CleanAtlantic

Tackling Marine Litter in the Atlantic Area

Evaluation of the potential harm caused to the marine environment by cigarette filters

Work Package 5.4

Cedre

Cefas

Instituto Español de Oceanografía

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Abbreviations

AA: Atlantic Area

AF: Accumulation Factor

ATR-FTIR: Attenuated Total Reflection Fourier-Transformed Infrared Spectroscopy

APCI: atmospheric pressure chemical ionization

CAS: Chemical Abstracts Service

CB: Cigarette butt

CEDRE: Centre of Documentation, Research and Experimentation on Accidental Water Pollution (France)

CEFAS: Centre for Environment Fisheries and Aquaculture Science (United Kingdom)

CETIS: Comprehensive Environmental Toxicity Information System

DP-TOF-MS: Direct inlet probe- Time of Flight mass spectrometry

DW: Dry Weight

EC_{50}: Half maximal effect concentration

EF: Enrichment Factor

ESI: Electrospray ionization

FWHM: Full width at half maximum

GC-MS: Gas Chromatography – Mass Spectrometry

GC-MS/MS: Chromatography – Tandem Mass Spectrometry

GLP: Good Laboratory Practice

ICES: International Council for the Exploration of the Sea

ICP-AES: Inductively Coupled Plasma - Atomic Emission Spectroscopy

ICP-MS: Inductively Coupled Plasma - Mass Spectrometry

IEO: Instituto Español de Oceanografía/Spanish Institute of Oceanography

IMAs: Initiatives, Measures and Actions

LC_{50}: Half maximal lethal concentration

LC: Liquid Chromatography

LC-HRMS: Liquid Chromatography – High Resolution Mass Spectrometry

LOD: Limit Of Detection

LOQ: Limit Of Quantification

LOEC: Lowest Observable Effects Concentration

MMO: United Kingdom Marine Management Organisation

***m/z*:** mass to charge ratio

NOEC: No Observable Effects Concentration

OCNS: Offshore Chemical Notification Scheme

OSPAR: Regional Sea Convention for the Protection and the Conservation of the North-East Atlantic and its resources

PAH: Polycyclic Aromatic Hydrocarbons

PNEC: Predicted No-Effect Concentration

RPM: Revolutions Per Minute

SPE: solid phase extraction

TSNA: Tobacco-specific N-nitrosamines

UK: United Kingdom

WW: Wet Weight

Executive summary

Cigarette filter are very common litter found under the form of cigarette butts (CBs), which are the combination of the filter and the remnants of a smoked cigarette. Worldwide, it is estimated that 4.5 trillion CBs are littered and end up in the environment every year and they are usually found in the marine environment. To address the environmental issue caused by CB pollution, several actions have been launched. At the international level, the OSPAR Commission has set up an action through its Regional Action Plan (RAP) (2014-2020) for the prevention and management of marine litter in the North-East Atlantic. This action (No 48) aims at assessing the potential harm caused to the marine environment by several types of litter including cigarette filters/butts and to develop proposals on the requirements for the removal, modification or adaptation of these potentially problematic items. More recently, on 21 May 2019, the European Union adopted several measures to reduce the impact of certain plastic products on the environment, including CBs. Measures targeting CBs are: marking requirements, extended producer responsibility and awareness raising **(**Directive EU 2019/904**)**.

In this context, the Interreg Atlantic CleanAtlantic project (2017-2023), which brings together 19 partners or associated partners representing France, Ireland, Portugal, Spain and the United Kingdom, including the OSPAR Commission and ministries in charge of the environment in the five countries, proposes to provide new knowledge on abundance, fate and potential impacts of cigarette butts on the marine environment and associated recommendations in order to support decision-making regarding this litter type.

To address this task, CleanAtlantic experts from Cedre (France), Cefas (United Kingdom) and IEO (Spain) conducted a collaborative study composed of 3 parts:

1) A targeted analyses of data obtained in CleanAtlantic work package (WP) 4.1 on the characterisation of beach litter pollution in the Atlantic Area (AA), to provide new information on CB abundance and distribution on beaches of the AA;

2) A literature review and an experimental study addressing CBs behaviour, degradation, toxicity and release of chemical contamination to provide new knowledge on fate and impacts of CBs in the marine environment. The study relies on experiments made on both (i) real butts collected fresh just after smoking or weathered on pavements in an urban beach environment and (ii) butts obtained from three types of cigarettes (a light, a medium strength and a strong) artificially smoked (herein after called artificial butts). Before use, artificial butts were characterized in term of mass and intrinsic contamination (organic and metallic) to ensure of there representativity. Four characteristics of cigarette butts were studied:

    - Behaviour, i.e. flotability in seawater, abitily to drift with wind and currents and / or to sink to the seafloor, was assessed in Cedre facilities.

    - Degradation was assessed by weathering CBs in outdoor conditions in Brest (France) in three different environments: (i) deposited on Cedre artificial sandy beach, (ii) on the seafloor, immerged in Brest marina and (iii) immerged on the floor of Cedre outdoor basin. Evolution of CB integrity, mass and intrinsic contamination (organic and metallic) was monitored.

    - Acute toxicity was assessed on 7 different species which can be found in coastal environment either in the seawater column (the bacteria *Aliivibrio fischeri,* the microalgae *Phaeodactylum*

tricornutum), on the bottom (the shrimp *Palaemonetes varians*, the oyster *Crassotrea gigas*) or within the sediment in interdital zones of sandy beaches (the worm *Arenicola marina,* the bean clam *Donax sp.* and the copepod *Corophium arenarium*). Most testings performed rely on international guidelines which are accepted and used by regulators and statutory authorities, such as ICES, UK Marine Management Organisation (MMO) and Offshore Chemical Notification Scheme (OCNS).

- Transfer of contaminants to the marine environment was studied both indirectly via Microtox© toxicity testing in CBs exposed seawater and directly via chemical analyses of exposed seawater and biota. Chemical analyses include (i) screening of substances associated with smoked cigarettes, (ii) targeted analyses of organic contaminants and (iii) targeted analyses of trace metals.

3) A review of existing initiatives, measures or actions to reduce CBs pollution in order to identify existing options to fight CBs pollution. This review includes initiatives identified in CleanAtlantic WP4.2 on identification of initiatives tackling marine litter in the AA.

Key results obtained during the study are summarized below:

- **CBs are abundant on Atlantic Area beaches**

Analysis of OSPAR beach litter monitoring data (CleanAtlantic WP4.1) indicates that CBs are the 5th most collected items on Atlantic area beaches over the period 2016-2019. Over the considered period, 25 183 CBs have been collected during the 922 surveys on the 62 survey sites located in the different parts of the AA. CBs have been found on 50% of surveys performed over the four-year period.

- **CBs have a complex behaviour and can reach every marine compartments**

Despite being predominantly made of cellulose acetate, a polymer denser than seawater, CBs have a complex behaviour due to their fibrous structure and associated air content. They can eather float and drift with wind and current, have neutral buoyancy, or sink to the seafloor. Thus, CBs can reach every marine compartment: shoreline, seafloor, sea surface and water column.

- **CBs appear to degrade in seawater but to persist longer on a sandy beach**

After one year on a sandy beach, CBs exhibit, visually, a negligible degradation (apart from the surrounding papers) suggesting a slow degradation rate in outdoor conditions despite sun and rain exposure. On the seafloor, degradation rate (though involved processes remain unknown) appears to be higher but dependent on filter composition.

- **CBs have a complexe chemical composition which includes toxic compounds**

The environmental risk associated with CBs primarily stems from their chemical composition. These chemicals can originate from the compounds initially present in the filter, the tobacco, or those formed during the combustion of cigarettes. Once in environments, CBs are likely to release potentially toxic compounds that can be categorized into two main groups: metals and organic chemicals such as nicotine and PAHs. Chemical analyses performed on artificial, fresh and weathered CBs confirmed the presence of a diversity of dangerous organic compounds (*e.g.* nicotine, PAHs) and metals (*e.g.* Zn). Among organic contaminants studied, nicotine appears to be the most abundant. Results suggests that CB contamination is higher for strong cigarettes than for light ones. CBs can also accumulate chemical compounds and metals in the environment. The adsorption capacity increased the chemical complexity of this litter type, and

potentially the risks associated with future releases into another environment during CBs weathering or after accidental ingestion by living organisms.

- **CBs contain soluble contaminants which are rapidly released in seawater**

CB contain soluble contaminants, such as nicotine, that can be released in water in a short period of time, suggesting CB represent a risk for water quality when in contact with water (seawater or freshwater, including rainwater).

- **In a short period of time, one CB can contaminate water or sediment at a level that may affect marine organisms**

This study showed that in 24h, a CB can contaminate a quarter of litter of seawater at a level affecting 50% of a population of water column organisms. When milled into powder and mixed with sediment (for more than 24h), a CB can contaminate about 1 kg of dry sediment at a level affecting 50% of a population of burrowing organisms. Overall, this study showed that leachates and sediments produced after 24h of contact with CBs showed toxic effects on a large number of aquatic organisms: bacteria *Aliivibrio fischeri* (artificially smoked CBs: EC_{50}= 3.4 – 5.6 CBs/L; unsmoked CBs: EC_{50}= 36.1-43.5 CBs/L; fresh smoked CBs: EC_{50}= 3.06 CBs/L; weathered smoked CBs: EC_{50}= 3.06 CBs/L), microalgae *Phaeodactylum tricornutum* (artificially smoked CBs: EC_{50}= 6.98 CBs/L), amphipods *Corophium arenarium* (artificially smoked CBs: LC_{50}=1.04 CBs/kg dry sediment), bean clam *Donax* sp. (artificially smoked CBs: EC_{50}=1.86 CBs/kg dry sediment), shrimp *Palaemonetes varians* (artificially smoked CBs: EC_{50}=1.04 CBs/kg dry sediment) and annelid *Arenicola marina* (fresh CBs: LC_{50}=0.345 CB/kg dry sediment; weathered CBs: LC_{50}= 1 CB/kg dry sediment). These results demonstrate that CBs can contaminate water or sediment at a level that may affect marine organisms in a short period of time and they could pose a risk for aquatic life by their capacity to release hazardous compounds in the surrounding environment.

- **There are numerous initiatives, measures and actions existing to combat cigarette butt littering but they are not all satisfactory**

Initiatives, measures or actions include environmental clean-up, selective collection, recycling, awareness raising, eco-conception, public policies... However, some of them such as environmental clean-ups and development of alternative filters that biodegrade in the environment are not satisfactory solutions as they only tackle the plastic pollution part of the problem but they do not prevent the transfer of chemical contaminants in the environment that occurs rapidly when CBs are in contact with water.

Overall, this study provides new knowledge on abundance, fate and potential impacts of cigarette butts in the marine environment. The study confirmed that cigarette butts are harmful for the marine environment and for aquatic environments in general. Their harmfulness is mainly due to their chemical composition with the presence of toxic contaminants that can rapidly be released in water at levels that can affect organisms. Despite their harmfulness, CBs are still thrown in huge quantity in the environment. They are regularly found during litter clean-ups or monitoring programs. In this context, it appears necessary to continue current efforts and take additional appropriate measures to eliminate CB pollution and associated impacts on the environment.

Introduction

Tobacco consumption is a well-known and documented threat to global health (**World Health Organization, 2017**). It kills more than 7 million people per year and is currently the world's single biggest cause of preventable death (**World Health Organization, 2017**). Nevertheless, their environmental associated problems were not identified as an issue until relatively recently (e.g. **Araújo, 2019; Green et al., 2022**).

Cigarette filters were introduced in the 1950s to make filtered cigarettes a 'healthier' alternative to cigarettes without filters (**Brandt, 2007**). Indeed, it has been well documented that cigarette companies responded to the 'lung-cancer scare' of the early 1950s by investing heavily in the design and marketing of filter-tipped cigarettes. Filters were added to cigarettes since the 1950s to retain chemical compounds during the smoking process and thereby reduce smokers' exposure to toxic compounds (**Novotny *et al.*, 2009**). Cigarette filters are generally composed of more than 15,000 fibers strands made of cellulose acetate with plasticized additives (**Belzagui et al., 2021**). Despite that, a team of researchers asserted in 2010 that "*the shift from non-filter to filter cigarettes appears to have merely altered the most frequent type of lung cancer, from squamous cell carcinoma to adenocarcinoma*" (**Novotny et al., 2009; Ito et al., 2011**). Epidemiological studies have not been able to demonstrate a public health benefit from modifications made to cigarettes over the past 50 years.

Cigarette butts (herein after CBs) which are the combination of the filter and the remnants of a smoked cigarette, contain several potentially hazardous chemical substances (**Baker, 2006**). The tobacco industry has improved cigarette filters to retain most chemicals that would be potentially released once the CBs are disposed of in the environment (**Harris. 2011**). So, this improvement has probably conducted to an increase in CBs potential toxicity in the environment (**Novotny & Slaughter, 2014**). Some researchers have suggested that CBs should be classified as hazardous waste (**Barnes, 2011; Rebischung et al., 2018**). A study performed by **Moriwaki et al., (2009)** found that arsenic, nicotine, polycyclic aromatic hydrocarbons (PAHs) and heavy metals are released into the environment by littered 'roadside waste' CBs. Most of these chemicals are not an original component of the filter; however, they tend to accumulate on it as a result of smoking.

The negative impacts of smoking on human health are well researched, however less is known about the potential impact's that CBs cause to the marine environment whereas CBs are among the most common forms of litter (**World Health Organization, 2017**). Worldwide, it is estimated that 4.5 trillion CBs are littered and end up in the environment every year and they are usually found in the marine environment (**Novotny and Slaughter, 2014, Araújo, 2019**). CBs are frequently left on the beach even after beach clean ups (**Loizidou et al, 2018; Araújo, 2019**). In 2018, about 2.4 million cigarette butts were collected from beaches during international shoreline clean-up campaigns, making this waste the top ten most collected wastes on beaches (**Addamo et al., 2017; Ocean Conservatory, 2018**). In addition to their direct discard into the environment by smokers, water channelled by sewer systems and streams acts to accumulate CBs in localised areas.

To address the environmental issue caused by CBs pollution, several initiatives have been launched. At the international level, the OSPAR Commission has set up an action through its Regional Action Plan (RAP) (2014-2020) for the prevention and management of marine litter in the North-East Atlantic. This action (No 48) aims at assessing the potential harm caused to the marine environment by several litter including cigarette filters/butts and to develop proposals on the requirements for the removal, modification or adaptation of

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3) A review of of existing initiatives, measures or actions to reduce CBs pollution in order to identify existing options to fight CBs pollution. This review includes initiatives identified in CleanAtlantic WP4.2 on identification of initiatives tackling marine litter in the AA.

Overall, this study provides new knowledge on abundance, fate and potential impact of CBs in the marine environment and associated recommendations in order to support decision-making. The structure of the study is synthesized in the Figure 1.

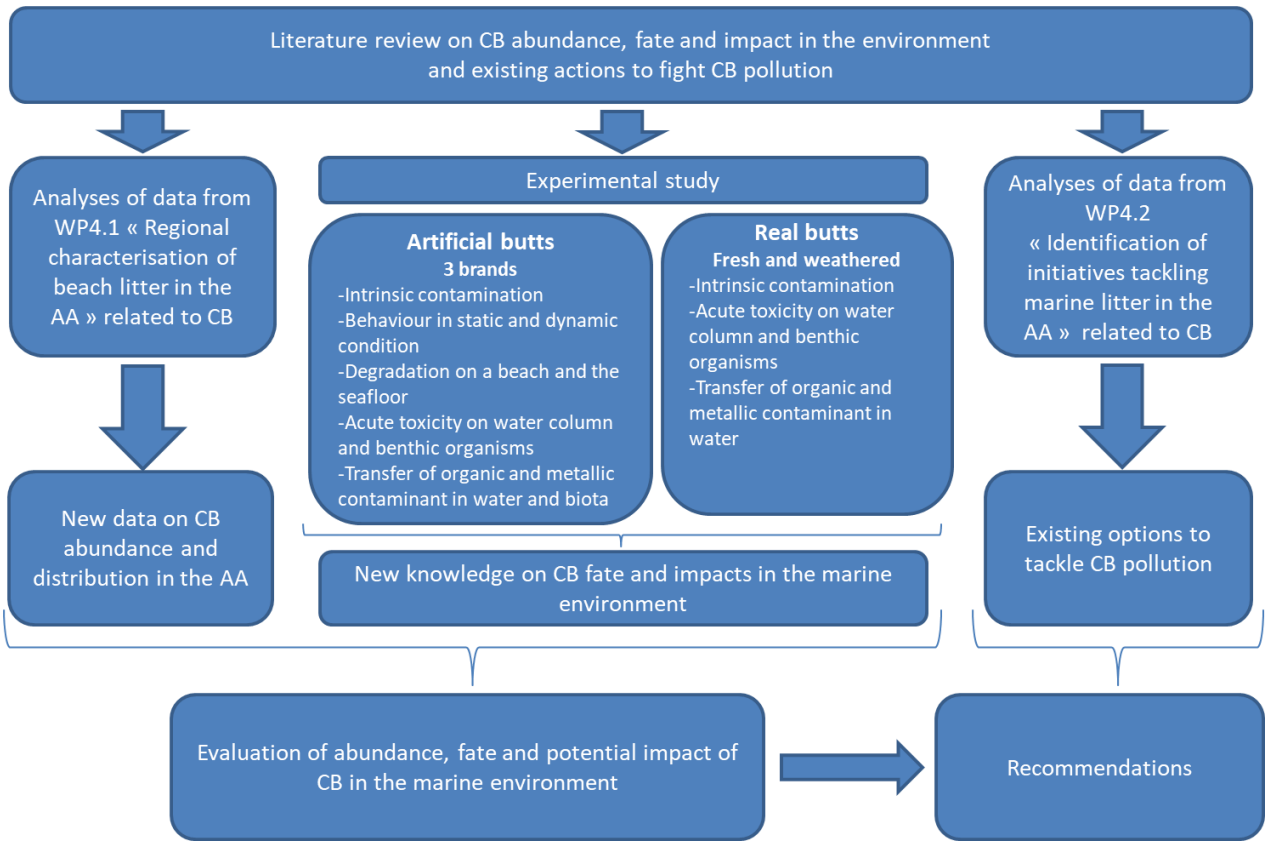


Figure 1: The CleanAtlantic approach to evaluate the potential harm caused to the marine environment by cigarette filters and proposed associated recommendations.

Materials and methods

1. Analyses of Cigarette Butts abundance and distribution on Atlantic Area beaches

The assessment of CBs abundance and distribution on AA beaches is based on the work performed in the first action of the WP 4 of CleanAtlantic project "*WP4.1: Regional characterisation of marine litter in the Atlantic Area*" which is described in the final report "*Overview of marine litter status in the Atlantic area: beach litter*" (**Cedre, 2020**).

This work relies on an analysis of OSPAR beach litter monitoring data over the period 2016-2019 (4 years). Data are collected using a standardized fit-for-purpose monitoring protocol consisting in collecting, identifying and counting all visible litter (> 0.5 cm) on the beach sand surface, four times a year, on fixed survey site of 100m in length (https://www.ospar.org/work-areas/eiha/marine-litter/assessment-of-marine-litter/beach-litter).

The assessment includes only data obtained on sites located in the AA. Data were downloaded from the OSPAR beach litter database (https://beachlitter.ospar.org/). The assessment includes data collected on 62 sites: 4 in Ireland, 18 in United Kingdom, 9 in France, 12 in Spain and 19 in Portugal. Based on work done for CleanAtlantic WP4.1, specific analyses were performed on CB and following indicators were calculated at country and Atlantic Area scales:

- Total number of CBs collected,
- Percentage of CBs collected in the total number of beach litter,
- Rank in the Top 5 of the most collected beach litter,
- Mean and median of CBs collected over a survey,
- Minimum and maximum of CBs collected over a survey,
- Number and percentage of surveys where CBs were found.

2. Experimental study on artificial Cigarette Butts

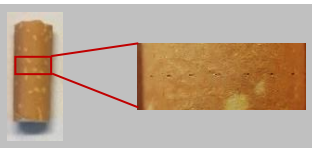
2.1. Preparation of artificial Cigarette Butts

2.1.1.Cigarette selection

The study was conducted on three types of cigarettes, differing in terms of filter ventilation, tar, nicotine, carbon monoxide emissions, length, and diameter. To select the cigarettes, readily available pre-made brands in France were chosen using data provided by the French Agency for Food, Environmental and Occupational Health & Safety (**Anses, 2019**), excluding menthol and roll-up cigarettes. Based on the Anses data, the three types of cigarettes were categorized as Light, Medium, and Strong.

The medium cigarette is one of the most sold pre-made cigarettes in France according to the French Observatory of Drugs and Drug Addiction and the strong cigarette is described as 100% tobacco and free of additives by the manufacturer. Emissions are proportional to cigarette strength, being highest for the Strong whereas ventilation is inversely proportional to cigarette strength, ranging from 80 % for the Light to 22 % for the Strong. Cigarette total length and diameter are similar for the different types but filter varies from 21 mm for the Medium to 27 mm for the Light. Characteristics (size, ventilation and emissions) of the three selected cigarettes are detailed in the Table 1.

Table 1: Characteristics of the three cigarettes selected for artificial smoking

Cigarette	Pictures of filter and ventilation holes	Filter ventilation (%)	Tar emission (mg/cig)	Nicotine emission (mg/cig)	Carbon monoxide emission (mg/cig)	Cig total length, filter length, filter diameter (mm)
Allowed maxima (Directive EU 2014/40)	-	-	10	1	10	-
Light		80	1	0,1	2	83 / 27 / 7.79
Medium		34	10	0,7	10	83 / 21 / 7.8
Strong		22	10	1	10	84 / 25 / 7.9

2.1.2.Preparation of artificial Cigarette Butts

Cigarette butts (CBs) were artificially prepared in the laboratory using a home-made smoking machine developed for the project (Figure 2). This equipment includes a vacuum pump (Buchi Vacuum Pomp V-700), which is controlled by an air flow meter (CôleParmer LPM AIR). The vacuum pump is connected to a vacuum bench (Interchim 518100) capable of accommodating up to 12 cigarettes, which are held in place by pipette tips without obstructing the ventilation holes of the cigarettes. A glass bottle containing water and a pipette filled with cotton were placed between the vacuum bench and the pump to avoid fouling of the latter.

Several tests were conducted to develop a smoking protocol that follows the ISO standard 3308:2012 and replicates standard smoking conditions as closely as possible. The protocol consists in a discontinuous smoking of 6 cigarettes simultaneously by making 8 puffs of 2 seconds while aspirating with each puff a volume of 35 ml per cigarette (flow rate set at 1L/min per cigarette, equivalent to a total flow of 6L/min), interspersed with 45 seconds of pause. For this smoking protocole, filter ventilation holes were left opened and filters were ventilated according to their ventilation rates.

To assess the impact of smoking conditions on CBs contamination and toxicity, two other smoking protocoles were also tested:

- A discontinuous unventilated smoking using the protocole described above but without ventilation of the filter by clogging ventilation holes,
- A continuous ventilated smoking consisting in smoking the 6 cigarettes with ventilation holes left opened in one continuous puff and stopping smoking 2 mm before the filter.

CBs consists in remaining tobacco and a filter surrounded by two folds of paper: one white and one orange (external). In this part of the study (using artificial CBs), remaining tobacco was removed from the CBs and

papers was cut close to the filter. CBs (filter +papers) were then individually wrapped in aluminium foil and stored at -20°C until use. To conduct certain tests, it was necessary to use cigarette butt powder. To obtain the powder, the surrounding papers were first removed from the CBs, which were then milled for five minutes using the Retsch® Mixer Mill MM 400. The resulting powders were carefully stored in individual glass containers at -20°C until use. In cases where virgin (unsmoked) cigarette filters were required for testing, the cigarettes were first cut close to the filter. Next, the tobacco was completely removed, while the papers surrounding the filter were retained for the experiment.

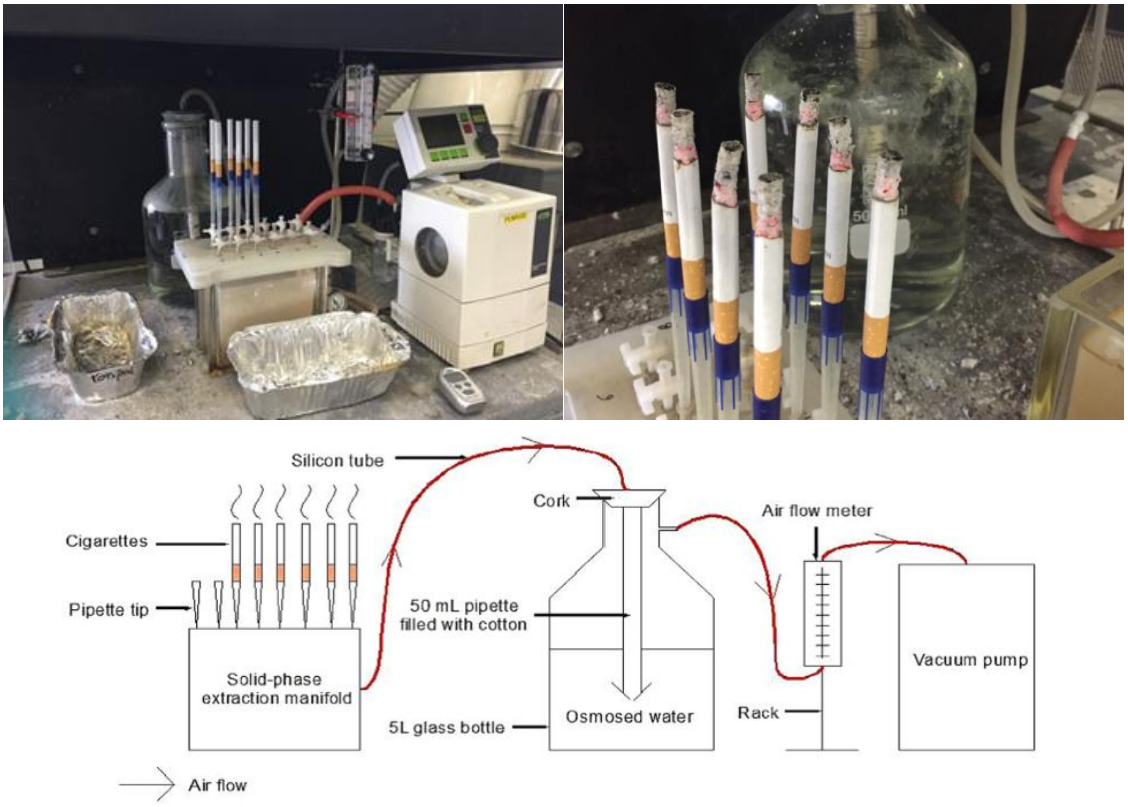


Figure 2: Equipment used to produce artificially-smoked CBs (©Cedre).

2.1.3. Characterization of artificial Cigarettes Butts

2.1.3.1. Physical parameters and polymer composition

After smoking, CBs dry weight, mass gain during smoking and humidity percentage were determined with a precision balance (Mettler Toledo® XP205, ±0.01 mg) after drying at 50°C.

CBs filter polymer composition were characterized by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) in a SMART iTX NICOLET iS20 (Thermo Scientific). The cigarette filters were placed on the ATR diamond crystal and FTIR absorption spectra were recorded with an average of 50 scans in the mid-infrared range 4000–525 cm^{-1}. The polymer type was identified based on the absorption frequencies for specific chemical bond types present in the relevant polymer samples. Spectrum software was used to analyse recorded IR spectra and the material was identified by a spectrum search in commercial and customised polymer libraries (Omnic 9). These libraries contained spectra of all common polymers, natural and weathered materials with different uses and colours among others.

2.1.3.2. Contamination assessment

CBs instrinsic contamination was also assessed *via* targeted chemical analysis of organic and metallic contaminants as described below.

- **Organic contaminants**

Several organic contaminants including nicotine, indole, PAHs, phenols and nitrosamines were selected to be analysed, according to the litterature. The list of contaminants is detailed in Table 2.

Light and medium CBs were analysed in triplicate. CBs were spiked with 8 internal standards (naphthalene d10, biphenyl d10, phenanthrene d10, pyrene d10, benzo[a]anthracene d12, benzo[a]pyrene d12, benzo(g,h,i)perylene d12 and 4-chlorophenol) in a glass tube and extracted with analytical-grade dichloromethane. The extracts were filtered on fiber glass and sodium sulfate to remove particulate matters and water residue. There were then evaporated with a rotary evaporator to 200 µL. Samples were then analysed by Gas Chromatography - Mass Spectrometry (GC-MS) using a gas chromatography system Agilent 7890A coupled to an Agilent 5975c mass spectrometer (Agilent Technologies) and equipped with a thermal desorption unit combined with a Cooled Injection System (Gerstel). Analytes were quantified relatively to internal standards using a calibration curve ranging from 0.5 to 50 ng injected. Results are expressed as ng analytes/CB. Limits of quantification (LOQ) were calculated by the calibration curve method and limit of detection (LOD) were estimated by dividing LOQ by 3. Benzo(b)fluoranthene/benzo(k)fluoranthene, m-cresol/p-cresol and isomers of hydroquinone, were quantified as sums, named benzo(b+k)fluoranthene, (m+p)-cresol and hydroquinone isomers, respectively, due to poor resolutions.

Table 2: List of organic contaminants analysed.

Molecules	Category	CAS number
Naphthalene	PAH - 2 rings	91-20-3
Acenaphthylene	PAH - 3 rings	208-96-8
Acenaphthene	PAH - 3 rings	83-32-9
Fluorene	PAH - 3 rings	86-73-7
Phenanthrene	PAH - 3 rings	85-01-8
Anthracene	PAH - 3 rings	120-12-7
Fluoranthene	PAH - 4 rings	206-44-0
Pyrene	PAH - 4 rings	129-00-0
Benzo[a]anthracene	PAH - 4 rings	56-55-3
Chrysene	PAH - 4 rings	218-01-9
Benzo[b+k]fluoranthene	PAH - 5 rings	205-99-2 and 207-08-9
Benzo[a]pyrene	PAH - 5 rings	50-32-8
Dibenz(a,h)anthracene	PAH - 5 rings	53-70-3
Indeno(1,2,3-cd)pyrene	PAH - 6 rings	193-39-5
Benzo(g,h,i)perylene	PAH - 6 rings	191-24-2
o-cresol	Phenols	95-48-7
(m+p)-cresol	Phenols	108-39-4 and 106-44-5
2-éthylphenol	Phenols	90-00-6
4-éthylphénol	Phenols	123-07-9
hydroquinone isomers	Phenols	123-31-9, 120-80-9 and 108-46-3
indole	Indole	120-72-9
nicotine	Nicotine	22083-74-5
N-nitrosoanatabine	Nitrosamines	887407-16-1
N-nitrosoanabasine	Nitrosamines	37620-20-5

- **Metallic contaminants**

All samples were prepared in Cedre facilities and transferred for analyses to the Instituto Espanol de Oceanografia (IEO) in Vigo (Spain). A series of metals and metalloids were analyzed: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), strontium (Sr), uranium (U), vanadium (V) and zinc (Zn). To do so, leachable metal concentrations in aqua regia were determined. For the latter, samples were placed in plastic centrifuge tubes containing a mixture of HNO_3 (3M) and HCl (2M) in a proportion of 1:3. Samples were stirred using an orbital shaker for 24 h at 100 rpm. Then, samples were centrifugated for 10 min (3500 rpm) and filtered using syringe filters. Filtered samples were stored (-20°C) until analysis. Analyses were carried out directly in the samples using an ICP-MS (7900 Agilent). In order to have a quality control of the analysis, blanks were included within the samples.

To evaluate the nature and rates of interactions between metals and CBs, the data were modelled using the two types of kinetics generally used for the adsorption onto solids of compounds dissolved in a liquid phase: the pseudo-first order kinetics proposed by **Lagergren (1898)** (Equation 1), and the pseudo-second order kinetics proposed by **Ho and McKay (1999)** (Equation 2). We followed the procedure described by **Sánchez and Cameselle (2017)** to model the sorption of metals in CBs. Adsorption and desorption rate constants (forwards and reverse) and equilibrium constants were derived from Equations (1) and (2) by assuming that equilibrium concentrations were represented by the mean of the final measurements in the timecourse **(Holmes et al., 2012)**.

Equation 1

$$q_t = q_e\,(1 - e^{-kt})$$

where q_e is the adsorption capacity in equilibrium (ng metal mg^{-1} CB), k is the first order adsorption rate constant (h^{-1}) and t is the time (h).

Equation 2

$$q_t = q_e^2 \frac{Kt}{(1 + kq_e t)}$$

where q_t is the adsorption capacity (ng metal mg^{-1} CB) at a time t (h), K is the pseudo-second order adsorption rate constant (mg ng^{-1} h^{-1}), and q_e is the adsorption capacity in equilibrium.

2.2. Study of Cigarette Butts leaching in seawater

2.2.1.Leachate preparation

Leachates were achieved by contaminating natural seawater with CBs (concentration = 10 CBs/L). CBs were incubated in seawater at 160 rpm for 24 hours and then the leachate was filtered using a vacuum pump on a nitrocellulose filter (0.45 µm). The leachate was then stored in glass bottles away from the light at 4°C for a maximum of 3 days in order to prevent degradation of the solution. To study the evolution of the CBs toxicity over time, some CB leachates were performed by putting the CBs back into clean seawater between agitation times (6, 24 and 72 h). CBs were kept to perform chemical analyses in order to compare the level of chemicals/metals between CBs and leachates over time.

2.2.2. Contamination assessment

Chemical and metal analyses in both cigarette butts and/or seawater were performed according to the protocols described in the part 2.1.3.2.

2.3. Study of Cigarette Butts behaviour in seawater

2.3.1. Behaviour in beaker

CB behaviour was first assessed in beaker under both static and dynamic conditions.

- **Static tests**

Static tests were realised in beakers filled with seawater. CBs (filter+white paper, orange paper was removed) were deposited on the seawater surface and left for 24h. Behaviour was monitored visually and CB seawater uptake was followed by weight measurement.

- **Dynamic tests**

Dynamic tests were performed similarly to static tests but under agitation on an orbital table. Two agitations were tested: 60 and 150 rpm.

2.3.2. Behaviour in a seawater column

Sinking experiments in the seawater column were performed in Cedre experimental column, a 5 m-high column filled with natural filtered seawater (Figure 3). Before testings, CBs were put in seawater to be humidified and remove all air inside. Wet CB were then deposited in the seawater at the top of the column and sinking was followed visually. The sinking speed (m/s) was assessed between two points located at different heights.

Figure 3: Cedre experimental column filled with natural filtered seawater (5-meter high) (©Cedre).

2.4. Cigarette Butts degradation

2.4.1. Weathering on Cedre artificial beach

In February 2019, 126 CBs individually weighed and tagged (42 per type of cigarettes) were deposited on Cedre artificial sandy beach (Figure 4). Cedre's beach is a 2,500 m^2 man-made beach designed to study pollution on sandy shore, for weathering experiments, equipment trials and training purposes. CBs were placed in a wood frame protected by a net to prevent them from flying away and being picked up by birds. A WatchDog® 2000 series weather station (Spectrum® Technologies, Inc.) was positioned close to the Cedre's

artificial sandy beach in order to take various measures: wind speed, wind direction, solar radiation, temperature, humidity, rainfall and dew point (Figure 4). The frequency of measurements was one measurement every hour. Data weas collected using SpecWare® 9 software (Spectrum® Technologies, Inc.).

All CBs were individually weighed before deployment to obtain the initial dry weight. The study lasted 465 days. Regularly, 6 CB per type of cigarettes were sampled and individually frozen in aluminum foil until analyses. Three CBs were kept to monitor the evolution of the toxicity of the CBs using a Microtox® test on their leachates (concentration of 8 CBs/L). The CBs were then rinsed with distilled water to remove salts, dried for 24 hours and weighed to assess the loss of mass of the CBs over time. The three others CBs were used to assess the evolution of chemical contamination over time by chemicals analyses and metals analyses. Chemical analyses were conducted on medium strength CBs after 7, 21 and 56 days of deployment. In total, 9 samplings were carried out during the experiment (the dates of sampling are detailed in the Table 3).

Figure 4: Tagged CB (left), CB deposited on Cedre artificial beach (middle) and view of the beach with CB weathering facilities and the weather station (right) (©Cedre).

Table 3: List of samplings during the weathering experiment on the beach.

Sample	Deposit date	Sampling date	Weathering duration
0	-	06/02/2019	-
1	06/02/2019	13/02/2019	7 days
2	06/02/2019	20/02/2019	14 days
3	06/02/2019	27/02/2019	21 days
4	06/02/2019	06/03/2019	28 days
5	06/02/2019	03/04/2019	56 days
6	06/02/2019	02/05/2019	85 days
7	06/02/2019	31/07/2019	175 days
8	06/02/2019	06/11/2019	273 days
9	06/02/2019	04/02/2020	366 days

2.4.2. Weathering on the seafloor in Brest Marina and Cedre outdoor basin

A field experiment of 1 year was conducted in the Brest “Marina du Château” and in Cedre facilities (march 2019 – march 2020) to monitor the degradation of CBs in marine environment. All CBs used for the experiment were weighed individually to obtain their initial mass. Then, CBs were immerged inside stainless steel cages (Figure 5). A total of 6 samplings were performed during the experiments (details in Table 4); for each date, 3 CBs per type of cigarettes were sampled and frozen indivudally in aluminium foil until analyses. The CBs degradation was studied by spectroscopy analyses (1) and mass measurement (2).

(1) The oxidation state of CBs was monitored by infrared spectroscopy (Nicolet Summit FTIR Spectrometer equipped with an ATR Everest Diamant) using the method described in the part 2.1.3.
(2) CBs were dried at room temperature to a constant weight after collection. This dry mass was monitored over time and compared to the initial weight before experiment.

In addition, chemicals analyses were assessed on medium strength CBs after 70 days of deployment to study the evolution of chemical contamination.

Figure 5: Photos of the deployment of CBs in stainless streel cages to monitor degradation processes over 1 year in Brest "Marina du Château" and in Cedre facilities (©Cedre)

Table 4: List of samplings during the degradation experiment on the Brest Marina and Cedre's basin.

Sample	Filing date	Sampling date	Time
0	-	07/03/2019	-
1	07/03/2019	16/05/2019	70 days
2	07/03/2019	04/07/2019	119 days
3	07/03/2019	05/09/2019	182 days
4	07/03/2019	29/11/2019	267 days
5	07/03/2019	04/02/2020	340 days
6	07/03/2019	09/03/2020	368 days

2.5. Cigarette Butts toxicity on marine organisms

2.5.1.Preparation of the exposure media

2.5.1.1. Contaminated sediment

The exposure of burrowing organisms was achieved by contaminating natural sediment (collected with the organisms) with CBs powder. The sediment is used directly after the addition of the powder and manual homogenization.

2.5.1.2. Contaminated seawater

The exposure of non-burrowing organisms was achieved by contaminating natural seawater with CBs leachate (concentration = 8 CBs/L). To do this, CBs were incubated in seawater at 160 rpm for 24 hours and then the leachate was filtered using a vacuum pump on a nitrocellulose filter (0.45 µm). The leachate was then stored in glass bottles away from the light at 4°C for a maximum of 3 days in order to prevent the degradation of the solution.

2.5.2. Microtox

Toxicity of CB leachates was determined using the *in vitro* Microtox® test (**ISO 11348-3:2009**). This test, known for its facility of implementation and speed of response, assesses the toxicity of a substance by measuring the percentage of inhibition of bioluminsence of the marine bacteria *Aliivibrio fischeri* or by determining the median effective concentration resulting in a 50% decrease of the bioluminescence (EC_{50}).

It responds to chemicals or combinations of chemicals that are toxic to cells or reduce their speed of replication.

To evaluate the toxicity of the three types of CBs and virgin filters, leachates were prepared in seawater at concentrations ranging from 0.5 to 8 CBs/L, with a 24-hour extraction time in dark condition at 160 rpm. The toxicity was assessed using the B-Tox mode. Aliquots of *A. fischeri* culture were reactivated and added to each test vial (volume = 100 µL). After 15 minutes, the bacteria bioluminescence was assessed using a portable toxicity analyser Microtox© FX (ModernWater, UK). Thereafter, the tested leachates (900 µL) were added in vials for 15 minutes exposures before a new measurement of the bacteria bioluminescence. The luminosity change of each sample is then measured and compared to the luminosity of the control, with the difference expressed as a positive or negative percentage difference from the control.

2.5.3. Microalgae test

The microalgae (*Phaeodactylum tricornutum*) were supplied by MicrobioTests Inc., in a kit form. The interest of this kit lies in the use of an "immobilized" strain of *Phaeodactylum tricornutum* which is easily reactivated through a 3-day incubation process. There is therefore no pre-culture phase of the algae, as is done in conventional algae tests.

Toxicity tests on algae were performed according to **ISO 10253:2016** using the Marine AlgalToxKits (MicrobioTests Inc., Belgium). The test consists in measuring the inhibition of algal growth when exposed to a toxicant. Algae growth is measured by UV spectrophotometry at a wavelength of 670 nm. The culture as well as the reading of the optical density is performed in 10 cm cells available in the kit. Microalgae were exposed for 72 hours to 6 concentrations of medium strength CB: 0, 0.3, 0.6, 1.5, 3.5 and 8 CBs/L.

2.5.4. Amphipod test

Adult amphipods (*Corophium arenarium*) (4-6mm) and sediments (granulometry <500 µm) were collected at low tide in the harbour of Argenton (Finistère, France) in February 2019.

The amphipod exposure protocol was based on the **ISO 16712:2005**. 360 individuals were exposed for 10 days to: (1) 5 concentrations of medium strengh CBs (27, 62, 142 and 746 mg CBs/kg dry sediment (DW)), (2) 1 concentration of light CBs (194 mg CBs/kg DW), (3) 1 concentration of strong CBs (194 mg CBs/kg DS), (4) 1 concentration of unsmoked medium strengh cigarette filter (123 mg CBs/kg DW) and (5) 1 control condition (0 mg CB/kg DW). These 9 treatments were carried out on 20 individuals in triplicates, except for the control for which 6 replicates were used (Figure 6).

Mortality was assessed after 10 days of exposure by couting live/dead amphipods, and a LC_{50} value was calculated for the medium strengh CBs. In addition, the burrowing capacity was studied after the 10 days exposure by recovering living individuals from each condition and placing them in crystallizers containing uncontaminated sediment and then by visual observation of amphipods behaviour after one hour.

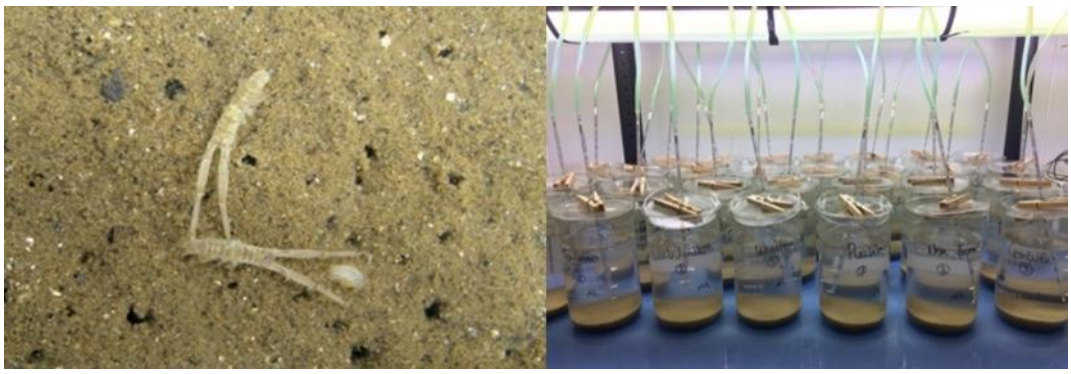


Figure 6: Photos of *Corophium arenarium* (left) and the experimental device for the exposure (right) (©Cedre).

2.5.5. Bean clam test

Adults bean clam (*Donax* sp.) (22-37 mm) and sediment (granulometry <1 mm) were collected at low tide on the beach of Blancs Sablons (Finistère, France) on March 2019. Individuals were acclimatised for 3 days at room temperature in a tank continuously supplied with natural seawater containing 5 cm of sand with a daily monitoring of the water parameters (temperature, salinity, pH and oxygen).

The exposure of the bean clam was adapted from a study by **Neuberger-Cywiak *et al*., (2003)**. Briefly, 42 individuals (7 individuals per condition) were exposed for 96 hours to 6 concentrations of medium strength CBs: 0, 25, 57, 132, 304, 700 mg CBs/ kg DW in 5 L beakers containing 2 kg of contaminated sediment, 4 L of natural seawater and an oxygenation system (Figure 7).

The burial behaviour of the individuals was studied by visual counting during an exposure of 96-hour. Observations were classified in 4 categories: (1) buried, (2) partially buried, (3) unburied and (4) dead. Individuals were considered dead when no response to mantle stimuli was detected.

Figure 7: Photos of *Donax* sp. (left) and the experimental device for the exposure (right) (©Cedre).

2.5.6. Shrimp test

Shrimps (*Palaemonetes varians*) (30-50 mm) were supplied by the “Museum National d'Histoire Naturelle of Concarneau” (Finistère, France) on April 2019. Individuals were acclimatised for 21 days at room temperature in a tank continuously supplied with natural seawater with daily monitoring of the water parameters and were fed with mussels every two days.

The shrimp exposure protocol was adapted from the **AFNOR NF T 90-349** standard. Briefly, 240 individuals (20 individuals per condition) were exposed for 6 hours to 6 concentrations of medium strength CBs: 0, 0.5,

1, 2, 4 and 8 CBs/L, in duplicate in 5L glass beakers containing 4L of natural seawater and an oxygenation system. The shrimp were then placed in tanks continuously supplied with natural seawater for 24 hours for decontamination (Figure 9).

A LC_{20} and LC_{50} were calculated at the end of the 24-hour recovery period by counting live individuals and a sublethal response was studied by visually counting the number of individuals exhibiting paralysis during the 6-hour exposure period.

Figure 8: Photos of *Palaemonetes varians* (left) and the experimental device for the exposure (right) (©Cedre).

2.5.7.Oyster exposure

Triploid adult oysters (*Crassostrea gigas*; number of animals: 104; size: 92 ± 7 mm) were supplied from an oyster farmer (Plouguerneau, Finistère, France) in March 2019. Once in Cedre facilities, individuals were acclimated for 7 days in a 300 L tank continuously supplied with naturally filtered seawater (salinity: 35.1; pH: 7.99) at room temperature (20 °C). During this period, the water parameters were monitored every day, the faeces were removed and oysters were fed every two days with a dose of 162 µL/ind of a concentrated microalgae solution (RGcomplete APBreed®). No oyster mortality was recorded during the acclimation period.

To assess potential toxicity of CBs on oysters, 25 individuals (5 individuals per condition) were exposed for 24 hours to 5 concentrations of medium strength CBs: 0, 1, 2, 4 and 8 CBs/L, individually in 1L glass beakers containing 900 mL of natural seawater and an oxygenation system (Figure 9). Two sub-lethal responses were studied in oysters:

(1) <u>The paralysis:</u> The paralysis was tested on open individuals after 4, 7 and 24 hours of exposure by lightly touching their mantles with a skewer pick. Valve closure time was measured by filming each test with a camera. An individual was considered to be paralyzed if no response to stimuli was detected after 1 minute.

(2) <u>The filtration:</u> After 24 hours of contamination, the oysters were replaced individually to the cleaned beakers and filled with 600 mL of uncontaminated seawater and a dose of food. 2 mL of seawater was removed at the time of feeding and after 5 hours. The filtration rate (Rf) in mL/h was calculated according to the following formula: $\mathbf{Rf= (V/t)*ln\ (C_0/C_t)}$, where V is the volume of the solution (mL), t is the time between C_0 and C_t (hour), C_0 is the starting algae concentration, and C_t is the final algal concentration (**Anandraj *et al.*, 2002**).

Figure 9: Photos of *Crassostrea gigas* (left) and the experimental device for the exposure (right) (©Cedre).

To assess potential bioaccumulation of metals by oysters, 36 individuals (18 per condition, 6 per tank) were exposed, in triplicate, for 7 days to the medium strength CBs leachate (1 CB/L) in glass tanks containing 10 L of naturally filtered seawater and an aeration system. During the exposure, seawater, stock solution (leachate) and food were renewed every two days and the faeces were removed. Oysters were fed with a dose of 162 µL/ind of a concentrated microalgae solution (972 µL per tank). A seawater control group (per triplicate) was processed at the same time. After 7 days of exposure and 2 days after the last feeding, 3 fasting individuals of each tank (i.e. 9 individuals per condition and a total of 18 individuals) were sampled. For each oyster, shells were opened and the body was weighed, then the gills and the digestive gland were dissected out using a metal scalpel before being weighed and stored individually in aluminium foil at -80°C. After dissection, tanks were cleaned and filled with 10 L of non-contaminated natural filtered seawater. The remaining oysters (18 individuals, 9 per condition) were left in clean seawater for an additional 7-day decontamination period. Oysters were fed with a dose of 162 µL/ind of a concentrated microalgae solution (486 µL per tank). After 7 days of decontamination and 2 days after the last feeding, the remaining individuals of each condition were sampled. For each oyster, shells were opened and the body was weighed, then the gills and the digestive gland were dissected using a metal scalpel before being weighed and stored individually in aluminium foil at -80°C.

Regarding the metal analyses, total metal concentrations were determined in samples consisting of pooled gills or digestive glands from 3 individuals of each condition (1 per tank), equivalent to approx. 3 g of wet weight (WW) for the gills and approx. 2 g WW for the digestive glands. These samples were acid digested in Teflon pumps inside a microwave digester using 6 mL of HNO_3 (suprapur, Merck) and a temperatura ramp of 75 min reaching a maximum of 180 °C and were finally made up to 25 mL. Finally, analyses were carried out directly in the samples using an ICP-MS (7900 Agilent). In order to have a quality control of the analysis, blanks and certified reference material (NIST-1566b, oyster tissue) were included within the samples. Recoveries for metals were (n = 3) 107 ± 7% (As), 104 ± 8% (Cd), 108 ± 9% (Co), 101 ± 6% (Cr), 104 ± 4% (Cu), 108 ± 9% (Fe), 97 ± 2% (Hg), 103 ± 8% (Mn), 101 ± 14% (Ni), 99 ± 17% (Pb), 98 ± 14% (Sb), 102 ± 5% (Sr), 111 ± 11% (U), 95 ± 11% (V) and 103 ± 3% (Zn).

3. Experimental study on real Cigarette Butts

3.1. Collection and Preparation of Cigarette Butts

3.1.1.Fresh cigarette butts

Fresh CBs were collected over the same time period from volunteers who were provided with clean (solvent rinsed) sample jars and instructions on creating a standardised CB. The CBs were collected in the jars immediately after smoking, and brought to the lab within one week, ensuring that they consisted of the filter, paper wrapper and approximately 5 mm of tobacco remaining on the cigarette. All Fresh CBs were readily available pre-made brands in the UK. Menthol and roll-up cigarettes were avoided. Once collected the sample jars were stored frozen. Immediately before tests were set up, the CBs were allowed to defrost and cut into approximately 5 mm strips before blending until homogenous. A sub-sample of CBs were weighed to establish a standard weight per CB.

3.1.2.Weathered cigarette butts

Weathered CBs were collected from pavements in an urban beach environment in the UK. Care was taken to select CBs that represented the parameters for the fresh CBs. Each one had a small amount of tobacco remaining, and no menthol or roll-up cigarettes were collected. Samples were immediately placed into clean sample jars and frozen before use. The same processes were carried out before testing as for the fresh CBs.

Figure 10: Homogenised CBs and stainless steel chopping/blending equipment (©Cefas).

3.2. Cigarette Butt Toxicity

3.2.1.Arenicola marina 10-day Acute Toxicity

The International Council for the Exploration of the Sea (ICES) guideline, "Biological effects of contaminants: Sediment bioassay using the polychaete *Arenicola marina*" (**Thain and Bifield, 2001**) was selected as a suitable test protocol because it is accepted and used by regulators and statutory authorities, such as ICES, Marine Management Organisation (MMO) and Offshore Chemical Notification Scheme (OCNS).

This test is primarily designed to measure impacts on mortality as an acute toxic endpoint. A secondary endpoint is the production of casts, which are a measure of feeding effort by *Arenicola marina* in the sediment. Feeding effort can decrease when worms detect toxic sediments. This may represent a reduction

in time spent feeding, a refusal to burrow into the sediment, or a reduction in worm numbers where ingestion of a toxic sediment has reduced the overall numbers of worms producing casts.

3.2.1.1. Sediment Collection and Preparation

Tests were carried out using a reference sediment collected from a previously identified uncontaminated marine site (Shoebury Sands, near Southend, Essex, UK). For each test concentration, half the required air-dried sediment was weighed into 5 L Nalgene bottles. The desired weight of homogenised CBs (either fresh or weathered) was added to the Nalgene bottle before adding the second half of sediment. Each bottle was topped up with artificial seawater. The bottles were shaken on an orbital platform shaker for 3 hours at 150 RPM.

Once removed from the shaker, approximately 3 cm depth of sediment was added to each test vessel and left to settle overnight. Vessels were topped up with artificial seawater the following day to approximately 3 cm above the sediment line then left to settle for a further 6 hours. Each vessel had an airline added once the sediment had settled (Figure 11).

3.2.1.2. Experimental design

Five healthy *A. marina* were added to each vessel. Weights of *A. marina* were recorded before addition. Observations were carried out every 24 hours and water quality parameters were measured daily.

Figure 11: *Arenicola* test set up for cigarette butt testing (©Cefas).

Due to the speed at which mortalities were occurring across the CBs dosed test vessels, the first test was terminated on day 3, whilst a mortality dose response was still clear. Toxicity profiles were noted to be similar between the fresh and the weathered CBs. The concentration range was adjusted, and the test was repeated using fresh CBs only. The second test was terminated on day 10 as per the ICES guideline. On test termination the number of surviving *A. marina* was recorded. Water and sediment samples from the initial test were taken for chemical analysis.

3.2.2.Microtox Analysis

The Microtox analysis (method available in the part 2.5.2) was carried out on water overlying the test sediments from *Arenicola* test one. Samples were taken after the CBs had been in the water and sediment test vessels for three days.

3.3. Cigarette Butt Chemical Analysis

Chemical analysis of CBs (fresh and weathered) and sediments (with 0, 0.56, 1.00 and 5.60 fresh CBs/kg and 5.60 weathered CBs/kg) used for *Arenicola* toxicity testing was undertaken on a limited number of samples as a screen to identify substances associated with smoked cigarettes which might potentially be responsible for toxic effects.

For organic substances, qualitative techniques based on high resolution mass spectrometry were applied in order to screen for known compounds previously reported in the literature, but also to potentially identify new suspect substances that are detected in both CBs and sediments. A high-level targeted screen using Direct inlet Probe – Time Of Flight Mass Spectrometry (DP-TOF-MS) was initially applied to weathered and fresh CBs in order to detect suspect substances present at relatively high concentrations. The CBs were separated into their filter, paper, tobacco components before analysis. Two of the three replicate sediment samples subjected to the *Arenicola* toxicity testing (with 0, 5.60 fresh CBs/kg and 5.60 weathered CBs/kg) were subsequently extracted and cleaned-up extracts were analysed by Liquid Chromatography – High Resolution Mass Spectrometry (LC-HRMS) alongside by DP-TOF-MS. Both targeted and non-targeted qualitative analyses were performed by LC-HRMS.

A range of trace metals typically monitored in the environment were also analysed quantitatively in both CBs (weathered and fresh, duplicate analysis) and the one replicate sediment samples from the *Arenicola* toxicity test (with 0, 0.56, 1.00 and 5.60 fresh CBs/kg and 5.60 weathered CBs/kg) in order to have an indication on whether the CBs used might be a source of trace metals.

3.3.1.Direct Probe – Time of flight mass spectrometry (DP-TOF-MS)

Only CB samples were submitted to DP-TOF-MS. Homogenization of the CB samples did not result in homogeneous samples, therefore sub-samples consisting of orange paper (Figure 12a), shag (tobacco; Figure 12b), and filter material (Figure 12c) were prepared for analyses.

Randomly selected parts of the three samples were analysed qualitatively in duplicate for both types of CB samples with DP-TOF-MS. Additionally, a few pieces of the three materials were transferred into chromatography glass vials to which approximately 1 mL methanol was added. To allow methanol to extract the compounds of interest, the vials were mixed on a rotary shaker and placed in an ultrasonic bath for 10 minutes. Subsequently, the extracts were also directly analysed qualitatively by DP- TOF-MS.

Qualitative DP-TOF-MS was performed with a microTOF II (Bruker Daltonics, Bremen, Germany) detector (with optimal mass accuracy <2ppm and resolution >16,500 FWHM) and equipped with an atmospheric pressure chemical ionization (APCI) source. Qualitative analyses were performed in both positive and negative ionization mode. The following substances previously detected in smoked cigarettes and reported elsewhere as potentially harmful in aquatic organisms were specifically screened: nicotine (CAS No. 54-11-5), ethylphenols (CAS No. 90-00-6, 620-17-7, 123-07-9), phenol (CAS No. 108-95-2), tobacco-specific N-nitrosamines (TSNA) such as N'-nitrosoanabasine (CAS No. 37620-20-5), N'-nitrosoanatabine (CAS No. 887407-16-1), N-nitrosonornicotine (CAS No 80508-23-2) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-

butanone (CAS 64091-91-4); and pyridines (i.e. 3-methylpyridine, 3,5-dimethylpyridine, 3,4-dimethylpyridine, 4-ethylpyridine, 3-acetylpyridine, 3-vinylpyridine), acetaldehyde (CAS No 75-07-0), formaldehyde (CAS No. 50-00-0).

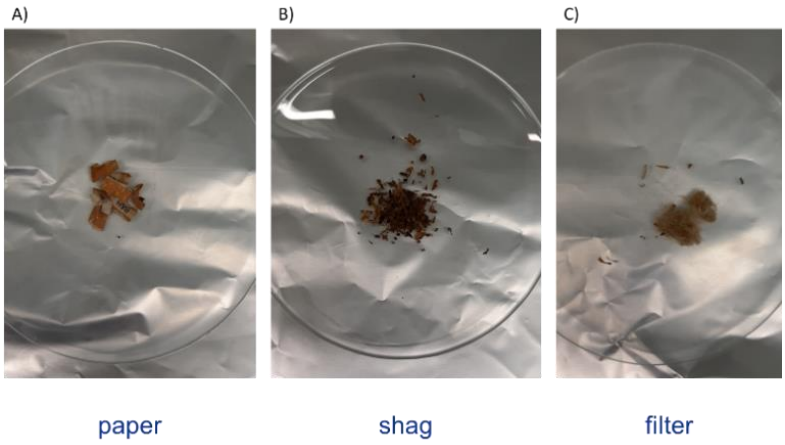


Figure 12: Parts of cigarette butts. a) paper b) shag (tobacco) and c) filter (©Cefas).

3.3.2.Liquid Chromatography – High Resolution Mass Spectrometric (LC-HRMS) analyses

Six sediment samples and the methanolic extract of both fresh and weathered CBs (prepared for DP-TOF-MS) were analysed by LC-HRMS. For the sediment samples, two duplicate control sediment samples, two duplicate sediment samples exposed to fresh CBs (5.6 CBs/kg sediment), and two duplicate sediment samples exposed to weathered CBs (5.6 CBs/kg sediment) were analysed. For this, approximately 10 g of each sediment sample was weighed into a 50 mL tube. After adding 10 mL acetonitrile, the samples were placed on a shaking device for 20 minutes. The samples were centrifuged at 5000 rpm for 10 min, the acetonitrile was collected in a fresh 10 mL tube. The extraction procedure was repeated with 5 mL acetonitrile. The extracts were evaporated to dryness under a gentle stream of nitrogen in a water bath at 45°C and reconstituted in 500 µL acetonitrile: H_2O (1:9, v/v).

Clean up of sample extracts was performed using a solid phase extraction (SPE) method described by **Beyer et al. (2007)**. Varian Bond Elute Certify cartridges (130 mg; 3 mL) were conditioned with 1 mL methanol followed by 1 mL purified water. After bringing the sample extract to the top of the column, the cartridge was washed with 1 mL purified water, followed by 1 mL 0.01 M aqueous hydrochloric acid. Elution of the compounds was performed with 2 mL methanol (fraction I) and 1 mL aqueous ammonia in methanol (2/98, v/v) (fraction II). Extracts were evaporated to dryness under a gentle stream of nitrogen on a water bath at 45°C, and reconstituted in 500 µL acetonitrile: H_2O (1:9, v/v) for analyses with LC-HRMS. The methanolic extracts of CB filter material, paper, and shag prepared for DP-TOF-MS were concentrated to dryness, and 100 µL acetonitrile was added. After mixing on a rotary shaker, 450 µL purified water was added to 50 µL of the acetonitrile extract in a glass liquid chromatography vial for analysis by LC-HRMS analysis.

The LC-HRMS analyses were performed on an Agilent 1290 Infinity LC coupled to a Bruker Compact quadrupole ToF-MS (LC/Q-ToF-MS) equipped with an Electrospray ionization (ESI) source. For LC separation, a Xselect® HSS T3 2.5 µm (2.1x150 mm) LC-column was used. The mobile phase consisted of 50 mM aqueous ammonium formate adjusted to pH 3.5 with formic acid (eluent A) and acetonitrile (eluent B). The column was equilibrated for 10 minutes with 90 % of eluent A and 10 % of eluent B at a flow of 400 µL/min. After injection of 1 µL of the sample extract the LC conditions were kept on the initial conditions for 2 min. In 5

min eluent B was increased to 80% at a flow of 0.6 mL/min, and hold stable for 2 min. Finally, eluent B was decreased to 10 % in 3 min at a flow of 0.4 mL/min. Extracts were analysed in both positive, and negative ionization modes.

All analyses performed were qualitative, and included suspect screening and non-targeted analysis. The following substances previously detected in smoked cigarettes and reported elsewhere as potentially harmful in aquatic organisms were specifically screened: nicotine (CAS No. 54-11-5), ethylphenols (CAS No. 90-00-6, 620-17-7, 123-07-9), phenol (CAS No. 108-95-2), tobacco-specific N-nitrosamines (TSNA) such as N'-nitrosoanabasine (CAS No. 37620-20-5), N'-nitrosoanatabine (CAS No. 887407-16-1), N-nitrosonornicotine (CAS No 80508-23-2) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (CAS No. 64091-91-4); and also a range of polycyclic aromatic hydrocarbons and pyridines (i.e. 3-methylpyridine (CAS No. 108-99-6), 3,5-dimethylpyridine (CAS No. 591-22-0), 3,4-dimethylpyridine (CAS No. 583-58-4), 4-ethylpyridine (CAS No. 536-75-4), 3-acetylpyridine (CAS No. 350-03-8), 3-vinylpyridine (CAS No. 1121-55-7), acetaldehyde (CAS No 75-07-0), formaldehyde (CAS No. 50-00-0).

3.3.3. Trace metals analysis

Sub-samples of the homogenised CBs (0.500 g ±0.025 g and a duplicate at 0.250 g ± 0.025 g) and sediment sample from the *Arenicola* toxicity test with 0, 0.56, 1.00 and 5.60 Fresh CBs/kg and 5.60 Weathered CBs/kg (2.0 g ± 0.1 g) were digested in a mixture of water and nitric acid using an enclosed vessel microwave (MarsXpress Microwave Reaction System, CEM Ltd, Buckingham, UK), the digest was made up in 1% nitric acid and further diluted in gold diluent prior to analysis by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent 7900, Agilent Technologies, Waldbronn, Germany) and Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES; Thermo iCAP 6500 Duo, Thermo Scientific, Hemel Hempstead, UK). Quantification of Al, As, Cd, Cr, Cu, Fe, Hg, Li, Mn, Ni, Pb, Rb, Se, Sr, V and Zn was done using a multi-point calibration with Indium as internal standard.

3.3.4. Quality control

For trace metals analysis, method blank and sediment certified reference materials (PACS-3 marine sediment, National Research Council Canada, Ontario, Canada and NWTH-2 harbour sediment, Environment Canada, National Water Research Institute, Ontario, Canada) were run within the sample batch for quality control purposes. Results are reported in mg/kg dry weight.

For DP-TOFMS, a mass calibration solvent was spiked on the probe of every single analyses run. A blank probe spiked with calibration solvent was analysed before every individual analyses. For LC-HRMS, alongside the extraction and analyses of the sediment samples a solvent blank was extracted and analysed. Five nicotine standard solutions (concentrations 25, 50, 100, 250, 500 ng/mL) were analysed alongside the samples for confirmation of the presence of nicotine, and for semi-quantitative determination of the concentration range of the presence of nicotine in the sediment samples.

4. Review of existing initiatives tackling Cigarette Butts

The review of existing initiatives, measures or actions to reduce CBs pollution was performed using web search engines. It also includes initiatives identified in the second action of the work package 4 of the CleanAtlantic project “WP4.2: Stakeholder characterization and initiatives tackling marine litter in the Atlantic area”and described in the final report “Overview of stakeholders and initiatives in the Atlantic Area in relation to marine litter” (**Cedre, 2021a and 2023a**).

Results

1. Cigarette Butts abundance and distribution on Atlantic Area beaches

Analysis of beach litter monitoring data from OSPAR (CleanAtlantic WP4.1) revealed that cigarette butts (CBs) are the 5th most commonly collected item on Atlantic Area (AA) beaches from 2016 to 2019. Across 922 surveys conducted on 62 survey sites in various parts of the AA, a total of 25,183 CBs were collected. CBs were found in 50% of the surveys conducted during the four-year period in the AA. On average, 27 CBs per 100 meters were collected during a survey in the AA, with the minimum being 0 and the maximum reaching 1,032 CBs per 100 meters in the Portuguese part of the AA. CBs represent 6% of the total litter collected during OSPAR beach litter monitoring in the AA in 2016-2019. The pollution caused by CBs is more significant on Spanish and Portuguese survey sites, with an average of 18 and 69 CBs per 100 meters collected during a survey, respectively. CB statistics in the different parts of the AA are detailed in the Table 5 below.

Table 5: CB statistics in the different parts of the Atlantic area (source: OSPAR beach litter monitoring programme, 2016-2019).

Part of Atlantic area	United Kingdom	France	Irland	Portugal	Spain	Whole Atlantic Area
Nb of sites considered	18	9	4	19	12	62
Nb of surveys considered	264	137	64	268	189	922
Total nb of CBs collected	1168	2200	23	18450	3342	25183
Percentage in the total nb of litter collected	1%	3%	1%	19%	8%	6%
Rank in the Top5 of most collected litter	Not in the Top5	Not in the Top5	Not in the Top5	1	4	5
Mean number collected over a survey (mean of all surveys) (CBs/100m)	4	16	0	69	18	27
Median number collected over a survey (median of all surveys) (CBs/100m)	0	0	0	11	2	1
Minimum nb collected over a survey (CBs/100m)	0	0	0	0	0	0
Maximum nb collected over a survey (CBs/100m)	159	572	7	1032	450	1032
Nb of survey where CBs were found	106	46	7	183	117	459
Percentage of survey where CBs were found	40%	34%	11%	68%	62%	50%

2. Experimental study on artificial Cigarette Butts

2.1. Characterisation of artificial Cigarette Butts

2.1.1. Polymer composition and physical characterisation

The characterisation using the ATR-FTIR showed that the main component of the CBs was the cellulose triacetate (Figure 13). The three types of selected CBs (light, medium strengh and strong) showed a similar spectrum. Likewise, weathering on beach (85 days) or in harbour (70 days) had no incidence on the spectral signatures of the CBs (Figure 14).

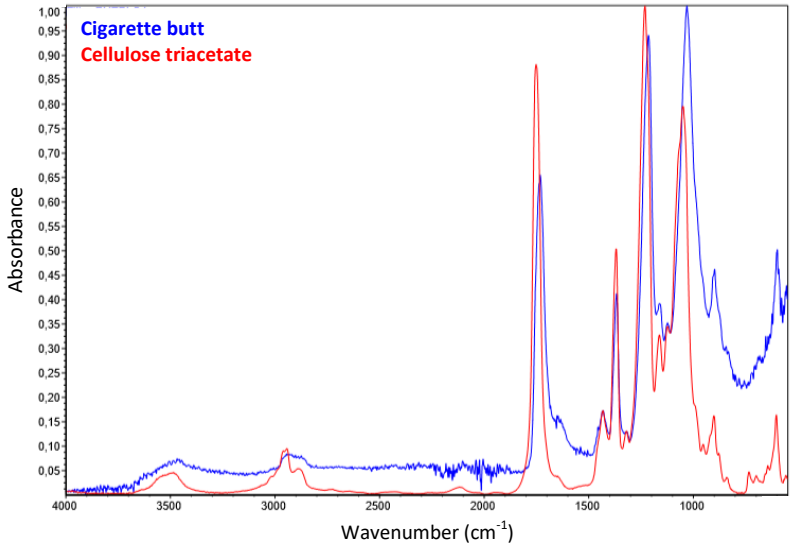


Figure 13: Typical absorbance spectrum for cellulose triacetate (red) compared to the spectrum shown by one of the CB used in the present study (©IEO). (See also Santos-Echeandía et al., 2021).

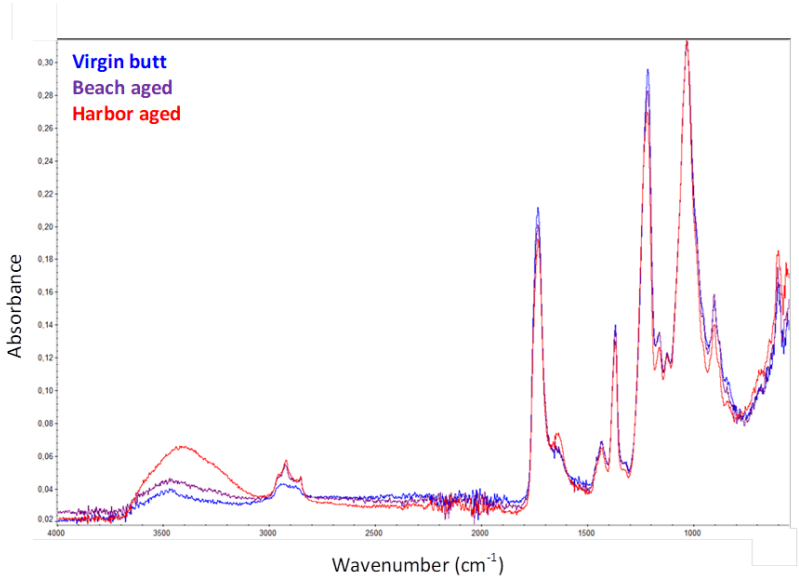


Figure 14: Absorbance spectrum for a fresh medium strength CB (blue) compared to the spectrum shown by the medium strengh CB aged on the beach for 85 days (purple) or aged in the harbour for 70 days (red) (©IEO). (See also Santos-Echeandía et al., 2021).

After the standard cigarette smoking, a coloration of filters was observed for each type of cigarettes traducing a contamination of the filter (Figure 15). This coloration increased according to the cigarette strength: Light

< Medium < Strong, indicating different contamination patterns between cigarette types. The same pattern, though more intense in terms of coloration was observed after a continuous smoking, indicating an influence of the smoking mode on the filter contamination (Figure 15).

Likewise, a mass evolution during smoking was observed with a gain (5-10 mg) in dry weight is observed for the three CB types (Table 6).

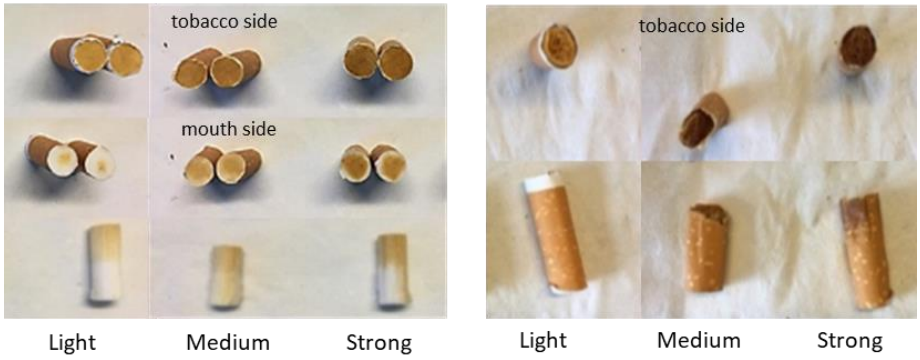


Figure 15: coloration of CB after discontinuous artificial smoking (left) and continuous artificial smoking (right) (©Cedre).

Table 5: Comparative table of parameters measured on the three types studied (mean ± standard deviation).

Cigarette types	Unsmoked filter dry mass (mg DW)	CB dry mass (mg DW)	Mass gain after smoking (mg)	CB humidity %	Continuous smoking time (min)
Light	209,86 ± 4,72	215,02 ± 4,88	5.16	2.4	2'08''
Medium	168,25 ± 2,41	173,25 ± 2,81	5	2.4	50''
Strong	182,7 ± 1,69	193,13 ± 1,51	10.43	1.7	2'08''

2.1.2. Intrinsic contamination

Organic compounds in "artificially smoked" cigarette butts

Chemical analyses performed on light and medium strength CBs are described in the Figure 16. Statistical analyses revealed no significant difference (*p*-values > 0.05) in the concentrations of nicotine (average value= 40.4 ± 24.4 µg/CB), phenols (average value= 3.3 ± 1.6 µg/CB), indole (average value= 3.1 ± 1.8 µg/CB) and PAH (average value= 1.3 ± 1.6 µg/CB) between the two cigarette types.

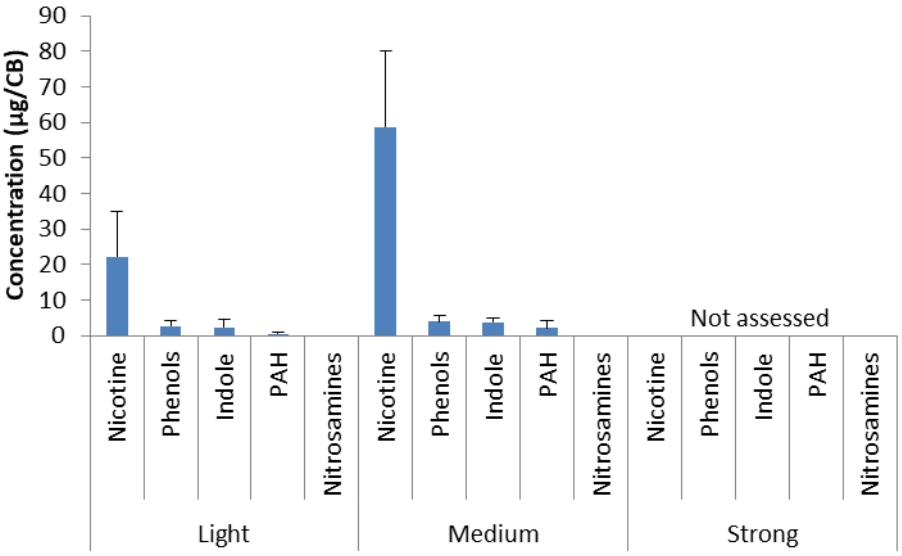


Figure 16: Intrinsic contamination (µg/CB) of fresh artificial butts obtained from the light and medium cigarettes (n=3). Statistical analyses revealed no significant difference (*p*-values > 0.05).

Metal concentration in unsmoked cigarette filters and "artificially smoked" cigarette butts

Leachable metal concentrations in unsmoked cigarette filters and artificially smoked CBs for the different brands are shown in Table 7. In the case of unsmoked cigarette filters, Fe was the element with the highest values measured (3.97–7.86 mg/kg) followed by Zn (0.470–0.900 mg/kg) and Cr (0.262–0.808 mg/kg) while the lowest values were measured for U (0.001–0.002 mg/kg) and Cd (0.001–0.003 mg/kg). Regarding smoked CBs, the highest levels were also measured for Fe (49.8–130 mg/kg) and Zn (3.05–5.26 mg/kg) while the lowest ones again for U (0.010–0.188 mg/kg) and Cd (0.002–0.037 mg/kg). Most of the elements showed a statistically significant increase (*p*-values < 0.05) in their levels for the smoked CBs (Cd, Co, Cu, Fe, Li, Mn, Sr, U, V and Zn). However, other elements significantly decreased (*p*-values < 0.05) their concentrations (As, Cr and Hg) or just showed no significant change in their levels (*p*-values ≥ 0.05) after the cigarettes were smoked (Mo, Ni, Pb and Sb). The highest change before and after being smoked was observed for Sr, U and V with an enrichment factor up to 100 times, while the smallest change was observed for Mo, Ni or Sb which barely doubled their levels (Figure 17). Finally, As, Cr, Hg or Sb reduced their levels by a quarter (Figure 17). A closer look at each of the brands separately revealed a higher enrichment factor in brand C for metals such as As, Co, Fe, Pb, U or V. On the other hand, metals like Cd, Cr, Cu, Hg, Mn, Sr or Zn were enriched in brand A compared to the other two brands. Finally, Li, Mo, Ni or Sb, showed similar enrichment factors in all brands (Figure 17).

Table 7: Trace element concentrations (ppm) and standard deviation (n=3) in unsmoked cigarette filter and smoked butts. A: Medium, B: Strong and C: Light (b.d.l= below detection limit). [a] = ppb. (See also Santos-Echeandía et al., 2021).

Element	Unsmoked filter A	Unsmoked filter B	Unsmoked filter C	CB-A	CB-B	CB-C
As	0.153 ± 0.014	0.149 ± 0.015	0.131 ± 0.010	0.025 ± 0.007	0.020 ± 0.002	0.080 ± 0.008
Cd	0.003 ± 0.001	0.003 ± 0.001	0.001 ± 0.001	0.037 ± 0.003	0.014 ± 0.001	0.002 ± 0.001
Co	0.003 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.017 ± 0.001	0.046 ± 0.004	0.076 ± 0.007
Cr	0.808 ± 0.073	0.430 ± 0.042	0.262 ± 0.020	0.215 ± 0.106	0.020 ± 0.002	0.050 ± 0.005
Cu	0.245 ± 0.011	0.055 ± 0.005	0.051 ± 0.004	1.78 ± 0.460	0.650 ± 0.059	0.630 ± 0.060
Fe	7.86 ± 0.707	3.97 ± 0.389	4.13 ± 0.318	49.8 ± 8.91	130 ± 11.8	104 ± 9.97
Hg[a]	2.46 ± 0.221	4.19 ± 0.411	7.18 ± 0.553	5.28 ± 0.300	3.45 ± 0.314	3.88 ± 0.372
Li	b.d.l	b.d.l	0.006 ± 0.001	0.085 ± 0.035	0.100 ± 0.009	0.060 ± 0.006
Mn	0.091 ± 0.008	0.056 ± 0.005	0.176 ± 0.014	2.33 ± 0.098	2.65 ± 0.241	1.34 ± 0.130
Mo	0.015 ± 0.001	0.012 ± 0.001	0.006 ± 0.001	0.016 ± 0.006	0.013 ± 0.001	0.016 ± 0.002
Ni	0.160 ± 0.140	0.140 ± 0.014	0.056 ± 0.004	0.175 ± 0.021	0.160 ± 0.015	0.150 ± 0.014
Pb	0.096 ± 0.009	0.151 ± 0.015	0.012 ± 0.001	0.125 ± 0.085	0.083 ± 0.008	0.684 ± 0.066
Sb	0.017 ± 0.001	0.014 ± 0.001	0.008 ± 0.001	0.015 ± 0.006	0.017 ± 0.002	0.014 ± 0.001
Sr	0.061 ± 0.005	0.066 ± 0.006	0.199 ± 0.015	9.25 ± 1.33	5.20 ± 0.473	4.80 ± 0.461
U	0.001 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.057 ± 0.009	0.010 ± 0.001	0.188 ± 0.018
V	b.d.l	0.005 ± 0.001	0.005 ± 0.001	0.145 ± 0.084	0.501 ± 0.046	0.208 ± 0.020
Zn	0.760 ± 0.068	0.900 ± 0.088	0.470 ± 0.036	5.26 ± 0.59	3.05 ± 0.278	3.80 ± 0.365

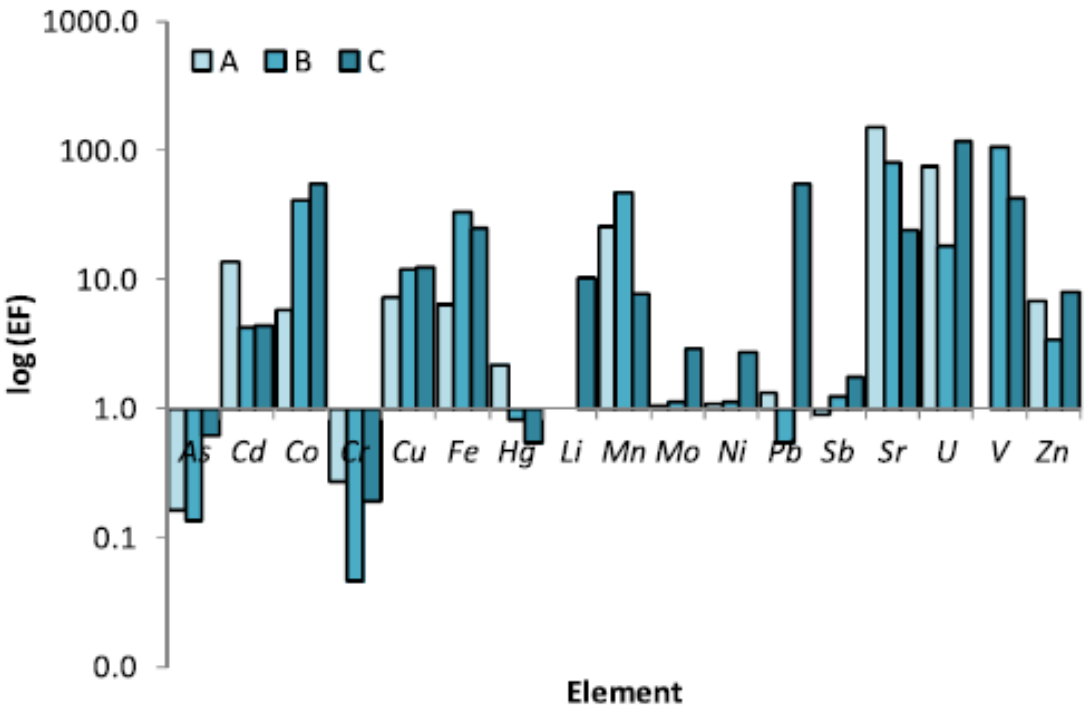


Figure 17: Enrichment factor (EF in logarithmic scale) for all the elements in the butts, after being smoked, of the three different brands (A, B, C) considered in the present study. A: Medium, B: Strong and C: Light. (See also Santos-Echeandía et al., 2021).

2.2. Study of Cigarette Butts leaching in seawater under experimental conditions

Leaching of organic compounds in "artificially smoked" cigarette butts

Leaching of organic compounds occuring in medium-strength CBs was tracked over 72h (Figure 18). In CBs, the amount of nicotine decreased signficantly from 6h of leaching (-83%; *p*-value = 0.005) and remained stable afterwards (*p*-value > 0.05). Consequently, in leachates, the amount of nicotine remained stable between 6 and 72h of leaching (mean value=34.2 ± 5.1 µg/L; *p*-value > 0.05).

The amount of phenols did not evolve significantly in CBs whatever the leaching time (mean value= 4.5 ± 3.2 µg/L; *p*-value > 0.05).

The amount of indole decreased signifantly from 6h of leaching (-53%; *p*-value = 0.006) and remained stable afterwards (*p*-value > 0.05). Consequently, in leachates the amount of indole remained stable between 6 and 72h of leaching (mean value= 1.5 ± 0.2 µg/L; *p*-value > 0.05).

The amount of PAHs decreased signifantly from 6h of leaching (-85%; *p*-value = 0.006and remained stable afterwards (*p*-value > 0.05). Consequently, in leachates, the amount of PAHs remained stable stable between 6 and 72h of leaching (mean value= 0.3 ± 0.03 µg/L; *p*-value > 0.05).

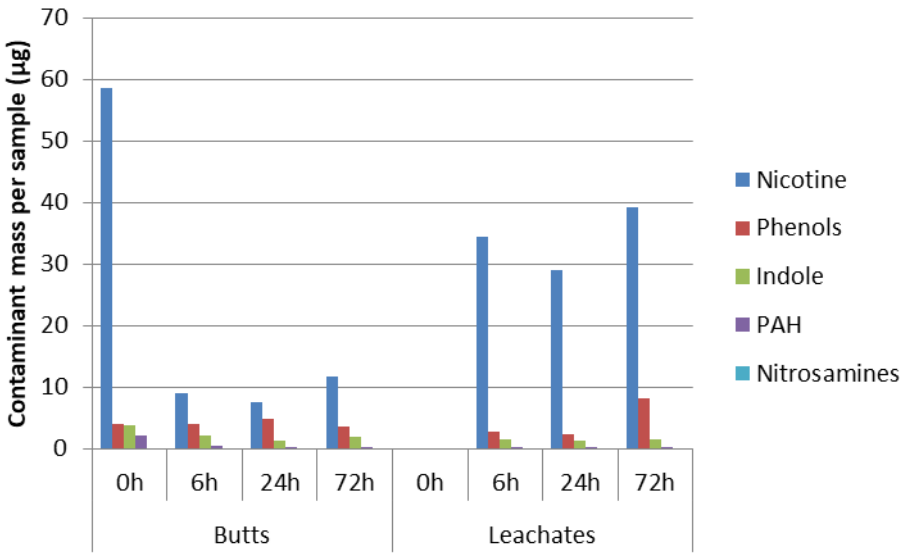


Figure 18. Average concentrations of organic compounds in fresh artificial butts (µg/CB; medium-strengh CB) or leachates (µg/L) after 6h, 24h and 72h of leaching in seawater (n=3).

Leaching of metals from artificially smoked cigarette butts

The release of trace metals from CBs in naturally filtered seawater was monitored through time (6, 24 and 72 h) showing a logarithmic decrease in metal concentrations of the CBs. A sharp initial decrease that tends to a constant value with time was observed (Figure 19, the parameters for the kinetic model can be found in Annex 1). Except for Li, which was not released from the CBs (Figure 19), all the elements showed a decrease in their concentrations in CBs. The percentage of desorption (24 h) for each metal and by cigarette brand is shown in Table 8. Copper was the element with the highest percentage of desorption (91 ± 3%) followed by Mn (89 ± 3%) and Zn (86 ± 2%). On the other side, Mo (36 ± 3%), Pb (39 ± 52%) and Sr (40 ± 0%) showed the lowest desorption percentages.

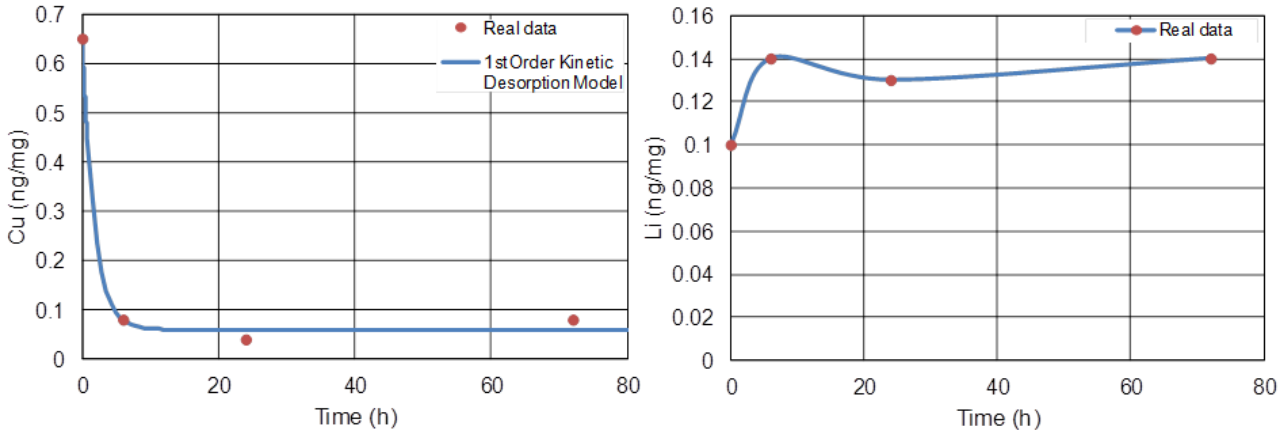


Figure 19: Copper (left) and lithium (right) concentration in CBs with time once placed in filtered seawater. (See also Santos-Echeandía et al., 2021).

Table 8: Percentage of desorption (24 h) for metals in the different smoked CBs once introduced in filtered seawater. Mean and standard deviation for each element is also shown. (See also Santos-Echeandía et al., 2021).

Element	**Brand (%)**			Mean %
	Light	Medium	Strong	Mean±St. Dev.
As	67	50	88	68±19
Cd	90	64	100	85±18
Co	81	83	88	84±3.6
Cr	-	-	-	-
Cu	90	94	89	91±2.6
Fe	83	80	83	82±1.7
Hg	79	74	74	76±2.9
Li	-	-	-	-
Mn	87	88	93	89±3.2
Mo	33	-	38	36±3.5
Ni	63	38	67	56±16
Pb	-	2	76	39±52
Sb	84	82	86	84±2.0
Sr	40	-	-	40±0.0
U	79	50	82	71±18
V	74	85	82	80±5.7
Zn	88	85	86	86±1.5

2.3. Cigarette Butts behaviour in seawater

2.3.1. Tests in beakers

In static condition, the tested medium-strengh CB float during the first 20 seconds (Figure 20a). At 40 seconds, the tested CB begins to sink slightly in the subsurface of the water (Figure 20b). And between 1 minute and 24 hours, the tested CB remains in the subsurface (Figure 20c). When manually saturated with seawater, the tested CB sinks. During the experiment, the white paper remains intact.

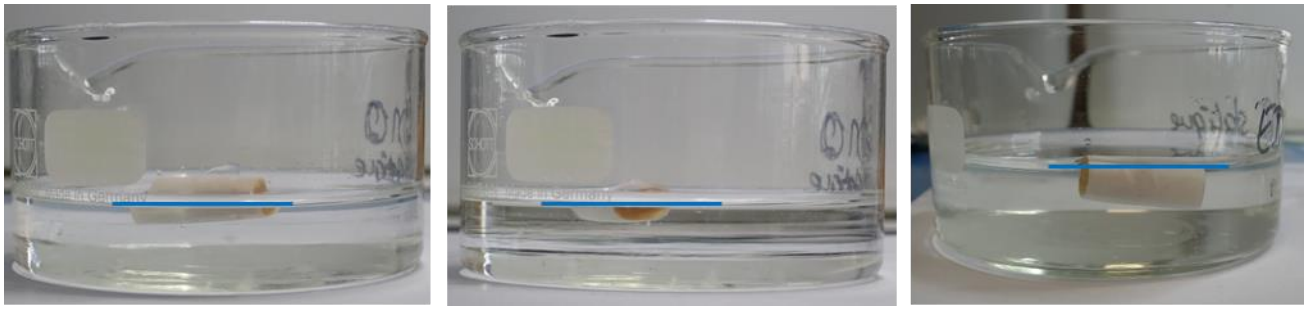


Figure 20: Behaviour of a medium-strengh CB in seawater in static condition at (a) 20 seconds, (b) 40 seconds and (c) 24 hours. The blue line represents the surface of the water.

For light CBs, after 20 seconds in static condition, the tested CB is slightly deeper on the surface of the water than for the two other types (Figure 21a). At 40 seconds, the tested light CB begins to position itself vertically in the crystallizer and floats (Figure 21b). Between 2 minutes and 24 hours, the tested CB floats vertically in the crystallizer (Figure 21c). When manually saturated with seawater, the tested CB sinks. During the experiment, the white paper remains intact.

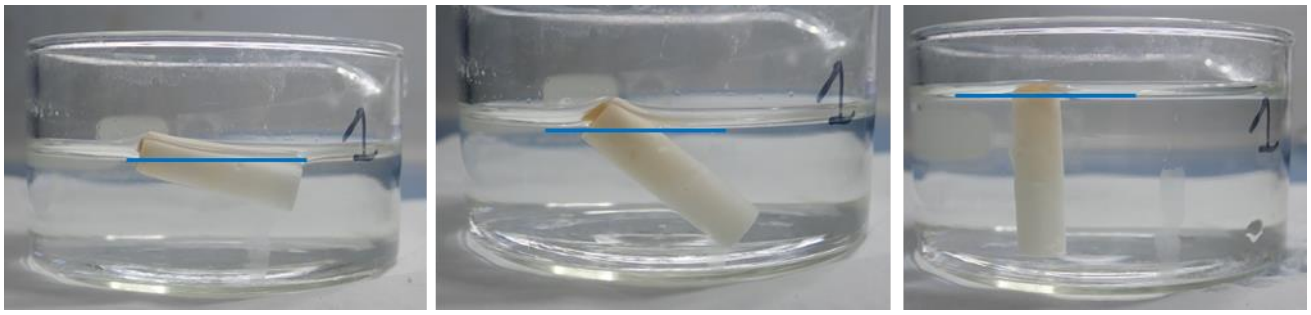


Figure 21: Behaviour of a light CB in seawater in static condition at (a) 20 seconds, (b) 40 seconds and (c) 24 hours. The blue line represents the surface of the water.

The evolution of the CB humidity rate over time showed some differences between the three types of CB. The light CB takes up water faster than the medium and strong CBs (Figure 22). At 20 seconds, the light CB has an average humidity rate of 58%, the medium-strengh CB of 37% and the strong CB of of 31%. After 120 minutes, the light CB reaches a humidity rate of 88%, the medium-strengh CB of 60% and the strong CB of 47%.

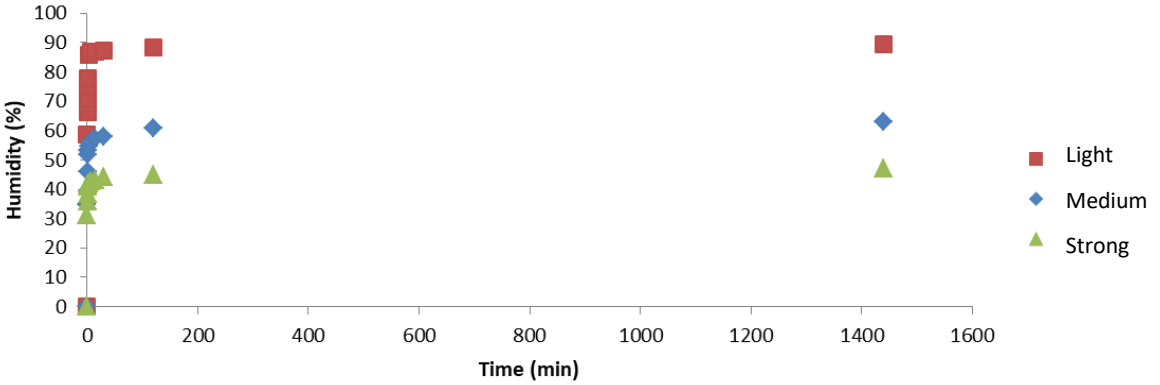


Figure 22: Evolution of the humidity rate (%) of the 3 types of CB over time, in seawater.

In dynamic condition, at 60 rpm, the tested medium-strengh CB floats during the first 10 seconds (Figure 23a). At 20 seconds, it begins to sink slightly in the subsurface of the water (Figure 23b). And between 40 seconds and 24 hours, it remains in the subsurface (Figure 23c). During the experiment, the white paper remains intact.

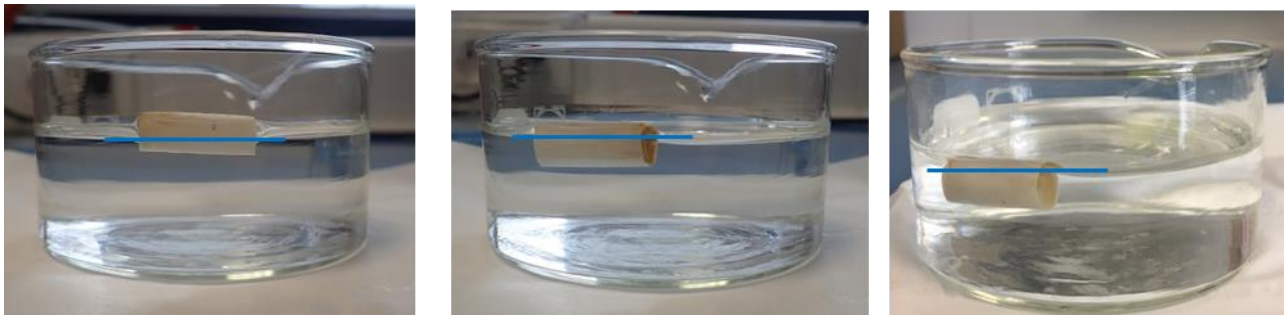


Figure 23: Behaviour of a medium-strengh CB in seawater at 60 rpm at (a) 10 seconds, (b) 20 seconds and (c) 24 hours. The blue line represents the surface of the water.

At 150 rpm, the tested medium-strengh CB slightly sinks in the subsurface during the first 10 seconds (Figure 24a). It remains in the subsurface during 24 hours (Figure 24b). However, the white paper starts to deteriorate after 20 minutes (Figure 24c).

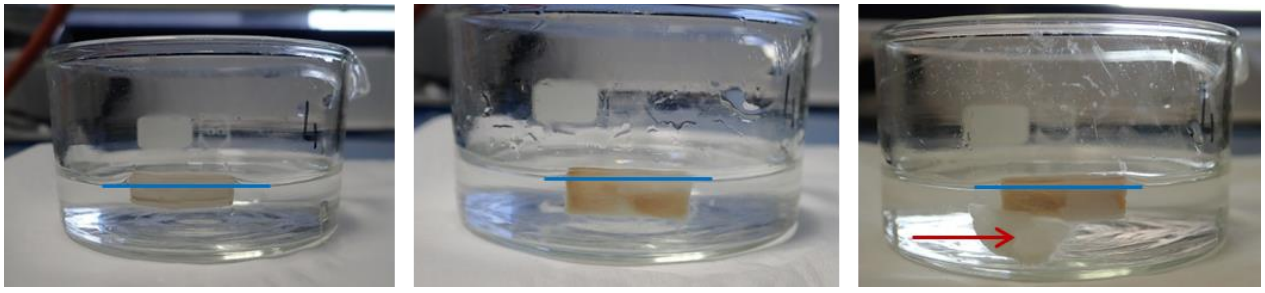


Figure 24: Behaviour of a medium-strengh CB at 150 rpm at (a) 10 seconds, (b) 24 hours and (c) 20 minutes with the white paper starting to deteriorate (red arrow). The blue line represents the surface of the water

The evolution of the CB humidity rate over time showed some differences depending on the intensity of the agitation. At 150 rpm, a medium-strengh CB takes up water faster than at 60 rpm (Figure 25). At 20 seconds it has an average humidity rate of 26% at 60 rpm and 41% at 150 rpm. After 120 minutes, it reaches a humidity rate of 64% at 60 rpm and 72% at 150 rpm.

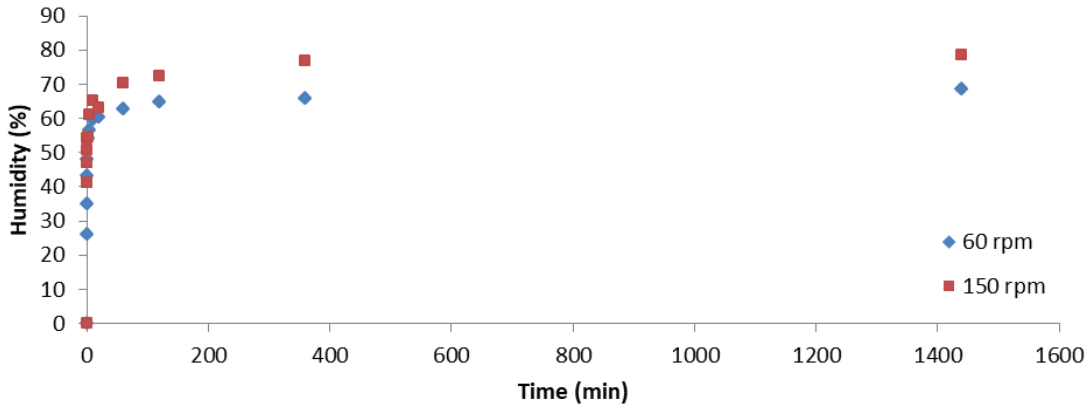


Figure 25: Evolution of the humidity rate (%) of medium-strengh CB according to the level of agitation (60 or 150 rpm).

2.3.2.Column tests

The experiment performed on different type of CB showed that, when saturated with water, CBs sink directly to the bottom of the seawater column at an average speed of 0.04 m/s (0,14 km/h) (Figure 26). No statistical difference was observed among CBs (*p*-value > 0.05; light CBs= 0.037 ± 0.006 m/s, medium-strengh CBs = 0.033 ± 0.010 m/s, strong CB = 0.039 ± 0.010 m/s); CBs need between between 3m30sec and 5 min to reach a 10 m depth.

Figure 26: Photos of a CB sinking in Cedre seawater column (©Cedre).

2.4. CB degradation

2.4.1.Degradation on the beach

After 1 year of ageing, a deterioration of CBs aged on Cedre artificial beach was observed visually with the erosion of the orange paper for the three types of CB (light, medium-strengh and strong). The degradation of the orange paper was more advanced for the light CBs (Figure 27).

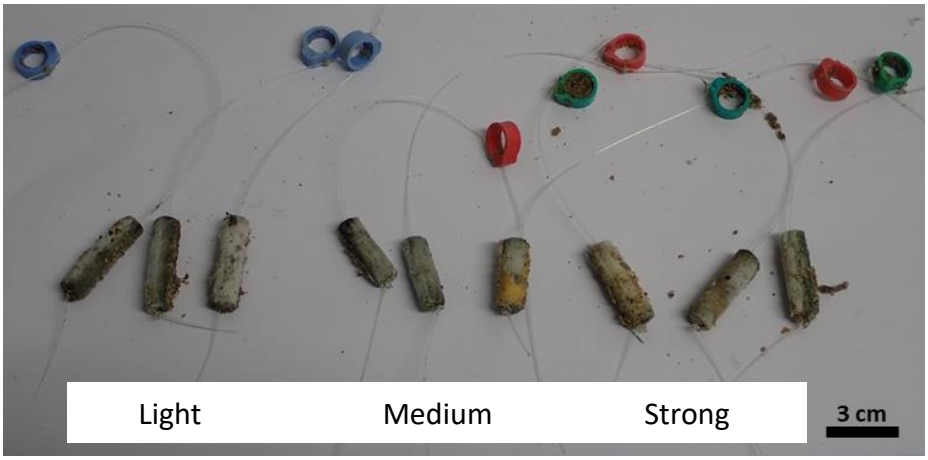


Figure 27: Photos of CBs after 1 year of ageing on Cedre artificial beach (©Cedre).

The study of the CB dry mass during the ageing period on Cedre artificial beach showed that after more than 1 year, the CB are still present with no significant change in their mass (*p*-value > 0.05) (Figure 28).

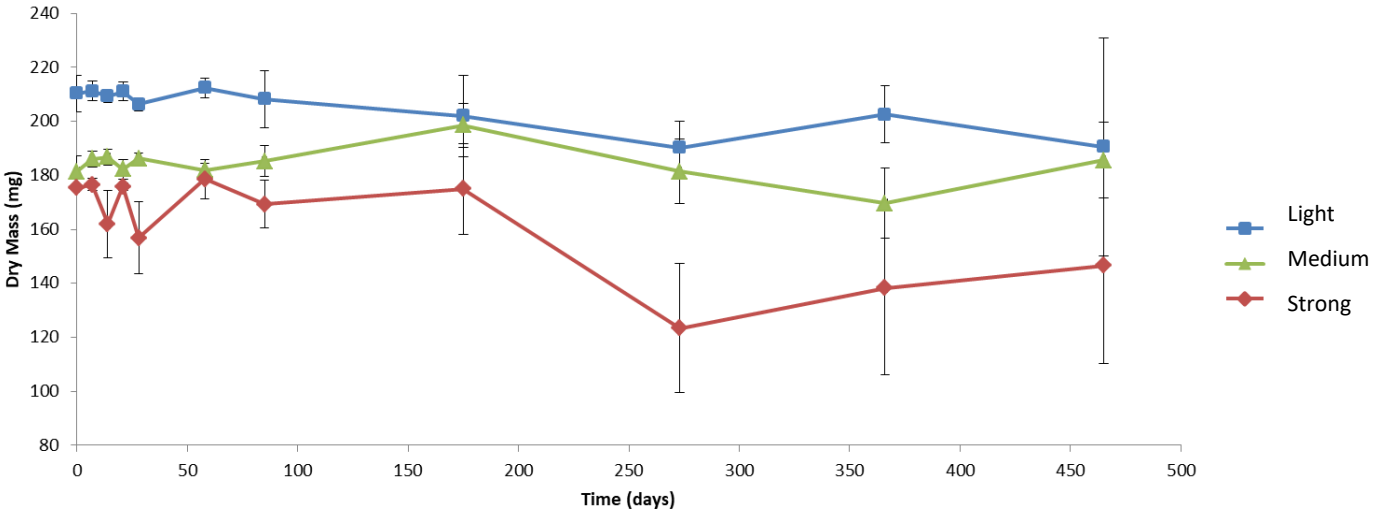


Figure 28: Average dry mass (mg) of the three types of CB during ageing on Cedre artificial beach (n=3, means ± SD).

The station's daily weather records indicated an average sunshine of 131 [min: 0; max: 1003] W/m² and an average rainfall of 0.14 [min: 0; max: 12.7] mm for the 365 days of the study period. Over this period, there were 4639 hours of sunshine and 1286 hours of rainfall over the 8760 hours of study.

2.4.2.Degradation on the seafloor in Brest Marina and Cedre outdoor basin

After 9 months of ageing, a deterioration of the CB aged on the Brest harbour seafloor was observed visually, by a complete degradation of CB surrounding papers and a partial degradation of the filter itself, though different depending on the CB (Figure 29). The greater degradation was observed for the medium-strengh CB.

Figure 29: The three types of CB after 9 months of ageing on the Brest harbor seafloor.

The study of CB ageing on the Cedre basin showed, visually, a slower degradation of CB compared to the study on the seafloor. Indeed, after 9 months of ageing, the CB surrounding papers started to degrade slightly (Figure 30).

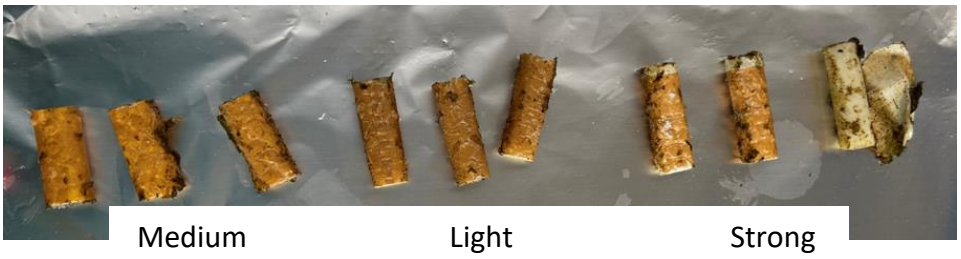


Figure 30: The three types of CB after 9 months of ageing in the Cedre's basin.

The study of the CB dry mass during the ageing period on the Brest harbour showed a significant loss of mass during the aging period (p-value < 0.05; Figure 31). By contrast, no significant difference was detected during the ageing in the Cedre's basin (p-value > 0.05; Figure 32).

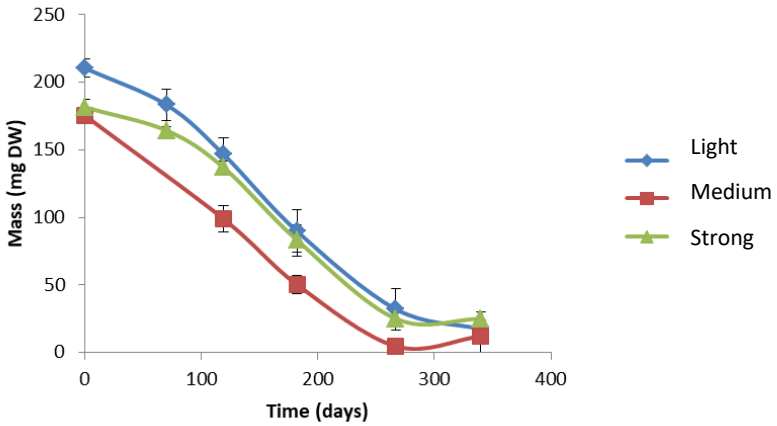


Figure 31: Average dry mass (mg) of the three types of CB during ageing on the Brest harbour. (n=3, means ± SD).

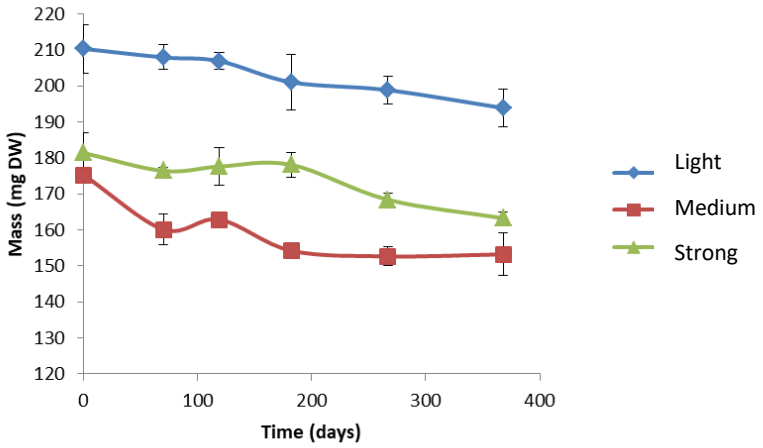


Figure 32: Average dry mass (mg) of the three types of CB during ageing in the Cedre's basin (n=3, means ± SD).

2.4.3.Influence of ageing on the intrinsic contamination

Exposure of *A. fischeri* to leachate (8 CBs/L) from the three types of CBs aged on Cedre's artificial beach revealed a significant decrease in the toxicity between 0 and 85 days, compared to unaged CBs (*p*-value < 0.05) (Figure 33). Inhibition of the bioluminescence of the bacteria decreases from 46% for leachates from unaged light and medium-strengh to 20% after 28 days of aging. The values then decrease to below 5% after 56 days of aging (Figure 33). For leachates from strong CBs, the inhibition of bioluminescence of the bacteria decreases from 63% for unaged CB to 38% after 7 days of aging. The values then decrease to 20% between 14 and 28 days of aging (Figure 33). After 56 days of aging, the bioluminescence inhibition values are below 10%.

The aging on Cedre's artificial beach had also repercussions on the amount of chemicals in CBs. The concentration of nicotine was reduced by 79% after 7 days of deployment (*p*-value < 0.05; Figure 34). No difference was detected in the amounts of nicotine and indole between 7 and 56 days of deployment. After 56 days, the amouts of phenols and PAHs analyzed were 0 µg/CB.

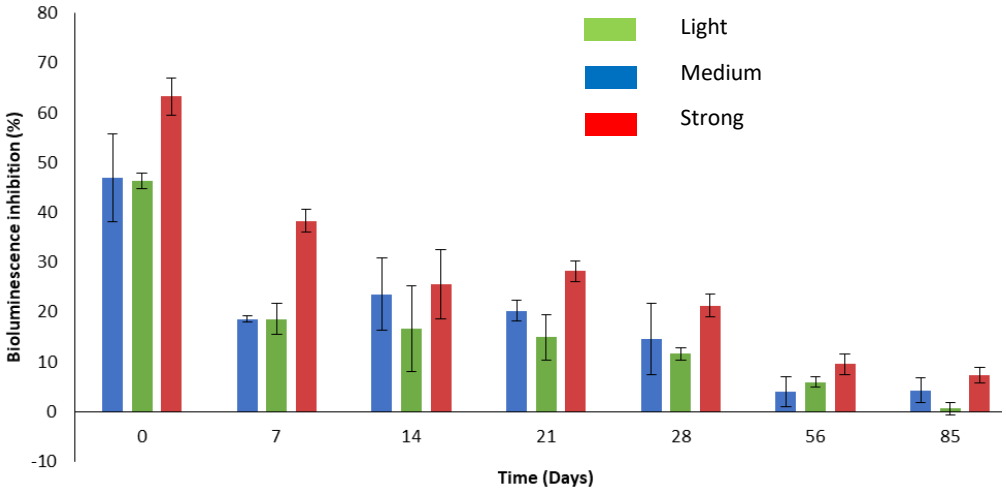


Figure 33: Average bioluminescence inhibition of *A. fischeri* (%) exposed to leachates from the 3 types of CBs, as a function of ageing time on Cedre's artificial beach (n=3, means ± SD).

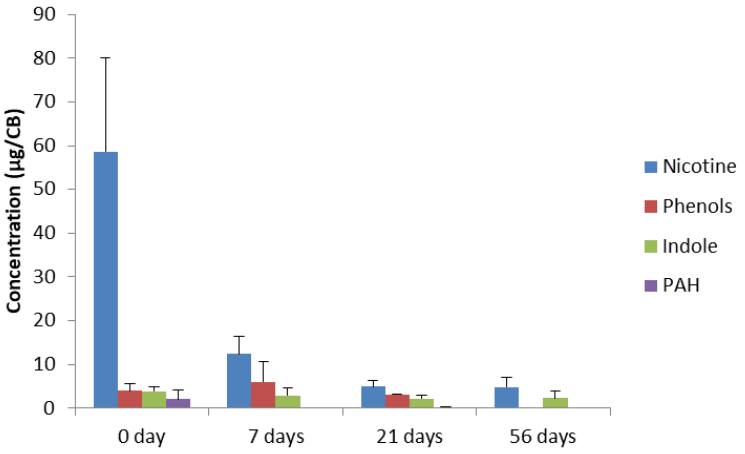


Figure 34: Concentrations of organic compounds in medium-strengh artificial butts (µg/CB) during 56 days of deployment on Cedre's artificial beach (n=3, means ± SD).

Regarding the aging on the seafloor in Brest Marina, the concentration of nicotine, phenols, indole and PAHs reched 0 µg/CB after 72 days of deployment (Figure 35).

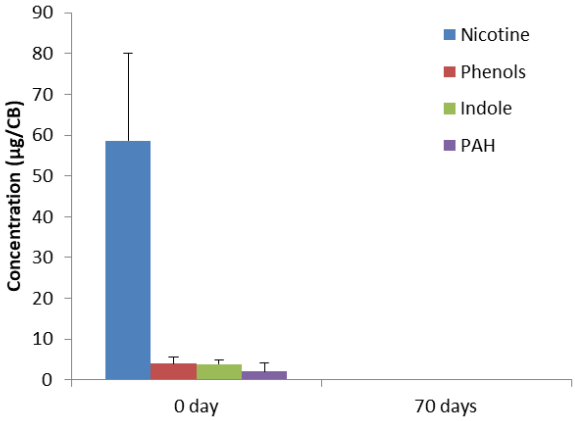


Figure 35: Concentrations of organic compounds in medium-strengh artificial butts (µg/CB) before and after 70 days of deployment on the seafloor in Brest Marina (n=3, means ± SD).

Regarding the metal accumulation in CBs, a significant increase (p-value < 0.05) in metal concentrations in the CBs was observed with time (at 14, 28 and 85 days) after being placed both in the sandy beach and on the seafloor in Brest Marina. In the case of the beach, most of the elements (except Mo and V), showed a logarithmic increase, that was sharp in the beginning and decreases with time tending to a constant value (Figure 36, the parameters for the kinetic model can be found in Table 9). A similar kinetic is expected for the CBs aged in the harbour; however, these CBs were sampled only once (70 days). What is undoubtedly is that the increase of metals in the CBs aged on the harbour is significantly greater (p-value < 0.05) than the one observed for the sandy beach for most of the metals except for Cd, Mn, and Zn. This fact can be observed by having a look at the 70-days enrichment factor shown in Figure 37. The highest enrichment factors in the harbour samples corresponds to Cr (60 000), followed by Mo (241) and Ni (233) while the lowest values were found for Mn (2), Cd and Hg (4). In the case of the sandy beach, the highest enrichment factors were observed for Cr (40), Cu (33) and Cd (24) and the lowest for Hg, Mo, U and V (2).

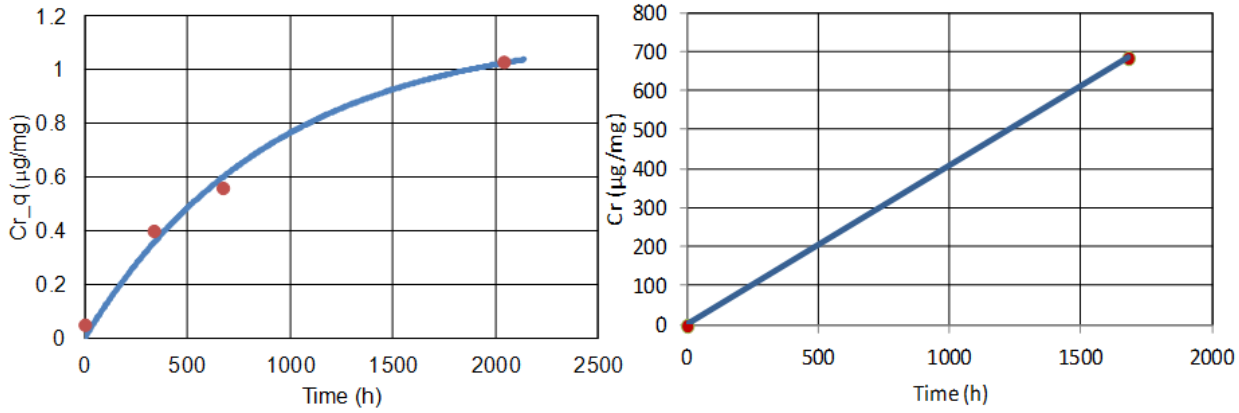


Figure 36: Chromium concentration in medium-strengh CBs with time once placed in the sandy beach (left) or in the harbour (right).

Table 9: Parameters calculated according to the first order kinetic model (k, q_e and R^2) and second order kinetic model (K, q_e and R^2) for desorption between metals and CB. (BF: medium-strengh CBs, CF: strong CBs). (See also Santos-Echeandía et al., 2021).

		First order kinetic model		
		k (h^{-1})	q_e (ng mg^{-1})	R^2
As	BF	0.0011 NS	0.667±0.551	0.9994
	CF	0.0032 NS	0.411 NS	0.9912
Cd	BF	0.0018 NS	0.119±0.076	0.96581
	CF	0.0014 NS	0.088±0.041	0.9997
Co	BF	0.0006 NS	0.604 NS	0.9917
	CF	0.0015 NS	0.334 NS	0.9493
Cr	BF	0.0002 NS	5.15 NS	0.9999
	CF	0.0011±0.0010	1.14±0.481	0.9935
Cu	BF	0.0003 NS	63.9 NS	0.9997
	CF	0.0013 NS	19.3±9.21	0.9842
Fe	BF	0.0007 NS	793 NS	0.9585
	CF	0.0016 NS	425 NS	0.9477
Hg	BF	-	-	-
	CF	0.0029 NS	0.008 NS	0.8707
Li	BF	0.0003 NS	1.76 NS	0.9878
	CF	0.0021 NS	0.512±0.337	0.9507
Mn	BF	0.0014 NS	19.7 NS	0.9878
	CF	0.0016 NS	12.1±9.88	0.9402
Mo	BF	lineal	lineal	lineal
	CF	-	-	-
Ni	BF	0.0018 NS	1.36 NS	0.9928
	CF	0.0052 NS	1.04 NS	0.9991
Pb	BF	0.0022 NS	2.57 NS	0.9998
	CF	0.0037 NS	3.26 NS	0.9950
Sb	BF	0.0008 NS	0.229 NS	0.9938
	CF	0.0023±0.0017	0.174±0.046	0.9973
Sr	BF	0.0042 NS	45.6 NS	0.9989
	CF	0.0027 NS	27.0 NS	0.9988
U	BF	0.0018 NS	0.052 NS	0.9785
	CF	-	-	-
V	BF	lineal	lineal	lineal
	CF	0.0021 NS	1.01±0.879	0.9364
Zn	BF	0.0015 NS	104±58.2	0.9999
	CF	0.0008 NS	67.9±41.3	0.9959

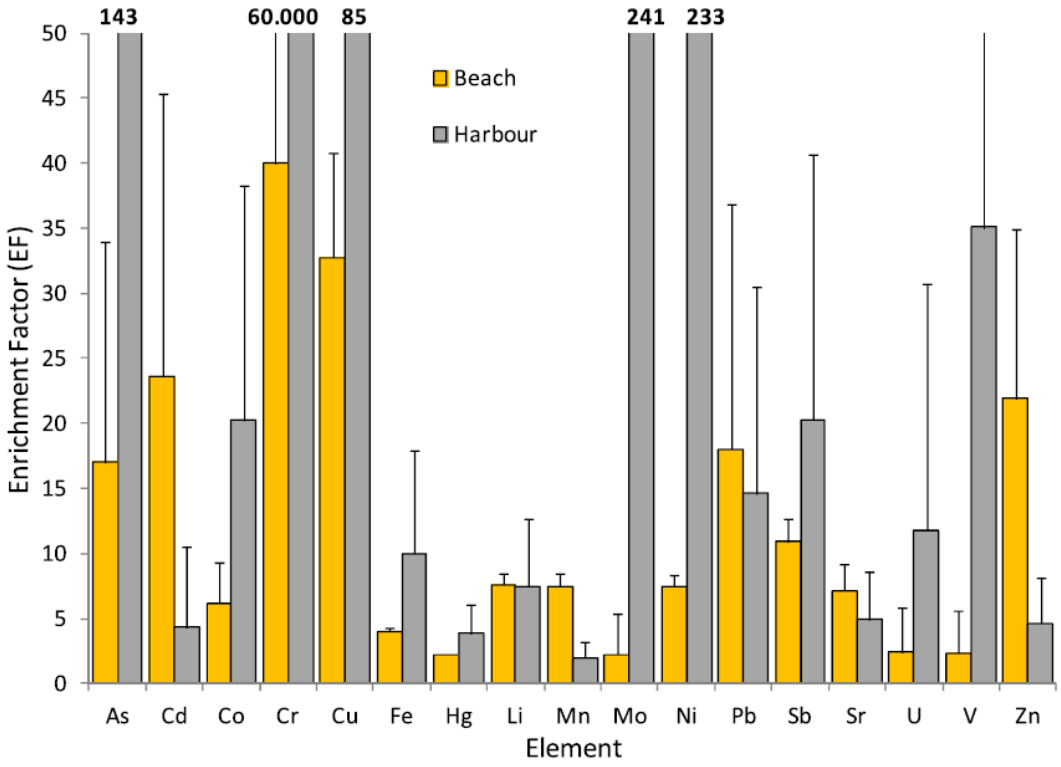


Figure 37: Metals enrichment factor (mean and standard deviation) of the different elements in the medium-strengh CBs after 70 days of weathering (Beach=Cedre artificial beach and harbour=Brest marina) (See also Santos-Echeandía et al., 2021).

2.5. Cigarette butt toxicity on marine organisms

2.5.1.On marine bacteria A. fischeri (Microtox© test)

At a constant concentration of 8 CBs/L, bioluminescence of *A. fischeri* inhibition tests on 24h-leachates of the 3 types of CBs with a classical, continuous or clogged smoking, show an increase of leachates toxicity from smoked the light to the strong CBs except for the clogged smoking where the toxicity of the three types of CB reach the same level (about 70% of inhibition) (Figure 38). The leachates of classical and continuous smoked light CBs lead to an inhibition of the bioluminescence of 48 and 80% respectively, the medium-strength CB leachates of 60 and 89% and the strong CB leachates of 72 and 94%. For the unsmoked CB, the light filter produces the greatest bioluminescence inhibition for *A. fischeri* (20%), followed by the strong (18%) and medium-strength filters (13%).

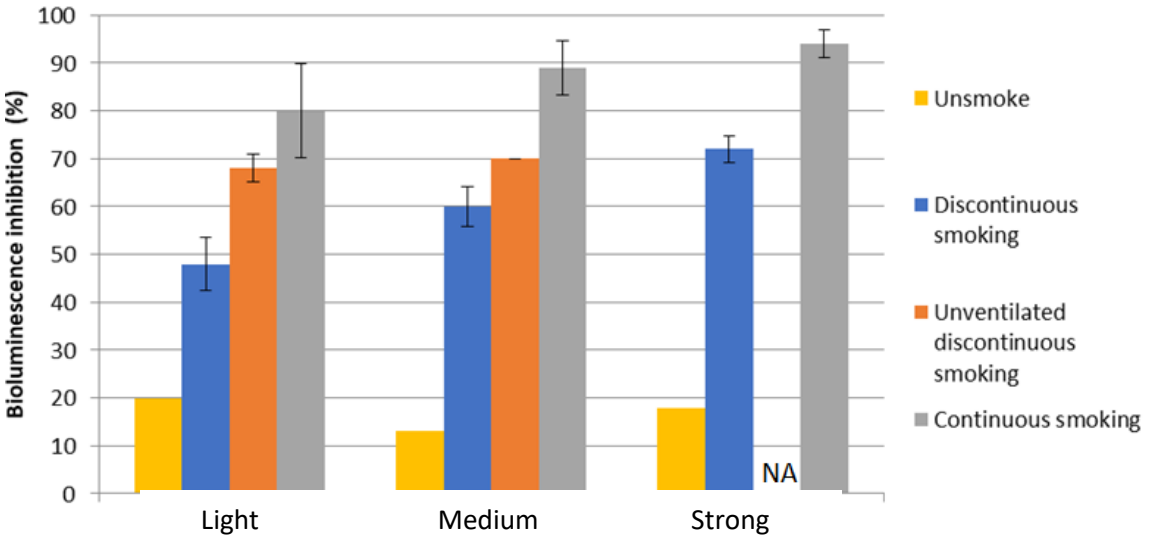


Figure 38: Bioluminescence inhibition (%) of *A. fischeri* for the three types of CB leachates (8 CBs/L) smoked or unsmoked with a classic, continuous or clogged smoking (n=2, means ± SD).

Regarding the EC_{50} of the leachate of the smoked CB and the estimated values for the leachate of the unsmoked CB, the obtained values were 5.6 and 36.1 CB/L respectively for light CBs, 3.4 and 43.5 CB/L for medium-strengh CBs and 3.9 and 36.8 CBs/L for strong CBs (Table 10).

Table 10: EC_{50} values obtained for the different types of CB (A, B and C) smoked and unsmoked on the marine bacteria *A. fischeri*.

	EC_{50}	
CB types	Smoked CB	Unsmoked CB
Light	5,6 CB/L	36,1 CB/L*
Medium	3,4 CB/L	43,5 CB/L*
Strong	3,9 CB/L	36,8 CB/L*

The exposure of *A. fischeri* to different types of medium-strengh CBs leachates (paper and paperless, continuous smoking, unsmoked CB) and at different leaching times resulted in differences in toxicity (Figure 39). Continuous smoking produced the greatest bioluminescence inhibition for *A. fischeri* (91%), followed by paperless CB (76.5%), then CB with paper (68%) and finally unsmoked CB (27%). Obtained results showed also that leachates toxicity increased rapidly and reached a stable level during the first 24h of leaching (Figure 39).

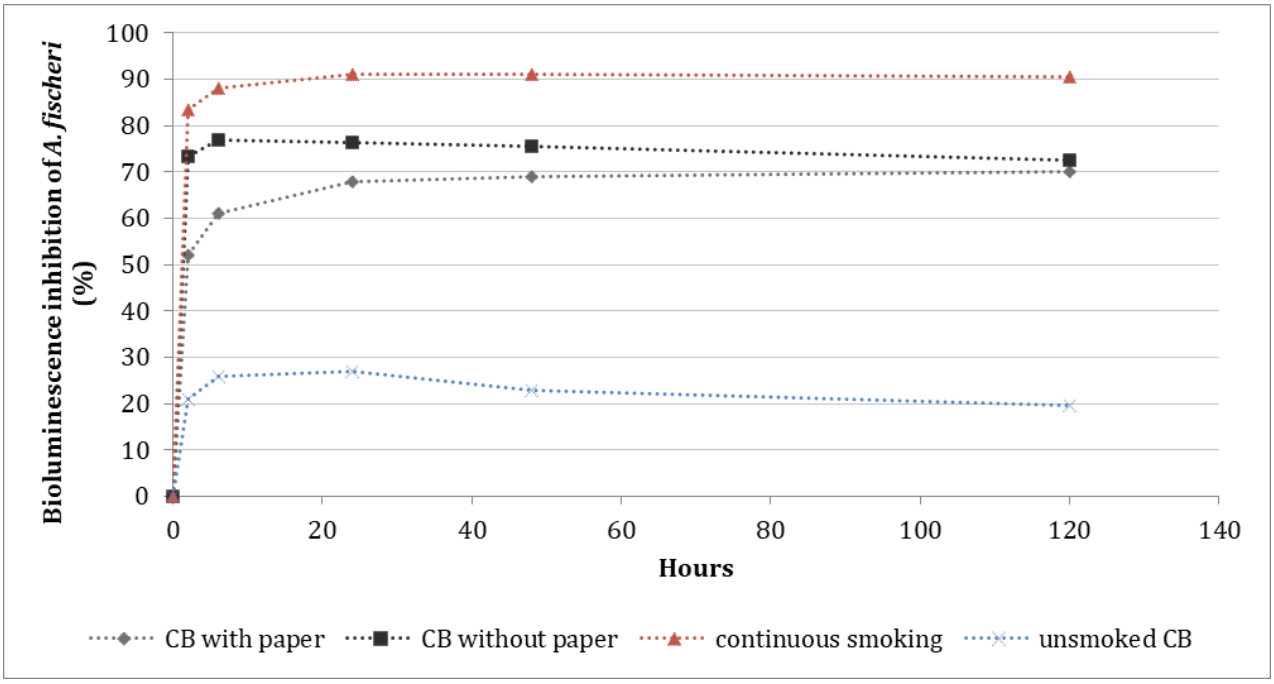


Figure 39: Mean bioluminescence inhibition (%) of *A. fischeri* by medium-strength CB leachate at a concentration of 10 CBs/L as a function of leaching time (hours) and type of CB used: with papers (n=1), without papers (n=2), continuous smoking (n=2), and unsmoked filters (n=2).

To assess toxicants leaching capacity over time, *A. fischeri* was exposed to leachates obtained by putting the same CBs in clean seawater after each toxicity measurement. Results showed that the leachate toxicity decreased over time with a maximum of toxicity after 24 hours of agitation indicating the leaching is maximum during the first 24h (Figure 40). After 72 hours of agitation, the toxicity reached a threshold around 20%. After 6 days of agitation, the toxicity was still around 20%.

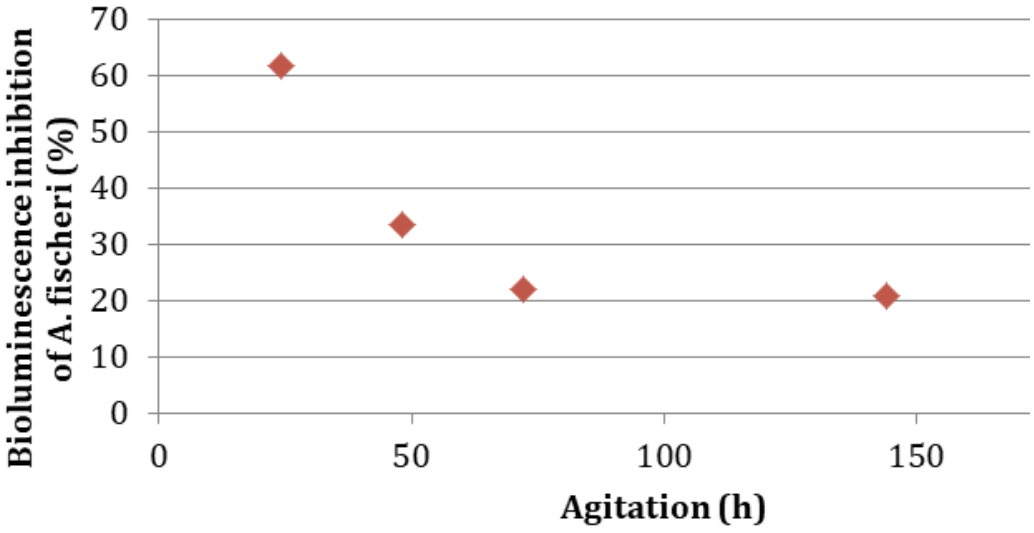


Figure 40: Evolution of the bioluminescence inhibition of *A. fischeri* exposed to medium-strength CBs leachates obtained by putting the same CBs in clean seawater after each toxicity measurement to assess the leaching capacity over time (n=2).

2.5.2.Microalgae

Exposure of *Phaeodactylum tricornutum* to the six selected concentrations of leachate from medium-strength CBs during 72 hours resulted in an estimated EC_{50} with a 95% confidence interval of 6.98 [6.63; 7.27] CBs/L (Figure 41).

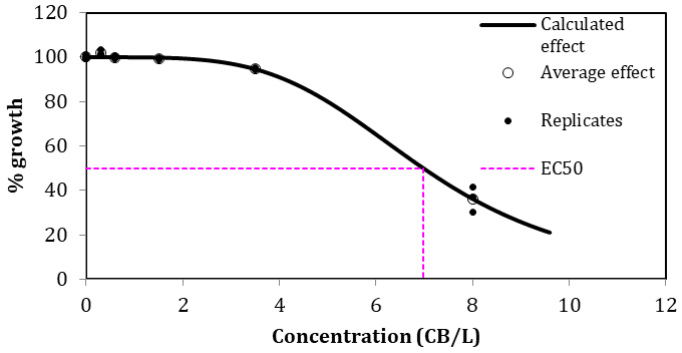


Figure 41: Percentage growth curve of *P. tricornutum* as a function of medium-strength CBs concentration in CBs/L.

2.5.3.Amphipod

Exposure of *Corophium arenarium* to five concentrations of medium-strengh CBs resulted in an estimated LC_{50} value with a 95% confidence interval of 123 [105; 144] mg CB/kg DS, corresponding to 1.04 [0.887; 1.22] CB/kg DW (Figure 42). At a concentration of 1.21 CB/kg DW, the mortality of *C. arenarium* for the three types of CB differed significantly (*p*-value < *0.05*) (Figure 42). Exposure to sediment contaminated with strong CBs powder resulted in 100% mortality, 58% for medium-strengh CBs and 27% for light CBs.

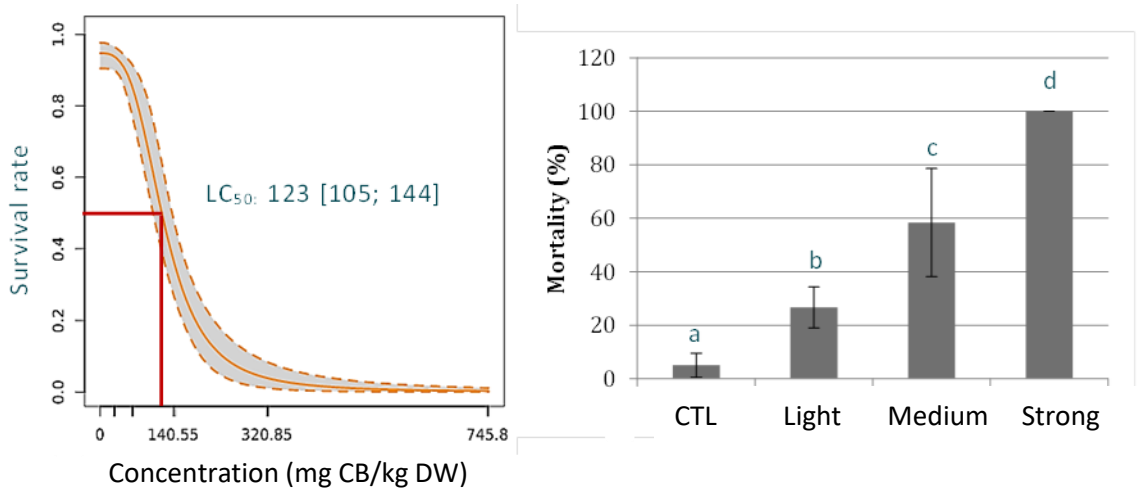


Figure 42: (a) Survival rate curve of *C. arenarium* as a function of medium-strength CBs concentration in mg CB/kg DW; (b) Mean mortality (%) of *C. arenarium* after exposure to uncontaminated sediment (CTL) and contaminated with the different types of CBs at a concentration of 1.21 CB/kg DW. Letters indicate a significant difference between conditions (n=3, means ± SD).

2.5.4.Bean clam

Monitoring of the burial behaviour of individuals during the exposure period showed that an increase in concentrations of sediment contaminated by medium-strength CBs resulted in a decrease in the percentage of individuals buried and an increase in the percentage of not buried relative to control (Figure 43). The EC_{50} calculated for the burial behaviour of the bean clam exposed to different concentrations of medium-strength

CBs resulted in an estimated value with a 95% confidence interval of 182 [69.3; 445] mg CB/kg DW, corresponding to 1.86 [0.966; 3.99] CB/kg DW.

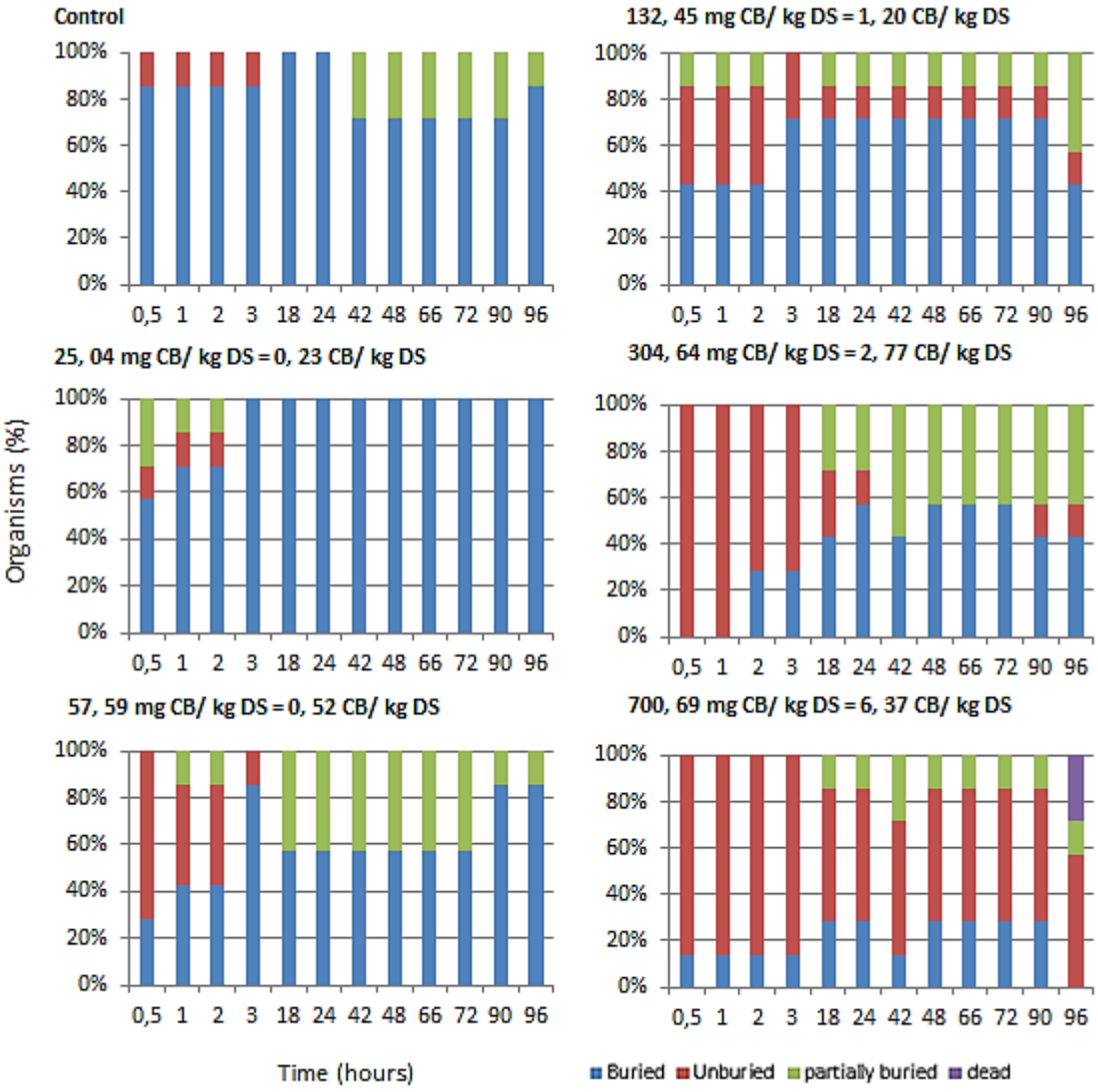


Figure 43: Percentage of buried, unburied, partially buried or dead individuals during an exposure to different concentrations of sediment contaminated by medium-strengh CBs (n=7). DS: Dry Sand = DW.

2.5.5.Shrimp

Exposure of *P. varians* to the five selected concentrations of leachate from medium-strengh CBs during 6 hours and 24 hours of decontamination resulted in an estimated LC_{20} with a 95% confidence interval of 7.83 [4.66; 10.6] CBs/L after 24 hours of decontamination (Figure 44). Extrapolation of values using the Mosaic® platform (https://mosaic.univ-lyon1.fr/), estimated an LC_{50} value of 11 [8.23; 21.9] CBs/L. In addition, paralysis with bleaching of some individuals was observed after 6 hours of contamination at the 8 CBs/L concentration. After transferring the individuals to the recovery tanks for 24 hours, the paralysis disappeared.

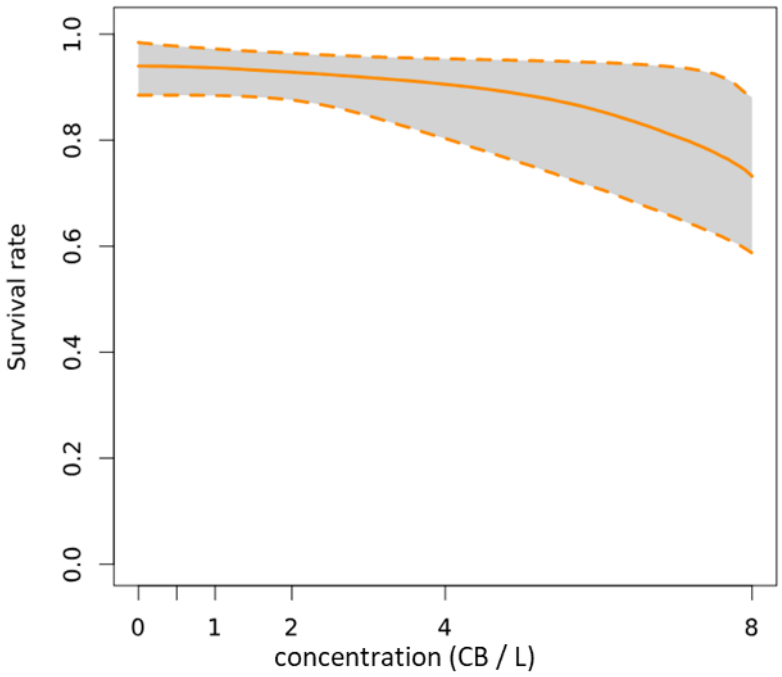


Figure 44: Survival rate curve of *P. varians* as a function of medium-strengh CBs concentration in CBs/L obtained with the Mosaic® platform.

2.5.6.Oyster

Experiments were conducted with a temperature of 11.2 ± 0.4°C, a salinity of 31.5 ± 0.5, a pH of 8.1 ± 0.1 and an oxygen level of 98.4 ± 1.4%. Filtration tests conducted after 24 hours of contamination by different concentrations of medium-strength CBs leachate showed no variation in the average filtration rate of the oysters between conditions (*p*-value > 0.05) (Figure 45). No paralysis was observed during the 24-hours exposure period to the various concentrations of leachate from medium-strength CBs.

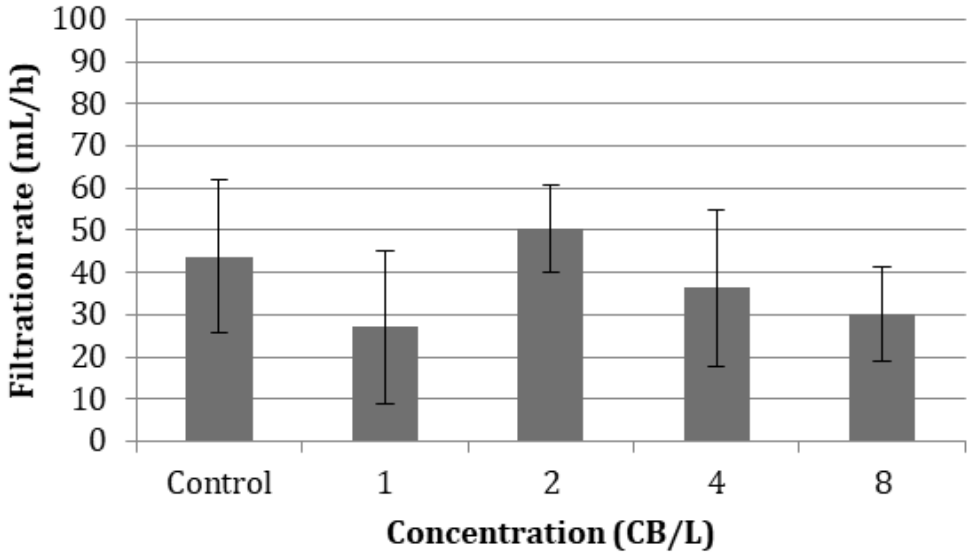


Figure 45: Oysters filtration rate (mL/h) after 24 hours of contamination at different concentration of medium-strength CB leachates (n=5, means ± SD).

Trace metal concentrations in oyster tissues (gills and digestive gland) before the exposure to the leachate from the medium-strength CBs, after 7 days of exposure and after 7 days of decontamination are shown in Table 11. The highest values among the elements were found for Zn (427–748 ng/mg) followed by Fe (52.4–103 ng/mg) and Pb (42.2–66.1 ng/mg). On the other side, the lowest values were found for Sb (0.005–0.017 ng/mg) and to a lesser extent for Co (0.035–0.098 ng/mg), Hg (0.023–0.061 ng/mg), Li (0.049–0.091 ng/mg) and U (0.052–0.126 ng/mg). If we differentiate by tissue, some elements showed higher concentrations in the gills (i.e. Hg, Li, Mn, Ni and Sr) while others in the digestive gland (i.e. As, Co, Cr, Fe, Mo, U and V). The third group of elements showed similar concentrations among tissues (i.e. Cd, Cu, Sb, Pb and Zn). Finally, the accumulation factor (AF) has also been calculated for the sum of both tissues after the 7 days period of exposure by dividing metal concentration after seven days by initial metal concentration. The AF value for most of the elements was between 0.83 and 1.21. However, AF for Mn and Sr were above these figures being 1.29 for the former and 1.35 for the latter (Table 11).

Table 11. Trace metal concentrations (ng/mg) for the different oyster tissues, treatments and time. Seven days accumulation factor (AF) for the whole tissue (gills + digestive gland) is also reported. (See also Santos-Echeandía et al., 2021).

Tissue	**Gills (ng/mg)**					**Digestive Gland (ng/mg)**					***AF***
Treatment	**Control**	**Control**	**Control**	**Exposed**	**Exposed**	**Control**	**Control**	**Control**	**Exposed**	**Exposed**	
Duration	**0 day**	**7 days**	**7 days**	**14 days**	**14 days**	**0 day**	**7 days**	**7 days**	**14 days**	**14 days**	
As	4.68	5.27	5.77	5.46	5.04	8.29	8.82	8.21	8.61	8.14	*1.08*
Cd	0.691	0.661	0.742	0.588	0.757	0.621	0.620	0.708	0.661	0.941	*0.95*
Co	0.037	0.035	0.036	0.036	0.037	0.079	0.077	0.078	0.079	0.098	*1.01*
Cr	0.162	0.163	0.158	0.163	0.185	0.23	0.236	0.237	0.284	0.367	*1.15*
Cu	16.0	16.4	15.4	17.5	24.1	14.0	14.7	10.9	18.7	20.0	*1.21*
Fe	57.0	56.6	59.9	52.4	63.5	84.0	86.8	84.5	83.2	103	*0.96*
Hg	0.062	0.046	0.061	0.044	0.048	0.053	0.029	0.026	0.023	0.031	*0.58*
Li	0.088	0.091	0.089	0.088	0.084	0.049	0.049	0.051	0.049	0.051	*0.94*
Mn	4.40	4.37	4.50	5.89	5.05	1.03	1.02	1.07	1.11	1.45	*1.29*
Mo	0.081	0.085	0.079	0.081	0.085	0.251	0.252	0.235	0.262	0.285	*1.04*
Ni	0.608	0.611	0.688	0.497	0.514	0.170	0.175	0.135	0.169	0.259	*0.83*
Pb	45.0	46.8	43.2	42.2	51.4	62.0	63.0	59.5	55.5	66.1	*0.91*
Sb	0.009	0.005	0.005	0.005	0.006	0.009	0.015	0.010	0.010	0.017	*1.04*
Sr	4.73	5.58	4.83	6.78	4.66	2.64	2.51	3.01	3.15	2.31	*1.35*
U	0.054	0.052	0.060	0.053	0.055	0.09	0.095	0.096	0.097	0.126	*1.00*
V	0.170	0.169	0.193	0.188	0.113	0.579	0.592	0.538	0.503	0.461	*0.92*
Zn	508	509	531	527	748	427	457	399	479	529	*1.08*

3. Experimental study on real cigarette butts

3.1. Cigarette Butt Toxicity

3.1.1.Arenicola marina

3.1.1.1. Arenicola marina Test 1 (stopped at day 3)

In the first *Arenicola* test, a concentration of 5 CBs/kg was tested. Over the first few days of the test, higher than acceptable mortality and very low cast numbers were observed in both the fresh and weathered CBs conditions. Due to the high mortalities, the test was terminated 3-days into the 10-day process whilst a dose response was still apparent.

Low mortality and good cast numbers in the control replicates demonstrated that the overall test conditions were good. The concentration of CBs required to reduce worm numbers by 50% was 0.345 fresh CB/kg and 1.0 weathered CB/kg. Casting was reduced to 50% at a concentration of less than 0.3 weathered or fresh CBs/kg.

A second test using lower amounts of CBs was then carried out.

3.1.1.2. Arenicola marina Test 2

The second test ran for a full 10 days as per the test protocol, using fresh CBs. Over 100 casts were observed in the control replicates over the 10 days. Similar numbers were also counted in the 0.046, 0.1, and 0.22 CB/kg treatments. For the 0.46 CB/kg treatment, vessel numbers were lower, at 75 casts and none were observed at 1.0 CB/kg, which was the highest concentration used in Test 2 (Figure 46). Mortality data followed a similar pattern with no discernible impact compared to the controls in concentrations up to 0.22, and then a partial response at 0.46 CB/kg and full 100% mortality at 1.0 CB/kg (Figure 46). The EC_{50} was 0.424 CB/kg (95% confidence interval: 0.340 – 0.528).

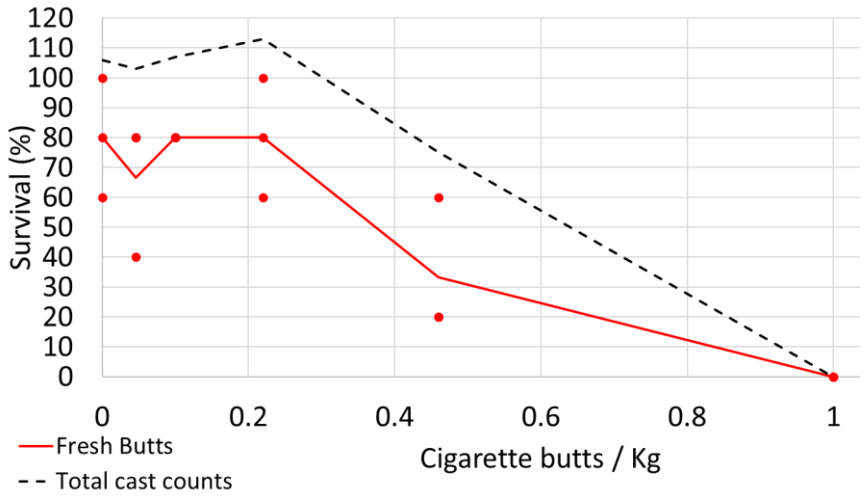


Figure 46: Exposure of *A. marina* to different concentrations of fresh CBs (from 0 to 1 CB/kg). Solid line represents % survival at the end of the test for each concentration. Dashed line represents the total number of casts. Solid circles are the individual replicate survival data for each concentration tested.

3.1.2. Microtox

The Microtox testing showed clear dose responses for the water overlying the test sediments dosed with fresh and weathered CB homogenates with an EC_{50} of 3.06 CBs/kg (Figure 47).

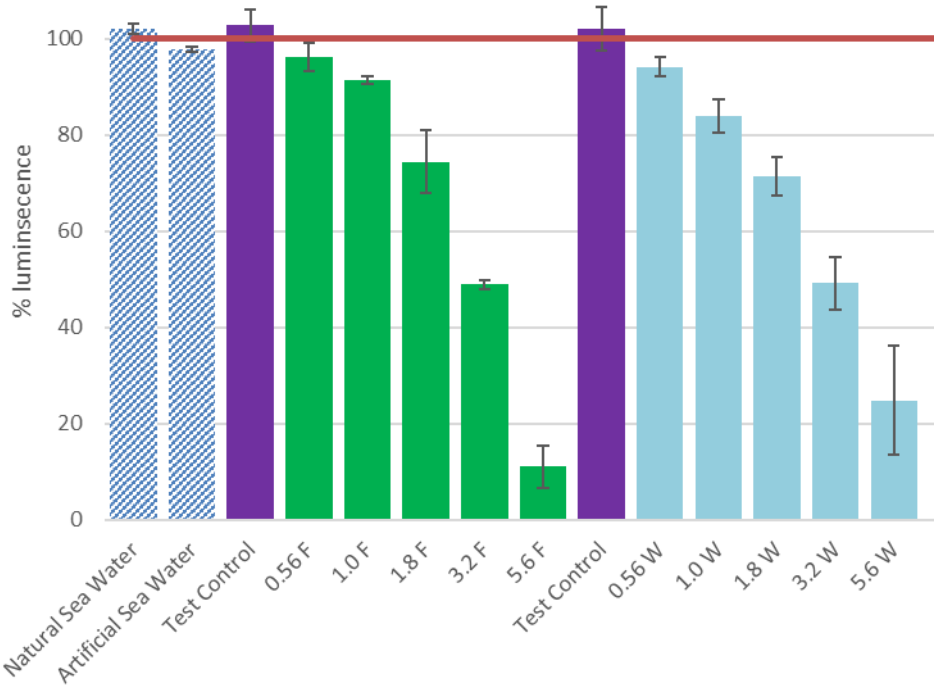


Figure 47: Microtox assessment of water overlying the *Arenicola* sediment test. F = Fresh CBs. W = Weathered CBs (n=3; means ± SD).

3.2. Cigarette Butt Chemical Analysis

3.2.1. Direct inlet probe- Time of Flight mass spectrometry (DP-TOF-MS) analysis (Vrije Universiteit, Amsterdam)

Filter, paper and tobacco materials from fresh and weathered CBs and their methanolic extracts were subjected to DP-TOF-MS for the targeted analysis of seventeen substances previously detected in smoked cigarettes and reported elsewhere as potentially harmful in aquatic organisms. Results are available in the Table 12. Nicotine was the only compound detected in all CB samples analysed, whether fresh or weathered, or whether directly in the materials or in the methanolic extracts. Ethylphenols were detected in fresh CBs (filter, paper, tobacco) and weathered CBs (paper and tobacco, but not in the filter element). For note, it is not possible to differentiate between 2-ethylphenol, 3-ethylphenol and 4-ethylphenol by DP-TOF-MS as their mass to charge ratio (*m/z*) is exactly the same.

3-vinylpyridine was the only other targeted suspect substances detected in the samples, but only in duplicate filter samples from fresh CBs and in the tobacco of weathered CBs. 3-vinylpyiridine was also detected in one replicate of fresh CB paper and one replicate of the filter of weathered CB. 3-vinylpyridine is formed from nicotine during the smoking of tobacco products.

DP-TOF-MS is a quick screening technique that allows the detection of compounds present at relatively high concentrations (in comparison to LC-HRMS) in samples with minimal analytical processing. Another 99 unknown *m/z* were detected (62 in positive mode and 37 in negative mode), 17 of which being detected in all samples analysed by DP-TOF-MS.

Table 12: Suspect screening by DP-TOF-MS of substances previously detected in smoked cigarettes in extracts of paper, filter and tobacco of fresh and weathered cigarette butts (M = Material; E = Methanolic Extract).

Suspect substances	Fresh cigarette butt						Weathered cigarette butt					
	Paper		Filter		Tobacco		Paper		Filter		Tobacco	
	M	E	M	E	M	E	M	E	M	E	M	E
Formaldehyde												
Acetaldehyde												
3-methylpyridine												
Phenol												
3-vinylpyridine	1/2		2/2						1/2		2/2	
3,4-dimethylpyridine 3,5-dimethylpyridine 4-ethylpyridine												
3-acetylpyridine												
2-Ethylphenol 3-Ethylphenol 4-Ethylphenol	2/2		2/2		2/2		2/2				2/2	
Nicotine	2/2		2/2		2/2		2/2		2/2		2/2	
N-nitrosonornicotine												
N'-nitrosoanatabine												
N'-nitrosoanabasine												
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone												

3.2.1. Liquid chromatography – high resolution mass spectrometric (LC-HRMS) analysis (Vrije Universiteit, Amsterdam)

The methanolic extract of both fresh and weathered CBs (prepared for DP-TOF-MS) and sediment samples from the *Arenicola* toxicity testing (control, 5.6 Weathered and 5.6 Fresh CBs/kg, all duplicates) were analysed qualitatively by LC-HRMS for a number of target compounds previously identified in smoked cigarettes, but also for non-targeted compounds.

Table 13 presents the results obtained for the targeted analysis of suspect compounds in the methanolic extracts of the fresh and weathered CBs. Although an indication of response intensity is presented (number of +s), the response will be dependent on the amount of each CB component extracted. Of the 17 suspect substances screened, three were detected in both fresh and weathered CBs. Both nicotine and 3-vinylpiridne were detected in all CB components analysed which is consistent with the results obtained by DP-TOF-MS.

3-methylpyridine was detected in all components of the weathered CBs, but only in the paper extract of fresh CBs. 3-methylpyridine is hazardous for aquatic organisms, the PNEC in the marine environment being 30 µg/L in marine water and 450 µg/kg dw sediment[1].

In contrast to the DP-TOF-MS results, no ethylphenols were detected by LC-HRMS, which is likely to be due to the additional analytical steps (extract clean up) required for LC-HRMS analysis or the LC separation column technology used (i.e. ethylphenols might not be retained or might not elute from the LC column with the mobile phase used).

[1] https://echa.europa.eu/brief-profile/-/briefprofile/100.003.307 accessed 14/12/2020

Table 13: Suspect screening by LC-HRM of substances previously detected in smoked cigarettes in methanolic extracts of paper, filter and tobacco of fresh and weathered CBs (purple highlight indicates substance detection; number of + indicate intensity of response on a peak area basis)

Suspect substances	Fresh cigarette butt			Weathered cigarette butt		
	Paper	Filter	Tobacco	Paper	Filter	Tobacco
Formaldehyde						
Acetaldehyde						
3-methylpyridine	+			++	+	+
Phenol						
3-vinylpyridine	++	++	+	+++	++	++
3,4-dimethylpyridine						
3,5-dimethylpyridine						
4-ethylpyridine						
3-acetylpyridine						
2-Ethylphenol						
3-Ethylphenol						
4-Ethylphenol						
Nicotine	+++++	++++	++++	+++++	+++++	+++++
N-nitrosonornicotine						
N'-nitrosoanatabine						
N'-nitrosoanabasine						
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone						

Table 14 presents the results of the targeted screening of suspect compounds in sediment samples analysed. Nicotine was the only compound detected in fresh and weathered sediments, including a small positive response in both duplicates from the control sediment. The nicotine concentration in sediments was determined semi-quantitatively using standard solutions of nicotine analysed alongside the samples. Nicotine concentration in sediments spiked with 5.6 fresh CBs/kg was *ca* 8.5 µg/kg sediment (on a wet weight basis) and 6.9 µg/kg for sediment with 5.6 weathered CBs/kg (wet weight). Working from this data, the lowest concentration of CBs in this test was 0.046 CBs/kg, which would represent about 0.069 µg/kg of Nicotine which aligns with the PNEC value for nicotine in marine sediment: 0.065 µg/kg sediment (on a dry weight basis). The indicative concentrations calculated in the CB spiked sediments represent scenarios from the PNEC and upwards.

Investigation into the non-target responses from LC-HRMS from fresh and weather CBs suggests the presence of several tobacco-based compounds in the filter, paper and tobacco components. These tentatively identified compounds include cotinine, nornicotine, N-Formyl-anatabine, N-Acetyl-nornicotine, and 4-(Acetylmethylamino)-1-(3-pyridyl)-1-butanone.

Nornicotine and cotinine are minor tobacco alkaloids which are structurally similar to nicotine (which accounts for 96–98% of the total alkaloid) (**Huang and Hsieh, 2007**). N-Formyl anatabine and N-Acetyl-nornicotine have been reported as alkaloid derivatives in burley tobacco (**Andersen et al, 1989**). (Acetylmethylamino)-1-(3-pyridyl)-1-butanone is used to add aroma to smoking tobacco. 4-(Acetylmethylamino)-1-(3-pyridyl)-1-butanone stands out as an outlier from the other detected chemicals as it was only found in all components of the weathered CB test but was not found in any of the fresh CBs (Table 15).

Table 14: LC-HRMS suspect screening in sediments of substances previously detected in cigarette smoke (red highlight indicates substance detection; number of + indicate intensity of response on a peak area basis)

Suspect substances	Sediment			Laboratory Blank
	Control 0	Fresh 5.6 CBs/kg	Weathered 5.6 CBs/kg	
Formaldehyde				
Acetaldehyde				
3-methylpyridine				
Phenol				
3-vinylpyridine				
3,4-dimethylpyridine				
3,5-dimethylpyridine				
4-ethylpyridine				
3-acetylpyridine				
2-Ethylphenol				
3-Ethylphenol				
4-Ethylphenol				
Nicotine	+	+++	++	
N-nitrosonornicotine				
N'-nitrosoanatabine				
N'-nitrosoanabasine				
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone				

Although these substances are linked to cigarette smoking, it is important to highlight that the identification of these has been done using accurate mass library searches, which also identify alternative substances (also provided in Table 15). Further substance confirmation techniques (e.g. using analytical standards) should be applied to increase confidence in the identity of these substances.

Table 15: Non-targeted LC-HRM analysis of extracts of paper, filter and tobacco of fresh and weathered cigarette butts (P = paper; F = filter; T =tobacco; red highlight indicates possible substance detection; number of + indicate intensity of response on a peak area basis).

Possible substance	Fresh cigarette butt			Weathered cigarette butt			Alternative substance
	P	F	T	P	F	T	
Cotinine	+++	+++	+++	++++	+++	+++	
nornicotine	++	++	++	++++	++	+++	
N-Formyl-anatabine	++	++	++	+++	++	++	Indolepropionamide Phenazone Vasicine
N-Acetyl-nornicotine	++	++	++	+++	++	++	Cytisine 4,4'-Dimethylaminorex 4-HO-AMT Indantadol 5-Methoxytryptamine 2-Methyl-5-hydroxytryptamine α-Methylserotonin N-Methylserotonin
4-(Acetylmethylamino)-1-(3-pyridyl)-1-butanone				+++	++	++	Pheneturide Phenylethylmalonamide

These five tobacco specific substances were also detected in sediment samples spiked with CBs (5.6 CBs/kg) from the Arenicola toxicity testing but not in the associated sediment controls (Table 16). These substances might have leached/desorbed from the CBs into the sediment/water phase, and/or component parts of the CBs might have been co-extracted with the sediments during analysis. Tryptophan was tentatively identified in the sediment samples exposed to CBs, but not in the control sediment or the CB components (Table 16).

Table 16: Non-targeted LC-HRM analysis of extracts of sediment samples (red highlight indicates possible substance detection; number of + indicate intensity of response on a peak)

Possible substances	Sediment			Laboratory Blank	Alternative Substances
	Control 0	Fresh 5.6	Weathered 5.6		
Cotinine		++++	+++		
nornicotine		+++	++		
N-Formyl-anatabine		+++	++		Indolepropionamide Phenazone Vasicine
N-Acetyl-nornicotine		+++	++		Cytisine 4,4'-Dimethylaminorex 4-HO-AMT Indantadol 5-Methoxytryptamine 2-Methyl-5-hydroxytryptamine α-Methylserotonin N-Methylserotonin
4-(Acetylmethylamino)-1-(3-pyridyl)-1-butanone		++	++		Pheneturide Phenylethylmalonamide
Tryptophan		++++	++		Ethotoin Fenozolone Idazoxan Nirvanol ORG-26576 3-Phenylazoacetylacetone Thozalinone

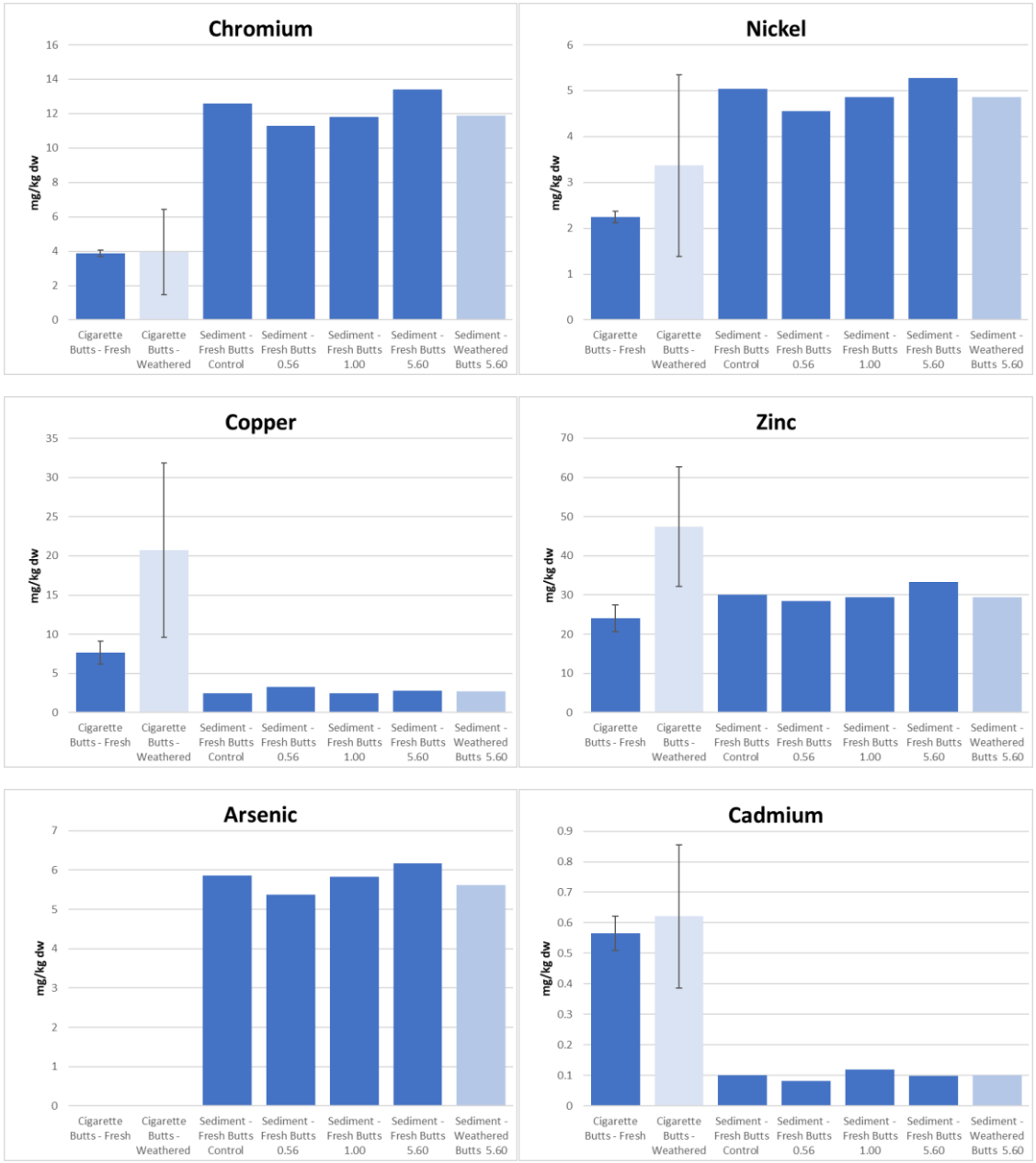
3.2.2. Trace metals in Sediment and cigarette butts

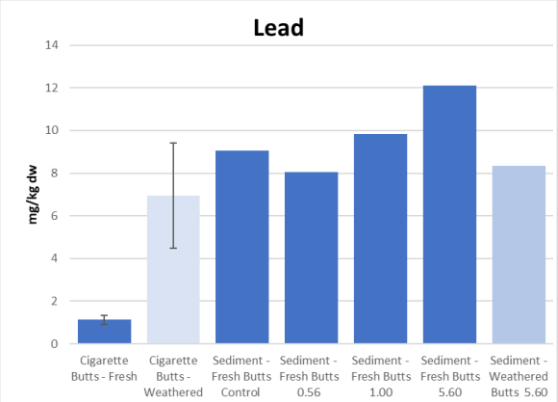
Levels of trace metals were determined in fresh and weathered CBs (in duplicate) and in one replicate of the *Arenicola* test sediments spiked with 0, 0.56, 1.00 and 5.60 fresh CBs/kg and 5.60 weathered CB/kg in order to evaluate potential trace metals contributions from CBs. Both weathered and fresh CBs were homogenised before analysis, but were difficult to break down due to the nature of the materials (such as the fibrous filter material), and fully homogeneous samples could not be obtained.

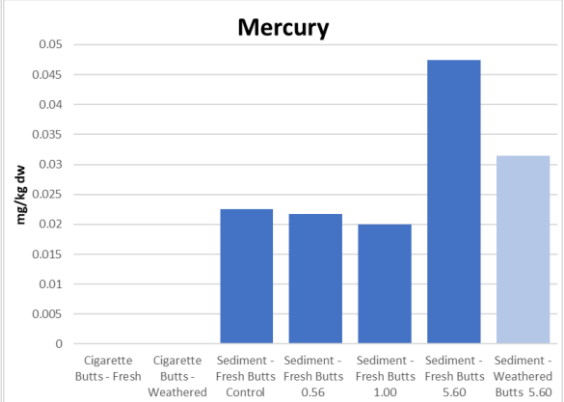
Trace metals results indicate that both fresh and weathered CBs analysed have greater cadmium and copper concentrations than any sediment samples tested (Figure 48). In leaching studies of metals from CBs artificially smoked, **Santos-Echeandia et al (2021)** reported that both copper and cadmium had high percentages of desorption from smoked CBs into seawater. Fresh CBs seem to also contain greater levels of rubidium to that of the sediment samples tested but this was not observed for Weathered cigarette butts. Arsenic and mercury were not detected above limits of detection (LOD) in either fresh (LOD 0.37 mg/kg and 0.034 mg/kg respectively) or weathered (LOD 0.95 mg/kg and 0.085 mg/kg respectively) CBs. This

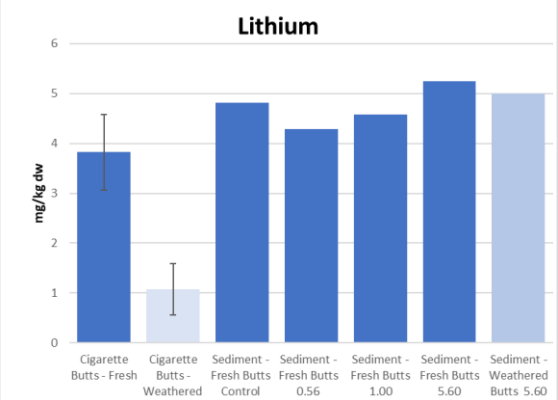
observation is consistent with **Santos-Echeandia et al (2021)** who indicate that these elements are likely volatilised during smoking.

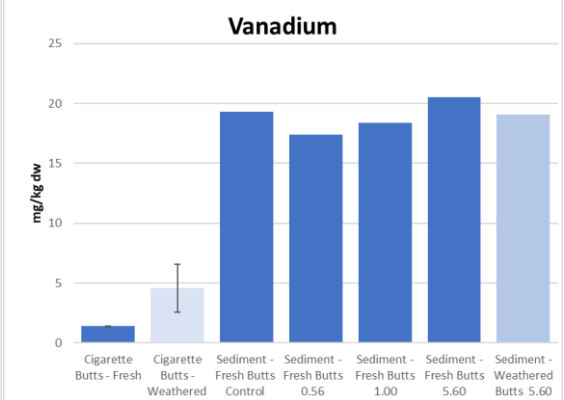
Significant variation in metals concentration were observed in the weathered CBs analysed in duplicate, which is likely to be linked to the difficulties encountered with CB sample homogenisation. The amounts of sediment sample available to complete both trace metals and organics analyses were limited which meant that replicate analyses in sediments were not possible and results obtained here are indicative of CBs being potentially a point source for some trace metals.

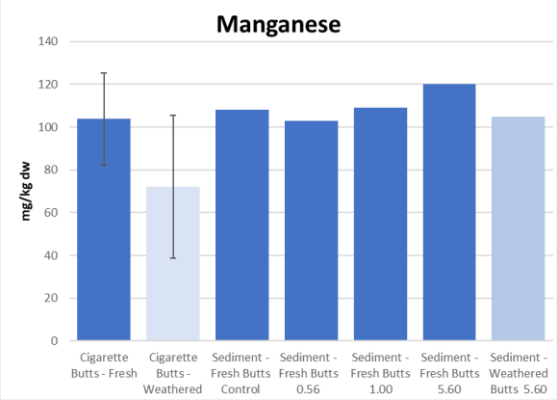


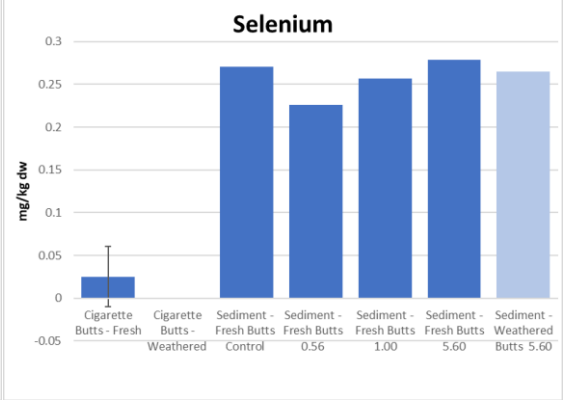

Lead
mg/kg dw
0
2
4
6
8
10
12
14
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60

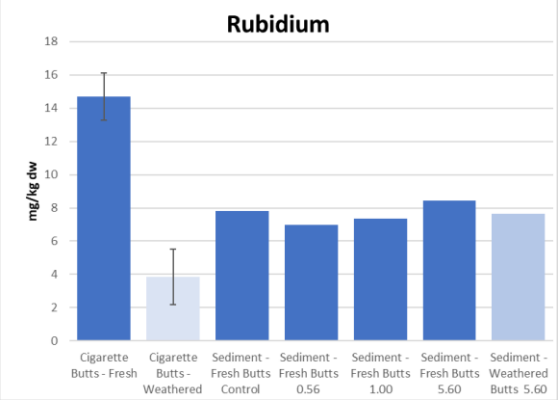

Mercury
mg/kg dw
0
0.005
0.01
0.015
0.02
0.025
0.03
0.035
0.04
0.045
0.05
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60

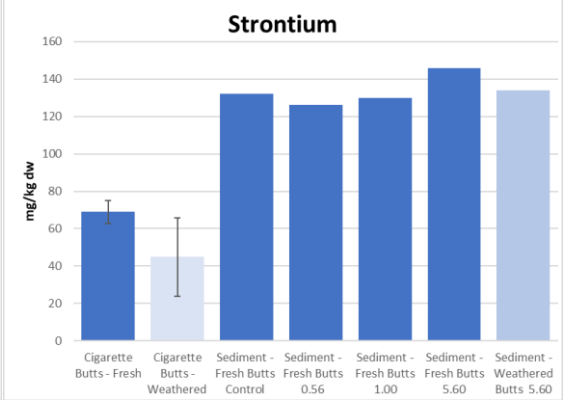

Lithium
mg/kg dw
0
1
2
3
4
5
6
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60


Vanadium
mg/kg dw
0
5
10
15
20
25
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60


Manganese
mg/kg dw
0
20
40
60
80
100
120
140
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60


Selenium
mg/kg dw
-0.05
0
0.05
0.1
0.15
0.2
0.25
0.3
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60


Rubidium
mg/kg dw
0
2
4
6
8
10
12
14
16
18
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60


Strontium
mg/kg dw
0
20
40
60
80
100
120
140
160
Cigarette Butts - Fresh
Cigarette Butts - Weathered
Sediment - Fresh Butts Control
Sediment - Fresh Butts 0.56
Sediment - Fresh Butts 1.00
Sediment - Fresh Butts 5.60
Sediment - Weathered Butts 5.60

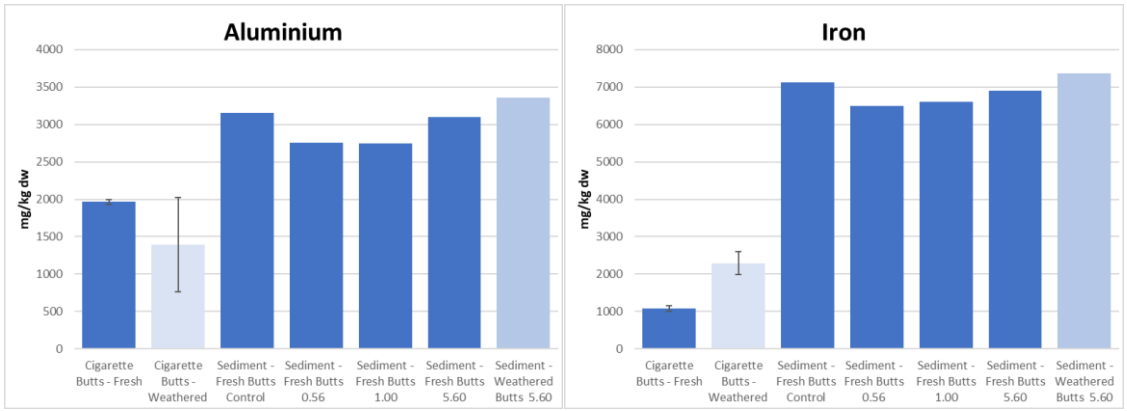


Figure 48: Trace metals concentrations (in mg/kg dw) in CBs (n=2) and sediment samples (n=1) spiked with CBs.

4. Review of existing initiatives, mesures and actions to reduce CBs pollution

Different types of initiatives, measures and actions (IMAs) targeting CB pollution were identified based on WP4.2 word **(Cedre, 2021a and 2023b)** and additional researchs. These IMAs can be classified under different categories that are presented below with some examples:

- **Policy:**

This category includes policy measures aiming at reducing CBs pollution. For exemple, on 21 May 2019, the European Union adopted several measures to reduce the impact of certain plastic products on the environment, including CBs. Measures targeting CBs are: marking requirements, extended producer responsibility and awareness raising **(**Directive EU 2019/904**)**. This category also includes local smoking bans in public places such as parks or beaches (example on Sables d'Olonnes in France: https://www.francebleu.fr/infos/sante-sciences/aux-sables-d-olonne-une-nouvelle-plage-sans-tabac-1659514796) or the introduction of fines for CBs thrown in the streets (e.g. in Paris, France).

- **Circular economy:**

This category includes several IMAs aiming at collecting and recycling cigarette butts to make new raw material and products **(e.g:** https://me-go.fr/, https://www.tree6clope.com/qui-sommes-nous-tree6clope/, https://imeko.cl, https://www.cigabrick.com/index.html, https://ecomegot.com/).

- **Clean-up/recovery:**

Numerous citizen clean-ups are organized to collect CBs on beaches or in urban areas. Some incentives can also be proposed to encourage people to collect CBs (e.g. gift vouchers or beer offered against a glass of CBs collected).

- **Awareness-raising:**

There are numerous awareness raising IMAs targeting CBs. They include the distribution of pocket ashtrays (such as the ones developed by CleanAtlantic partner DRAAC in WP8). They also include awareness campaign launched by municipalities, other authorities or organisations (e.g. https://www.landerneau.bzh/listes/landerneau-ville-zero-megot/, https://zerodechetlyon.org/le-megot-un-petit-dechet-mais-un-gros-probleme/) with in some cases, the use of nudges to influence smokers' behaviour (ex: installation of voting ashtrays or plates "here begins the sea" near rainwater drains. They include also the development of awareness raising games as the ones developed in CleanAtlantic WP8: the "Cluedo Butt" and the "Litter Eater" **(Cedre, 2021b and 2023c).**

- **Other:**

Among other IMAs, the development of biodegradable filters to replace conventional filter was identified to reduce the persistence of CB pollution and the release of microplastics (e.g: https://www.greenbutts.com/; https://www.koerber-technologies.com/en/news-stories/future-trend-biodegradable-filters, https://www.cerdia.com/en/company.html). Another initiative identified proposed to provide expert assistance to governments, communities, organizations and individuals seeking to reduce the environmental burden of cigarette butt waste (https://www.cigwaste.org/).

Discussion

1. Cigarette butts, an abundant litter items in the Atlantic Area

The data analyses achieved in the current report based on the OSPAR beach litter monitoring data showed that CBs are the 5th most commonly collected item on AA beaches from 2016 to 2019. Over the period, more than 25 000 CBs were collected by the monitoring program. These results demonstrate that CBs are abundant in the litter of the AA coastline. These results are in line with other regions of the world. Every year, it is estimated that 6 trillion cigarettes are smoked and that 4.5 trillion of them end up in the environment (**Novotny and Slaughter, 2014**) and in particular in the marine environment. According to the Report on Marine Litter Assessment in the Mediterranean (UNEP/MAP MEDPOL, 2015), the main groups of items found on beaches in the Mediterranean are sanitary items (mostly cotton bud sticks) and CBs, as well as packaging items and bottles (**JRC, 2015**). According to the Surfrider Fondation (https://www.surfrider.org/programs/beach-cleanups), CBs are the most frequently collected item during beach clean-ups promoted by the group. In 2018, about 2.4 million cigarette butts were collected from beaches during international shoreline clean-up campaigns, making this waste one of the top ten most collected wastes on beaches[2].

CBs found in environment can have different origins. These are either dumped directly on beaches or by terrestrial additions via runoff and rivers or by deliberate or accidental discharges at sea. Smokers are frequently reported as discarding their cigarette butts improperly (**Novotny and Slaughter, 2014**). According to a report by Arcadis, recreational and tourism activities are responsible for the majority of marine litter (**Arcadis report, 2012**). In the same way, on Californian beaches, cigarette butts have been found in large quantities, mainly related to tourism activities (**Santos *et al.,* 2005**). People are unaware of the pollution caused by littering cigarette butts in the environment and see this action as a natural extension of smoking. In addition, since the early 1980s there has been increasing concern about the health consequences of passive smoking and thus anti-smoking laws have been implemented, banning smoking in enclosed areas of places of work and public places. These anti-smoking laws are designed to protect people from exposure to tobacco smoke and reduce the health problems associated with it. However, these laws had a negative incidence to the environment, increasing the littered cigarette butts to the urban environment (**Novotny *et al.,* 2009; Roder Green *et al.,* 2014**).

2. Cigarette butts can be artificially produced in the lab

The artificial cigarette smoking method developed in this study allowed to obtain, in a simple and rapid manner (preparation of 6 CB in less than 7 minutes), representative CBs comparable to those used in the literature. However, the device used does not entirely comply with the criteria of the ISO 3308: 2012 standard, as it was not possible to respect both the number of puffs and the pause time between puffs because it burned the filters. To effectively fulfill these criteria, the decision has been made to decrease the interval between puffs from 1 minute to 45 seconds, ensuring the recommended number of puffs is maintained (i.e. 8 puffs). An increase in the number of puffs increased the smoking intensity as well as the

[2] https://oceanconservancy.org/blog/2018/08/31/tobacco-butts-pack-poisonous-punch-people-ocean/

toxicity of the butts (**Adam et al., 2010**). Furthermore, reducing the number of puffs would lead to a decrease in smoking intensity, potentially causing an underestimation of the toxicity of the studied cigarette butts.

3. Cigarette butts have a complex behaviour

In aquatic environments, the initial positioning of plastic debris is determined by the density of the polymer. Cigarette butts (CBs) primarily consist of cellulose acetate, a polymer denser than freshwater or seawater, with a density ranging from 1.27 to 1.34 g cm^{-3}. This characteristic implies that CBs have the potential to contaminate and accumulate in benthic environments (**Kane & Clare, 2019**). However, the findings of the current study demonstrate that CBs do not sink immediately but instead float on the water surface. This outcome can be attributed to the presence of air in cigarette filters. As time progresses, CBs gradually become saturated with water. This water uptake can lead to a change in behavior, leading to a sinking of CBs, as observed in the seawater column assay conducted during this study. The speed at which CBs become water-saturated depends on environmental conditions such as swell, wind, and current. In calm conditions, the saturation process is notably slow, with CBs remaining at the surface of seawater even after 24 hours. However, when agitation was introduced in Cedre's wave tank, the floating equilibrium of CBs was disrupted, resulting in their presence either in the subsurface or at the bottom of the tank. This ability to persist on the water's surface for extended periods enables CBs to be dispersed over long distances through rivers and currents (**Dobaradaran et al., 2021**).

The results of the present study highlight the complex behavior of CBs in aquatic environments, which is influenced by environmental conditions rather than solely the nature of the polymer. CBs possess the capacity to contaminate the entire water column, spanning from the water surface to benthic environments, thereby posing a potential risk of interaction with a large range of aquatic organisms and the potential release of their constituents throughout these habitats. Furthermore, the behavior of CBs in aquatic environments evolves as they become colonized by aquatic organisms such as bacteria and microalgae (**Nikolopoulou et al., 2023**). This colonization process can impact the interactions between CBs and aquatic fauna, as well as affect their positioning within the water column. The presence of biofilm on CBs can release substances that facilitate chemical interactions with animals and increase the density of the colonized items. Consequently, this can cause CBs to descend within the water column and ultimately settle on the sediment (**Savoca et al., 2016; Galloway et al., 2017**).

4. Cigarette butts degrade in the marine environment

CBs pose an environmental concern resulting from their relative persistence in the environment. The filters of cigarettes are composed of cellulose acetate, a type of plastic derived from the acetylation of cellulose (**Puls et al., 2011**). These filters consist of a complex and densely intertwined network of cellulose fibers, combined with plasticizers, which contributes to their slow and challenging degradation process (**Puls et al., 2011**).

The current study on the ageing of CBs in the marine environment has revealed distinct patterns of degradation depending on the conditions, such as the air, seawater, and an artificial seawater basin. During the 465-day deployment on Cedre's beach, the dry mass of the three tested CBs remained unchanged. However, **Bonanomi et al. (2015)** conducted a two-year investigation into CB degradation under laboratory and field conditions, including sand dunes and grasslands. They observed a mass loss ranging from 30% to 35% across all conditions by the end of the experiment. The disparity between the two studies could be

attributed to weather differences between Brest (current study) and Portici (Italy), particularly the discrepancy in sunshine duration, which was 1.4 times higher in Bonanomi's study compared to the present study. It is worth noting that while CBs are generally known for their slow degradation, they do exhibit photodegradability. Some additives, such as titanium dioxide, are incorporated into cigarette filters to modify their properties. These additives, used as whitening agents, can enhance the photo-degradability of cellulose acetate (**Puls et al., 2011**).

In terms of degradation in the marine environment, the findings revealed a significantly higher degradation of cigarette butts (CBs) in the Brest Marina compared to Cedre's basin. A mass loss exceeding 90% was observed for all three types of CBs in the Brest Marina, whereas no mass loss was recorded in Cedre's basin. This discrepancy can be attributed to the contrasting environmental conditions between the two locations. Cedre's basin represents a notably sheltered area with treated waters to prevent algae development, whereas the Brest Marina has natural and biologically rich waters and it experiences greater agitation, including factors like currents, which could rapidly deteriorate the paper surrounding the CBs. The higher biological richness and agitation could lead to a more rapid degradation or disintegration of the filters, thereby leading to the observed mass loss.

It is important to acknowledge that degradation can occur through various processes (such as biodegradation, hydrolysis, or the production of micro- or nanoplastics), which were not specifically investigated in this study. Although the biodegradation of CBs was not included in this project, it is a crucial parameter that should be addressed in future studies. Biodegradation has the potential to offer an industrial-level solution for the disposal of CBs. Currently, there is an ongoing debate regarding the concept of biodegradability when it comes to CBs. Biodegradability can be defined as the "*microbial-initiated conversion of a substrate in a biologically active environment into carbon dioxide (aerobically), methane (anaerobically), cell wall material, and other biological products*" (**Puls et al., 2011**). Although cellulose acetate, the main component of CB filters, is known for its slow degradation rate, it is technically considered to be biodegradable by the scientific community, despite its sluggish breakdown.

5. Cigarette butts can create harmful environments for marine organisms

Owing to the high persistence and accumulation of CBs in aquatic environments, it is necessary to assess their potential effects on aquatic life at different level of organization (*e.g.* molecular, cellular, individual, populations) with several trophic levels from benthic and pelagic environments as CBs can float or sink and release their chemicals in all compartments.

The bioluminescence inhibition test conducted on *A. fischeri* using leachates from the three types of unsmoked CBs revealed an inhibition rate ranging from 13% to 20% across all tested CBs. However, the toxicity of smoked CBs was found to be approximately 3 to 5 times higher compared to unsmoked CBs across all three types. These findings suggest that while many of the chemical compounds responsible for toxicity are typically formed during the combustion process (**Baker, 2006**), potentially toxic substances may already be present in the filters of cigarettes even before smoking. This toxicity could be associated with the presence of additives, adhesives, or other compounds introduced during the manufacturing of the filters.

Toxicity of CBs depends on the type of cigarettes. Indeed, bioluminescence inhibition tests of *A. fischeri* at a concentration of 8 CBs/L for all three types of smoked CBs showed values of 48% for light CBs leachate, 60% for medium-strength CBs leachate and 72% for strong CBs leachate. It appears that the light CBs are less toxic than the medium-strength and strong CBs. The same observation was made on CBs aged on Cedre's artificial

beach with a significantly higher toxicity of strong CBs than light CBs after 21 days of ageing for a concentration of 8 CBs/L (28% and 15% bioluminescence inhibition respectively). These results were also observed on *C. arenarium* with a lower percentage of mortality in contact with sediment contaminated by light CBs (27%), than with contamination by medium-strength (58%) or strong CBs (100%) for a concentration of 1.21 CB/kg. This can be explained by the differences in composition between the three types of CB, with strong CBs having the highest levels of tar, nicotine and carbon monoxide emissions. **Micevska et al. (2006)** also studied the toxicity of different types of CBs and found a difference in toxicity of 2.9 to 8 times between the CB containing the least tar and those containing the most for *C. dubia* and *A. fischeri* respectively. The percentage of ventilation of the three types of CBs could also partly explain the results obtained. Indeed, the light filters have a higher percentage of ventilation (80%) compared to the medium-strength (34%) and strong filters (22%) filters. Filter ventilation is represented by the presence or absence of ventilation holes of varying sizes on the surface of the papers surrounding the filter, allowing the volume of smoke passing through the filter during aspiration to be reduced and diluted (**Adam et al., 2010**). In addition, light CBs were observed to have less intense filter colouring than medium-strengh and strong CBs after smoking, suggesting lower contamination of the light CBs. Indeed, results showed that there is a correlation between the colour intensity of filters and the estimated amount of tar (**Pauly et al., 2009**). Smoking intensity should also be taken into account when assessing the toxicity of CBs. Indeed, it was shown in this work that continuous smoking produced toxicity approximately 1.5 times higher than classical smoking for the three types of CBs studied. Bioluminescence inhibition of *A. fischeri* at a concentration of 8 CBs/L showed values about 70% for clogged light and medium-strength CBs confirming the role of smoke dilution through the ventilation holes and show that with clogged ventilation holes, less toxic butts such as the light CBs, become more toxic. Real CBs could therefore be more toxic if smokers have a habit of clogging the ventilation holes.

Experiments with the marine benthic polychaete Arenicola marina showed the most sensitivity in these studies with an EC_{50} of 0.424 CB/kg, and perhaps more importantly showed that the overlying water, which had not directly received any CB material, also became toxic during the test with an EC_{50} to *A. fischeri* of around 3.06 CB/kg.

Bioluminescence inhibition measurements using *A. fischeri* result in EC_{50} values ranging from 614 to 1250 mg CB/L whether with artificial, fresh or weathered CBs (3.4-5.6 CB/L). These values are in the same range as those obtained in a study by **Rebischung et al. (2018)**, which also employed *A. fischeri* but utilized CBs recovered from ashtrays along with residual tobacco. In their study, the authors reported EC_{50} values ranging from 870 to 3730 mg CB/L. Another study by **Micevska et al. (2006)** using artificially smoked CBs also demonstrated similar toxicity levels with EC_{50} values for *A. fischeri* ranging from 104 to 832 mg CB/L. These results validate that CBs generated artificially in the laboratory are comparable to those obtained through other smoking methods or from actual smokers. The slight differences in toxicity observed among the studies may be attributed to variations in sample preparation. For instance, **Micevska et al. (2006)** noted the retention of 1 cm of residual tobacco for each CB, which has been shown in several studies to increase CB toxicity (**Slaughter et al., 2011; Register, 2000**). The relatively lower toxicity values observed in the **Rebischung et al.** study could be attributed to the use of wet CBs, which may have allowed for the potential release of certain compounds through leaching. Additionally, variations in the Microtox© test protocol, particularly the incubation time of the bacteria with the samples, may also contribute to the observed variations and differences between studies. Overall, the results of toxicity assays performed on bacteria, microalgae, amphipods, clams, worms and shrimps displayed EC_{50}/LC_{50} between 0.42 and 11 CBs/L or CBs/kg

which are in line with the results available in the literature regarding the toxicity of CBs on aquatic organisms (see review by **Dobaradaran et al., 2021**). For instance, EC_{50}/LC_{50} values ranged from 1.8 to 4.3 CBs/L in marine or freshwater fish species **(Slaughter *et al.*, 2011)** and from 0.05 to 2 CBs/L for a small freshwater crustacean (*Daphnia magna*) **(Register, 2000)**. CBs have also been shown to cause effects on the development of embryos (**Lee & Lee, 2015; Parker & Rayburn, 2017**) and behavioural changes in gastropods (**Booth et al., 2015**). For instance, a 96h-LC_{50} of 1.2 CBs/L and a 96h-EC_{50} (malformation) of 0.90 CBs/L were calculated for frog (*Xenopus leavis*) embryos exposed to CBs leachates (**Parker & Rayburn, 2017**). By contrast, no acute toxicity was observed on oysters' behavior in the present study. Effects on oyster filtration rate could be appared for higher exposure duration than the one used in the present study (1 day). Indeed, a 3-fold decrease in the filtration mussel (*Mytilis edulis*) was observed after 5-days exposure to 1 CB/L (**Green et al., 2021**).

In this study, the analyses focused solely on the leachates, representing one source of impacts associated with cigarette butts (CBs) and other plastic debris. However, another potential source of impact is linked to the accidental ingestion of plastic items, as reviewed by **Paul-Pont et al. (2018)**. For instance, **Tourinho et al. (2010)** identified various debris, including cigarette butts, in the stomachs of green turtles along the southern Brazilian coast, with a frequency of occurrence of 6%. In future studies, it would be valuable to investigate the impacts of CBs ingestion, particularly focusing on the potential release of chemicals, micro- and nanoplastics during the weathering process of CBs in aquatic environments.

6. Cigarette butts have a complex chemicals composition, responsible for their toxicity

The environmental risk associated with CBs primarily stems from their chemical composition. These chemicals can originate from the compounds initially present in the filter, the tobacco, or those formed during the combustion of cigarettes. A study by **Marcilla et al. (2012)** demonstrated that numerous chemical compounds present in cigarette smoke can pass through filters during the smoking process and become trapped there. Additionally, it has been established that cigarette smoke contains over 5,000 chemical compounds, with 50 known to be carcinogenic to humans (**Slaughter et al., 2011; Hoffmann et al., 1997**). Among these compounds are carbon monoxide, polycyclic aromatic hydrocarbons (PAHs), phenol, tar, nicotine, arsenic, heavy metals, and various additives. Chemical additives are incorporated into tobacco products to enhance their appeal to consumers. Certain additives, such as humectants used to make smoke milder and easier to inhale or extend the shelf life, may have carcinogenic properties for humans (**Slaughter et al., 2011**). During the smoking process, cigarettes are exposed to temperatures exceeding 950°C and varying levels of oxygen, leading to incomplete combustion and the release of a substantial quantity of chemical compounds in cigarette butts (**Baker, 2006**).

The fact that CBs are not enriched in the same proportion for all the elements after being smoked suggests not only that the metal concentration or the additives included by the manufacturer in the paper and tobacco are significant, but also that the chemistry of each element is going to play a role. This is the case of volatile elements (i.e. As and Hg). Although their concentrations are higher in the tobacco than in the filter, As and Hg are not enriched in the filter after smoking because a great part of them may be volatilized during the smoking action due to the high temperatures reached. The As or Hg levels are reduced in CBs after being smoked.

Once in the environment, CBs are likely to release potentially toxic compounds that can be categorized into two main groups: metals and organic chemicals (such as nicotine and PAHs). Analysis of the evolution of CBs toxicity during ageing in the environment, assessed by measuring the inhibition of *A. fischeri* bioluminescence for the leachates of the three types of CB, showed a significant loss of toxicity of about 25% compared to non-aged CBs C after 7 days of ageing on the beach. These results confirm that the chemicals responsible for the toxicity of the CBs were released into the environment, potentially into the sand, in rainwater and/or air. A gradual decrease in toxicity was then observed. After 56 days of ageing, the toxicity of the CB reached a level below that of unsmoked CB, with values between 1 and 10% loss of bioluminescence.

Roder Green et al. (2014) conducted a study on the release of chemical compounds from cigarette butts into deionized water. They found that nicotine, which is highly soluble in water, was detected in water after only 30 minutes of soaking. Another study investigated the release of various metals present in cigarette butts, including aluminum, barium, cadmium, chromium, copper, iron, manganese, nickel, lead, strontium, titanium, and zinc. After 24 hours of soaking, all of these compounds, with the exception of cadmium, were quantifiably recovered in the aqueous solution (**Moerman and Potts, 2011**). This phenomenon was confirmed in the present report. Indeed, the leaching experiment demonstrated the capacity of CBs to leach the majority of their component over a short period including nicotine (the main component detected in all analyses CBs), phenols, indole, PAHs. For instance, the amount of nicotine in CBs was reduced by 83% after 6h of leaching time in seawater. This fact was also observed in natural environment, whether on Cedre's artificial beach or in the Brest marina; e.g. the concentration of nicotine, phenols, indole and PAHs were below LOQ in CBs after 72 days of deployment in the Brest Marina. Similarly, metal analysis demonstrated an overall important capacity of CBs to leach their metals rapidly, for instance the minimum percentage of desorption was 36%. These findings highlight the potential for cigarette butts to rapidly release a range of toxic compounds into aquatic environments. **Lozano-Rivas *et al.*, (2020)** measured that the leaching of CBs littered annually upon roads and sidewalks in nightlife areas in Bogota (Colombia) can add about 51.6 tons/year of cadmium and 1.81 tons/year of arsenic to the Bogota River.

The high desorption capacity of CBs can explain their toxicity to aquatic organisms. However, due to the high diversity of chemicals/metals measured/detected in artificial, fresh and weathered CBs in the present study and in the literature, it is highly difficult to attribute an effect to a specific compound. Indeed, the toxicity of compounds from plastic debris can be linked to a cocktail effect (**Tallec et al., 2023**). Nevertheless, in certain cases it is possible to propose hypothesis based on the knowledge on chemical compounds independently. For instance, **Micek (2020)** studied the impact of nicotine exposure on an amphibian larvae species (*Lithobates catebeianus)*. The results showed a direct correlation between increasing nicotine concentration and increasing mortality rates with a 100% mortality rate within 24 hours of exposure, for a concentration of 3 mg of nicotine/L of water. The threshold nicotine level was between 1.2 – 1.5 mg of nicotine/L of water. Here, experimentation carried out by seawater contamination on *P. varians* revealed paralysis of a few individuals after 6 hours of contamination under conditions with the highest concentrations of CBs (8 CBs/L). Paralysis has been identified when individuals were positioned on their backs with movements of their appendages. It should be noted that once placed in the decontamination tanks, the shrimp saw their paralysis disappear. This paralysis could be explained by the presence of nicotine in leachates. Nicotine is a substance present in significant quantities in cigarettes, between 8 and 20 mg per cigarette (**Alkam and Nabeshima, 2019**). Nicotine has been used since the 17th century as an insecticide (**Ujvári, 1999**), however its use has been severely restricted in the United States, Canada and Europe because of its toxicity to aquatic organisms

(**Roder Green *et al*., 2014**). Nicotine is a strong neurotoxin that acts on the nervous system of vertebrates and invertebrates by binding to nicotine acetylcholine receptors (**Ujváry, 1999**). Work on the nematode *Caenorhabditis elegans* has shown that nicotine uptake causes contractions of the muscle wall of the body and paralysis in individuals (**Wolf and Heberlein, 2003**). Another study on the marine worm *Hediste diversicolor* showed that nicotine present in CBs resulted in inhibition of the landfill capacity of the worm after 96 hours of exposure to a CBs leachate at 2 CBs/L (**Wright *et al*., 2015**).

In contrast to the desorption mechanism, CBs can also accumulate chemical compounds and metals in the environment. Indeed, we showed an increase in the concentrations of all metals in CBs after a deployment on the Cedre's artificial beach or in the Brest marina, the enrichment factors were between 2 and 60 000. The adsorption capacity increased the chemical complexity of this waste, and potentially the risks associated with future release into another environment as the butt ages or after accidental ingestion by an animal.

Although a single CB would not inflict serious environmental damage, the cumulative effect of many CBs littered in a specific area may present a significant threat to local organisms (**Araújo, 2019**). In this sense, **Quéméneur et al. (2020)** demonstrated that the presence of CBs in marine sediments increased metal dissolved concentrations in the surrounding waters. Considering this, it is likely that organisms such as bivalves will be exposed to CB released metals and chemicals. Some compounds in CBs are likely to be found in marine organisms. In his work, **Wei (2018)** showed that there is bioaccumulation of certain compounds from cigarette butts in the mussel *Mytilus galloprovincialis* after a 28-day exposure to a CB leachate. The present study investigated the desorption of metals by CBs and the risk of bioaccumulation by oysters (*C. gigas*) with laboratory analyses. Except for Sr and Mn, and to a lesser extent Sb and U, metal concentration in oyster tissues after exposure to CBs leachates did not show a significant increase. Marine organisms in general and marine bivalves in particular, use some of these elements (*i.e.* Co, Cu, Fe, Zn, Mo, V) for their metabolic functions, which make them essentials for their survival. Consequently, they tend to accumulate these elements in their tissues to use them as micronutrients. **Suami et al. (2019)** analyzed trace metals in oysters from the Atlantic Ocean finding concentrations up to 2 µg/g for Co, 16 µg/g for Cu, 460 µg/g for Fe and 113 µg/g for Zn. These values are very similar to the ones found in the present study. In addition to the high natural values present for these elements in oyster tissues, the potential accumulation of the metals derived from the CBs is relatively low. Taking into account the metal content in the CBs, the percentage of desorption, the total volume added to the experimental tanks where the oysters were placed, the volume of these tanks, the number of oysters and the mean weight of each tissue (gills and digestive gland), the maximum concentration that could be accumulated in the oysters´tissues are shown in Table 11. Considering these values, only Fe, Mn, Sb or Sr could increase their concentration in the tissues with respect to the natural values. All these elements showed moderately increased concentrations in oyster gills and/or digestive gland after being exposed to CB leachateS. **Dobaradaran et al. (2017)** observed that once in the environment, CBs accumulate more metals than after being smoked. This process has been confirmed in the present study, both for the CBs weathered on the beach and in the harbour. If these CBs reached clean waters, the metals could be released to reach equilibrium and thus be potentially available for marine organisms. The accumulation of these elements is more feasible in oysters, as filter-feeding animals; they filter high quantities of water accumulating all the elements. Oysters may have more chances to accumulate a higher concentration of metals released from weathered CBs than from non weathered ones. Not only higher concentrations for the metals but also more elements (i.e. As, Cr, Cu, Ni, Pb, U or V) could potentially increase their concentrations in oyster tissues under this new scenario.

7. Existing options to reduce CBs pollutions are not all satisfactory

It appears that numerous IMAs have been implemented to reduce CB littering issues. They include environmental clean-up, selective collection, recycling, awareness raising, eco-conception, public policies... However, some of them such as environmental clean-ups and development of alternative filters that are biodegradable in the environment are not satisfactory solutions as they only tackle a part of the problem. They address the plastic pollution but they do not prevent the transfer of chemical contaminants in the environment that occurs rapidly as soon as CBs make contact with water.

Conclusion

Overall, this study confirmed that cigarette butts are harmful for the marine environment and for aquatic environments in general even in low numbers. Their harmfulness is mainly due to their chemical composition including toxic contaminants that can rapidly be released in water at levels that can affect organisms. Despite their harmfulness, CBs are still thrown away in huge quantities into the environment and are regularly found during litter clean-ups or monitoring programs. In this context, it appears necessary to continue current efforts and take additional appropriate measures to reduce the presence and associated impacts of CBs on the environment.

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