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Exploring the influence of washing activities on the transfer and persistence of fibres in forensic science

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ABSTRACT

In forensic science, a robust and sound interpretation and evaluation of transferred fibre evidence requires an understanding of the principles and mechanisms that underpin fibre transfer, yet existing research lacks consistency and repeatability. This study investigates the impact of washing activities on both the release of fibres into wastewater and the transfer of constituent fibres from donor garments to receiver swatches. Using a low-cost friction tester and automated data collection through photography and ImageJ image processing software, controlled conditions were maintained for repeated experiments. Results indicated significant fibre release during wash cycles, with load size and donor garment history playing crucial roles. The donor garments subjected to repetitive washes exhibit a progressive decrease in the number of fibres transferred, independently of the load size. This study underscores the importance of considering a garment's washing history in forensic science contexts, but also for consistency in the way that data are collected.

1. Introduction

Textiles and fibres are omnipresent in modern society, extending beyond clothing to everyday items such as upholstery, surfaces such as carpets and curtains, and vehicles. The ubiquity and diversity of fibres make them valuable in forensic science for studying transferred fibres at crime scenes or on individuals to determine their characteristics and potential sources. Fibre transfer can occur through direct contact between a textile and a surface, leading to primary transfers, as well as indirectly between two surfaces. This indirect fibre transfer refers to the exchange of evidence with no direct contact between the original source and the location or surface on which it is retrieved. The number of indirect steps is generally unknown when referring to indirect transfer, but can be referenced as, for example, secondary transfer when it is established that just one single step occurred after the initial deposit.

In forensic science, the transfer and persistence of fibres has been studied by many authors over the past 50 years, with a focus on primary transfer [1–12] and secondary transfer [3,13–15]. From these studies, several parameters were identified to have a potential impact on the transfer and persistence of fibres, such as the fibre type, their morphology and thickness, the fabric texture and manufacture, the method of transfer and the applied pressure associated with the transfer event and the post-transfer activities (such as wear and tear and cleaning). Despite a relative consensus on the impact of these parameters on the transfer of fibres, the analysis of the literature highlighted a large variety of materials (i.e. textiles) and methodologies: a wide range of transfer activities have extensively been investigated, employing various techniques such as manual pressure [15,16], rolled up and twist [17], shake [3] and wrapping [11]. Systems testing conditions close to real-world scenarios have also been examined to gain insights into activities encountered in casework [3,7,9,12,18,19], including simulated physical assault, simulated smothering, sleeping, sitting on car seats and chairs, and wearing of items. However, a large majority of the experimental methods provide insufficient details which, combined with a paucity of data [20], cause issues for the reproducibility and replicability of the works. Both reproducibility and replicability are essential to scientific data integrity and validity as they enhance the reliability of the results while also posing challenges to making meaningful comparisons.

A relatively unexplored area in forensic science is the impact of washing activities on fibre transfer and persistence. Although

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publications in forensic science commonly address the persistence of biological fluids [21–24] and the persistence of fibres on difference surfaces [3,15–17], there is minimal available information on the effects of washing cycles on fibre transfer and persistence. One study by Bresee and Annis [5] is dedicated to the effect of the use of softener in the transfer of fibres. This study holds a pioneering status as it was the first publication to explore the effects of washing in fibre transfer. The authors used softener but did not use detergent, which differs from typical washing practices where detergent is commonly used. This makes it challenging to identify the different parameters of the washing process that impact the transfer in real-life scenarios

To address these gaps, this work aimed to design a universal experimental protocol (i.e., repeatable, practical, and transparent) for data collection and analysis for future research in fibres transfer and persistence in forensic science. This study focusses on the validation of this method by studying the effect of repetitive washes on the transfer of constituent fibres from donor to receiver garments. In addition, the research addresses the impact of washing activities on the release of constituent fibres from a garment into the wastewater associated with the washing cycle. Szewcow et al. [15] and by Watt and Roux [25]; however, it was not the primary focus of their studies. Consequently, this research aims to specifically address secondary transfer, thereby enhancing the understanding of fibre transfer dynamics in various contexts.

2. Material and methods

2.1. Donor garments

The donor garments selected were identical round neck knitted jumpers of 100 % cotton, colour red, women's UK size 16 (n= 7) and 12 (n=12), see Fig. 1-A. The average number of stitches per cm^2 was found to be approximately 63. Each garment was given an identification number attached to its label. One garment was kept as a control garment (i.e., never washed). A total of 10 distinct contact areas (CA) of 20 cm × 3 cm strips, shown in Fig. 1-A, were identified on each donor garment using a paper template of an overall size of 27 cm × 46 cm. Five contact areas had their length aligned parallel to the rib of the knit of the garment (annotated CA1 to CA5) and 5 perpendicular to the rib of the knit of the garments (annotated CA6 to CA10). Fig. 1-B shows the parallel and the perpendicular orientation.

2.2. Receiver garments

The receiver textile selected for all the series was a plain (white) weave fabric, made of light to medium weight 100 % cotton of approximately 111 g/m^2, see Fig. 2-A. For the transfer experiments, the receiver textile was cut into 5 cm × 5 cm and attached to one face of a Perspex cube using double-sided sticky tape, as presented in Fig. 2-B. To preserve the textile properties such as strength, flexibility and elasticity, each piece of textile was tied to the Perspex cubes without being stretched. The samples were cleaned with a spray duster after being made and stored in metal boxes to prevent contamination and electrostatic effects. Each sample was only used once per experiment.

A positive control (receiver swatch) was created by performing a transfer from the control donor textile (as received and never washed) with an 800 g weight for a 20 cm length (see transfer experiment section for the full experimental protocol). The negative control was a Perspex block with receiver textile, but with no transfer performed. Both controls were stored in the same box to explore storage-related cross-contamination; this investigation did not reveal any significant cross-contamination.

For the secondary transfer experiments, the receiver textile (identical to the one used for the transfer series (Fig. 2-A) was cut into 31 cm × 31 cm squares and stored in large plastic boxes to prevent contamination and covered with paper to prevent an electrostatic effect. A total of 15 receiver samples were prepared for this set of experiments.

2.3. Washing procedure

The washing machine used for this study was a Montpellier, model MW7140S, 7 kg load. Two different cycles were selected for the experiments, one to wash the garments (60 min - daily wash programme of the machine, 40 °C, 1200 rpm) and one to clean the washing machine after each washing cycle (15 min- rapid wash programme of the machine, no spin, no set temperature). The rinsing cycles, performed 4–6 times, were necessary to remove the large majority of fibres remaining in the washing machine after the donors were washed, ensuring that any remaining fibres were not significant and did not affect the results

The donor garments were washed in separate series without laundry detergent, under the same washing condition, as described above. After each wash, the donor garments were left to air dry for a minimum of 20 h, on a drying rack covered with brown paper to limit possible contamination.

A total of three different sets of repetitive washing were performed,

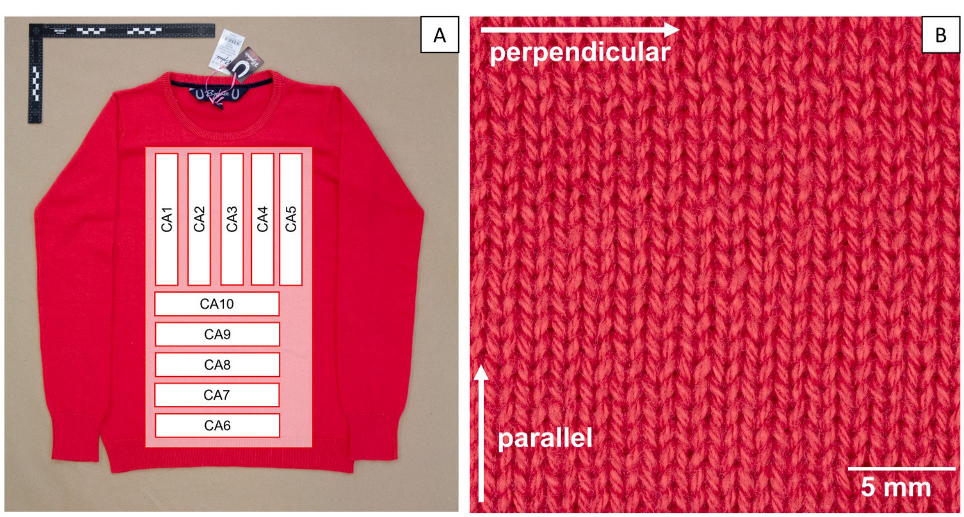

Fig. 1. 100 % cotton donor garment, with (A) the full garment with the location of 10 contact areas (CA), (B) details of the mesh, the arrow showing the perpendicular and parallel orientations on the mesh.

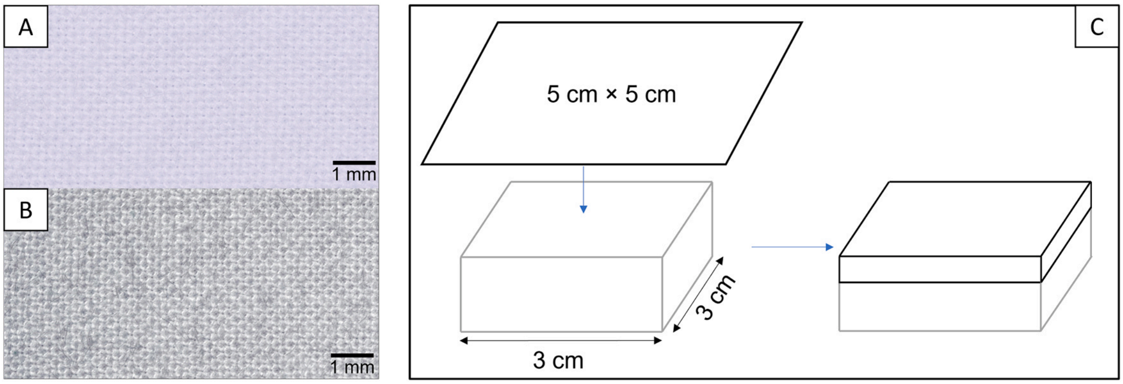

Fig. 2. Receiver textile, in (A) details of the mesh, (B) details of the mesh with digital enhancement for improved visualization, (C) Receiver swatches: a piece of receiver garment is placed on top of a 3 cm × 3 cm Perspex block.

as presented in Table 1. The repetitive wash cycles were carried out until a number of constituent transferred fibres (see photography and fibre counting section for more details) reached a plateau between subsequent wash cycles. All the donor garments were individually labelled using letters on the collar label to facilitate identification after each wash.

2.4. Wastewater volume and fibre release

A water filtering system was designed to collect fibres from the wastewater, as shown in Fig. 3-A. Once the wash cycle was over, the volume of wastewater was measured on a graduated scale placed on the side of the barrel (uncertainty calculation for the graduation of the barrel is available in section I of the supplementary information). After each wash cycle, the wastewater was manually stirred to homogenise the distribution of the fibres and filtered using a stainless-steel filter (Spectra Mesh®, 90 mm diameter, 105 µm, see Fig. 3-C) clamped in a purposely designed Perspex holder (Fig. 3-B). Once dry, the filters were weighed with a precision laboratory balance (Mettler AT200), which was calibrated regularly. The mass of the filters was recorded alongside a reference filter (unused and kept as received) before and after each filtration. The mass of fibres was calculated as the difference between the mass of the filter before and after filtration, normalised to the reference filter. An example of mass recording for a given experiment as well as the uncertainty calculation is available in the supplementary information (section II).

2.5. Secondary transfer experiments

This experiment consisted of studying the secondary transfer of the red fibres from the 5 red jumpers previously washed together (medium load, 2^{nd} series), present in the washing machine, to new receiver textiles during a wash cycle. Once a plateau in the number of transferred fibres between wash cycles was reached, no rinse cycle was performed. A total of 15 identical receiver textiles (31 cm × 31 cm, white, 100 % cotton) were then used as receiver material and were washed one at a time, one after the other, under the same washing conditions (60 min cycle, 40 degrees, 1200 rpm). After the wash, the receiver textile was left to dry flat on the drying rack as previously described.

Table 1
Overview of the three series of repetitive washing with controlled parameters (i. e., 40 °C, 1200 rpm) and load size.

Series	Number of donor Garments	Size	Number of washes	Mass (g)	Load size
1	1	16	15	343	Small load
2	5	16	51	1543	Medium load
3	12	12	41	3075	Filled washing machine

2.6. Transfer experiments

A low-cost transfer device was built, based on the Arduino-Based uniaxial Tensile Tester developed by Arrizabalaga *et al.* [26]. The transfer device was made of a wooden frame with a sliding rail on each side to guide the moving platform on which a load cell was mounted, see Fig. 4. A load cell was connected to an Arduino Uno microcontroller (physical programmable circuit board), itself connected to an Integrated Development Environment (IDE). A linear actuator, controlled with Double Pole Double Throw (DPDT) 3 positions momentary rocker switch was attached to the moving central platform to allow linear up and down displacement. A metallic chain was used to connect the load cell to the square frame purposely designed to surround and pull the Perspex block with its receiver swatch. A pulley plumb to the load cell allowed the linear upward movement to be directly translated into a horizontal displacement, a straight-line motion in one direction. The list of components, software codes to read data from the Arduino apparatus and instructions are available in the supplementary information (section III-V).

For each single transfer, a receiver swatch was placed on top of the donor garment (textile on textile), with the pulling frame fitted around the Perspex block. The metal chain was positioned and aligned to the top of the block. An 800 g weight, representing an 80 kg individual sitting on a chair, was added on top of the receiver swatch. The Arduino interface (IDE) was then run to record the time and the load during the transfer. Once the data recording was launched, the rocker switch was flipped to start the linear actuator before being switched off once the 3 cm × 3 cm Perspex block travelled the full 20 cm (end stop of the linear actuator, average travelling speed 33 mm/s). The receiver swatch was then removed from the pulling frame and securely placed back in a metal box (2 samples per box).

For the first wash series (i.e., 1 single garment washed), all ten contact areas were used for transfer after each wash. For the second wash series (i.e., 5 garments washed together) all 5 garments were used for transfer, with some of the contact areas used for transfer after each wash, while others were subject to transfer every 2 washes, 5 washes or every 10 washes. For the third wash series (i.e., 12 garments washed together), transfer data were collected after each wash from the ten contact areas on the same 3 garments, the intended purpose of the other 9 garments being to fill the washing machine. Full details are available in section VI of the supplementary information.

In parallel to the transfer performed between washes, 100 repetitive transfers were performed on 4 different contact areas of one control garment (used as received and unwashed), 2 contact areas parallel to the rib of the knit of the garment (CA1 and CA4) and 2 perpendicular (CA6 and CA8), for a total of 400 transfers. In addition, 15 repetitive transfers

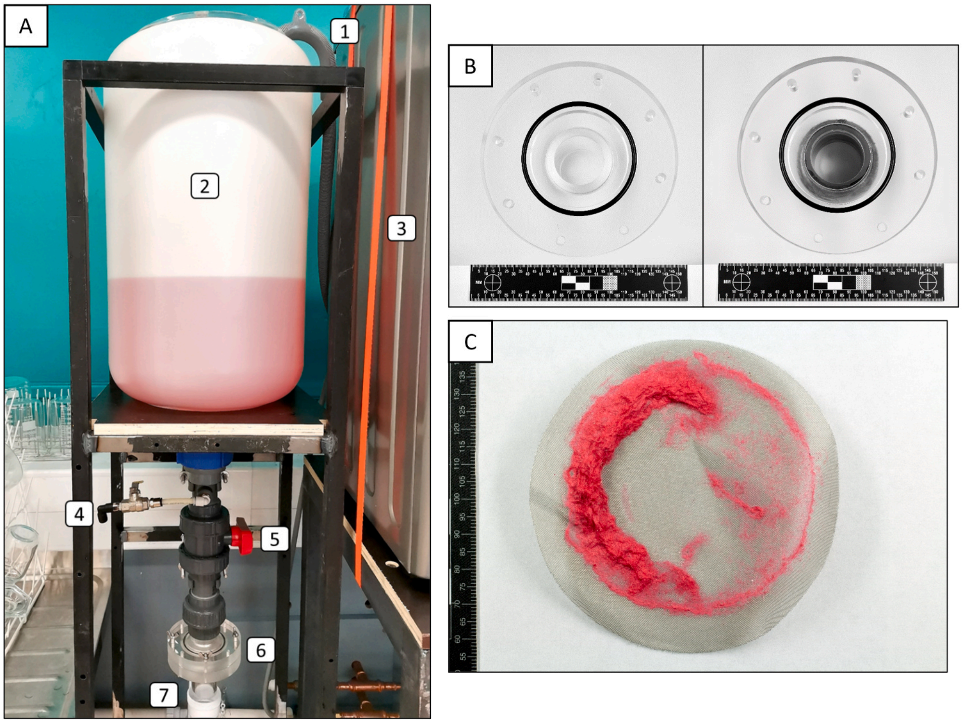

Fig. 3. Waster water filtration system. (A) waste water collection apparatus: (1) washing machine waste pipe, (2) barrel to collect waste water, (3) washing machine (only the side panel is visible), (4) small valve for water sample collection, (5) big valve to wastewater filter holder, (6) filter holder, (7) general waste pipe. (B) Filter holder - two rings joined and held in place by 4 bolts (not shown). (C) Stainless steel filter with red fibres collected from the wastewater of a wash.

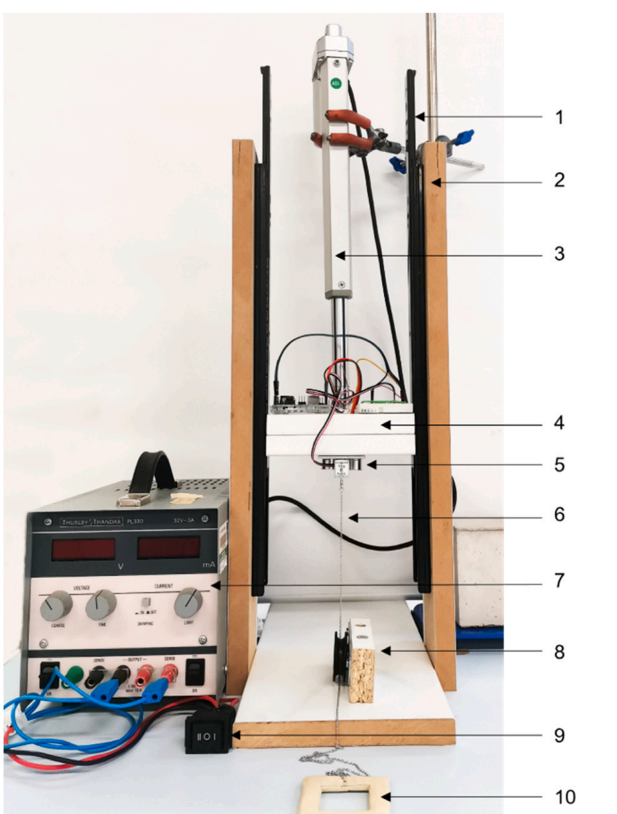

Fig. 4. Transfer device, with in (1) Drawer slides, (2) Wooden frame, (3) Linear actuator, (4) Central loading apparatus, (5) 5 kg load cell, (6) Metal chain, (7) Generator, (8) Pulley, (9) DPDT 3 position momentary rocker switch, (10) pulling frame.

were conducted on one garment washed on its own only once, and one garment washed on its own 15 times (on two contact areas parallel to the rib of the knit of the donor and two perpendicular, as for the control garment).

2.7. Photography

A Nikon D5600 Digital SLR Camera coupled to a macro lens (Nikon 60 mm f2.8 D AF Micro Nikkor Lens) was used to take photos of the receiver swatches (transfer experiments) and the receiver textile (secondary transfer experiment). The camera was powered using an external power supply and mounted on a Kaiser Copy Stand. The receiver swatches/textiles were illuminated by a LED lighting unit (Kaiser – 2 × 27 W, 5600 K). To prevent camera shake, all the photos were taken using a remote shutter release (Nikon MC-DC2). The camera settings used for the acquisition of all the photos were ISO 100, aperture f/16 and shutter speed1/80 s.

For the transfer experiments, a classic nano ColorChecker® (24 colour patch, 24 × 40 mm) was photographed prior to photographing a series of receiver swatches/textiles (background level and after transfer). In addition, a negative control swatch (a receiver swatch with no known transferred fibres) and a positive control swatch (a receiver with a known number of transferred fibres, manually deposited) were also photographed to look at the acquisition conditions and to subsequently check the analysis procedure.

For the secondary transfer experiments, a matrix of 100 images of 3 cm × 3 cm areas was collected for each side of the receiver garment. To avoid image overlapping a system was created and composed of cubes (L: 3 cm, W: 3 cm, H: 1.5 cm) and a Perspex sheet (L: 35 cm, W: 35 cm, H: 0.5 cm) with the receiver textile placed on top (illustrated supplementary information – section VII). Starting from the upper left corner of the receiver textile, a series of images were captured by systematically eliminating cubes in a sequential manner. The Perspex plate was then rotated by 180 degrees and the acquisition sequence repeated for the other half of the receiver sample. The same steps were carried out again for the second side of the receiver garment. The entire acquisition sequence resulted in a total of 200 photos per receiver sample, and 400 images per experiment (i.e., before and after wash).

2.8. Fibre counting

Fibre counting was performed by analysing the photographs of the receiver swatches/textile using ImageJ software after calibration with the nano ColorChecker® and the Adobe Bridge software. A script was written to automatically crop the photographs, define a colour threshold, and count the fibres (See supplementary information, section VIII). The L*a*b* colour space was chosen for the analysis, and Fig. 5 shows an example of a receiver swatch before and after being processed in ImageJ.

All receiver swatches/textiles were photographed prior to the transfer/secondary transfer experiments and after the transfer/secondary transfer were performed. Despite cleaning all receiver swatches/textiles with a spray duster, this method was not 100 % efficient, therefore some background fibres present on the receiver swatches before the transfer were inevitable and important to consider. As a result, the number of fibres was determined by subtracting the number of fibres detected with ImageJ on the images before transfer/secondary transfer from the number of fibres detected on the images after the transfer/ secondary transfer.

Data processing was carried out using R (version 4.3.1) and RStudio (Version 2023.06.2), the code is available via: https://doi.org/10.5281/zenodo.10809788 [27]

3. Results

3.1. Volume of water per wash

Fig. 6 shows the volume of wastewater during each of the three conducted series of experiments: washing a single garment (1st series), washing five garments together (2nd series), and washing twelve garments together (3rd series). The full dataset is available in the supplementary information provided via the persistent identifier [27]. The average volume of wastewater released was 21.95 $\pm$ 0.70 L (mean $\pm$ standard deviation), 23.88 $\pm$ 1.91 L and 23.40 $\pm$ 1.47 L for 1st, 2nd and 3rd series, respectively. The wastewater volume released during each wash remained relatively stable when washing just one garment (1st series), however the 2nd and 3rd wash series exhibited significant fluctuations, as evidenced by the higher standard deviations.

3.2. Mass of fibres in the wastewater

Fig. 7 shows the mass of fibres (mg) released in the wastewater normalised to the weight of the garment, as a function of the wash number, for the three tested conditions: one garment (343 g), 5 garments (1543 g) and 12 garments (3075 g). Descriptive statistics are presented in Table 1 and the raw data are available in the supplementary information provided via the persistent identifier [27]. A Pearson correlation coefficient (PCC) was calculated to measure the strength of the linear association between the mass of fibres released in the wastewater and the volume of water used to wash the donor garments. The PCCs were as follows: 0.22 (p-value = 0.42) for the 1st series (wash with just 1 garment), 0.36 (p-value = 0.011) for the 2nd series (i.e., wash with 5 garments), and 0.05 (p-value = 0.77) for the 3rd series (i.e., wash with 12 garments) (See supplementary information, section IX). These results indicated a positive correlation between the mass of fibres released in the wastewater and the volume of water used in the three experiments. However, the correlation was significant only for the 2nd experiment (p-value $<$ 0.05).Table 2

3.3. Secondary transfer experiments

Fig. 8 shows the number of fibres transferred from the washing machine to receiver swatches during a wash, for each of 15 subsequent washes, performed after the 51 washes of the 5 donor garments (i.e., 2nd Exp, medium load). A total of 897 red fibres were retrieved on the first receiver textile following the first wash S001 (both sides combined). The number of secondary transferred fibres decreased to 210 after the second wash (S002), a 76.6 % decrease from S001. A less pronounced decay is then observed for the remaining washes to S015 observed throughout the entire series.

3.4. Transfer experiments

3.4.1. Washing series vs control garment

Transfer experiments were carried out from donor garments to receiver swatches, using the transfer device outlined in the methodology section. Donor garments from the three washing series and the control garment were used (see material and methods and section VI of the supplementary information). The average number of constituent fibres transferred from the donor garment to the receiver swatch was calculated by including all garments and averaging the number of contact areas per washing series. The results for the three washing series and the control garment are presented in Fig. 9.The number of transferred fibres is expressed as the difference between the number of fibres on the swatches after transfer to the number of fibres detected on the swatches

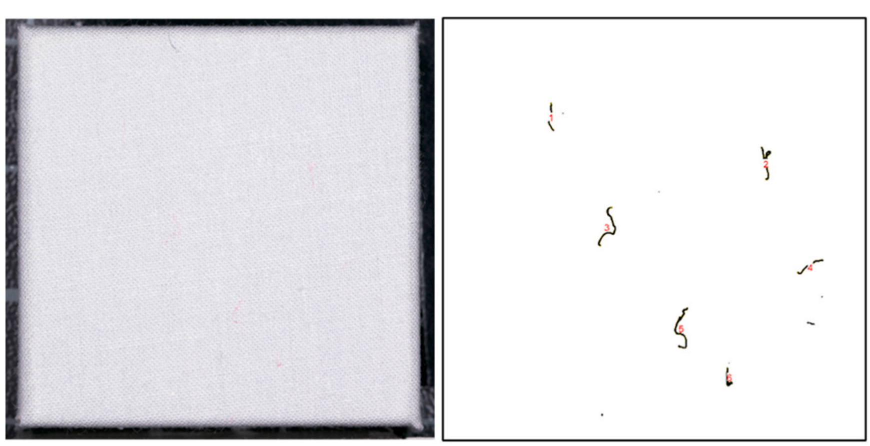

Fig. 5. Fibre counting with ImageJ. On the left, a photo of the 3 × 3 cm receiver swatch after a transfer with the red donor. On the right, the same sample after proceeding in ImageJ.

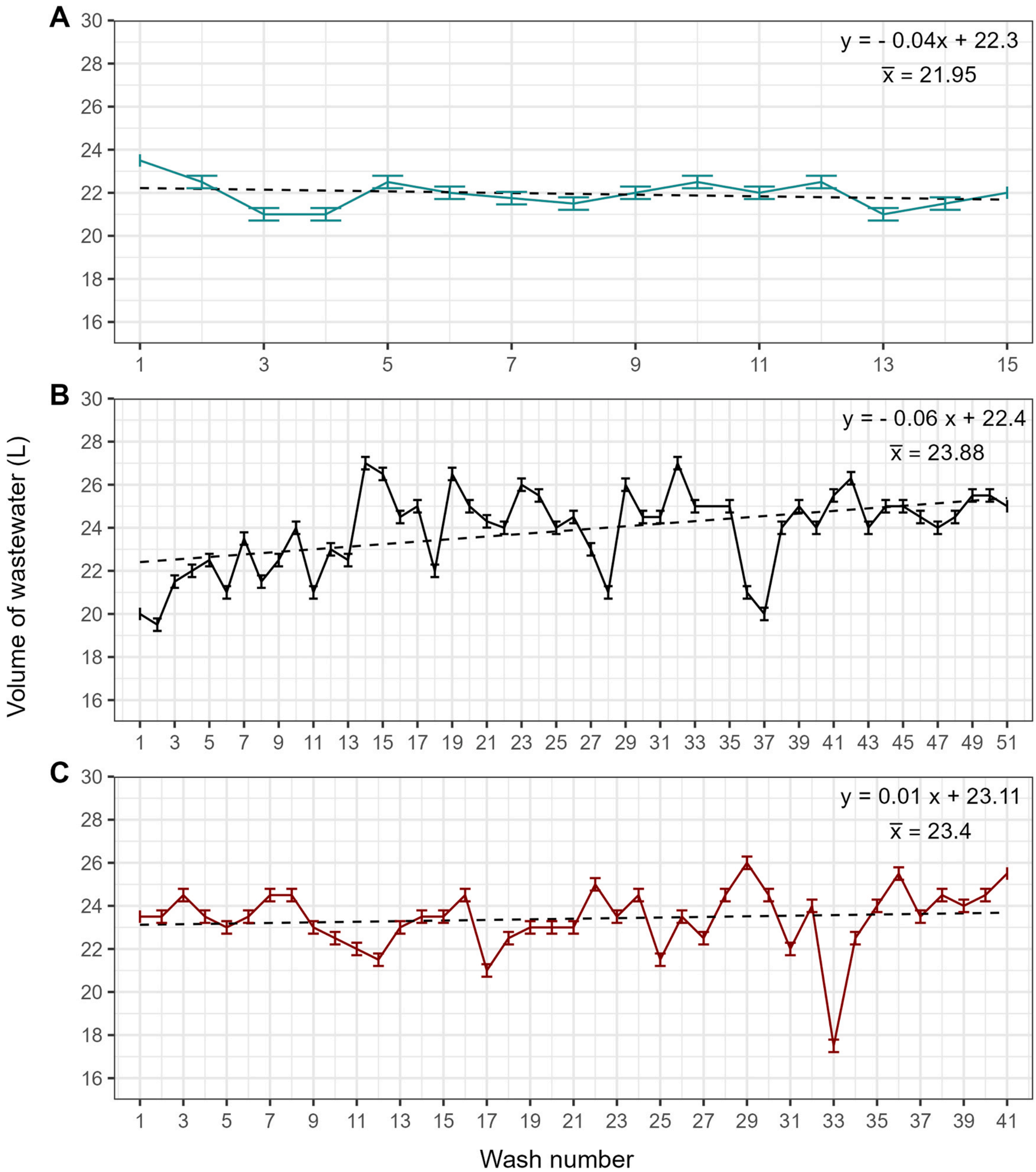

Fig. 6. Wastewater volume released (L) during the washing cycle series, A) results obtained with washing one garment 15 times, B) results obtained with washing five garments 51 times and C) results obtained with washing twelve garments 41 times. Wash number 34 is not represented in B) due to an issue during data collection. The dashed lines represent the linear regressions, and each equation is displayed in the top right. The error bars displayed correspond to uncertainty linked to the calibration of the barrel and the reading standard uncertainty (± 2 U). The averages ($\bar{x}$) are displayed on the top right of each plot.

before transfer, fibre counts as determined by processing the images with ImageJ. The choice of polynomial order was determined for the purpose of visualisation.

In the first wash series (only 1 garment washed), the average number of transferred fibres initially increased from 5.9 ± 2.0 fibres (mean ± standard deviation) before the first wash (i.e. W000) to 9.2 ± 4.3 fibres after the first wash (W001). This is followed by an exponential decay in subsequent data points, reaching a plateau after the sixth wash. For the second wash series (5 garments washed together), the number of transferred fibres increases for the first 5 data points, from an average of 9.2 ± 4.3 fibres before the first wash to 24.3 ± 6.4 fibres on average after the 4th wash. Subsequent data points show an exponential decay before reaching a plateau after the 30th wash. In the third washing series with 12 garments, a similar trend is observed with an increase in the number of fibres transferred from 5.5 ± 3.1 before the first wash to a peak at 15.4 ± 5.2 fibres after the second wash. This is then followed by a slow decline before reaching a plateau after the 27th wash (2.4 ± 2.3).

For the transfer obtained with the control garment that was never washed (first 52 data points only shown in Fig. 9, all data points available in supplementary information, section X), the results show a nearly constant fibre transfer throughout the series, ranging between 1 fibre (repetitive transfer 41) and 7.5 fibres (repetitive transfer 12). At a low wash number, the average number of fibres transferred from the control garment was found to be lower than in the three wash series, while the opposite was observed when reaching a high wash number (e.g. greater than 30).

Repetitive transfers over the same four contact areas were conducted on three donor garments with different washing history: on the control

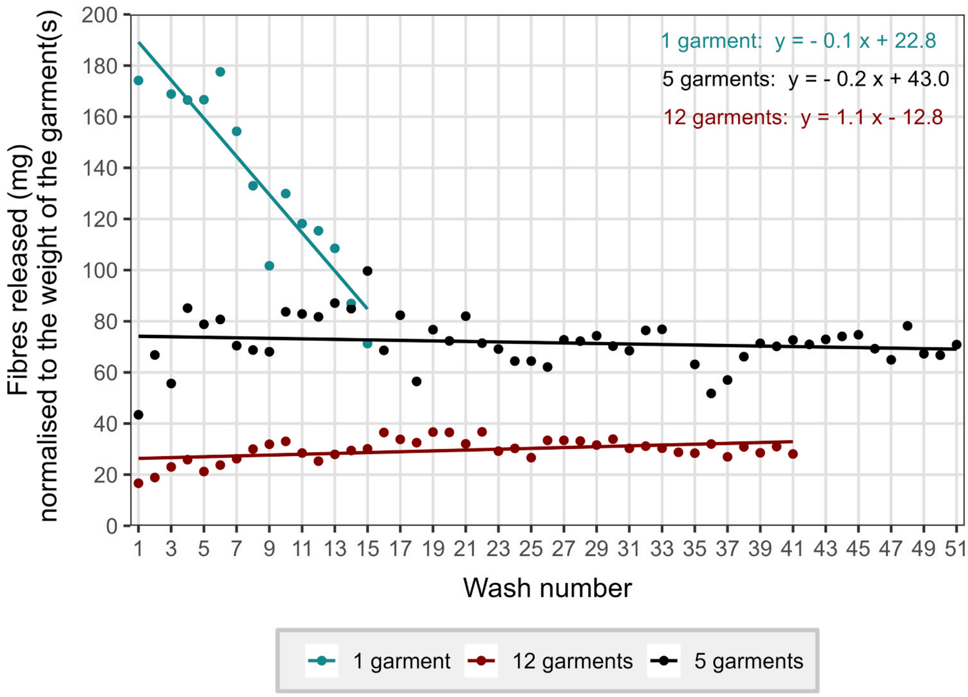

Fig. 7. Mass of fibres (mg) released in the wastewater normalised to the weight of the garment, as a function of the wash cycles performed, for each series: the 1st series in green (one garment - 343 g), the 2nd series in black (5 garments –1543 g) and the 3rd series in red (12 garments - 3075 g). The lines represent the linear regressions, and the equations are displayed in the top right.

Table 2
Mass of fibres (mg) released in the wastewater for each series. In brackets and bold is indicated the mass of fibres released in the wastewater normalised to the weight of the garments.

	1 garment	5 garments	12 garments
Wash cycle	n =15	n = 51	n = 41
minimum (mg)	24.44 (**71.25**)	67.00 (**43.42**)	51.18 (**0.04**)
maximum (mg)	72.56 (**211.55**)	153.78 (**99.66**)	112.94 (**36.73**)
Average (mg)	47.67 (**138.97**)	110.51 (**71.62**)	91.07 (**28.86**)
Median (mg)	45.62 (**133.00**)	109.78 (**71.15**)	93.06 (**30.10**)
SD* (mg)	13.40 (**39.06**)	14.98 (**9.71**)	13.95 (**6.47**)
SEM** (mg)	3.46 (**10.09**)	2.12 (**1.37**)	2.18 (**1.01**)

* SD corresponds to the standard deviation.
** SEM corresponds to the standard error of the mean.

donor garment never washed, one garment washed on its own only once and one garment washed on its own 15 times (i.e. after the 15 washes for the one garment washing series depicted in Fig. 9). The results are illustrated in Fig. 10. The number of fibres from consecutive transfers varied, ranging from 2 fibres to 7.5 for the unwashed (control) garment, from 0 fibres to 6 for the garment washed 15 times, and from 1 fibre to 26 for the garment washed only once. The highest average number of transferred fibres ($\bar{x} = 13.07$) was observed for the garment washed only once, a result consistent with the previous observation made in Fig. 9, which also showed a maximum peak in the number of transferred fibres from the donor garments after the first wash. The average results for the other two garments (unwashed: $\bar{x} = 4.25$ and washed 15 times: $\bar{x} = 1.07$) are also in agreement with the observations seen in Fig. 9. It is also noteworthy that the number of fibres transferred from the donor garments remains mainly constant throughout the entire repetitive series (15 successive transfers on the same area) implying there is no observable fibre depletion. This was also noted for the unwashed garment over 50 transfers (Fig. 9). Finally, the average number of fibres was higher for the two contact areas with the knit perpendicular to the transfer direction compared to the two parallel contact areas, with a ratio ranging from 1.5 to 2.2.

3.4.2. *Variability between and within garments*

A comparative analysis between parallel and perpendicular contact areas both within garments (intra-garment variability) and between garments (inter-garment variability), was performed. First, for the inter-garment variability for the wash series with 5 and 12 garments washed together, the average numbers of fibres (from all contact areas, parallel and perpendicular to the rib of the knit) for each garment at each wash are shown in Fig. 11. The inter-garment variability for the washing series performed with one garment alone is not considered, as no comparison can be made with another garment washed under the same conditions. A consistent pattern is observed for the transfer results collected from garments washed together for both wash series, although some small differences could be noted between the average fibres transferred from parallel and perpendicular contact areas. The pearson correlation coefficients (PCC) between the donor garments were calculated, using the average number of fibres retrieved per parallel and perpendicular contact areas, across the washing series (n=51). The PCC obtained ranged from 0.86 to 0.97 (all p-values < 0.001) in the washing series involving 5 garments (full details available in the supplementary information, section XI). In the washing series with 12 garments (of which only three were involved in the transfer), the correlation coefficients ranged from 0.83 to 0.91 (all p-values < 0.001). The averages of the PCC were found to be slightly higher for the 2nd series (i.e., 5 garments washed together) compared to 3rd series (i.e., 12 garments washed together), respectively of 0.92 and 0.87.

For the intra-garment variability, the average numbers of fibres per contact area (from all garments per washing series), are shown in Fig. 12. For the washing series involving one single garment, the fibres counts are simply the individual numbers recorded after each wash cycle. Similar trends are noted between the three wash series, with matching results between contact areas across the garments. The frequency at which the transfer of fibres was carried out, either every wash, two, five or even ten washes, performed on specific contact areas, do not seem to have an influence on the number of transferred fibres. As expected, the calculated PCC for the number of fibres transferred between contact areas (parallel and perpendicular) confirmed the strong and

Fig. 8. Number of fibres secondarily transferred from the washing machine to the receiver garments, in 15 subsequent washes. Data collected following the 51st repetitive wash series done with the red donor garments in the 2nd Exp (medium load),.

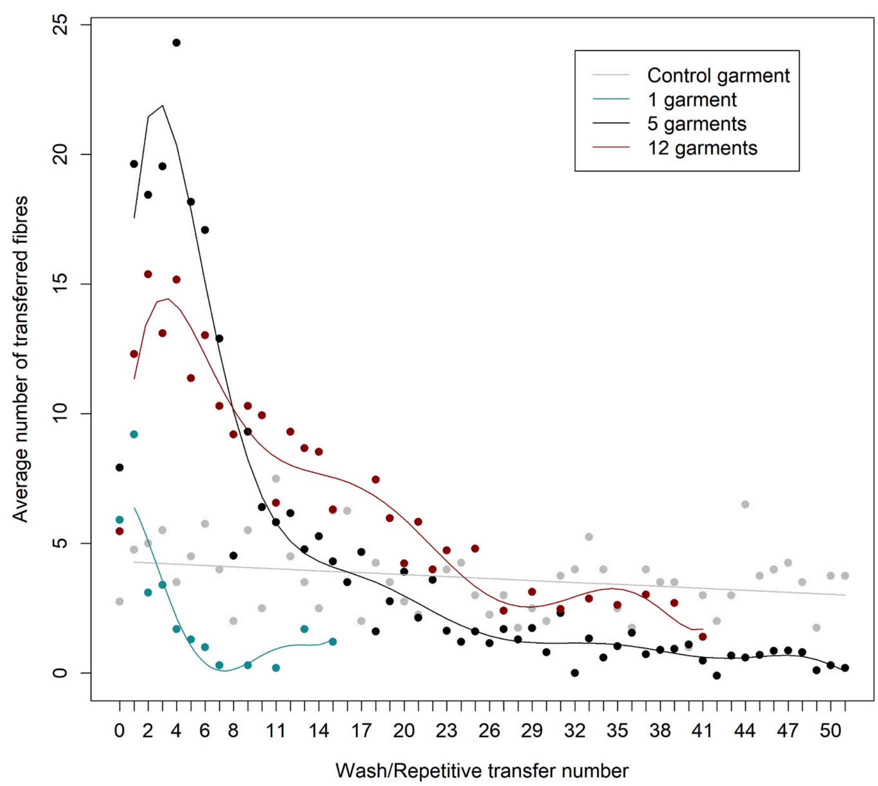

Fig. 9. Comparison between the repetitive transfer from the control garment and the three washing series. The grey line represents the linear regression for the repetitive transfer from the control garment, the green line the fifth order polynomial for the wash series with 1 garment, the black line the ninth order polynomial for the wash series with 5 garments and the red line the seventh order polynomial for the wash series with 12 garments. The choice of polynomial order was determined for the purpose of visualisation.

positive relationship between the contact areas (full details available in the supplementary information, section XI), with 0.67 (p-values $<$ 0.001) for the garment washed alone, values ranging from 0.92 to 0.97 (all p-values $<$ 0.001) for the 5 garments wash series to 0.84 – 0.88 (all p-values $<$ 0.001) for the 12 garments wash series.

3.5. *Fibres in wastewater vs fibres transferred*

A Pearson correlation analysis was conducted between the mass of fibre released per litre of wastewater (mg/L) and the average number of fibres transferred from donor to receiver garment across the washing series. The results are presented in Fig. 13. For the wash with just one

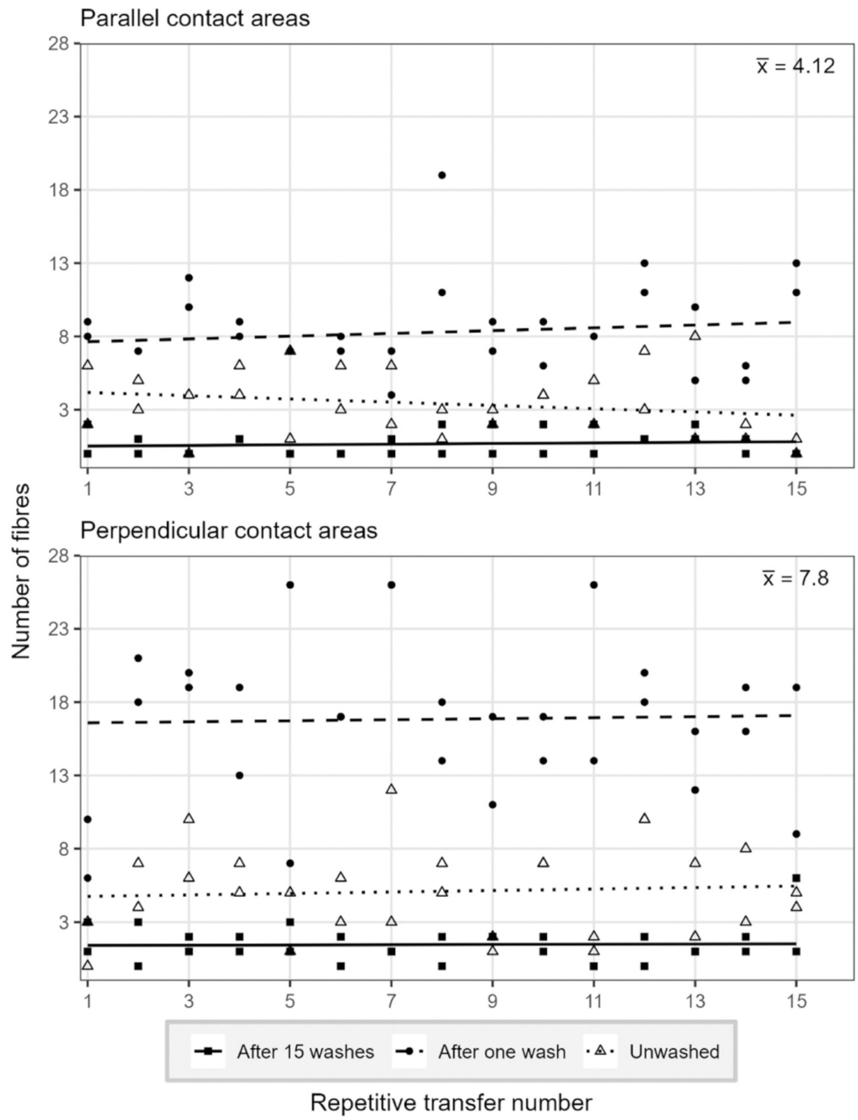

Fig. 10. Fibre count for the 15 repetitive transfers performed on two contact areas parallel to the rib of the knit of the donor and two perpendicular, on three donor garments. The black squares with the solid regression line show the repetitive transfers performed on the donor garment washed 15 times (1st Exp), the triangles with the dotted regression line shows the repetitive transfers performed on the control donor garment never washed and the black dots with the dashed regression line shows the repetitive transfers performed on a donor garment washed once. Individuals values are used for each plot. The averages for each orientation are displayed in the right hand corner.

garment (i.e., 1st series), the PCC was 0.96 (p-value = <0.0001), which indicated a very strong positive correlation between the mass of fibres released per litre of wastewater and the number of fibres transferred. For the 5 garment wash series a strong positive correlation (PCC = 0.77, p-value = <0.0001) was observed between the two variables. Finally, for the simulated normal load with 12 garments (i.e., 3rd series), there was a moderate positive correlation (PCC =0.63, p-value = <0.0001), indicating a positive relationship between the two variables. The p-values obtained were all below the typical significance threshold (e.g., 0.05), indicating that all reported correlations were statistically significant.

4. Discussion

For the release of fibres in water when garments are washed in a washing machine, the results for the first wash cycle indicated that the smallest load size (1 garment only) generated the highest mass of fibres per kg of garment, with 174.17 mg/kg. This was compared to 43.42 mg/kg for a half load (5 garments washed together) and 16.64 mg/kg for the normal load (12 garments washed together). These results agree with the findings of Scheid *et al.* [28] which reported that a reduced load led to a significant increase in mechanical action in a washing machine and that load size impacted on fibre release. The study by Scheid *et al.* [28] specifically used cotton, further aligning with the findings of the current study. Conversely, Kelly *et al.* [29] found that higher mechanical action did not lead to a higher release of polyester microfibres (i.e., extremely light artificial fibres that are used to make cloth [30]); instead the crucial factor was the volume of water. The results obtained in this study showed a large variation in the volume of water used per wash, ranging between 19.5 L and 27 L, which, according to Kelly *et al.* [29], could impact the release of fibres. However, two out of the three Pearson correlation did not show evidence that the water volume had an impact on the number of fibres released in wastewater, which aligns more closely with the observations reported by Scheid *et al.* [28].

Kelly *et al.* [29] observed a decrease in numbers of microfibres released from cotton garments with repeated washes, averaging 124.37 ± 14.40 mg/kg of fibres in the first cycle decreasing to 45.57 ±

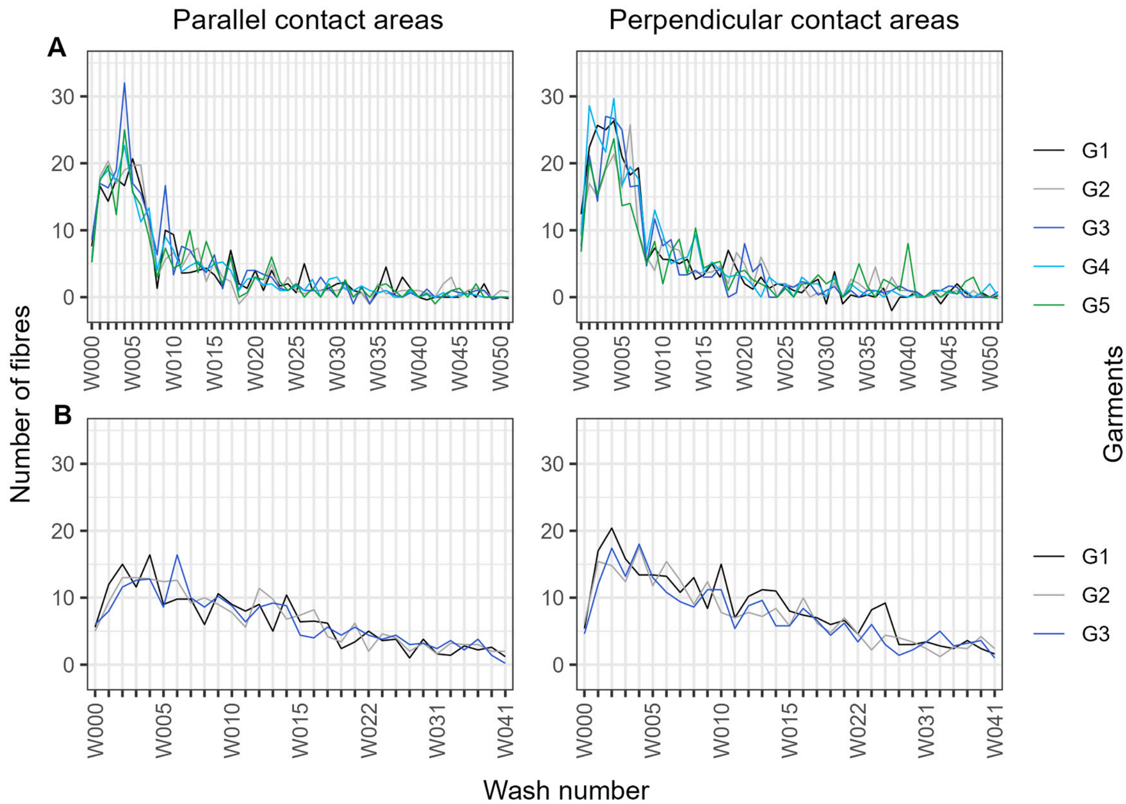

Fig. 11. Number of fibres recovered on receiver swatches per garment, parallel and perpendicular contact areas combined per garment. A) results obtained with the series of 5 garments washed together, annotated G1 to G5. B) results obtained with the series of 12 garments washed together, three used for the transfer series annotated G1 to G3.W000 correspond to the number of fibres recovered after transfer before the first wash.

2.43 mg/kg by the fourth cycle. A similar result was also reported in the study by Zambrano *et al.* [31], noting a decrease from 100 mg/kg for the first cycle to 60 mg/kg for the third one, using cotton textile cut to represent a 1.8 kg load. Cesa *et al.* [32] also reported a decrease but with higher values of fibre release in water, from 551.72 mg/kg for the first wash cycle and 154.48 mg/kg for the third cycle, using cotton t-shirts (145 g) washed in a 1 kg capacity, top load, mini washing machine. These findings from the literature are in part consistent with the observations seen in this work for the wash experiment with just one garment, which saw a decrease in the number of fibres released into the wastewater throughout the entire wash series. However, Kelly *et al.*, Zambrano *et al.* and Cesa *et al.* [29,31,32] commonly noted that the release of microfibres reached a steady state after 4–10 wash cycles, much quicker than the 15 wash cycles applied to one garment in this work, but still consistent with the results of the other two wash series with multiple garments, both of which indicated that fibre release remained almost constant under those washing conditions.

While the mass of fibres retrieved in the three series remained similar to the mass of microfibres released from the study of Kelly *et al.* [29] and Zambrano *et al.* [31], it was notably lower than the numbers reported by Cesa *et al.* [32]. The discrepancies observed in the existing literature and the 2nd (medium load, 5 garments) and 3rd (normal load, 12 garments) series of experiments of this work could be attributed to the type of washing machine and the type of garments used but also variations in the ratio of water volume per mass of garment in each wash cycle. This information is however, not available in the cited literature making a comprehensive assessment difficult. This study underscores the importance of sharing all data (e.g., wastewater volume, weight of fibres released per kg of textile, load size, etc.) including details that go beyond what is required for any given project so that existing work and data can be built upon and further expand our knowledge.

Although significant masses of fibres were retrieved in the wastewater, the investigation of secondary transfer during washing indicated that fibres persisted in the washing machine and can be secondarily transferred in a subsequent wash cycle. These results are in agreement with Watt *et al.* [25] who examined coloured textile fibres in domestic washing machines, recovering a total of 4,695 secondary transferred fibres from five front-load machines. It is not known where these fibres tend to accumulate in the washing machine although fibre clusters were observed in the filter/pump area. Also, it is yet to be determined if any of these fibres can redistribute within the washing machine between two washes and if they can be carried by the water back to the washing machine drum after migration into the filter and pump areas. These questions hold particular significance for forensic practitioners, especially in scenarios involving victims or suspects who have washed their garments. The results of this study revealed that it is possible to recover fibres located in a washing machine by performing an additional wash, by collecting them from the wastewater, using blank textiles, or a combination of both. The secondary transfer results showed that 897 fibres could be detected on a blank textile after just one wash cycle, to a mean of 57 (wash cycle 5–15). It is however important to be reminded that the work presented here only involved investigating fibres from donor garments and that the retrieved fibres (from the donor textile) only represented a fraction of what was collected from the wastewater. Szewcow et al. [15] observed the transfer of target fibres from a donor garment to accompanying undergarments during washing, despite this not being the primary focus of their experiment. Building upon the findings of this study and that of Szewcow et al. [15], a pertinent experiment would involve examining the secondary transfer of extraneous fibres (originating from another garment) already present on a donor garment prior to washing. For comparable textiles (cotton, wool, polyester etc.) it would be reasonable to assume that the donor textile would contribute the most to the overall number of transferred fibres in a washing machine, and thus if only interested in the fibres from

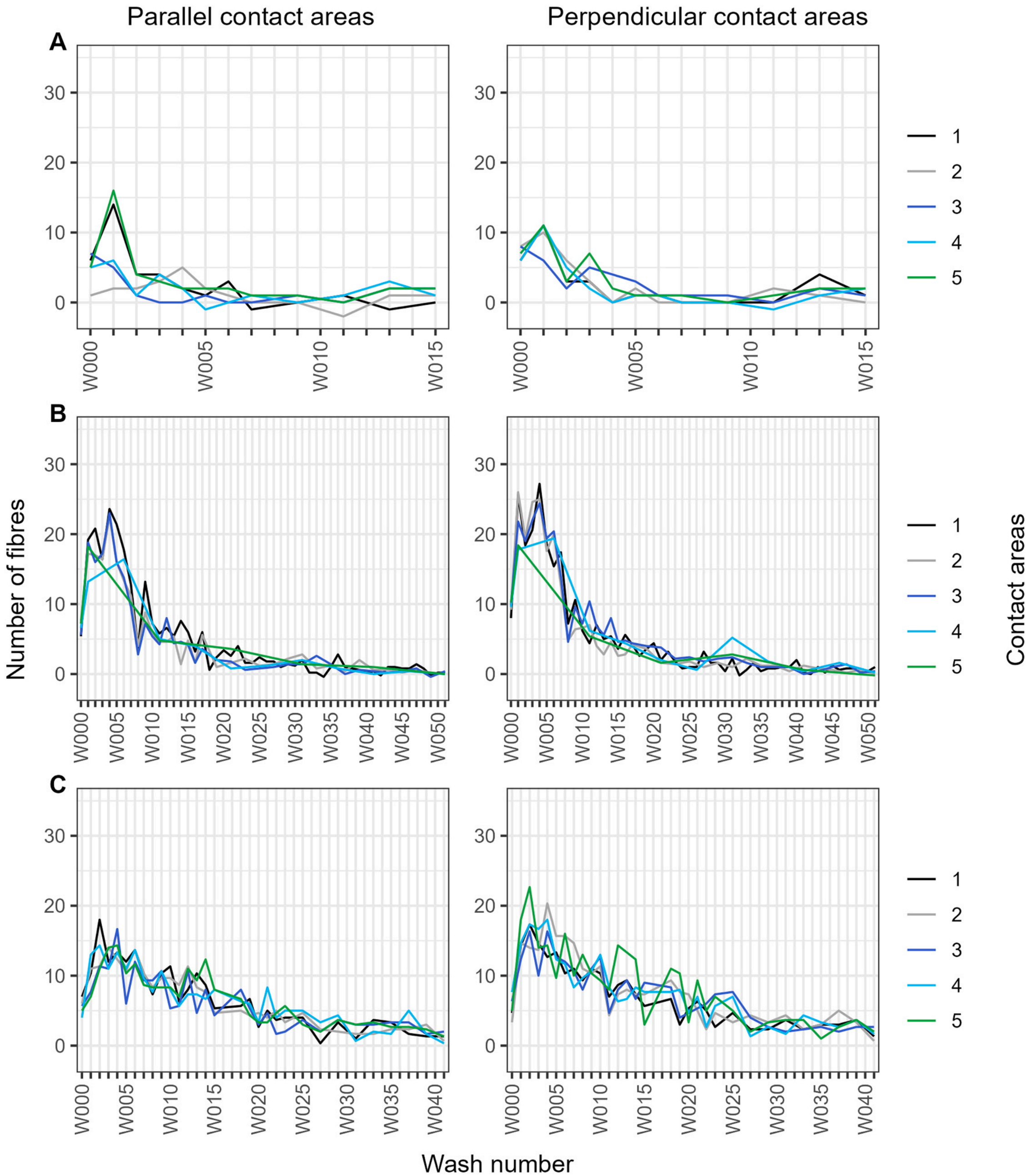

Fig. 12. Number of fibres recovered on receiver swatches for each of the contact areas, all garments combined. A) results obtained with the series of 1 garment washed alone. B) results obtained with the series of 5 garments washed together. C) results obtained with the series of 12 garments washed together. W000 correspond to the number of fibres recovered after transfer before the first wash.

an external origin (transferred from another garment), these fibre counts would likely be very small. More importantly, while this experiment would indeed be highly relevant to forensic casework, it is crucial to recognise that a fundamental understanding of fibre transfer cannot be overlooked. Previous observations have highlighted the need to investigate the effect of various common parameters, whether related to using a washing machine (e.g., loading in the washing machine, washing cycle, detergent concentration, contact mass, motion, etc.) or not, to better understand the transfer and persistence of fibres.

One example illustrated in this work is the variation in the number of fibres at the different stages of the washing history. A higher number of transferred fibres occurred after washing the garment once compared to being unwashed or after 15 wash cycles. This was only done for the first wash series (wash with one garment) as this requires redoing the same experiment numerous times with a new (unwashed and used as received) garment but stopped at a different stage to carry out the repetitive transfers. While requiring additional effort and resources, the same approach should be tested on the other series especially since higher fibre counts were observed. Nonetheless, this clearly demonstrates that a simple and yet controllable experimental condition such as the number of washing cycle can easily affect the results.

In their study on smothering scenarios involving pillows, Schnegg *et al.* [12] reported the transfer of cotton fibres on skin ranging from 1.01 to 5.78 following sleeping, and between 2.13 and 19.46 following smothering (numbers normalised to a 9 cm^2 surface area). In their methodology, Schnegg *et al.* [12] mentioned that the newly purchased pillowcases (six orange and six burgundy) were machine-washed by colour and without any other fabrics, using a regular cycle (40 °C, 60 min) with 100 ml of laundry detergent. Since washing was not considered a parameter in the results, it is reasonable to question

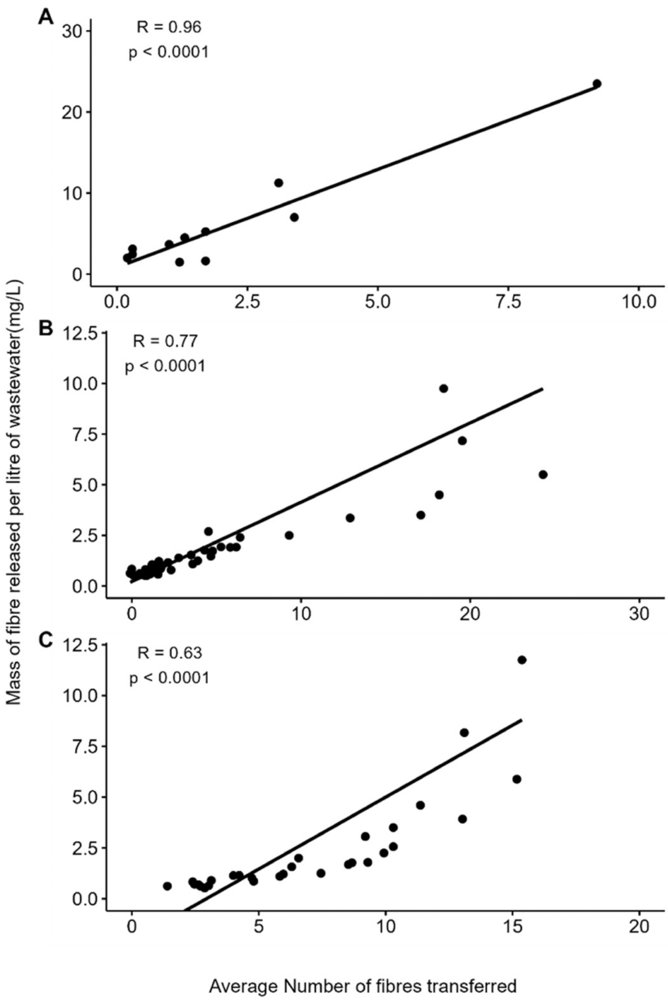

Fig. 13. Mass of fibre released per volume of wastewater (mg/L) as a function of the average number of fibres transferred from donor to receiver garment, across the entire washing series. Pearson correlation coefficients (R) and corresponding p-values are presented for three distinct load sizes: A) 1st series - one garment, 15 washes, B) 2nd series - 5 garments, 51 washes, and C) 3rd series - 12 garments, 41 washes.

whether the washing activity and the sequence of experiments in Schnegg et al. [12] could have influenced the observed differences in fibre transfer between the smothering and legitimate activities. While it can be inferred that the authors washed the pillowcases only once, the frequency of washing is not explicitly stated, leaving room for interpretation. The work by Schnegg et al. [12] has clear merits and presented interesting results, and this discussion is not intended as criticism. However, the lack of detailed published data in the study of Schnegg et al. [12] makes it challenging to ensure the precise sequence of events, which could have a significant impact on the observed outcomes.

The current study indicated that irrespective of the load size, washing activity or repetitive transfer activities, the transfer orientation can affect the transferred fibre count. It is acknowledged that the number of transferred fibres can vary based on the pressure applied during transfer, as highlighted by various authors such as Pounds and Smalldon [1], as well as Palmer [17], Salter et al. [33], and Roux et al. [7]. While this is a consensus, the wide variety of parameters also makes comparison between works reported in the literature a lot more challenging. One such example is pressure described as being manually applied [15,16], rolled up and twist [17], a shake [3], wrapping [11], applied with an abrasion tester [2,4–6]. While variability in methodological approach can be expected between studies as they are intended to address specific questions, the absence of experimental details significantly weakens the scientific argument.

The experimental protocol described in this work does not claim to be comprehensive but certainly provides a strong reference point for other research to build upon. While highly controlled experiments risk underrepresenting the random variance possible in the complexities of real-world scenarios, it is essential to understand all parameters that can impact fibre transfer under controlled conditions. This is crucial for gaining a better understanding of the mechanisms that result in fibre transfer, establishing a strong baseline necessary to support casework interpretation, and reinforcing future studies involving real-life scenarios.

5. Conclusion

This study investigated the influence of washing activities on the release and transfer of fibres. The results highlighted that the load size played a significant role in the release of fibres, with a smaller load leading to a higher initial release of fibre per mass of garment. While this study revealed substantial variation in water volume per wash, the results suggested that the release of fibres is primarily influenced by mechanical action rather than the water volume.

The investigation of secondary fibre transfer within washing machines demonstrated that fibres can persist in the washing machine and transfer to receiver garments, even after numerous washes following the last cycle involving the donor materials. These results hold particular significance for forensic applications, emphasising the importance of understanding fibre dynamics within washing machines and its implications for evidence collection and analysis. Further research that incorporates more parameters, such as varying the spin speed and temperature, and subsequently the use of detergent and softener, could provide a more comprehensive understanding.

This study also investigated the influence of repetitive washing cycle on the transfer of fibres. With the donor textiles being subject to more washing, the results indicated a reduction in the transfer of fibres from the donors made of 100 % cotton to receiver swatches. The findings underscored the significance of the load size, indicating a higher transfer of fibres in the initial wash cycles with a regular load compared to medium and small loads. These observed variation in transfer fibre counts at different stages of the washing process emphasises the role of damage generated during wash cycles. This underscores that washing, as a form of mechanical action, is a critical contributor to the evolution of garment shedding behaviour, which is a crucial parameter to consider in forensic examinations, particularly if the garment of interest is discovered long after a crime was committed. The orientation of the transfer whether it was parallel or perpendicular to the knit of the donor garments also appeared to have an impact on the quantity of fibres being transferred. This study suggests that future research should consider different contact areas and orientations on garments to gain a comprehensive picture on the transfer of fibres.

The use of the transfer device demonstrated advantages in providing controlled conditions and precise measurements, resulting in repeatable experiments. The use of photography, coupled with automated fibre counting, proved to be efficient in rapidly quantifying large amounts of receiver swatches with fibres. The integration of such software has the potential to enhance the accuracy and reliability of research outcomes while conserving valuable time and resources. Furthermore, emphasising the importance of comprehensive data collection and sharing is essential in advancing the understanding of fibre release and transfer dynamics. By providing detailed information on experimental protocols, researchers can contribute to a collective repository of knowledge that enables meaningful comparisons and facilitates the refinement of experimental methodologies.

While adding to the existing knowledge, this research also lays a foundation upon which future studies can be developed. Overall, this study presented a controlled approach to investigate the transfer of fibres encountered in forensic science, so far focussed on just 100 % virgin cotton. To broaden its scope and robustness, further investigations should encompass various fibre types including wool, polyester, acrylic, and recycled fibres. Moreover, involving participants in controlled activities would increase the available data and enable a comparison between laboratory findings and real-world scenarios, thereby strengthening the link between research and forensic casework.

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CRediT authorship contribution statement

Hervé Ménard: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Stephanie Wilson:** Investigation. **Niamh Nic Daéid:** Writing – review & editing, Funding acquisition, Conceptualization. **Patricia Dugard:** Writing – original draft, Formal analysis. **Chris Gannicliffe:** Writing – original draft. **Virginie Galais:** Writing – original draft, Methodology, Investigation, Formal analysis.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2024.112078.

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