

# **The ecological impacts of discarded cigarette butts**

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## Glossary

**Cellulose acetate:** The overwhelming majority of cigarette filters currently in use are made of cellulose acetate. It is derived from plant-based cellulose but converted into a plastic by industrial processes (acetylation, hydrolysis and the application of a plasticiser). Modelling suggests that cellulose acetate cigarette butts may take up to 14 years to decompose in the environment.

**Cigarette butts:** The filter or end through which cigarettes have been smoked. Machine-manufactured cigarettes have contained filters for over 70 years and we define “butt” as the used filter once the cigarette is smoked. Cigarette butts present a unique challenge because they are ubiquitous and resistant to decomposition when littered. The smoked butt may contain paper, unsmoked and scorched tobacco, ash and contains chemicals some of which can exert deleterious effects on biota.

**Ecotoxicology:** The study of the presence and impacts of toxic (usually synthetic) substances in non-human biota.

**Leachate:** Technically defined as water that has percolated through a substance or substrate, we use the term in this review to refer to water in which cigarette butts have been soaked for the purpose of ecotoxicological research. Cigarette butt leachate has typically (though not exclusively) been used to evaluate the impacts of discarded butts in aquatic organisms, but compounds can be leached from cigarette butts in terrestrial habitats as well.

**Single-use plastics:** Plastic items intended to be used once before they are disposed of or recycled. Given that cellulose acetate cigarette butts are made of plastic, they constitute single-use plastics.

**Xenobiotics:** Chemicals detected in the environment or in organisms that would not naturally be present in substantially higher concentrations than would otherwise be expected. Cigarette butts have been found to contain hundreds of chemicals. When cigarette butts are littered, these

xenobiotic chemicals may potentially enter the environment and have been shown to affect biota.

## **Abstract**

Cigarette butts, one of the most littered items globally present a unique challenge to ecosystems due to their ubiquity, persistence and potential for harm. Over 35 studies have examined the toxicity of cigarette butts in biota from aquatic and terrestrial habitats from microbes to mice, but many organisms and habitats have not been tested. Two thirds of studies are on aquatic organisms and lethal effects were common. Research on the impacts on terrestrial life is lagging behind. Cigarette butts can affect the growth, behaviour and reproductive output of individual organisms in all three habitats, but research on wider effects on biodiversity and ecosystem functioning is lacking. Here we summarise the ecotoxicological concerns and identify important knowledge gaps for future research.

## **A unique and ubiquitous combination of physical and chemical pollution**

Given the ubiquity, toxicity and persistence of discarded **cigarette butts**, the poorly understood contribution of cigarette butts to microplastic pollution [1] and the global biodiversity crisis, there is a growing interest in the potential impacts of cigarette butt pollution in terms of toxicity on biota and, ultimately, how it may affect ecosystems. Approximately 1.13 billion smokers worldwide consumed 7.41 trillion tobacco-equivalents [2], which comprised of more than 6 trillion cigarettes in 2019, with up to three-quarters of them littered [3]. Discarded cigarette butts can be carried via surface run-off, ultimately entering watercourses and oceans [3-5]). Cigarette butt litter is recognised as a problem globally [4, 6-9] with recently reported maximum densities of 130 butts per m<sup>2</sup> in some cities, 150 per km of suburban road and 1600 per 100 m of beach [10-12]. The Ocean Conservancy, a US-based non-profit organisation,

76 routinely reports cigarette butts as the most encountered item of beach litter during its annual  
77 'International Coastal Clean-up' [3], and cigarette butts comprise up to 33%, 24% and 15% of  
78 all litter in Argentinian coastal cities, on Brazilian beaches and in Balinese coastal villages  
79 respectively [6, 13-14].

80 Cigarette butts are made of tightly packed microfibre bundles of **cellulose acetate** [15].  
81 Cellulose acetate is categorised as a 'bioplastic' because it is based on plant-derived cellulose  
82 which has been treated with acetic acid [16-17]. Although machine-measured levels of tar in  
83 simulated smoking experiments are lowered when filters are added to cigarettes [18],  
84 epidemiological data do not support them as beneficial to human health [19-20]. The  
85 effectiveness of filters at reducing the exposure of smokers to harmful substances is at best  
86 dubious and at worst potentially responsible for minimising public perceptions of the health  
87 risks of smoking [16, 21-22]. Cigarette filters are not regarded by the global public health  
88 authority to be a protective device [3]. Since the 1950s, most machine-manufactured cigarettes  
89 have contained filters, although the proportion of 'roll-your-own' smokers adding standalone  
90 filters to their cigarettes is not well understood [23].

91 Modelling suggests that cigarette butts take up to 14 years to fully decompose, likely due to  
92 the resistance to microbial degradation imparted by acetylation, and influenced by conditions  
93 such as temperature, moisture, the nature of the substrate and potentially also by the nitrogen  
94 content of smoked cigarette butts [24-25]. What makes cigarette butt pollution unique as an  
95 environmental challenge is that, in addition to the plastic pollution posed by its considerable  
96 persistence and ubiquity, butts can also be highly toxic. Smoked cigarette butts are infused with  
97 chemicals from smoking, >40 of them harmful to aquatic organisms. These include polycyclic  
98 aromatic hydrocarbons (PAHs), metals, phthalates, nicotine and volatile organic compounds  
99 [26], which can be released as **leachate** in water. The physicochemical properties of water (e.g.

pH, salinity, ionic strength) affect the leaching rate of some potentially toxic metals [27]. This phenomenon could lead to differential effects on marine and freshwater organisms.

Inappropriately disposed cigarette butts, therefore, are **single-use plastics** of questionable human health benefit which are contaminated with various **xenobiotics**. The impacts on biota following exposure to cigarette butts are wide-ranging and include lethal (i.e. increased mortality) and sublethal effects (e.g. affecting growth, reproduction and behaviour). Since the first published and peer-reviewed study in 2006 [28], a total of 36 investigations have been published in the scientific literature to date investigating the **ecotoxicology** of cigarette butts across a range of endpoints and on various biota (Supplemental information online). The biota studied include microbes, plants, invertebrates, fish, amphibians, birds and mice (Figure 1) and current evidence indicates that cigarette butts potentially exert more deleterious impacts in aquatic systems compared to terrestrial environments. For example, investigations into aquatic life have reported relatively greater acute effects including mortality, and the toxicity of cigarette butts in water may exceed that of littered cigarette butts in terrestrial environments possibly due to the slower release of toxicants in terrestrial environments, e.g. [29-30].

However, to date, aquatic biota has been subject to greater ecotoxicological investigation than terrestrial systems.

It is important that research on the environmental toxicity of cigarette butts is done as environmentally realistic as possible. For example, studies on organisms inhabiting lotic or marine aquatic environments should be subjected to flow-through experiments as opposed to static tanks to more accurately represent the flow of water that occurs in such habitats. Much research has been done in standardised, static simulations within laboratories, which provided valuable information, but is difficult to translate “to the field”. In this review, we highlight the current gaps of knowledge to fully understand how cigarette butts affect aquatic and terrestrial

ecosystems. The findings are summarised in a heatmap (Figure 2) to provide a colour-coded graphical depiction of the intensity of research effort and significant findings thus far.

### **How has the ecotoxicity of cigarette butt litter been assessed?**

Published research has focussed on different levels of biological organisation, from experiments using molecular analyses to observations of species assemblages (Figure 2), which we have categorised: for each publication, we recorded (i) which endpoints were measured, (ii) in which category/categories of organism/s and, out of those, (iii) whether the responses were statistically significant, compared to controls (i.e., conspecific subjects not exposed to cigarette butts). Although not displayed in the heatmap, it should be emphasised that any synthesis of the literature on ecotoxicological research on cigarette butts undertaken to date is compounded by differences in study design, particularly concentrations used and exposure duration. In addition, some studies have compared effects of: (i) smoked *vs.* unsmoked cigarettes, (ii) whether remnant tobacco was retained alongside the cigarette butt or removed, (iii) ‘regular’ *vs.* flavoured (e.g. mentholated) cigarettes, (iv) different cigarette brands and (v) cigarettes containing conventional cellulose acetate butts *vs.* those containing alternative, biodegradable butts (supplemental information online). Examples of such studies are discussed in this review. Freshwater organisms have been commonly used as ecotoxicological research subjects: 26 aquatic-only, peer-reviewed studies with 15 freshwater, seven marine and four covering both freshwater and marine and ten terrestrial-only studies (supplemental information online). The four most important take-home messages from Figure 2 are: (i) There has been a greater research focus on aquatic (particularly freshwater) organisms, compared to terrestrial life, with deleterious effects following cigarette butt exposure regularly reported in aquatic systems. (ii) The majority of research has used cigarette butt leachate rather than whole butts, thus the physical component of this contaminant is understudied (iii) Very few studies have examined

wider ecological effects of cigarette butts such as communities and those that did were on microbes and ectoparasites. No studies thus far have addressed their wider impacts on ecosystem functioning and services. (iv) sublethal effects are still understudied, including changes in behaviour and reproduction.

#### **How toxic are cigarette butts and associated substances for aquatic biota?**

Most aquatic tests have focused on invertebrates (crustaceans, molluscs and freshwater insect larvae) and vertebrates (fish and amphibians), with a smaller number of studies involving microbes, algae and plankton (Figure 2). Cigarette butts may exert greater lethal effects in aquatic life in comparison with terrestrial biota: mortality for treatment subjects has been observed in most of the aquatic studies, i.e., in foraminifera [31], invertebrates [1, 29, 32-37] and vertebrates [38-40], with many significant effects reported. Mosquitoes have an aquatic and terrestrial life stage, and six studies have reported lethal effects of cigarette butt leachate on mosquitos during their aquatic life stages, but not much is known on their terrestrial life cycle. As mosquitoes are vectors of zoonotic disease, this research effort has implications for the use of collected cigarette butts as vector control [32-34, 41], but also as to whether the global ubiquity of cigarette butts may facilitate selection pressure for nicotine resistance in mosquitoes [37].

Aside from lethal effects, cigarette butts can illicit important sublethal effects such as alterations to behaviour and changes to reproductive output of a range of aquatic organisms. Animal behaviour is a sensitive and important variable in research to aquatic organisms [42] and, indeed, significant alterations to behaviour were found in most studies which tested it in response to cigarette butt leachate. The movement of marine [29] and freshwater [35] gastropods was reduced when exposed to high concentrations of cigarette butt leachate. Marine polychaetes [43] and freshwater bivalves [44] took longer to create burrows and, in the case of

the bivalves, also dug shallower burrows. Behavioural endpoints are, however, often overlooked in ecotoxicological assessments [45], but alterations to behaviour can impact the fitness of an individual (e.g. via predator avoidance and feeding), with consequences for population dynamics, species interactions and ecosystem functioning [46].

Lima *et al.* [47] found that the reproductive rates of marine copepods (*Nitokra* sp.) exposed to leachate of just 0.1 or 0.01 butts L<sup>-1</sup>, in water or sediment respectively, were reduced by >50% relative to controls. The effects of cigarette butts on reproduction, however, have predominantly been assessed in mosquitos with mixed results (either decreases in hatching success; [32]; no effect on hatching success of fecundity; [34] or some increases in hatching success; [37]). Given the link of reproductive success to the stability of populations, we need to understand the effects of cigarette butts on reproduction of a broader range of organisms.

Differences between aquatic studies may occur due to differing experimental set up. Realistic exposure durations are important for any environmentally-relevant assessment of ecotoxicity. Conditions for animals inhabiting large and dynamic aquatic environments (e.g., rivers and oceans) can be simulated using realistic flow-through, as opposed to static, water systems or in-situ experiments. To date, however, there are only two studies that has used continuously flowing water: Green *et al* [48] reported that smoked conventional butts decreased clearance rates of blue mussels (*Mytilus edulis*) (at 1 butt L<sup>-1</sup>) 2.5 times and reduced the biomass of benthic microalgae (at 0.25 and 1 butt L<sup>-1</sup>) by up to 3 times. On the contrary, Werdel *et al* [49] found that biofilm formation, in terms of algal biomass and diatom health, was unaffected in an experiment using a flowing stream with agar plates made with various concentrations of cigarette butt leachate. Such systems are relevant to understand ecotoxicological effects of cigarette butts, but standardisation is required to facilitate comparison between different ecosystems.



Given that cigarette butts can persist in the environment for many years, a better understanding is needed of how their ecotoxicological impacts can change over time. An apparent temporal decline in cigarette butt toxicity has also been observed for marine bacteria [25], terrestrial snails [30] and mosquito larvae [32]. Notably, an experiment which tested the effects of leachate from cigarette butts which had been aged for 5 years found that the growth of a freshwater microalga (*Raphidocelis subcapitata*) was the most strongly inhibited by recently smoked cigarette butts (i.e., less than 30 days post-smoking) [25]. In this long-term experiment, after 30 days most compounds in leachate they had detected earlier were no longer detectable and there was a rapid decline in nicotine concentrations. However, despite this apparently fast decline in toxicity, leachate of butts that had been decomposing for five years were still toxic to *R. subcapitata* [25]. It is also possible that microfibrils, a type of microplastic, are released during degradation. There is a growing body of evidence of how microplastics affect aquatic biota [50], including animals and primary producers [51]. Studies assessing the impact of cigarette butts on primary producers, however, have been limited with only three studies on microalgae [25, 49, 52], one study on a marine macroalga [48] and no studies on plants in either habitat. Understanding impacts at the base of aquatic food webs would enable better predictions of impacts across multiple trophic levels in both freshwater and marine systems, but also in terrestrial habitats.

#### **Studies of impacts of cigarette butts in terrestrial systems are limited**

Amongst terrestrial biota, three studies to date have focused on plants (Figure 2) [11, 53-54]. The effects reported in terrestrial plants include physiology: chlorophyll [11], root water content [54], growth (root [54] and shoot length/biomass [11]; cell growth and overall toxicity, comprising cytotoxicity, i.e., cell toxicity and mutagenicity [53]. Green *et al.* [11] investigated effects on perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) following

224 exposure to regular vs. mentholated cigarette butts including smoked butts with remnant  
225 tobacco attached and unsmoked filters. The germination of *T. repens* was especially negatively  
226 impacted: 27% fewer seeds germinated with smoked cigarette butts present. Reductions in  
227 shoot length, root biomass and changes to chlorophyll content in both plants occurred  
228 regardless of whether butts were unsmoked, smoked or contained remnant tobacco. The plants  
229 used are model organisms for important grassland ecosystems where they are at the basis of  
230 the food web. Another example of detrimental effects on plant health is reported by Mansouri  
231 *et al.* [54] who found that almost 50% seeds of *Vicia faba* did not germinate when exposed to  
232 leachate from cigarette butts, and ash had even a stronger impact with no seeds able to  
233 germinate. Furthermore, the uptake of chemicals associated with cigarettes, such as nicotine,  
234 into plant tissues as found by Selmar *et al.* [55] could lead to cascading effects on foodwebs,  
235 including plant communities which are important for sustaining biodiversity and for supporting  
236 insect pollinators.

237 Three studies [56-58] so far have investigated the effects of cigarette butts directly on terrestrial  
238 vertebrates. Cardoso *et al.* [56] examined predator avoidance behaviour in laboratory mouse  
239 (*Mus musculus*), growth and physiological responses (food and water consumption, locomotor  
240 activity, visual, auditory and olfactory functions) following provision drinking water  
241 containing leachate. A significant reduction in avoidance behaviour in response to the presence  
242 of domestic cats (*Felis catus*) or corn snakes (*Pantherophis guttatus*) was observed for exposed  
243 mice. For example, when a snake was present the mice spent ~ 50% more time in a safe place  
244 when not exposed to cigarette butt leachate (an avoidance response) than when exposed to  
245 environmentally realistic concentrations of leachate, suggesting that the cigarette butt leachate  
246 may make them more prone to predation. Another ecotoxicological concern is the interaction  
247 of birds with cigarette butts. House finches (*Carpodacus mexicanus*) were found to incorporate  
248 littered cigarette butts into the lining of their nests [58-59]. The reasons for this are not fully

established, but levels of nicotine and other chemicals in butts correlate with reduced numbers of parasitic arthropods in nests [58-59]. Hatching/fledging success and nestling immune response of house finches were positively correlated with the proportion of cigarette butt material in the nest. However, long-term genotoxic harm in nestlings increased with the proportion of cigarette butts in nest material, indicating that nestlings were at heightened risk of DNA damage, but lower parasite burden [57-59]. Despite potential positive impacts outlined above, the possibility of chicks ingesting cigarette butt litter introduced into their nests is of concern. Due to their small size and fast metabolism, birds absorb chemicals faster, meaning they require less toxins to cause harm and so are at particular risk from ingestion of cigarette butts [60], although research on ingestion rates is lacking.

Not many studies have investigated the effects of cigarette butts on terrestrial invertebrates, but research has been done on ectoparasites in nests [58-59], snails [30] and earthworms [61]. Gill *et al.* [30] reported short-term behavioural changes in snails (*Anguispira alternata*) in the presence of smoked mentholated cigarette butts. At the start of a three-week experiment, six times more snails were associated with sections of mesocosms containing no cigarette butts and were less likely to occupy those sections containing the maximum quantity of butts. However, this difference was not there at the end of the experiment. Given that food provision did not differ between sections, it is possible that the toxicity of butts declined over time (as suggested previously). Similarly, despite a relatively lengthy experiment period of 70 days, Korobushkin *et al.* [61] reported no mortality effects for earthworms (*Eisenia fetida*) in experimental microcosms containing both smoked and unsmoked butts. Total worm biomass in microcosms containing smoked butts was, however ~2 times greater than that for controls, with the presence of worms correlating with lower quantities of cigarette paper, thus worms appeared to consume the cigarette paper wrapping, though not the cellulose acetate butt itself.

## **Material matters: cigarette butts are complex pollutants and more than just leachate**

Most ecotoxicological studies have focused on the chemical component of cigarette butts, using extracted leachate (28 of a total of 36 peer-reviewed studies), with five studies using whole butts, and two exposing organisms to a combination of leachate and microfibres from butts. In addition, just one study has compared the impact of leachate from cigarette ash vs. that of smoked butts, reporting higher phytotoxicity associated with leachate from ash [54]. When a cigarette butt is littered, the used plastic filter, ash, remnant tobacco, microfibres and leachate all enter the environment. By only testing the effect of leachate we are missing crucial information about the wider impact of this unique pollutant. For example, when cellulose acetate microfibres were present in smoked cigarette butt leachate, it was more toxic to water fleas (*Daphnia magna*) with 50% immobilisation (as a proxy for mortality) occurring at 0.017 butts L<sup>-1</sup> compared to 0.067 butts L<sup>-1</sup> in leachate without microfibres [1]. On the contrary, Wright *et al.* [43] found no significant effects on growth rates, burrowing time and DNA damage in ragworms (*Hediste diversicolor*) when exposed to smoked cigarette microfibres in sediment, with subjects accumulating 13 times less nicotine following fibre exposure compared to those which were exposed to leachate. Ragworms exposed to leachate in seawater had >30% weight loss, twice the DNA damage and >10 times longer burrowing times compared with controls. Investigations into the effects of cigarette butt microfibres in a greater range of taxa is desirable given their importance as a form of microplastic litter. Belzagui *et al.* [1] reported that smoked butts in water release approximately 100 microfibres (<0.2 mm) per day, equating to 0.3 million tons of microfibres released per year.

The plastic (cellulose acetate) filter itself, even when unsmoked, can cause detrimental effects possibly due to plasticizers, such as diethyl phthalate (a known toxicants to plants [62] and animals [63]). Leachate from unsmoked cellulose acetate filters can be toxic to marine and freshwater fish [38], amphibians [39] and freshwater microalgae [25] and unsmoked filters can

decrease germination and growth of plants [11] and alter microbial communities in marine sand [64].

Current moves to reduce single-use plastics may lead to a shift to alternative butts composed of biodegradable cellulose instead of cellulose acetate. There have been concerns in the past that biodegradable filters would not be acceptable to smokers due to poor taste, short shelf life and physical instability during smoking [4]. However, recent advances in filter technology, combined with shifting consumer opinions [65], may improve uptake of biodegradable filters in the future as pressure to reduce plastic litter mounts. The decomposition rates and ecotoxicological impacts of biodegradable cigarette butts remain largely unknown. It was estimated that smoked biodegradable cellulose butts would take 2.3–13 years to disappear in compost and at the soil surface, respectively, whilst smoked conventional cellulose acetate butts would take 7.5–14 years to disappear [24]. As well as uncertainty of the persistence of biodegradable butts, there is also very little known about their ecotoxicological impacts. An experiment simulating a marine environment found that smoked biodegradable butts caused no significant impacts, whilst smoked conventional butts affected clearance rates of *M. edulis* and reduced the biomass of benthic microalgae [48]. On the contrary, leachate from either biodegradable or conventional smoked butts caused equal mortality of freshwater pond invertebrates at 5 butts L<sup>-1</sup> and a reduction in their movement at 1 butt L<sup>-1</sup>[35]. In addition, leachate from biodegradable butts may have relatively higher concentration of metals than conventional butts possibly leading to alterations to microbial communities [66]. Whether biodegradable filters play an important role in the future or not, they contain toxic compounds from smoking, and it is possible they will be preferentially littered due to their perceived biodegradability [67], therefore they pose a similar threat to the environment as conventional butts do.

## **Concluding Remarks and Future Directions**

The majority of studies investigating cigarette butt ecotoxicity have focused on individual organisms from aquatic habitats and many have found lethal effects in enclosed systems. In contrast, sub-lethal endpoints (e.g. reproduction, behaviour, physiology) have received less attention, but there is evidence that ecosystem engineers (such as bivalves and plants) can be affected by environmentally realistic concentrations of cigarette butts and this could lead to wider ecological impacts. Very few investigations have been made into the wider ecological effects of cigarette butts on populations or species diversity, and no studies have focused on ecosystem functioning. Whilst evidence of ecological impacts is often needed to justify policy reform, there is already strong evidence from lower levels of biological organisation that cigarette butts pose a hazard to the environment and require tighter legislation. Finally, study design is important; to accurately predict the impacts of cigarette butts in the real environment we need environmentally realistic experiments in relation to exposure duration, contaminant concentrations and the use of flow-through systems for biota associated with habitats containing moving water.

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## **Supplemental Information**

Supplemental information associated with this article can be found online.

## Tables

Table 1. Definitions of the endpoint categories used in this review to summarise the cigarette waste (i.e., butts, leachate, microfibres and ash) ecotoxicological tests reported in the literature to date

Endpoint category	Study Definition
Mortality	Tested whether subjects died/were immobilised.
Toxicity	Tested whether sublethal responses such as cytotoxicity, immunotoxicity, genotoxicity and mutagenicity were expressed.
Physiological	Tested physiological responses, such as feeding rates, filtering rates, or chlorophyll content of plants/algae.
Reproduction	Tested whether the subject's reproductive output was altered. This included germination for plants and hatching success for egg-laying organisms.
Growth/Malformation	Tested whether growth rates were affected or malformation occurred during development.
Behavioural	Tested whether the subject's behaviour in terms of movement (speed, frequency of movement, latency to move, predator avoidance, burrowing) were altered.
Population/Diversity	Tested impacts at the population level of a species or at the level of species diversity.

## Figure captions

**Figure 1. Diagram to illustrate exemplar organisms and their habitats** (1: terrestrial, 2: freshwater, 3: marine; organisms not to scale) in which the effects of cigarettes butts have been studied at various endpoints. 1a: The songbird house finch (*Haemorhous mexicanus*); 1b: The earthworm *Eisenia fetida*; 1c: the plant white clover (*Trifolium repens*); 1d: the grass *Lolium perenne*; 1e: the snail *Anguispira alternata*; 1f: terrestrial bacteria (various strains); 1g: the mouse *Mus musculus*; 2a: the freshwater fish Nile tilapia (*Oreochromis niloticus*); 2b: the freshwater shrimp *Thamnocephalus platyurus*; 2c: freshwater bacteria (various strains); 2d: the freshwater microalgae *Raphidocelis subcapitata*; 2e: the frog species *Hymenochirus curtipes*; 2f: the zebra mussel (*Dreissena polymorpha*); 3a: marine bacteria (various strains); 3b: the marine microalgae *Dunaliella teriolecta*; 3c: the copepod (genus *Nikotra*); 3d: the marine fish topsmelt (*Atherinops affinis*); 3e: the marine snail *Austrocochlea porcata*; 3f: the blue mussel *Mytilus edulis*.

Image credits: Earthworm icon: [naakila.blogspot.com](http://naakila.blogspot.com) (CC BY 4.0); Clover icon [Piotr Siedlecki](#) (CC BY 4.0); Land snail silhouette [Piotr Siedlecki](#) (CC BY 4.0); bacteria icon [Metro Science Natural Sciences Icons](#) (Icons8); Microalgae icon [Hanna Vernydub](#) (CC BY 4.0); Copepod icon [Pham Thanh Loc](#) (CC BY 4.0); Fish icon [The Noun Project](#) (CC BY 4.0); Sea snail icon [iconsout](#) (licence); Mussel icon [The Noun Project](#) (CC BY 4.0); Shrimp icon [Free Icons Library](#); Frog icon [SVG SILH](#) (CC BY 4.0). All other images are free for commercial use with no attribution required.

**Figure 2.** Heatmap summarising research focus to date on the effects of cigarette butts in different habitats. The colour of the columns headed as "Tested" relates to the top left value, which is the number of overall experiments/tests done as reported in the literature, and the colour of the column headed "Sig." is based on the number of tests which were reported significantly different from a concurring control. The bold value in the middle of a cell in the "Tested" column represent the number of papers published which report a test in a particular habitat measuring a response variable in the corresponding category. Many papers report multiple independent observations, thus



the number of tests is often greater than the number of papers. For example, there were 22 tests done on freshwater invertebrates, of which 16 reported significant differences from the control, but the results have been published in nine papers.

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Figures

Figure 1

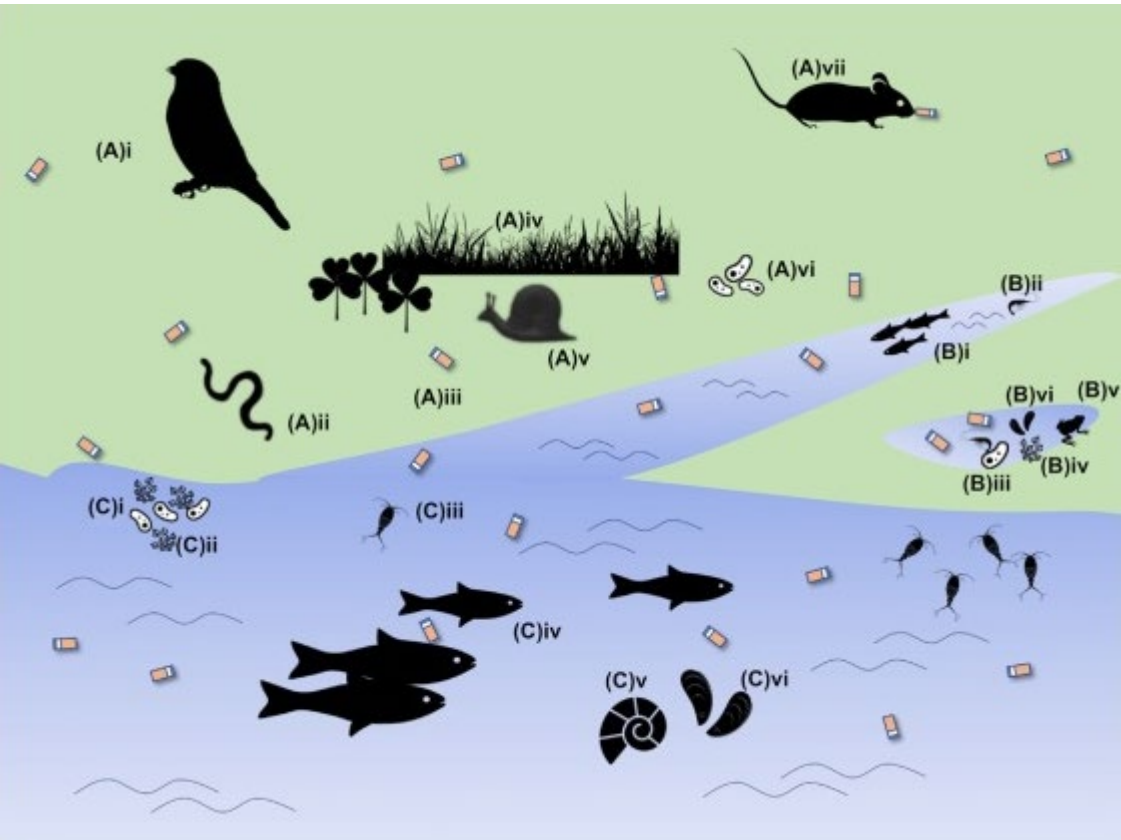


Figure 2

Habitat	Taxa	Mortality		Toxicity		Physiological		Reproduction		Growth/Malfor.		Behavioural		Pop./Diversity		Count				
		Tested	Sig.	Tested	Sig.	Tested	Sig.	Tested	Sig.	Tested	Sig.	Tested	Sig.	Tested	Sig.					
Freshwater	Microbe									2	2					0				
	Algae/plant					2	2	0		3	2	1				1				
	Invertebrate	22	9	16	3	1	3	8	7	6	4	4	3	2	2	1	2			
	Vertebrate	5	3	5				5	2	5		5	2	5	1	1	1	3		
Marine	Microbe	3	1	3				5	2	5					3	1	3	4		
	Algae/plant									4	3	3					5			
	Invertebrate	8	4	8	1	1	1	5	2	4	2	1	2	5	4	3	7	2	6	
	Vertebrate	1	1	1														7		
Terrestrial	Microbe														2	1	0	8		
	Plant				3	2	7	7	2	7	3	2	3	6	2	5		16		
	Invertebrate	2	2	0				1	1	0				2	2	1	1	1	1	3
	Vertebrate				2	1	2	5	1	0		2	1	2	7	2	1			22