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Supplementary Information for

Human pollution exposure correlates with accelerated ultra-structural degradation of hair fibers

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Supplementary Information Text

Population sampling and image acquisition for comparison of fibers between Dalian and Baoding. Within the frame of an already published study [1], two populations of 102 subjects from two different cities in China were recruited. The exposure of the subjects in their daily live to UV can be assumed comparable, while they were exposed to different levels of pollution. As an inclusion criterion an expert validated that no coloration, no hair perm and no relaxing treatment was applied on the hair before. From the 102 subjects available per region in the in-vivo study, 40 were selected in a first approach (as outlined in Fig. 1) as a representative subpopulation. The selection strategy was based on design of experiment (space filling design) based on an expert assessment with the aim to get a subset of subjects uniformly distributed in the descriptor space. The overall sampling scheme is presented in Fig. S5.

From the swatches taken from those 40 subjects, one random fiber was taken and oriented from root to tip. On each fiber, the root region corresponding to the first centimeter available was sampled. A second region 20 cm from the end of the root region was also taken. Each piece of each fiber taken from the initial samples received a new ID ensuring that the rest of the study would be conducted in a blind way.

In order to section hair fibers for TEM observation, it is necessary to embed them in an Epon-812 resin. To this end, we designed a protocol for fiber embedding that allows to maintain the identification of an isolated fiber across all preparation and observation steps.

A simple flat embedding mold was designed using three layered Aclar sheets (as shown in Fig. S8). Two full sheets of Aclar of 500 µm thickness sandwich a third one on which rectangular grooves that have been pierced using a punch (3mm/10mm). Hairs are taped inside the groove one by one on a piece of double sided tape with precision tweezers. To allow for identification of each sample, the order of the fibers is recorded for each bloc. A larger fiber (nylon string) is also placed, marking the start of the line of fibers. Once the 5 hairs are placed on the tape, a label identifying the bloc is added and the Epon resin is poured inside the groove. The top sheet of Aclar is added on top of the construction. The whole setup is then transferred between two glass microscopy slides and pressed together using bulldog clips. The setup is left in an 65°C oven for the night to polymerize. Once polymerized, resin blocs containing the organized fibers are separated from the montage and ready to be sectioned. Transversal sections of the resin blocs are made using a PTX ultra microtome (RMC Instruments) at 75 nm thickness. Sections are recovered on butvar coated slot grids.

All fibers were observed under a Hitachi HT7700 120kV transmission electron microscope. For all sections deposited on a separate EM grid, the observation steps go as follows:

- Low magnification observation to identify the nylon fiber position.
- Acquisition of random images of the cuticles (x2) and cortical regions (x2). To prevent observation bias, the regions are as much as possible selected randomly. The low resolution camera is used to hover over a unresolved region of the fiber, then, acquisition of an image at 3K magnification is done without any other kind of movement of the stage. This ensures that the image acquired is not selected on any other kind of criterion than being over a cuticular or cortical region. This methodology helps lowering the observation bias during acquisition.
- Low magnification mode is then used to localize the next fiber and repeat the whole process of acquisition of random images. All images are stored under the ID given to each specimen at the start of the preparation ensuring that anonymity is kept during the whole process.

Observation conditions were optimized and kept similar for all samples to allow for imaging of the samples without additional staining. This allows for quicker treatment and suppresses a possible additional bias source for quantitative analysis.

Population sampling and image acquisition for comparison of fibers with different pollution profiles and UV treatment. In the rest of the study (for Fig 2, 3 and 4), the same protocol as above was applied with some deviations in the sampling. The exact sampling schemes for the different experiments are outlined in Fig S6 and S7 in detail. The main difference is the addition of a random independent sampling step to select the fibers from the initial swatches. Hairs were embedded in a classical way as a bundle rather than with the flat embedding technique due to the large amount of fibers present per block. Those schemes aim to lower observer bias in a stereological approach to assess damage inside the hair fiber using our cortical damage analysis.

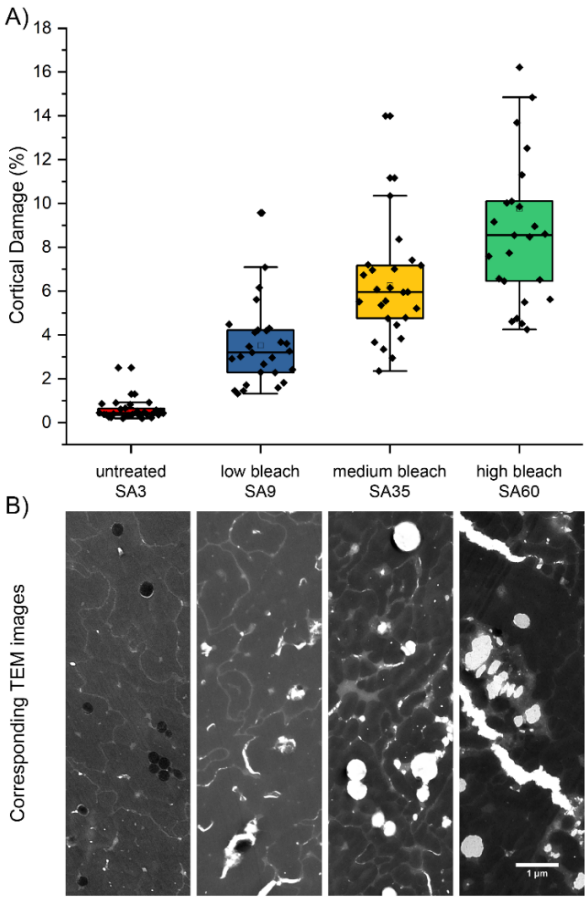

Fig. S1: Cortical damage in chemically treated hair. A) Cortical damage measured in a homogenized swatch of Chinese hair, bleached with different intensities. The hair was bleached with a low, medium and high bleaching intensity (see SI Text and SI Table 2) and the related damage was assessed using the alkaline solubility test. The SA value of this test is a measure for the weight loss of the hair fibers after treatment with NaOH solution (SI Table 2). The SA3 was measured on the non-treated sample, for the three treated samples, average SA values of 9, 35 and 60% were detected. The graph plots the cortical damage value measured on the TEM images in dependency of the SA value. N=30. B) TEM images corresponding to the non-treated and the three treated samples.

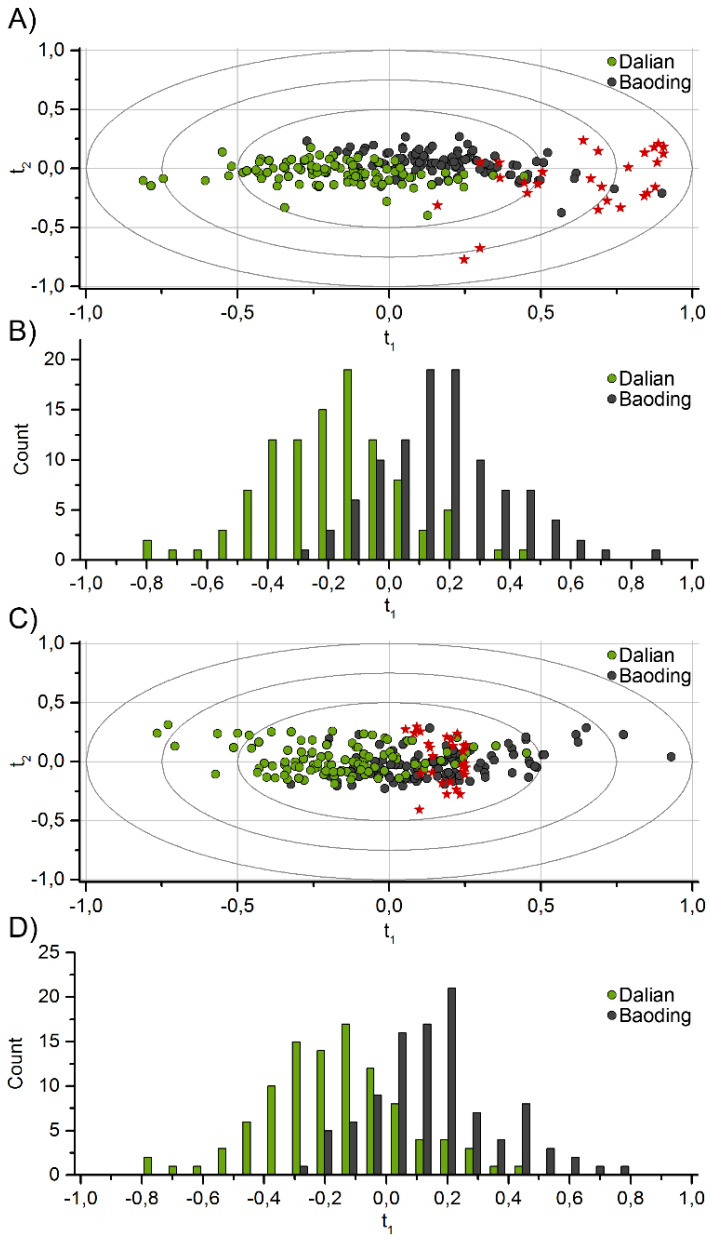

Fig. S2: Definition of fibers with similar pollution profiles. A) Partial Least Squares Discriminant Analysis (PLS-DA) of different PAH concentrations found in individual fibers as already published in [1] the loadings of the PAHs are shown as red stars. Each point corresponds to the hair fibers of an individual, individuals from Dalian are colored green, individuals from Baoding are shown in dark grey. N=204 B) Histogram along the first principal component t1 of the PLS-DA. C) Principal component analysis (PCA) of the same data showing similar results to the PLS-DA. D) Histogram along the first principal component t1 of the PCA shown in C)

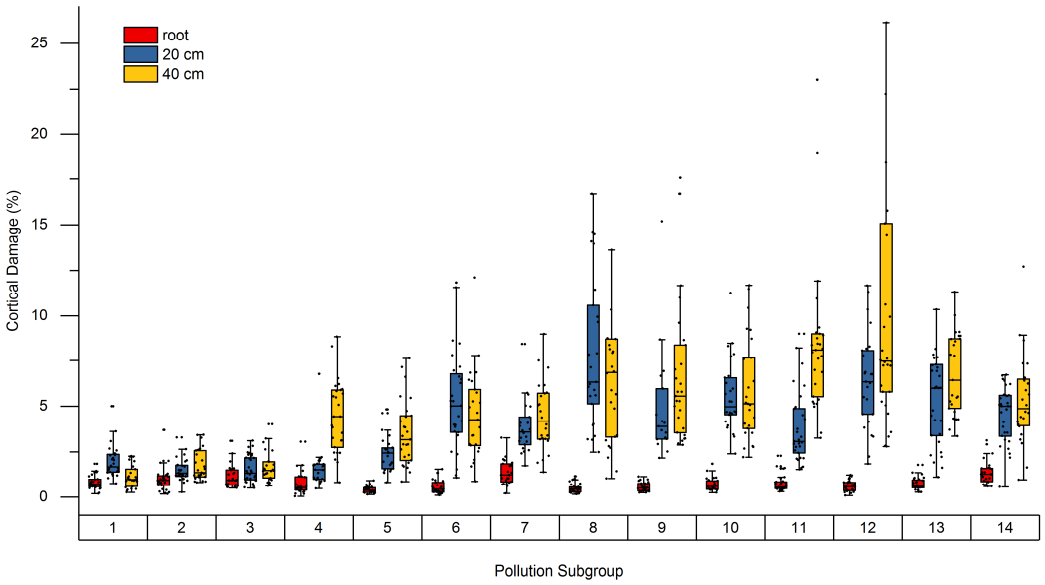

Fig. S3: Cortical damage quantification as illustrated in Fig 1D for the 14 different pollution subgroups as defined in Fig 2A. For each subgroup three different zones along the hair fiber were analyzed, at the root, at 20 cm (corresponding to 1.5 years of hair growth) and at 40 cm distance (corresponding to 3 years of hair growth).

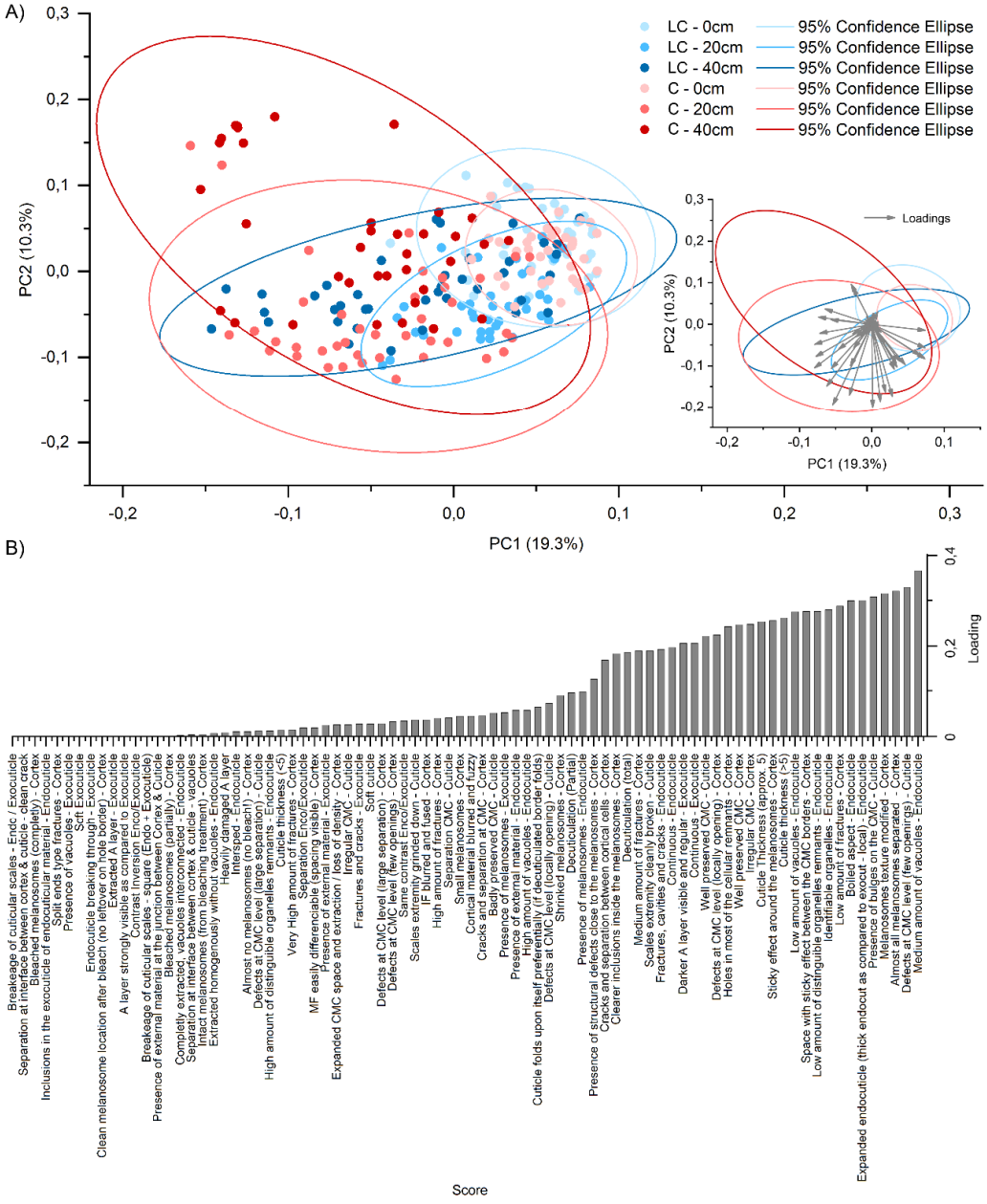

Fig. S4: Hair damage typology of hair fibers from two different pollution groups via expert scoring performed on images taken at different zones along the hair fiber. A) Principal component analysis of 82 scores shown in B). Each score is answered with 0 or 1 and has equal weight. Samples from the less contaminated group (LC) are shown in blue, samples from the contaminated group (C) are shown in red, the color becomes darker for analyzed zones with increasing distance from the root. The spread of the points increases for both groups with increasing distance along the fiber. For the distances at 20 and 40 cm, the spread of the points is larger for samples coming from the contaminated group as compared to the less-contaminated one, indicating that more fibers are found with additional damage scores. The inset shows the different loading vectors for the different scores. B) Length of the loading vectors for the different scores. The higher the values, the more the score has an influence on the distribution in the biplot shown above. Some scores with the highest loading are exemplified in Fig 3.

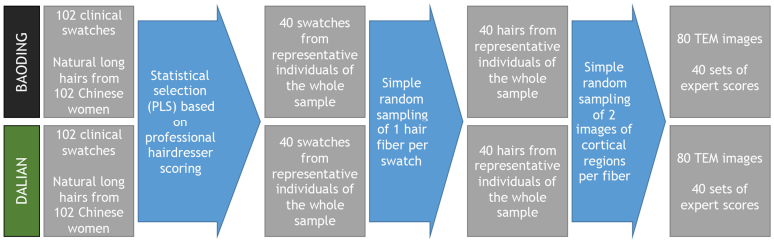

Fig. S5: Sampling scheme to compare hair microstructure of two representative subpopulations from Dalian and Baoding respectively as analyzed in Fig 1.

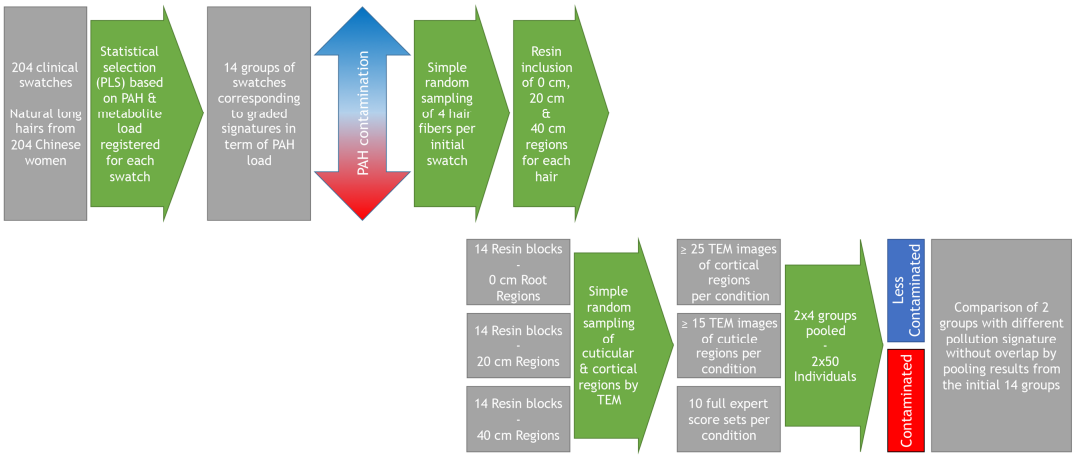

Fig. S6: Sampling scheme to analyze the hair microstructure in dependency of the PAH and PAH metabolite concentrations detected in the fibers. This sampling was used for the data and images shown in Fig 2 and Fig 3.

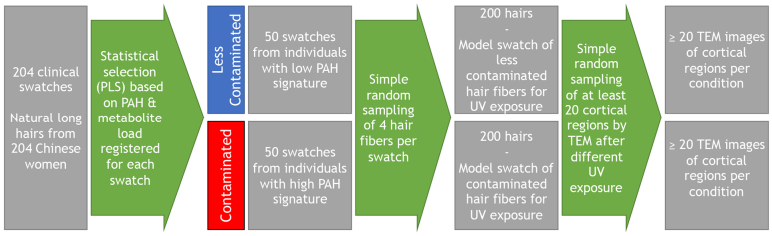

Fig S7: Sampling scheme to analyze the effect of controlled UV irradiation on hair fibers with different pollution profiles. This scheme was used to obtain data shown in Fig 4.

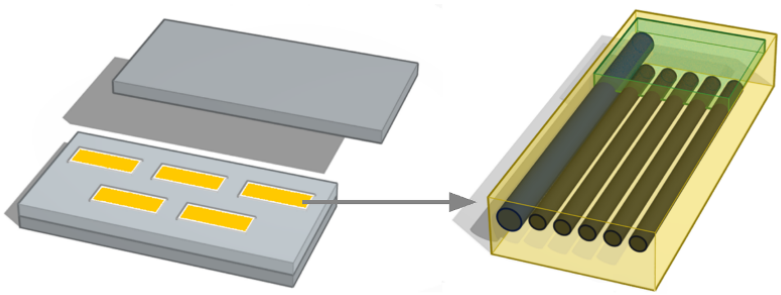

Fig. S8: The left shows an Aclar embedding mold: the three Aclar sheets are figured in gray, the resin-filled grooves in yellow. Right pictures the resin bloc after polymerization and separation from the mold: hair fibers are figured in black, nylon string in blue, double face tape and label in green and resin in yellow. The nylon string allows to keep the right orientation of the bloc; each bloc is labeled.

Table S1. Average concentrations and statistics of PAH and their metabolites of the less contaminated (LC) and contaminated (C) groups

Pollutant	NB (%)	Min	Max	Min Corr	LC Moy Cor	C Moy Cor	Increase Moy	LC Median	C Median	Exact Prob>|W|	Asymp. Prob>|W|
acenaphtylene	72	0,02	301,69	0,01414	2,60511	13,89361	5,33323	1,085	5,36	6,39E-05	1,31E-04
fluorene	85	0,57	95,13	0,40305	13,54767	29,34444	2,16601	11,455	27,195	1,69E-05	4,26E-05
phenanthrene	100	13,41	650,77	9,4823	77,2796	204,169	2,64195	70,02	181,645	1,24E-14	9,93E-10
anthracene	95	0,09	29	0,06364	3,66756	12,7576	3,47849	3,34	10,565	3,38E-14	1,19E-09
fluoranthene	96	1,48	95,24	1,04652	12,66672	54,1546	4,27534	12,02	52,275	1,78E-15	7,79E-10
pyrene	100	4,52	55,98	3,19612	11,6712	34,4904	2,95517	10,855	33,325	1,78E-15	7,79E-10
benz a anthracene	100	0,31	5,46	0,2192	0,6188	2,3496	3,79703	0,55	2,035	1,78E-15	7,79E-10
chrysene	100	0,93	16,5	0,65761	2,5138	8,5474	3,40019	2,355	7,91	1,78E-15	7,79E-10
B b fluoranthene	100	0,14	2,81	0,09899	0,4266	1,5908	3,72902	0,385	1,58	1,78E-15	7,79E-10
B k fluoranthene	100	0,14	4,26	0,09899	0,485	1,8312	3,77567	0,455	1,72	1,78E-15	7,78E-10
B a P	89	0,03	1,81	0,02121	0,19987	0,8254	4,12975	0,175	0,78	1,78E-15	7,78E-10
I 1,2,3-cd pyrene	85	0,07	2,19	0,0495	0,36457	1,03858	2,84877	0,4	1,06	1,05E-11	7,15E-09
Benzo g,h,i perylene	93	0,05	4,52	0,03536	0,39915	1,1546	2,89265	0,365	1	5,44E-11	1,46E-08
1-OH-Naph	100	0,37	33,42	0,26163	0,7104	1,734	2,44088	0,61	0,98	1,34E-06	6,25E-06
2-OH-Naph	100	0,68	200,21	0,48083	3,6812	13,5294	3,67527	2,27	4,565	4,52E-06	1,53E-05
9-OH-Fluorene	96	1,41	44,38	0,99702	6,27896	13,8828	2,211	4,865	10,13	4,65E-05	9,62E-05
3-OH-Fluorene	76	0,05	1,21	0,03536	0,07791	0,40911	5,25071	0,335	0,05	1,23E-13	1,60E-09
2-OH-Fluorene	96	0,06	1,09	0,04243	0,14739	0,3738	2,53606	0,155	0,33	1,28E-12	3,26E-09
4-OH-Phenanthrene	100	0,08	0,6	0,05657	0,1634	0,3058	1,87148	0,16	0,29	1,67E-13	1,74E-09
9-OH-Phenanthrene	100	0,27	0,89	0,19092	0,4356	0,5356	1,22957	0,425	0,495	3,63E-04	5,48E-04
1-OH-Anthracene	99	0,34	7,11	0,24042	1,12181	1,4034	1,25102	1,015	0,945	0,77683	0,77679
3-OH-Phenanthrene	96	0,02	0,58	0,01414	0,03573	0,1768	4,94803	0,03	0,145	1,78E-15	7,73E-10
1-OH-Phenanthrene	98	0,06	1,14	0,04243	0,1501	0,446	2,97141	0,14	0,385	1,78E-15	7,76E-10
2-OH-Phenanthrene	97	0,06	1,89	0,04243	0,10835	0,5736	5,29417	0,1	0,475	1,78E-15	7,77E-10
1-OH-Pyrene	84	0,05	0,49	0,03536	0,09261	0,22371	2,41567	0,08	0,205	2,52E-12	4,12E-09

Concentration values are given in pg/mg, only pollutant detected in more than 60% of the population are considered (NB%). Exact probabilities and asymptotic probabilities were computed using a Wilcoxon Signed Ranks Test between polluted and less polluted groups. Values under 0.05 are considered significant.

Table S2. Protocol for bleaching hair with different intensities. The columns list the used concentrations for the three different bleaching intensities low, medium and high. After bleaching an alkaline solubility test was performed and the corresponding results are given.

Machine Bleaching Intensity	**Low**	**Medium**	**High**
Temperature for phases 1-3	37°C	30°C	37°
Bath Ratio	1g of hair for 50 ml of solution		
1. Washing Phase	15 minutes under agitation in a solution of 63.55ml Ammonium lauryl sulfate (180g/L) in 5.45L distilled water Rinsed abundantly for 15 min with tap water then distilled water.		
2. Bleaching Phase	45 minutes under agitation in a solution of 0.454 kg of sodium persulfate, 9.078 g EDTA, 0.275 L of oxygenated water (110 volume), 0.150 L ammoniac in 4.319 L of distilled water. Rinsed abundantly for 15 min with tap water then distilled water.	45 minutes under agitation in a solution of 1.362 kg of sodium persulfate, 9.078 g EDTA, 0.826 L of oxygenated water (110 volume), 0.454 L ammoniac in 3.713 L of distilled water. Rinsed abundantly for 15 min with tap water then distilled water.	
3. Neutralization Phase	30 minutes under agitation in a solution of 0.454 L Ammonium lauryl sulfate (180g/L), 0.303 L of buffer solution, 0.027 L of sodium thiosulfate (500g/L) in 4.54 L of distilled water. Rinsed abundantly for 1 hour with tap water then distilled water. Dried with hair drier and combed.		
Alkaline Solubility Measurement	100 mg of homogenized hair are taken and dried for 16 h at 105 °C. After measuring their weight (P0) they are treated for 30 min at 65 °C with a 100 mM NaOH solution. After washing with demineralized water and subsequent drying at 105 °C for 16 h the fibers are weighted again (P1). The loss of material gives the SA factor defined by SA [%] = (P0-P1)/P0*100%, which depends on the initial hair state and on the treatment strength. This measure quantifies the bleaching induced damage within the hair fiber, usually in cosmetic treatments SA values around 10 represent light bleach, 20-30 represent medium bleach, and values above 40 represent a strong bleach.		
Measured Alkaline Solubility Values (SA)	9	35	60

References

1. Palazzi, P., et al., *Exposure to polycyclic aromatic hydrocarbons in women living in the Chinese cities of BaoDing and Dalian revealed by hair analysis.* Environment International, 2018. **121**: p. 1341-1354.