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The impact of algae growth on forensic evidence: Considerations for latent fingermark visualisation strategies☆

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ABSTRACT

The recovery of latent fingermarks from wetted items can present a challenge to forensic scientists. This study investigated the effect of submersion in a natural water environment in the United Kingdom on mark recoverability, exploring fingermark age prior to submersion and length of time submerged. In total, 5400 natural fingermarks were donated. These were deposited on three types of non-porous samples that were then aged for three different lengths of time, before half were submerged in a lake for three different durations. Fingermarks of identifiable quality were only recovered from samples submerged for 24 h. Samples that were submerged for longer became heavily contaminated with algae, which had a significant and detrimental effect on fingermark recovery. Only 0.7% of the marks deposited on submerged samples were visualised to be of identifiable quality, compared to 51% for the non-submerged samples. Superglue Fuming was slightly more effective than Powder Suspension on these sample types, although the difference was only statistically significant on the non-submerged stainless-steel samples and due to the low numbers of marks recovered; the results for the submerged samples should be treated with caution. This study highlights the requirement for further field work to understand critical factors in localised environments.

1. Introduction

Becker and Nordby noted that “Criminals often seek a watery repository for weapons and other evidence of wrongdoing” [1], either to dispose of the item and/or to destroy forensic evidence deposited on it. Water sources used for this purpose can be natural environments, such as lakes and rivers, as well as sources encountered in domestic and commercial settings including tap water and drains.

Fingermarks are a key piece of forensic evidence, and can persist on submerged, non-porous, items longer than touch DNA [2,3]. However, the recovery of latent fingermarks on items exposed to water environments can be challenging. Some visualisation processes target water-soluble constituents that may be washed away [4] and marks could be physically degraded by the aquatic environment.

There are numerous variables of interest that merit investigation when considering fingermark recovery from submerged items. Published studies have explored the longevity of submerged fingermarks [3, 5–7], with 12 week recovery times reported [6].

A variety of visualisation techniques have also been investigated in order to show efficacy for mark visualisation on wetted non-porous surfaces, including Lipid Dyes [6], Fingerprint Powders [3,8,9], Powder Suspensions [10–13], Small Particle Reagent [5, 14–16], Superglue Fuming [7, 17–20], and some novel techniques [21–24].

Another crucial factor that has been the subject of research is the type and location of water. One approach has been to submerge fingermarks in containers of collected water [3,16,20,25,26]. This allows

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[1] At the time of the study.
[2] Note: The Home Office Centre for Applied Science and Technology was integrated into Dstl on 1st April 2018.

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for control over more of the variables but does not truly replicate real world conditions. Other studies have been carried out in various field environments including the sea [6,13,19], rivers [6,19,27], lakes [6,9] and ponds [24]. These studies found that the longer the fingermarks were submerged, the lower the recovery rate. They also report that recovery rates from items submerged in field environments were lower than from their non-submerged control counterparts [9,13,27]. Studies have also suggested that water temperature may play a role in the speed of mark degradation [3,6].

Field studies are important for operational validity, and only two of the previous field studies found were based in the United Kingdom (UK) [6,19]. As water temperature (and therefore climate) is a contributing factor in the success of fingermark recovery, one of the aims of this study was to carry out field research in the UK to provide further data to inform local forensic strategies.

It can be difficult to compare or interpret the results of previous studies meaningfully due to differences in experimental approach. Many of the studies used groomed, sebaceous rich fingermarks [6,9,20,22,26,27], and it has been estimated that the total sebaceous mass of a groomed mark can be over represented by a factor of six [28]. Groomed marks are likely to have a much higher lipid content, and be less water soluble, enabling them to survive submersion for extended time periods, compared to the natural fingermarks that would be present on forensic evidence. Therefore, this study aimed to gain a better understanding of the persistence of natural fingermarks submerged in water. This also aligns with 'persistence and recovery' research considerations published by the Forensic Science Regulator for England and Wales, which include aspects such as determining the possibility of recovering fingermarks in various circumstances [29].

Studies were scarce that mentioned the effect of fingermark age before submergence [30]. This is an important consideration, because it is reasonable to expect that not all evidential fingermarks will be deposited immediately prior to submergence. Changes in latent fingermark residue occur over time [31–35] and if these changes significantly impact upon the subsequent visualisation of marks that are then immersed in water, then it is likely to be of interest to a forensic practitioner.

To gain a better understanding of the variables that can affect fingermark recovery following submersion in a field environment, three non-porous surfaces, with three different ages of naturally deposited fingermarks, were submerged in a lake for three different time periods during late summer in the United Kingdom (UK). The experimental work itself was carried out in 2016, but the data collected is still deemed relevant as the rate of climate change since this date would not have significantly altered the results. This paper also reflects on published research conducted after this date when discussing the results to fully consider the impact of the study.

During the course of this study a substantial algal bloom occurred in the lake [36]. Algae occur naturally in inland waters such as rivers, streams, and lakes, and when conditions are ideal for growth, an algal bloom can occur. During a bloom, algae cause the water to become less clear, block sunlight and deplete the oxygen [37]. This created a specific, although fairly common, field environment and the algae growth significantly influenced the outcomes of the study.

2. Methods and materials

2.1. Substrate preparation

A4 sized (210 × 297 mm) sheets of the following substrates were prepared:

- Stainless-steel (grade 304) purchased from Alloy Sales Ltd. (Hatfield, Herts, UK);
- Black high-density polyethylene (HDPE) purchased from RS Components Ltd. (Corby, Northants, UK);
- Black bin bags (40 x Tie Top Refuse Sacks, Item code 1370935), purchased from Sainsbury's Supermarkets Ltd. (Welwyn Garden City, Herts, UK).

These substrates were selected because they represent the material of items that are commonly encountered in operational casework, such as knives (stainless-steel), wheelie bins and other rigid plastic items (HDPE) and bags made from low density polyethylene (LDPE) (bin bags).

Holes were drilled into each corner of the stainless-steel and HDPE sheets to allow them to be fastened into crates ready for water submergence. To remove unwanted fingermarks or contamination from the surface of the sheets, they were cleaned before use. This involved pre-soaking the item in warm tap water with washing-up detergent, then wiping the surface with a new, soft, non-abrasive cloth, saturated with the detergent solution. The surface was then rinsed thoroughly with warm running water, and then rinsed with ethanol and wiped with disposable tissue. Lastly, the surface was rinsed with deionised water and left to air dry.

Due to the difficulties of cleaning the LDPE, a new roll of bin bags was purchased, and the handled outer layer of the roll was discarded. Sections of the bag were cut and attached to sheets of plastic mounting board. The mounting board had holes drilled into each corner before the bag sections were attached, to allow them to be secured into the crates.

2.2. Fingermark collection

Fingermark collection was carried out in line with published guidance [38,39]. Ten participants of varying age, gender, and ethnicity (demographic data not collected) each provided a depletion series of five natural, consecutively deposited fingermarks on to each sample. A mixture of known 'good', 'average' and 'poor' quality fingermark donors were used, based on results from previous studies using routine detection methods. Donors were asked not to wash their hands for 30 min prior to donation and were asked to thoroughly rub their hands together to create an even distribution of constituents across the fingers. Then they were asked to deposit marks on the samples using gentle pressure, under guidance.

The marks for each of the three ageing periods (Section 2.3) were deposited on the same day and to minimise a reduction in mark quality over the course of the donation session, each session was sub-divided. Donors used one finger per sample to deposit five marks, in a depletion series, on nine samples. They were then asked to wait a few minutes and rub their hands together before this was repeated on a further nine samples. Then, after a break of at least one hour, the entire process was repeated on the remaining 18 samples for that age group. This process was repeated, using the same donors, seven and 14 days later in order to collect fingermarks for all variables under investigation. In total, 108 samples were prepared and 5400 fingermarks were deposited for this study, shown in Fig. 1.

2.3. Ageing and submersion of fingermarks

Two sample sets were created, one designated 'non-submerged' (control) and one designated 'submerged' (experimental), with each set containing 2700 fingermarks. Sutton et al. [6] have shown that submerging items in laboratory conditions can give very different results to those submerged in the field, so the decision was made not to submerge the control samples. This allows the impact of submersion on fingermark recovery to be assessed in comparison to non-submerged samples. Another benefit of this approach is that the effectiveness of fingermark visualisation processes on non-submerged samples can also be determined in parallel to the submerged dataset.

Both sets of fingermarks were aged in the laboratory for one of three time periods: four hours, one week or three weeks before the experimental samples were secured to plastic crates, as shown in Fig. 2, ensuring space between each sheet to allow the circulation of water.

The crates were submerged into a water-sports activity lake in Hertfordshire, UK and were secured under the water to a floating dock on the lake, which was approximately 0.5 m deep at that location. Each time a crate was submerged or removed, the temperature and pH of the water were recorded. The recorded temperature values of the lake water ranged from 19.7°C to 24.0°C, with an average of 21.8°C, and the pH ranged from 8 to 9.

Samples were submerged during August and September for one of three time periods: 24 h, one week or three weeks, before being removed and allowed to air dry naturally in the laboratory. The non-submerged sample set was left in a laboratory storeroom with no natural light for equivalent periods of time, plus the initial time period during which the marks were ageing (Fig. 1). The temperature in the laboratory storeroom was monitored once a week and ranged between 12.7°C and 25.5°C during the study.

2.3.1. *Removal of contaminants*

Fig. 3 shows the condition of crates and substrates after removal from the lake for examination.

Substrates with visible silt or algae were washed prior to the application of fingermark visualisation techniques, in an attempt to remove any contamination. This involved soaking the substrates in clean, tepid water under gentle agitation, before rinsing under gently running cold tap water [40] and allowing to dry before chemical treatment. This cleaning method was selected as the additional water exposure would be unlikely to detrimentally affect the marks if performed with minimal force. It was selected as an alternative to dry brushing, which had proved unsuccessful and potentially damaging during a pilot trial. The approach used was only marginally successful, and the surfaces remained heavily contaminated.

2.3.2. *Visualisation processes*

Two sequential processing pathways were used on both the submerged and non-submerged items (Fig. 4), based on the two routes recommended in 'Chart 1', for Non-porous items in the Fingermark Visualisation Manual (FVM) (2014) [40]. The Visual Examination, Fluorescence Examination and Vacuum Metal Deposition steps at the start of the sequential pathway in Chart 1 were omitted from this study due to time constraints.

Several types of Powder, including magnetic, granular, flake and fluorescent varieties, were trialled on test samples. However, due to high background development or poor contrast, neither the HDPE nor LDPE were deemed suitable surfaces for powder application. This decision was supported by previous research, which suggested that in general Powders are not suitable for use on plastic bags [12]. Black granular Powder

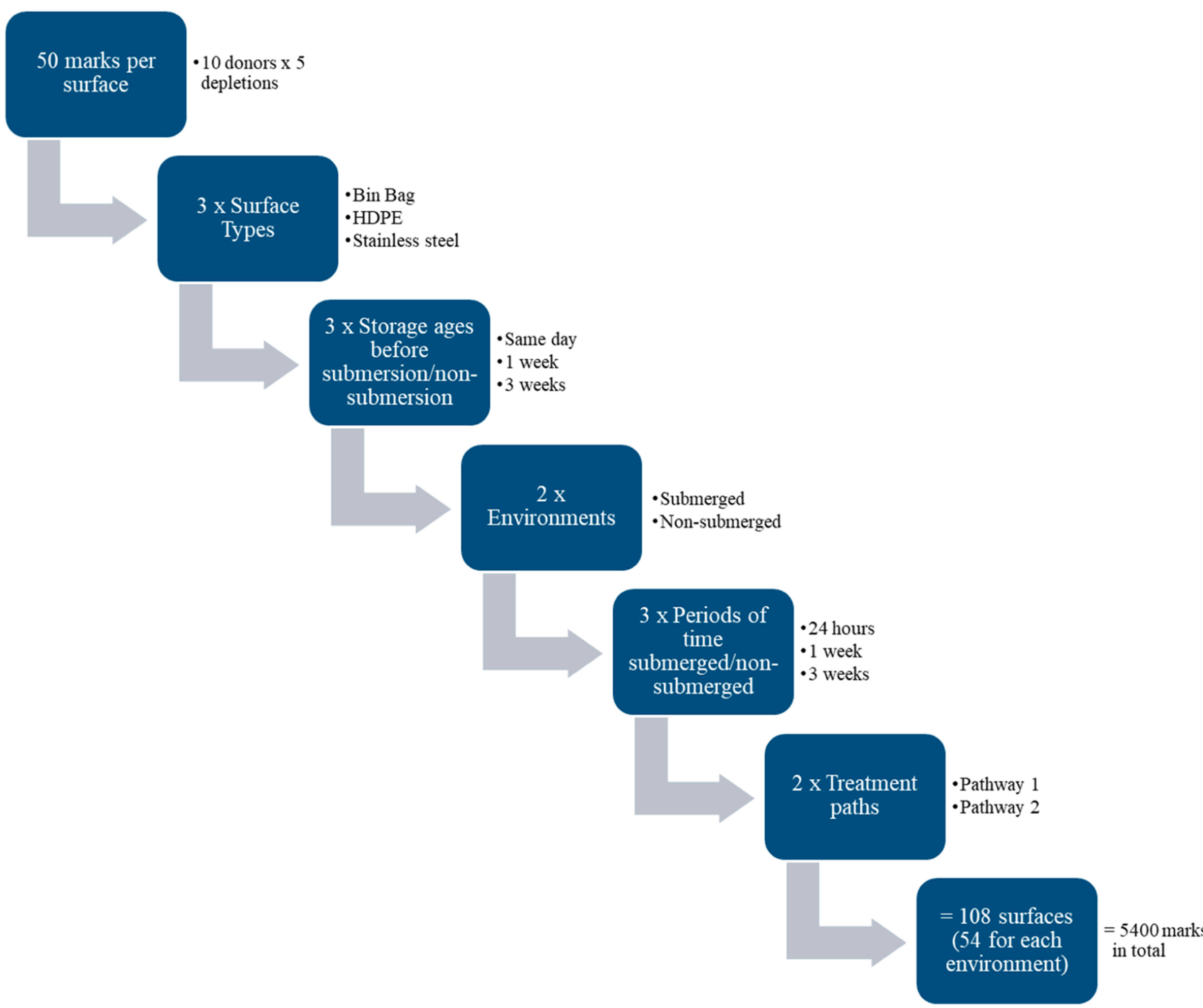


Fig. 1. Schematic showing the breakdown of the sample set.

was used on the stainless-steel samples, as it was found to be the most effective on the preliminary test samples. To achieve the best contrast, black Iron Oxide based Powder Suspension (IOPS) was applied to the stainless-steel samples and white Titanium Dioxide Powder Suspension was used on the LDPE and HDPE samples. Natural Yellow 3 was used in place of Basic Violet 3 to provide a contrast on the dark substrates. Details of the processes and visualisation methods used are given in Table 1.

2.4. Examination, grading and photography

All samples were examined and graded using a ‘linen tester’ eye glass with x5 magnification (as required) and then photographed using a Sony α77 digital camera with a Sony 2.8/50 mm macro lens, mounted on a copy stand, to create a record of the marks’ appearance. Details of light sources and viewing filters used are given in Table 1.

Visualised fingermarks were assessed by the researcher using a numerical grading scheme as shown in Table 2. Marks of grades 3 and 4 are generally considered to contain sufficient ridge detail for an identification to be made, while marks of grade 2 are occasionally ‘identifiable’ depending on the detail present [38]. Example fingermarks for each grade can be seen in Fig. 5. A total of 5400 fingermarks were deposited for this study, however, as the samples were subjected to sequential processing, the same marks were regraded leading to a total of 12600 grades. The number of additional marks graded 3 or 4 that were not deemed sufficient quality by the previous process have been also recorded. These results have been presented as a separate data set showing the unique marks recovered by sequential processing.

2.5. Mark grading - quality check

Once all grading was complete, 10% of the fingermarks were reassessed by the original grader after their final visualisation process, to check for consistency. Eleven samples (containing 550 marks), with a mix of mark ages and surface types, were selected and regraded (3 x HDPE, 5 x black bin bags and 3 x stainless-steel). The results were compared to the original grades to check if the regraded marks were within one grade of the original result.

2.6. Statistical analysis

To assess whether there was a statistical difference between the quality of fingermarks for submerged vs. non-submerged marks, or for factors of interest within these conditions (age of mark, time submerged, visualisation process, sequential pathway, and sample surface type), the grade 3 and 4 marks were compared using a difference of proportion test [42,43]. This was carried out using the 'prop.test' (two−sided) in R v4.4.1 [44] and all p − values were adjusted for multiple testing using the Bonferroni correction (multiplied by the number of tests). A closed testing approach was used in this case: if no statistically significant difference was found between the factors of interest, no further tests were carried out; if a statistically significant difference was found between the factors of interest, additional pairwise comparisons of the differences in proportions of grade 3 or 4 within each of the factor of interest were then carried out. In the event of further pairwise comparisons, additional Bonferroni correction was applied accordingly depending on the number of pairwise comparisons required.

Fig. 2. Samples secured into a crate before submersion into the lake.

3. Results and discussion

3.1. Non-submerged VS. submerged fingermarks

From the non-submerged samples, 37% of all fingermarks graded were assigned a grade 3 or 4 (2355 out of 6300 grades). From the submerged samples only 0.4% of all fingermarks graded were assigned grade 3 or 4 (24 out of 6300 grades). The breakdown of the data for non-submerged and submerged samples is available in Appendix 1. The statistics show a significantly greater proportion of grade 3 and 4 fingermarks were recovered from non-submerged samples overall. This was also the case when considering the factors of interest; age of mark, time submerged, visualisation process, sequential pathway, and sample surface type, ($p < 0.001$ in all cases).

This shows that the lake environment was very detrimental to fingermark recovery. As the controls in this study were not submerged, it is not known how much of the reduction in fingermark recovery from the lake samples can be attributed solely to the marks being submerged in water, and how much was due to the algal contamination. This study would need to be repeated to determine the cause of the low recovery rate, however, due to the extent of the algal contamination, it is thought likely that this had a significant impact on fingermark visualisation.

3.2. Non-submerged fingermarks

The purpose of the non-submerged fingermarks was to provide a control to allow assessment of the impact of submersion on fingermark recovery. However, the chemical treatment of the non-submerged fingermarks has also generated data about the effectiveness of visualisation processes on these sample types. This data could be used to support decision making when selecting visualisation processes for non-wetted surfaces (Fig. 6).

3.2.1. HDPE samples

Superglue Fuming with Basic Yellow 40 Dye Stain was the most successful chemical treatment process on the HDPE samples, with 60% of the 450 marks visualised with this process giving grade 3 or 4, compared to 56% for Powder Suspension, 62% with Natural Yellow after Superglue Fuming and 46% with Natural Yellow after Powder Suspension (Fig. 6). However, the small differences between these results were not statistically significant, so either sequential pathway could be successful on HDPE. This aligns with the Fingermark Visualisation Manual Second Edition ‘Process Selection’ chart recommendations, although Powder Suspension is stated to be generally more effective on rigid plastics [4]. It has been found that surface chemistry and roughness can have a significant impact on the performance of Powder Suspensions [45,46]. The HDPE used in this study had a slightly textured surface, which could have trapped the Powder Suspension particles causing background staining (Fig. 7), whereas the vapour deposition method of Superglue Fuming would not have been impeded by this texture.

However, it should also be noted that the Iron Oxide based Powder Suspension formulation in the Fingermark Visualisation Manual Second Edition [4] has been revised due to environmental legislation since this study was undertaken [47].

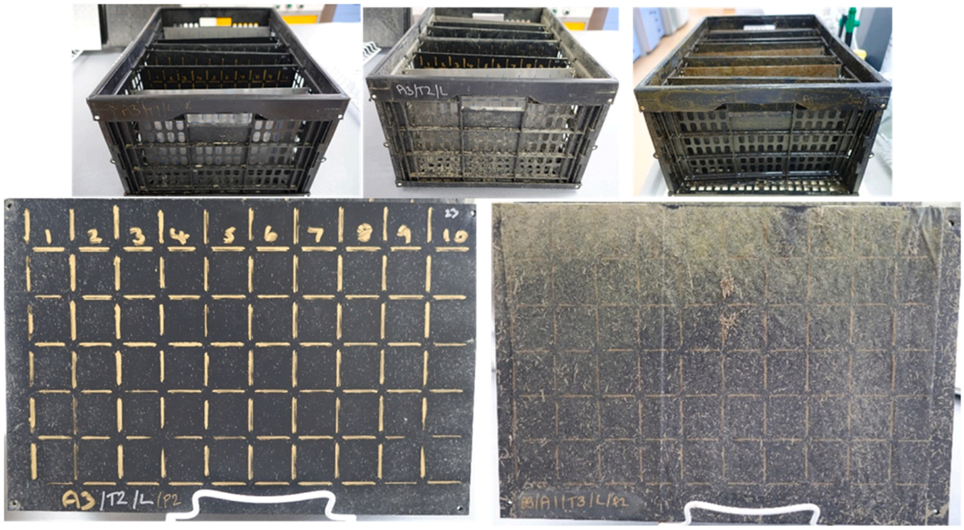


Fig. 3. Top row: Algal contamination on crates submerged for 24 h (left) one week (middle) and three weeks (right). Bottom row: Examples of algae contamination on a sample submerged for one week (left) and three weeks (right).

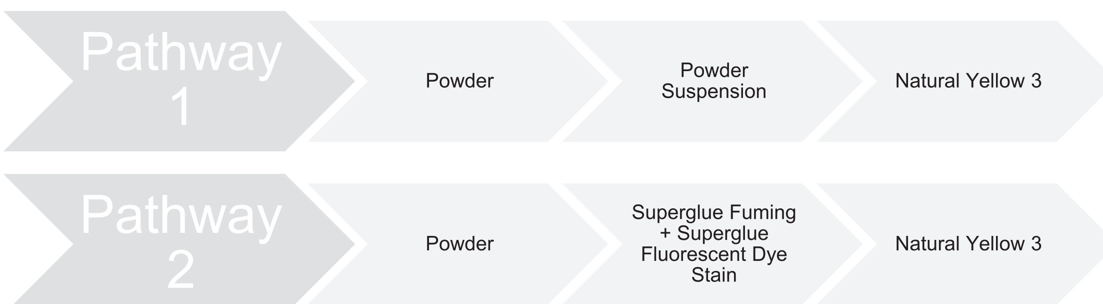


Fig. 4. The two sequential processing pathways used. Note: Powder was only applied to the stainless-steel samples.

3.2.2. Bin bag samples

No significant difference was detected between the proportions of grade 3 and 4 marks recovered by Powder Suspension (61%) or Superglue Fuming (60%) on the bin bag samples. This aligns with recommendations in the FVM Second Edition [4], specifically the 'Plastic Packaging (Soft)' sequential processing chart [4] as well as other studies reporting comparable numbers of fingermarks on flexible plastic films, such as plastic bags [12]. Both Powder Suspension and Superglue Fuming recovered significantly more marks than Natural Yellow ($p < 0.001$ for both comparisons). Natural Yellow caused substantial background staining on this surface, which could account for the lower number of marks recovered and shows the importance of spot testing processes before use where possible.

3.2.3. Stainless-steel samples

Stainless-steel was the most challenging substrate as it had the lowest recovery of 'identifiable' fingermarks. Fig. 6 shows that Powder (26% grade 3 or 4 marks) and Superglue Fuming followed by Basic Yellow 40 (24% grade 3 or 4 marks) were the most effective treatments. Both processes recovered significantly more grade 3 and 4 marks than Powder Suspension ($p < 0.001$) or Natural Yellow ($p < 0.001$), suggesting that Powder followed by Superglue Fuming may be a more successful route for stainless-steel. For 'Untreated Metals', the Fingermark Visualisation Manual Second Edition [4] states it is not known which sequential route (Superglue Fuming or Powder Suspension) is the most effective. This could be due to the wide variety of metals covered by this chart, so studies such as this one which include specific metals help inform operational guidance.

3.2.4. Added value of sequential processing

Fig. 8 shows the success rate of each additional visualisation process by displaying only the new marks graded 3 or 4 following the chemical treatment. Overall, 1365 (51%) of the 2700 marks deposited but not submerged were visualised to be of identifiable quality. 63% of the marks deposited on the HDPE samples and visualised via pathway 2 were deemed 'identifiable' (grade 3 or 4), the highest percentage out of all three surface types and sequential pathways. Corresponding difference of proportion tests identified that for stainless steel the proportion of grade 3 or 4 fingermarks for the P1 pathway (24%) versus P2 pathway (38%) was statistically significant (Bonferroni corrected $p < 0.0001$). However, for bin bags and HDPE, the corresponding proportions for P1 versus P2 pathways were not statistically significant (60.7% versus 60.0%; Bonferroni corrected $p > 0.999$, and 57.1% versus 63.1%; Bonferroni corrected $p = 0.230$ respectively).

For all of the sequences, the first visualisation process visualised the most grade 3 or 4 fingermarks, and the subsequent processes offered additional unique marks but to a much lesser extent. This shows the benefit of sequential processing in an operational context, in order to obtain the full evidential value from the exhibit.

3.3. Submerged fingermarks

3.3.1. Effectiveness of visualisation processes

Fig. 9 and Appendix 1 show the impact that submersion had on fingermark visualisation. Only 19 unique grade 3 or 4 marks were recovered from the submerged samples. This represents a recovery rate of only 0.7% of the 2700 marks in the submerged sample set. As the

Table 1
The fingermark visualisation processes, chemicals and equipment used in this study. Unless stated, the processing methods within the Fingermark Visualisation Manual (FVM)(2014) [40] were followed.

Fingermark Visualisation Process	Sample surface type	Chemicals	Processing conditions and equipment	Visualisation equipment
Powders	Stainless-steel	Black Dusting Powder (Keygrove Marketing Limited, Dunstable, UK)	-	White light illumination
Iron oxide-based Powder Suspension (IOPS)	Stainless-steel	*Working solution* 20 g Iron (II/III) Oxide (Fisher Scientific, Loughborough, UK) 20 mL Powder Suspension Stock Detergent Solution. *Powder Suspension Stock Detergent Solution* 350 mL Ethylene Glycol (Honeywell, Bracknell, UK & Sigma Aldrich, Gillingham, UK) 250 mL Triton® X-100 (Sigma Aldrich, Gillingham, UK) 400 mL Deionised water (In-house Sartorius water purification system)	-	White light illumination
White Powder Suspension	HDPE Bin bags	Wet Powder™, White (Kjell Carlsson Innovation, Sweden).	-	White light illumination
Superglue Fuming	Stainless-steel HDPE Bin bags	4 g Cyanobloom (Foster and Freeman, Evesham, UK)	20 min fuming time at 80% Relative Humidity (RH) in the MVC5000 Superglue Fuming cabinet (Foster and Freeman, Evesham, UK)	N/A. Visualised after Basic Yellow 40 Superglue Fluorescent Dye Stain only.
Basic Yellow 40 (BY40) Superglue Fluorescent Dye Stain (hereafter referred to as Basic Yellow 40 Dye Stain)	Stainless-steel HDPE Bin bags	2 g Basic Yellow 40 powder (35869–60–4) (Town End, Leeds, UK) 1 L Ethanol Absolute (99.8%) (Sigma Aldrich Gillingham, UK)	-	Blue Crime-Lite 82 s, 420–470 nm (Foster and Freeman, Evesham, UK) Schott GG495AG (476 nm) viewing goggles and camera filter (Foster and Freeman, Evesham, UK)
Natural Yellow 3 Lipid Dye.	Stainless-steel HDPE Bin bags	10 g (approx. 70%) Curcumin (Natural Yellow 3, CI 75300) 1 L Propylene Glycol Methyl Ether (PGME) 500 mL 1% Sodium Chloride solution in water 500 mL 1% Dioctyl Sulfosuccinate (DOSS) solution in water (All chemicals from Sigma Aldrich Gillingham, UK. Water from in-house Sartorius water purification system)	Formulation and method as used by Perry & Sears [41]. Samples were immersed in the working solution for 1 min.	Blue/Green Crime-Lite 82 s, 460–510 nm (Foster and Freeman, Evesham, UK) Schott OG550AG (529 nm) viewing goggles and camera filters (Foster and Freeman, Evesham, UK)

Table 2
The numerical grading scheme used to assess the quality of recovered fingermarks. Source: [38].

Grade	Level of detail
0	No evidence of a fingermark
1	Weak development. Evidence of contact but no ridge detail
2	Limited development - up to $^1/_3$ ridge detail
3	Strong development - between $^1/_3$ and $^2/_3$ ridge detail
4	Very strong development - more than $^2/_3$ ridge detail

same marks were graded after each sequential treatment, a total 24 marks graded 3 or 4 were recovered, which represents only 0.4% of all fingermarks graded (24 out of 6300 grades). Due to the small numbers of marks, conclusions about the effect of visualisation processes must be treated with caution.

Within the limited results Fig. 9 shows Powder was the most effective visualisation process on the submerged samples, with a statistical pairwise comparison of the differences in proportions of grade 3 and 4 fingermarks showing that Powder visualised a significantly greater proportion of grade 3 and 4 marks than any other process ($p = 0.030$ with Superglue Fuming and Basic Yellow 40 Dye Stain, $p = 0.012$ with Powder Suspension and $p = <0.0001$ with Natural Yellow).

Other studies have also had success using Powders to recover wetted marks [3,8,9,25], and it has been suggested that the Powder may adhere to sebaceous, non-water-soluble, components in the fingermark [48,49] which could survive the submersion.

The success of Powder on the submerged samples in this study could be because Powder was used only on the stainless-steel samples and, in general, these samples had the least algal growth. This could also account for the fact that stainless-steel samples had the highest recovery rates of all the submerged sample types, in contrast to the non-submerged samples, where it had the lowest recovery rates (Fig. 6). However, due to the low number of marks recovered, there was no significant difference in proportions of grade 3 and 4 marks between any of the surface types for submerged samples ($p = 0.123$).

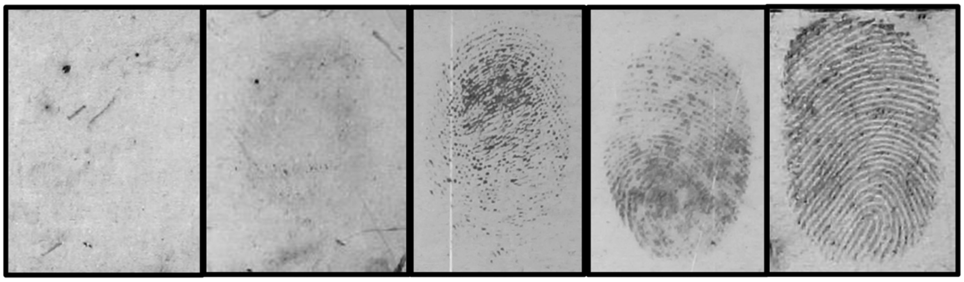
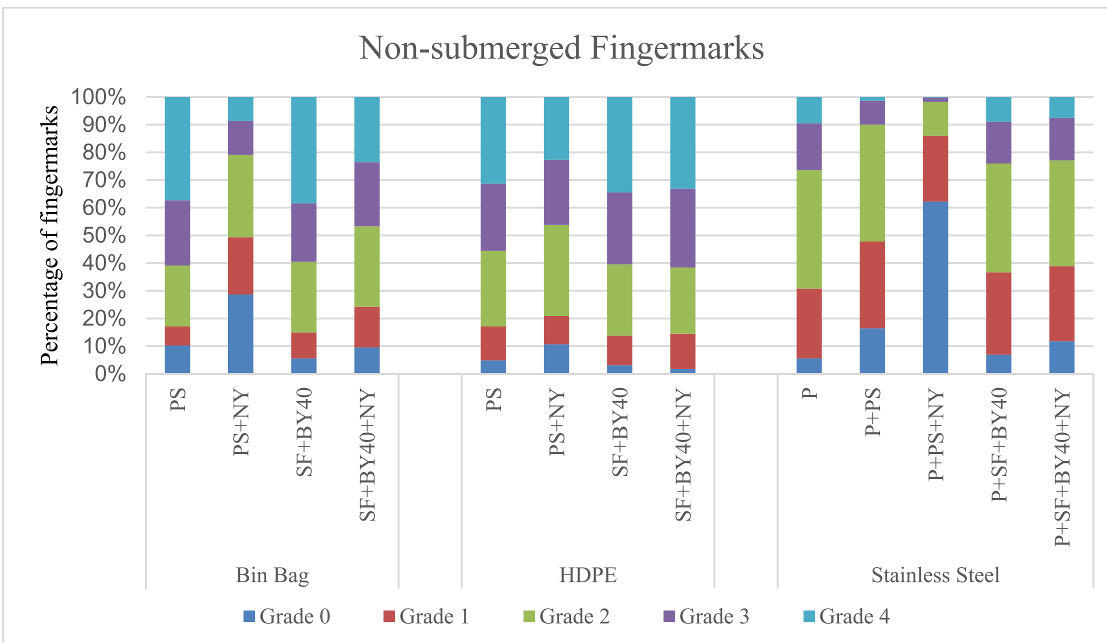


Fig. 5. Example marks for each grade. From left to right grade 0, 1, 2, 3, 4. Images taken from stainless-steel non-submerged samples, treated with Powder.



	Bin Bag					HDPE					Stainless Steel				
Percentage of marks	PS	PS+NY	SF+BY40	SF+BY40+NY		PS	PS+NY	SF+BY40	SF+BY40+NY		P	P+PS	P+PS+NY	P+SF+BY40	P+SF+BY40+NY
Combined Grade 3+4	60.9	20.9	59.6	46.7		55.6	46.2	60.4	61.6		26.4	10.0	1.8	24.0	22.9
Grade 4	37.3	8.7	38.4	23.6		31.3	22.7	34.4	33.1		9.6	1.3	0.2	8.9	7.6
Grade 3	23.6	12.2	21.1	23.1		24.2	23.6	26.0	28.4		16.9	8.7	1.6	15.1	15.3
Grade 2	22.0	29.8	25.6	29.1		27.3	32.9	25.8	24.0		42.8	42.2	12.2	39.3	38.2
Grade 1	6.9	20.7	9.3	14.7		12.2	10.2	10.7	12.7		25.2	31.3	23.8	29.8	27.1
Grade 0	10.2	28.7	5.6	9.6		4.9	10.7	3.1	1.8		5.6	16.4	62.2	6.9	11.8

Fig. 6. The effectiveness of the visualisation processes on each sample type for non-submerged substrates showing the percentage for all grades of fingermarks visualised following each process. The number of fingermarks graded per bar is 450, except for Stainless Steel Powder where 900 marks were graded (this data has been combined for simplicity as the trend was consistent between P1 and P2 datasets). Due to sequential visualisation, individual marks may have been represented more than once in this figure and differences in totals may be affected by rounding. PS = Powder Suspension, NY = Natural Yellow, SF = Superglue Fuming, BY40 = Basic Yellow 40 dye stain, P = Powder.

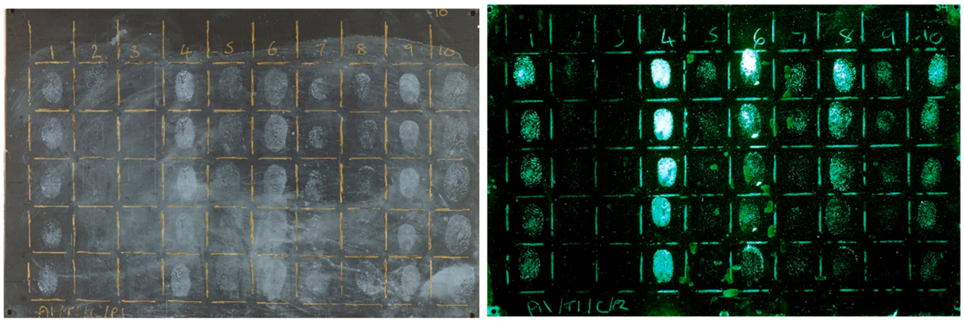


Fig. 7. An example of background staining observed with Powder Suspension (left) and no background staining with Superglue Fuming (right) on non-submerged HDPE samples.

Fig. 9 shows Superglue Fuming with Basic Yellow 40 Dye Stain was the next most successful process on the submerged samples, after Powders, although it was not significantly more effective than the other processes. The visualisation ability of Superglue Fuming was not anticipated as the water-soluble components of the fingermark that are thought to initiate the polymerisation process are likely to be washed away. A red indicator symbol to denote the negative effect of water on the process was depicted in the Fingermark Visualisation Manual (FVM) (2014) at the time of the study, and text noting it is "generally ineffective on items or surfaces exposed to water as it only targets water soluble components" [40]. However, this does depend on the mark properties and the environment to which it's exposed. Older marks, or those exposed to salt water (rather than fresh water), are more likely to be recovered [30].

The reason for the relative success of Superglue Fuming on the submerged samples may just be that the process was more suited to these sample types generally, as overall more fingermarks at grades 3 and 4 were found with Superglue Fuming than Powder Suspension on the non-submerged samples too (Fig. 6). However, several other studies have also found Superglue Fuming to be an effective process to recover marks that have been submerged in water. Although their methodology varied and often used groomed, sebaceus rich marks, so the results cannot be directly compared to this study [3, 7, 17–20, 50, 51]. Needham et al. [7] attributed the success of Superglue Fuming over Iron Oxide Powder Suspension on groomed, sebaceous rich fingermarks to the survival of Superglue polymerisation factors within the sebaceous deposits on the fingermarks. Due to the growing evidence that Superglue Fuming can be effective on wetted items, the FVM Second Edition (2022), published after this study was completed, has downgraded the effect of water on Superglue Fuming to an amber indicator symbol [4].

Powder Suspension was expected to be one of the more successful processes in this study. It is thought to be less detrimentally affected by water than Powders or Superglue Fuming, as it may target constituents held within a water insoluble matrix [4]. However, in this study, it was found that the submerged samples treated with Powder Suspension often had a high degree of background staining, potentially limiting its success by obscuring marks. The ability of Powder Suspensions to strongly develop traces of plant matter (such as grass stains) has been observed in previous studies on footwear marks [52], so it is possible the process was interacting with algal contamination on the samples (see Section 3.3.3). Although Superglue Fuming and Basic Yellow 40 Dye Stain also gave some background staining, it was of a lesser extent, with mark visualisation being further enhanced by fluorescence, as shown in Fig. 10. Also, as noted in Section 3.2, surface texture and chemistry can affect the performance of Powder Suspension, therefore it may be that the surface types used in this trial were not suited to the use of Powder Suspension.

Fig. 9 shows Superglue Fuming produced a slightly higher proportion

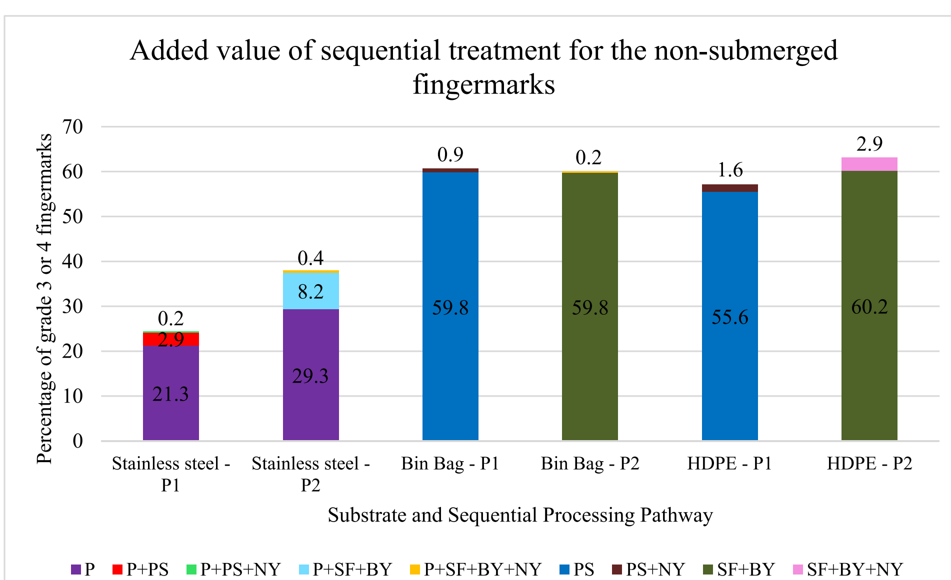
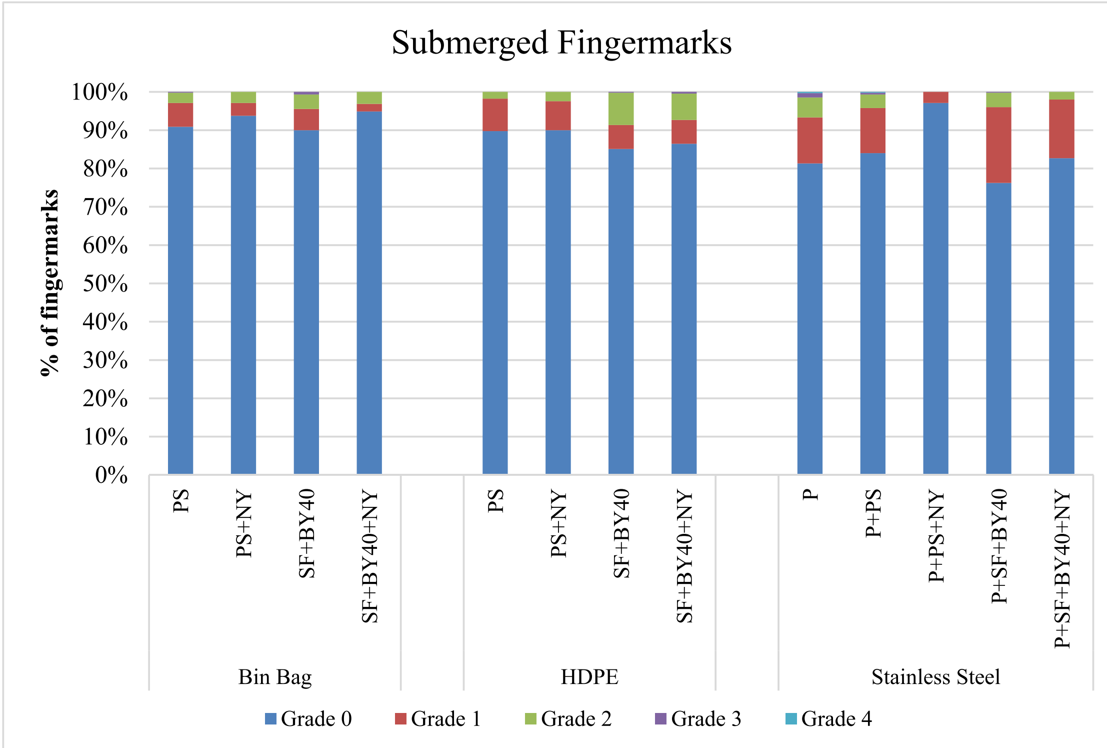


Fig. 8. Added value of sequential treatment for the non-submerged substrates showing the results for the percentage of fingermarks graded 3 or 4 per pathway. Individual marks are counted only the first time they are graded 3 or 4, so only unique additional marks are shown with each subsequent technique. P1 = Pathway 1, P2 = Pathway 2, PS = Powder Suspension, NY = Natural Yellow, SF = Superglue Fuming, BY40 = Basic Yellow 40 dye stain, P = Powder.



	Bin Bag				HDPE				Stainless Steel				
Percentage of marks	PS	PS+NY	SF+BY40	SF+BY40+NY	PS	PS+NY	SF+BY40	SF+BY40+NY	P	P+PS	P+PS+NY	P+SF+BY40	P+SF+BY40+NY
Combined Grade 3+4	0.2	0.0	0.7	0.0	0.0	0.0	0.2	0.4	1.4	0.7	0.0	0.2	0.0
Grade 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.0
Grade 3	0.2	0.0	0.7	0.0	0.0	0.0	0.2	0.4	1.1	0.4	0.0	0.2	0.0
Grade 2	2.7	2.9	3.8	3.1	1.8	2.4	8.4	6.9	5.2	3.6	0.0	3.8	2.0
Grade 1	6.2	3.3	5.6	2.0	8.4	7.6	6.2	6.2	12.0	11.8	2.9	19.8	15.3
Grade 0	90.8	93.7	90.0	94.8	89.7	90.0	95.1	86.4	81.3	84.0	97.1	76.2	82.6

Fig. 9. The effectiveness of the visualisation processes on each sample type for submerged fingermarks. The number of fingermarks graded per bar is 450, except for Stainless Steel Powder where 900 marks were graded (this data has been combined for simplicity as the trend was consistent between P1 and P2 datasets). Due to sequential visualisation, individual marks may have been represented more than once in this figure and differences in totals may be affected by rounding. PS = Powder Suspension, NY = Natural Yellow, SF = Superglue Fuming, BY40 = Basic Yellow 40 dye stain, P = Powder.

of grade 3 and 4 marks than Powder Suspension as a single visualisation process. However, when the results for the two sequences (Fig. 4) for the submerged samples were considered as a whole, there was no statistically significant difference in proportions of grade 3 and 4 fingermarks between the two pathways ($p > 0.999$).

3.3.2. *Effect of mark age prior to submersion*

The grades of fingermarks recovered from samples submerged in the lake, for each age of mark before submersion, are presented in Appendix 1.

Identifiable fingermarks were recovered from all three mark ages, although recovery was significantly lower than the non-submerged marks. Only five identifiable fingermarks were recovered from the fingermarks aged for four hours, fourteen from those aged one week and five from those aged for three weeks.

A previous study found that the effectiveness of Superglue Fuming depended on the age of the fingermark prior to wetting, with older marks being more likely to develop than fresh marks [30]. Therefore, an aim of this study was to see if this trend was observed. However, when comparing the proportions of grade 3 and 4 fingermarks on the submerged samples, no statistically significant differences were found between the different mark ages ($p = 0.203$). These results may have been affected by exposure to varying levels of algal contamination. Due to the logistics of the study, not all samples were in the lake at the same time, meaning they were exposed to slightly different environmental conditions, and increasing levels of algae as time went on. The samples aged for three weeks, which were submerged last, were likely to have been more affected by algal contamination than samples submerged at the beginning of the study, likely adversely affecting the results of the oldest marks. In future studies, consideration should be given to ensuring more consistent environmental conditions for the different aged samples.

3.3.3. Effect of length of time submerged

The grades of fingermarks recovered from samples submerged in the lake, for each length of time submerged are presented in Appendix 1. The shorter timeframe of submersion led to significantly more ($p < 0.001$) grade 3 and 4 fingermarks being recovered. In total, 24 grade 3 and 4 marks were recorded after 24 h, (19 of these were unique and 5 were visualised by more than one chemical process), whereas no fingermarks were recovered after one or three weeks of submersion.

The lack of identifiable fingermarks recovered from the samples submerged for one week or three weeks was not anticipated when this study was designed, as other researchers have recovered marks after longer periods of submersion, including after 28 or 30 days [7,15], 45 days [26] and even up to 95 days [6]. However, researchers have also found that mark numbers and mark quality decrease with increasing periods of immersion [16,25,26], which supports the findings of this study.

The specific field environment is critical to explaining the difference in findings between the studies. It is known that water type can affect fingermark recovery; for example salt water has been found to be less damaging to marks than fresh water [4,18,50,53], and other factors such as current [27], position [9], or temperature may play a role, with lower temperatures slowing mark degradation, and increasing the chances of fingermark visualisation [3,6]. During this study the water temperature in the lake was quite warm, between 19.7°C and 24.0°C, so the higher temperature may have contributed to more rapid mark degradation.

Most impactfully for this study, the fingermark samples were contaminated by the presence of algae in the lake. The algae coated the samples, potentially obscuring marks and causing background development when chemical processes were applied. The longer the samples were submerged, the greater the algal contamination (Fig. 3). Therefore, it is not possible to say whether the marks had been destroyed or were 'washed away' by the water, or if they were present on the samples but were obscured under a layer of algae contamination, despite attempts to remove it. Further work to determine which situation occurred could include more aggressive algae removal techniques, or potentially chemical profiling of the samples using Mass Spectrometry techniques to determine if fingermark residues are present.

Interestingly, some of the samples submerged in the lake for 24 h showed preferential algae growth on the background surface. It is unlikely this is a film coating the surface during submersion due to the uneven appearance, and instead it is postulated that the fingermark residue initially inhibited the attachment or growth of the algae. These voids in the algal growth may explain why fingermarks could still be visualised in this sample set. Examples of this can be seen in Fig. 11. Samples that were in the lake for longer periods became more uniformly coated in algae and this pattern was not observed.

Other studies have also commented on algae or other microflora growth when substrates were submerged in water tanks [6,54], noting that it may have affected fingermark visualisation, so this issue is not unique to field environments. Unless the effect of algae is the intended subject of the work, future work should be planned carefully to minimise the potential for algal growth.

At a different time of year, a different climate, a different location, or if there was no algal bloom the results of this study may have been markedly different. This highlights the difficulties of extrapolating the results, not only from laboratory experiments to operational field environments, but also from one field environment to another. It also illustrates the importance of more extensive field research in varying conditions, to gain a better understanding of the factors that affect mark quality [9] and persistence [29].

3.4. Mark grading - quality check

Of the 550 marks that were regraded, 65 marks (11.8%) were found to be one grade different from the original, and no marks were more than two grades different. The small amount of variability found is consistent with work by Fritz et al., which found that when researchers were asked to regrade marks, 78% of grades were identical to their original score. The study by Fritz et al. concluded that inter- or intra-grader variability did not cause significant differences to data and that a grading scale was an appropriate and consistent technique for assigning values to fingermark samples [55]. This provided confidence in the data gathered throughout the duration of the study.

4. Conclusion

The results of the study have shown that submerging fingermarks in a lake in the UK had a significant impact on the recovery rates. A total of 2700 fingermarks were deposited onto black bin liners, HDPE and stainless-steel samples which were then submerged in the lake and subjected to sequential treatment. Only 0.7% of all marks deposited on submerged samples were of identifiable quality, in comparison to 51% of the fingermarks deposited on the non-submerged samples. The results from the non-submerged samples provides data about the effectiveness of the visualisation processes trialled on these sample types, which could be used to support practitioners' decision making when selecting visualisation processes for non-wetted surfaces. However, the small number of marks recovered from submerged samples means guidance on the most suitable techniques is not possible with any degree of confidence, and the results obtained should be interpreted with caution.

The results showed that for the submerged HDPE and bin bag samples, there was no significant difference between the two sequential processing paths offered in the FVM (2014) [40] for non-porous surfaces. However, when considering individual processes, Superglue

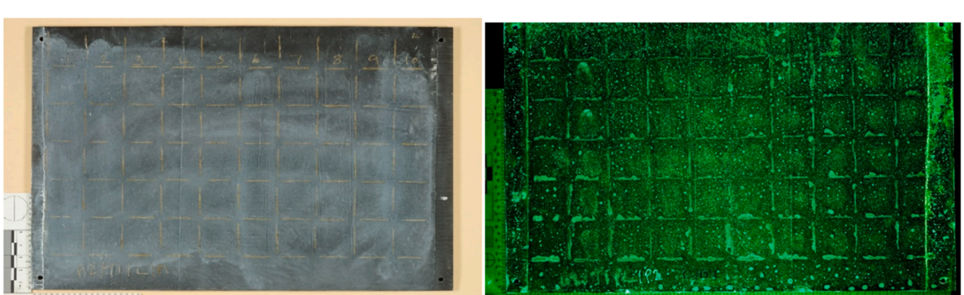


Fig. 10. An example of differential background staining observed. Powder Suspension (left) and Superglue Fuming with Basic Yellow 40 Dye Stain (right) on bin bag samples that had been submerged in the lake for 24 h.

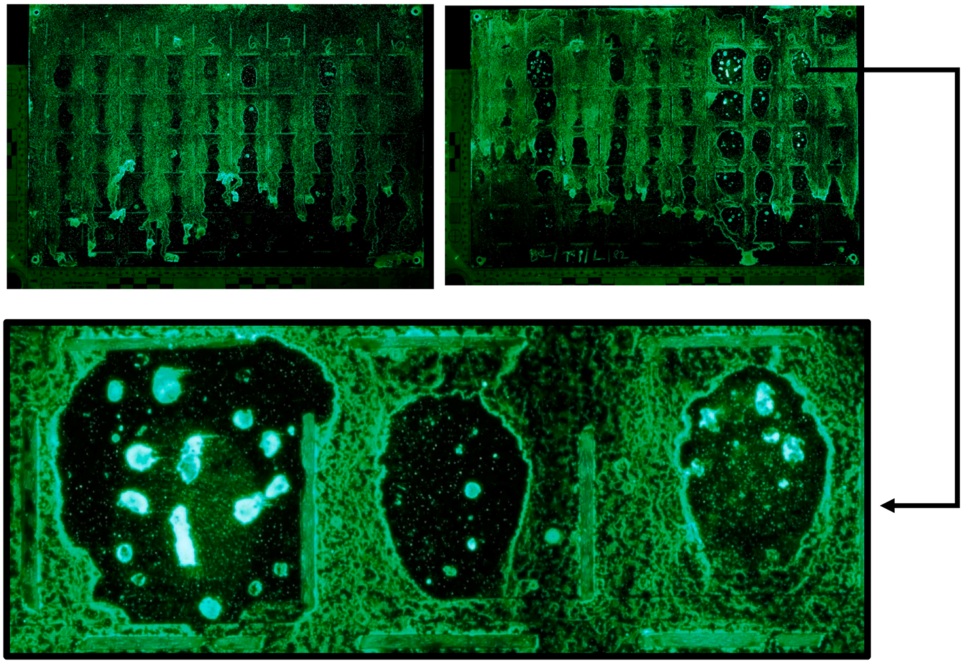


Fig. 11. Top row: Images of stainless-steel (left) and HDPE (right) samples submerged in the lake for 24 h and treated with Superglue Fuming and Basic yellow 40 Dye Stain. Bottom row: close up of marks on the HDPE sample, showing preferential algae growth on the surface surrounding the marks. Visualised using Blue Crime-Lite 82 s, 420–470 nm (Foster and Freeman, Evesham, UK) with Schott GG495AG (476 nm) viewing goggles and camera filter (Foster and Freeman, Evesham, UK).

Fuming with Basic Yellow 40 Dye Stain produced slightly more grade 3 and 4 marks than Powder Suspension on both the submerged and non-submerged samples. It should be noted that the difference between the two was only found to be statistically significant on the non-submerged stainless-steel samples ($p < 0.001$). The recovery of marks from the submerged samples treated with Superglue Fuming was not expected due to the water soluble residue which the process preferentially targets [40]. However, due to the growing evidence (including this study) that suggests water is not as destructive as previously thought for this technique, UK guidance has been subsequently updated [4].

One aim of this study was to investigate whether the age of a latent fingermark before wetting affected the quality of recovered marks in a natural water environment, however no significant trend was observed. These results were significantly affected by algae growth, a largely unreported feature in water-based studies. Algae growth resulted in recovery of identifiable marks only from the samples submerged for 24 h. Further work could include investigations into more successful methods from removing algal contamination and gaining a better understanding of the effects of water on Superglue Fuming.

This study demonstrates how variable the field environment can be, making cause and effect more difficult to attribute than a laboratory trial. Other factors, such as currents, debris, animals, plants or climate conditions, were not investigated in this study but are likely to impact operational results. This highlights the requirement for further work based on common case circumstances in localised environments.

CRediT authorship contribution statement

Maishman Thomas: Writing – review & editing, Formal analysis. **Sarah Fieldhouse:** Supervision. **Sears Vaughn G:** Supervision. **Cutler Deborah Marie:** Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hussey Laura Joy:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1

Data for non-submerged and submerged samples for each mark age and length of time submerged (all sample types and fingermark visualisation processes combined). NOTE: After ageing in the laboratory, the 'submerged' samples were submerged in the lake. The non-submerged samples remained in storage for the equivalent length of time

Experimental condition	Mark age	Length of time subjected to experimental condition (after ageing)	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Number of marks graded
Non-submerged	4 h	24 h	116	128	161	138	157	
Non-submerged	4 h	1 week	100	143	213	149	95	

(continued on next page)

(*continued*)

Experimental condition	Mark age	Length of time subjected to experimental condition (after ageing)	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Number of marks graded
Non-submerged	4 h	3 weeks	88	137	280	108	87	
		Sub-total:	304	408	654	395	339	2100
Non-submerged	1 week	24 h	83	108	194	133	182	
Non-submerged	1 week	1 week	113	122	227	137	151	
Non-submerged	1 week	3 weeks	75	159	192	141	83	
		Sub-total:	271	389	613	411	416	2100
Non-submerged	3 weeks	24 h	56	136	220	137	151	
Non-submerged	3 weeks	1 week	62	117	264	107	150	
Non-submerged	3 weeks	3 weeks	130	119	202	105	144	
		Sub-total:	248	372	686	349	445	2100
		Total (non-submerged):	823	1169	1953	1155	1200	6300
Submerged	4 h	24 h	506	144	45	5	0	
Submerged	4 h	1 week	683	17	0	0	0	
Submerged	4 h	3 weeks	698	1	1	0	0	
		Sub-total:	1887	162	46	5	0	2100
Submerged	1 week	24 h	506	111	69	10	4	
Submerged	1 week	1 week	669	31	0	0	0	
Submerged	1 week	3 weeks	698	1	1	0	0	
		Sub-total:	1873	143	70	10	4	2100
Submerged	3 weeks	24 h	434	152	109	5	0	
Submerged	3 weeks	1 week	612	80	8	0	0	
Submerged	3 weeks	3 weeks	700	0	0	0	0	
		Sub-total:	1746	232	117	5	0	2100
		Total (Submerged)	5506	537	233	20	4	6300
							Total (All marks)	12600

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