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Emerging Concern from Short-Term Textile Leaching: A Preliminary Ecotoxicological Survey

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Abstract Textile dyes and their residues gained growing attention worldwide. Textile industry is a strong water consumer potentially releasing xenobiotics from washing and rinsing procedures during finishing processes. On a decentralised basis, also final consumers generate textile waste streams. Thus, a procedure simulating home washing with tap water screened cotton textiles leachates ($n = 28$) considering physico-chemical (COD, BOD_5, and UV absorbance) and ecotoxicological data (*Daphnia magna, Pseudokirchneriella subcapitata* and *Lepidium sativum*). Results evidenced that: (i) leachates presented low biodegradability levels; (ii) toxicity in more than half leachates presented slight acute or acute effects; (iii) the remaining leachates presented "no effect" suggesting the use of green dyes/additives, and/or well established finishing processes; (iv) no specific correlations were found between traditional physico-chemical and ecotoxicological data. Further investigations will be necessary to identify textile residues, and their potential interactions with simulated human sweat in order to evidence potential adverse effects on human health.

Keywords Dye · Textile · Cotton · Leachate · Ecotoxicity

Contaminants are considered as "emerging" if they have not been historically present in the environment on a global scale, even because nobody looked at them intentionally. They can be commonly derived from both treated and untreated wastewater indicating that they are mostly produced at industrial scale, currently used also in everyday homecare activities, and easily and unintentionally dispersed into the environment. Some recent examples are pharmaceutical and personal care products (Carotenuto et al. 2014; Lofrano et al. 2016), engineered nanomaterials (Libralato 2014), tannins (Lofrano et al. 2008a; Libralato et al. 2011), micro- and nano-plastics (Mattsson et al. 2015). Despite their continuous release worldwide, dyes and their residues on sold textile goods still remain a great problem and their impact has been scarcely evaluated. Consumers involuntarily leach chemicals from textiles during normal wear (e.g. sweat) and washing (e.g. hand washing or washing machine). This risk increases when finishing steps are not applied properly especially in developing countries (Khatri et al. 2015; OEKO 2016). Thus part of chemicals used for textile production could be still present in textile goods and their characterization and proper management is becoming a challenging responsibility for textile manufacturers. Water use from public water supply ranged within 72–400 L per person day^{-1} in EU (EUROSTAT 2016) and 300–380 L per person day^{-1} in USA (USGS 2016).

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At present, many developing countries such as Indonesia (Kerstens et al. 2016), Pakistan (Ensink et al. 2004), Ghana (Keraita and Drechsel 2004), and Senegal (Faruqui et al. 2004) (i.e. estimated consumption of up to 20 L person day^{-1} in Africa (IWfA 2016)) struggle to provide water and wastewater services, while textile residues are discharged without any treatment into surface water (Lofrano et al. 2008b). In India, millions of people wash their clothes directly into rivers turning them into giant launderettes (Robinson 2015). In Europe, about 80 % of the population is connected to wastewater treatment, whereas in the South-East Europe (e.g. Turkey, Bulgaria and Romania) only approximately 40 %; the remaining part of untreated wastewater flows into the closest receiving water bodies (EEA 2012).

Several studies demonstrated the toxicity of textile dyes (Bae and Freeman 2007; Ballesteros et al. 2006; Bertanza et al. 2013; Bazin et al. 2012; De Souza et al. 2007; Loos et al. 2007; Meriç et al. 2005; Novotny et al. 2006; Srivastava et al. 2004; Suryavathi et al. 2005; Wang et al. 2002) showing potential adverse effects on human health and the environment (Dave and Aspegren 2010). High molecular synthetic textile auxiliaries and dyes can produce wastewater containing large amounts of refractory COD. Respirometric measurements showed that dye carriers could exert high toxicity causing serious inhibition of microbial respirometric activity (Alaton et al. 2006).

This study aimed at evaluating cotton textile leachates in home washing simulated conditions integrating toxicity data (*Daphnia magna, Pseudokirchneriella subcapitata*, and *Lepidium sativum*) with basic wastewater information (BOD_5, COD, UV–VIS absorbance) in order to provide a preliminary overview of their effects.

Materials and Methods

Colored cotton textile goods (n = 28) were randomly purchased at different prices in Avellino-Salerno area (Italy). All textiles, colored by staining (i.e. no patterned pictures on them), were new and unwashed when purchased. Leachates were produced immersing textiles (1 m^2) in 2.5 L of cold tap water from Salerno aqueduct (100 mg $CaCO_3$/L, pH = 7.50, Cl < 0.5 mg/L) at 25 °C for 30 min in static conditions (i.e. 1 textile in 2.5 L in 1 replicate) using a surface-to-volume ratio (s/v) of 0.4 similarly to Dave and Aspegren (2010) (i.e. the maximum s/v). Neither soap nor other detergents were used to observe background effects avoiding any washing product potential interference to the final wash out quality. Leachates were stored in 0.5 L glass bottles (no air space between the sample and the lid) and kept refrigerated at 4 °C. Samples were labeled as summarized in Table 1. Total chemical oxygen demand (COD) was determined according to APHA (1998). The biological oxygen demand after 5 days (BOD_5) was measured by manometric pressure difference (OxiTop, ISCO, IT) and after seeding activated sludge taken from a municipal wastewater treatment plant. The BOD_5/COD ratio identified leachate biodegradability (Chamarro et al. 2001) making leachates with values >0.4 suitable for biological treatment (Loos et al. 2007). Absorbance at 254 and 280 nm was determined in triplicate using UV–VIS spectrophotometer (1 cm quartz tube, Lambda 12 Model, Perkin Elmer, Waltham, Massachusetts), quantifying double bonds and aromatic structures. A battery of toxicity tests was considered including the 72 h chronic test with *P. subcapitata*, the 24 h mortality test with *D. magna*, and the 72 h acute test with *L. sativum* (Libralato et al. 2016a, b; Lofrano et al. 2016). Before toxicity testing, pH values were measured (perpHecT LogR meter, model 330, Orion, Beverly, MA, USA) and reported in Table 1. Acute toxicity tests with *D. magna* were carried out according to ISO (2013). Newborn daphnids (<24 h old, n = 5) were exposed in four replicates for 24 and 48 h at 20 ± 1°C under continuous illumination (1000 lx). Before starting the test, they were fed with *P. subcapitata* (300,000 cells/mL) *ad libitum*. Toxicity was expressed as percentage of dead organisms. The chronic growth inhibition test with *P. subcapitata* was carried out according to ISO (2012). Cultures were kept in Erlenmeyer flasks. The initial inoculum contained 10^4 cells/mL. The specific growth inhibition rate was calculated considering 6 replicates exposed at 20 ± 1°C for 72 h under continuous illumination (6000 lx). Effect data were expressed as percentage of growth inhibition. The acute bioassay with *L. sativum* evaluated the potential toxicity considering the root elongation according to OECD (2006). Experiments were conducted in triplicate (n = 10) at 25 ± 1°C for 72 h in aqueous solutions. Seed germination and root elongation inhibition normalized on negative control data were expressed as percentage of effect, and the germination index (GI) was calculated as reported in Libralato et al. (2016a). The significance of differences between average effect values of different experimental treatments and controls was assessed by the analysis of variance (ANOVA) considering a significance threshold level always set at 5 %. When ANOVA revealed significant differences among treatments, post-hoc tests were carried out with Dunnett's method and Tukey's test. The assessment of macrophyte phytotoxicity endpoints was carried out with ImageJ (Schneider et al. 2012). Whenever possible, toxicity was expressed as EC50 along with 95 % confidence limit values. Otherwise, toxicity was expressed as percentage of effect (PE, %) or toxic unit (TU). Principal component analysis (PCA) and biplot representation were proposed for data integration and interpretation. Statistical analyses and graphs were carried out using Microsoft® Excel 2013/XLSTAT©-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA). Toxicity data were integrated

Table 1 Physico-chemical characterization of textile leachates

Samples	Colors	BOD_5 (mg/L)	COD (mg/L)	BOD_5/COD	UV_{254} (1/m)	UV_{280} (1/m)	pH
TW water	Colorless	0	<5	0	0	0	7.50
T1	Red	<10	29	–	0.0513	0.0366	7.63
T2		10	47	0.21	0.1569	0.0986	7.52
T3		<10	17	–	0.0687	0.0503	7.57
T4	White	12	56	0.21	0.2227	0.1766	7.61
T5		<10	28	–	0.1434	0.1202	7.49
T6		<10	27	–	0.1705	0.1482	7.53
T7		10	33	0.30	0.0578	0.0465	7.59
T8		13	118	0.11	0.1570	0.1360	7.99
T9		13	35	0.37	0.0332	0.0302	7.67
T10	Yellow	15	66	0.23	0.2297	0.1815	7.63
T11		15	147	0.10	3.2566	0.9602	7.59
T12	Fuchsia	<10	7	–	0.0238	0.0074	7.61
T13		<10	12	–	0.1102	0.0800	7.60
T14		<10	17	–	0.0569	0.0481	7.62
T15	Blue	10	128	0.08	0.0978	0.0794	7.63
T16		10	76	0.13	0.2159	0.1651	7.62
T17		<10	10	–	0.0761	0.0622	7.62
T18		22	73	0.30	0.1846	0.1319	7.50
T19		11	43	0.26	0.1686	0.1260	7.50
T20		13	207	0.06	0.3967	0.3206	7.49
T21		20	51	0.39	0.1077	0.0803	7.48
T22	Cream	20	78	0.26	0.0945	0.0457	7.51
T23		<10	27	–	0.2582	0.092	7.55
T24	Black	18	73	0.25	0.1045	0.0722	7.56
T25	Pink	<10	10	–	0.0643	0.0398	7.68
T26	Light-blue	18	43	0.40	0.2147	0.1759	7.53
T27	Light-green	10	66	0.15	0.2102	0.1608	7.52
T28	Blue-gray	20	79	0.25	0.0801	0.0634	7.51

TW tap water

according to Libralato et al. (2010) and Persoone et al. (2003) considering the class weight score system.

Results and Discussion

Physical and chemical data of textile leachates were reported in Table 1. COD values greatly changed (from 10 to 207 mg/L), while BOD_5 was ≤22 mg/L. COD values increased in the following order: Blue > Yellow > Grey > Cream > Black > White > Red > Light blue > Fuchsia. The highest COD value (207 mg/L) was associated to a blue textile; however, COD from blue goods fell within 10–207 mg/L. Leachate from Fuchsia textiles (T12–14) showed the lowest COD (Table 1). Leachate biodegradability (Chamarro et al. 2001) was always very low ($BOD_5/COD \leq 0.4$). UV–VIS measurements were higher than tap water and varied in a wide range due to their different chemical structures (Table 1). Although there was a strong correlation (0.947) between UV_{254} and UV_{280}, a limited correlation was noticed between COD and UV–VIS values (COD/UV_{254} with $p = 0.592$ and COD/UV_{280} with $p = 0.575$, $\alpha = 0.05$). The pH values of leachates did not significantly change compared to the initial leaching tap water (7.50). Toxicity data were summarized in Fig. 1 including *D. magna* 24 h (Fig. 1a), *D. magna* 48 h (Fig. 1b), *P. subcapitata* (Fig. 1c), and *L. sativum* (Fig. 1d) effects. The analysis of toxicity data from Fig. 1a evidenced the presence of two main groups of samples considering a threshold value ≤ 10 % for the effects significantly different from the negative control. Most samples presented effects below the established threshold except for T5, T9, T11, T15, T19, T20 and T25, showing toxicity up to 50 % (T11). After 48 h exposure (Fig. 1b), the number of samples presenting toxicity effects ≤ 10 % decreased evidencing that contact time plays an important role in toxicity definition. Except

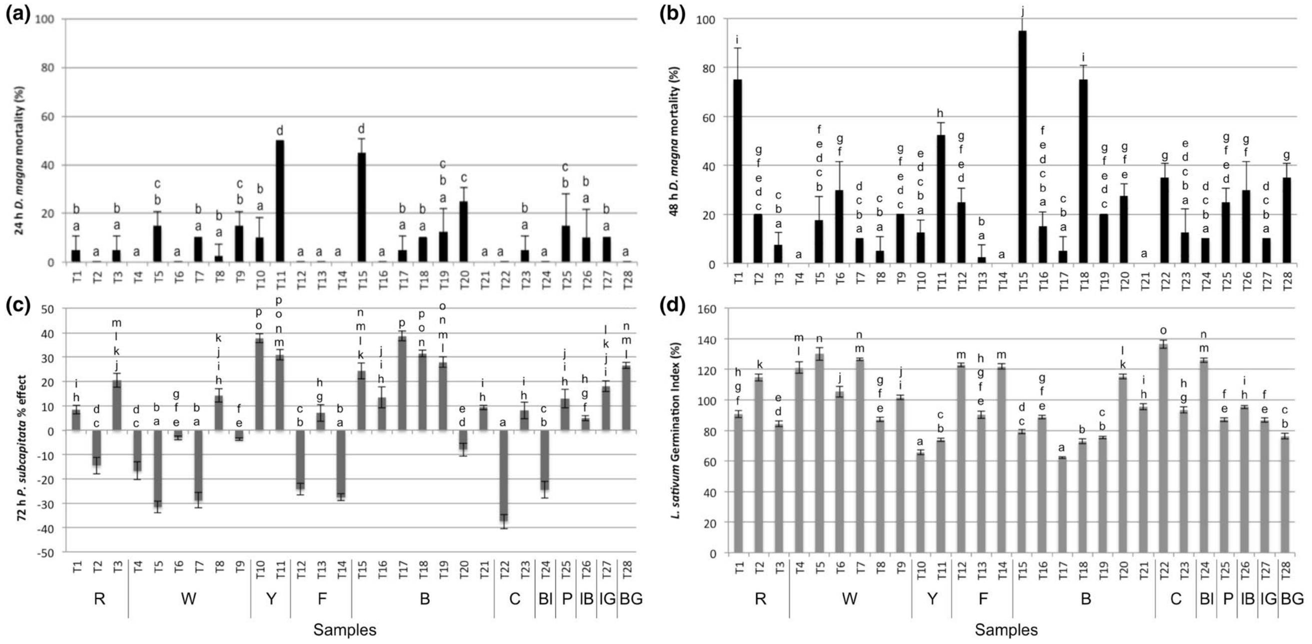

Fig. 1 Textile leachate data (T1–28) with *D. magna* (24 h) (**a**), *D. magna* (48 h) (**b**), *P. subcapitata* (72 h) (**c**), and *L. sativum* (72 h) (**d**); data with *different letters* (*a–n*) are significantly different (Tukey's, $p < 0.05$); *R* red, *W* white, *Y* yellow, *F* fuchsia, *B* blue, *C* cream, *Bl* black, *P* pink, *lB* light-blue, *lG* light-green, *BG* blue-gray

for 10 samples (T3, T4, T7, T8, T13, T14, T17, T21, T24, and T27), all others presented immobilization effects up to 95 % (T15) in the exposed population. Keeping the same threshold set for *D. magna*, the effects of leachates on *P. subcapitata* after 72 h exposure (Fig. 1c) can be clustered into three main groups: (i) no effect ($-10\% \leq PE \leq 10\%$); (ii) biostimulation growth effect ($PE < -10\%$); (iii) and inhibitory growth effects ($PE > 10\%$). Only 8 samples (T1, T6, T9, T13, T20, T21, T23, and T26) presented no effect at all. Comparing *D. magna* 48 h and microalgae results, only T13 and T21 were deemed as not toxic. Biostimulation was observed in 8 samples (T2, T4, T5, T7, T12, T14, T22, and T24) up to −38 %. All other leachates presented microalgae growth inhibition effects up to 39 %.

According to Libralato et al. (2016a), *L. sativum* GI (%) (Fig. 1d) data can be clustered into three main groups: (i) no effect ($80\% \leq GI \leq 120\%$); (ii) biostimulation ($GI > 120\%$); and (iii) inhibition ($GI < 80\%$). Several samples presented no effect (T1, T2, T3, T6, T8, T9, T13, T16, T20, T21, T23, T25, T26, T27). Samples T13 and T21 were confirmed to be not toxic also by *L. sativum*. A group of 7 samples (T4, T5, T7, T12, T14, T22, and T24) showed stimulatory effects up to 136 % of GI and another group of 7 samples (T10, T11, T15, T17, T18, T19, and T28) showed inhibitory effects up to 62 % of GI. Leachate toxicity data from *P. subcapitata* and *L. sativum* linearly correlated suggesting ($y = 101.214 (\pm 0.492) - 0.929 (\pm 0.020) x$; $R^2 = 0.986$) that leachates composition acted very similarly in autotrophic photosynthetic organisms. According to Libralato et al. (2008, 2010, 2016a, b), toxicity data from a battery of toxicity tests should be integrated providing a final judgment on leachate quality. Considering the class weight score (CWS) system (Persoone et al. 2003), toxicity data (*D. magna* (48 h), *P. subcapitata* and *L. sativum*) were combined and ranked into three main groups of samples as summarized in Fig. 2: (i) no acute toxicity ($CWS < 0.4$); (ii) slight acute toxicity ($0.4 \leq CWS < 1$); and (iii) acute toxicity ($1 \leq CWS < 10$). The CWS approach confirmed that T13 and T21 presented no acute toxicity because bioassays' results were ≤10 %. Other samples averagely presented no acute toxicity like T3, T4, T8, T10, T16, T17, T23, T25, T26 and T27. Anyway, their toxicity values taken singly reached a maximum of 20 % effect according to CWS approach. The total amount of no toxic samples accounted for 43 % of the total investigated samples. Approximately 39 % of samples were ranked as slight acute toxic (T1, T2, T5, T6, T7, T9, T14, T19, T20, T24 and T28) and 18 % as acute toxic (T11, T12, T15, T18, and T22). This means that 57 % of leachates presented effects able to generate some adverse ecotoxicological consequences.

A biplot summarizing PCA results concerning chemical and ecotoxicological data is shown in Fig. 3. The first two principal components accounted for 38.02 and 19.62 % of variation, respectively. Therefore, the two-axis ordination diagram can depict 57.64 % of variation. The biplot regarding component loadings suggested that the F1 scores are influenced by high values of COD, UV_{254} and UV_{280} as well as 24 h *D. magna* toxicity data. The loading of *P. subcapitata*

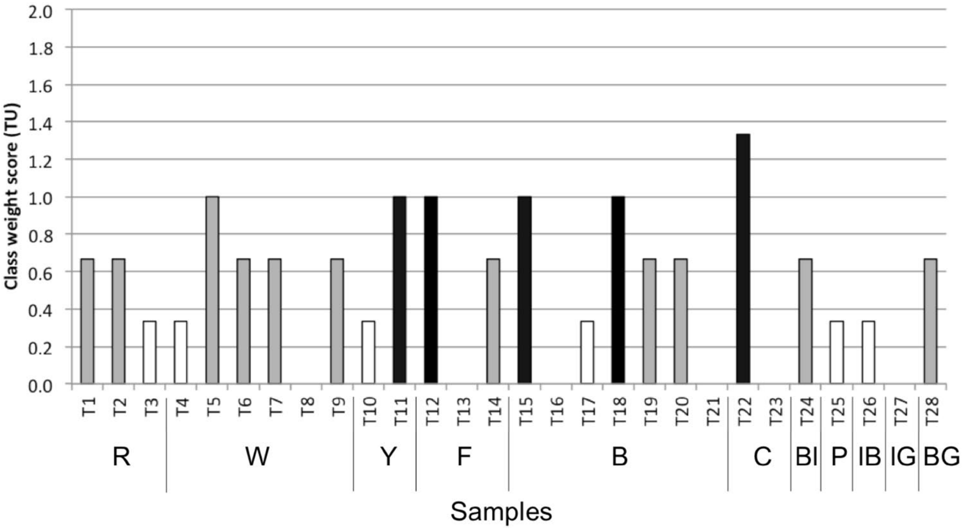

Fig. 2 Hazard classification for textile leachates (T1–28) potentially discharged into the aquatic environment according to Persoone et al. (2003); *white*, no acute toxicity (TU < 0.4); *dark gray*, slight acute toxicity (0.4 ≤ TU < 1); and *black*, acute toxicity (1 ≤ TU < 10); *R* Red, *W* White, *Y* yellow, *F* fuchsia, *B* blue, *C* cream, *Bl* black, *P* pink, *lB* light-blue, *lG* light-green, *BG* blue-gray

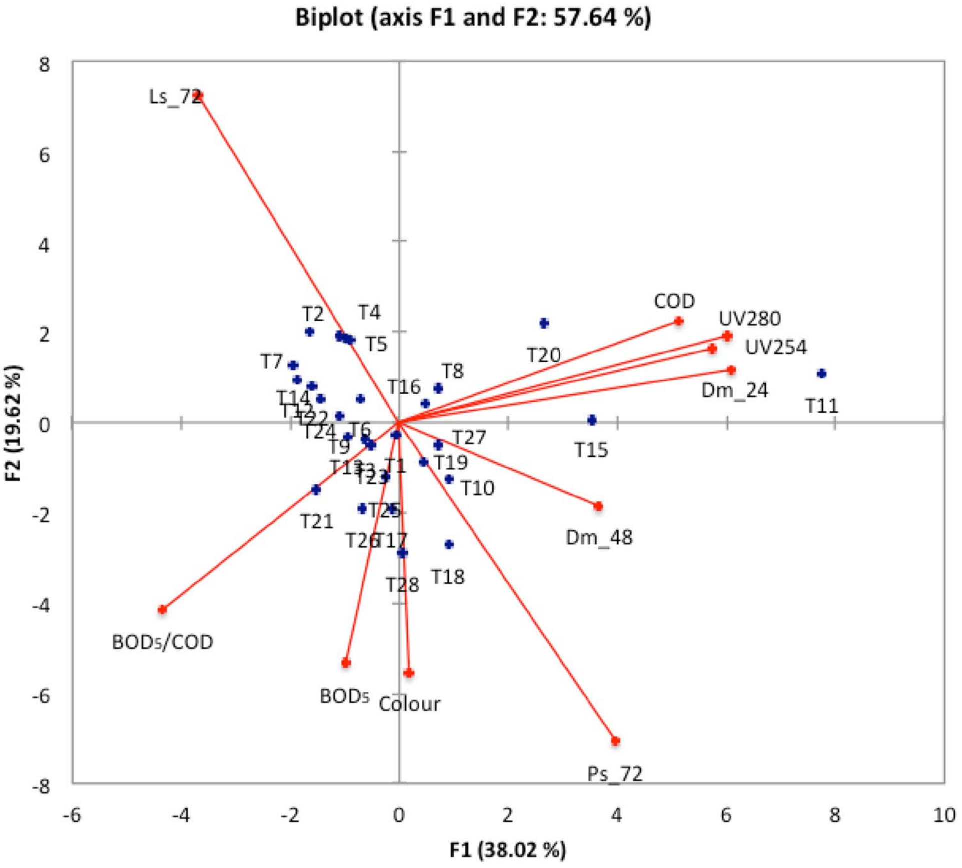

Fig. 3 Principal component analysis (PCA) as *biplot* representation with loadings and scores in the coordinates of the first two principal components (F1 and F2) of wastewater samples; Dm_24 = *D. magna* 24 h toxicity data, Dm_48 = *D. magna* 48 h toxicity data, Ps_72 = *P. subcapitata* 72 h toxicity data; Ls_72 = *L. sativum* 72 h toxicity data

and *L. sativum* toxicity data mainly influenced F2 scores. Looking at the ordination plot of component scores in F1 and F2 biplot, it was found that wastewater samples could be clustered into two main groups according to eigenvalues: (1) T11, T15 and T20 (F1); and (2) all the remaining. It seems that only T11, T15 and T20 are correlated with COD, UV_{254} and UV_{280} data presenting under an ecotoxicological viewpoint acute (T11 and T15) or slightly acute (T20) toxicity. The values of BOD_5, BOD_5/COD and color showed slight/no correlations within the dataset. Dave and Aspegren (2010) reported similar results on textile goods, but considering various fibers other than cotton, identifying significantly higher toxicity for printed rather than unprinted textiles. Textile goods releasing unknown chemicals to the aquatic environment can become a wide emerging concern. This study proposed a survey of potential effects related to cotton textile house washing. Results evidenced: (i) chemical residues in tap water leachates related to dyes and dyes' additives presenting a low biodegradability; (ii) toxicity in more than half of the investigated leachates samples presented from slight acute to acute effects; (iii) the remaining part of leachate samples presented no effects according to the selected battery of toxicity tests suggesting the use of green dyes (and/or additives), and/or well established finishing processes; (iv) no specific correlation were found between traditional physico-chemical (COD, BOD_5, UV_{254}, and UV_{280}) and ecotoxicological data. Further studies could investigate which kind of dyes and additives residues are responsible for toxicity effects, identify the best finishing processes enabling low or no toxicity effects in textile leachates normalizing data on textile weft considering the number of fibres per unit area. Moreover, due to the ecotoxicological effects highlighted, it would be interesting assessing the potential interactions between textile residues and simulated human sweat in order to evidence potential adverse effect on human health.

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