use of calibration standards, quantitative analysis can be performed resulting in exact elemental concentrations. The disadvantage, however, is that it is destructive and requires extensive sample preparation and a substantial quantity of fibres. In this analysis, cotton and wool textiles were washed and then analysed using the Agilent 7500 ICP-MS. However, during the washing and preparation of repeat samples, the Agilent 7500 ICP-MS experienced technical difficulties that were unable to be fixed. As a result, the repeat samples had to be analysed using another instrument, the Thermo iCAP RQ ICP-MS. Due to this, the concentrations calculated for the repeat samples are affected by two variables: their washing conditions (while the number of washes and detergents used were the same for all samples in the repeats, the date of washing and the washing loads were different for the two repeat samples) and the instrument used.

Variance in sample

Table 5-9: Average %RSD for each sample

Textile	Agilent (Average %RSD)	Thermo (Average %RSD)	Combined (Average %RSD)
Blank Cotton	3	45	32
White Cotton	67	42	34
SL White Cotton	N/A	57	57
Pink Cotton	4	37	28
Yellow Cotton	7	65	44
Blue Cotton	4	48	36
Red Cotton	3	37	29
SL Red Cotton	N/A	21	21
Blank Wool	3	31	26
White Wool	5	30	20
Red Wool	3	45	35
Average	11	42	33

The average % RSD for each sample on both instruments was calculated and recorded in Table 5-9. The samples were measured ten times on the Agilent ICP-MS, and three times on the Thermo ICP-MS. The %RSD calculated on the Agilent was generally significantly smaller than that on the Thermo with an average %RSD of 11% compared to 42% RSD on the Thermo ICP-MS. This is due predominantly due to the difference in their sample sizes. The larger sample size in the Agilent measurements resulted in a significantly smaller %RSD value.

Variance between the same sample (Repeatability)

Agilent ICP-MS

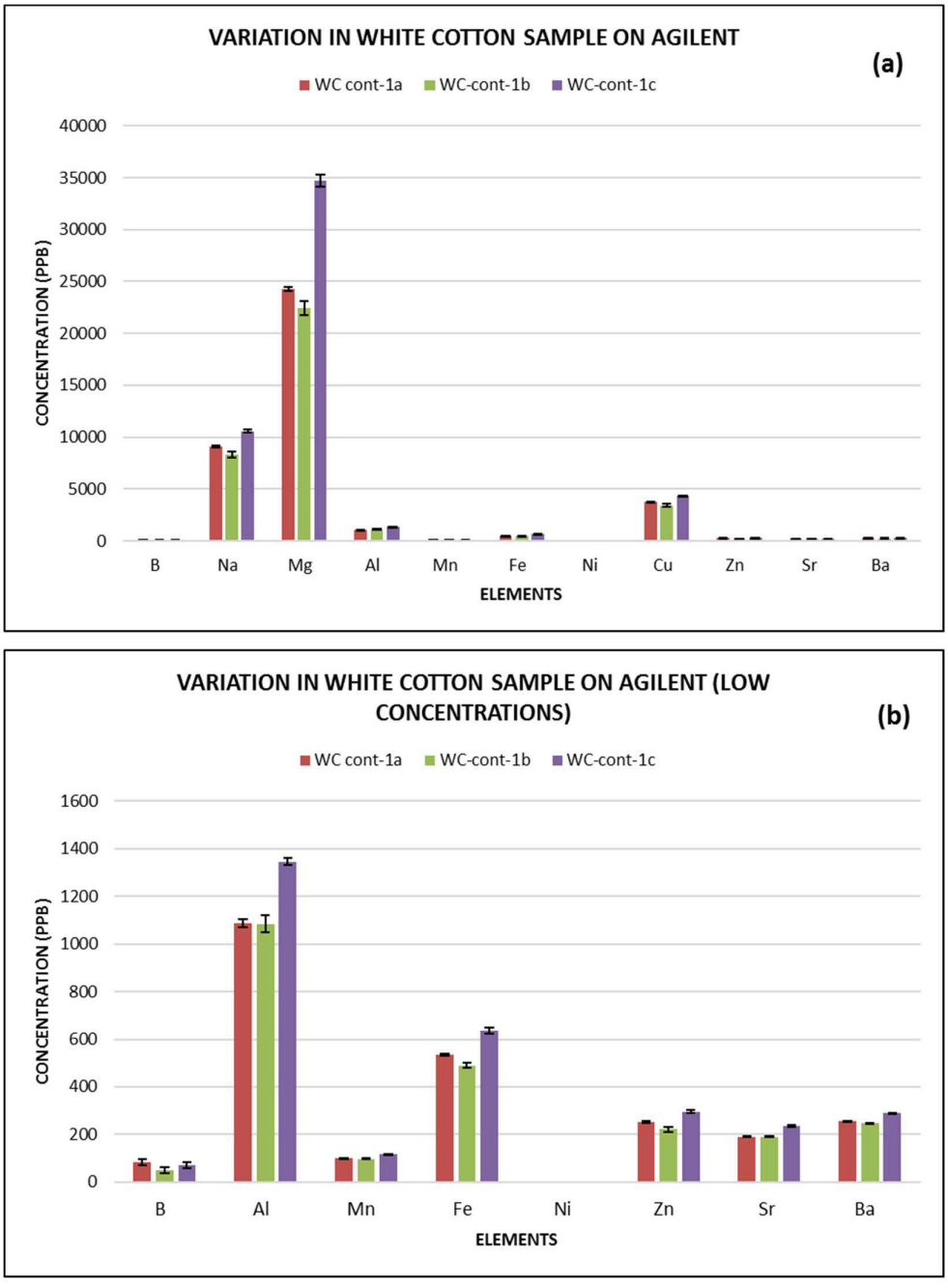


Figure 5-12: a) total and b) low concentration elemental composition of the same white cotton control sample

Each textile sample was digested and diluted to 10 mL and transferred to a 5 mL sample vial for analysis in the Agilent ICP-MS. In Figure 5-12, 1a and 1b are the same sample vial analysed on two different days, while 1c is the second 5 mL aliquot which was analysed at the same time as the 1b sample. The same method and calibration standards were used for all three samples. As they are from the same source material, it would be expected that they would have the same elemental composition. In Figure 5-12 it can be seen that for the majority of the elements, the average concentration and the standard deviation error bars for each sample overlap, supporting that the samples have the same concentration of those elements and so are likely to have come from the same source. However, a discrepancy is found between the 1a and 1b aliquot sample and the 1c sample for sodium, magnesium, aluminium and iron. For these elements, the 1a and 1b samples are found to correlate while the 1c sample tends to have a larger concentration of each element. This reinforces the idea that the sample solutions are not entirely homogenous, resulting in a higher concentration of elements at certain points in the solution. As 1c was the bottom aliquot from the original source solution, it is possible that the higher concentrations seen in this sample are due to the settling of dissolved solute to the bottom of the tube, although the cause of this difference is not definitely known. The close correlation between the average concentrations of elements in 1a and 1b, along with the small %RSD values within a sample (when using the Agilent ICP-MS) shows that there is some degree of repeatability with this technique.

Table 5-10: Average, standard deviation and %RSD for elemental concentrations in 1a, 1b and 1c

Element	Average (ppb)	SD (ppb)	% RSD
^{11}B	69.37	13.81	20
^{23}Na	9334.73	939.82	10
^{24}Mg	27131.25	5416.74	20
^{27}Al	1172.04	122.65	10
^{55}Mn	104.07	7.94	8
^{56}Fe	553.56	61.83	118
^{60}Ni	0.00	0.00	0.00
^{63}Cu	3824.59	371.56	10
^{66}Zn	256.22	30.73	12
^{88}Sr	205.40	21.32	10
^{137}Ba	262.97	18.19	7

Table 5-10 reflects the variation seen in the elemental concentrations of the white cotton control 1a, 1b and 1c samples, with %RSD values ranging between 7 and 20%. These high RSD values show that, due to inconsistencies in the source solution, there can be complications in finding the precise concentration. While the exact reason for the difference in the concentration of 1c to 1a and 1b is not known, thoroughly mixing the samples before transferring them to the sample vial could improve the homogeneity of the solution. Multiple repeat analyses of the samples would have to be performed to confirm this theory.

Thermo iCap RQ ICP-MS

Table 5-11: Average and %RSD values for elemental composition in three repeat samples of blank cotton control, white cotton control and pink cotton control

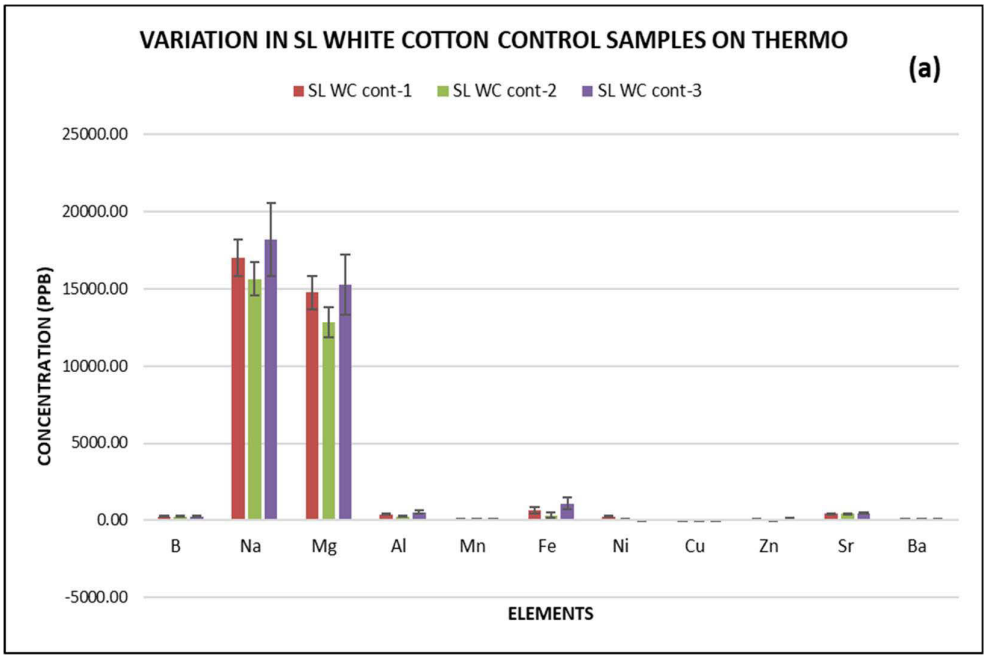
	Blank Cotton Control		White Cotton Control		Pink Cotton Control	
	Average	**%RSD**	**Average**	**%RSD**	**Average**	**%RSD**
^{11}B	38.84	141	79.86	141	126.0849	141
^{23}Na	11990.74	14	11618.34	2	39245.52	16
^{24}Mg	3751.99	7	37289.29	3	11672.62	10
^{27}Al	762.66	53	1269.26	12	333.8314	82
^{55}Mn	35.23	141	67.96	110	8.863475	141
^{56}Fe	340.66	77	390.65	76	692.4884	21
^{60}Ni	0.00	n/a	0	n/a	0	n/a
^{63}Cu	22.86	141	19.71	141	11.95546	141
^{66}Zn	27.62	141	58.05	n/a	21.88145	141
^{88}Sr	41.60	141	316.81	27	972.296	8
^{137}Ba	34.99	141	280.96	16	26.16319	141

The same blank cotton control, white cotton control and pink cotton control aliquots were analysed in three different runs on the Thermo iCap RQ ICP-MS. % RSD values were calculated in Table 5-11. % RSD values over 100% were calculated for samples where an elemental concentration was detected and measured in one run, but was below the limit of detection one or more runs. This generally occurred with elements with very low concentrations. Without taking those values into account, blank cotton control had % RSD values that ranged from 7 % (magnesium) to 77 % (iron). The high values show that there is a large degree of variation between runs on the Thermo iCap RQ ICP-MS which limits the repeatability of the instrument. The white cotton control samples had far smaller

% RSD values with two of the elements (sodium and barium) having values below 5 % while the highest degree of variation was seen for iron with 76%. Similarly, the pink cotton control sample had %RSD ranging from 8 to 82 %.

These results show that the variation between the samples is not consistent for every element, with some elements having only a very small amount of variation (magnesium) and others having a lot of variation (boron and aluminium). The results also show that the degree of variation was not consistent across the samples with blank cotton control having far more variation than the white cotton and pink cotton samples. The variation between the samples can be linked to two main factors. Firstly, for each run new calibration standards had to be created. These new standards would result in the creation of different calibration curves for each element and thus result in a degree of variation between the two runs. Reusing the same calibration standards is not possible with the Thermo iCap RQ ICP-MS as it is with the Agilent ICP-MS as the instrument requires a larger volume for analysis. The second cause for the variation between the two runs is lack of homogeneity of the dissolved samples in the sample tube. This is supported by the results in Figure 5-12 which also saw a high degree of variation between two samples from the same aliquot.

The lack of repeatability present in the Thermo iCap RQ ICP-MS indicates that, to ensure the accuracy and validity, the elemental composition of fibres should only be compared if analysed on the same run. It should also be noted that small differences in the elemental composition of two fibres does not reliably exclude fibres from the same source.



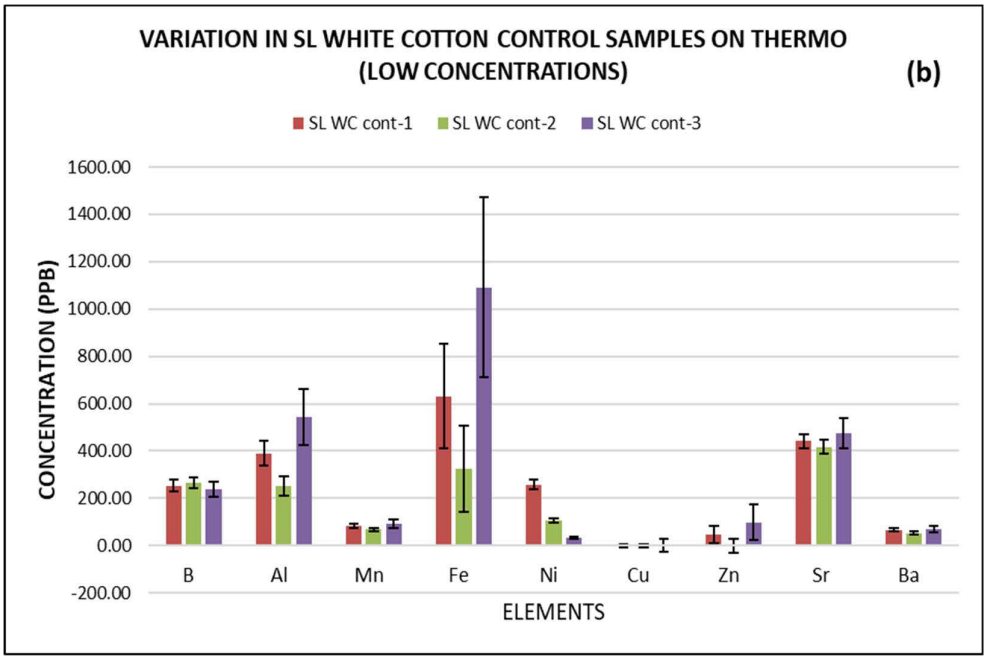


Figure 5-13: a) total and b) low concentration elemental composition of the SL white cotton control on the Thermo iCAP RQ ICP-MS

Figure 5-13 shows the elemental concentrations measured in three samples of SL (from Spotlight) white cotton control samples on the Thermo iCAP RQ ICP-MS. These samples were from adjacent areas of the same source material. The comparison of the average concentration of each element and their standard deviations show that all the elements have similar concentrations across the three samples, but not all are found to correlate

visually. In general, SL white cotton control-1 and SL white cotton control-3 were found to have very similar concentrations, resulting in an overlap of their standard deviations for all elements. SL white cotton control-2, on the other hand, had a lower concentration of several elements including sodium, magnesium, aluminium and iron. Overall, the three samples were only found to not correlate with regards to aluminium, iron and nickel. These elements must therefore must not be homogenously distributed over the source textile, possible due to contamination or natural variation in the textile. These results, however, show that there is a possibility that the samples can be linked to a common source as there is a high degree of correlation between the majority of the elements. However, as three elements were not found to correlate, this link cannot be definitively proven.

Table 5-12: Average, standard deviation and %RSD for elemental concentrations in SL white cotton control-1,2 and 3

Element	Average (ppb)	SD (ppb)	% RSD
^{11}B	252.08	11.49	5
^{23}Na	16948.40	1054.21	6
^{24}Mg	14289.37	1055.52	7
^{27}Al	395.35	118.76	30
^{55}Mn	81.59	10.78	13
^{56}Fe	681.62	315.18	46
^{60}Ni	133.03	93.12	70
^{63}Cu	0.28	0.39	141
^{66}Zn	48.73	40.29	83
^{88}Sr	444.18	22.87	5
^{137}Ba	63.17	7.40	11

The %RSD values calculated in Table 5-12 reflect the results in Figure 5-13. Large %RSD values were found for elements that were not found to correlate such as aluminium (30%), iron (46%) and nickel (70%). The high RSD value of 83% was found for zinc as (in the

initial diluted sample measured by the machine), the concentration of zinc in SL white cotton control-2 was found to be below the limit of detection on the calibration curves and so was recorded as a negative value. Due to this, an accurate undiluted concentration was unable to be found, and so the concentration was given as 0, resulting in a large variation. Copper also had a similar limitation as the concentrations in SL white cotton control-1 and 2 were below the threshold, while SL white cotton-3 had a very low undiluted concentration of 0.07 ppb. The low concentration along with the large standard deviation resulted in a very large RSD value of 141%. Due to these factors, the %RSD values of copper and zinc will be neglected in this analysis as they are not indicative of the actual deviation in the sample.

Table 5-13: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for SL White Cotton control on the Thermo ICP-MS

	ANOVA (p-value)	**p-test (p value)**		
		1 & 2	**2 & 3**	**1 & 3**
^{11}B	0.48	3.49×10^{-2}	0.07	0.10
^{23}Na	0.24	3.36×10^{-3}	0.08	0.22
^{24}Mg	0.16	6.31×10^{-4}	4.69×10^{-2}	0.40
^{27}Al	0.01	3.87×10^{-3}	2.22×10^{-2}	0.07
^{55}Mn	0.12	8.20×10^{-3}	0.05	0.15
^{56}Fe	0.04	7.60×10^{-3}	2.33×10^{-2}	0.04
^{60}Ni	2.97×10^{-6}	2.57×10^{-3}	1.38×10^{-3}	2.15×10^{-3}
^{63}Cu	7.93×10^{-5}	2.14×10^{-3}	6.83×10^{-3}	0.01
^{66}Zn	0.02	1.07×10^{-3}	2.23×10^{-3}	0.14
^{88}Sr	0.36	2.03×10^{-2}	0.10	0.26
^{137}Ba	0.13	9.34×10^{-4}	0.06	0.41

Statistical analysis of the data using ANOVA: Single Factor and t-Test: Paired Two Sample for Means in Table 5-13 show that all three white cotton samples can statistically correlate for barium, boron, sodium, magnesium, manganese and strontium. Reflecting the visual analysis of Figure 5-13, the t-test analysis shows there is no correlation for any of the elements between SL white cotton control 1 and 2, while there was a high degree of correlation between SL white cotton control 2 and 3.

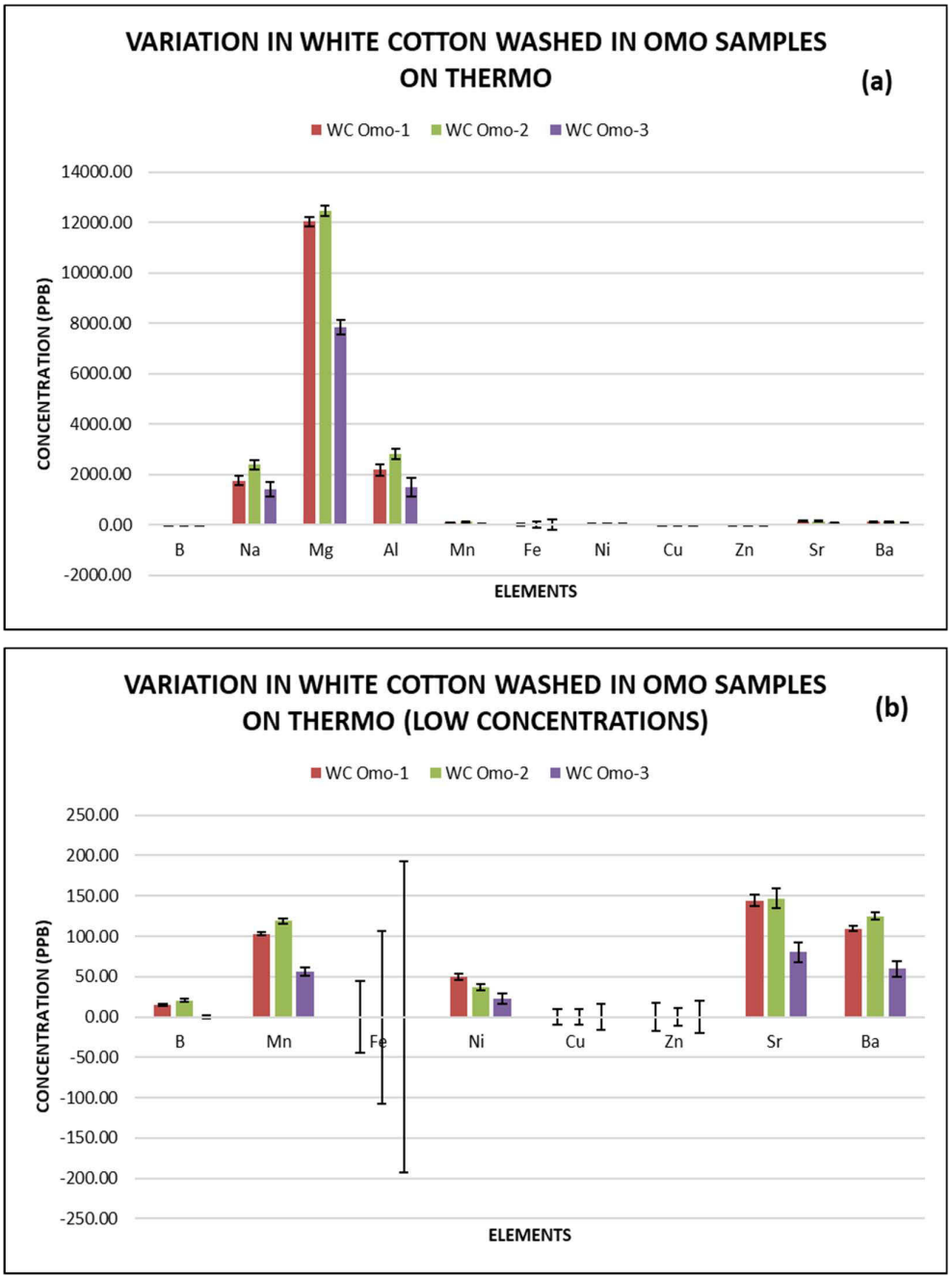


Figure 5-14: a) total and b) low concentration elemental composition of white cotton washed in Omo on the Thermo iCAP RQ ICP-MS

Figure 5-14 displays the elemental concentrations detected in white cotton that was washed with Omo detergent. The three samples came from three white cotton swatches that were cut from the same white cotton source material and were washed at the same time. These samples are analysed to determine whether there is much variability between samples that come from the same textile and are washed the same way but are not from

adjacent areas. WC Omo-1 and 2 have very similar concentrations while WC Omo-3 had elemental concentrations that were consistently smaller. The average concentration and standard deviation of all three samples did not overlap for any of the elements, though WC Omo-1 and 2 can be seen to overlap for boron and strontium.

Table 5-14: Average, standard deviation and %RSD for elemental concentrations in white cotton control washed in Omo

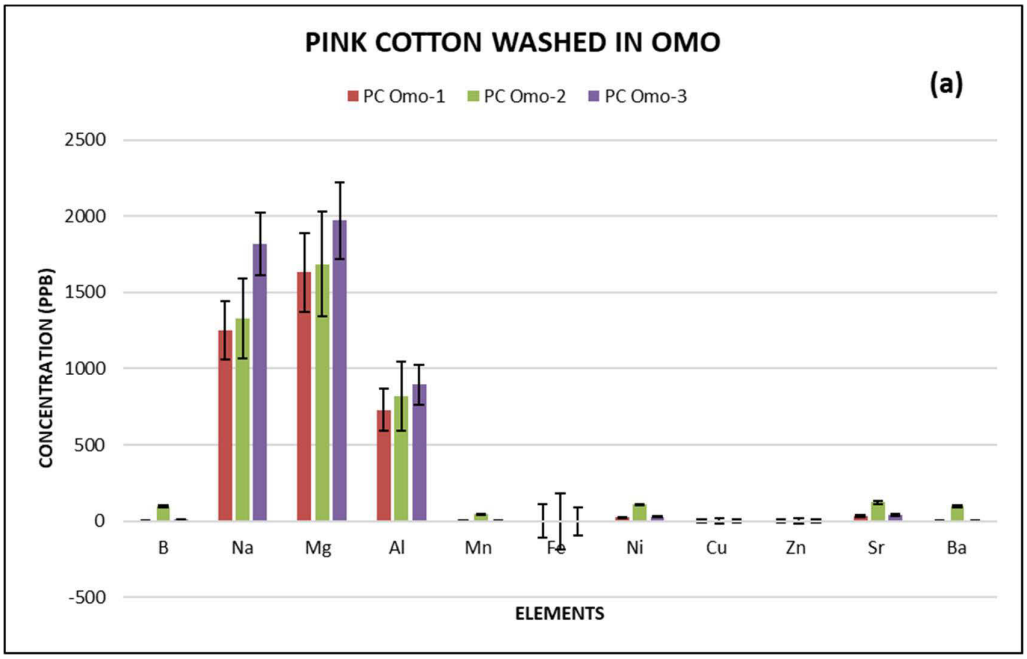
Element	Average (ppb)	SD (ppb)	% RSD
^{11}B	11.79	8.64	73
^{23}Na	1841.61	408.52	22
^{24}Mg	10774.75	2082.04	19
^{27}Al	2164.57	534.78	25
^{55}Mn	92.53	26.54	29
^{56}Fe	0.00	n/a	n/a
^{60}Ni	36.14	10.91	30
^{63}Cu	0.00	n/a	n/a
^{66}Zn	0.00	n/a	n/a
^{88}Sr	123.72	30.86	25
^{137}Ba	97.95	27.82	28

The %RSD for the three samples in Table 5-14 ranged between 19 and 73%. The high %RSD of 73% for boron is due to the concentration of boron in WC Omo-3 being below the LOD. The high %RSD values again reflect that consistent concentrations of the elements are not found throughout the three samples and, and a result, it may be falsely concluded that two fibres do not come from the same textile. The variation between the three samples could be due to how each sample experienced the wash cycles. Although the three swatches were washed at the same time and in the same laundry bag, the textiles were found to fold up and clump to each other during the wash, varying the exposure each sample had to both the dissolved detergent and the water itself.

Table 5-15: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for White Cotton washed in Omo

	ANOVA (p-value)	p-test (p value)		
		1 & 2	**2 & 3**	**1 & 3**
^{11}B	1.39×10^{-3}	0.17	1.14×10^{-4}	0.02
^{23}Na	0.01	0.03	3.31×10^{-3}	0.02
^{24}Mg	0.02	0.69	0.01	0.02
^{27}Al	0.01	0.08	0.01	0.02
^{55}Mn	0.02	0.31	0.01	0.02
^{56}Fe	1.03×10^{-3}	0.32	8.60×10^{-4}	0.01
^{60}Ni	1.76×10^{-3}	0.09	3.85×10^{-3}	0.01
^{63}Cu	4.59×10^{-3}	0.63	1.42×10^{-3}	0.02
^{66}Zn	0.01	0.10	2.93×10^{-3}	0.03
^{88}Sr	0.01	0.85	0.01	0.01
^{137}Ba	0.01	0.34	0.01	0.01

The statistical analysis of the elemental composition of the three white cotton samples washed in Omo in Table 5-15 support the earlier conclusion based on the visual analysis of Figure 5-14. White cotton-Omo 1 and 2 were found to be significantly similar, with a p-value below 0.05 only being calculated for one element, sodium. Neither of these samples, however, were found to correlate to White Cotton-Omo-3 at all. As discussed previously, the reason for this difference is unknown and analysis on significantly larger samples sizes washed at the same time would need to be done to explore this further.



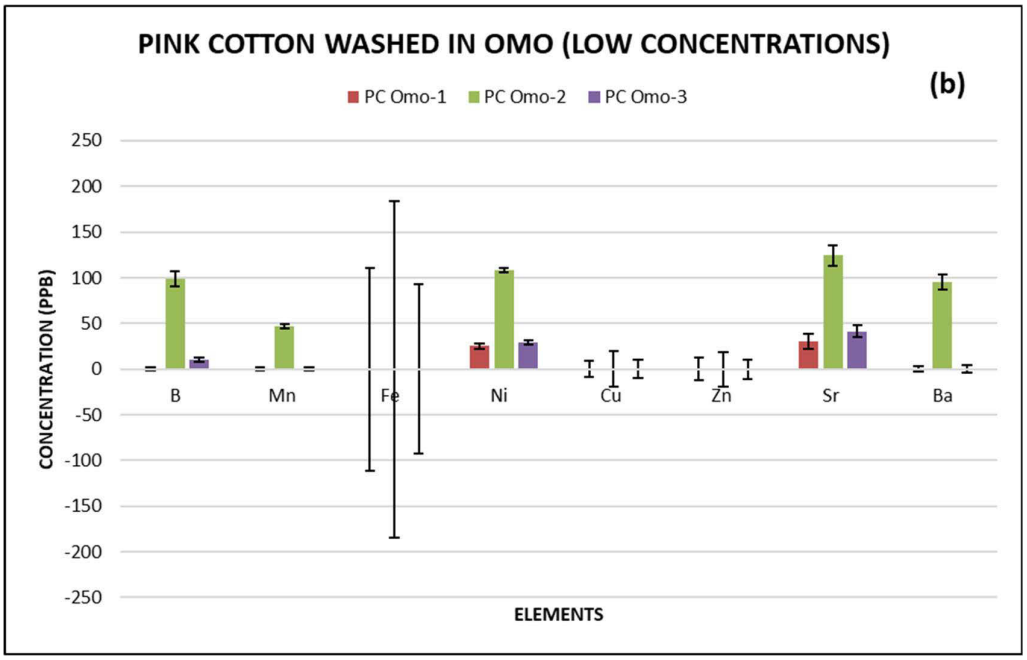


Figure 5-15: a) total and b) low concentration elemental composition of pink cotton washed in Omo on the Thermo iCAP RQ ICP-MS

Table 5-16: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Pink Cotton washed in Omo

Element	ANOVA (p-value)	p-test (p-value)		
		1 & 2	**2 & 3**	**1 & 3**
^{11}B	4.48×10^{-7}	1.07×10^{-3}	1.57×10^{-3}	4.84×10^{-6}
^{23}Na	0.04	0.21	4.43×10^{-3}	3.71×10^{-4}
^{24}Mg	0.36	0.38	0.05	2.50×10^{-3}
^{27}Al	0.53	0.23	0.35	0.01
^{55}Mn	3.23×10^{-9}	2.92×10^{-4}	4.21×10^{-4}	3.12×10^{-4}
^{56}Fe	0.02	0.10	0.02	1.66×10^{-3}
^{60}Ni	4.59×10^{-8}	9.24×10^{-5}	1.45×10^{-4}	2.23×10^{-3}
^{63}Cu	9.01×10^{-5}	2.33×10^{-3}	4.70×10^{-3}	1.72×10^{-4}
^{66}Zn	1.10×10^{-4}	8.58×10^{-4}	4.95×10^{-3}	3.79×10^{-4}
^{88}Sr	2.25×10^{-5}	4.23×10^{-4}	9.45×10^{-4}	3.20×10^{-3}
^{137}Ba	3.84×10^{-7}	6.99×10^{-4}	6.97×10^{-4}	7.99×10^{-4}

The analysis of pink cotton washed in Omo (Figure 5-15) showed similar results, with only magnesium and aluminium found to relate across all three samples. In general Pink Cotton-Omo-1&2 were found to be more similar than Pink Cotton-Omo-3. What these results do show is that caution should be taken when intepreting the elemental composition of evidence. This data should be used to distinguish a washed sample from control sample, as will be talked about further, rather than to link a washed textile to another washed textile.

The large degree of variation both within a sample, and between samples from the same source, show that the concentration of elements in the sample is not consistent. As a result of this, the sample size being tested is signficant. The larger the sample size, the more this variation is taken into account, creating a represntative concentration for the sample. When a small sample size is used (for example one fibre) it may be found to be statistically different from a fibre that came from the same source, leading the source fibre to be falsely excluded. This can create problems in case work where often only small sample sizes are available. However, further research into optimising this technique and performing a wide scale analysis of small samples sizes should be conducted.

Variance between instruments

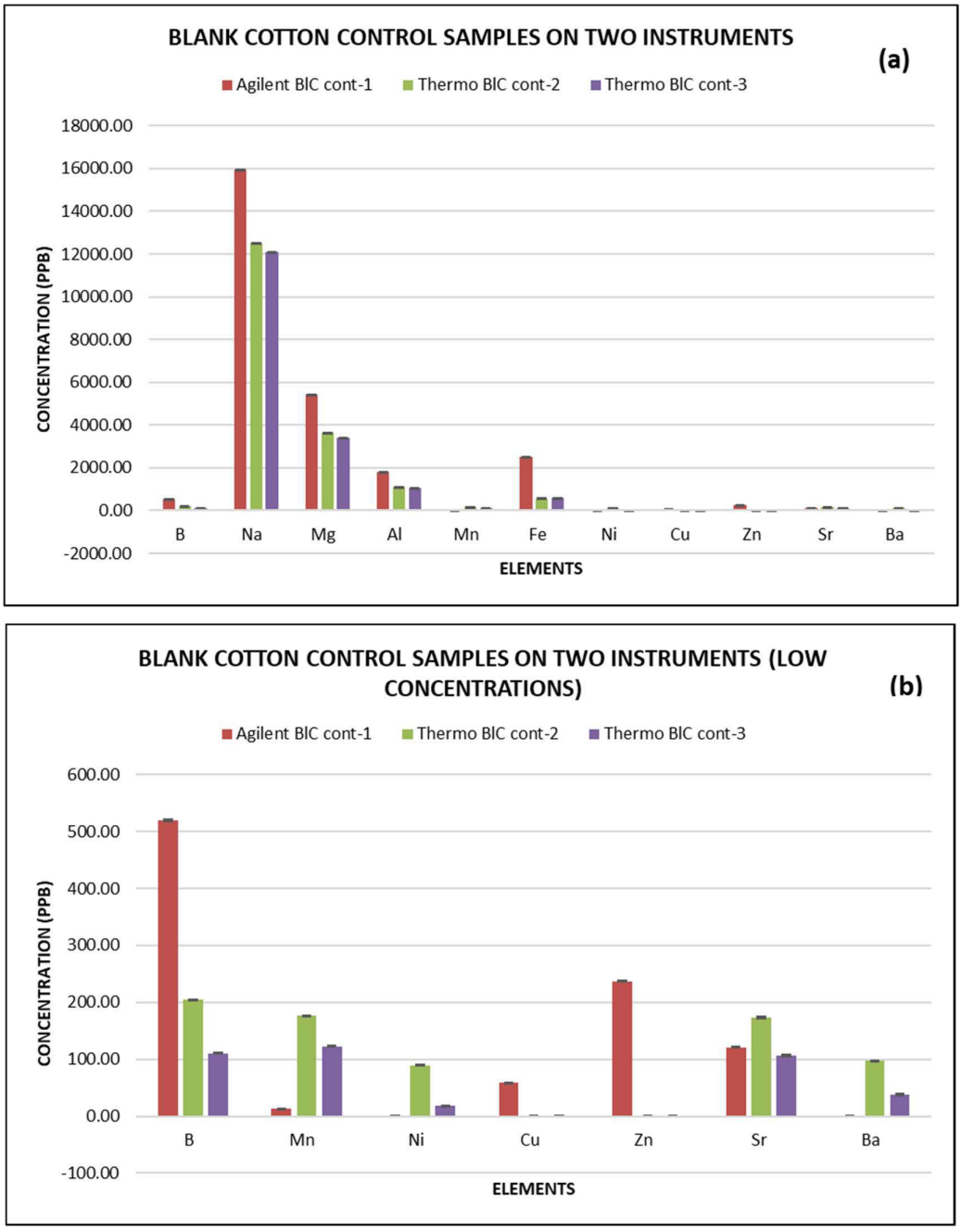
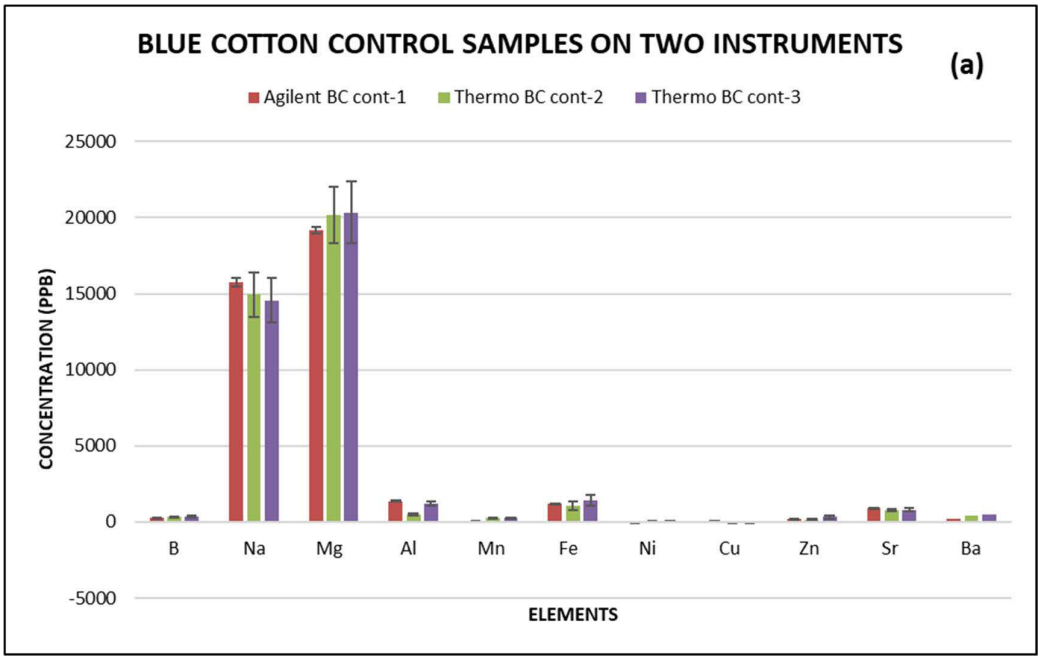


Figure 5-16: a) total and b) low concentration elemental composition of blank cotton on the Agilent and Thermo iCAP RQ ICP-MS

As discussed earlier, due to instrumental problems, most repeat samples had to be run on a different ICP-MS instrument to the original samples. Figure 5-16 displays the elemental concentrations and standard deviations measured for blank cotton control samples that

came from adjacent areas of the same source textile. BlC cont-1 was analysed using the Agilent ICP-MS while BlC cont-2 and 3 were analysed on the Thermo iCAP RQ ICP-MS. It should be noted that a fresh set of calibration standards were also created for the set run on the Thermo instrument. While BlC cont-2 and 3 had very similar concentrations for all elements, BlC cont-1 had concentrations that were consistently higher for the majority of elements. The exceptions to this are manganese and strontium (which had very low concentrations) and nickel and barium (which were below the threshold). It is significant to note that BlC cont-1 was not found to correlate with either BlC cont-2 or 3 for any element.



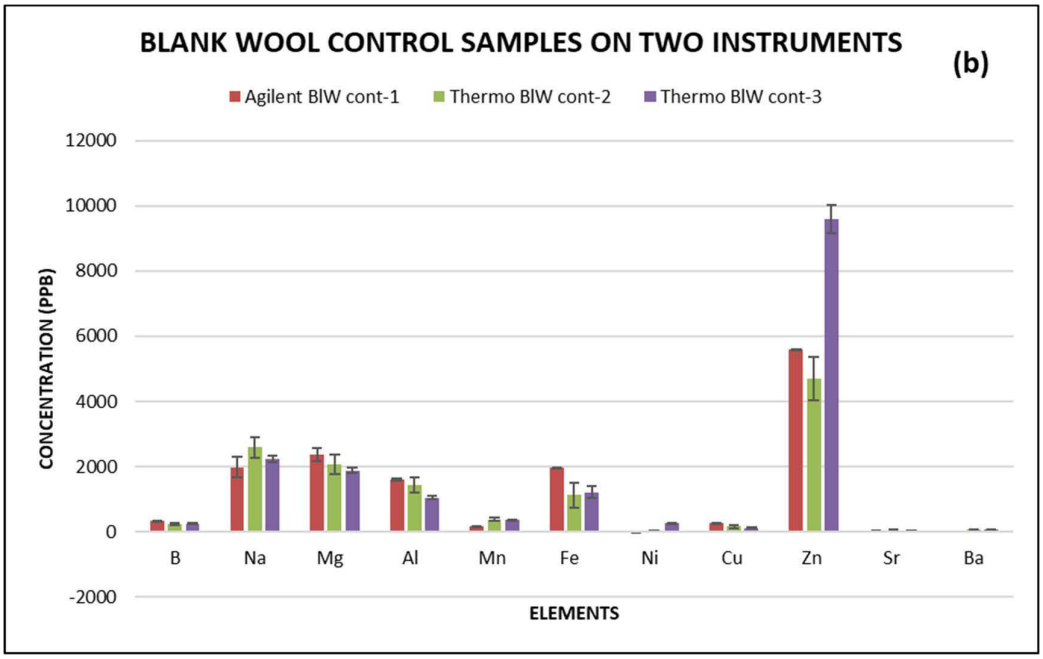
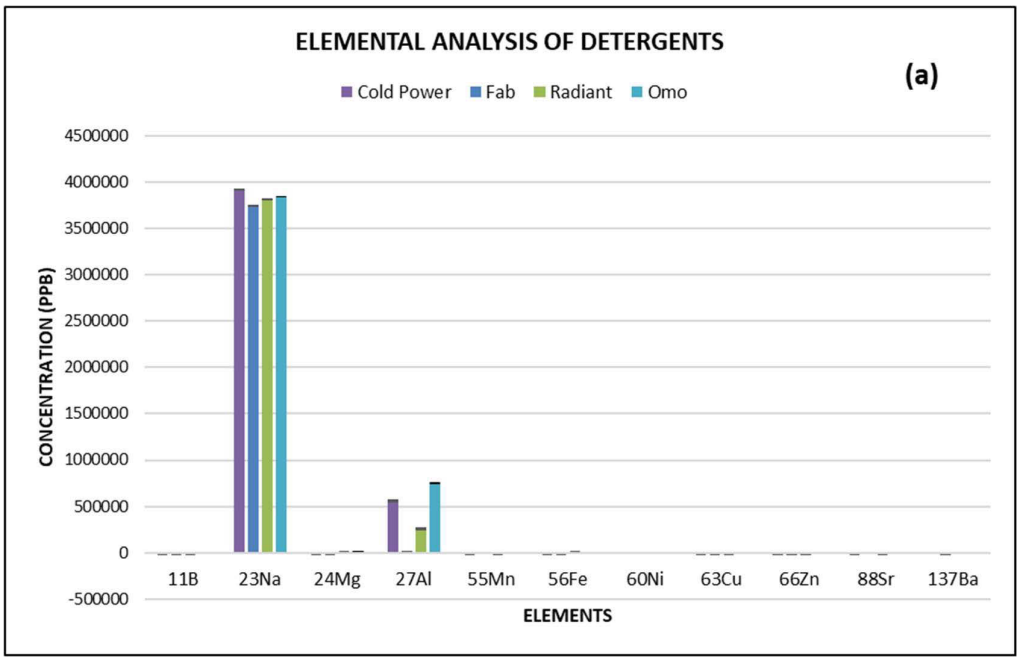


Figure 5-17: a) blue cotton and b) blank wool analysed on the Agilent and Thermo iCAP RQ ICP-MS

Despite the variability seen in the blank cotton control samples over the two instruments, far more similar elemental concentrations are seen for the majority of the other control samples, such as blue cotton and blank wool controls in Figure 5-17. In this figure it can be seen that the control samples were found to have far more consistent elemental concentrations measured using both the Agilent ICP-MS and the Thermo ICP-MS for the majority of the elements. The largest variation in elemental concentration in the blank wool sample can be seen for zinc. This difference in elemental concentration is greatest between the two samples analysed on the Thermo ICP-MS, with the Agilent analysed sample, Agilent BLW cont-1, having a zinc concentration which almost overlaps with the Thermo analysed sample, Thermo BLW cont-2. The only other control sample found to have the same degree of variation between the two instruments was the white wool control samples. This indicates that the variability seen in Figure 5-16 is likely due to other factors such as contamination, or uneven elemental composition of the original source material.

The consistency between the controls in Figure 5-17 indicates that this technique is repeatable over different instruments as long as external calibration standards are used. To ensure the most accurate results, however, samples should be analysed on the same machine if possible, to remove any potential external factors that may cause variability.

Elemental analysis of laundry detergents



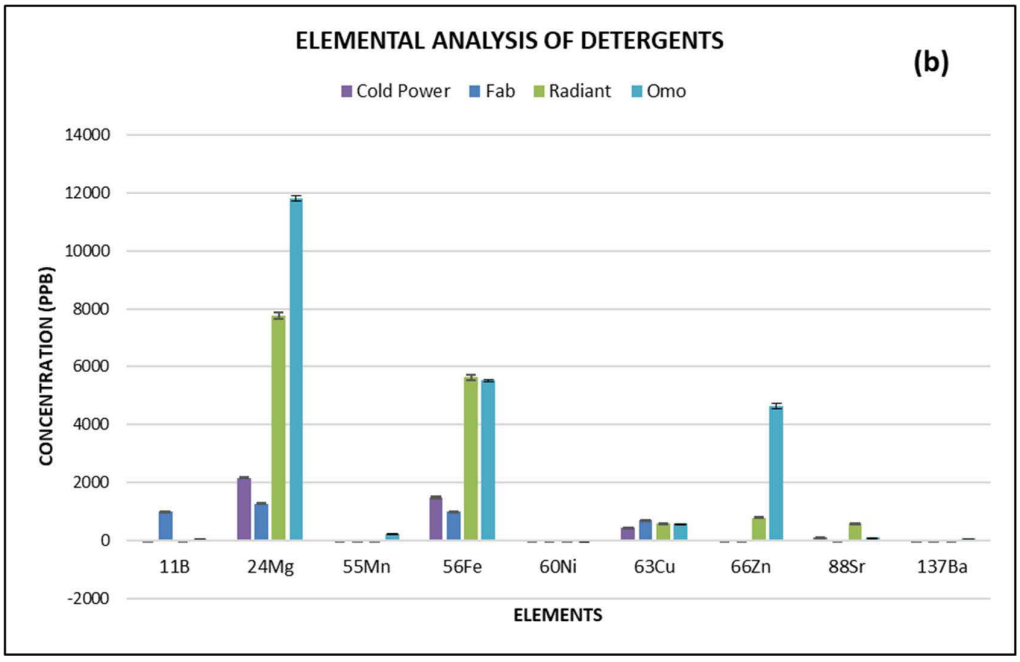


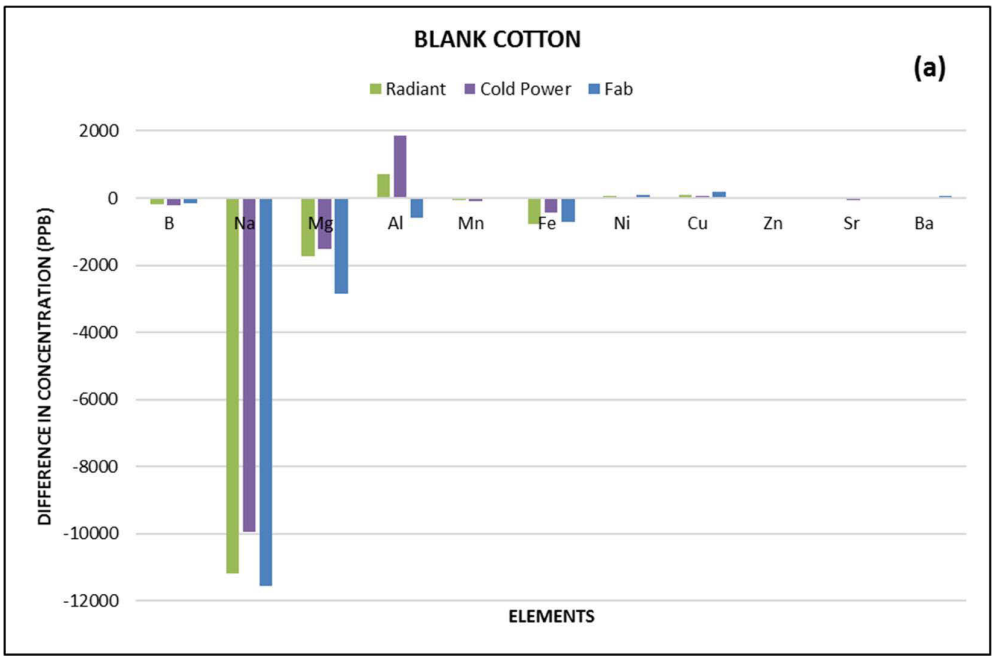
Figure 5-18: a) total and b) low concentration elemental composition of Cold Power, Fab, Radiant and Omo detergents on the Thermo iCAP RQ ICP-MS

The elemental composition of Fab, Radiant, Cold Power and Omo detergents are shown in Figure 5-18. The main component of all four detergents is sodium, due to the large

number of sodium salts in commercial laundry detergents (Table 1-1). The second main element is aluminium which was found to have variable concentrations among the four detergents with Fab having no aluminium detected at all. This is an expected result as, unlike the other detergents, Table 1-1 shows that Fab contains no aluminosilicate zeolites or any other form of aluminium. Magnesium, iron and zinc are also found in all four detergents, especially in Omo and Radiant detergents, thought at far lower concentrations. The source of these elements cannot be determined from the ingredients list of each detergent.

The results show that while there is some variation in the elemental composition of the detergents, most notably with the lack of aluminium in the Fab sample, the overall elemental composition of all four detergents is very similar.

Comparison of washed and unwashed samples



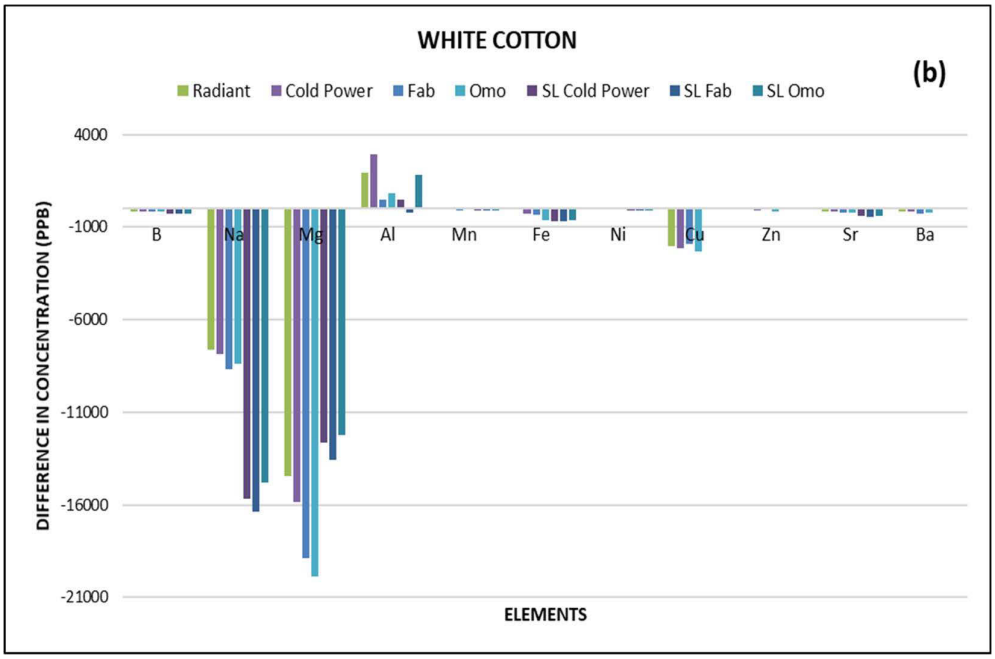


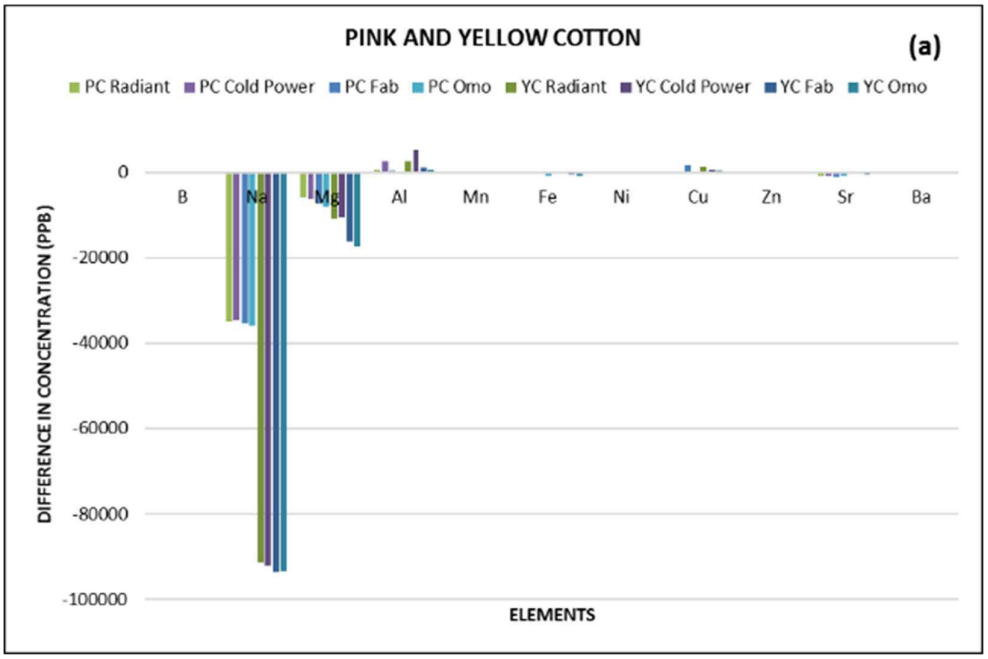
Figure 5-19: Difference between concentrations of elements in washed and unwashed a) blank and b) white cotton

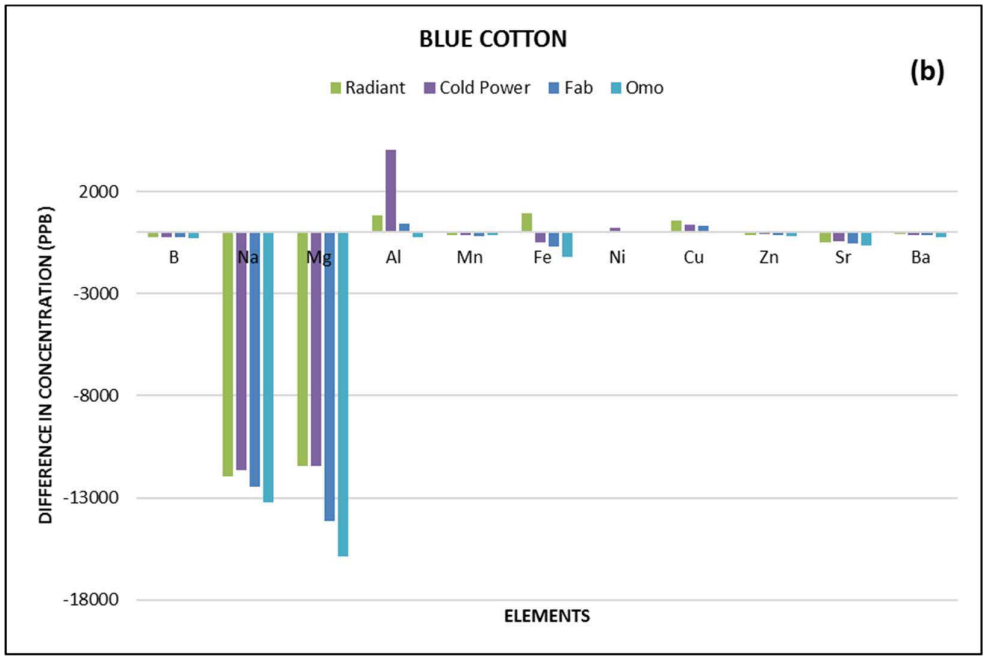
The average concentration of each element in each sample was found. The average concentration of the control samples was then subtracted from the washed samples, in order to identify whether there was an increase or decrease of each element after washing and whether this could be found to be consistent. The results are portrayed in Figure 5-19 where a trend for each element can be identified. As blank cotton has not gone through any processing steps, all elements detected in the cotton control are from either the cotton fibres (and originally from the cotton plant) or from machinery used to weave the textile. Elements including potassium, magnesium, calcium, sodium, iron, manganese, copper, zinc and lead have all been identified in cotton plants [125]. In blank cotton, all elements except aluminium and copper are found to decrease after washing. The most significant differences are sodium and magnesium which are found to decrease substantially. Iron is also found to decrease to a lesser extent. This is most likely attributed to the elements above being washed from the fabric. A slight decrease in boron, manganese, zinc and strontium is also observed while there is an increase in aluminium, nickel and copper. The increase in aluminium is likely due to its presence in the laundry detergents in the form of zeolites like sodium silicoaluminate which are ion-exchange water softening builders. The water used to wash the samples can also be linked to the increase in copper and aluminium, as both elements are present in the Sydney water supply. A report from

Sydney Water (see Figure A- 7) gives the aluminium concentration in the Prospect North water system as 0.01-0.018 ppm and the copper concentration as 0.006 to 0.038 ppm. This is consistent with the concentrations (0.029 ppm and 0.032 ppm respectively) that were measured when a sample of tap water was analysed using the Agilent 7500 ICP-MS.

White cotton shows the same trends with the exception of copper which was found to decrease instead of increase in the white cotton bought at Lincraft (Radiant, Cold Power, Fab and Omo in Figure 5-19). There was no significant increase or decrease in the Spotlight white cotton control copper concentration. This is likely because the original copper concentration in the white cotton from Lincraft was quite high at around 2000 ppb, compared to the blank cotton control that had a low concentration of 19 ppb. As a result, more copper is likely to be removed through the washing process than added. The large decrease in magnesium in the white cotton samples is likely due to its higher concentration in the white cotton control. The Lincraft white cotton control had an average magnesium concentration of 30665 ppb while the Spotlight white cotton control sample had an average concentration of 14289 ppb. The blank cotton control samples only had a magnesium concentration of 4160 ppb, leading to a much smaller decrease in concentration after washing. While the presence of magnesium is often controlled during the cotton textile processing due to its interference with steps such as dying, it is often added in the form of magnesium sulphate during the bleaching stage to act as a stabiliser to hydrogen peroxide [123].

While most of the detergents exhibited the same trend in elemental concentration, one exception was the change in aluminium concentration of samples washed in Fab detergent. In Figure 5-18 it was determined that Fab was the only detergent of the four analysed that did not contain aluminium and in Figure 5-19 it can be seen that Fab was the only detergent to result in a decrease in aluminium in both the blank cotton and Spotlight white cotton samples. This characteristic has the potential to be used to determine whether a washed textile was washed in Fab detergent, however as Lincraft white cotton washed in Fab resulted in an increase in aluminium concentration it can not be used as a reliable indicator.


PINK AND YELLOW COTTON
(a)
PC Radiant
PC Cold Power
PC Fab
PC Omo
YC Radiant
YC Cold Power
YC Fab
YC Omo
0
-20000
-40000
-60000
-80000
-100000
DIFFERENCE IN CONCENTRATION (PPB)
B
Na
Mg
Al
Mn
Fe
Ni
Cu
Zn
Sr
Ba
ELEMENTS


BLUE COTTON
(b)
Radiant
Cold Power
Fab
Omo
2000
-3000
-8000
-13000
-18000
DIFFERENCE IN CONCENTRATION (PPB)
B
Na
Mg
Al
Mn
Fe
Ni
Cu
Zn
Sr
Ba
ELEMENTS

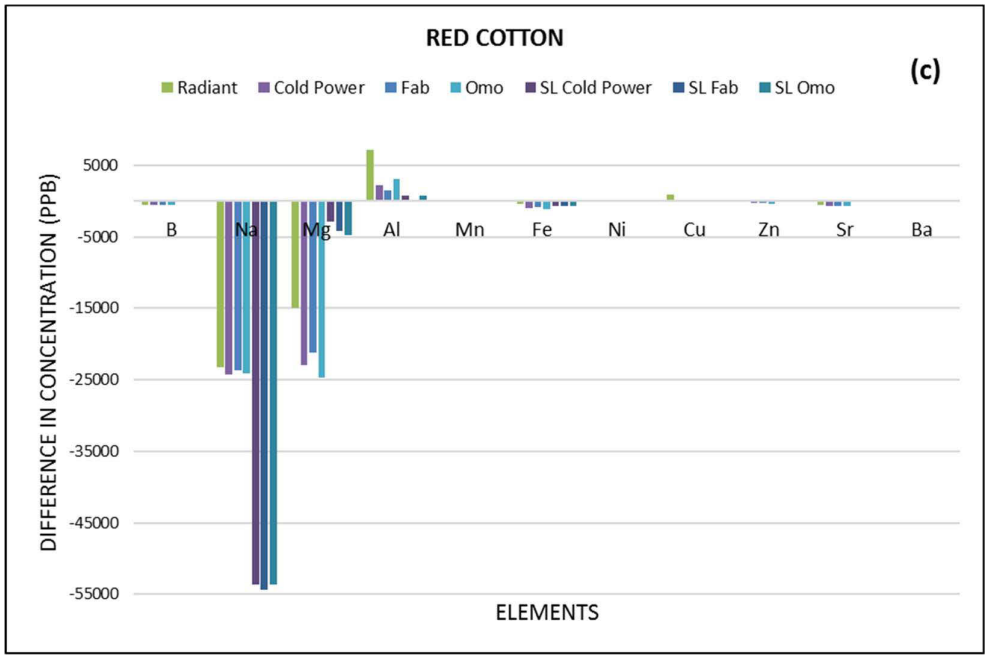
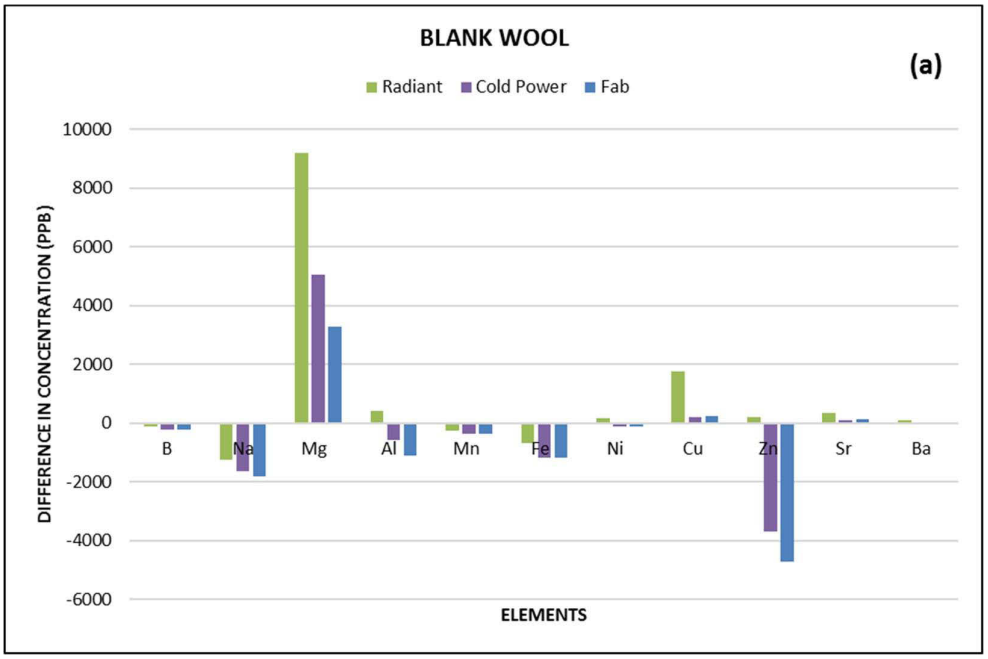
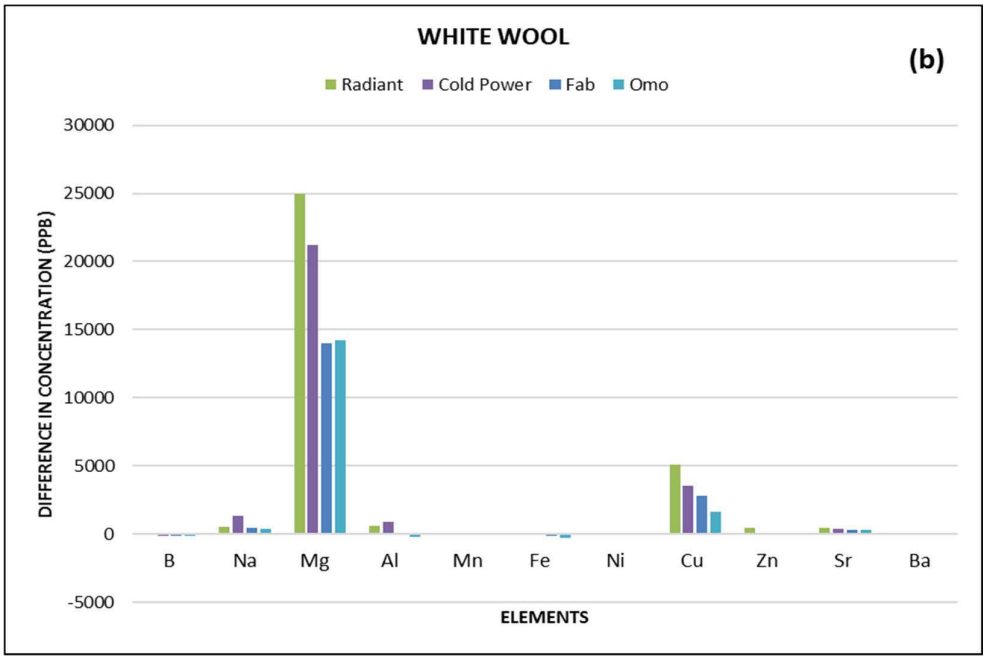


Figure 5-20: Difference between concentrations of elements in washed and unwashed a) pink and yellow, b) blue and c) red cotton

The dyed cotton samples (pink, yellow, blue and red cotton) shown in Figure 5-20 again all follow the same trend discussed above regarding the main concentration changes in sodium, magnesium, aluminium and iron. One main difference seen is that samples washed in Fab detergent also exhibited an increase in aluminium concentration. All dyed samples showed a very small increase in copper concentration, however, it not a significant increase. The red cotton purchased from Spotlight (SL) saw a smaller decrease in magnesium concentration than that bought at Lincraft. This is again due to it having a substantially lower concentration of magnesium in its control sample. The Lincraft red cotton had a concentration of 37000 ppb in the control which reduced to an average of 16000 ppb in the washed samples. The red cotton from Spotlight, on the other hand, had an initial concentration of 7000 ppb that dropped to an average of 3000 ppb after washing.

The dyed cotton samples washed in Fab did not show the same decrease in aluminium that was seen in Figure 5-19, though it did not increase to the same degree as the other detergents. This indicates the limitations of elemental analysis in identifying the exact detergent used.


BLANK WOOL
(a)
Radiant
Cold Power
Fab
10000
8000
6000
4000
2000
0
-2000
-4000
-6000
DIFFERENCE IN CONCENTRATION (PPB)
B
Na
Mg
Al
Mn
Fe
Ni
Cu
Zn
Sr
Ba
ELEMENTS


WHITE WOOL
(b)
Radiant
Cold Power
Fab
Omo
30000
25000
20000
15000
10000
5000
0
-5000
DIFFERENCE IN CONCENTRATION (PPB)
B
Na
Mg
Al
Mn
Fe
Ni
Cu
Zn
Sr
Ba
ELEMENTS

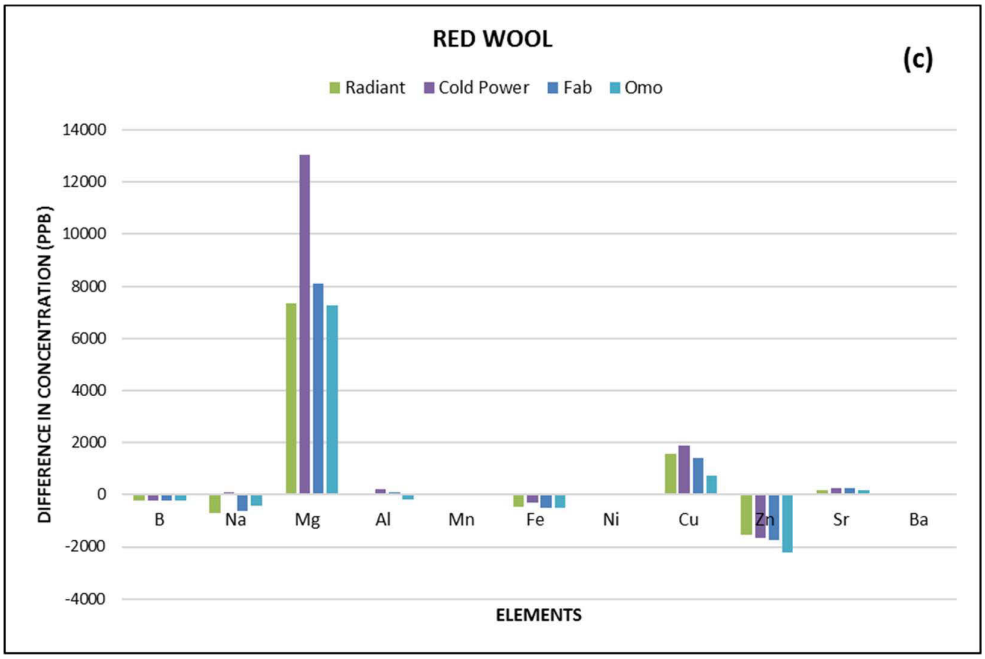


Figure 5-21: Difference between concentrations of elements in washed and unwashed a) blank, b) white and c) red wool

Wool showed very different trends to cotton when the element concentrations in washed and control wool samples were compared in Figure 5-21. The trends that were consistent for all three wool samples (blank, white and red) were that the magnesium and copper concentration increased after washing. Though magnesium is naturally found in wool, it is present in far lower concentrations than in cotton. During the rinsing cycle in the washing machine when they are exposed to hard water without detergent builders, wool fibres can be found to absorb magnesium increasing the concentration of magnesium found in the washed fibres [117]. Copper concentrations were quite low (around 100 ppb) in the wool control samples, even though copper is one of the more prevalent minerals found in sheep wool due to its importance in the growth and keratinisation of hair and wool. It is also reported that copper and zinc bind tightly to wool proteins and are only partially removed by prolonged washing with strong chelating agents [126]. Its low concentration must, therefore, be a result of effective scouring processes in the wool textile processing. Due to the small concentration of copper in the control samples, a substantial increase could, therefore, be seen when the textiles came into contact with the rinse water, absorbing copper.

The blank wool sample produced interesting results in which the concentration of aluminium, nickel, zinc and barium were all found to decrease in the samples washed in Cold Power and Fab but increase in Radiant. As the samples were unable to be washed in the three detergents at the same time, the washing of these samples was spread out over a month and with different items of clothing. As a result of this, it is unclear whether it is the detergent itself that created this difference in concentration, or something else in its washing cycle. This difference was not observed in any of the other samples.

Unlike cotton which tended to display the same trends regardless of whether the sample was dyed or not, wool samples show fairly different trends over the three wool samples. Sodium saw a decrease after washing in the blank wool and red wool but an increase in the white wool. This can again be linked to their original concentrations in the controls, with blank wool and red wool having higher concentrations (2400 and 1400 ppb) compared to white wool (1100 ppb). After washing, the blank and red wool samples had sodium concentrations averaging 837 and 828 ppb while the white wool had a slight increase to a concentration of 1716 ppb. Zinc also had variable results, with a substantial decrease in the red wool, while there was the mixed trend in the blank wool and a very slight increase in concentration in the white wool.

By identifying the elements that undergo the most change during washing, these elements can be used as target elements when comparing two fibres to determine whether they have been washed or not. There is no variation between the effects of different detergents on the elemental composition of cotton that could be used to identify the detergent used. There is a slight anomaly with the effect of radiant detergent on blank wool. However this does not apply to both the white or red dyes, and further repetition would need to be performed to determine whether this trend continues.

Statistical Analysis of Data

ANOVA: Single Factor and t-Test: Paired Two Sample for Means

ANOVA single factor and t-test: Paired two samples for Means were performed comparing control samples of each textile to two washed samples. The concentration values used for each group were compiled from three measurements taken from two

repeat samples, resulting in six values being compared for each group. This comparison was performed with a confidence interval of 95%. When p-values were calculated to be above 0.05, the null hypothesis (that the means of the concentration for each element is equal for each sample) was accepted. The null hypothesis was rejected when p-values were below 0.05.

Table 5-17: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Blank Cotton Control, Blank Cotton-Radiant and Blank Cotton-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	6.44×10^{-4}	1.33×10^{-3}	8.09×10^{-4}	0.04
^{23}Na	1.81×10^{-17}	3.32×10^{-8}	5.14×10^{-8}	0.77
^{24}Mg	1.05×10^{-6}	1.12×10^{-4}	3.79×10^{-5}	0.29
^{27}Al	3.72×10^{-5}	0.02	3.30×10^{-6}	0.05
^{55}Mn	1.35×10^{-4}	3.69×10^{-3}	1.14×10^{-4}	0.11
^{56}Fe	1.22×10^{-4}	9.81×10^{-5}	5.97×10^{-4}	0.67
^{60}Ni	0.01	5.12×10^{-5}	4.67×10^{-4}	0.39
^{63}Cu	6.99×10^{-6}	5.40×10^{-7}	4.91×10^{-4}	4.91×10^{-4}
^{66}Zn	2.16×10^{-6}	2.46×10^{-7}	0.20	0.20
^{88}Sr	0.02	0.87	8.85×10^{-4}	0.03
^{137}Ba	0.02	0.11	4.32×10^{-4}	0.02

The statistical comparison of the control and blank cotton samples washed in Radiant and Cold Power was performed and the p-values are shown in Table 5-17. The ANOVA tests for correlation between all three samples by testing the null hypothesis that the three samples mean concentration for each element are equal. As no p-values were above the confidence interval of 0.05, the null hypothesis was rejected for every element, indicating that the control sample and the washed samples do not have elements present in the same concentration.

The t-test compared the means of the control with each washed sample, as well as the two washed samples to each other. The control and Radiant samples were found to only have the same concentration of barium and strontium while the control and Cold Power sample had the same concentration of Zinc. The washed samples, on the other hand, were found to have six elements (sodium, magnesium, manganese, iron, nickel and zinc) with the same mean concentration at the 95% confidence interval. These results reflect the change in concentration in Figure 5-19 which saw that the concentration of zinc, barium and strontium changed minimally after washing. The results support that the control and the washed samples can be statistically distinguished based on their elemental composition. The number of elements that the washed samples have in common show that the blank cotton washed fibres are unable to be distinguished based on which detergent they were washed in.

These results show that, despite the variation that has been seen in repeat samples analysed on the Thermo iCap RQ ICP-MS, there is a statistically significant difference between the elemental composition of the repeated wash samples and the repeated control samples that they are able to be reliably distinguished from each other.

Table 5-18: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for SL White Cotton Control, SL White Cotton-Omo and SL White Cotton-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Omo	Control + Cold Power	Omo + Cold Power
^{11}B	1.44 x10^{-22}	6.76 x10^{-10}	9.24 x10^{-9}	0.69
^{23}Na	4.35 x10^{-20}	5.10 x10^{-10}	7.30 x10^{-9}	0.04
^{24}Mg	5.38 x10^{-19}	1.53 x10^{-9}	2.37 x10^{-8}	0.26
^{27}Al	1.11 x10^{-5}	5.84 x10^{-4}	2.61 x10^{-3}	0.01
^{55}Mn	1.85 x10^{-14}	1.40 x10^{-8}	1.64 x10^{-7}	0.84
^{56}Fe	2.49 x10^{-7}	4.58 x10^{-5}	8.60 x10^{-5}	0.10
^{60}Ni	2.00 x10^{-3}	0.03	0.02	0.39
^{63}Cu	0.43	0.35	0.95	0.07
^{66}Zn	1.72 x10^{-10}	1.68 x10^{-6}	4.20 x10^{-6}	0.04
^{88}Sr	3.48 x10^{-18}	3.08 x10^{-11}	3.39 x10^{-9}	0.02
^{137}Ba	2.17x10^{-9}	3.44 x10^{-5}	2.46 x10^{-7}	2.46 x10^{-7}

The analysis of the SL white cotton samples in Table 5-18 reveal similar results to the blank cotton samples. Copper was the only element that was found to have the same concentration in the control sample and both washed samples, using the ANOVA test. T-test analysis of the control sample with both washed samples produced p-values below 0.05 for every other element, rejecting the null hypothesis. The t-test comparing the SL white cotton washed in Omo to the sample washed in Cold Power produced p-values above 0.05 for six elements (boron, sodium, manganese, iron, nickel and copper). These results show a significant difference between the control sample and the washed samples, allowing them to be distinguished using solution ICP-MS. The strong correlation between the two washed samples show that it is not possible to identify the detergent used.

Table 5-19: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Pink Cotton Control, Pink Cotton-Radiant and Pink Cotton-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	1.45×10^{-19}	4.09×10^{-8}	4.05×10^{-8}	9.35×10^{-5}
^{23}Na	3.15×10^{-14}	4.74×10^{-6}	4.52×10^{-6}	6.03×10^{-4}
^{24}Mg	1.33×10^{-11}	4.21×10^{-5}	1.95×10^{-5}	0.09
^{27}Al	1.29×10^{-3}	0.24	0.02	0.02
^{55}Mn	1.74×10^{-5}	2.68×10^{-3}	0.01	1.48×10^{-15}
^{56}Fe	1.38×10^{-3}	7.27×10^{-4}	0.02	0.63
^{60}Ni	0.01	0.01	0.01	0.06
^{63}Cu	3.11×10^{-9}	8.77×10^{-6}	3.33×10^{-5}	1.88×10^{-3}
^{66}Zn	0.08	0.67	5.51×10^{-4}	0.16
^{88}Sr	9.90×10^{-16}	1.21×10^{-6}	9.62×10^{-7}	0.02
^{137}Ba	0.02	0.12	0.11	0.06

The statistical analysis of the pink cotton samples in Table 5-19 shows a closer relationship between the control and the washed samples than the blank cotton and white cotton samples. While zinc is the only element that had an ANOVA p-value greater than 0.05, the control sample had three elemental concentrations in common with the Radiant sample (barium, aluminium and zinc) and one in common with the Cold Power sample (barium). The strongest correlation is between the two washed samples, with 5 elements that have p-values that accept the null hypothesis. This analysis indicates that when determining if a sample has been washed or not, elements such as iron, nickel and magnesium should be focused on as they have a significant difference in concentration between the control and the washed samples.

Table 5-20: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Blue Cotton Control, Blue Cotton-Radiant and Blue Cotton-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	3.47 x10^{-13}	4.30 x10^{-6}	1.88 x10^{-6}	0.27
^{23}Na	1.27 x10^{-14}	1.04 x10^{-6}	1.00 x10^{-6}	3.20 x10^{-4}
^{24}Mg	2.40 x10^{-11}	1.86 x10^{-6}	1.10 x10^{-5}	0.03
^{27}Al	0.63	0.55	0.59	0.74
^{55}Mn	1.21 x10^{-11}	1.30 x10^{-6}	9.56 x10^{-6}	0.99
^{56}Fe	3.37 x10^{-7}	2.45 x10^{-5}	9.70 x10^{-4}	0.39
^{60}Ni	0.03	0.04	0.06	0.07
^{63}Cu	7.96 x10^{-9}	5.11 x10^{-5}	3.87 x10^{-4}	1.83 x10^{-5}
^{66}Zn	2.74E-08	1.76E-04	9.20 x10^{-4}	0.69
^{88}Sr	7.25E-09	7.38E-06	6.14E-05	0.13
^{137}Ba	1.35x10^{-8}	3.40 x10^{-6}	3.32 x10^{-5}	2.30 x10^{-3}

In Table 5-20, the blue cotton samples are found to have the same aluminium concentration in both the control and washed samples, according to the ANOVA test results. T-test analysis found that the control sample had no other elemental concentrations in common with the blue cotton sample washed in Radiant, but that it had a statistically similar nickel concentration as the blue cotton sample washed in Cold Power. The washed samples however had 5 elements (strontium, zinc, iron, manganese and boron) that were found to have same concentration in both the sample washed in Radiant and the sample washed in Cold Power. This strong correlation between the washed samples shows that the blue cotton fibres generally exhibit the same change in elemental concentration after being washed, regardless of the detergent brand they were washed in.

Table 5-21: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Yellow Cotton Control, Yellow Cotton-Radiant and Yellow Cotton-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	2.49×10^{-10}	7.54×10^{-5}	1.30×10^{-4}	0.18
^{23}Na	1.15×10^{-12}	1.25×10^{-5}	1.64×10^{-5}	4.28×10^{-3}
^{24}Mg	1.43×10^{-7}	2.42×10^{-4}	1.33×10^{-3}	0.40
^{27}Al	4.75×10^{-10}	1.25×10^{-5}	8.52×10^{-6}	0.27
^{55}Mn	1.51×10^{-8}	1.19×10^{-4}	8.51×10^{-4}	0.17
^{56}Fe	6.60×10^{-4}	0.01	0.03	0.20
^{60}Ni	0.07	0.08	0.53	0.02
^{63}Cu	1.46×10^{-10}	1.96×10^{-5}	1.86×10^{-5}	1.17×10^{-4}
^{66}Zn	0.03	0.22	0.23	1.01×10^{-4}
^{88}Sr	2.87×10^{-6}	6.44×10^{-4}	0.01	0.69
^{137}Ba	0.15	0.74	0.22	0.02

The analysis of the yellow cotton samples in Table 5-21, as with the pink and blue cotton samples, shows a higher correlation between the two washed samples than the control and the washed samples. The control sample was found to have its barium, nickel and zinc concentrations in common with both the yellow cotton samples washed in Radiant and Cold Power. The Radiant and Cold Power samples, on the other hand, had six element concentrations in common (boron, magnesium, aluminium, manganese, iron and strontium). As these six elements were found to vary between the control sample and the washed samples, they are good target elements to use when analysing yellow cotton samples to determine whether the sample has been washed or not.

Red cotton

Table 5-22: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Red Cotton Control, Red Cotton-Radiant and Red Cotton-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	2.69 x10^{-12}	1.57 x10^{-5}	1.82 x10^{-5}	2.47 x10^{-4}
^{23}Na	6.16 x10^{-15}	1.28 x10^{-6}	1.31 x10^{-6}	3.51 x10^{-5}
^{24}Mg	1.40 x10^{-10}	2.01 x10^{-6}	2.76 x10^{-6}	2.37 x10^{-5}
^{27}Al	6.41 x10^{-8}	2.87 x10^{-5}	2.99 x10^{-3}	3.09 x10^{-3}
^{55}Mn	2.95 x10^{-5}	0.39	3.81 x10^{-3}	6.76 x10^{-6}
^{56}Fe	8.00 x10^{-8}	6.61 x10^{-4}	1.49 x10^{-4}	7.91 x10^{-5}
^{60}Ni	2.16 x10^{-3}	0.13	0.08	0.01
^{63}Cu	1.07 x10^{-11}	6.40 x10^{-6}	6.93 x10^{-5}	5.10 x10^{-6}
^{66}Zn	2.10 x10^{-5}	0.04	0.03	6.95 x10^{-6}
^{88}Sr	9.18 x10^{-11}	6.05 x10^{-7}	1.82 x10^{-4}	0.85
^{137}Ba	3.98x10^{-7}	0.04	5.47 x10^{-7}	1.08 x10^{-3}

The statistical analysis of the red cotton samples in Table 5-22 indicated that the samples have little in common with each other in terms of the concentration of their elements. The ANOVA test produced p-values under 0.05 for every element, showing that there was no correlation between all three samples. The control sample was found to have the same concentration of nickel as both the washed red cotton samples, however the t-test comparing the concentration of nickel in both washed samples produced a p-value of less than 0.05, rejecting the null hypothesis and proving that they did not have the same concentration of nickel as each other. The control sample was also found to have the same concentration of manganese as the Radiant washed sample. As there were more elements that were found to have dissimilar concentrations between the control and the washed

samples than similar concentrations, the elemental composition of an unknown red cotton sample can be used to determine if it has been washed or not.

The two washed samples, however, were only found to have one element whose p-value accepted the null hypothesis, strontium. The lack of elements in common between the two washed samples, compared to other samples analysed that were found to have six elements in common, indicates that the elemental composition could also be used to distinguish between two samples washed in different detergents, Radiant and Cold Power.

Table 5-23: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Blank Wool Control, Blank Wool-Radiant and Blank Wool-Cold Power

Element	**ANOVA (p value)**	**p-test (p value)**		
		Control + Radiant	**Control + Cold Power**	**Radiant + Cold Power**
^{11}B	6.49×10^{-8}	0.01	9.94×10^{-7}	0.01
^{23}Na	9.60×10^{-9}	1.07×10^{-3}	8.39×10^{-6}	0.01
^{24}Mg	2.69×10^{-8}	1.35×10^{-4}	2.16×10^{-5}	1.70×10^{-3}
^{27}Al	2.29×10^{-4}	0.17	0.01	6.12×10^{-4}
^{55}Mn	4.40×10^{-11}	4.52×10^{-5}	1.58×10^{-6}	1.58×10^{-6}
^{56}Fe	2.49×10^{-7}	0.02	3.64×10^{-5}	2.81×10^{-4}
^{60}Ni	1.34×10^{-3}	3.27×10^{-5}	0.07	4.17×10^{-3}
^{63}Cu	2.86×10^{-8}	3.41×10^{-4}	1.99×10^{-4}	4.26×10^{-4}
^{66}Zn	2.32×10^{-3}	0.81	0.01	5.10×10^{-5}
^{88}Sr	1.71×10^{-11}	4.29×10^{-6}	4.46×10^{-5}	1.54×10^{-5}
^{137}Ba	4.69×10^{-6}	0.01	3.09×10^{-6}	1.34×10^{-3}

The blank wool samples in Table 5-23 had very little relationship to each other in terms of their elemental composition. The control and Radiant sample had two elements in common, aluminium and zinc, while the control and Cold Power samples had one element in common, nickel. The two washed samples, however, had no elemental concentration

in common. This reflects the results in Figure 5-21 which showed a significant difference between the trend in the Radiant and Cold Power detergents after washing, with the two detergents showing opposing trends for some of the elements. It should be noted, however, that the Cold Power and Fab detergents showed a far more similar trend. This data shows that the elemental concentrations measured for the blank wool samples are quite varied, and that the washed samples have more in common with the control samples than with each other. There is enough variation between the control and the washed samples to be able to say that the washed samples are statistically different to the control samples, and thus use that data to determine if a sample has been washed or not. The difference between the two washed samples concentration imply that there is not an elemental concentration that is specific to the washed samples.

Table 5-24: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for White Wool Control, White Wool-Radiant and White Wool-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	3.42×10^{-10}	4.74×10^{-8}	5.86×10^{-5}	0.45
^{23}Na	2.21×10^{-6}	1.81×10^{-4}	3.02×10^{-4}	0.13
^{24}Mg	1.31×10^{-9}	4.44×10^{-6}	7.60×10^{-5}	0.75
^{27}Al	0.14	0.15	0.92	0.05
^{55}Mn	2.78×10^{-12}	5.98×10^{-6}	3.52×10^{-7}	1.98×10^{-4}
^{56}Fe	1.00×10^{-3}	0.08	1.95×10^{-3}	0.11
^{60}Ni	0.06	0.53	0.14	1.87×10^{-3}
^{63}Cu	4.83×10^{-10}	3.18×10^{-6}	5.63×10^{-4}	1.82×10^{-3}
^{66}Zn	2.55×10^{-10}	6.32×10^{-5}	0.03	1.09×10^{-4}
^{88}Sr	5.80×10^{-5}	2.79×10^{-3}	4.71×10^{-4}	0.69
^{137}Ba	5.28×10^{-10}	8.32×10^{-5}	6.13×10^{-8}	0.01

The ANOVA analysis of the white wool samples in Table 5-24 show that all three samples correlate in regard to aluminium and nickel. As a result, these two elements cannot be used to distinguish the samples. The concentration of iron was also found to be similar in the two washed samples, and between the control and the white wool sample washed in Radiant.

The Radiant and Control samples had six elemental concentrations that varied (boron, sodium, magnesium, aluminium, iron and strontium). All these elements, with the exception of iron, which was found to be the same in the control sample, could be used to distinguish a washed white wool fibre from an unwashed white wool fibre. The large degree of correlation between the two washed samples, however, shows that the samples cannot be distinguished based on detergent type.

Table 5-25: p- values for ANOVA: Single Factor and t-Test: Paired Two Sample for Means at α=0.05 for Red Wool Control, Red Wool-Radiant and Red Wool-Cold Power

Element	ANOVA (p value)	p-test (p value)		
		Control + Radiant	Control + Cold Power	Radiant + Cold Power
^{11}B	3.86×10^{-11}	1.63×10^{-5}	6.58×10^{-5}	0.89
^{23}Na	1.26×10^{-4}	2.72×10^{-5}	0.04	0.02
^{24}Mg	2.92×10^{-9}	1.09×10^{-5}	8.00×10^{-5}	0.16
^{27}Al	0.75	0.60	0.47	0.81
^{55}Mn	3.42×10^{-8}	7.98×10^{-5}	7.70×10^{-5}	0.01
^{56}Fe	2.15×10^{-4}	9.19×10^{-4}	1.12×10^{-3}	0.32
^{60}Ni	0.01	0.03	0.06	0.11
^{63}Cu	2.23×10^{-8}	3.90×10^{-5}	1.33×10^{-4}	0.03
^{66}Zn	7.83×10^{-6}	4.60×10^{-5}	1.78×10^{-4}	0.03
^{88}Sr	1.65×10^{-9}	3.33×10^{-5}	2.06×10^{-5}	0.88
^{137}Ba	3.78×10^{-6}	1.64×10^{-5}	3.16×10^{-3}	0.13

The red wool samples in Table 5-25 show a large degree of correlation between the two washed samples, with seven of the 11 elements having p-values over 0.05. The concentration of aluminium was found to be consistent across all three samples, and therefore it can be seen that the concentration of aluminium does not change significantly after washing. The control sample was also found to have the concentration of nickel in common with the Cold Power washed sample. These results show that the elemental composition of red wool changes significantly after washing, and that this trend can be seen to be consistent regardless of which detergent, Radiant or Cold Power, is used to wash the samples.

The ANOVA and t-test analysis of the elemental concentrations measured using solution ICP-MS showed that generally, there was a high degree of correlation between the two washed samples, but very little concentrations in common between the control and the washed samples. As a result, this supports the conclusion that solution ICP-MS can be used to distinguish washed from unwashed samples, but not distinguish the samples based on detergent. There were several elements that could be used to distinguish control from washed samples, such as sodium and magnesium. However, the element that most consistently was found to be similar in both washed samples but not the control samples, and therefore can be used as a clear indicator that the sample has been washed, was iron. Iron was found to have a p-value over 0.05 for five out of the six t-test analyses of washed cotton. This was followed by magnesium and manganese which were similar in four of the six cotton samples. For the wool samples, boron and magnesium were found to be similar in both washed wool samples in two of the three wool types tested. From this, it is clear that it should be ensured that these elements are targeted when performing the analysis.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a form of multivariate analysis that finds patterns in large data sets and visually represents the data in the form of score plots. The Minitab 10 statistical software was used to perform PCA on the data collected using ICP-MS.

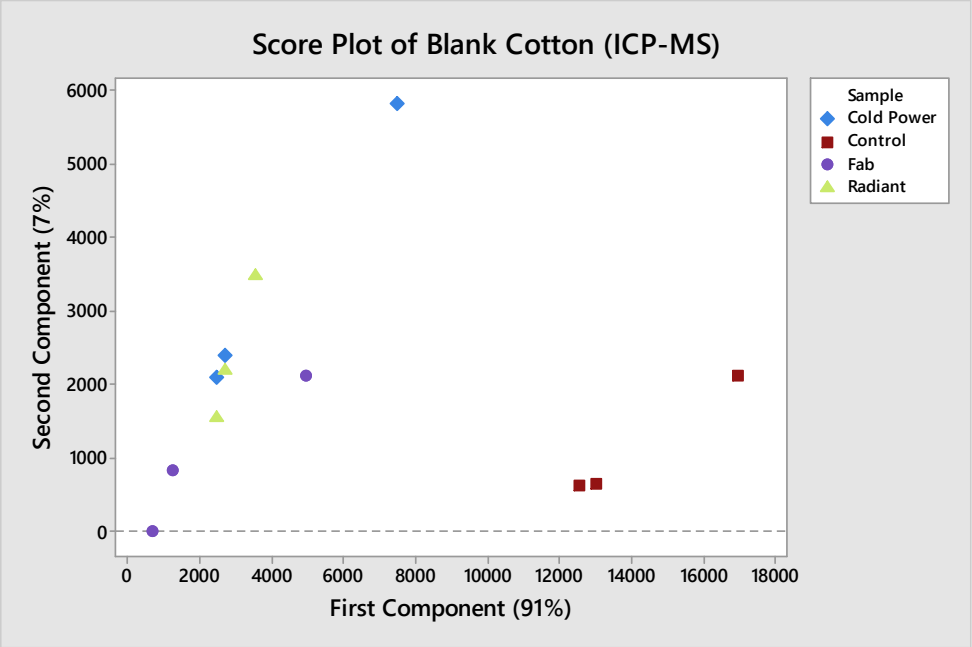


Figure 5-22: Score plot of Blank Cotton

Covariance PCA analysis was conducted on the blank cotton samples, creating the plot in Figure 5-22. These results show that there is a clear divide between the blank control samples (to the right of the first component scale) and the washed samples (distributed up the second component). Blank cotton control 2 and 3, which were analysed on the Thermo iCAP RQ ICP-MS, are clustered closely together. The blank cotton control-1, which was analysed on the Agilent instrument, is not as closely aligned to this cluster. Similar results can be seen with the washed samples where sample pairs 2 and 3 of blank cotton washed in Fab, Radiant and Cold Power are more closely situated to each other than blank cotton Fab-1, Rad-1 and CP-1 (which were washed in a separate washing stage and analysed on the Agilent instrument not the Thermo). This reinforces that there is a degree of variation between the samples on the two different instruments. However, the washed 2 and 3 samples are not clustered as closely as the control 2 and 3 samples, reflecting that there is a small degree of variation between the two samples that were washed at the same time and analysed with the same instrument. The PCA loading plot (Figure C- 1) shows that the element responsible for distribution along the first component is sodium while aluminium is the main element influencing the second component. This reflects the results discussed in Figure 5-16 where the control samples were found to have a high concentration of sodium which had decreased in all the washed cotton. Figure 5-19

also shows that the largest variation between the washed samples was in the aluminium concentration, hence why this element resulted in a spread of the washed samples up the second component.

As the samples are not clustered based on the detergent used, PCA cannot be used to identify the detergent used to wash a sample; however the divide between the control and the washed samples means it can be used to discriminate between washed and unwashed blank cotton textiles.

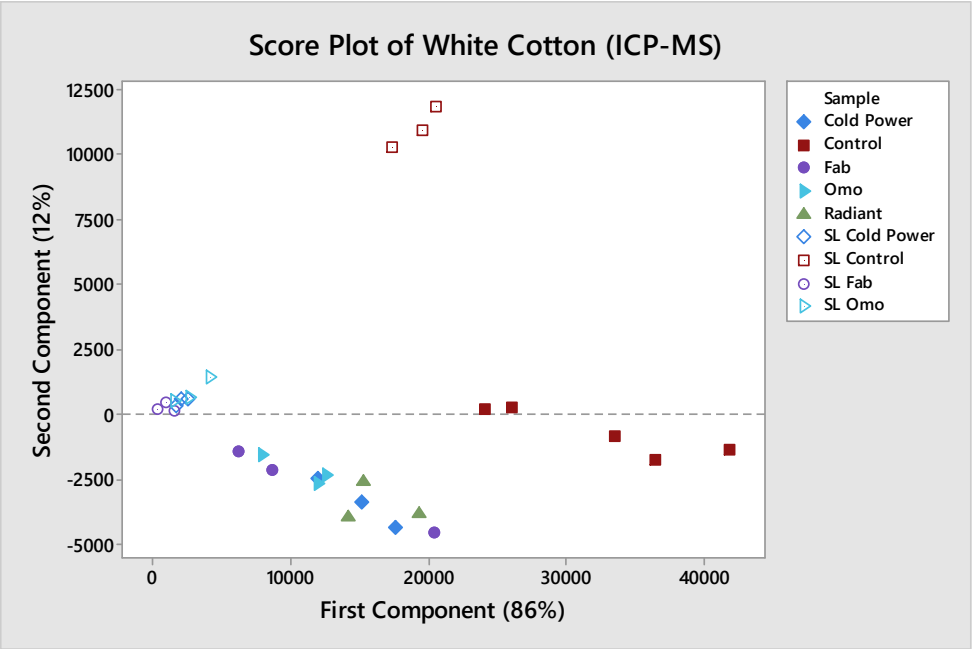


Figure 5-23: Score plot of White Cotton (ICP-MS)

The covariance PCA plot of washed and unwashed white cotton purchased from Lincraft and Spotlight is represented in Figure 5-23. In this plot, it can be seen that the samples are separated based not only on whether they are washed or unwashed but also based on where the white cotton was purchased. All the control samples are to the right of the first component while the washed samples are to the left. The white cotton control samples are then further separated along the second component depending on whether they were bought from Spotlight or Lincraft. This distribution is reflected in the washed samples with the washed cotton samples from Spotlight are placed further up the second component than the Lincraft samples. The PCA loading plot for this data (Figure C- 2) shows that this separation is based on the concentration of magnesium along the first

component and the concentration of sodium and aluminium along the second component. As discussed in Figure 5-19, both the Spotlight and the Lincraft white cotton controls have a high magnesium concentration compared to the washed cotton samples. As a result, they both appear further to the right of the first component. The Spotlight white cotton control samples had a higher sodium concentration than the Lincraft white cotton (around 17000 ppb compared to 10000 ppb) resulting in the Spotlight control samples being distributed further up the second component than the Lincraft samples. The washed cotton samples are plotted to the left of the first component due to their low magnesium concentration. Further separation occurs between the Spotlight and Lincraft washed cotton samples due to their aluminium concentrations. Lincraft washed white cotton, with their higher aluminium concentration, fall further down the second component than the Spotlight White Cotton.

In Figure 5-23 it can be seen there is one cluster formed between the Spotlight White Control Samples and another tighter cluster between the washed Spotlight white cotton samples. The white cotton samples from Lincraft are less clustered and show no identifiable clustering based on detergent brand. The tightness of the Spotlight white cotton clusters could be because all the Spotlight white cotton samples in a particular detergent were washed at the same time and analysed on the same instrument (Thermo). However, it can also be noted that three of the white cotton control samples (1a,1b and 1c) which as discussed earlier were from the same digested control sample, are also widely spread along the first component. The Lincraft white cotton samples washed in Omo were also all washed at the same time and analysed on the same instrument and again are not tightly clustered. This reflects that a large amount of variation can exist within a sample as well as between samples, and so clustering of these samples without overlap is unlikely to occur.

Despite the lack of detergent-based clustering, the divide between the control and washed samples means that ICP-MS along with PCA can be used as a tool to discriminate between white cotton washed and unwashed samples, as well as between white cotton samples from different brands.

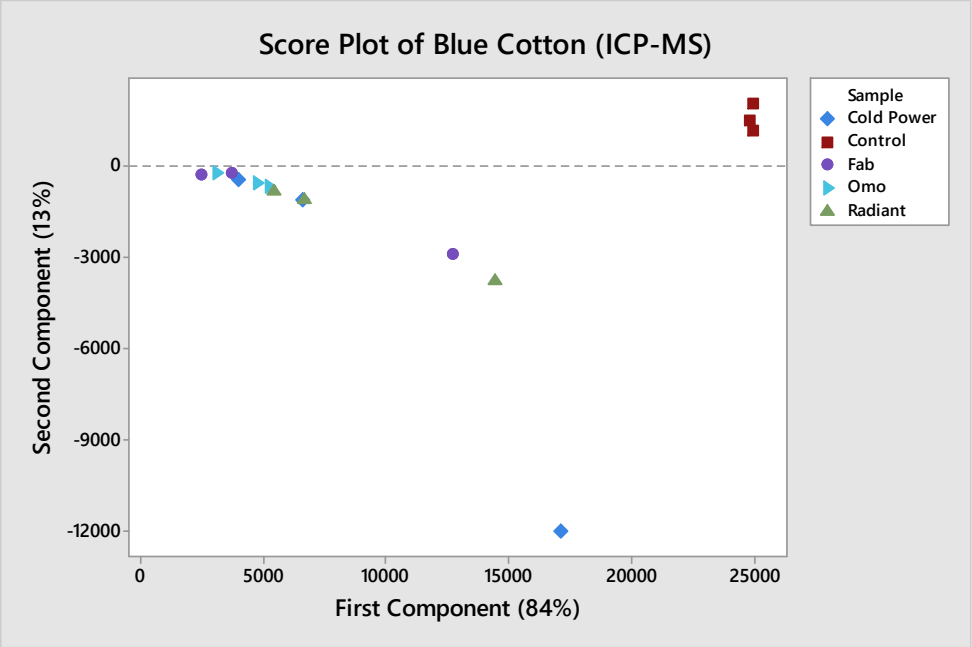


Figure 5-24: Score plot of Blue Cotton (ICP-MS)

The covariance PCA plot of blue cotton in Figure 5-24 shows that all three control samples are closely grouped in the top right quartile, while the washed blue cotton samples that were part of the second stage of sample washing and were analysed on the Thermo iCAP ICP-MS are closely clustered in the top left quartile. The samples that were washed in the first stage and analysed on the Agilent ICP-MS (Fab-1, Rad-1 and CP-1) are all set apart from the cluster, with CP-1 sitting as an outlier at the bottom of the second component.

Referring to the PCA loading plot (Figure C- 3) it can be seen that the position of the blue cotton control samples is due to their high sodium and magnesium concentration compared to the washed control samples. The Fab-1, Rad-1 and CP-1 samples are further down the second component than the rest of the clustered samples due to a higher aluminium, iron and copper concentration. The graph of element concentrations in blue cotton (Figure C- 4) shows that while the samples analysed on the Agilent had a high iron concentration (in the 10^3 ppb magnitude), the samples analysed on the Thermo ICP-MS had levels that were below the LOD. It is unclear whether this variation is due to instrumental differences or factors in the wash. The separation between the control and washed samples means that, again, it can be determined whether a sample has been washed or not. However the detergent brand is unable to be determined.

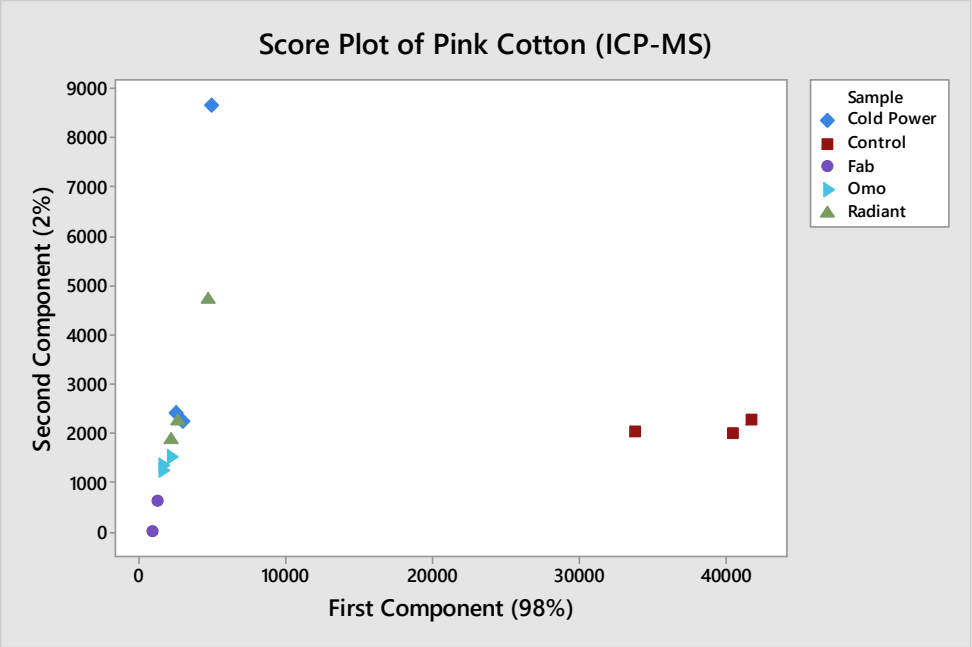


Figure 5-25: Score plot of Pink Cotton (ICP-MS)

Figure 5-25 depicts the covariance PCA plot for pink cotton control and washed samples. There is a clear clustering of control samples in the bottom right quartile, separate from a cluster of washed pink cotton samples in the bottom left quartile. As with the blue cotton in Figure 5-24, the pink cotton samples washed in Fab, Radiant and Cold Power which were analysed using the Agilent ICP-MS sit further up the second component from the cluster of samples analysed using the Thermo ICP-MS. The PCA loading plot (see Figure C- 5) for this score plot shows that the position of the pink cotton control samples on the first component are due to their high sodium concentration. The second component is influenced by predominantly aluminium concentrations, followed by iron and magnesium. The Agilent samples all have higher concentrations of these elements than the samples analysed on the Thermo, explaining why they are distributed higher up the second component than the other samples.

There is a slight clustering of the Thermo ICP-MS analysed samples based on their detergents, with the two Fab samples down the bottom, followed by the three Omo samples and then the Radiant samples which overlap with the Cold Power sample at the top. Due to the close proximity of these clusters, it is highly likely that any unknown samples analysed could be placed in the wrong cluster, ending in a false identification. In order to test this, clusters should be further defined by performing multiple replicates of

washed samples followed by in-depth testing with test sample to identify false and positive inclusion and exclusion ratios of samples into clusters.

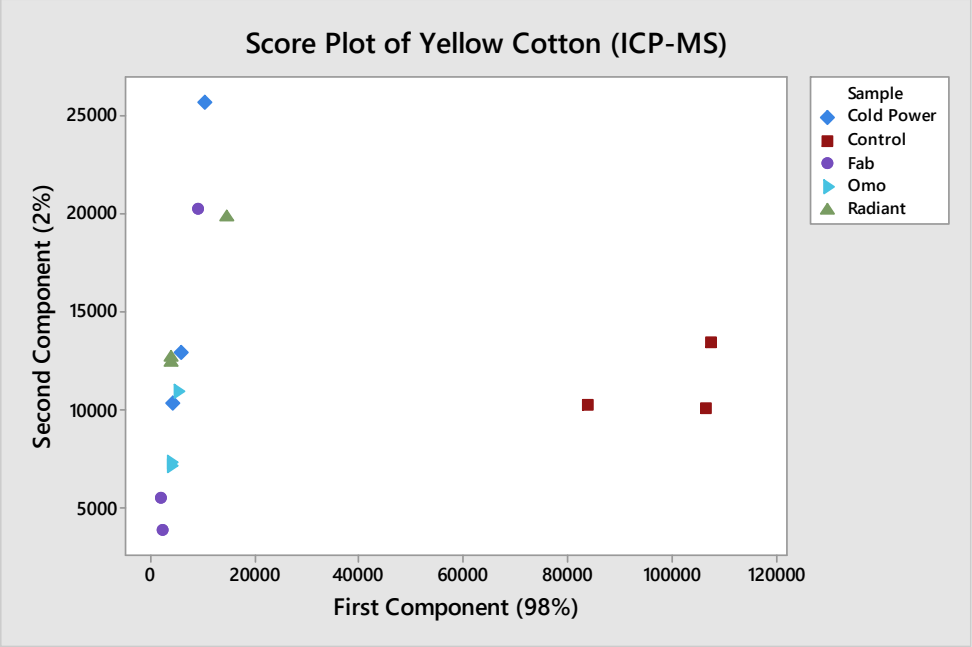


Figure 5-26: Score plot of Yellow Cotton (ICP-MS)

The yellow cotton covariance PCA score plot in Figure 5-26 shows very similar clustering of samples to both the blue and pink cotton in Figure 5-24 and Figure 5-25. The yellow cotton control samples are grouped to the right of the first component scale, while the washed samples are spread up the second component. The PCA loadings plot (Figure C-6) shows that the distribution of the control samples to the right of the first component is again due to their high sodium concentration, while the washed sampls, with their lower sodium concentration, are at the axis of the first component. The Agilent analysed samples are again clustered further up the second component than the other washed samples due to their higher concentrations of magnesium, aluminium, copper and iron. As with the pink cotton samples, the two other Fab samples (analysed on the Thermo ICP-MS) are again together at the bottom of the second component axis. However, unlike the pink samples, there is far more overlap of the other detergents and, as a result, no clusters based on the detergent brand can be formed.

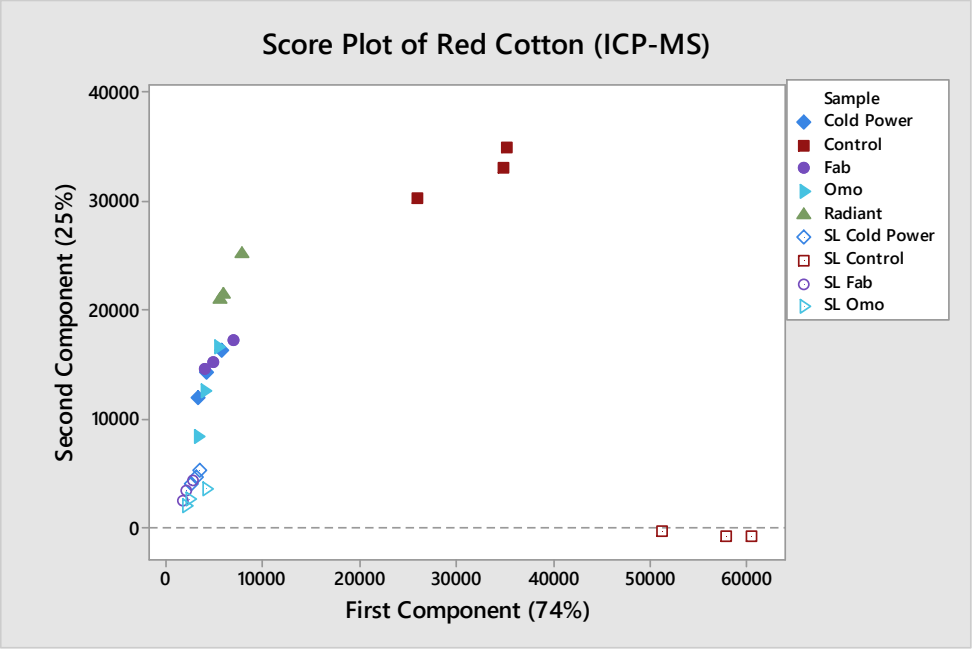


Figure 5-27: Score plot of Red Cotton (ICP-MS)

As with the white cotton samples in Figure 5-23, the Lincraft and Spotlight red cotton samples in Figure 5-27 again show a clear separation between washed and unwashed samples as well as the Lincraft and Spotlight samples. The Spotlight red cotton controls are clustered in the bottom right quartile while the washed Spotlight samples are in the bottom left quartile. Similarly, the Lincraft red cotton control samples are in the top right quartile while the washed samples are in the top left quartile of the plot. In this score plot, the main elements attributing to the first component are sodium (to the right) and aluminium (to the left) while the second component has magnesium (at the top). It was seen in Figure 5-20 that the sodium concentration in washed samples was found to decrease while aluminium increased. It is as result of this that the control samples are distributed to the right of the plot while the washed samples are further to the left. The Lincraft red cotton control samples had a higher concentration of magnesium than the Spotlight samples, hence why they are further up the second component. This difference in magnesium concentration between the two brands of cotton also affects the distribution of the washed sample, with the washed Lincraft samples again appearing at the top of the second component while the Spotlight samples are at the bottom.

In this plot, the Lincraft samples washed in radiant are clustered away from the other detergents at the top of the second component (due to having higher magnesium concentrations than any other samples) however no other detergent clusters have formed.

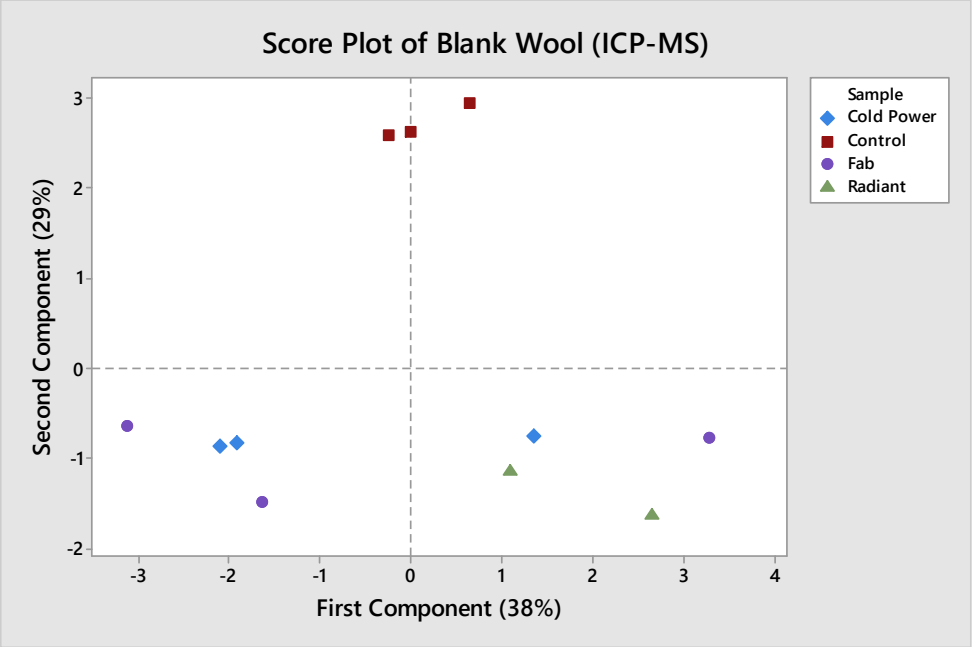


Figure 5-28: Score plot of Blank Wool (ICP-MS)

The correlation PCA score plot of blank wool samples is shown in Figure 5-28. The PCA plot shows a clear separation between the control samples in the top half of the plot and the washed samples in the bottom half. Two separate groups have been formed in the washed samples, however, these groups are only partly based on the detergent used. The group on the left is comprised of the blank wool samples that were washed in Fab and Cold Power in the second stage and analysed using the Thermo ICP-MS. The group on the right has the blank wool samples that were washed in Radiant and analysed on the Thermo ICP-MS along with the Fab and Cold Power samples that were washed in the first stage and analysed on the Agilent ICP-MS. There was no blank wool sample washed in Radiant that was analysed on the Agilent. These results show that variance within a sample group can lead to the overlapping of groups.

The PCA loading plot (Figure C- 8) reveals that, due to the correlation PCA analysis used, there are several elements that contribute to the placement of the scores on the first and second component. The control samples have higher concentrations of boron, manganese

and iron, causing them to sit further up the second component than the washed samples. Likewise, the washed samples have higher magnesium, copper and strontium concentrations (as seen in Figure 5-21), contributing to their lower placement in the second component.

The separation of the washed samples along the first component is predominantly due to the difference in the concentration of aluminium. The two radiant samples along with the Fab and Cold Power sample on the right-hand side of the plot are clustered as they have a larger concentration of aluminium than the other washed samples.

This PCA plot can be used to discriminate between washed and unwashed wool samples, however the detergent clusters are not defined enough to separate samples based on the detergent used.

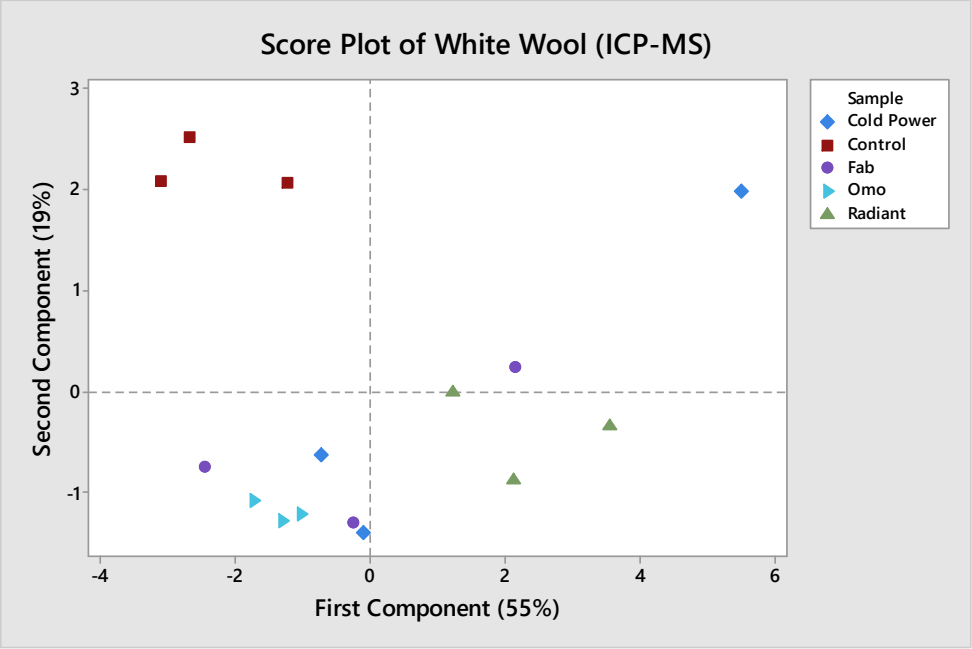


Figure 5-29: Score plot of White Wool (ICP-MS)

The correlation PCA score plot of white wool in Figure 5-29 shows a similar separation to the blank wool plot in Figure 5-28. Again, the control samples are clustered in the top left quartile while the washed samples are spread along the first component in the bottom half of the plot. As with the other textiles, the white wool washed in Fab and Cold Power and analysed on the Agilent ICP-MS are distributed further away from the rest of their

detergent samples. The sample washed in Radiant and analysed using the Agilent instrument (WW Rad-1) on the other hand is situated nearby the other Radiant samples, showing there is not as much variation between these samples as the other detergent washed samples. A tight cluster is made up of the white wool samples washed in Omo, however the other detergents are spread out, limiting the use of the PCA plot to identify the detergent used.

Based on the PCA loading plot (Figure C- 9) the position of the white wool control samples in the top half of the plot is predominately due to their concentration of boron compared the washed samples, whole the washed samples placement in the bottom half of the plot is based on their higher strontium and magnesium concentrations. The separation of the washed samples along the first component is due to the contribution of sodium, with samples that have a higher sodium concentration being further to the right of the plot.

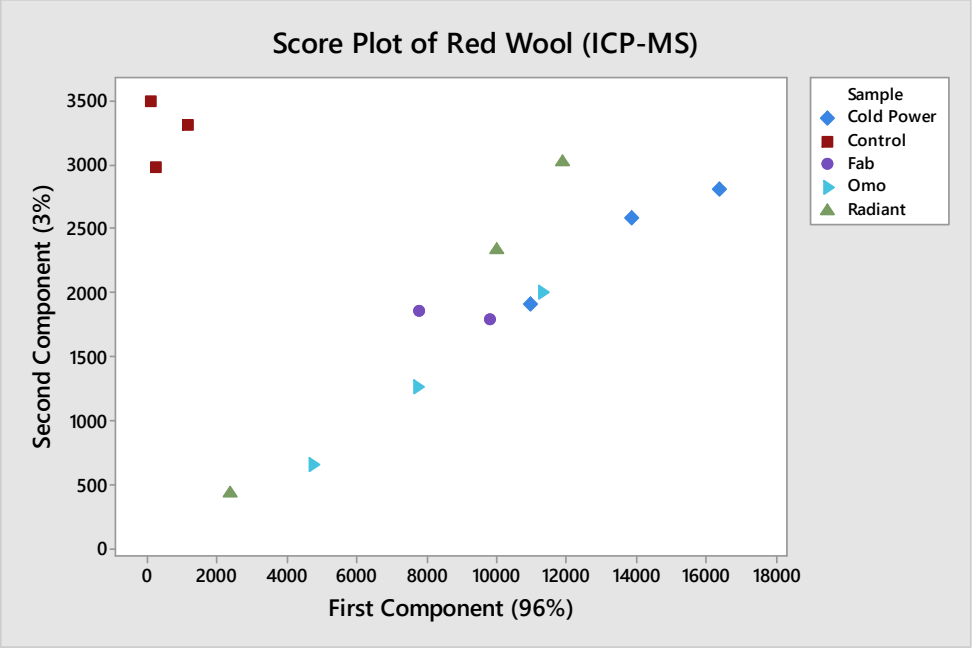


Figure 5-30: Score plot of Red Wool (ICP-MS)

Figure 5-30 shows the covariance PCA score plot of red wool. The red wool control samples are situated in the top left quartile while the washed samples are spread along both the first and second component. The detergent brands are spread along the washed sample group and so no detergent clusters are formed.

The PCA loading plot (Figure C- 10) shows that the second component has several elements contributing to the scores of the samples. Higher concentrations of sodium and zinc lead to the control samples' position at the top of the second component. The distribution of the washed samples along the first component are a result of their magnesium and copper concentrations. As the concentration of these elements was found to generally increase after washing, there is also a partial separation of washed and control samples along the first component.

As with the other samples analysed using ICP-MS, there is no clustering based on the detergent the samples were washed in. The clear and consistent difference in their elemental concentrations does allow for the separation of washed red wool samples from control red wool samples

5.4.3 Conclusion

Quantitative analysis of the concentration of elements in both washed and control samples using solution ICP-MS revealed that the elemental composition of a sample does consistently change during the washing process.

The standard deviations of each sample show that there is a degree of variation within a sample which can limit the ability to correctly determine if samples come from the same source. This variation has been linked partly to the lack of homogeneity in the digested sample solutions.

A large degree of variation has also been identified when the same sample was analysed on the Thermo iCap RQ ICP-MS on three different days. The significant variation indicates that the technique has limited repeatability, even when calibration standards are used.

Variation was also seen in the elemental concentrations of three samples prepared from the same source textile (both control and washed) and analysed at the same time. Statistical analysis of these samples showed that, in the case of the SL white cotton control sample, only 2 of the three samples were found to statistically correlate. This variation

indicates that there are limitations in the use of solution ICP-MS to compare fibres as samples from the same source can be found to be statistically different.

Comparison of the elemental composition of control and washed samples show that there was a consistent trend for each textile. Cotton samples predominantly showed a large decrease in sodium and magnesium and an increase in aluminium concentration after washing. Wool samples typically showed an increase in magnesium and copper concentrations which can be linked to the water used during the washing process. As elemental compositions of water supplies vary geographically (based on water supply and type of pipes), future work should involve comparison of these results with samples washed both in ultra-pure water and water in other locations to determine whether this trend continues.

Principal Component Analysis using the Minitab 10 software allowed easier visualisation of the different samples by taking multiple variables (the element concentrations) into account. Using this form of analysis, it was found that control samples of each textile can easily be distinguished from washed textiles, however the brand of detergent cannot be determined. There is a large avenue for future work looking at PCA. This includes performing the analysis on multiple replicates to determine whether more defined clusters can be formed or whether the samples become more dispersed. Samples that have been washed a different number of times can also be analysed to see whether separate groups are formed. The value of this form of analysis can also be tested by applying new samples to the PCA score plots and determining the ratio of false and positive inclusions and exclusions of these samples into the control or washed group. From this it can be determined whether the benefit of ICP-MS analysis along with multivariate analysis outweighs the cost and time that this sort of analysis requires.

Chapter 6:

Blind Tests

Chapter 6: Blind Tests

6.1 Introduction

In order to test the conclusions of the previous chapters, blind sample analysis was performed. For this purpose, eight pairs of blind samples of the same fibre, colour and dye were compared based on their fluorescence and elemental composition to determine whether they could be excluded as having come from the same source. As it cannot be determined how many times a sample has been washed, or which detergent was used, the same source in this context is defined as a textile with the same washing history. For this reason, these techniques can be used to exclude a fibre from a source but cannot be used to confirm they came from the same source. These experiments also aimed at testing whether the proposed SOP can indicate if samples had been washed or not.

These comparisons were performed using fluorescence microspectrophotometry and solution ICP-MS. While it is known how the fluorescence of the textiles used in this project appear before and after washing, this will be disregarded to simulate how this step would be used in forensic casework, where there are no positive and negative control samples for comparison.

6.2 Results and Discussion

6.2.1 Fluorescence Microspectrophotometry

Table 6-1: Conclusions from fluorescence microspectrophotometry analysis of blind samples

Textile	Blind Sample	Samples excluded from same source	Sample with more washes
Red Cotton	1A	Y	1B
	1B		
Blue Cotton	2A	N	n/a
	2B		

Red Cotton	3A	N	n/a
	3B		
Yellow Cotton	4A	Y	4B
	4B		
Red Wool	5A	N	n/a
	5B		
Red Wool	6A	Y	6A
	6B		
Blank Wool	7A	N	n/a
	7B		
Red Polyester	8A	N	n/a
	8B		

The first step in the blind sample analysis was to analyse each sample using the Craic microspectrophotometer with UV light to determine if fluorescence was detected from each blind sample which could be indicative of it having come in contact with optical brighteners. If the fibres have the same fluorescence, it will be unable to be determined whether it came into contact with the optical brighteners during the manufacturing process or during washing with laundry detergents. If the fluorescence is different, however, it could be determined that one sample has likely encountered additional optical brighteners in the washing process. If the samples have different fluorescence intensities or different fluorescence spectra, it will be concluded that they have likely come from different sources. The conclusions drawn from this analysis are shown in Table 6-1.

Table 6-2: MSP fluorescence images of red cotton blind samples 1A and 1B

	
1A	**1B**

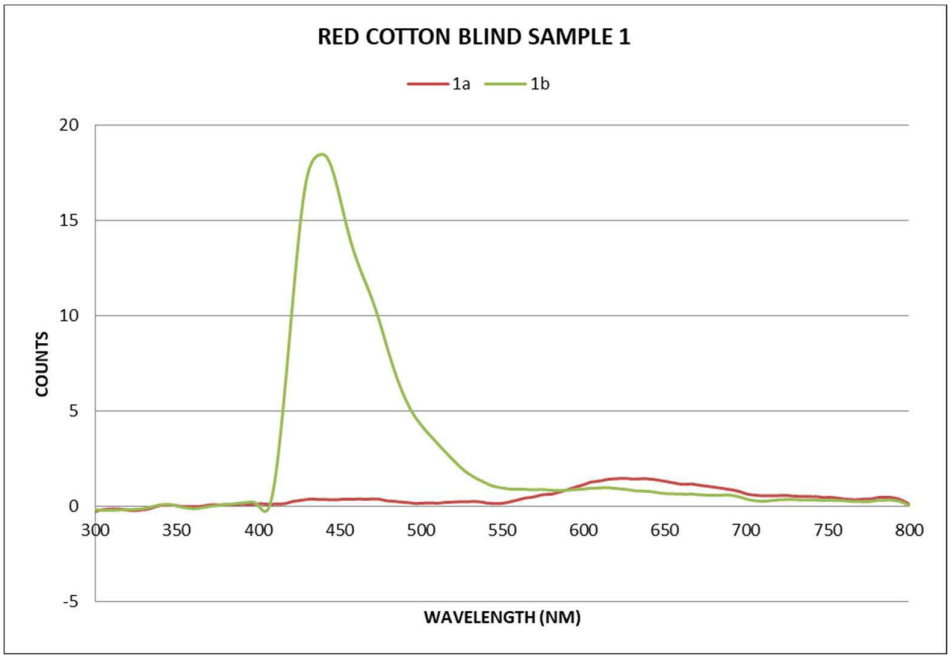


Figure 6-1: Fluorescence spectra of red cotton blind samples 1A and 1B

Analysis of the fluorescence spectra of the red cotton blind sample pair 1 in Figure 6-1 found no fluorescence was detected from sample 1A except for a small peak between 600 and 700 nm, which is due to the red colour of the cotton. 1B, on the other hand, exhibited a strong fluorescent peak in the blue wavelength region between 400 and 600 nm. From this comparison, it can be determined that 1 A has not come in contact with optical brighteners while 1B has. This is supported by the fluorescence images (Table 6-2) which showed only fluorescence from the 1b sample. Fluorescent analysis of red cotton fibres in this project found that fluorescence of washed red cotton was usually inconsistent along the fibre length and could not reliably distinguish between control and washed samples. However, analysis of all fibres that made up the blind samples (about ten fibres each) found that while fluorescence could not be found on any of the 1a fibres, fluorescence was present in multiple areas of all the 1b fibres. The difference between the two samples was significant enough that the two samples can be excluded as having come from the same source.

Table 6-3: MSP fluorescence images of blue cotton blind samples 2A and 2B

	
2A	**2B**

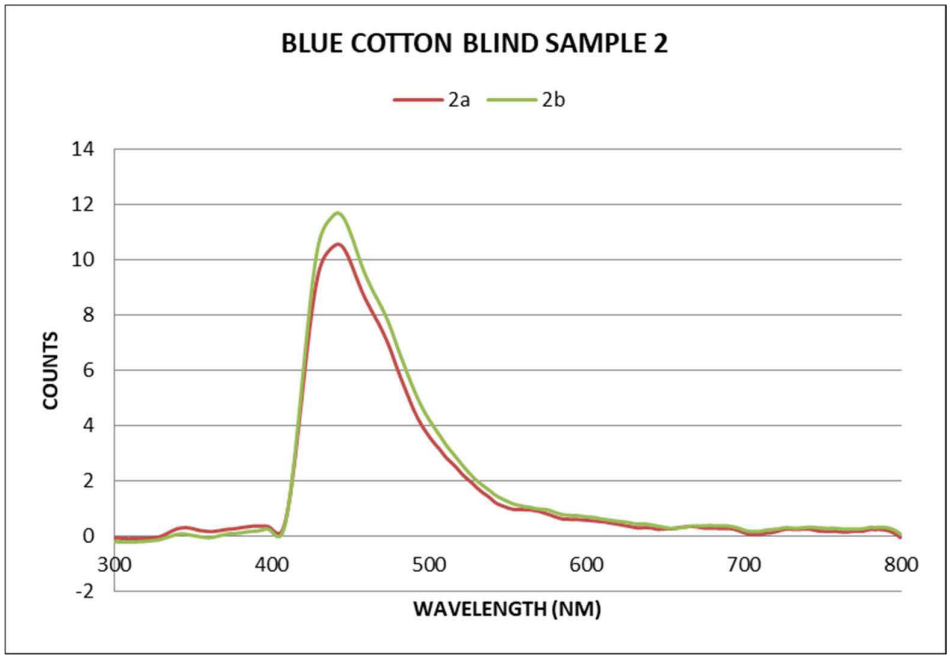
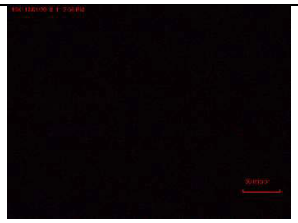
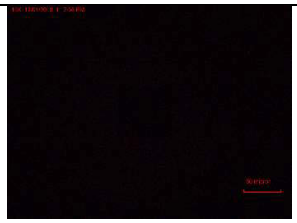


Figure 6-2: Fluorescence spectra of blue cotton blind samples 2A and 2B

Both blue cotton blind samples (blind sample 2A and 2B) in Figure 6-2 emit fluorescence between 600 and 700 nm with intensities ranging between 10 and 12 counts, while fluorescence images of both samples in Table 6-3 showed low-intensity blue fluorescence emitted by both samples. While previous analysis of blue cotton fibres indicates that this fluorescence is from optical brighteners applied during the manufacturing process and is not intense enough to be from washing, as positive and negative controls are not available in case work this would not be known. As the fluorescence of the samples is the same, the fibres cannot be excluded as having come from the same source. Additionally, it cannot be determined whether the fluorescence observed is from contact with optical brighteners in the manufacturing process, or optical brighteners in the washing process and so it is not known if the source sample had been washed or not.

Table 6-4: MSP fluorescence images of red cotton blind samples 3A and 3B

	
3A	**3B**

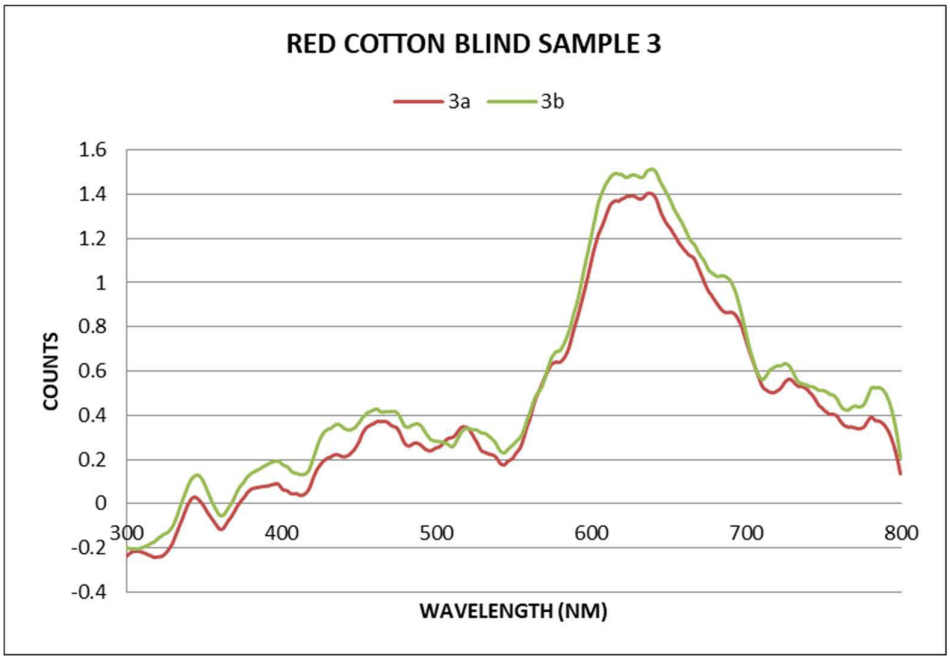


Figure 6-3: Fluorescence spectra of red cotton blind samples 3A and 3B

Analysis of the second red cotton blind sample pair (3A and 3B) revealed no fluorescence was detected from either sample except for the small peak between 600 and 700 nm which is known to be due to the red colour of the sample (Figure 6-3). Fluorescence images of the samples in Table 6-4 also revealed no visual fluorescence could be seen from either sample. As a result, it can be concluded that neither sample has come in contact with optical brighteners and therefore they cannot be distinguished based on their fluorescence.

Table 6-5: MSP fluorescence images of yellow cotton blind samples 4A and 4B

	
4A	**4B**

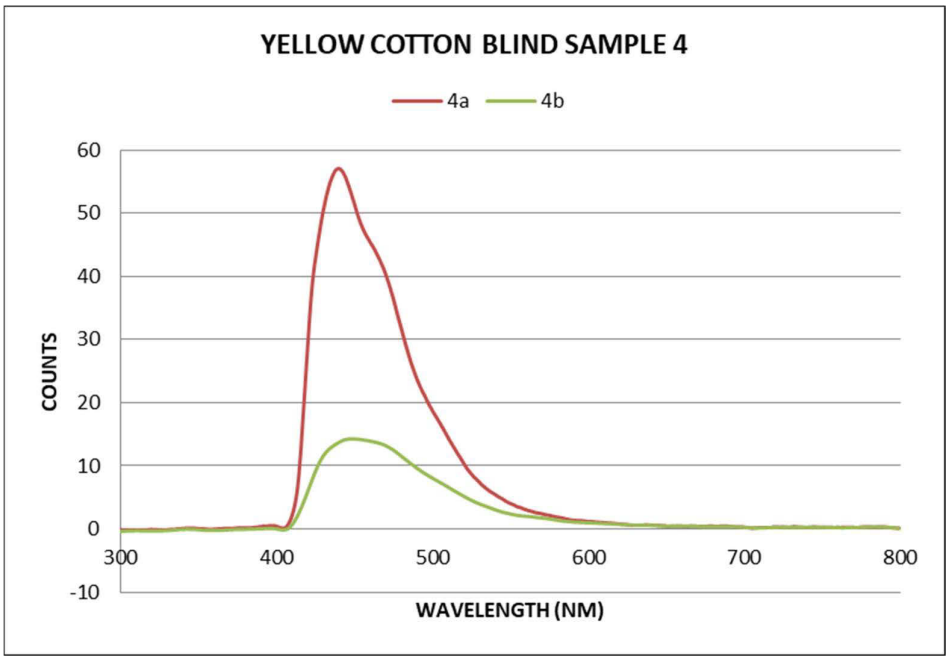
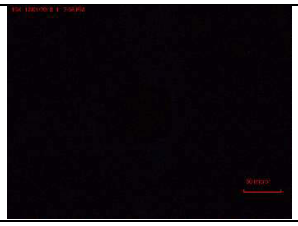


Figure 6-4: Fluorescence spectra of yellow cotton blind samples 4A and 4B

Fluorescence images of the yellow cotton blind samples (4A and 4B) in Table 6-5 showed that blue fluorescence was emitted by both samples but was much more intense in the 4A sample. This was reflected in the fluorescence spectra in Figure 6-4 where, while both samples had a fluorescence peak between 400 and 600 nm, the shape and intensity of the fluorescence peaks were significantly different. As a result, the yellow cotton blind samples can be excluded as being from the same source. Additionally, it can be determined that the 4A sample has been washed more than the 4B sample, due to the higher intensity of its fluorescence.

Table 6-6: MSP fluorescence images of red wool blind samples 5A and 5B

	
5A	**5B**

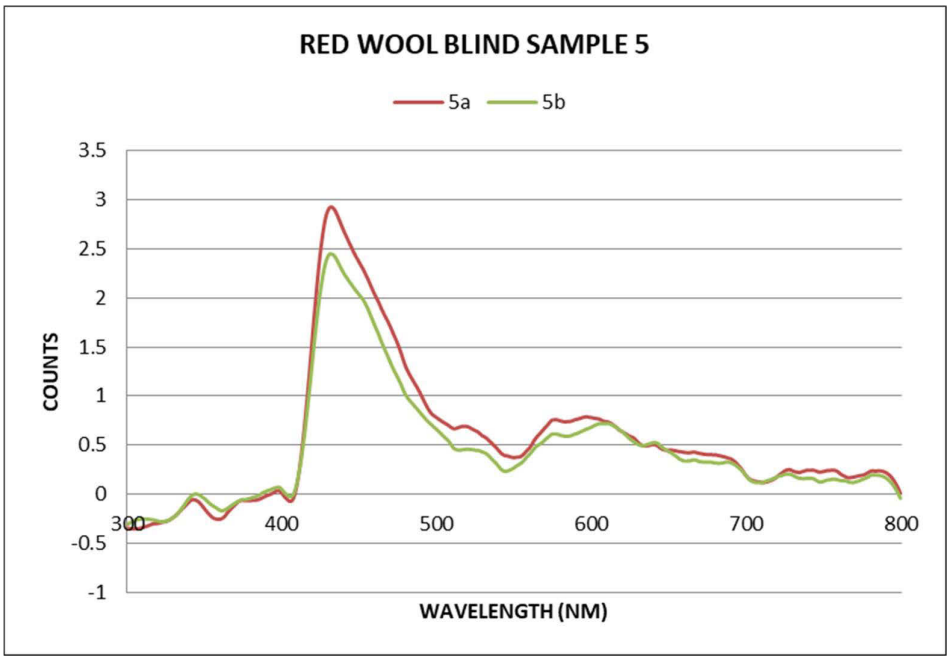
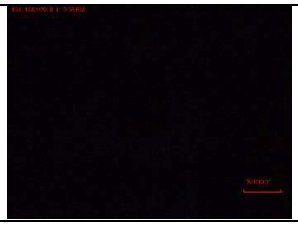
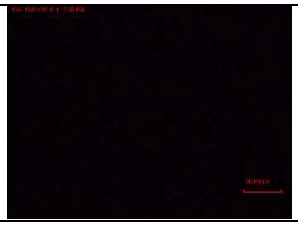


Figure 6-5: Fluorescence spectra of red wool blind samples 5A and 5B

Fluorescence images of the red wool blind samples 5A and 5B in Table 6-6 showed no fluorescence on any of the sample fibres. The fluorescence spectra (Figure 6-5), however, showed a sharp fluorescence peak between 400 and 550 nm for both samples, indicating that both red wool samples had come in contact with optical brighteners. As a result, it can be determined that both 5A and 5B have been washed and cannot be excluded as having come from the same source.

Table 6-7: MSP fluorescence images of red wool blind samples 6A and 6B

	
6A	**6B**

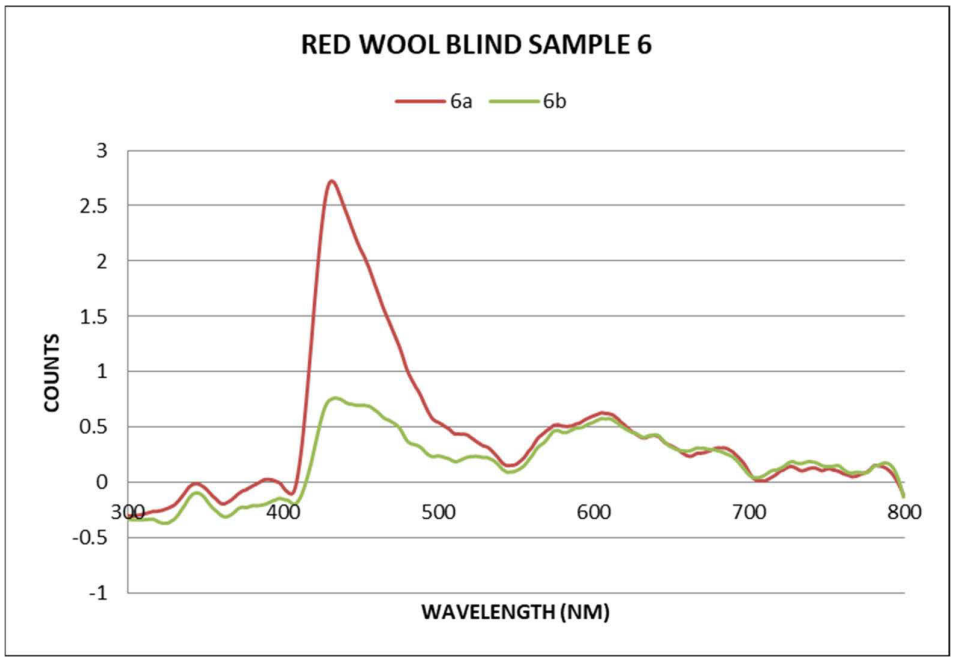


Figure 6-6: Fluorescence spectra of red wool blind samples 6A and 6B

The second pair of red wool blind samples (6A and 6B) showed similar fluorescence images to the 5A and 5B pair, with no fluorescence seen from either sample (Table 6-7). The fluorescence spectra of both samples in Figure 6-6 are significantly different, however, allowing for the fibres to be distinguished. The 6A sample showed a sharp fluorescence peak between 400 and 550 nm, as was seen in the 5A and 5B samples. The 6B sample also has a peak in this range however it is shallower and noisier. From these results, it can be concluded that the two samples are from different sources and that 6A sample has been washed while the 6B sample has not.

Table 6-8: MSP fluorescence images of blank wool blind samples 7A and 7B

	
7A	**7B**

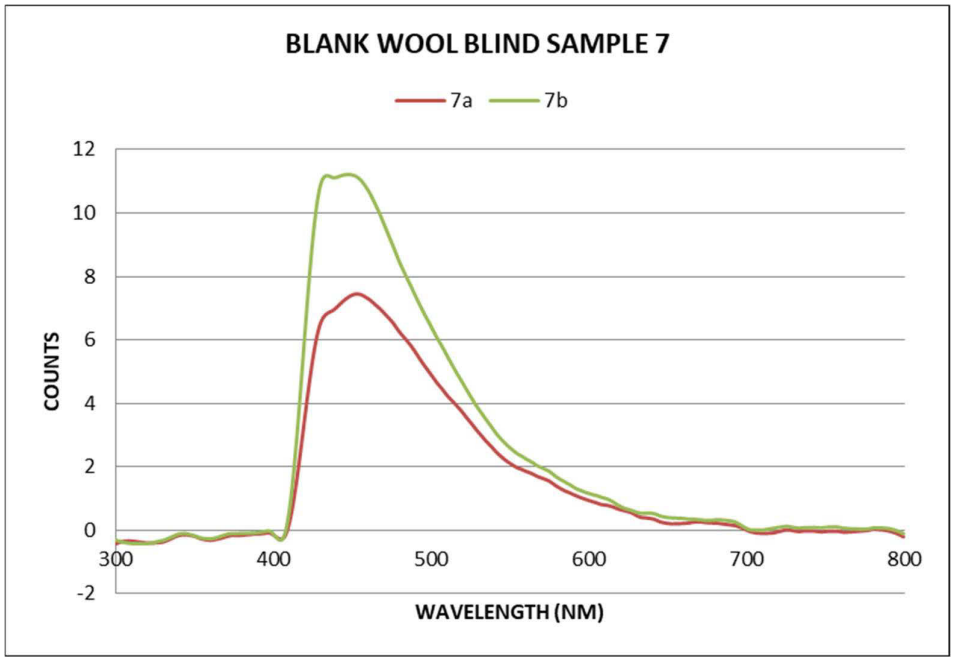
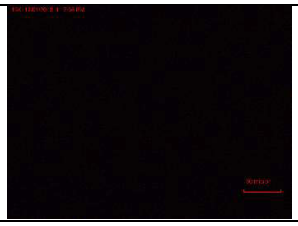
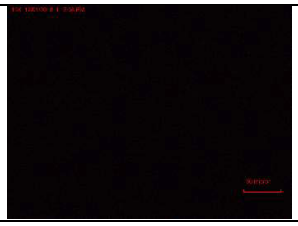


Figure 6-7: Fluorescence spectra of blank wool blind samples 7A and 7B

Low-intensity fluorescence was seen in the fluorescence images of both blank wool blind samples (7A and 7B) in Table 6-8. The fluorescence spectra of these samples both have peaks between 400 and 700 nm and so the samples are unable to be distinguished by fluorescence microspectrophotometry (Figure 6-7). Blank wool samples are known to have a natural fluorescence (as seen in Figure 3-27) and therefore the fluorescence of the textiles cannot be used to indicate whether the samples have been washed or not.

Table 6-9: MSP fluorescence images of red polyester blind samples 8A and 8B

	
8A	**8B**

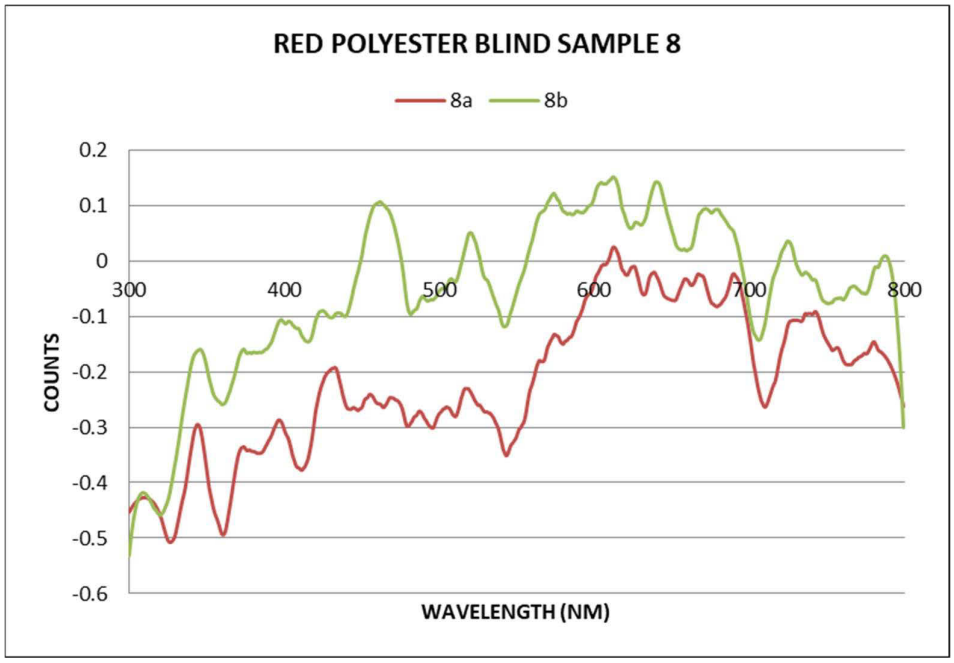


Figure 6-8: Fluorescence spectra of red polyester blind samples 8A and 8B

The last pair of blind samples (8A and 8B) were red polyester samples. No fluorescence could be detected either visually or spectrally (Figure 6-8 and Table 6-9). Due to this, the blind samples cannot be excluded as coming from the same source.

Using fluorescence microspectrophotometry, three of the eight pairs of blind samples can be excluded as coming from the same source (pairs 1, 4 and 6).

The next stage of the analysis was to compare the elemental composition of the samples that cannot be distinguished using solution ICP-MS. The red polyester blind sample was not examined using this method as it was found in this project that red polyester was unable to be dissolved using the acid digestion method implemented in the project.

6.2.2 Solution ICP-MS

Table 6-10: Conclusions from solution ICP-MS analysis of blind samples

Blind Sample Pair	Samples excluded from same source (Y/N)	Sample with more washes
2	Inconclusive	n/a
3	Y	3B
5	N	n/a
7	Y	7B

In Chapter 5 it was found that the elemental concentration of textiles significantly changed after washing and that the increase or decrease in elements was consistent for each textile type. It was concluded that there was a potential to use this technique to compare fibres by determining if there was a significant change in concentration which correlated with the trends seen in Chapter 5.4. High levels of variation between fibres from the same source indicated that statistical comparison of the samples using t-test: Paired two sample for means could not be used as a test to determine if the fibres came from the same source. While statistical correlation between samples is a strong indicator that the fibres are likely from the same source, lack of correlation cannot be used as an indicator that they are from different sources as, due to sample variation, samples from the same source can also be found to be statistically different. Solution ICP-MS is used to analyse these blind samples to test whether it can be used to accurately compare unknown fibres.

The elemental concentrations of each blind sample pair (consisting of about 0.01 g of fibres per sample) was compared to determine whether there was a significant difference in the elemental concentrations and whether the difference in the concentrations followed the trends observed in Chapter 5.4. If this difference is seen, it can be concluded whether the fibres can be included or excluded from the same source. Additionally, it can also be determined which sample has been washed more than the other. Without a control sample for comparison, it is unable to be determined if a fibre has never been washed before. The sample size analysed was significantly smaller than the sample sizes used in the project

(0.01 g compared to 0.2 g) in order to more accurately reflect the sample sizes encountered in case work. As a result, the samples analysed are less representative of the source material as a whole. The conclusions made from the solution ICP-MS analysis of the blind samples are displayed in Table 6-10.

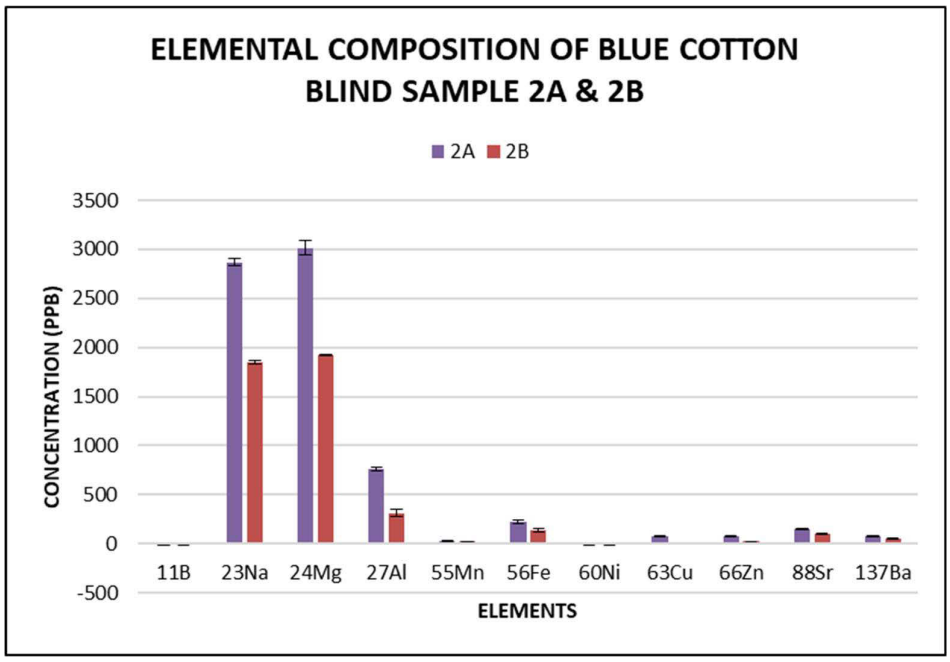


Figure 6-9: Elemental composition of blue cotton blind samples 2A and 2B

Comparison of the blue cotton control blind samples 2A and 2B in Figure 6-9 showed that the 2B sample had consistently smaller concentrations of every element and, as a result, could not be found to correlate. It is uncertain, however, whether the difference in the concentrations can be linked to one of the source materials being washed. Analysis of the elemental trends for blue cotton in Figure 5-20 found that, after washing, magnesium and sodium concentrations decreased substantially, while the aluminium concentration was found to increase slightly. The 2A and 2B blind samples do not show that trend, with the aluminium concentration decreasing in the 2B sample by the same amount as the sodium and magnesium concentrations. The difference in the concentration of the samples could also potentially be due to natural variation in the samples, or human or instrumental error. As a result, solution ICP-MS is unable to to conclusively include or exclude the fibres as coming from the same source. Due to this, the fluorescence microspectrophotometry results alone will be used to conclude that the fibres likely came from the same source.

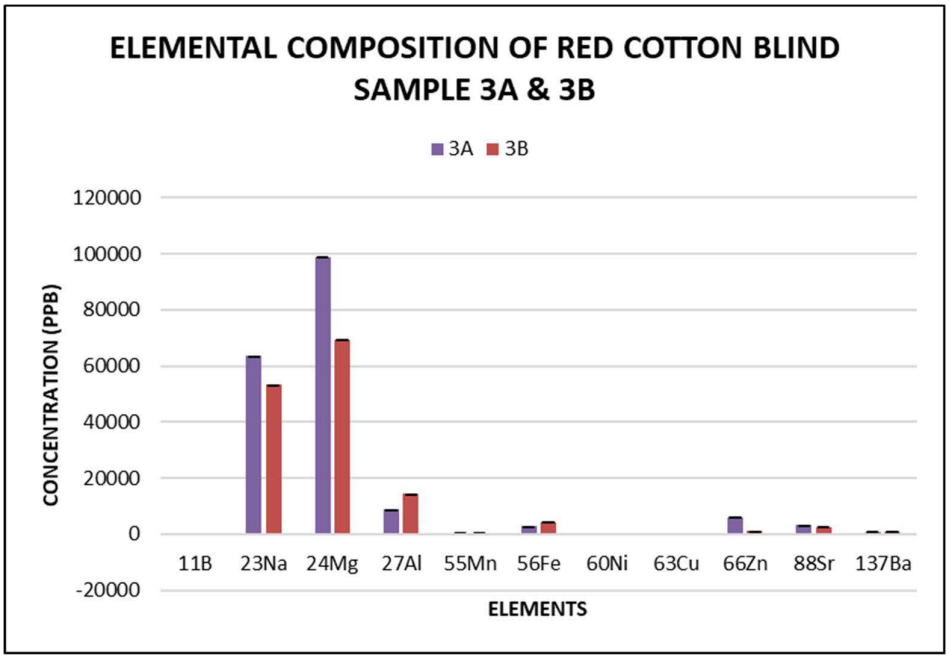


Figure 6-10: Elemental composition of red cotton blind samples 3A and 3B

The red cotton blind samples, 3A and 3B, also had different element concentrations (Figure 6-10), however, unlike the blue cotton blind samples, the difference in concentration was not consistent. While the difference in concentration was minor, the blind sample 3B was found to have lower sodium and magnesium concentrations than the 3A sample, and a higher aluminium concentration, consistent with the trends seen in washed red cotton in Figure 5-20. As a result, it can be concluded that the fibres are likely from different sources and that the 3B sample has been washed more than the 3A sample.

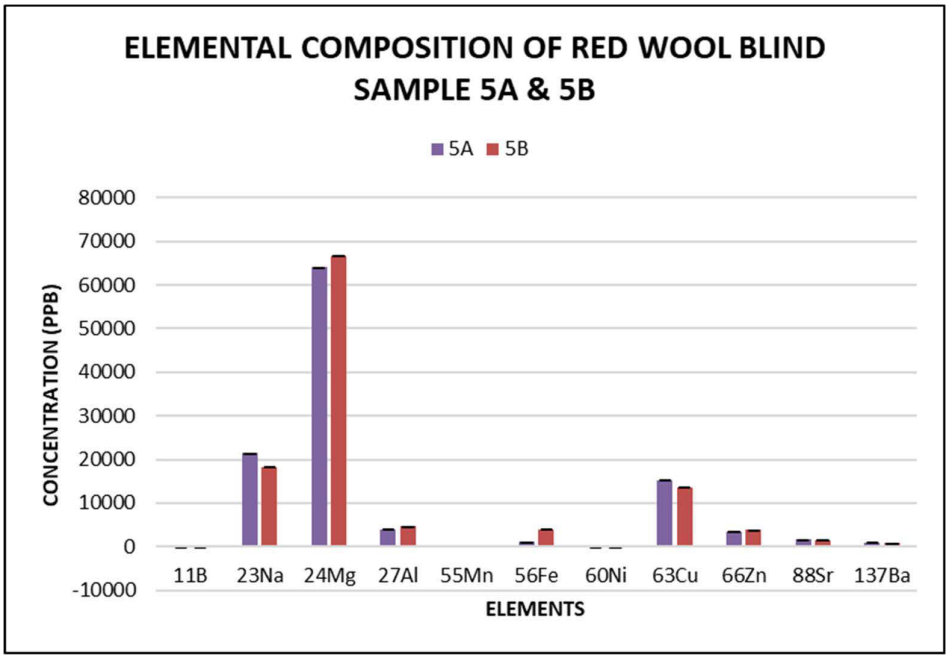


Figure 6-11: Elemental composition of red wool blind samples 5A and 5B

Table 6-11: p- values for t-Test: Paired Two Sample for Means at α=0.05 for red wool blind samples 5A and 5B

Element	t-test (p-value)
^{11}B	1.33×10^{-4}
^{23}Na	8.09×10^{-3}
^{24}Mg	0.27
^{27}Al	0.14
^{55}Mn	0.31
^{56}Fe	0.03
^{60}Ni	0.03
^{63}Cu	9.01×10^{-3}
^{66}Zn	0.05
^{88}Sr	3.24×10^{-3}
^{137}Ba	1.97×10^{-3}

The two red wool blind cotton samples were found to be very similar for all elements (Figure 6-11) and were found to statistically correlate using the t-test: paired two sample for means for three elements (magnesium, aluminium and manganese) in Table 6-11. The fluorescence microspectrophotometry analysis of the samples revealed that both fibres had come into contact with optical brighteners and so had likely been washed. The statistically similar elemental concentrations of the two fibres is, therefore, a strong indicator that the fibres came from the same washed material as more elemental concentration variation would be expected from fibres that came from different washed red wool textiles.

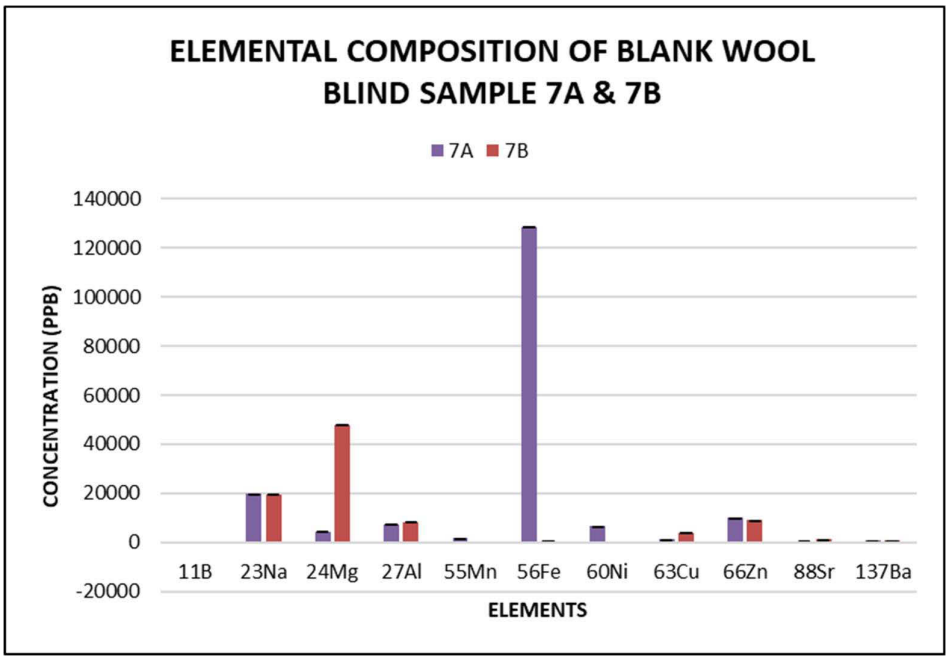


Figure 6-12: Elemental composition of blank wool blind samples 7A and 7B

A clear difference could be seen between the two blank wool samples, 7A and 7B, in Figure 6-12. The 7B fibres had a significantly higher magnesium concentration than the 7A fibres, and also had a significantly smaller iron concentration. The change in these concentrations were both trends that were seen for washed blank wool samples in Figure 5-21. From these results, it is indicated that the 7A blank wool fibres came from an unwashed textile, while the 7B blank wool fibres came from a blank wool sample that has been washed.

Table 6-12: Comparison of overall conclusion with correct identification of blind samples

Blind Experiment	Exclusion	Sample with more washes	Ground Truth
1	Y	1B	1A: Lincraft red cotton unwashed 1B: Lincraft red cotton washed 16 X
2	N	n/a	2A: Blue cotton unwashed 2B: Blue cotton unwashed
3	Y	3B	3A: Lincraft Red cotton unwashed 3B: Lincraft Red cotton unwashed
4	Y	4A	4A: Yellow cotton washed 4x 4B: Yellow cotton unwashed
5	N	n/a	5A: Red wool washed 16x 5B: red wool washed 16x
6	Y	6A	6A: Red wool washed 16x 6B: Red wool unwashed
7	Y	7B	7A: Blank wool unwashed 7B: Blank wool washed 16x
8	inconclusive	n/a	8A: Red polyester washed 16x 8B: Red polyester unwashed

After the blind samples were compared, analysed and an assessment was made about whether the fibres could have come from the same source, the results were then compared to the actual identities of the blind samples in Table 6-12 to determine if the assessments were correct. Experiments shaded in green were correctly identified as being excluded from the same source, or unable to have been excluded and the correct samples with more washed was identified. Experiments shaded in red were incorrectly identified and the experiment shaded in orange was inconclusive.

The red polyester blind sample 8 was found to be inconclusive as there was no fluorescence from either sample, and it was unable to be analysed by solution ICP-MS. Of the remaining seven sample pairs, six were correctly identified.

It was found that all conclusions made were correct, except for that of the red polyester blind sample (8A and 8B) and the red cotton blind sample pair (3A and 3B) which were incorrectly excluded from the same source.

Assessing the process used to examine the blind samples, it can be seen that fluorescence microspectrophotometry was the most accurate method for comparing the fibres. Of the five blind sample pairs that were found to have the same fluorescence spectra (2, 3, 5, 7 and 8), three of these were confirmed to be from the same source. The blank wool and red polyester samples were unable to be distinguished as the fluorescence of the samples is not affected by washing.

The solution ICP-MS analysis of the blind samples was found to be problematic due to variation in the samples themselves. Both samples in blind sample pairs 2 and 3 were found to have different concentrations that were found to not statistically correlate. The analysis of the blue cotton pair was determined to be inconclusive and so the fluorescence microspectrophotometry results were used to correctly conclude the fibres were from the same source. As the red cotton samples exhibited a difference in concentration which was consistent with a sample that had been washed, it was incorrectly concluded that the samples were from different sources. The variation in the element concentration was likely due to natural variation in the sample, rather than variation due to being washed.

Where solution ICP-MS analysis was found to be helpful was in the analysis of the blank wool sample pair (7A and 7B). It is known from the fluorescence analysis of blank wool in this project that the fluorescence of blank wool is consistent both before and after washing and therefore cannot be used to distinguish blank wool fibres. The significant difference in the elemental concentration seen when comparing the blank wool blind samples in Figure 6-12, as well as the ways in which the element concentrations were different, allowed for the samples to be correctly excluded from the same source and for the 7B blind sample to be identified as having been washed more than the 7A sample.

6.3 Conclusions

The use of fluorescence microspectrophotometry and solution ICP-MS proved to be an effective tool to distinguish washed and unwashed samples which originally came from the same source material and thus could not be excluded based on fibre type, dye or colour.

Fluorescence microspectrophotometry proved to be a quick, non-destructive that was able to accurately, easily and reliably distinguish fibres from different sources. The results from the blind test show that it is a valuable process that should be incorporated into the SOPs of forensic trace laboratories after the comparison of fibres based on their fibre type and colour.

The solution ICP-MS analysis did prove that there are limitations in comparing fibres based on their elemental concentration, due to variation in concentration of samples from the same source. The technique cannot be used to perform a simple statistical analysis of the concentrations of two samples to determine if they came from the same source. Only significant changes in concentration that reflect the trends observed in elemental concentration after washing should be used as an indicator that the fibres came from different sources. Therefore, while solution ICP-MS can be used to distinguish between fibres based on their washing history, due to the subjective nature of the analysis and the statistically high degree of variation within samples from the same source, it cannot be used to accurately exclude fibres and therefore currently has limited application in forensic casework.

The application of ICP-MS in fibre comparison can be further tested by using the technique to compare a large number of blind samples and to determine the success rate the technique has both on its own, and when interpretation is paired with fluorescence examination of the samples.

Chapter 7: General Discussions and Conclusions

Chapter 7: General Discussions and Conclusions

7.1 General Discussion and Conclusion

The work presented in this thesis aimed to investigate the forensic question of whether the analysis of laundry detergents on textiles can be used as an acquired characteristic, to aid in the comparison of fibres and improve their forensic value. Fibre evidence is a valuable tool that allows investigators to recreate events behind crime scenes and make connections between people, objects and locations. However, textiles are mass produced items, and hence, there is a 'natural' limit to what a standard analysis focusing on the manufacturing characteristics can achieve.

This project was conducted by washing textiles in several commercially available laundry detergents which were then analysed to collect three types of data: fluorescence, chemical composition and elemental composition.

Fluorescence examination of washed and control textiles concluded that, depending on the textile type and colour, the fluorescence of the fibres significantly changed after washing. This allowed for fibres that had not been washed to be distinguished from those that had. There was, however, no difference in the fluorescence of samples washed in different brands in the detergents, nor was there any increase in the fluorescence of the samples after four wash cycles. As a result, the potential for added discrimination based on such analyses is limited.

Raman spectroscopy was able to produce unique Raman spectra for each detergent based on their Raman spectra. Unfortunately, the technique was unable to detect evidence of these detergents on washed textiles, with the exception of a small peak on the washed blank cotton samples that could be attributed to optical brighteners. As a result, Raman spectroscopy was unable to be used to compare the chemical composition of textiles before and after washing.

The elemental analysis of the samples was investigated using three different techniques, SEM-EDS, LA-ICP-MS and solution ICP-MS. Solution ICP-MS was determined to be the only valid technique that could be used to analyse the samples.

Solution ICP-MS proved to be the most valuable technique though had the disadvantage of being destructive. Elemental concentrations were able to be collected and, through the comparison of control and washed textiles, the effect of the washing cycle on the elemental composition of textiles was able to be determined. Through this study, it was found that the majority of the changes in the elemental concentrations could be attributed to the loss of elements from the control textile or the gain of elements from the water source. There was little evidence that proved elements from the detergents themselves were being detected on the textiles. This, however, could enable samples to be compared based on the geographical location they were washed in, which is a topic for future research. One limitation in the use of solution ICP-MS is the large degree of variance that is present in samples from the same source.

Principal component analysis (PCA) proved to be a useful tool for both the fluorescence and elemental analysis of the samples. This multivariate statistical procedure allowed for the reduction of a large number of variables (such as wavelengths or elements) to determine which variables accounted for the most variation between the samples. The value of this tool is that it removes much of the subjectivity that comes from visually and qualitatively comparing data, allowing for differences in samples to be easily observed. The limitation with the use of this statistical analysis in this project is that, especially in the case of fluorescence analysis, the sample size the analysis was applied to was not big enough to produce significant results.

Fluorescence microspectrophotometry and solution ICP-MS was tested by analysing eight pairs of blind samples of known fibre type and colour. Fluorescence microspectrophotometry was able to correctly link or exclude six of the eight samples. Solution ICP-MS was then used to analyse sample pairs that gave the same fluorescence response. It was found that while it was able to correctly distinguish the blank wool sample pair due to a significant variation in element concentration which reflected the behaviour of wool samples after washing, it also had many limitations. A large variation

in concentration was also found between samples fibres that were from the same source and so, using this technique alone, the fibres would have been incorrectly excluded.

The two techniques that produced the most valuable information were fluorescence microspectrophotometry and solution ICP-MS. It is recommended that fluorescence microspectrophotometry be conducted alongside visual microspectrophotometry of fibres (which is routinely used in fibre examination for colour analysis and comparison). If fibres are unable to be distinguished based on their fibre type, colour, composition and fluorescence characteristics, it is then recommended that solution ICP-MS be performed to determine if there is a significant difference in the samples elemental composition which might be indicative of a difference in washing history.

This project revealed that using fluorescence and elemental composition, it is possible to distinguish a fibre that has never been washed in laundry detergents from one that has. However, the brand of detergent used, or the number of washing cycles the sample has been through cannot be identified. As a result, the use of laundry detergents as an acquired characteristic can only be used as an exclusionary technique, rather than to confirm as fibres as coming from the same source, or to identify the exact washing conditions of the source textile (i.e. laundry detergent used).

This data allows many types of forensic questions to be addressed. The first and most significant question is the determination of the source of a textile. The techniques used to add another dimension to the comparison of evidence fibres to a suspect fibre or garment. Currently, this comparison is based on the type and colour of the fibre and the dye used. In the case of fibres that are mass produced, discrimination of fibres based on these characteristics is limited. If, however, one of the fibres being compared has never been washed and the other fibre has, then discrimination of the fibres can occur using fluorescence and elemental analysis.

This information can also be applied to investigations at the activity level. One application could be determining if a suspect garment has been washed. For example, if it is known that an evidential fibre came from an unwashed suspect garment through means other than fibre examination, but fluorescence and elemental analysis reveal the suspect

garment has been washed, this information can support the proposition that the suspect washed the garment, possibly to cover up evidence of the crime.

This project also brings to light several areas of potential future research. This includes expanding on the research that was involved in this study, as well as exploring further the topic of acquired characteristics on fibres.

This study focused on the effect of detergents on 100 % textiles such as cotton and wool where it was found that the textile type had a large effect on factors such as fluorescence and changes in elemental composition after washing. Future work should investigate the effect of washing on mixed blend fabrics to determine whether different trends can be observed.

One of the main limitations of this research was that samples were washed in a domestic washing machine in loads comprising of a variety of clothing. While this served to recreate the washing cycle that casework samples would also go through, it also means that the impact of these clothing on the results is not known. Changes in the elemental concentration of the washed samples in chapter 5 could be due to contamination from these other clothing items. There is, therefore, an area for future work to look at washing samples in a more controlled environment to determine whether similar trends could be detected.

The fluorescence analysis was conducted on textiles that were kept in dark storage, where it was found that after three years there was no noticeable change in fluorescence. However, as optical brighteners are UV sensitive and do degrade, further investigation is needed into how this fluorescence degrades in various conditions to determine whether it is worthwhile to perform fluorescence analysis if a period of time has passed between the collection of the evidential fibres and the suspect material.

Raman spectroscopy was able to differentiate the four detergents analysed in their pure form, with characteristic peaks identified that would enable their identification. From these results, it can be seen that discrimination of pure detergent is possible. The problem lies in detecting the residues of these detergents on the washed textiles themselves. In all of the samples analysed, the only peaks found that linked to the presence of the detergents

was that of the optical brightener at 1600 cm^{-1}. This identified the presence of detergent but did not discriminate between detergents. The lack of other identifiable peaks may mean that no other detergent residues were present on the textile, or it may be that the Raman was not sensitive enough to pick up these residues.

An exploration into the optimisation of Raman spectroscopy for acquired characteristics on textile fibres, exploring different wavelengths and Raman analysis techniques, has the potential to yield further information than could be discovered in this study. Several studies [109, 127, 128] have shown that the amount of fluorescence produced by a fibre under a particular wavelength is dependent on the colour of the fibre. As a result, while general trends were discovered (i.e. that the 633 nm wavelength generally produced poor spectra), there is not one wavelength that produces the best results across all fibres. It was also found that different peak detail can be observed under different wavelengths. Future studies examining each sample under multiple wavelengths would produce the best results. Applying a fluorescence suppression technique such as Surface Enhanced Raman Spectroscopy (SERS) has been shown to increase the Raman spectrum of samples and therefore decrease the effect of fluorescence [128]. This technique could be applied to washed textiles to attempt to increase the signal of low concentration analytes such as any possible detergent residues.

LA-ICP-MS is a valuable tool as it is mostly non-destructive; however, it was found to yield limited information in this study due to the lack of quantitative data. An investigation into the use or development of suitable internal or external standards would greatly increase the value of this technique.

Solution ICP-MS analysis of washed and control samples revealed that the elemental composition of the water used to washed samples most likely had a larger effect on the textiles than the detergent itself. Comparison of textiles washed in pure water as well as water from other geographical locations would provide more information about the extent of the impact the water has, as well as whether samples can be separated based on where they were washed.

There are also many areas of future work that could involve the use of Principal Component Analysis. This includes performing the analysis on ICP-MS data from

multiple replicates to determine whether more defined clusters can be formed or whether the samples become more dispersed. The value of this form of analysis can also be tested by applying new samples to the PCA score plots and determining the ratio of false and positive inclusions and exclusions of these samples into the control or washed group. From this, it can be determined whether the benefit of ICP-MS analysis along with multivariate analysis outweighs the cost and time that this sort of analysis requires. It can also be applied to solution ICP-MS data of samples washed a varying number of times to investigate whether clusters form based on how many times a sample has been washed. PCA could also be applied to other forms of data such as fluorescence and Raman spectra.

The main question behind this project was to determine whether acquired characteristics on fibres can be used to aid in their comparison and increase their evidential value. This research projects focused on the use of laundry detergents as acquired characteristics. However, textiles can come into contact with many things throughout their lifetime that can also be investigated as an acquired characteristic. These can include cigarette smoke, soil, paint, cosmetics, toiletries and so on. The use of acquired characteristics for fibre comparison is a relatively new area of research, and there are many potential opportunities as highlighted by David Stoney in his work on very small particles attached to fibres [89].

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Appendices

Appendices

Appendix A

Safety Data Sheet – Cold Power Laundry Detergent Powders

1. Identification

GHS Product Identifier	Cold Power Laundry Detergent Powders
Company Name	Henkel Australia (ABN 82 001 302 996)
Address	135 to 141 Canterbury Road, Kilsyth. Victoria 3152
Telephone	Australia: 03 9724 6444 New Zealand: 09 272 6710
Fax	Australia: 03 9728 7828 New Zealand: 09 272 6711
Emergency Phone	Australia: 1800 638 556, New Zealand: 0800 764 766
Recommended use of the chemical and restrictions on use	Powder laundry detergent
Other Names	Cold Power Regular Laundry Powder Cold Power Sensitive Laundry Powder Cold Power with a Touch of Cuddly Laundry Powder
Other Information	New Zealand Address: 2 Allens Road, East Tamaki

2. Hazard Identification

Classification of the product	Classified as Hazardous according to the Globally Harmonised System of Classification and labelling of chemicals (GHS) including Work, Health and Safety regulations, Australia. Not classified as Dangerous Goods according the Australian Code for the Transport of Dangerous Goods by Road and Rail (7th Edition)
GHS Classification	Eye damage/Irritation – Category 2A
Signal Word	WARNING
Hazard Statement	H319 Causes serious eye irritation
Pictogram	
Precautionary Statement – Prevention	P264 Wash contaminated skin thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection/face protection
Precautionary statement – Response	P305+P351+P338 IF IN EYES: Rinse coutiously with water for several minutes.Remove contact lenses if present and easy to do so. Continue rinsing. P337+P313 If eye irration persists get medical attention/advice

Issued By: Henkel Australia Date Issued: April 15, 2015

Safety Data Sheet – Cold Power Laundry Detergent Powders

3. Composition/information on ingredients

Ingredients	Name	CAS	Proportion
	Sodium carbonate	497-19-8	40 to 50%
	Sodium sulfate	7757-82-6	20 to 30%
	Sodium dodecylbenzene sulfonate	25115-30-0	10 to 20%
	Sodium aluminosilicate	1344-00-9	5 to 10%
	Alcohols, C12-14, ethoxylated	68439-50-9	1 to 5%
	Sodium silicate	1344-09-8	1 to 5%
	Ingredients determined not the be hazardous, including water		Balance

4. First- Aid Measures

Inhalation	If inhaled, remove affected person from contaminated area. Keep at rest until recovered. If symptoms develop and/or persist seek medical attention.
Ingestion	Do not induce vomiting. Wash out mouth thoroughly with water. Seek medical attention.
Skin	Wash affected area thoroughly with soap and water. If symptoms develop seek medical attention.
Eye Contact	If in eyes, hold eyelids apart and flush the eyes continuously with running water. Remove contact lenses. Continue flushing for several minutes until all contaminants are washed out completely. Seek medical attention.
First Aid Facilities	Eyewash, safety shower and normal washroom facilities.
Advice to doctor	Treat symptomatically
Other Information	For advice in an emergency situation, contact a Poison Information Centre (131 126) or a doctor at once.

5. Fire Fighting Measures

Suitable extinguishing media	Use appropriate fire extinguisher for surrounding environment.
Hazards from Combustion Products	Non-combustible material
Specific hazards arising from the product	This product is non-combustible. However, under fire conditions, the product may decompose and/or burn emitting toxic and/or irritating fumes.
Decomposition Temperature	Not available
Precautions in connection with Fire	Fire fighters should wear Self-Contained Breathing Apparatus (SCBA) operated in positive pressure mode and full protective clothing to prevent exposure to vapours or fumes. Water spray may be used to cool down heat-exposed containers.

Figure A- 1: Cold Power MSDS

SAFETY DATA SHEET

FAB SUNSHINE FRESH ULTRA HDD

Infosafe No.: LQ4FX
Version No.: 1.0
ISSUED Date: 07/05/2015
ISSUED BY Colgate-Palmolive Pty Ltd

1. IDENTIFICATION

GHS Product Identifier
FAB SUNSHINE FRESH ULTRA HDD

Company Name
Colgate-Palmolive Pty Ltd (ABN 002 792 163)

Address
Australia: Level 14, 345 George Street, Sydney
NSW 2000 Australia

Telephone/Fax Number
Tel: AUS (02) 9229 5600 NZ: 04 576 6700
Fax: AUS (02) 9229 5700, NZ: 04 568 8835

Emergency phone number
AUS: 131 126, NZ: 0800 764766

Recommended use of the chemical and restrictions on use
Laundry washing detergent.

Other Information
New Zealand Address: Level 4, 45 Knights Road, Lower Hutt.

2. HAZARD IDENTIFICATION

GHS classification of the substance/mixture
Classified as Hazardous according to the Globally Harmonised System of Classification and labelling of Chemicals (GHS) including Work, Health and Safety regulations, Australia
Not classified as Dangerous Goods according to the Australian Code for the Transport of Dangerous Goods by Road and Rail. (7th edition)

GHS Classification:
Eye damage/irritation - Category 2A

Signal Word (s)
WARNING

Hazard Statement (s)
H319 Causes serious eye irritation.

Pictogram (s)
Exclamation mark

Precautionary statement – Prevention
P264 Wash contaminated skin thoroughly after handling.
P280 Wear protective gloves/protective clothing/eye protection/face protection.

Precautionary statement – Response
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 If eye irritation persists: Get medical advice/attention.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Ingredients

Name	CAS	Proportion
Sodium carbonate	497-19-8	50-55 %
Sodium sulphate	7757-82-6	25-30 %
Sodium dodecyl benzene sulphonate	25155-30-0	5-10 %
Sodium aluminosilicate	1344-00-9	1-5 %
Sodium silicate	1344-09-8	1-5 %
Ingredients determined not to be hazardous, including water.		Balance

4. FIRST-AID MEASURES

Inhalation
If inhaled, remove affected person from contaminated area. Keep at rest until recovered. If symptoms develop and/or persist seek medical attention.

Ingestion
Do not induce vomiting. Wash out mouth thoroughly with water. Seek medical attention.

Skin
Wash affected area thoroughly with soap and water. If symptoms develop seek medical attention.

Figure A- 2: Fab Sunshine Fresh MSDS

Unilever

MATERIAL SAFETY DATA SHEET

1. IDENTIFICATION OF THE PRODUCT

Product Name/s:	Variant/s	Manufacturer's Product Codes	
		Finished Goods Code:	Formula Code:
Omo & Persil Concentrate Powder	Top Loader	All pack sizes	21165898

Recommended Use:	Laundry washing powder	
Supplier:	Unilever Australasia	
Address:	219 North Rocks Rd, North Rocks, NSW 2151, Australia	
	103 Carlton Gore Road, Newmarket, Auckland 1023, New Zealand	
Telephone:	+61 2 9869 6100 (AUS)	+64 4 5666949 (NZ)
Facsimile:	+61 2 9869 6150 (AUS)	+64 4 5683396 (NZ)
Consumer Relations Department:	Mon–Fri 9:00 am – 5:00 pm	
Telephone:	1 800 888 449 (AUS)	0 800 900 029 (NZ)
Website	www.unilever.com.au	www.unilever.co.nz
Poison Information Centre (24 hour)	131 126 (AUS)	0 800 764 766 (NZ).

2. HAZARDS IDENTIFICATION

STATEMENT OF HAZARDOUS NATURE FOR AUSTRALIA

HAZARDOUS SUBSTANCE – NON-DANGEROUS GOOD
According to the Criteria of NOHSC, and the ADG Code

Hazard Category:	Xn: Harmful
Risk Phrase(s):	R22 Harmful if swallowed
	R41 Risk of serious eye damage
	R38 Irritating to skin
Safety Phrase(s):	S2 Keep out of reach of children
	S22 Do not breathe dust
	S24 Avoid contact with skin
	S25 Avoid contact with eyes
	S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
	S46 If swallowed, seek medical advice immediately and show this container or label.

Unilever

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS No.	Proportion %
Sodium Carbonate	497-19-8	30-<60%
Anionic Surfactant	25155-30-0	10-<30%
Alcohols, C12-18, ethoxylated	68213-23-0	<10%
Sodium Silicate	-	<10%
Fluorescer	-	<1%
Enzymes	-	<1%
Fragrance Ingredients	-	<1%
Other ingredients determined not to be hazardous	-	To 100%

4. FIRST AID MEASURES

Ingestion:	Remove product from mouth. Drink 1 or 2 glasses of water (or milk). If large amount swallowed or symptoms develop obtain medical attention. DO NOT induce vomiting.
Eye contact:	Hold eyes open, flood with water for at least 15 minutes and obtain medical attention.
Skin contact:	Remove contaminated clothing and wash the skin thoroughly with water. If symptoms occur seek medical advice.
Inhalation:	Remove from source of exposure to fresh air. Obtain medical attention immediately.
Symptoms:	Symptoms may vary depending on the level of exposure
Notes to physician:	Symptomatic and general supportive treatment should be given.
Treatment:	Treat Symptomatically

5. FIREFIGHTING MEASURES

Suitable extinguishing media:	Extinguish with water, CO_2, foam or dry chemical. Fire fighters should wear self-contained breathing apparatus for risk of exposure to decomposition products.
Specific hazards:	Not flammable, but may give off toxic fumes if involved in a fire.
Hazards from decomposition products:	Burning can produce CO_2, CO, water vapour, and sulfur oxide.

Figure A- 3: Omo MSDS

Radiant Mixed Colour Wash - Laundry Powder

PZ Cussons Australia Pty Ltd

Chemwatch Hazard Alert Code: 3

Chemwatch: 40-6784
Version No: 3.1.1.1
Safety Data Sheet according to WHS and ADG requirements

Issue Date: 06/17/2016
Print Date: 06/27/2016
S.GHS.AUS.EN

SECTION 1 IDENTIFICATION OF THE SUBSTANCE / MIXTURE AND OF THE COMPANY / UNDERTAKING

Product Identifier

Product name	Radiant Mixed Colour Wash - Laundry Powder
Synonyms	Not Available
Other means of identification	Not Available

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	Linear alkylbenzene sulfonates (LAS) are, by volume, the most important group of synthetic anionic surfactant today. LAS are mainly used in laundry detergents and cleaning agents. LAS are frequently used as the sodium salts as the sole surfactant in a formulation or in conjunction with other anionic, nonionic or cationic surfactants. LAS consist of an alkyl chain attached to a benzene ring in the para position to the sulfonate group. Use according to manufacturer's directions. Laundry powder - main wash.

Details of the supplier of the safety data sheet

Registered company name	PZ Cussons Australia Pty Ltd
Address	Building A, Level 1, 13-15 Corpark Circuit Mulgrave VIC 3170 Australia
Telephone	1800 809 282
Fax	+61 3 8545 2799
Website	www.pzcussons.com
Email	Not Available

Emergency telephone number

Association / Organisation	Poisons Information Centre (Aus)
Emergency telephone numbers	13 11 26
Other emergency telephone numbers	Not Available

SECTION 2 HAZARDS IDENTIFICATION

Classification of the substance or mixture

Poisons Schedule	Not Applicable
Classification [1]	Acute Toxicity (Inhalation) Category 4, Skin Corrosion/Irritation Category 2, Serious Eye Damage Category 1, Specific target organ toxicity - single exposure Category 3 (respiratory tract irritation), Acute Aquatic Hazard Category 2
Legend:	*1. Classified by Chemwatch; 2. Classification drawn from HSIS ; 3. Classification drawn from EC Directive 1272/2008 - Annex VI*

Label elements

GHS label elements	
SIGNAL WORD	DANGER

Hazard statement(s)

H332	Harmful if inhaled.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H335	May cause respiratory irritation.
H401	Toxic to aquatic life

Precautionary statement(s) Prevention

P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

P261	Avoid breathing dust/fumes.
P273	Avoid release to the environment.

Precautionary statement(s) Response

P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
P362	Take off contaminated clothing and wash before reuse.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.

Precautionary statement(s) Storage

P405	Store locked up.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.

Precautionary statement(s) Disposal

P501	Dispose of contents/container in accordance with local regulations.

SECTION 3 COMPOSITION / INFORMATION ON INGREDIENTS

Substances

See section below for composition of Mixtures

Mixtures

CAS No	%[weight]	Name
497-19-8	30-60	sodium carbonate
7757-82-6	10-30	sodium sulfate
8046-53-5	10-30	(linear)alkylbenzenesulfonic acid, sodium salts
13870-28-5	1-10	sodium disilicate
Not Available	<1	perfume
9014-01-1	<1	protease
9012-54-8	<1	cellulase
Not Available	<1	speckles - green/red
9000-92-4	<0.2	amylase
	balance	ingredients determined not to be hazardous

SECTION 4 FIRST AID MEASURES

Description of first aid measures

Eye Contact	If this product comes in contact with the eyes: ▸ Immediately hold eyelids apart and flush the eye continuously with running water. ▸ Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids. ▸ Continue flushing until advised to stop by the Poisons Information Centre or a doctor, or for at least 15 minutes. ▸ Transport to hospital or doctor without delay. ▸ Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.
Skin Contact	If skin contact occurs: ▸ Immediately remove all contaminated clothing, including footwear.

Figure A- 4: Radiant MSDS

VS **Gardner Kimberley <Kimberley.Gardner@pzcussons.com> on beh**
18/08/2016 12:41 PM

To: Rachel Morison

Hi Rachel,

My apologies for the delay in getting back to you with this information - our Radiant 'know-it-all' has been on leave until recently. He knows everything there is to know about our Radiant products and he has advised that the opti brightener that we use in this range is "Disodium Distyrylbiphenyl Disulfonate",

I hope this information is helpful to you and we wish you all the best with your studies.

Kind regards,

Kimberley Gardner
Consumer Services Co-ordinator
PZ Cussons Australia Pty Ltd
Building A, Level 1, 13-15 Compark Circuit,
Mulgrave, Victoria, 3170

Ph: +61 3 8545 2717
Internal Ext: 2717

Figure A- 5: personal communication with PZ Cussons

Cold Power - CS201608-001389

L **lhc.consumeraffairs.anz@henkel.com <lhc.consumeraffairs.anz@h**
3/08/2016 8:32 AM

To: Rachel Morison

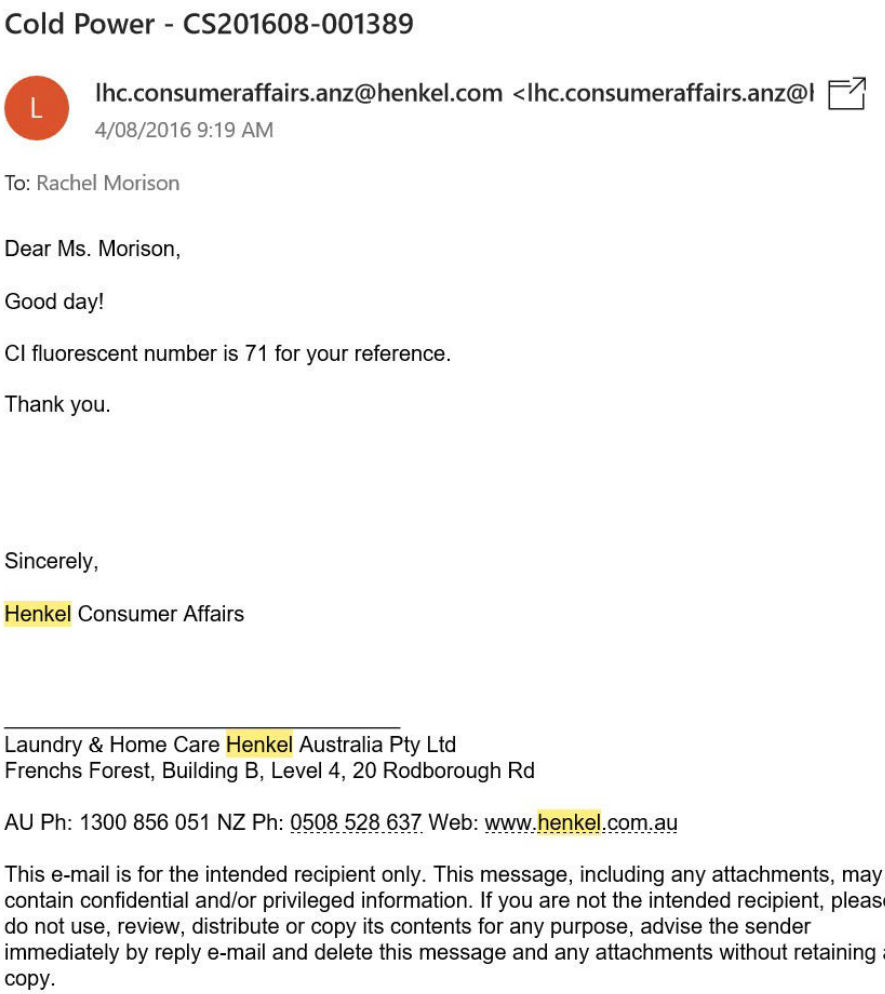
Dear Ms. Morison,

Thank you for your time and interest in choosing our product.

For your reference optical brightener ingredient used is CI Fluorescent for Cold Power Regular Powder and for Fab Sunshine Fresh Powder.
Again, thank you for contacting Henkel.

Sincerely,

Henkel Consumer Affairs



Cold Power - CS201608-001389

L lhc.consumeraffairs.anz@henkel.com <lhc.consumeraffairs.anz@l
4/08/2016 9:19 AM

To: Rachel Morison

Dear Ms. Morison,

Good day!

CI fluorescent number is 71 for your reference.

Thank you.

Sincerely,

Henkel Consumer Affairs

Laundry & Home Care Henkel Australia Pty Ltd
Frenchs Forest, Building B, Level 4, 20 Rodborough Rd

AU Ph: 1300 856 051 NZ Ph: 0508 528 637 Web: www.henkel.com.au

This e-mail is for the intended recipient only. This message, including any attachments, may contain confidential and/or privileged information. If you are not the intended recipient, please do not use, review, distribute or copy its contents for any purpose, advise the sender immediately by reply e-mail and delete this message and any attachments without retaining a copy.

Figure A- 6: Personal communication with Henkel Australia

Sydney WATER

Water analysis: Prospect North water supply system

Parameter	Units	ADWG* Health	ADWG* Aesthetic	Prospect North (10^{th} – 90^{th} percentile range)
Physical characteristics				
True colour	TCU or HU	na	15	<2 - 4
Turbidity	NTU	na	5	0.1 – 1.2
Total dissolved solids	mg/L	na	600	100 – 136
pH	pH units	na	6.5 - 8.5	7.8 – 8.2
Conductivity	mS/m	na	na	19 – 23
Total hardness	mg $CaCO_3$ /L	na	200	49 – 62
Calcium hardness	mg $CaCO_3$ /L	na	na	30 – 40
Magnesium hardness	mg $CaCO_3$ /L	na	na	17 – 23
Alkalinity	mg $CaCO_3$ /L	na	na	31 – 40
Temperature	degrees C	na	na	14 - 22
Dissolved oxygen	% saturation	na	>85%	98 – 125
Disinfectants				
Free chlorine	mg/L	5	0.6	<0.04 – 0.76
Monochloramine	mg/L	3	0.5	0.08 – 1.42
Disinfection by-products				
Trihalomethanes	mg/L	0.25	na	0.039 – 0.191
Inorganic chemicals				
Aluminium	mg/L	na	0.2	0.010 – 0.018
Ammonia (as NH_3)	mg/L	na	0.5	<0.001 – 0.40
Arsenic	mg/L	0.01	na	<0.001
Cadmium	mg/L	0.002	na	<0.001
Calcium	mg/L	na	na	11.1 – 16.9
Chloride	mg/L	na	250	28.6 – 39.6
Chromium (Cr as VI)	mg/L	0.05	na	<0.0004 – 0.0005
Copper	mg/L	2	1	0.006 – 0.038
Cyanide	mg/L	0.08	na	<0.005
Fluoride	mg/L	1.5	na	0.98 – 1.10
Iron	mg/L	na	0.3	0.010 – 0.030
Lead	mg/L	0.01	na	<0.001
Nickel	mg/L	0.02	na	<0.001
Magnesium	mg/L	na	na	4.3 – 5.2
Manganese	mg/L	0.5	0.1	<0.001 – 0.002
Mercury	mg/L	0.001	na	<0.0001
Nitrate (as NO_3)	mg/L	50	na	0.66 – 1.15
Nitrite (as NO_2)	mg/L	3	na	<0.001 – 0.03
Phosphorous	mg/L	na	na	0.007 – 0.009
Potassium	mg/L	na	na	1.8 – 2.3
Reactive silica (as SiO_2)	mg/L	na	<80 mg/L	2.5 – 5.1
Selenium	mg/L	0.01	na	<0.003
Silver	mg/L	0.1	na	<0.003
Sodium	mg/L	na	180	13.0 – 18.0
Sulfate	mg/L	500	250	7.4 – 8.8
Zinc	mg/L	na	3	<0.005
Organic compounds				
Chlorinated, polynuclear aromatic, aromatic hydrocarbons		various	various	nd
Chlorophenols		various	various	nd
Pesticides		various	various	nd

Legend: na = no published health or aesthetic guideline value, **nd =** reported results are non-detectable (less than the limit of detection), ***ADWG =** *Australian Drinking Water Guidelines 2011*

Figure A- 7: Elemental analysis of water supply from Prospect North

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 612

Trace Elements in Glass

This Standard Reference Material (SRM) is intended to facilitate development of chemical methods of analysis for trace elements in glass. The nominal mass fractions of 61 elements added to the glass matrix are in the range of 10 mg/kg to 80 mg/kg. A unit of SRM 612 consists of four wafers, sliced to 3 mm thickness from a hand-pulled rod. The wafers are of oval to circular cross-section with nominal diameter of 12 mm to 14 mm.

Certified Mass Fraction Values: Certified values for 15 elements of SRM 612 are reported in Table 1 as mass fractions [1]. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [2].

Reference Values: Reference values for four elements are reported in Table 2 as mass fractions [1]. The normalized isotope atom ratio for strontium is also included. Reference values are non-certified values that are the best estimates of the true values based on available data; however, the values do not meet the NIST criteria for certification [2] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Values: Information values for 13 elements are reported in Table 3 as mass fractions. Also reported is the isotope atom fraction of uranium-235. An information value is considered a value that will be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value [2].

Expiration of Certification: The certification of **SRM 612** is valid indefinitely, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). Accordingly, periodic recalibration or recertification of this SRM is not required. The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this material over the period of its certification. If substantive technical changes occur that affect the certification, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of technical measurements for certification of SRM 612 was performed by W.R. Shields of what is now the NIST Analytical Chemistry Division. Coordination of technical measurements for updates of values was performed by W.C. Davis and J.R. Sieber of the NIST Analytical Chemistry Division.

Analyses for the original characterization were performed by R.W. Burke, T.E. Gills, E.J. Maienthal, L.W. Masters, T.C. Rains, and B.A. Thompson of what is now the NIST Analytical Chemistry Division. Analyses for the initial update of values were performed by E.L. Garner, J.W. Gramlich, L.A. Machlan, J.R. Moody, L.J. Moore, T.J. Murphy, P.J. Paulsen, and K.M. Sappenfield of what is now the NIST Analytical Chemistry Division. Analyses for the current update of values were performed by W.C. Davis and J.R. Sieber of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 06 April 2012
Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

Analyses for the original characterization were performed by the following collaborating laboratories and analysts: United States Geological Survey, Denver, CO, C. Hedge and M. Tatsumoto; Australian National University, Canberra, ACT, Australia, W. Compston; and University of Ghent, Ghent, Belgium, F. Bellemans.

Statistical consultation for this SRM was provided by S.D. Leigh, A.L. Pintar, and A.M. Possolo of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

To relate analytical determinations to certified values, a minimum sample quantity of 250 mg is recommended (see "Preparation and Analysis"). Each wafer surface should be cleaned before use. To prepare a wafer for analysis, wipe it clean with ethanol, then give it a mild surface cleaning (not etch) in dilute (1:10) HNO_3. The acid wash is recommended to remove potential copper contamination from cutting with a copper-bonded diamond wheel. The material should be stored in its original container in a cool, dry location.

Preparation and Analysis: Sixty-one trace elements were added to the glass support matrix, which has a nominal composition of 72 % SiO_2, 14 % Na_2O, 12 % CaO, and 2 % Al_2O_3 (mass fractions). A list of 29 elements that were added but for which no values have been assigned is provided in Table 4. The material was prepared in rod form and then sliced into wafers. Considerable effort was invested in the manufacturing of the material to ensure sufficient homogeneity to yield a ≤2 % relative repeatability of measurement when an entire wafer is used. Spatial heterogeneity exists within each wafer, which may adversely affect repeatability of microanalysis techniques. Values were assigned using the analytical methods listed in Table 5.

Certified Value Assignment: For antimony, arsenic, barium, cadmium, chromium, manganese, and selenium, the certified value and uncertainty were determined during recertification. Their certified values are weighted means of the mass fractions determined using the methods listed in Table 5. The form of the weights was introduced in reference 3 and described further in reference 4. Their expanded uncertainties are the half widths of symmetric 95 % parametric bootstrap confidence intervals [5] with expansion factor $k = 1.96$, and are consistent with the ISO Guide [6].

The certified values for iron, lead, nickel, rubidium, silver, strontium, thorium, and uranium are as assigned in the original certificate of this material. These values have not been updated, and are qualified with the original statement of measurement uncertainty, which is equal to the entire range of values measured for individual samples or to the 95 % confidence interval, whichever is greater. The user can treat such uncertainty assessments as half widths of 95 % confidence intervals based on Gaussian, Type A evaluations using no more than five measured values each.

Table 1. Certified Mass Fraction Values for SRM 612

Constituent	Mass Fraction (mg/kg)
Antimony	34.9 ± 2.2
Arsenic	37.4 ± 2.2
Barium	38.6 ± 2.6
Cadmium	29.9 ± 4.2
Chromium	35.0 ± 3.3
Iron	51 ± 2
Lead	38.57 ± 0.2
Manganese	37.7 ± 3.8
Nickel	38.8 ± 0.2
Rubidium	31.4 ± 0.4
Selenium	16.1 ± 1.6
Silver	22.0 ± 0.3
Strontium	78.4 ± 0.2
Thorium	37.79 ± 0.08
Uranium	37.38 ± 0.08

Figure A- 8: NIST SRM 612 glass standard concentrations

Appendix B

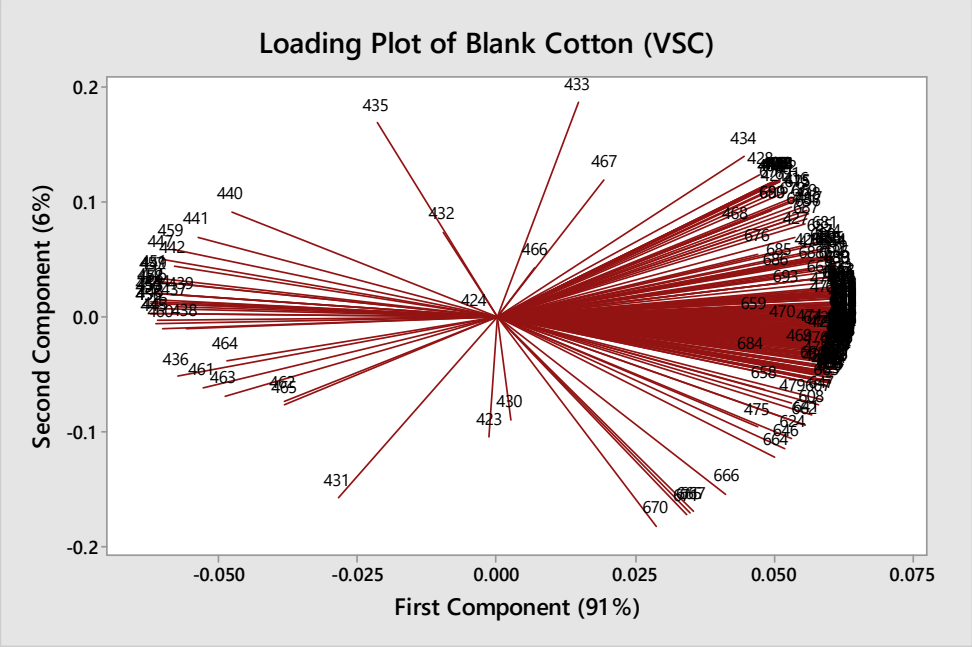


Figure B- 1: Loading plot of Blank Cotton (VSC)

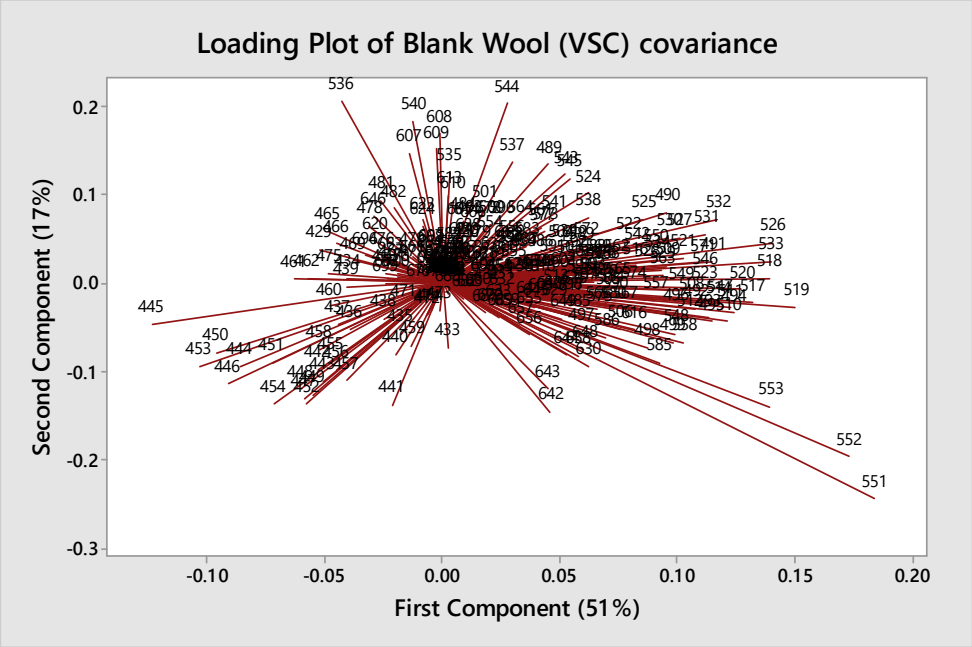


Figure B- 2: Loading plot of Blank Wool (VSC)

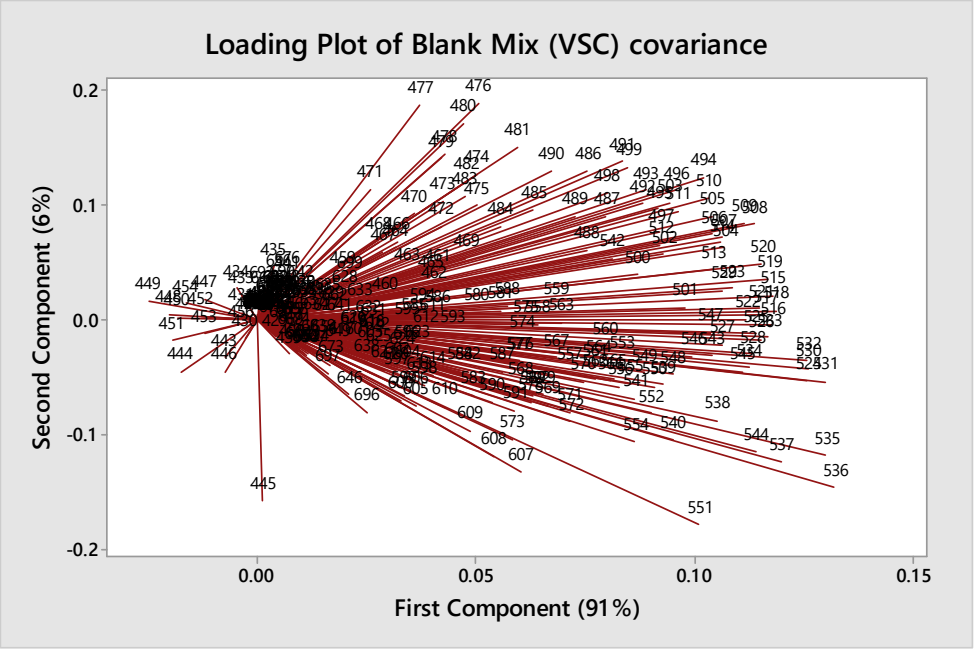


Figure B- 3: Loading plot of Blank Mix (VSC)

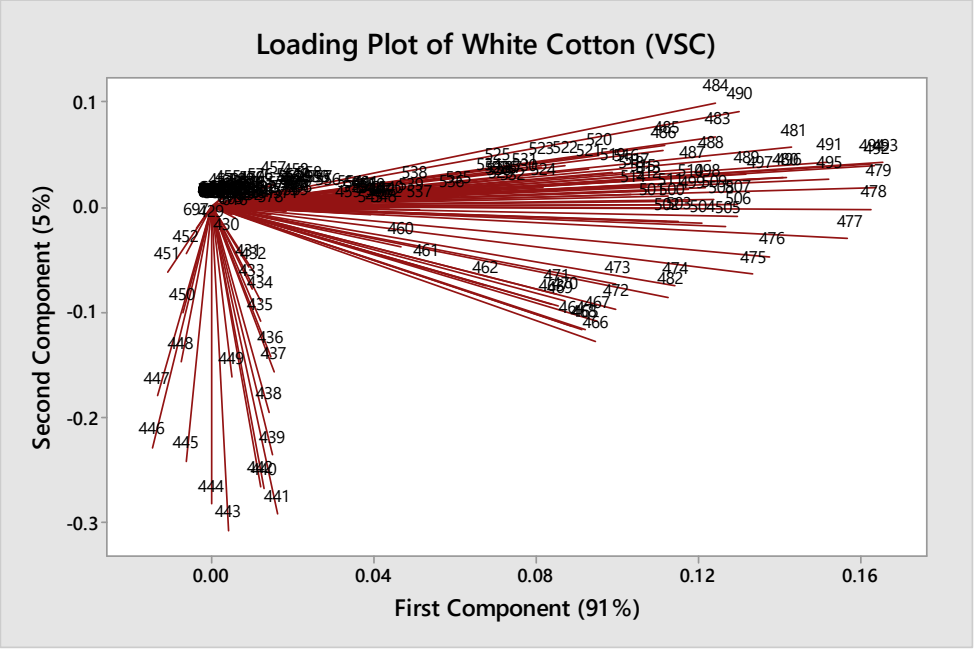


Figure B- 4: Loading plot of White Cotton (VSC)

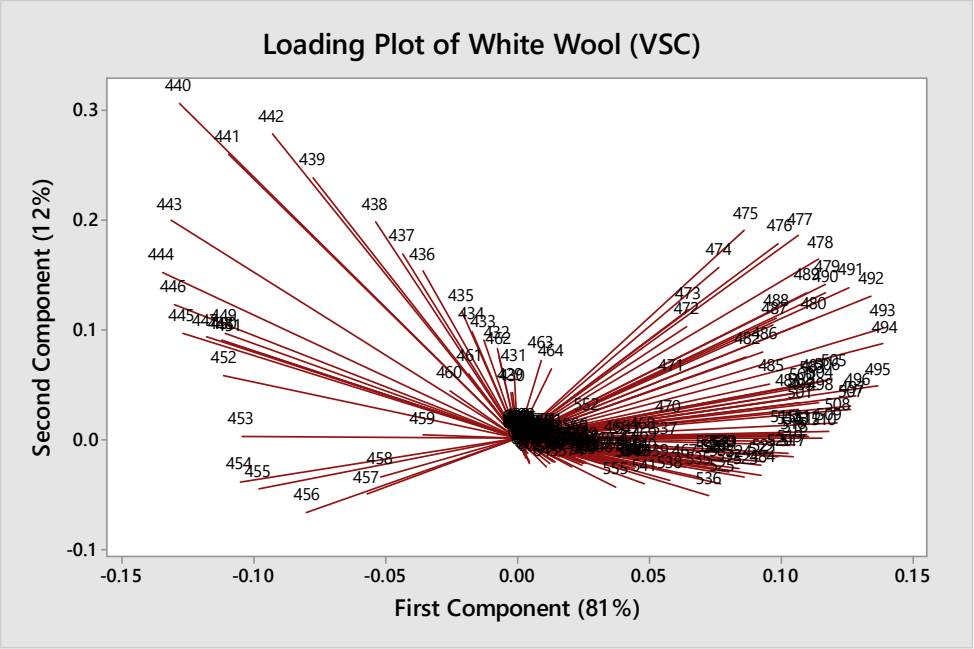


Figure B- 5: Loading plot of White Wool (VSC)

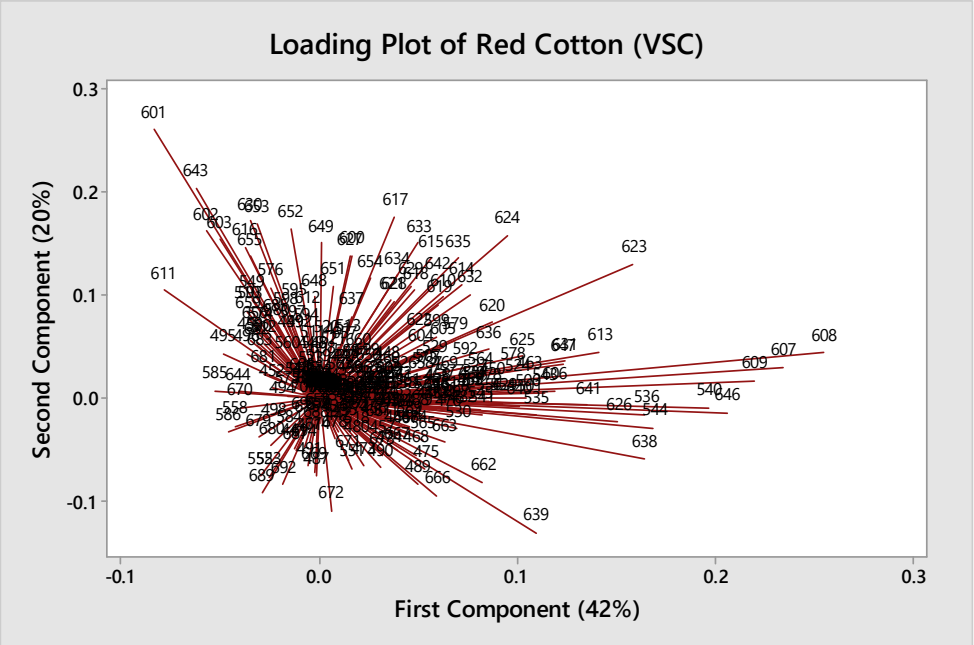


Figure B- 6: Loading plot of Red Cotton (VSC)

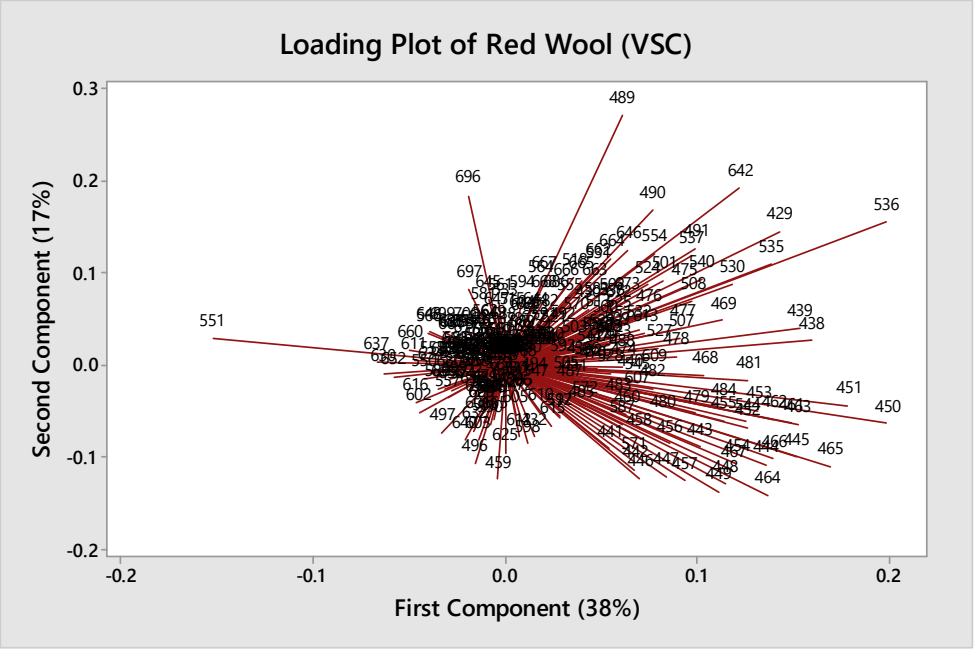


Figure B- 7: Loading plot of Red Wool (VSC)

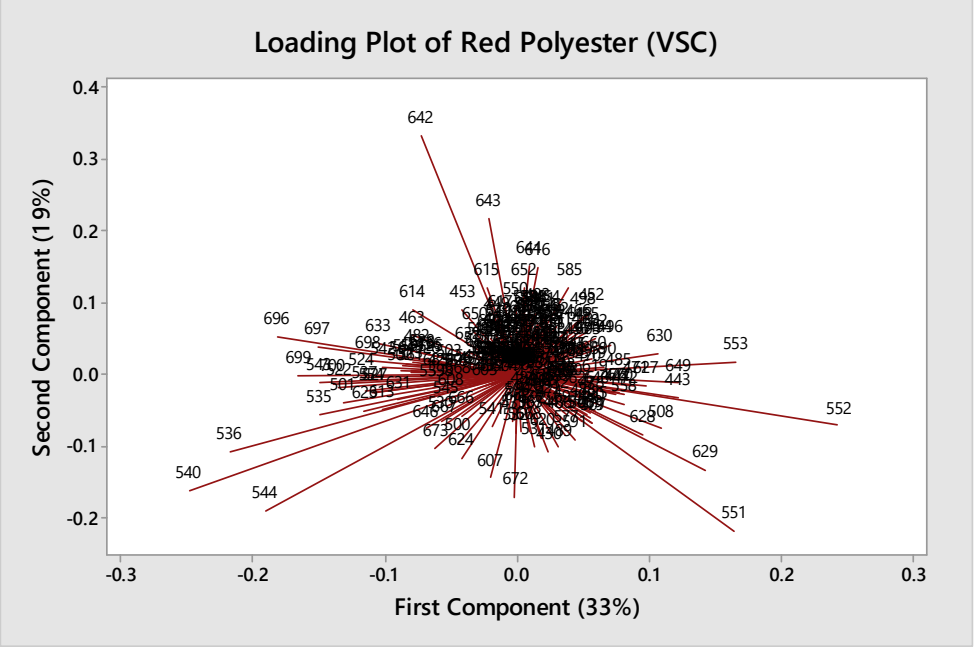


Figure B- 8: Loading plot of Red Polyester (VSC)

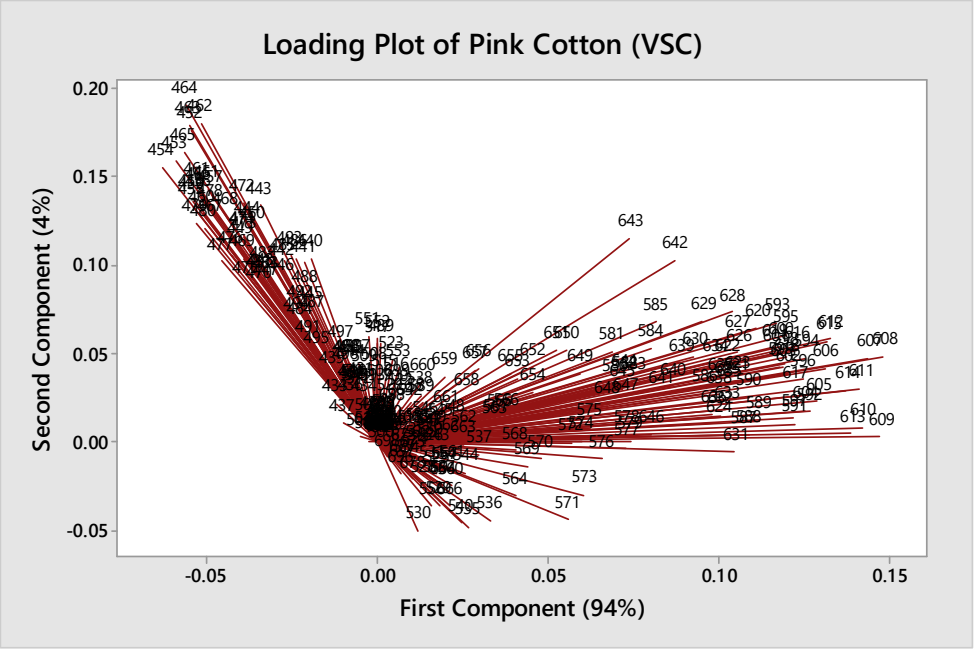


Figure B- 9: Loading plot of Pink Cotton (VSC)

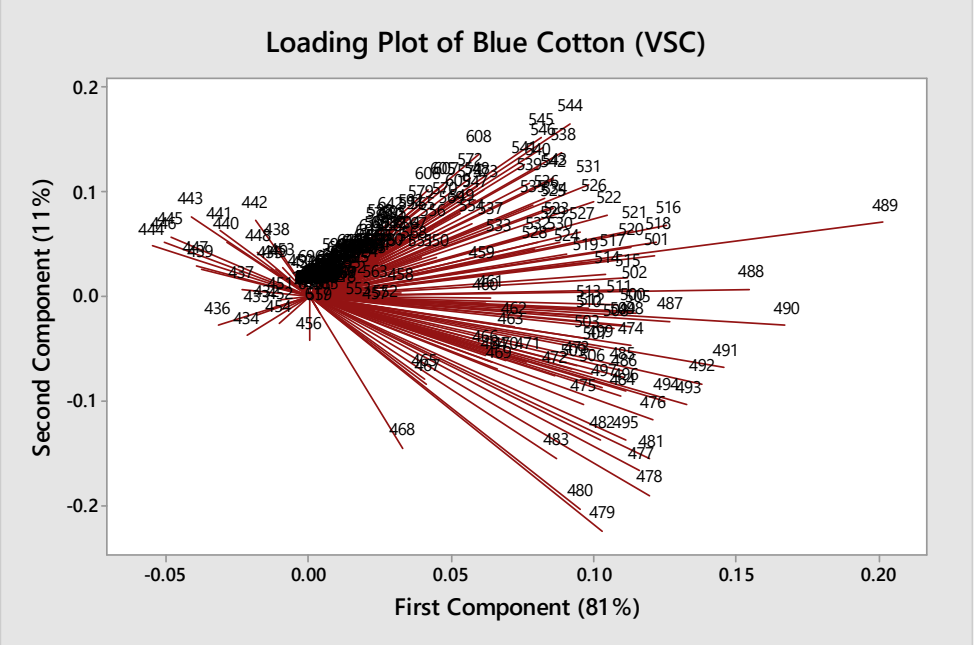


Figure B- 10: Loading plot of Blue Cotton (VSC)

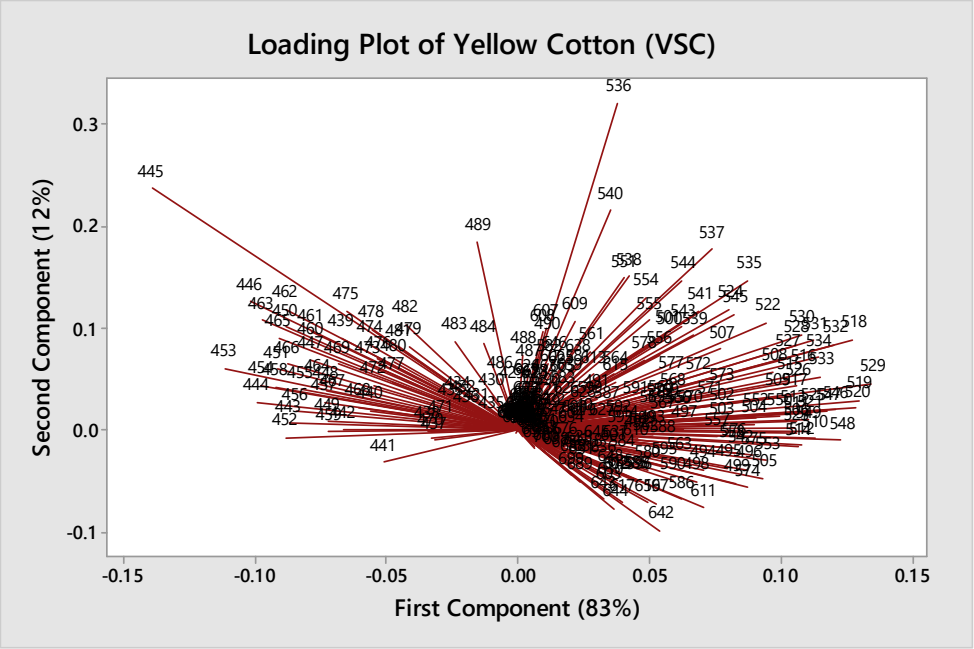


Figure B- 11: Loading plot of Yellow Cotton (VSC)

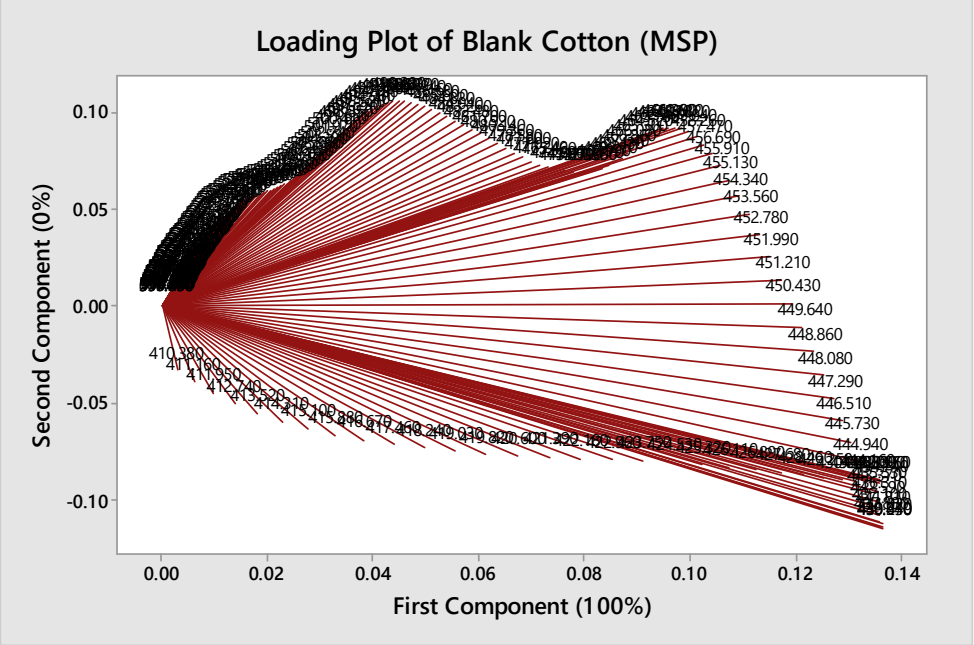


Figure B- 12: Loading plot of Blank Cotton (MSP)

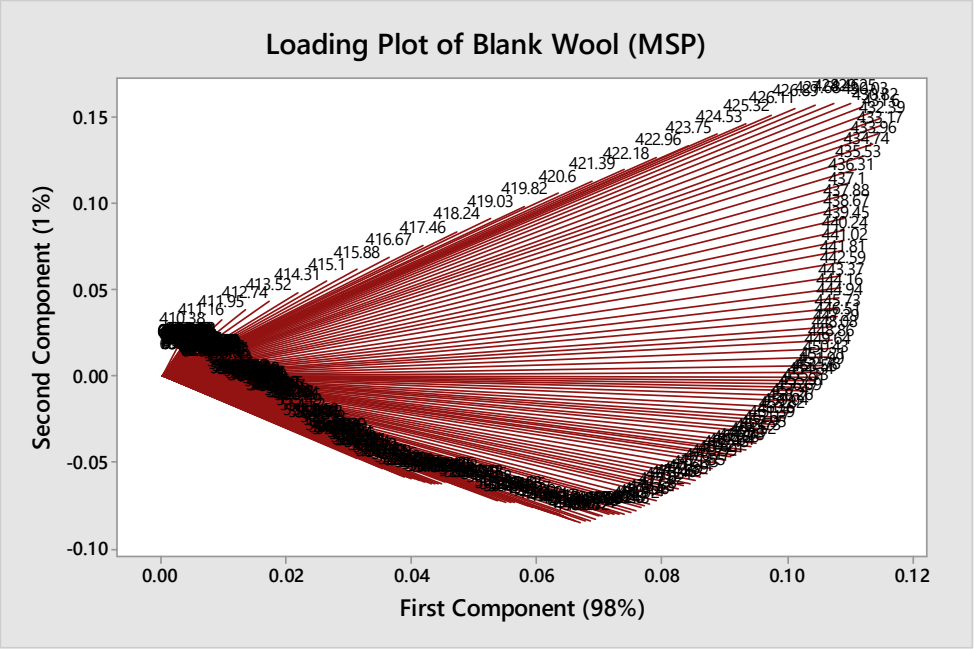


Figure B- 13: Loading plot of Blank Wool (MSP)

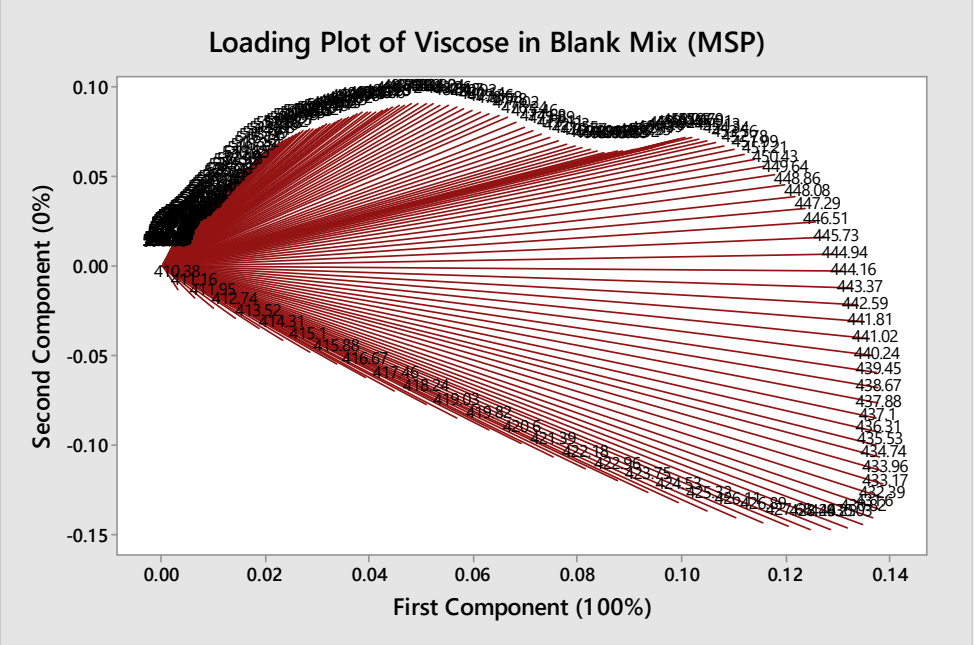


Figure B- 14: Loading plot of Viscose in Blank Mix (MSP)

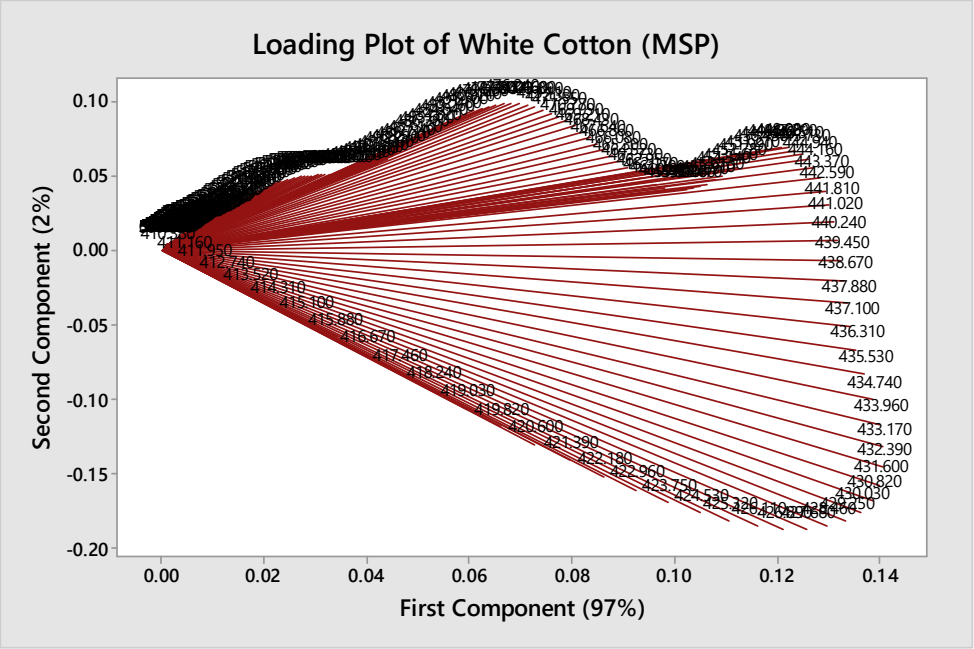


Figure B- 15: Loading plot of White Cotton (MSP)

Figure B- 16: Loading plot of White Wool (MSP)

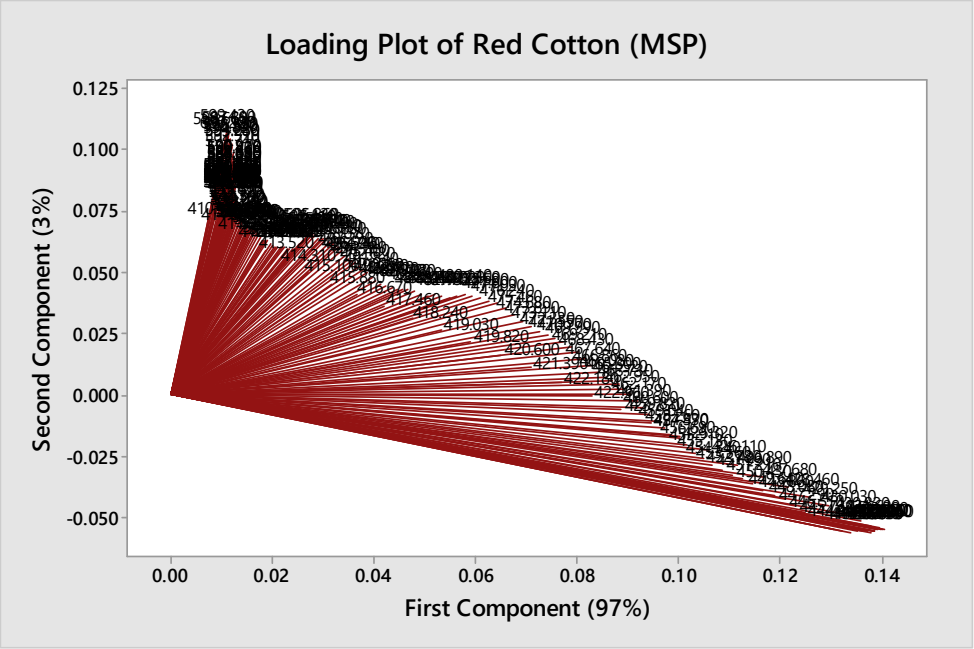


Figure B- 17: Loading plot of Red Cotton (MSP)

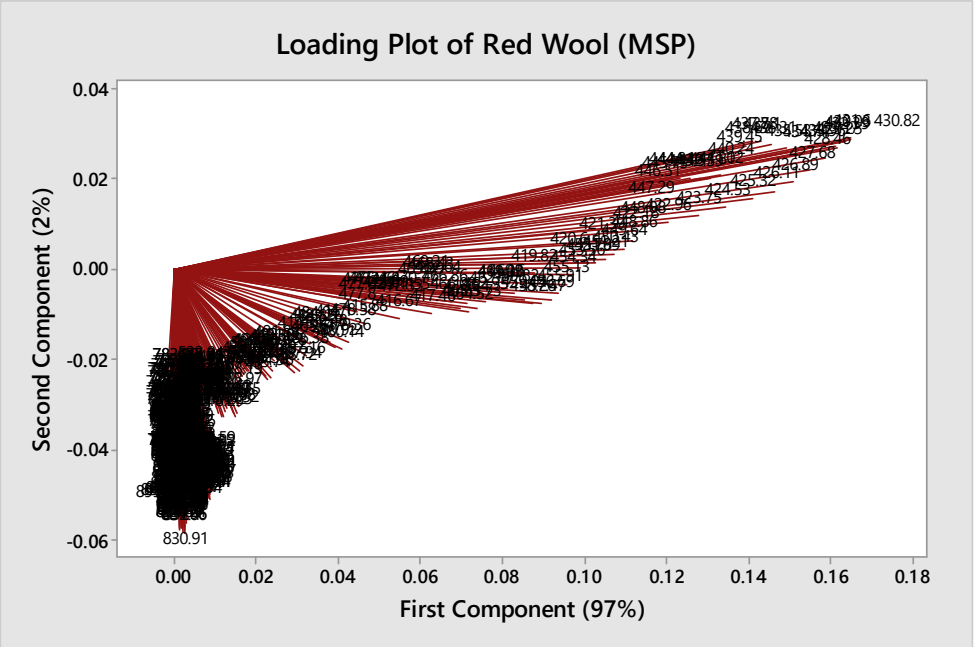


Figure B- 18: Loading plot of Red Wool (MSP)

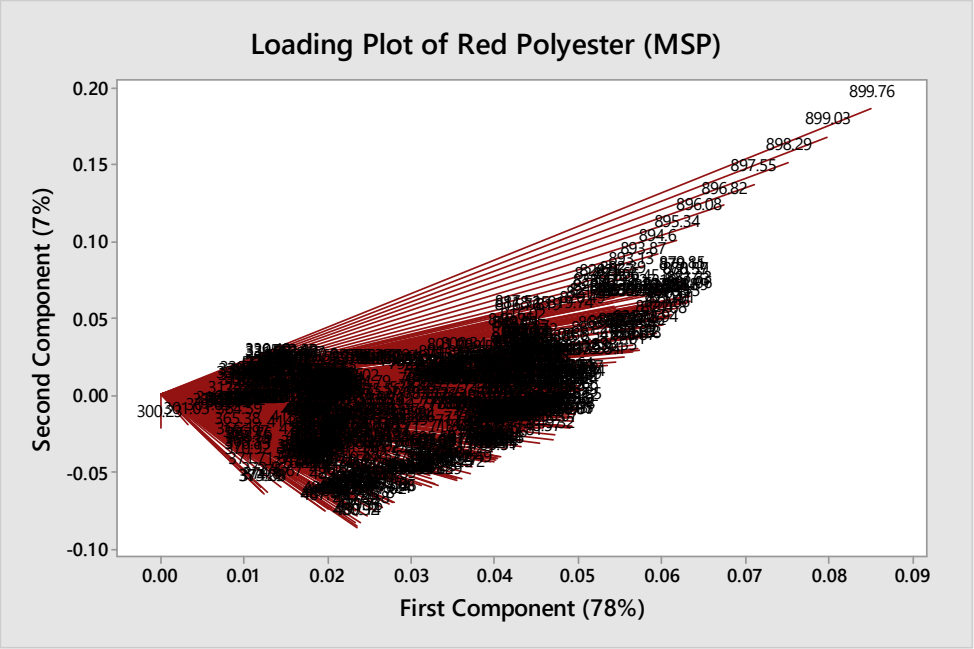


Figure B- 19: Loading plot of Red Polyester (MSP)

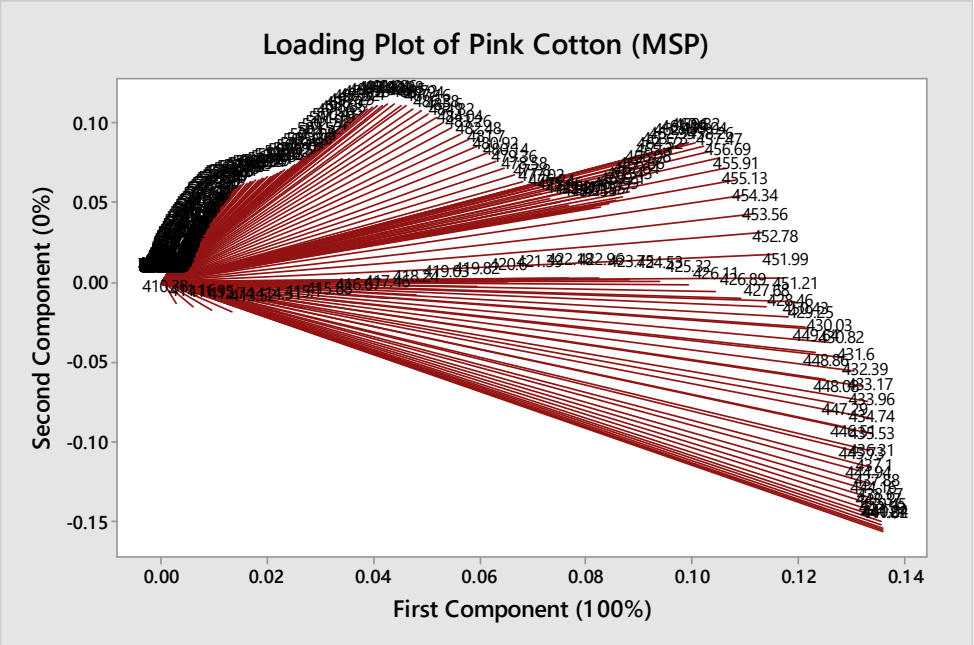


Figure B- 20: Loading plot of Pink Cotton (MSP)

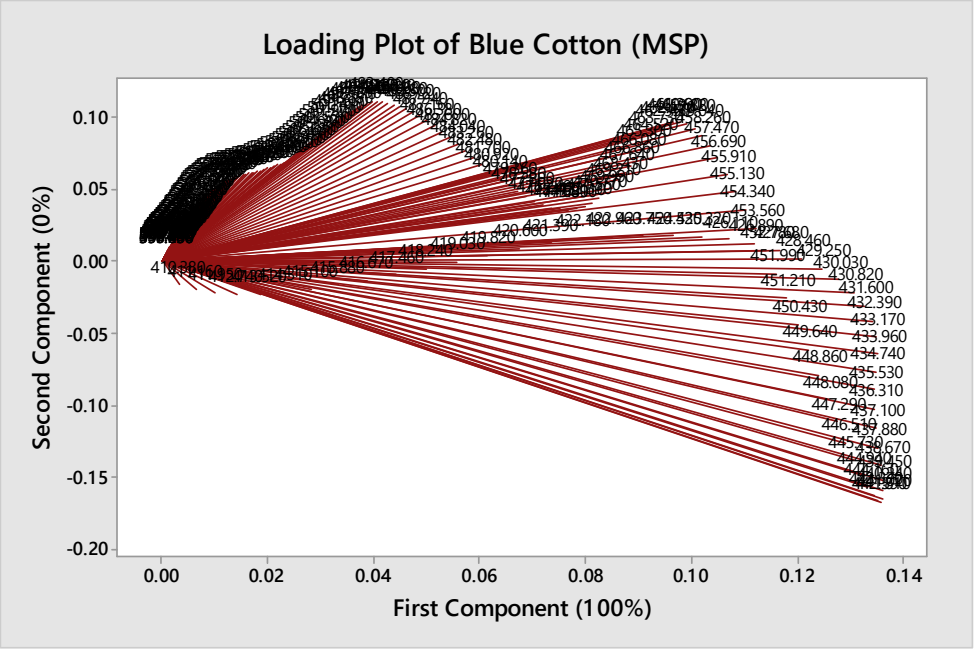


Figure B- 21: Loading plot of Blue Cotton (MSP)

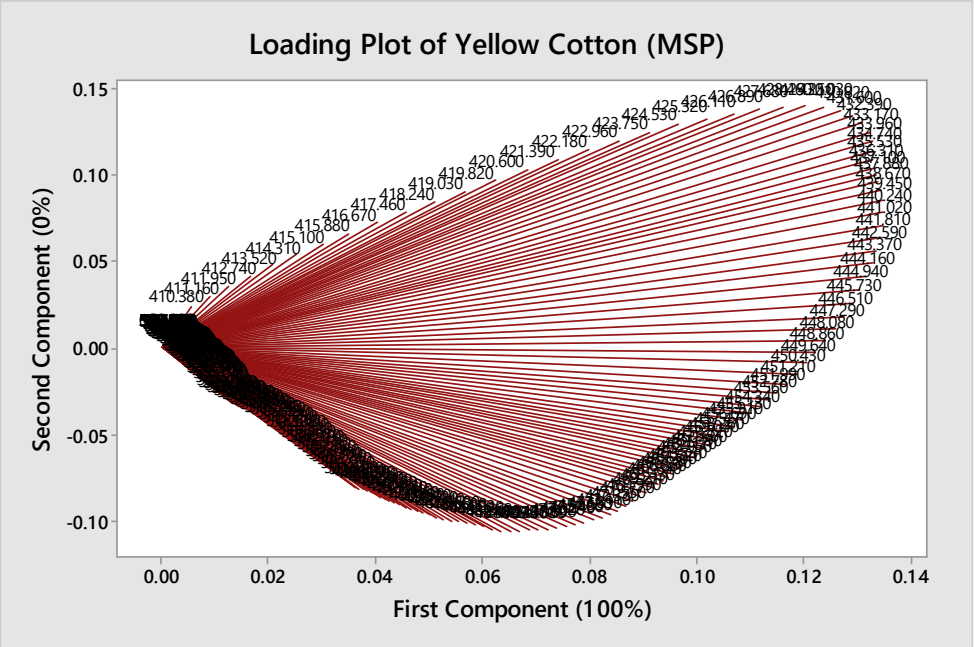


Figure B- 22: Loading plot of Yellow Cotton (MSP)

Appendix C

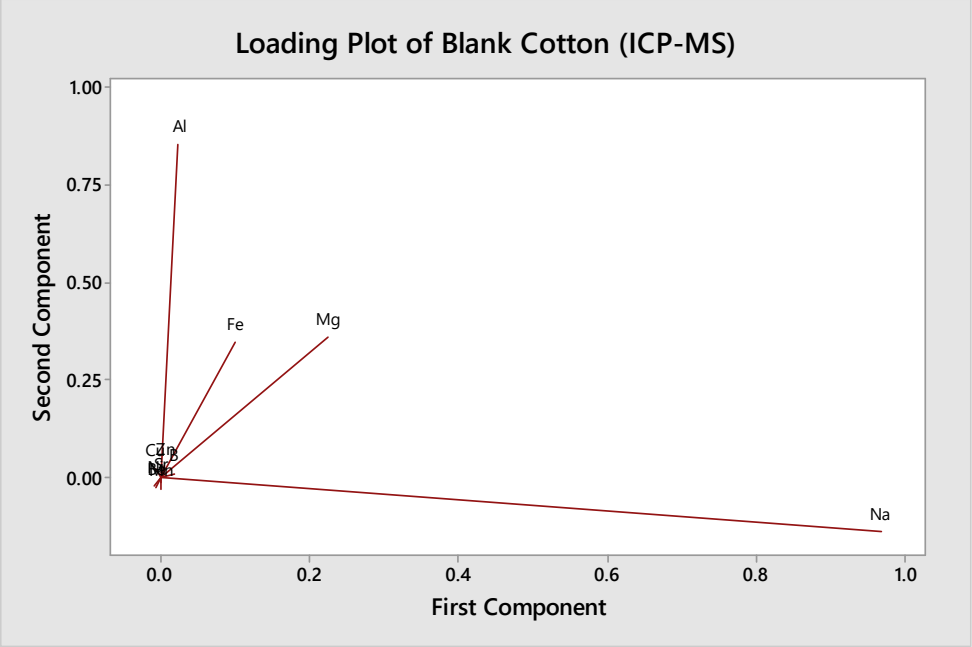


Figure C- 1: Loading Plot of Blank Cotton (ICP-MS)

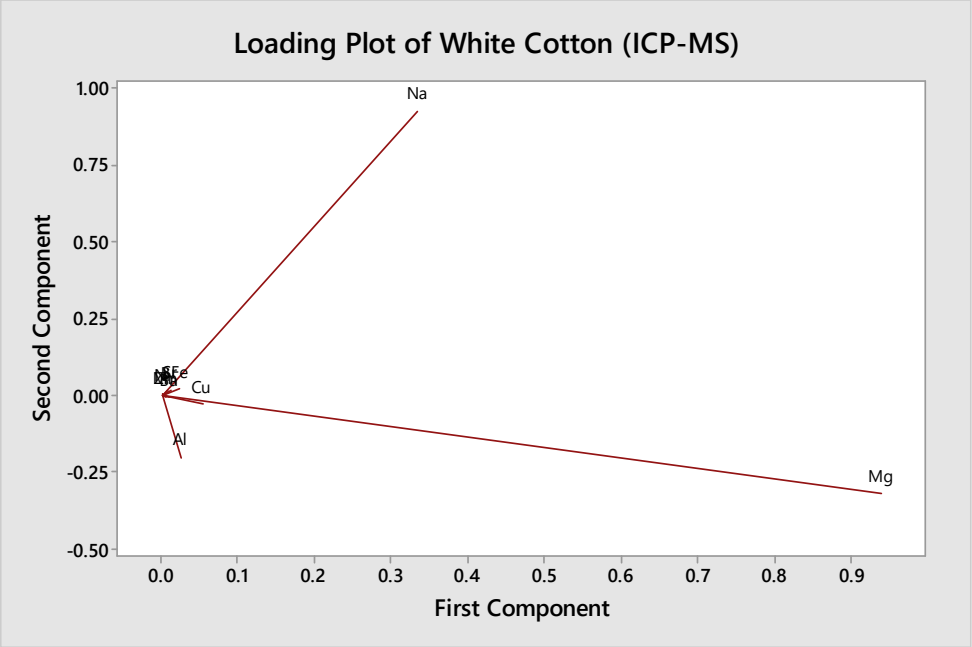


Figure C- 2: Loading Plot of White Cotton (ICP-MS)

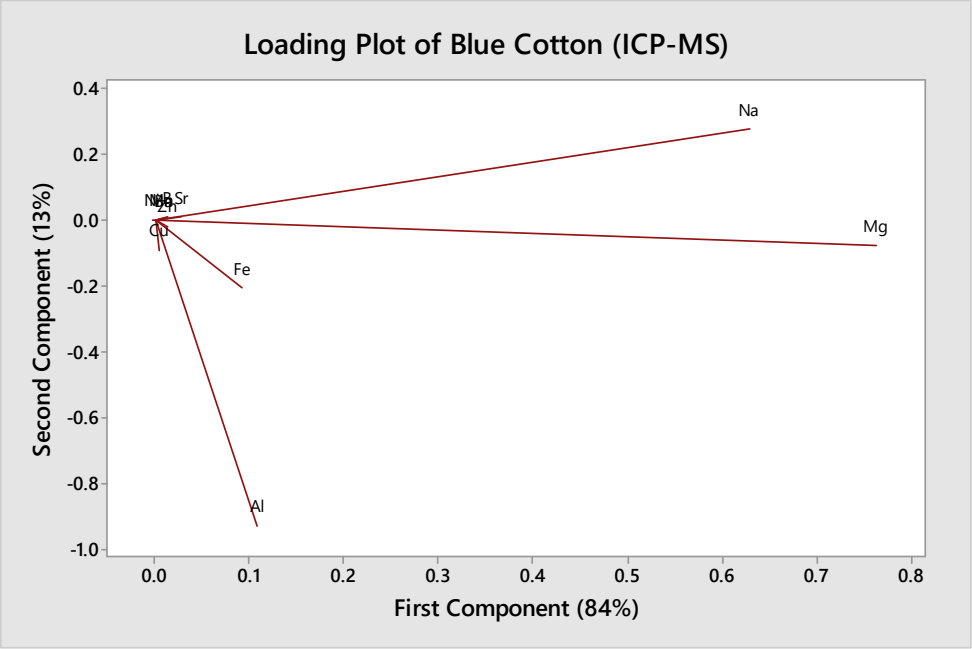


Figure C- 3: Loading Plot of Blue Cotton (ICP-MS)

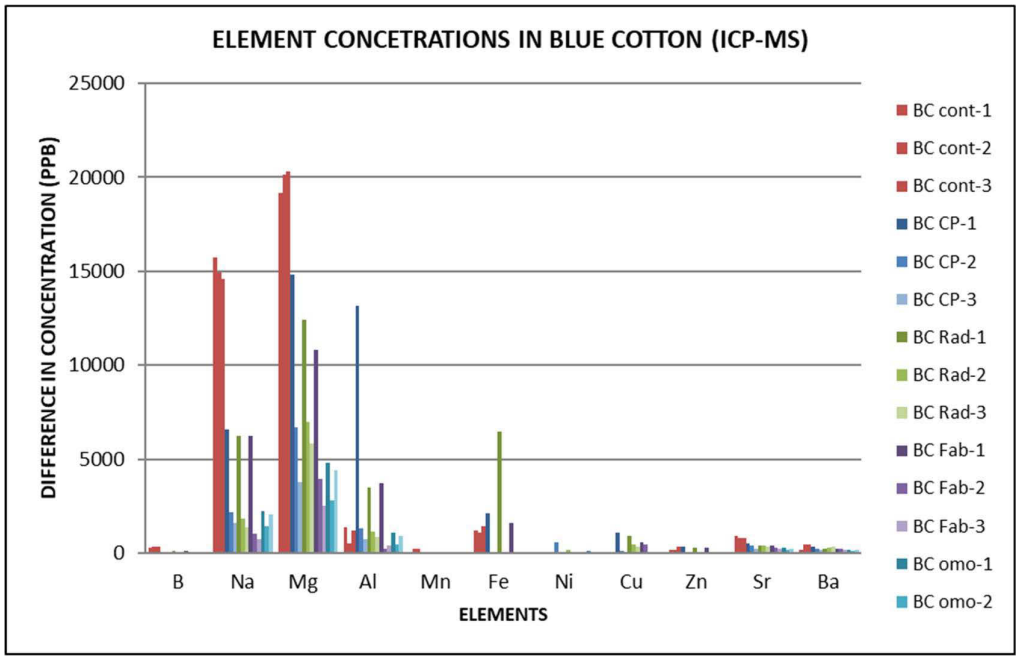


Figure C- 4: Element concentrations in blue cotton samples (ICP-MS)

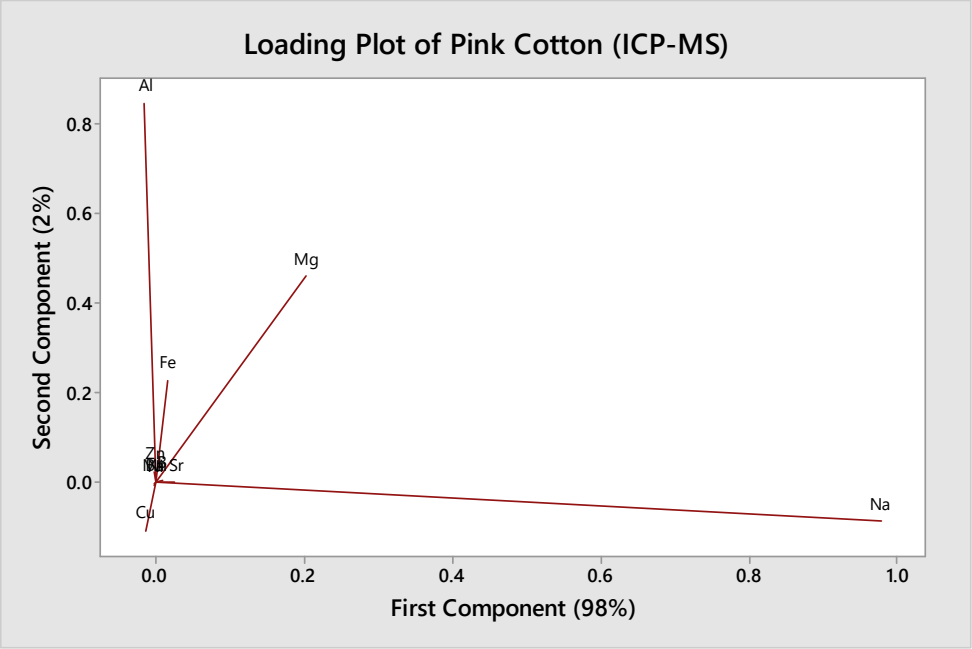


Figure C- 5: Loading Plot of Pink Cotton (ICP-MS)

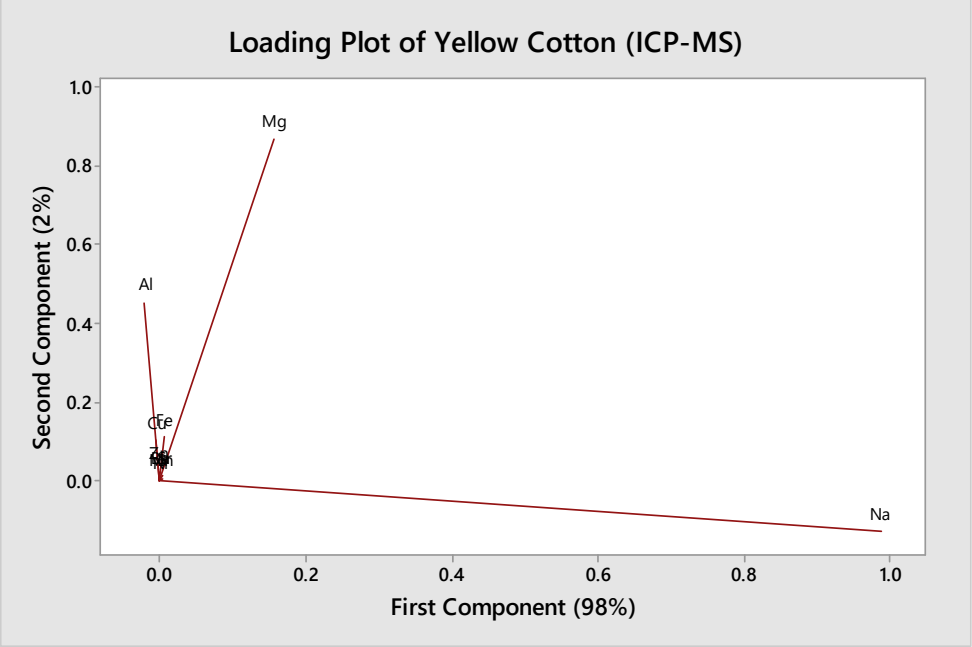


Figure C- 6: Loading Plot of Yellow Cotton (ICP-MS)

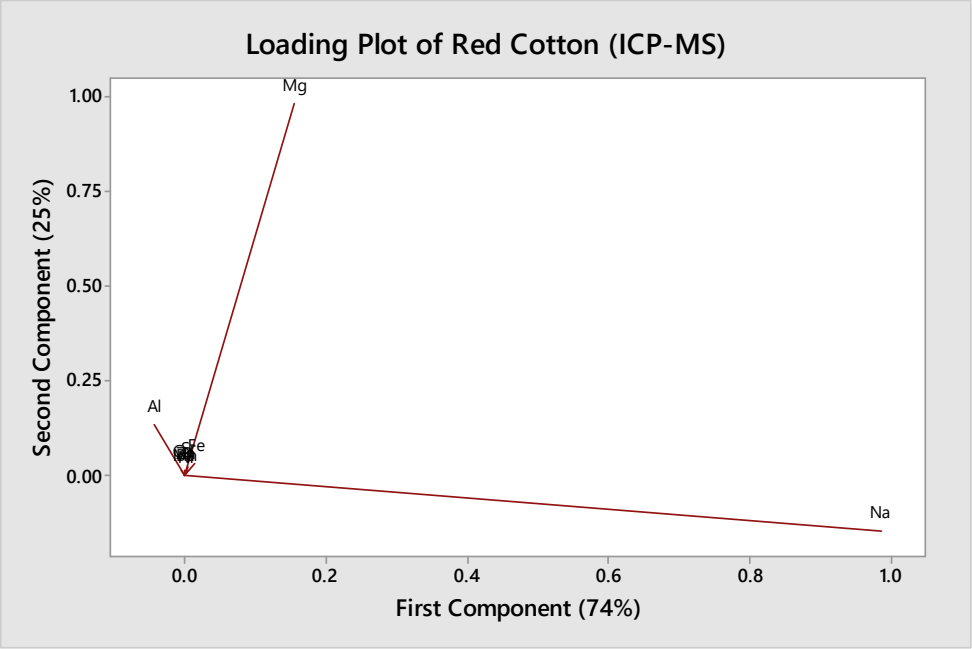


Figure C- 7: Loading Plot of Red Cotton (ICP-MS)

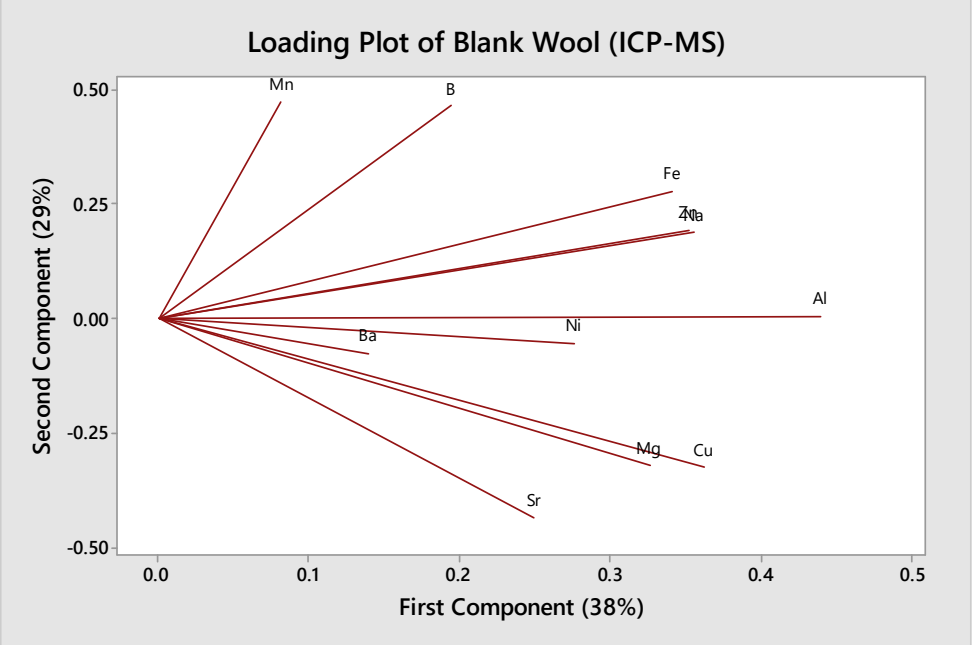


Figure C- 8: Loading Plot of Blank Wool (ICP-MS)

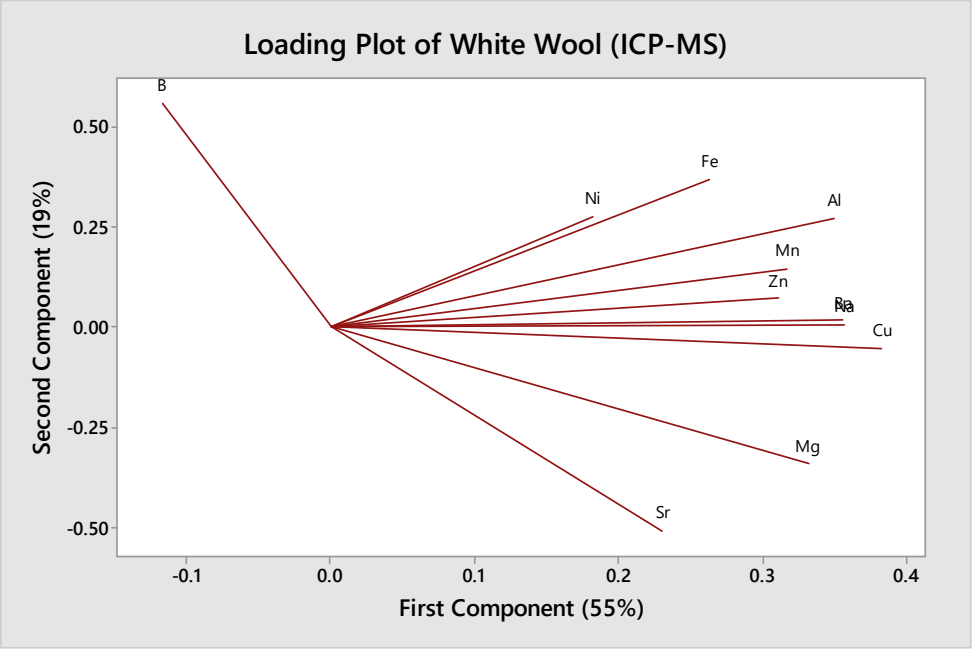


Figure C- 9: Loading Plot of White Wool (ICP-MS)

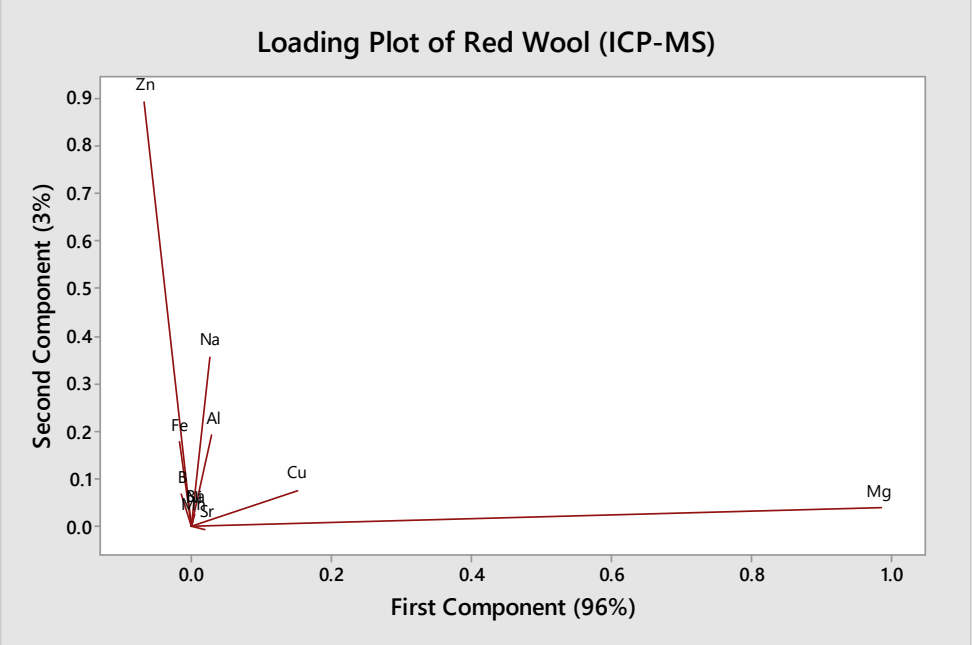


Figure C- 10: Loading Plot of Red Wool (ICP-MS)

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