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# Smokers' behaviour and the toxicity of cigarette filters to aquatic life: a multidisciplinary study

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**Abstract**

Cigarettes are the most littered item in public spaces. Smokers who litter are leaving a trace of toxic waste that adds to the global plastic pollution due to harmful chemicals and semisynthetic microfibres that compose cigarette filters. Here we present a multidisciplinary study aiming to assess i) predictors of cigarette littering, and ii) the toxicity of semisynthetic filters to the freshwater invertebrate *Chironomus riparius*, including iii) the potential driver of toxicity. Unobtrusive observations of 597 smokers at public places were analysed using logistic regression, which showed that *age* (negatively) and *group setting* (positively) are personal predictors, and the *number of present ashtrays* (negatively) is a contextual predictor of cigarette littering. In addition, we assessed acute and chronic aquatic toxicity of cigarette filters in standardized ecotoxicity tests on several lethal and sublethal effects, using both smoked and unsmoked filters. Following 48-h exposure, concentrations of 2 filters/L from smoked and unsmoked filters caused 36–100% and 75–100% larvae immobility, respectively. We further demonstrated that cigarette filter fibres seem to add to the toxicity of filter leachates. Seven-day exposures that used either contaminated water or sediment (3 weeks leaching time, eq. 1 filter/L water and 1 filter/166.5 ml sediment) showed exposures via sediment caused more frequent and severe effects on the larvae than exposures via water. Larvae exposed to contaminated sediment (smoked and unsmoked filters) exhibited > 20% higher mortality, > 1.5-fold decrease in growth, and > 80% decreased development, compared to larvae in control conditions. Moreover, we found that cigarette filters have the potential to be teratogenic to freshwater invertebrates. Our results could be used to support litter prevention efforts, advisably via integrated educational campaigns. The campaigns could account for the societal and environmental complexity of cigarette littering by being tailored to the determined littering predictors and using ecotoxicity results as content.

**Keywords** Littering, Unobtrusive observation, Field study, Group influence, Plastic pollution, Microfibres, *Chironomus riparius*

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## Introduction

Cigarettes are one of the most found litter items in public spaces [1–7]. The average cigarette litter concentration was found to be 2.7 items/m$^2$ (max. 49 items/m$^2$) in Berlin, Germany [8] and 0.21 items per meter city passage (max. 53 items) in Qazvin, Iran [7]. Cigarettes are also one of the most frequently found litter items at beaches worldwide [9–11] and depending on the area, even make up for the most found items [12], especially at the Mediterranean coast [13, 14]. Thereby, these tremendous litter amounts

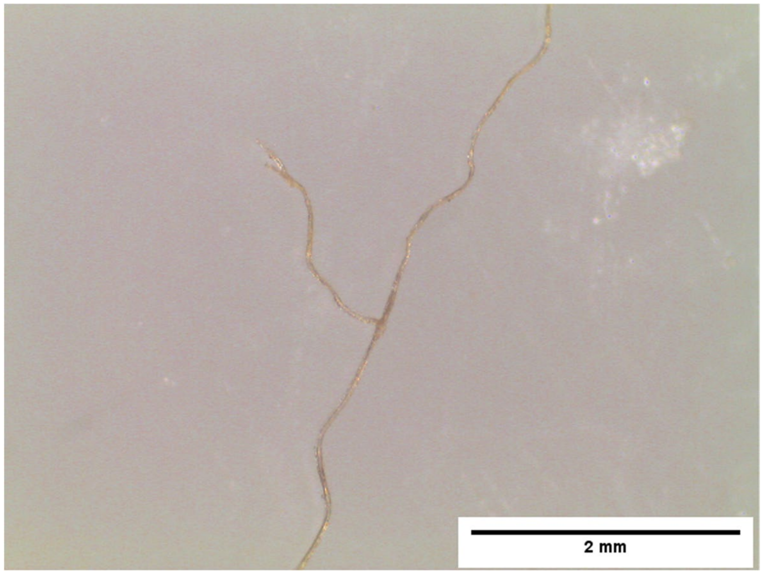


**Fig. 1** Light microscope image showing the Y-shape of one fibre from smoked cigarette filters

are adding to the global plastic pollution due to the cigarette filter that is 2–3 cm long and made of over 12,000 densely packed, Y-shaped fibres that are commonly made from cellulose acetate [15, 16] (Fig. 1). Cellulose acetate is a natural, semisynthetic polymer [17], but can be considered as plastic material due to its high degree of modification [18]. Cellulose acetate filters have one of the fastest fragmentation rates amongst common plastic products with 15% weight loss per year (in seawater condition) and can fragment three times faster than polyethylene terephthalate (PET) bottles [19]. However, cigarette filters are in general not fully biodegradable, and their degradation depends strongly on the disposal environment [20]. Studies report degradation times ranging from 5 years in different laboratory and field conditions [21] to 7.5 years in compost and 14 years on soil surface [20].

Cigarette filter litter eventually ends up in aquatic environments [22] through wind, rain and the drainage system or storm water sewers [23]. Once there, cigarette filter litter can leach multiple chemicals that derive from tobacco harvesting and processing (e.g. pesticide residuals [24–26]), from the filter production (e.g. titanium dioxide, triacetin [15]), or the combustion process (e.g. metals [16]). Smoked cigarette filters can leach polycyclic aromatic hydrocarbons (PAHs)- especially naphthalene [27], nicotine [8], ethylphenol [28], benzene, toluene, ethylbenzol, xylene (BTEX) [29], and heavy metals [16] into water. In turn, dissolved PAHs [30–32], nicotine [33], BTEX [32, 34] and heavy metals [16, 35] can accumulate in the tissue of aquatic biota. Overall, cigarette filter litter is classified as hazardous waste [36] with a long-term hazardous potential [37], under the EU Waste Framework Directive [38]. This classification is based mainly on the nicotine content, but also on the ecotoxic potential [36]. The ecotoxic potential was predominantly assessed by investigating the effects of chemical leachates on aquatic model species [28, 35, 39–47]. For example, leachates with a concentration of only 1.8 smoked filters/L are sufficient to cause mortality (LC50) in marine fish *Atherinops affinis* in a 96-h exposure [39]. However, the sensitivity to cigarette filter leachates is species-specific and ecotoxic effects on one species cannot predict effects for other species [28, 43].

Another risk for the aquatic environment is the release of loose fibres from cigarette filters. Within 14 days, one smoked cigarette filter releases about 100 fibres per day in slowly agitating, distilled water (2 cycles per s) [48]. Thereby, most detached fibres are smaller than 0.2 mm and range in the microplastic scale of 0.001 – 1 mm [18]. But only a few studies investigated the effects of microfibres (physical fibre effect) from cigarette filters in the aquatic environment [48, 49] and more studies are necessary to quantify their ecotoxic potential.

Cigarette littering occurs more frequently compared to the littering of other items. A study in the US found a littering rate of 65% for cigarette litter but only 17% for general litter [3]. Worldwide, high cigarette littering rates can be found [4, 5, 50]. The cigarette litter problem is essentially a problem about human behaviour. Psychological research suggests that behaviour change is driven by capability, opportunity, and motivation [51]. Behavioural motivation is thereby influenced by the perception of a person that will put more effort in anti-littering behaviour, if they perceive the behavioural shift as their own will [52]. But to this day, smokers seem to have unrealistic perception of their behaviour and its harmfulness. Although smokers think they litter one out of 10 cigarettes, littering observations revealed that smokers littered two out of three cigarettes [53]. Most importantly, studies from the US and Germany confirmed that the majority of smokers (57 – 71%) do not know that cigarette filters are made from plastic material [54, 55]. This was also the case among adolescents and young adults that were considered to be part of a more eco-conscious generation [56].

So to further encourage the shift to anti-littering behaviour it is important to understand potential influences on cigarette littering [57]. One method is to investigate personal and contextual littering predictors through unobtrusive (i.e. hidden) observations of smokers [3, 4]. The influence of contextual predictors is undeniably important and higher for cigarette littering than for any other type of littering [3]. Additionally, littering predictors are site-specific and there is little consistent evidence for predictors that were identified so far [3, 4, 50, 53, 58]. More research is needed to allow for general conclusions based on local observations.

To account for the societal and environmental complexity of cigarette littering, we designed a multidisciplinary study with both human behavioural and ecotoxicological perspectives. Our study aimed at (i) investigating personal and contextual littering predictors at local scale, (ii) investigating the potential acute and chronic toxicity of semisynthetic cigarette filters to a freshwater model organism, and (iii) determine the potential drivers of toxicity. Based on the results from both the human behavioural observations and ecotoxicological testing, we discuss implications for litter prevention measures.

## Material and methods

### Cigarette littering observations

#### *Observation procedure*

To investigate cigarette littering predictors on a local scale, we conducted unobtrusive observations of smokers in Gothenburg, Sweden, which counts approximately 571,868 citizens and 40,000 daily smokers [59]. First, we carried out a pilot study by observing smokers at 10 different test sites in the city centre for 30 min each. The aim of the pilot study was to select the observation sites, to define the site boundaries, to fine-tune the littering protocol and to include site-specific variables. The final four observation sites were chosen based on their high number of data points that were obtained in the pilot study and on their size of the area. The area of an observation site needed to be within the observer's visual field but also allow for a high level of anonymity. The four observation sites consisted of three well-frequented public squares close to tram stops (hereafter site 1, site 2 and site 3), and one entrance

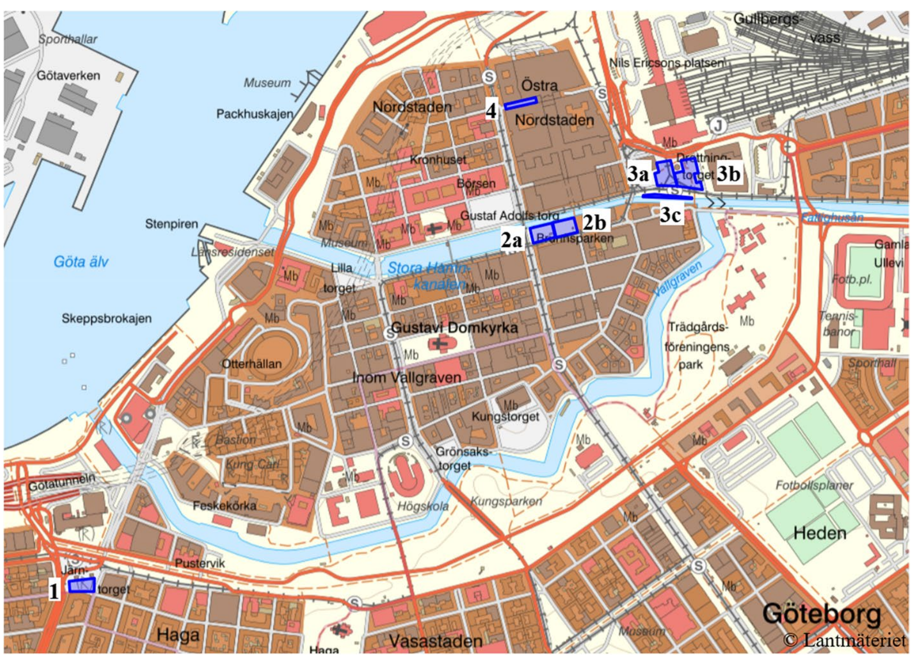


**Fig. 2** Overview map showing observation sites 1–4 in the city centre of Gothenburg, Sweden [60]. Sites are outlined by a blue line. Site 1 = public square *Järntorget*, site 2 = public square *Brunnsparken*, site 3 = public square *Drottningtorget*, site 4 = entrance to shopping centre *Nordstan*

to a shopping mall (site 4). Due to their large areas, sites 2 and 3 were in turn divided into separated sites (Fig. 2). Aerial photos of the observation sites including their defined boundaries and available disposal bins are shown in Figs. S1 – 4 of the Supplementary Information.

Based on the "Litter Behavioral Understanding Guide" from Action Research [61] and our pilot study, we developed the location description sheet and the littering protocol. Purpose of the location description sheet was to track contextual variables of the observation sites: observation site type, weather (*sunny/ cloudy/ rainy*), temperature, pre-existing general litter and cigarette litter in a scale from 0 (*not at all littered*) to 5 (*extremely littered*), number of ash receptacles, number of planters, number of tree pits, existing anti-smoking and anti-littering signage (*yes/ no*). The littering protocol tracked: gender, age, disposal method, group setting (*alone/ in group*), number of additional people in the group, number of additional smokers in the group, number of people in the setting (anonymity), distance to closest passer-by and closest ashtray at the disposal. Gender, age, and distances were visual estimates. All data were gathered on the observer's smartphone using the software Qualtrics. Before the start of the observation, the location description sheet was filled in at each site. After the start, observations were made for one hour straight, using a structured single-observer method i.e., one observer per site. Thereby, the observations were made from an unobtrusive lookout point within the sites. Only at site 3c were smokers observed from an external lookout point at the other site of the water canal. The observer was trying to be unobtrusive by checking their phone, drinking coffee, or pretending to wait by sitting on benches or walking around. After one hour, the observations changed onto the next observation site. Thereby the order of sites 1 to 4 was always kept constant and observations were conducted at a similar time of the day (morning, noon, afternoon, evening). Observations were included if smokers either littered or in other ways disposed of their cigarette within the site boundaries. Littering was defined as any disposal of cigarettes that did not end up in the available ashtrays, trash or recycling bins. Observations ended after an equal number of one-hour observations were made at each site and at least 580 cases were obtained. The total sample size was indicated by a power analysis using G*Power [62] (t-test, A priori: Tails = 2, $d = 0.27$, $\alpha = 0.05$, $1\text{-}\beta = 0.90$, $df = 1$), based on a desired statistical power of Cohen's $d = 0.27$. To estimate the effect size $d$, we calculated the average across overall effects of meta-analyses on pro-environmental behaviour ($n = 6$) [63–68]. Finally, seven one-hour observations were conducted at each site within March and April 2021, from Monday to Friday. In a total observation time of 49 h, 597 valid observations were made.

#### *Statistical analysis of results from cigarette littering observations*

The observations of 597 smokers were analysed by conducting a binary logistic regression model using SPSS version 28. The model tested whether *cigarette littering* i.e., the binary dependent variable (0 = *no littering*, 1 = *littering*), can be predicted based on different predictor variables. We included seven predictor variables that we hypothesized to have an influence on the likelihood of cigarette littering, as was shown elsewhere: *age* [3, 5], *number of present ashtrays* [3], *gender* [5, 50], *pre-existing general litter* [3], *pre-existing cigarette litter* [58], and *time of the* day [4]. We also included *group setting* as predictor variable and hypothesized that it is related to cigarette littering. The model was performed using a forced entry method. We used the likelihood ratio Chi-square test, Cox-Snell and Nagelkerke R squared and the Hosmer–Lemeshow goodness-of-fit test to evaluate the overall model fit. Moreover, we used the Wald Chi-square test to evaluate the contribution of one predictor variable to the prediction of cigarette littering. We approved that the model contains no obvious signs of bias by interpreting the model residuals. Although the model contains potential outliers (standardized residuals), there are no influential cases (Cook's distance, leverage values, and DFBetas). Finally, we confirmed non-multicollinearity among the predictor variables by running a linear regression model including collinearity statistics (tolerance values and variance inflation factors) and the same dependent and predictor variables as in the logistic regression model. All data used for the analysis are available in the Supplementary Data.

### Ecotoxicity testing of cigarette filters on *Chironomus riparius*

To study the effects of cigarette filters on the aquatic environment, we used *Chironomus riparius* larvae in 48-h acute and 7-day chronic exposures. *C. riparius* is suitable for this purpose, since it is sensitive (shows effects) and an established ecotoxicity model species, for which the toxicity assays are validated in test guidelines (e.g. OECD). Moreover, *C. riparius* is considered to be ecologically relevant (widespread distribution, numerical abundance, importance as prey for fish) and representative of the entire sensitivities of aquatic invertebrates [69]. The larvae were obtained from freshly hatched egg masses originating from our in-house culture.

We differentiated the toxicity between smoked and unsmoked cigarette filters. Smoked filters were collected from public ashtrays in the city centre at a random point in time (unknown time difference between disposal and collection). We intended this approach to obtain smoked filters from varying cigarette brands and products and to show-case the effects of a representative littered (smoked) filter. From the collected smoked filters, charcoal-containing filters (activated carbon filters) and filters with burn holes were excluded. Unsmoked filters were obtained from commercially available cigarettes (*L&M Blue Label* cigarettes) and incorporated to investigate the toxicity of substances originally present in virgin filters. For the filters used in experiments, wrapping paper and tobacco were removed manually with tweezers and cutter. In the chronic exposure, filters were left attached to the wrapping paper (only tobacco was removed).

### Acute exposure

The acute exposure aimed at investigating the toxicity of semisynthetic cigarette filters and its drivers by distinguishing between the effects of the whole filter, its chemicals, and its fibres (physical effect). Therefore, we performed three 48-h immobilisation experiments with the two cigarette filter types at five test concentrations each, using first instar larvae (i.e. < 24-h old). The experiments followed the OECD test guideline 235, "Chironomus sp., Acute Immobilisation Test" [70]. In a first step, we dipped the cleaned filters into liquid nitrogen and ground them using pestle and mortar. The fibres were used to test the effects of whole cigarette filters in experiment one (unwashed fibre experiment). Moreover, a fibre sample equivalent to one filter (according to the average filter weight) was placed in 500 ml dechlorinated tap water and stirred for 24 h on a shaking board with a frequency of 50 rounds per minute. Mean weight of the filters was $0.14 \pm 0.01$ g ($\pm$SD) among 20 smoked filters and $0.09 \pm 0.02$ g ($\pm$SD) among 10 unsmoked filters. After 24 h, fibre suspensions were vacuum filtered through WhatmanTM membrane filter paper (pore size 12 µm). The obtained leachates were used to test the chemical effects in experiment two (leachate experiment) and the obtained fibres were dried and used to test the fibre effect of filters in experiment three (washed fibre experiment).

Leachates were complemented with salts to recreate chironomids culture medium (dechlorinated tap water and salts) and to produce the leachate stock (final concentrations of 66.2 mg/L $CaCl_2$, 61.4 mg/L $MgSO_4$, 96 mg/L $NaHCO_3$ and 63 mg/L $CaSO_4$), following AFNOR guidelines [71]. The final concentrations were obtained by diluting the leachate stock in culture medium: eq. 2, 1, 0.5, 0.25 and 0.125 filters/L.

For the unwashed and washed fibre experiments, we mixed and combined several filter samples (smoked: $n = 10$, unsmoked: $n = 5$) to one fibre mass in order to increase the representativity of the filter sample that was then used in the experiments and collected according to the average filter weight. In the unwashed experiment, the average filter weight was 0.14 g for smoked filters and 0.11 g for unsmoked filters. In the washed experiment, the average filter weight was 0.12 g for the smoked filters and 0.09 g for the unsmoked filters. Additionally, fibre length was determined by microscopy image analysis using the ImageJ® software, and means were calculated on 30 random fibres per filter type and experiment. Mean length of the unwashed fibres was $1.8 \pm 0.9$ mm ($\pm$SD) for smoked filters and $2.4 \pm 1.6$ mm ($\pm$SD) for unsmoked filters. Mean length of the washed fibres was $1.3 \pm 0.6$ mm ($\pm$SD) for smoked filters and $1.4 \pm 0.9$ mm ($\pm$SD) for unsmoked filters. Each filter sample was placed in 500 ml culture medium right before the start of the experiments. The used concentrations were equivalent to the concentrations in the leachate experiment and were obtained by mixing the respective volumes of fibre suspension and culture medium.

In each of the three (independent) experiments, 10 larvae were introduced per Petri dish containing 10 ml of exposure medium. Each experiment included 11 exposure groups composed of 10 treatment groups (two filter types, each tested at five concentrations) and one control group (culture medium). The exposure groups comprised five replicates (Petri dishes) each. Only the washed fibre experiment had an unbalanced design and a varying number of replicates, due to five missing observations. After 48 h, the number of immobile larvae in each replicate was counted following the OECD guideline [70] i.e., by gently poking each larva and waiting 10 s for a potential response.

### Chronic exposure

The chronic exposure aimed at testing the toxicity of cigarette filter leachates (no testing of filter fibres) and the potential chemical partitioning between the water phase or the sediment phase. The exposure was based on the sediment–water chironomid OECD test guidelines 218 [72] and 219 [73]. At first, three glass jars (control, smoked and unsmoked filter types, respectively) were filled with silica sediment (Fontainebleau sand) and dechlorinated tap water (1:6; V:V), and covered with aluminium foil. For the smoked and unsmoked filter types, two filters per jar (eq. 1 filter/L of water and 1 filter/166.5 ml of sediment) were half buried in sand. The filters from smoked cigarettes were previously wiped with 70% ethanol to limit potential bacterial development during the leaching phase. In contrast to the acute exposure, we used whole filters for the leaching stage of the chronic exposure. After three weeks of leaching, filters were removed, and the sediment and water phases separated. The water phases were complemented with salts to recreate chironomids culture medium as previously described. The sediment and water phases from the leaching stage were then immediately used for organism exposure.

Chironomid larvae were exposed in glass beakers containing 50 ml of sediment and 300 ml of water phase, and were provided gentle aeration, light–dark cycle (16:8) and constant temperature (20 ± 1 °C). For each filter type, beakers were filled with either clean sediment and water from the leaching stage, or sediment from the leaching stage and clean, dechlorinated tap water complemented with salts as previously described. Ten 48-72 h old larvae were introduced per beaker and fed ad libitum every day. Each exposure group comprised seven replicates (beakers). After seven days, larvae were collected, photographed under microscopic conditions, and stored in 70% ethanol before further processing. Mortality was assessed based on the number of surviving larvae. Larvae body length and head capsule size were measured (ImageJ® software) to assess larval growth and development, respectively. Larvae instars (development) were determined based on guidelines from Government of Canada [74].

Teratogenicity was assessed following microscopic observation of larvae mouthparts, as described by Dias et al. [75]: mouthpart deformities were assessed and rated according to Warwick and Tisdale [76] and Vermeulen et al. [77]. Exposure groups were compared based on the occurrence of deformities and their severity (deformity scores).

#### Statistical analyses of results from ecotoxicity testing

All statistical analyses were performed using the R software version 4.1.2 [78] with a 0.05 level of alpha. For each acute exposure experiment, immobility was compared between exposure groups using one-way ANOVA that included all groups as separate levels ($a = 11$, $n = 5$, $N = 55$, washed fibres: $a = 11$, $n_1 \neq n_x$, $N = 50$). Following, we conducted Dunnett tests to compare the control group to each treatment group using the *DescTools* package [79]. To check if there are any differences between the smoked and unsmoked filter type, we ran full factorial two-way ANOVA models of *Concentration* ("2 filters/L", "1 filter/L", "0.5 filters/L", "0.25 filters/L" and "0.125 filters/L") by *Filter type* ("smoked" and "unsmoked") without the control ($a = 5$, $b = 2$, $n = 5$, $N = 50$, washed fibres: $a = 5$, $b = 2$, $n_1 \neq n_x$, $N = 45$). For that purpose, we used normalized immobility values that had been divided by the average immobility in the control groups (average across the replicates). To identify the drivers of cigarette filter toxicity i.e., the differences between the experiments, we ran a full-factorial three-way ANOVA with *Concentration*, *Filter type* and *Experiment* ("unwashed fibres", "leachate", "washed fibres") without the control ($a = 5$, $b = 2$, $c = 3$, $n_1 \neq n_x$, $N = 145$). For that purpose, we used normalized immobility values that had been divided through the average immobility in the control groups to allow for the comparison between the three different datasets. Following the multifactorial ANOVA models, we tested for pairwise comparisons and factor interactions by using estimated marginal means (also known as least-squares means) and their contrasts with the *emmeans* package [80]. This method allowed for post hoc analyses irrespectively of the unbalanced design in the washed fibre experiment. For the chronic exposure, mortality, larvae body length and mouthpart deformity scores were compared between exposure groups ("Control", "Water (sm.)", "Water (un.)", "Sediment (sm.)", "Sediment (un.)") using one-way ANOVA. Beforehand, individual body lengths and mouthpart deformity scores from a same replicate were averaged to account for individual variability. Following the ANOVA, we conducted Tukey HSD tests to analyse pairwise differences between groups, when relevant. Differences between groups for larval instars proportions and occurrence of mouthpart deformities were analysed with Pearson's chi-square tests. Additionally, we calculated effect size Cohen's *f* for all ANOVA models and Cramer's phi ($\phi_C$) for all chi-square tests. For all ANOVA models homogeneity of variances were confirmed with Levene tests (*car* package [81]), and normality of the model residuals with q-q plots and histograms. Further, chi-square test assumptions were approved by calculating the expected frequencies and checking for values smaller than five. Results were plotted using the *ggpubr* package [82]. All data used for the analyses are available in the Supplementary Data.

## Results

The following results are expressed in language of evidence using *p*-values from statistical significance testing as evidence measures, as recommended by Muff et al. [83].

#### Cigarette littering observations

In the total sample of 597 smokers, 66% were male, and 34% female. Observed ages ranged from 15 to 75 ($\bar{x} = 38$, $SD = 15$), and 66% of the smokers were alone at the moment of disposal. The clear majority littered their cigarettes (80%, 475/597). Observations were made at different times of the day, with 11% made in the morning before 11:00, 14% around noon between 11:00 and 14:00, 48% in the afternoons between 14:00 and 17:00, and 27% in the evening after 17:00. At the observation sites were either no, one, two, three or five ashtrays present. Further, cigarette litter was always present to some extent, ranging in scales 1 "little" to 4 "much" ($\overline{x}=2.76$, $SD=0.69$). Other types of visible litter (general litter) were less frequent and ranged in scales 0 "not at all" to 3 "moderately" ($\overline{x}=1.23$, $SD=0.58$).

The regression model showed strong evidence for containing variables that are related to cigarette littering, $\chi^2(7, N=597)=49.56$, $p<0.001$. Moreover, the Hosmer and Lemeshow chi-square test indicated good model fit, $\chi^2(8)=6.36$, $p=0.61$. The model correctly classified 4% of the 122 *no littering* and almost 100% of the 475 *littering* cases; the overall classification accuracy was 80%. Based on the included predictor variables, the model could explain 13% (Nagelkerke $R^2$) of variance in *cigarette littering* (Table 1).

Out of the seven predictor variables, only three variables showed strong evidence of being related to cigarette littering. There was very strong evidence that *group setting* is positively predictive of cigarette littering ($B=1.16$, $p<0.001$, odds ratio $=3.19$, 95% CI [1.82, 5.60]), with smokers in a group littering more than smokers who are alone at the moment of disposal. The littering rate was 91% for smokers in a group and 74% for individual smokers. Further, there was strong evidence that *age* is negatively predictive of cigarette littering ($B=-0.24$, $p=0.001$, odds ratio $=0.79$, 95% CI [0.68, 0.91]), with young smokers littering more than older smokers. Across all age groups, the clear majority of smokers littered their cigarette with littering rates steadily decreasing from the youngest smokers (93%), aged 15 to 25 years, to the oldest smokers (65%), aged 56 to 65 and 66 to 75 years. Moreover, there was strong evidence that *number of present ashtrays* is negatively predictive of cigarette littering ($B=-0.27$, $p=0.011$, odds ratio $=0.77$, 95% CI [0.62, 0.94]), with more ashtrays reducing the likelihood of littering. The highest littering rates occurred at sites with two and three ashtrays present (85% and 86%) and the lowest at sites with five ashtrays present (52%).

There was no evidence that either *gender* ($p=0.258$), *pre-existing cigarette litter* ($p=0.951$), or *time of the day* ($p=0.548$) was related to cigarette littering. However, the data revealed weak evidence for the influence of *pre-existing general litter* ($p=0.088$). We encourage readers to interpret this result in the light of past research, consequently showing that littering behaviour increases in littered compared to clean environments (e.g., Bateson et al. [84], Dur and Vollaard [85], Bergquist et al. [86]).

### Ecotoxicity testing of cigarette filters on *Chironomus riparius*

Following acute exposure, we found evidence that cigarette filters increased larvae immobility in concentrations from 0.25 filters/L and up of unsmoked filters ($p=0.002$) and from 1 filter/L and up of smoked filters ($p=0.043$, see Fig. 3A). Concentrations of 2 filters/L from smoked and unsmoked filters caused 36–100% and 75–100% larvae immobility, respectively. Mean immobility in the control group was 4%.

By checking the effects of chemical leachates, we only found evidence for increased larvae immobility from smoked filter leachate in the highest concentration compared to the control ($p=0.006$, see Fig. 3B). Filter leachates in concentrations of 2 filters/L from smoked and unsmoked filters caused 33–70% and 20–44% larvae immobility, respectively. Mean immobility in the control group was 16%.

By checking the physical fibre effect, we found strong evidence that filter fibres increased larvae immobility in all treatment groups compared to the control ("0.125

**Table 1** Logistic regression model predicting the likelihood of cigarette littering

| | *B (SE)* | Odds Ratio | 95% C.I. for Odds Ratio | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Group setting at disposal | 1.16*** (0.29) | 3.19 | 1.82 | 5.60 |
| Age (classified) | -0.24** (0.07) | 0.79 | 0.68 | 0.91 |
| No. of present ashtrays (categorical) | -0.27* (0.11) | 0.77 | 0.62 | 0.94 |
| Gender | -0.26 (0.23) | 0.77 | 0.49 | 1.21 |
| Pre-existing general litter (scale 0–5) | -0.33 (0.19) | 0.72 | 0.50 | 1.05 |
| Pre-existing cigarette litter (scale 0–5) | -0.01 (0.15) | 0.99 | 0.73 | 1.34 |
| Time of the day (classified) | 0.07 (0.11) | 1.07 | 0.86 | 1.34 |
| Cigarette littering | 2.62 (0.71) | 13.76 | | |

*Note.* $R^2=0.08$ (Hosmer–Lemeshow), 0.08 (Cox-Snell), 0.13 (Nagelkerke). Model $\chi^2(7)=49.56$, $p<0.001$

For each predictor variable, the unstandardized regression coefficient (*B*-value) is shown including its standard error. *P*-values are shown as evidence measure for predictor variables that have an influence on cigarette littering with $p<0.05$ for (*), $p<0.01$ for (**), and $p<0.001$ for (***). Moreover, the odds ratio and its 95% confidence intervals are presented

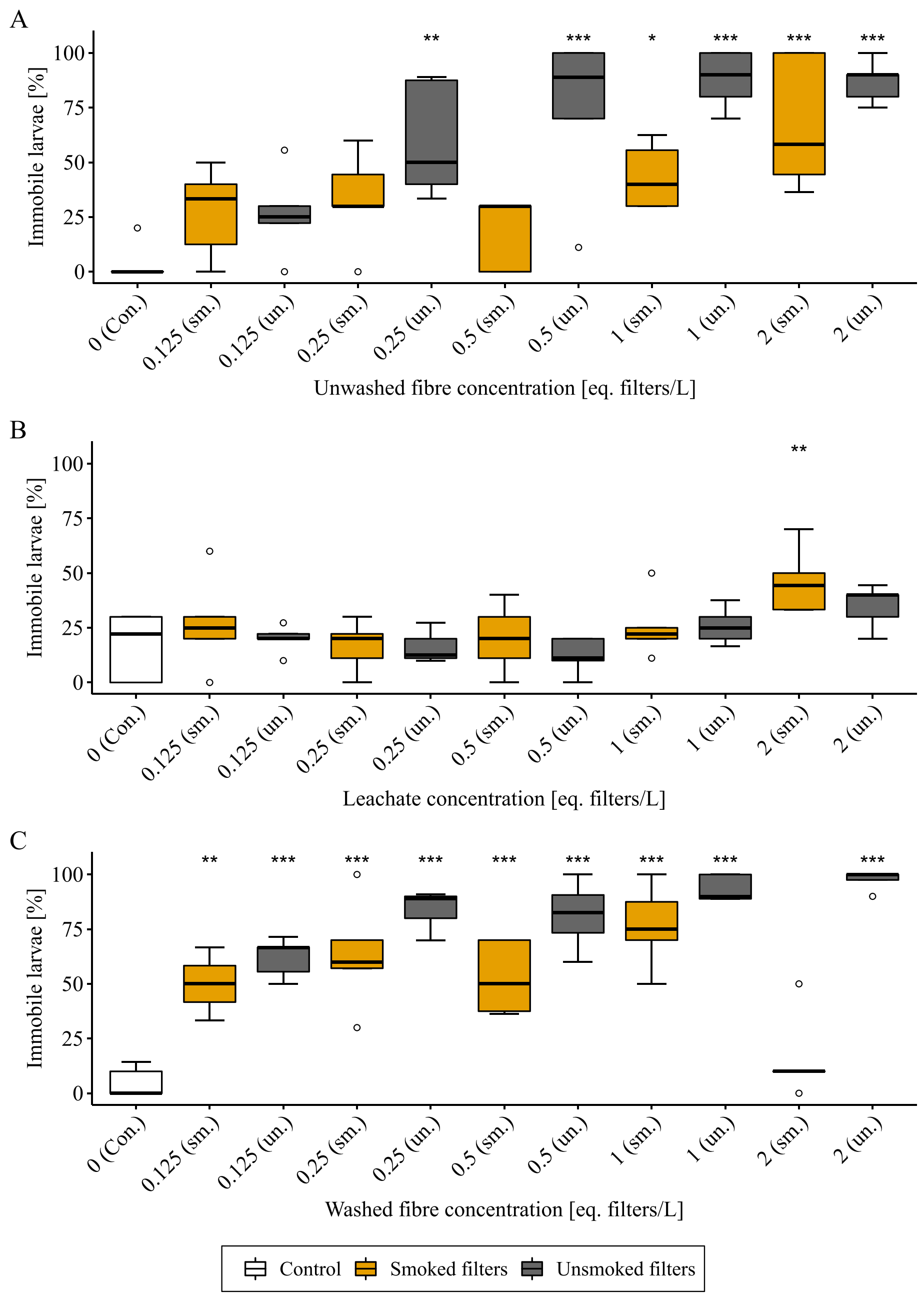


**Fig. 3** Immobility of first instar larvae following 48-h exposure experiments. Immobility is presented as the percent immobility in a replicate ($n = 5$) for all exposure groups ($a = 11$, $N = 55$) within the unwashed fibre experiment (**A**), leachate experiment (**B**) and washed fibre experiment (**C**). The washed fibre experiment had an unbalanced design ($a = 11$, $n_1 \neq n_x$, $N = 50$) due to five missing observations. The boxes display the 25%- and 75%-quartile, dark lines the median, and the whiskers extend to one and a half times the interquartile range. White circles represent outliers. The asterisks indicate evidence for increased immobility in the treatment groups compared to the control with $p < 0.05$ for (*), $p < 0.01$ for (**), $p < 0.001$ for (***), based on Dunnett test results

(sm.)": $p = 0.009$, All others: $p < 0.001$, see Fig. 3C). Only exemption was the group of smoked filter fibres in the highest concentration, for which we found no evidence of increased immobility compared to the control ($p = 0.855$). Filter fibres in concentrations of 2 filters/L from smoked and unsmoked filters caused 0–50% and 90–100% larvae immobility, respectively. Mean immobility in the control group was 5%.

We found strong evidence that smoked filters caused lower immobility than the unsmoked filters in the unwashed and washed fibre experiment (contrasts of estimated marginal means: both experiments $p < 0.001$). Contrarily, we found no evidence for differences between smoked and unsmoked filters in the leachate experiment ($F(1,40) = 2.22$, $p = 0.144$, $f = 0.24$). Furthermore, there was strong evidence for differences in larvae immobility between the three experiments ($F(2,115) = 72.18$, $p < 0.001$, $f = 1.12$). Larvae immobility was higher in the washed fibre experiment, compared to the unwashed fibre experiment, compared to the leachate experiment.

Following chronic exposure, only the sediment treatment group of the smoked filters showed evidence for increased larvae mortality compared to the control ($p = 0.017$, see Fig. 4). Larvae exposed to contaminated sediment exhibited on average > 20% higher mortality, compared to larvae in control conditions. There was no evidence for differences in mortality between the other treatment groups and the control ("Sediment (un.)": $p = 0.061$, "Water (sm.)": $p = 0.881$, and "Water (un.)": $p = 0.943$).

Moreover, there was strong evidence for larval growth inhibition in the sediment treatment groups (both filter types $p < 0.001$) compared to the control (Fig. 5). Larvae exposed to contaminated sediment (from both filter types) exhibited on average > 1.5-fold decrease in growth, compared to larvae in control conditions. On the contrary, there was no evidence for differences in body length between the water treatment groups and the control (smoked: $p = 0.659$, unsmoked: $p = 1.000$).

There was also strong evidence that larvae development was much more delayed in the sediment treatment groups compared to the control, with $\chi^2(1, N = 104) = 73.90$, $p < 0.001$, $\phi_C = 0.84$ for smoked and $\chi^2(1, N = 107) = 77.19$, $p < 0.001$, $\phi_C = 0.85$ for unsmoked filters (Fig. 6). Larvae exposed to contaminated sediment (from both filter types) exhibited > 80% decrease in development, compared to larvae in control conditions.

Mouthpart deformities were only assessed in larvae that reached the last development instar. Since so few larvae reached this instar in the sediment treatment groups, a considerable finding in itself, teratogenicity was assessed in the control and water treatment groups only. There was strong evidence that the occurrence of deformities (all deformities considered) was higher in the water treatment groups compared to the control with $\chi^2(1, N = 113) = 15.00$, $p < 0.001$, $\phi_C = 0.36$ for smoked filters and $\chi^2(1, N = 118) = 24.81$, $p < 0.001$, $\phi_C = 0.46$ for unsmoked filters (Fig. 7A). The frequency of deformities almost duplicated from 40% deformities in the control to 78% and 86% in the water treatment groups of smoked and unsmoked filters, respectively. The analysis of the severity showed very strong evidence for an increase in deformity scores in the water treatment groups compared to the control with $p = 0.008$ for the smoked and $p = 0.001$ for the unsmoked filter

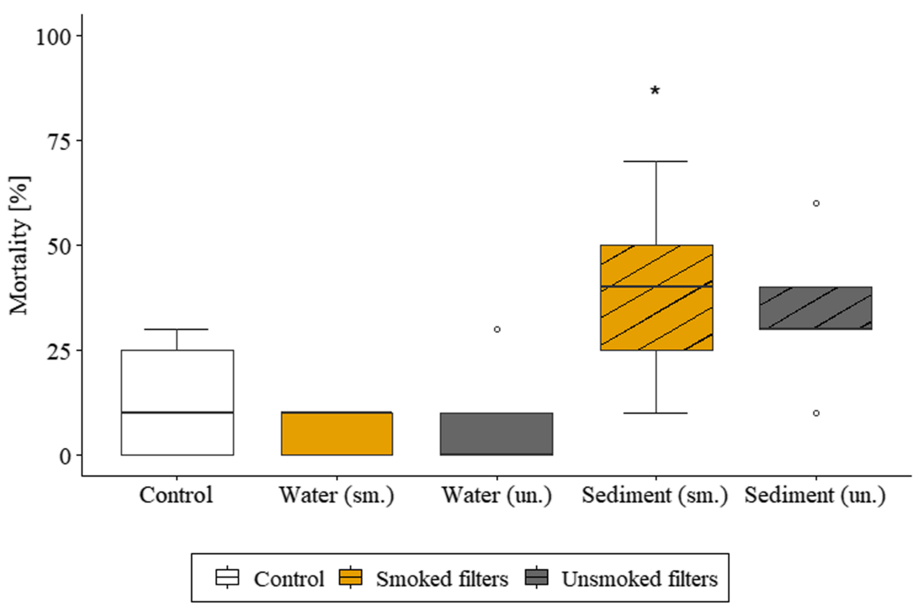


**Fig. 4** Larval mortality following the 7-day exposure. Mortality is presented as percent mortality in a replicate ($n = 7$) for all exposure groups ($a = 5$, $N = 35$). The boxes display the 25%- and 75%-quartile, dark lines the median, and the whiskers extend to one and a half times the interquartile range. Striped boxes represent the sediment treatment groups; white circles represent outliers. The asterisk indicates evidence for increased mortality in the treatment group compared to the control with $p < 0.05$ for (*), based on Tukey HSD results

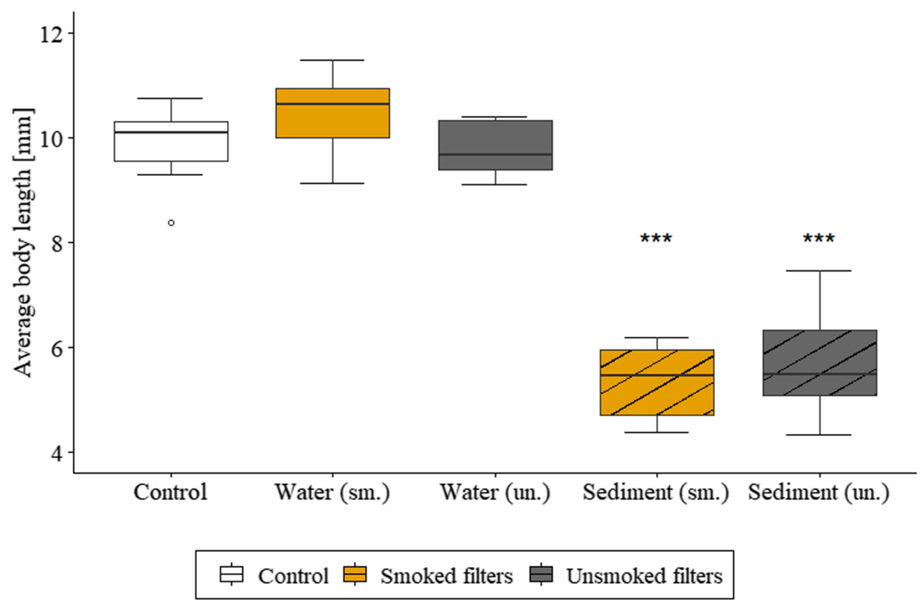


**Fig. 5** Larval growth following the 7-day exposure. Larval growth is shown as mean body length per replicate ($n=7$) for all exposure groups ($a=5$, $N=35$). The boxes display the 25%- and 75%-quartile, dark lines the median, and the whiskers extend to one and a half times the interquartile range. Striped boxes represent the sediment treatment groups; white circles represent outliers. The asterisks indicate evidence for growth inhibition in the treatment groups compared to the control with $p<0.001$ for (***)

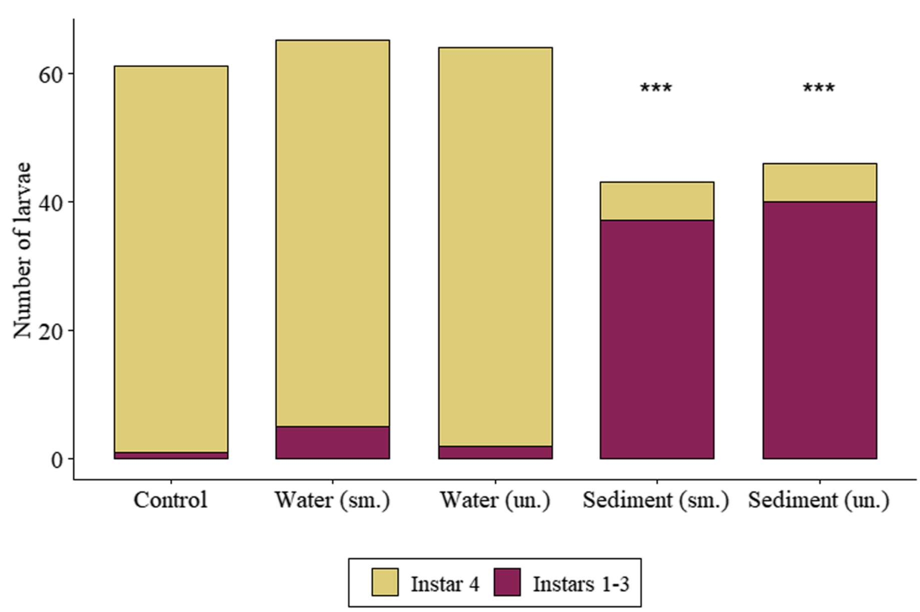


**Fig. 6** Larval development following the 7-day exposure. Larvae development is shown as larval proportions that reached instar four or instars one to three within each exposure group ($a=5$, $N=279$). Results shown are from exposures via water or sediment, using smoked (sm.) or unsmoked (un.) filters. The asterisks indicate evidence for larvae development inhibition in the treatment groups compared to the control with $p<0.001$ for (***)

contamination (Fig. 7B). Deformity scores of the water treatment groups were on average twice as high as the scores from the control. Light microscopy images showing examples of chironomid mouthpart deformities can be seen in Figure S5 of the Supplementary Information.

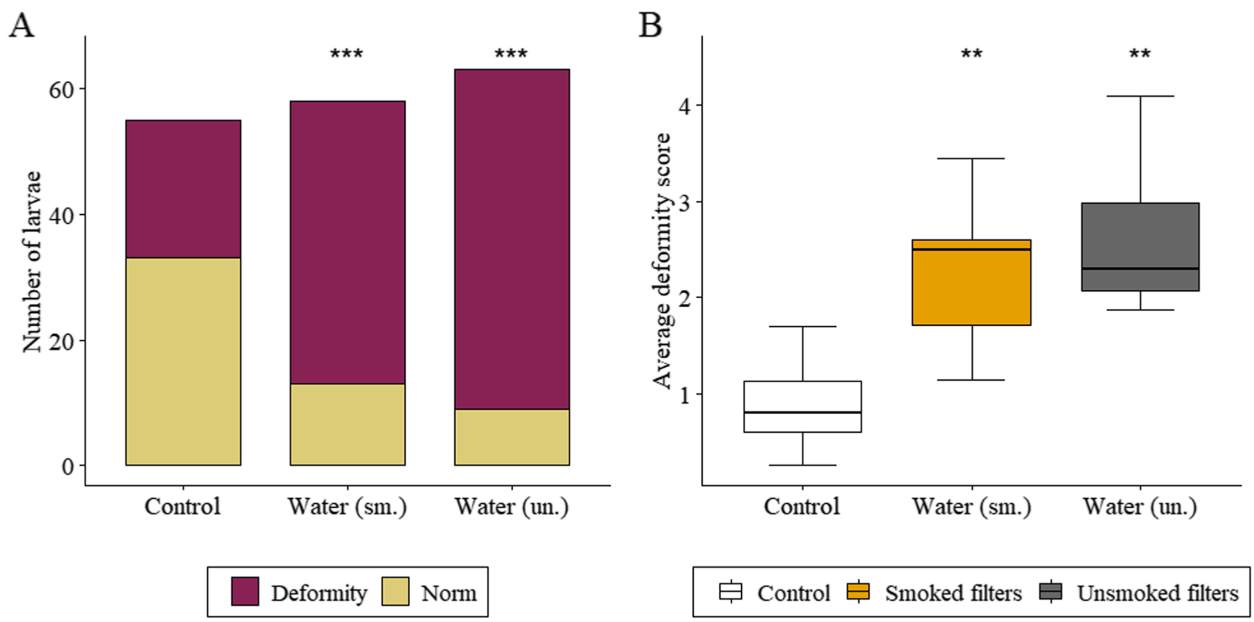


**Fig. 7** Teratogenicity of cigarette filter leachates following the 7-day exposure. Occurrence of mouthpart deformities (**A**) is shown as the proportion of larvae presenting mouthpart deformities in the control and water treatment groups ($a=3$, $N=176$). Mouthpart deformity scores (**B**) are shown as mean deformity scores per replicate ($n=7$) for the control and water treatment groups ($a=3$, $N=21$). The boxes display the 25%- and 75%-quartile, dark lines the median, and the whiskers extend to one and a half times the interquartile range. Results shown are from exposures via water, using smoked (sm.) or unsmoked (un.) filters. The asterisks indicate evidence for increased frequency (**A**) and severity (**B**) of mouthpart deformities in the treatment groups compared to the control with $p<0.01$ for (**) and $p<0.001$ for (***)

Furthermore, no difference between smoked and unsmoked filter leachates was detected, for any of the studied endpoints in the chronic exposure.

## Discussion

### Cigarette littering observations

We found that the majority of smokers littered their cigarette (80%), which is consistent with the finding of Patel et al. [4] (77%), who also observed smokers specifically at crowded places in the city centre of Wellington, New Zealand. Other observational studies that included a variety of different site types, partially outside the city centre, found lower littering rates (65% [3], 67% [5]), indicating that cigarette littering might be especially high at city centres. Therefore, we suggest implementing litter prevention measures specifically for city centres. Most importantly, our results suggest that cigarette littering is strongly influenced by the *age* and *group setting* of a smoker and the *number of present ashtrays*. We found that the likelihood of cigarette littering decreases with increasing age of a smoker, which is consistent with other studies [3, 5]. One reason for this is probably that young people have weaker personal norms against littering, as was shown elsewhere [87, 88]. Another reason could be the group influence (peer pressure) in cigarette littering that we identified in this study. More research is needed on which other factors determine cigarette littering of specifically young smokers and, we advise to target the 15- to 35-year-old smokers (the median age of those who littered in our study was 34 years).

The group influence seems to hold across the three youngest age groups of smokers ranging between 15 and 45 years with extremely high littering rates of 95–97%. From the age of 46, the group influence seems to be less determining. The 46- to 55-year-old and 66- to 75-year-old smokers who were in a group had even lower littering rates than same-aged smokers who were alone at the moment of disposal (70% vs 77% and 25% vs 70%, respectively). Since we did not analyse the interaction of the predictors *age* and *group setting*, no further interpretation is possible, and we encourage further research to include this specific interaction term in analyses of cigarette littering observations. One reason for the group setting to be strongly predictive of cigarette littering could be a link between positive self-evaluation and conformity of the group's norm. Christensen et al. [89] showed that positive emotions arise when conforming with the normative behaviour of a group, as long as the group is important for the individual's identity. To the best of our knowledge, our study is the first to demonstrate that the *group setting* stimulates cigarette littering and more research is needed to understand the coherence of group influences. Future research should investigate how the group influence can be used (as normative information) to stimulate anti-littering behaviour.

Our findings are suspects to limitation in form of unequal sample sizes in the regression data. The bias is mainly relevant for the *number of present* ashtrays that seems to be driven by the smallest sample size and needs to be interpreted with caution. Nevertheless, *the number of present ashtrays* is consistent with the findings of other studies that additionally identified the distance to the closest receptacle as predictor of cigarette littering [3, 5]. Therefore, we suggest that higher ashtray density throughout city centres can support litter prevention. Smokers who littered their cigarette were on average 9.45 m away from the nearest ashtray [3] and littering evidently increased from distances of 5 m and more [5]. In addition to the density, ashtrays should be well-positioned, and made easily accessible and identifiable. It was shown that receptacles with anti-littering signs (written prompts) or persuasive design can reduce littering [87]. Especially anti-littering signs with personal and social norm-activating prompts are known to reduce littering [87, 90–92]. Examples are the personal norm-activating prompt “Do you leave your litter lying around?” [87], and social norm-activating prompt “Pitch In!” (cooperation) [92]. Although findings on the most effective wording of prompts are inconsistent, often positively worded stimuli are more effective than negatively worded ones [91, 93]. Moreover, a study on pro-environmental behaviour (not specifically littering) suggests that the combination of positively and negatively associated sign content is most effective [94]. This implies e.g. positively worded text and negatively valenced emoticons or vice versa [94]. Eventually, any anti-littering sign seems to reduce littering compared to settings without signs [92]. Unfortunately, littering behaviour of young people (under the age of 25 [91]) is not much influenced by prompting [87, 91], and it remains unknown if certain ashtray designs can appeal to young smokers. Future research should investigate persuasive ashtray designs by differentiating between different age groups.

Overall, the ascertained predictors are important factors that should be considered for future litter prevention measures by targeting smokers in groups, young smokers aged 35 years and under, and by increasing the ashtray density and visibility in city centres. Moreover, research is needed on long-term effects of such measures once they are implemented.

### Ecotoxicity testing of cigarette filters on *Chironomus riparius*

The results of ecotoxicity testing suggest acute and chronic, aquatic toxicity of cigarette filters in form of different lethal and sub-lethal effects on *C. riparius.*

Our results indicate that smoked and unsmoked filter leachates similarly affected the larvae (acute and chronic exposure). This finding suggests that substances that are produced during the combustion processes while smoking and substances that are originally present in virgin filters, both play an important role in the toxicity of cigarette filters. During filter production, plasticisers such as triacetin [95, 96], diethyl phthalate [97], and delustrant titanium dioxide [96] are applied. Triacetin was, however, assessed to have low toxicity to aquatic invertebrate *Daphnia magna* [98] and therefore, might not play a major role in the toxicity we observed on *C. riparius*. Furthermore, titanium dioxide was assessed to have only low potential of aquatic toxicity [99] since it is insoluble in water and most likely to be transported as nanoparticles in cigarette smoke [100]. Diethyl phthalate though, was detected in smoked cigarette leachate (soaking time 24 h) and suspected to essentially contribute to the aquatic toxicity of cigarette filter litter [101]. Furthermore, diethyl phthalate is a toxicant to other aquatic species, including marine sea snail *Haliotis diversicolor supertexta* [102].

The results of the acute exposure indicate that loose filter fibres pose a considerable threat to *C. riparius*. Cigarette filter fibres seemed to add to the toxicity of cigarette filter leachates. But we did not perform chemical analysis and cannot further distinguish between chemical and physical fibre effect. It is possible that cigarette filter toxicity is driven by accumulation of the physical fibre effect and less water-soluble chemicals (e.g., PAHs), and this issue should be investigated further. In the filter leachates, water soluble chemicals like ethylphenol (tobacco flavouring agent) and nicotine [28] could have caused toxicity to chironomids. However, we observed only low effects of chemical leachates (see Fig. 3B) which could be explained by the potential degradation of chemicals. Indeed, nicotine has a relatively fast degradation rate in water (half-life: 3 days) [103]. Unlike fibres in the unwashed and washed fibre experiment, filter leachates were not used immediately for exposure but were stored in cold conditions (4 °C) with a two weeks-delay between leachate preparation and the start of the exposure. The enhanced toxicity in exposures with fibres is consistent with the findings by Belzagui et al. [48], who showed that leachates containing smoked filter microfibres were evidently more toxic to *Daphnia magna* in a 48-h exposure than leachates without microfibres (filtered leachates). They suggest that the ingestion of microfibres and their continuous leaching of harmful chemicals during the exposure, could be reasons for the discernible difference. By contrast, Wright et al. [49] showed that 28-day sediment exposures to smoked filter microfibres at concentrations up to eq. 8 filters/L had no impact on marine ragworm *Hediste diversicolor*. Differences could be due to different chemical partitioning between the water and sediment phase.

The results of the chronic exposure with water and sediment phases suggest that partitioning of toxic chemical leans towards the sediment phase, likely due to lower water solubility (higher octanol/water partition coefficient $Log\ K_{ow}$) of these substances. For the endpoints mortality, growth and development, sediment exposures caused more frequent and severe effects on the larvae compared to water phase exposures, which highlights the risk potential to benthic species. This is supported by the findings of King et al. [37] who demonstrated the long-term hazardous potential of cigarette butt leachates to benthic fauna by detecting nicotine, β-nicotyrine, myosmine, cotinine, linear alkanes, and xylenol in contaminated marine sediments that were detectable for 60 days, until the end of the exposure. The similar toxic effects of smoked and unsmoked filters in sediment are consistent with the findings by Quéméneur et al. [40]. They observed that both filter types decreased pH levels, and altered bacterial structures and metal distribution in marine sediment incubations. Moreover, we demonstrated the teratogenic potential of cigarette filter leachates (both smoked and unsmoked) in form of more frequent and severe mouthparts deformities following our chronic exposure. We therefore hypothesize that cigarette filter constituents interfere with the endocrine system of the larvae. Our findings are consistent with Parker and Rayburn [44] that showed the teratogenicity of smoked cigarette butt leachates on the development of *Xenopus laevis* embryos in 96-h exposures. The EC50 of malformations e.g., loose gut coiling or facial malformations, was 0.34–1.21 butts/L for two clutches of embryos. Additional to the teratogenic potential, the mutagenic potential of cigarette filters was evidenced by Montalvão et al. [35] in form of DNA damage in freshwater mussels exposed over 14 days to leachates from smoked filters in concentrations rounded to 0.023 butts/L and 0.230 butts/L.

Our used methods are subjects to restrictions that concern mainly the acute exposure. Although we used several filters to obtain more homogenous filter effects, with twice the number of smoked filters than unsmoked filter, unsmoked filters from the same brand and product were much more standardized than smoked filters. The random collection of smoked filters from different brands and products makes our study difficult to reproduce. Since the time difference between disposal and collection of smoked filters and their chemical load and composition is unknown, we cannot conclude that unsmoked filters are more toxic to the larvae than smoked filters. Toxic compounds from smoked filters could have been degraded or leached out already. Furthermore, the comparison between the three acute exposure experiments should be cautiously interpreted. Washed fibres might not be more toxic than unwashed fibres. The leaching time of 24 h could have been too short to solve a representative amount of chemicals that are usually trapped in smoked or unsmoked filters. Most effect-causing chemicals could have still been attached to the washed fibres. Thus, leaching of chemicals might have continued during the 48 h of the acute exposure in the washed fibre experiment. Again, since we did not conduct any chemical analysis, we cannot draw further conclusions. Another important restriction of the acute exposures with filter fibres is the accumulation of fibres in the test vessels (unequal distribution) and associated differences in direct exposure of the larvae [104].

Overall, our results indicate that cigarette filter litter has the potential to be toxic to freshwater invertebrates, either due to chemicals or fibres. Future research should further investigate the harmfulness of microfibres from cigarette filters (physical fibre effect). It should be determined if ‘clean’ fibres that have been purified of chemicals using additional solvent extractions, confirmed by chemical analyses, influence aquatic life.

### Implications of our multidisciplinary study for litter prevention

The results of both perspectives highlight the ubiquity (littering observations) and severity (ecotoxicity testing) of the cigarette littering problem and confirm the demand for action. To combat cigarette filter litter, a variety of policy and law initiatives have been established worldwide. Besides common and efficient indoor smoking bans, survey studies revealed that the extension of the ban to selected outdoor public spaces is perceived as an effective measure [105, 106] to reduce cigarette litter and substantially supported by the global public [107–110]. By way of example, smoking was banned at all beaches and parks in the state of Hawai’i [111], at public transportation platforms and in immediate proximity to entrances of public buildings in Queensland [112] and Sweden [113]. Another important policy is the Directive (EU) 2019/904 [114] (single-use plastic directive) that includes cigarette filters and aims to drastically increase their harmfulness to the environment. Furthermore, the directive obliges the extended producer responsibility on the tobacco industry, which is seen as an essential principle to help reduce cigarette filter litter [115]. Part of this obligation could be per-pack litter fees that are perceived as effective measure [105] by compensating public cleaning costs and potentially reducing cigarette sales [23, 54]. Under the principle of the extended producer responsibility, researchers have been arguing for years to ban the sale of filtered cigarettes [56, 115–117]. Ultimately, filters do not make smoking cigarettes any less harmful [116, 117]. Filters only reduce machine smoked tar and nicotine yields, leave the deceptive impression of a milder taste, and facilitate taking bigger puffs while smoking [116]. A recent clinical trial investigated the impacts of switching from filtered to unfiltered cigarettes on behaviour and toxic exposures of smokers [117]. Smokers perceived unfiltered cigarettes to have greater nicotine effects and less pleasant taste characteristics, and unfiltered cigarettes were smoked at a lower rate than filtered cigarettes [117]. Following, the ban of filtered cigarettes might make smoking less attractive and force the tobacco industry to implement actual solutions to lower the tar and nicotine yield in human smoked cigarettes. Finally, the ban would lead to less cigarette filter litter, thus decrease environmental harm.

Nevertheless, it was shown that policies alone cannot reduce marine and cigarette filter litter [54, 111, 118]. Law enforcement like cigarette littering fines [106] can potentially enhance the impact of waste-abatement and smokefree policies [111]. Cigarette littering fines are for example implemented in Sweden [119] and the UK [120]. Moreover, in order for policies to initiate individual behaviour change, they need to be accompanied by education and awareness campaigns [54, 111, 118]. Patel et al. [54] showed that smokers believing cigarette filter litter is harmful for the environment and not biodegradable, were more likely to support policies. For instance, to increase support for the single-use plastic directive, the regulation (EU) 2020/2151 [121] is applied since July 2021 and introduces marking specification for packages of cigarette filter products. Thus, cigarette packages sold within the EU contain a label informing the consumer that cigarette filters are made from plastic material. Its future impact should be monitored and investigated.

Overall, it was shown that smokers are less likely to support restrictive policies [108] and prefer educational measures [106]. Awareness and educational campaigns are evidently effective in reducing marine litter [118, 122], and increasing commitment to anti-littering behaviour [123]. Therefore, we suggest that our results can form the base of an integrated campaign that is tailored to both personal and contextual littering predictors (e.g. target group, place of distribution) and uses ecotoxicity results as content. We advise educational campaigns to target young smokers at e.g. schools, vocational colleges, universities and work places, in line with our suggested target group of the 15- to 35-year-old smokers. The campaigns could comprise information about cigarette filter litter as source of toxic plastic pollution. More specifically, they could inform that cigarette filters have the potential to be toxic to freshwater invertebrates, which are a substantial component of aquatic food webs. Future research should investigate the impact such an educational campaign could have in reducing cigarette filter litter.

Finally, policy measures could target excluding toxic chemicals from the filters and the semisynthetic polymer materials as a mean for reducing introduction of such compounds into the environment. There are numerous calls from researchers and other stakeholders to reduce the numbers and toxicity of chemicals in plastics as a means of increasing sustainability and increasing circularity [124, 125].

## Conclusions

We have presented a multidisciplinary study of cigarette littering predictors on a local scale and the toxicity of semisynthetic cigarette filters to aquatic life. Based on logistic regression analysis ($N = 597$), we showed that *age* (negatively) and *group setting* (positively) are personal predictors, and the *number of present ashtrays* (negatively) is a contextual predictor of cigarette littering. Thereby we are the first to demonstrate the group influence on cigarette littering. The identified predictors should be considered when designing future litter prevention measures. Litter prevention measures could use the group influence as normative information, target smokers aged 35 years and under, and increase the ashtray density and visibility throughout city centres.

Based on standardized ecotoxicity testing using *Chironomus riparius* larvae, we demonstrated that cigarette filter litter has the potential to be toxic and teratogenic to freshwater invertebrates. Both chemicals that occur during combustion processes and chemicals that are originally present in virgin cigarette filters seem to play an important role in the aquatic toxicity of cigarette filters. Furthermore, loose filter fibres seem to pose a considerable threat to *C. riparius*. Additionally, we showed that specifically benthic species might be at risk.

The results of this multidisciplinary study highlight the importance of adequate cigarette disposal and demonstrate the societal and environmental matter of cigarette littering in a comprehensive matter. Our results could be used to support litter prevention efforts, advisably via integrated educational campaigns. The campaigns could be tailored to the ascertained personal and contextual littering predictors and use ecotoxicity results as content.

**Abbreviations**

BTEX Benzene, toluene, ethylbenzol, xylene
PAHs Polycyclic aromatic hydrocarbons

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-022-00050-2.

**Additional file 1.** Supplementary Data

**Additional file 2: Figure S1.** Site boundaries and available disposal bins at observation site 1 (1). Site 1 = public square *Järntorget*. **Figure S2.** Site boundaries and available disposal bins at observation site 2 (1). Site 2 = public square *Brunnsparken*. **Figure S3.** Site boundaries and available disposal bins at observation site 3 (1). Site 3 = public square *Drottningtorget* next to the tram station *Centralstationen*. **Figure S4.** Site boundaries and available disposal bins at observation site 4 (1). Site 4 = entrance to shopping centre *Nordstan*. **Figure S5.** Examples of chironomid mouthpart norm and mouthpart deformities. Light microscopy image (A) shows the considered norm of chironomid mouthparts, (B) enlarged teeth, (C) additional tooth (indicated with a cross). On the control image (A), although present, the most outer teeth are not visible due to focus at high magnification.

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### Authors' contributions
TN and BCA developed the idea for this study. TN, AB, MBergquist and BCA supported the conceptualization and design. The experiments were performed by TN (littering observations, acute exposure), and MBlanchard and FM (chronic exposure), under supervision of AB, MBergquist and BCA. The data were analysed by TN, MBlanchard, and FM and data visualization was done by TN. TN wrote the first draft of the manuscript with support from AB (chronic exposure) and AB, MBergquist and BCA substantively revised it. All authors read and approved the final manuscript.

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### Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

## Declarations

### Ethics approval and consent to participate
Ethical approval was not requested since no smoker was personally approached or can be identified according to the obtained data. Smokers were simply observed in public places. Moreover, no ethical permit was required for the usage of the invertebrate ***Chironomus riparius.***

### Competing interests
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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## Publisher's Note