UNIVERSITÀ POLITECNICA DELLE MARCHE

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Corso di Laurea Magistrale

BIOLOGIA MARINA

IL MOZZICONE DI SIGARETTA COME RIFIUTO AMBIENTALE E MARINO: IMPATTO E MITIGAZIONE DEL PROBLEMA MEDIANTE AZIONI DI CITIZEN SCIENCE.

CIGARETTE BUTT AS ENVIRONMENTAL AND MARINE WASTE: IMPACT AND MITIGATION OF THE PROBLEM THROUGH CITIZEN SCIENCE ACTIONS.

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1. Introduzione e obiettivo del lavoro

La parte di sigaretta non fumata e il filtro sono comunemente chiamati "mozzicone di sigaretta" (in questa tesi abbreviato con CBs). I CBs sono rifiuti pericolosi solitamente gettati dai fumatori e sono tra i rifiuti prevalenti nell'ambiente urbano e idrico.

Nei mozziconi si possono trovare molti inquinanti chimici come sostanze tossiche, nocive, irritanti e cancerogene come componenti in fase gassosa e componenti in fase di particolato (ad esempio nicotina, polonio-210, ammoniaca e acido cianidrico).

I mozziconi contengono molte sostanze chimiche tossiche. È necessario però considerare i CBs non solo come un inquinante a livello chimico ma anche come un vero e proprio oggetto fisico e rifiuto che può causare danni agli organismi. A causa del lungo tempo di degradazione (da 1 a 5 anni) i mozziconi rimangono nell'ambiente per anni.

Nel presente lavoro, i CBs sono considerati come un rifiuto emergente e l'obiettivo della tesi di laurea si concentra sul problema dell'abbandono dei mozziconi di sigaretta che sarà analizzato secondo due aspetti.

Il primo approccio si concentra su un'analisi ecotossicologica su tre specie selezionate di foraminiferi bentonici (*Rosalina globularis, Textularia* *agglutinans* e *Quinqueloculina* spp.) che saranno sottoposti a un test acuto di 48 ore per valutare l'effetto tossico della nicotina presente nei mozziconi.

Il secondo approccio è legato alla Citizen Science e alla raccolta dati.

Purtroppo, non esistono dati quantitativi completi ed esaustivi sul contenuto di agenti chimici nei mozziconi, anche perché essi dipendono dalla posizione geografica di produzione del tabacco, dagli additivi utilizzati dalle industrie e dal modo di fumare del fumatore né soprattutto dati quantitativi sulla concentrazione di nicotina in acque costiere.

Con il fine di ottenere dei dati reali sulla quantità di nicotina e di altre sostanze tossiche che vengono rilasciate in ambiente ma soprattutto di sensibilizzare la popolazione con una più ampia divulgazione scientifica sull'abbandono dei rifiuti, con i colleghi di Ingegneria Informatica dell'Università Politecnica delle Marche è stata realizzata un'applicazione pilota per smartphone; tale app permetterà alla comunità scientifica di mappare la distribuzione dei mozziconi di sigaretta in ambiente (sia esso urbano, rurale o litorale) e di valutarne la quantità per area. Lo scopo finale dell'app **CliccalaCicca** sarà quello di quantificare le sostanze tossiche, soprattutto nicotina, che potenzialmente fluiranno nelle acque marine costiere. Tali valori di riferimento potranno essere utilizzati in future analisi *in vivo* e *in vitro* per valutare l'impatto di questo inquinante sull'ecosistema e sugli organismi marini.

1. Risultati: Foraminiferi

I parametri chimico fisici (T, S e pH) dell'acqua di mare sintetica utilizzata come mezzo di coltura sono stati misurati prima e dopo l'esperimento. I parametri sono rimasti stabili eccetto il pH che ha subito una leggera diminuzione da circa 8.35 a 8.25.

Il test ecotossicologico LC50 acuto di 48 ore è stato eseguito utilizzando due concentrazioni, una letale e una subletale, determinate durante un precedente esperimento di LC50 in cui le stesse specie di foraminiferi sono state incubate con il percolato di mozziconi di sigaretta. In questo caso, i foraminiferi sono stati intossicati *in vitro* con tali concentrazioni ma di nicotina sintetica per 48 ore. Questo passaggio dal percolato di CBs alla nicotina sintetica è stato fatto perché in Caridi et al. (2020), le analisi chimiche hanno stabilito che nel percolato c'è una quantità di nicotina pari a 62.5 mg di nicotina/L.

Alla fine dell'esperimento, la vitalità dei foraminiferi è stata valutata mediante l'utilizzo di una sonda colorante fluorescente (CTG-Cell Tracker Green) che si attiva metabolicamente soltanto se l'organismo è vitale.

Le tre specie di foraminiferi utilizzate per l'esperimento hanno risposto diversamente alle concentrazioni letale e subletale di nicotina sintetica. *Rosalina globularis* è la specie calcarea perforata che è più sensibile e la sua vitalità diminuisce fino al 20% se sottoposta alla concentrazione letale di soli

3.72 mg/ L di nicotina. L'80% della specie agglutinante *Textularia agglutinans* sopravvive se sottoposta ad una concentrazione letale, pari a 10.98 ml/ L di nicotina. Infine, la specie calcarea imperforata *Quinqueloculina* spp. risulta la specie più resistente con una diminuzione della vitalità del 34% con una concentrazione di nicotina di 14.11ml/ L. Tutte le specie alla fine dell'esperimento non sembrano avere segni evidenti di dissoluzione del guscio o di parte del guscio.

2. Risultati: Sviluppo app

Il prototipo di app **CliccalaCicca** segue lo schema di altre app con tematiche simili (i.e., Litterati). Lo sviluppo dell'app permette quindi di far registrare l'utente e di rispondere facoltativamente ad alcune domande relative alle abitudini (fumatore o non fumatore) e al contesto in cui si trova (urbano, spiaggia etc.). Quello che viene chiesto obbligatoriamente all'utente è di scattare una foto che permetta alla comunità scientifica che acquisisce l'informazione, di elaborare il dato in termini di: numero di mozziconi e geolocalizzazione dell'immagine.

3. Discussioni e Conclusioni

I risultati del test ecotossicologico confermano che i foraminiferi bentonici sono organismi marini sensibili al rilascio di nicotina nel mezzo di coltura. La nicotina, come contaminante emergente presente nei CBs, è in grado di disperdersi in mare e quindi potrebbe contribuire ad amplificare l'effetto tossico, tutt'ora già causato da altri contaminanti di origine antropica anche in termini di acidificazione delle acque.

I dati di questa tesi di laurea confermano che i CBs e la relativa nicotina presente, hanno un impatto sugli organismi marini e questo quadro è una conseguenza diretta dell'abbandono indiscriminato dei rifiuti nell'ambiente.

Per quanto riguarda il secondo aspetto di questa tesi di laurea, è importante tener presente che al giorno d'oggi lo smartphone è uno degli strumenti più potenti per la socializzazione e per la circolazione di informazioni/dati ed è per questo che è stato preso in considerazione come modello di divulgazione scientifica; è stata creata quindi un'app che potesse unire contemporaneamente una grande raccolta di dati e la sensibilizzazione della popolazione tramite attività di Citizen Science.

Tutti i dati vengono raccolti dall'app **CliccalaCicca** (foto con la loro posizione e tempo, numero di mozziconi, contesto e densità) all'interno di una banca dati esaminata dalla comunità scientifica. Da questa banca dati sarà possibile, in futuro, elaborare ed ottenere una mappa delle zone più inquinate d'Italia e stimare flussi di nicotina che arrivano in mare. Questi dati potranno quindi essere utilizzati per stimare la concentrazione di nicotina rilasciata e i suoi effetti su organismi marini target mediante esperimenti *in vivo* e *in vitro*.

È fondamentale quindi che la collaborazione tra mondo scientifico e cittadini continui nel tempo per rafforzare la connessione appena creata e per migliorare a livello pratico il progetto.

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1. Introduction

The not-smoked cigarette portion and the filter are commonly called a "cigarette butt" (CB) (Figure 1). CBs are hazardous wastes usually littered by smokers and are of the prevalence wastes in urban and water environment.

Figure 1. Cigarette butts on the beach.

In the butts you can find many chemical pollutants such as toxic, harmful and carcinogenic substances like gas phase components and particulate phase components (nicotine, polonium-210, ammonia and hydrogen cyanide ext.) (Table 1).

Gas phase components	Average concentration / cigarette	Particulate phase components	Average concentration / cigarette
carbon monoxide	17.0	total dust	40
toluene	0.15	nicotine	1.8
formaldehyde	0.09	phenol	0.08
acrolein	0.08	catechol	0.23
acetone	0.18	hydroquinone	0.2
hydrogen cyanide	0.45	lactic acid	0.12
ammonia	0.1	glycolic acid	0.08
nitrogen oxides	0.4	benzo[a]pyrene	0.07
acetic acid	0.57	nickel	0.03
benzene	0.28	arsenic	0.013
pyridine	0.09	polonium-210	0.5
acetaldehyde	0.7	cadmium	0.002

Table 1. Some chemical agents emitted during the combustion of a cigarette, expressed in mg / cigarette. Spiridozzi S, Fioravanti S, Muzi M. Hygienic and technical aspects of the anti-smoking legislation. Insert of ISL - Hygiene and Safety at work n. 5/2004.

From the chemical point of view, taking into account all the harmful substances present in CBs (Table 1) and due to its high consumption and widespread detection in the aquatic reservoirs, nicotine has been used as a chemical marker for anthropogenic contamination processes (Martinez Bueno et al., 2010; Senta et al., 2015; van Wel et al., 2016).

During the combustion of the cigarette, part of the chemical products is inhaled by the smoker, a portion is retained by the filter, a portion is dissipated in the external (second-hand smoking), and another part consisting of the ashes is placed in the environment forever (Figure 2. Lombardi et al., 2009).

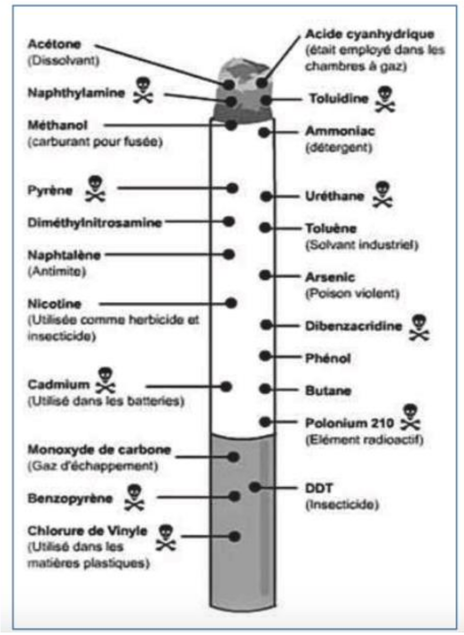


Figure 2. Toxic substances present in a cigarette (Lombarti et al., 2009).

At a chemical level, nicotine in tobacco products is rapidly absorbed by oral and intestinal mucosa, and alkalinization enhances absorption; nicotine-related symptoms develop rapidly (<4hours) after ingestion. These may represent a wide variety of symptoms including nausea, vomiting, salivation and diaphoresis; with severe poisoning, there may be convulsions, bradycardia with hypotension, cardiac and respiratory depression (Vig et al., 1990).

Previous studies have shown that chemicals in CBs leachate can be acutely toxic to aquatic organisms including microorganisms like *Vibrio fischeri* (Micevska et al., 2006), *Daphnia magna* and *Ceriodaphnia dubia* (Micevska et al., 2006; Register, 2000; respectively), tidepool snails (Booth et al., 2015), benthic foraminifera (Caridi et al., 2020) and others (Parker and Rayburn, 2017; Wright et al., 2015, Hiki et al., 2018).

It is necessary to consider CBs not only as a pollutant at a chemical level but also as a real physical object that can cause damage to organisms. Due to the long degradation time (see Table 2) the CBs remain in the environment for years.

Type of waste	Degradation time
Cigarette butts	from 1 to 5 years
Plastic bags	from 10 to 20 years
Nylon products	from 30 to 40 years
Aluminum cans	500 years
Glass bottles	1000 years
Plastic bottles	undefined time

Table 2. Degradation time of principal litter items (Stanley K., Stabenau E., Landry A. Debris ingestion by sea turtles along the Texas coast. In Eighth ANNUAL workshop on sea Turtle Conservation and Biology. Schroeder, B.A., Ed. NOAA Technical Memorandun: Fort Fischer, N.C: USA 1988: 119-121.).

Cigarette butts, defined as CBs in this work, are commonly discarded onto beaches, sidewalks, streets and many other public places where children, domestic animals and wildlife (e.g., birds and turtles) may be exposed to risk of ingestion.

In the present work, CBs are considered as an emerging waste and the aim of the degree thesis is focused on the problem of the abandonment of cigarette butts that will be analyzed according to two aspects. In view of the results presented by Caridi et al. (2020), **t**he first aspect is focuses on an ecotoxicological test on three species of benthic foraminifera (*Rosalina globularia*, *Textularia agglutinans* and *Quinqueloculina spp*.) that will be subjected to an acute 48h test to verify the toxic effect of synthetic nicotine present in CBs. The specimens were exposed to 16, 8, 4, 2, and 1 CB/ L concentrations (obtained by dilution of a prepared 32 CBs/ L stock solution corresponding to 62.5 mg of nicotine/ L). Starting from 4 CBs/ L (about 8 mg of nicotine/ L), 2 calcareous genera showed shell decalcification, and death of almost all the individuals, except for the more resistant agglutinated species.

An Italian study, considering that each cigarette contains an average of 10-15 mg of nicotine, of which 20% passes into the smoke and 50% is degraded during combustion, revealed that this toxicant cannot be ignored. In this case, can assume that 4.5 mg of nicotine is trapped in the butt. And since in Italy 72 billion cigarettes are consumed a year with butts, about 324 tons of nicotine are released into the environment (Lombardi et al., 2009).

From a recent study the average cigarette contains is 0.8–1.9 mg of nicotine. This delivers a human dose when smoked of 10–30 μg kg−1 based on an average adult weight of 68 kg, resulting in average peak plasma levels of 10–50 ng ml−1 (Wrigth et al., 2015). In Senta et al. (2015) compounds in raw wastewater of 13 Italian cities have been investigated (Figure 3).

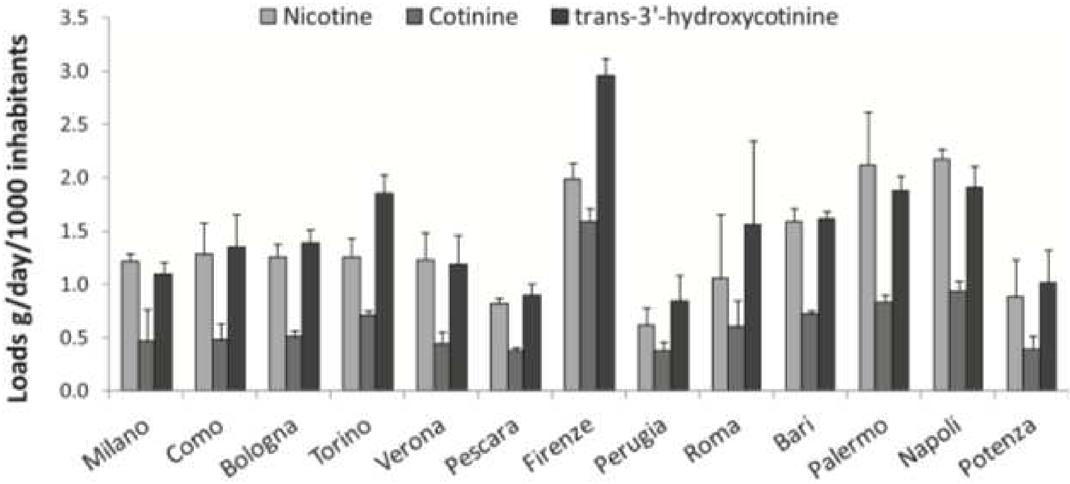


Figure 3. Normalized mass loads (g/day/1000 inhabitants) of caffeine, nicotine and their metabolites in 13 Italian cities. Results from the September-October 2012 sampling campaign (Senta et al., 2015).

The mass loads of nicotine, cotinine and trans-30-hydroxicotinine varied in the different cities and ranged between 0.5 and 3 g/day/1000 inhabitants. Nicotine itself was found in larger amounts than expected considering only urinary excretion. This is in accordance with the hypothesis that other sources of nicotine, such as the improper disposal of ash and CBs or pharmacological use, may contribute to the total amount of nicotine in wastewater (Senta et al., 2015).

The normalized mass loads of nicotine and metabolites were higher in the south than in the center and north of Italy (Table 3.). The nicotine mean mass load in the south was 1.7 ± 0.6 g/day/1000 inhabitants, higher than in the north (1.2 ± 0.02 g/day/1000 inhabitants, $p < 0.01$ by Dunn's multiple comparison test) and center (1.1 ± 0.6 g/day/1000 inhabitants, $p < 0.001$ by Dunn's multiple comparison test) (Senta et al., 2015).

Cities and region		Nicotine
NORTH	Milan	2.4 ± 0.92
	Como	2.97 ± 0.72
	Bologna	6.08 ± 1.00
	Turin	3.19 ± 0.43
	Verona	4.48 ± 1.05
CENTER	Pescara	2.31 ± 0.20
	Florence	3.21 ± 0.55
	Perugia	3.77 ± 1.03
	Rome	1.36 ± 0.71
SOUTH	Bari	6.87 ± 0.48
	Palermo	6.00 ± 1.39
	Naples	6.43 ± 0.71
	Potenza	2.20 ± 1.00

Table 3. Mean concentrations (mg/ L) ± standard deviation (SD) of the selected compounds in wastewater in Italy. (Senta et al., 2015).

Finally, nicotine data in marine aquatic habitats are still limited (Roder Green et al., 2014; Huerta-Fontela et al., 2008).

Therefore, the rationale of this work was to improve the knowledge about the effect of nicotine on benthic marine organisms (foraminifera) by acute toxicological test. In the meantime, the aim was to estimate the amount of nicotine. Namely, the average amount of nicotine per butt is known; therefore, by estimating the quantity of CBs distributed by urban or coastal area, it could be possible reconstructing the nicotine quantity that can be potentially dispersed in the sea. This quantity will be the reference for subsequent toxicological tests on benthic marine organisms including foraminifera that are at the base of the trophic chain.

An accurate estimate of nicotine flows and its quantity will also allow the scientific community to understand the real impact of harmful substances in the marine environment.

For this reason, the second aspect is related to citizen science. With researchers from the Department of Information Engineering of UNIVPM, I have implemented a pilot smartphone app that will allow the scientific community to map the abandonment of discarded CBs with the aim to quantify toxic substances (mainly nicotine) that can be used in future experimental tests. In fact, there are no complete and exhaustive quantitative data on the content of chemical agents in butts, also because it depends on the geographical location of tobacco production, from the additives used by industries and by the way of smoking of the smoker. The app will be developed and will help scientific community to metadata recovery. In the next paragraphs I will deepen the main tools I have used for my degree thesis.

1.1 Cigarette butt as urban litter

Currently, the smoker, thanks to the lack of laws, appropriate ashtrays and lack of awareness of the dangers of this material, gets rid of butts by throwing them wherever they happen, without any attention and precaution, releasing a complex mixture of toxins (see Table 1).

At the urban level, CBs are difficult to manage, as they remain stuck in all the interstices where the brooms and the mechanical means of sweeping cannot reach. At least 50% of all waste of urban areas are related to tobacco products: cigarette butts, cellophane, lining paper and container packages (EcoRecycle: www.ecorecycle.vic.gov.au).

According to article 232-bis, low 221 28/12/2008 (waste produced by smoking): "the municipalities should install special bins in the streets, parks and places of high social gathering for the collection of butts of smoking products" (Figure 4).

Figure 4. Cigarette case in a garbage bucket.

Furthermore, in the Art. 232-ter (prohibition of abandonment of very small waste): “in order to preserve the urban decor of inhabited centers and to limit the negative impacts deriving from the uncontrolled dispersion into the environment of very small waste, such as receipts, paper handkerchiefs and chewing gum, the abandonment of such waste is prohibited. on the ground, in the waters, in the drains and drains”.

Considering an average consumption of 15 cigarettes per day per capita, 195 million butts are produced every day in Italy, corresponding to 72 billion butts a year (Lombardi et al., 2009). Since there are no rules governing the disposal of this waste, most of these are abandoned without any criteria and attention in all possible places.

In Italy at the urban level, it is enough to take a look outside the shops, bars, restaurants, bus stops, parks, to see an authentic carpet of butts thrown in bulk by those who consume their own cigarette and then discard them (Figure 5) (La Repubblica 28/11/2008).

Figure 5. Cigarette case in a garbage bucket (not the correct use).

1.2 Cigarette butt as a marine litter

Marine litter is a global environmental problem. Among others, smoked cigarette filters are the predominant element of coastal waste (Table 4); it is estimated that 4.5 trillion are left in the environment each year, representing a source of bioplastic microfibers (cellulose acetate) and toxic substances harmful to marine environment (Wright et al., 2015).

Rank	Debris item	Number of debris item	Percentage of total debris items
1	cigarette/ cigarette filters	2177931	19%
2	food wrappers/ containers	1140222	10%
3	beverage bottle (plastic)	1065171	10%
4	bags (plastic)	1019902	9%
5	caps, lids	958893	9%
6	cups, plates, forks, knives, spoons	692767	6%
7	straws, stirrers	611048	6%
8	beverage bottle (glass)	521730	5%
9	beverage cans	339875	3%
10	bags (paper)	298332	3%
	TOP 10 total debris items collected	8765871	80%
	TOTAL DEBRIS items collected worldwide	10957338	100%

Table 4. Top 10 marine debris items collected, international coastal cleanup. Source: Ocean Conservancy, 2012: http://www.oceanconservancy.org/our-work/international-coastal-cleanup/top-10-items-found-1.html

Focus and *Legambiente* have found on the basis of the data collected during last campaigns "Clean beaches ", which every square meter of sand cleaned contains 2 cigarette butts, 2.5 plastic or metal caps, 1 straw, 1 ice cream stick.

Extending the results to the entire Italy it is possible to hypothesize that about 12.4 million CBs are abandoned on Italian beaches every year (Lombardi et al., 2009).

A study conducted by Vlachogianni et al. (2018) on the "Marine litter on the beaches of the Adriatic and Ionian Seas", abundance, composition and sources of marine litter were determined in 31 sites around the all countries of the Adriatic-Ionian macroregion, namely Albania, Bosnia and Herzegovina, Croatia, Greece, Italy, Montenegro and Slovenia (Figure 6.).

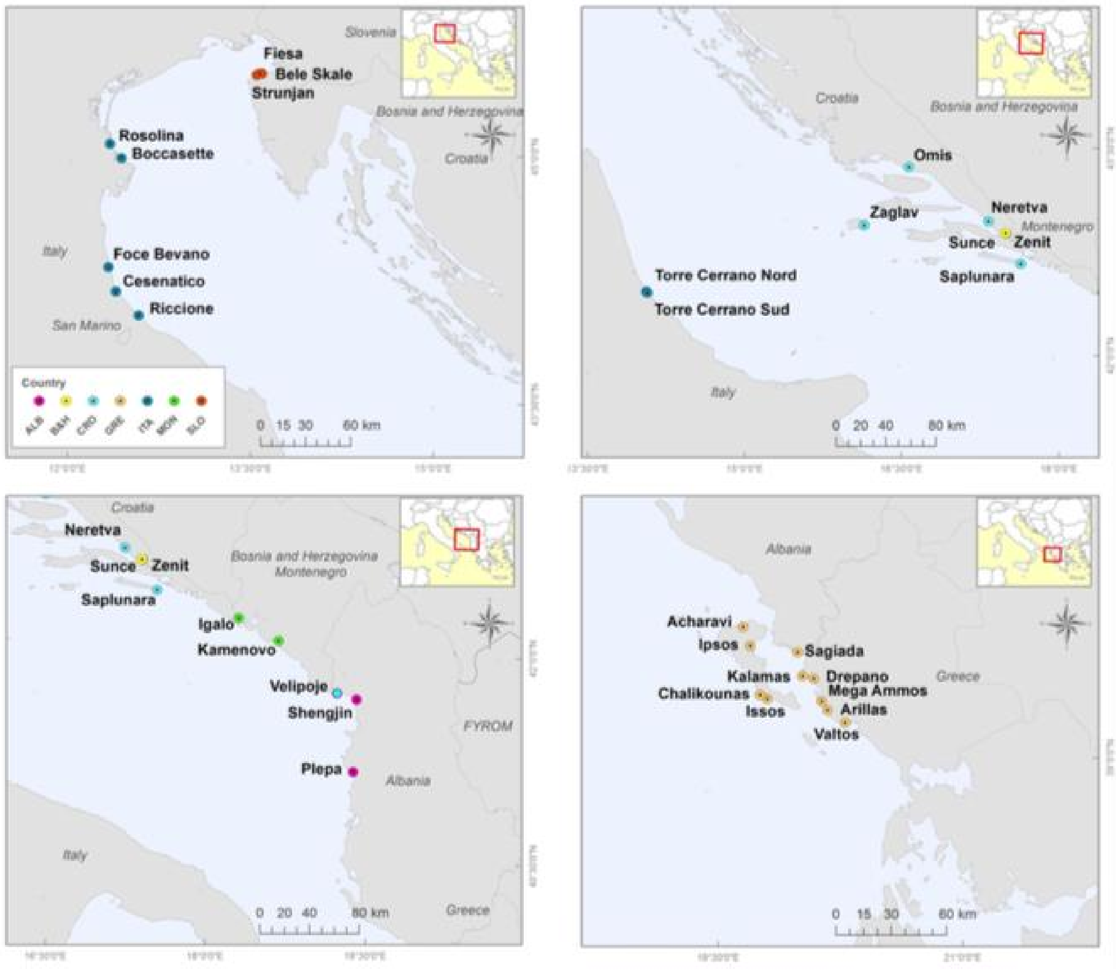


Figure 6. Maps of the 31 study sites located on the coastline of the Adriatic and Ionian Seas, in the seven countries participating in the study: Albania (ALB), Bosnia and Herzegovina (B&H), Croatia (CRO), Greece (GRE), Italy (ITA), Montenegro (MON) and Slovenia (SLO) (Vlachogianni et al. 2018).

In the 31-sites, where in total 180 surveys were performed, a total of 70,581 marine litter items were classified and removed.

The total abundance of litter items at Zaglav, Vis (Croatia) was found to be extremely high in comparison to the abundance of litter items recorded in the rest of the sites, with the average number of items being 11 items/m2 (1055 items/100 m). The second highest abundance of litter items was recorded at Ipsos (Greece) with the average number of items being 0.91 items/m2 (455 items/100 m), followed by Strunjan (Slovenia) with 0.83 items/m2 (828 items/100 m), Foce Bevano (**Italy**) with 0.55 items/m2 (549 items/100 m), Kamenovo (Montenegro) with 0.52 items/m2 (524 items/100 m), Bele Skale (Slovenia) with 0.49 items/m2 (490 items/100 m), Neretva (Croatia) with 0.48 items/ m2 (479 items/100 m), and Arrilas, Thesprotia (Greece) with 0.43 items/m2 (426 items/100 m). The lowest abundances of litter items were found on the beaches of Issos, Mega Ammos, Chalikounas and Kalamas, all located in Greece, with an average number of items for all surveys ranging between 0.08 and 0.09 items/m2 (92–177 items/ 100 m) (Vlachogianni et al., 2018).

From these analyzes CBs emerges as one of the most present waste in the monitored beaches (Table 5).

	Code	Items name	Total counts	%
1	G79	Plastic pieces 2.5 cm > < 50cm	14040	19.89
2	G82	Polystyrene pieces 2.5cm > < 50cm	8422	11.93
3	G95	Cotton bud sticks	6475	9.17
4	G21	Plastic cups/lids from drinks	4705	6.67
5	G27	Cigarette butts and filters	4660	6.60
6	G23	Plastic cups/lids unidentified	1743	2.47
7	G45	Mussel nets, oyster nets	1716	2.43
8	G30	Crisps packets/sweets wrappers	1492	2.11
9	G208	Glass or ceramic fragments > 2.5 cm	1368	1.94
10	G124	Other plastic/polystyrene items (identifiable)	1350	1.91

Table 5. Top 10 items found in the 31 surveyed beaches of the Adriatic and Ionian coastline, calculated on an aggregated basis of total litter counts in all beaches (Vlachogianni et al., 2018).

In particular, CBs rank among the top four marine litter in Italy (6.8% of total litter), being most abundant on the beaches of the western Mediterranean Sea where they reached the maximum density of 1480 pieces / 100 m (average density 1⁄4 15 items / 100 m). In the Adriatic Sea, the maximum value reached by cigarette butts was 531 pieces / 100 m (average density 1⁄4 5 pieces / 100 m). In contrast, in the Ionian and central Mediterranean Sea, the maximum value was 249 elements / 100 m, but in most beaches the density was close to zero. The percentage contribution to within group similarity of CBs increased from spring to autumn, thus after the touristic season. Indeed, CBs median density was 5 items/100 m in spring, while it doubled in autumn (10 items/100 m) (Fortibuoni et al., 2021).

Human influence - direct or indirect – is a strong determinant of CBs accumulation on beaches. According to Novotny et al. (2009), the number of CBs found on beaches is not necessarily related to the cigarettes smoked in situ.

Transport to the sand from the urban settings, roads and drains can also occurs along a range of distances.

CBs can float for long periods before becoming saturated with water and sinking, allowing their transport by rivers and currents (Engler, 2012).

Rivers can transport various objects including CBs, these with transport then tend to accumulate at the mouth of rivers where the transport speed is minimal, constituting a source of pollution.

Environmental factors contribute to the transport of this type of litter since they are lightweight and can easily be transported by wind. The winds in fact, based on their origin, move the waste with the possible aim of releasing it on the beaches and in the sea.

The position of beaches in relation to the prevailing winds (leeward or windward) is very important in the accumulation of marine debris (Araújo and Costa, 2019), and ultimately litter. In general, windward areas tend to accumulate more debris (Blickley et al., 2016; Scisciolo et al., 2016; Wilson and Verlis, 2017).

Indeed, inside the cigarette filter there is a cellulose acetate plastic substance that is photodegradable but not bio-degradable. Therefore, if the cigarette butt is considered as a plastic waste (microplastics), it’s possible assume that regarding composition, debris on windward coasts tends to contain a greater proportion of plastic objects, of which a greater proportion falls into the smaller meso- (2-25 mm) and micro-debris (<2 mm) (Corbin and Singh, 1993; Debrot et al., 1999, 2013; do Sul and Costa, 2007; Ribic et al., 2012).

Figure 7. Urban areas as possible sources of cigarette butts to beaches and coastal environments (Araujo et al., 2019).

In according with Fortibuoni et al. (2020), in the 2015 and 2016 surveys, 58 beaches were monitored, while since 2017, 6 more beaches were included in the monitoring programs. As it shows in the image below, the problem of the arrival of CBs in the sea is not only a problem of beach accumulation along coastal areas. In fact, the stub that is released from a site can travel great distances due to sea currents (Figure 8).

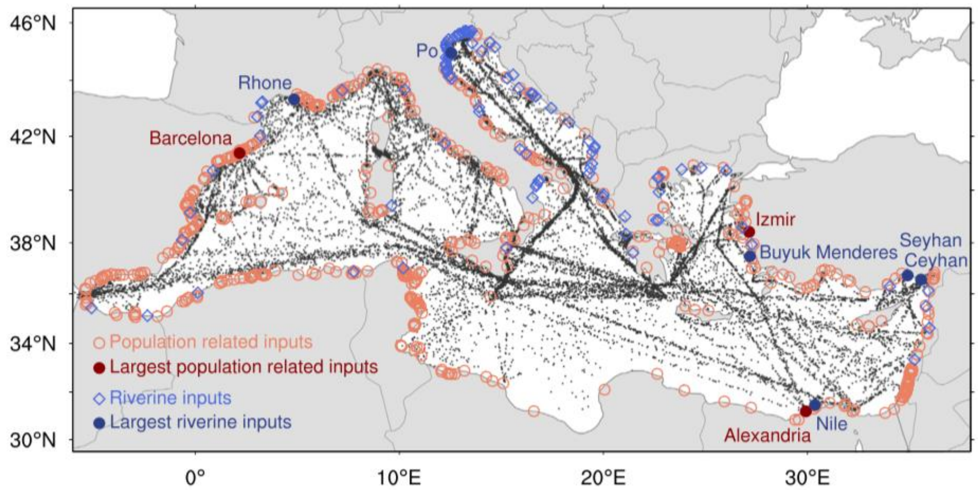


Figure 8. Spatial distribution of the floating debris inputs into the Mediterranean Basin: shipping lanes (graydots), rivers (open blue diamonds), the largest rivers (closed blue circles), cities (open red circles), and the largest cities (closed red circles) (S. Liubartseva et al., 2018)

1.3 Target organism: foraminifera

Given the emergency due to the presence of this pollutant in the environment and especially in the marine environment, in my degree thesis I tried to investigate the effect of one of the most abundant toxicants present in CBs: nicotine that has been analyzed in a group of organisms at the base of the trophic chain through an ecotoxicological study in culture.

The target organism chosen for this study is a group of marine unicellular organisms: foraminifera, heterotrophic amoeboid eukaryotic protozoa, both benthic and planktonic.

They are organisms that are part of the meiofauna as regards the dimensions (from 30 µm and 1 mm). They have a short life cycle and therefore react quickly to environmental changes, the taxonomic identification is relatively simple and thanks to foraminifera, other sensitive and opportunistic species can be identified.

Foraminifera can have a shell made up exclusively of protein material (i.e., organic or pseudochitinous), or they can have an agglutinated shell; in this case the foraminifera choose the material with which to build the shell and then glues the small pieces that it collects on the back through a sticky substance. Others, on the other hand, secrete a shell of calcium carbonate and therefore are excellent indicators of the past and ecology.

These organisms can have a calcium carbonate shell without pores (imperforate or porcelanaceous) or with pores (perforate or hyaline) and this difference is reflected in a different type of biomineralization.

Foraminifera with pseudochitinous organic shells have an outer layer of very fine sedimentary material: clay plates that the foraminifer glues onto a first organic layer. The exclusively organic shell has a low state of fossilization, and this does not allow a study by geologists or paleontologists.

Some species among agglutinated foraminifera are able to select the material by size, size and composition. In some cases, the selection depends on the type of material available in the sediment; in other cases, there is an exclusive selection of some material, and it is not known why they operate this extreme selection.

In the perforate calcareous foraminifera, the calcification mechanism starts from the formation of the so called primary organic membrane (POM), a thin layer of organic matrix partially isolating the organism from its environment. POM acts s template for the nucleation and precipitation of $CaCO_3$ (Sabbatini et al., 2014). In the imperforate calcareous foraminifera, individuals precipitate their needle-shaped calcite crystals (usually high Mg calcite) within intracellular vacuoles.

Foraminifera colonize both the water column and the sediment and therefore contribute to the distribution of organic matter from the benthic to the pelagic domain and vice versa. Foraminifera, non-symbionts, make up 30-50% of the benthic biomass, have a high diversity and a wide geographical distribution that covers both the continental and oceanic margins. They are important because they also have a high fossilization potential, particularly those with carbonate shells.

Benthic foraminifera are increasingly utilised as bioindicators, with particular interest for areas affected by strong anthropogenic impacts.

Since the 1950s, numerous studies have demonstrated the value of benthic foraminifera in detecting ecosystem contamination (Frontalini et al., 2011).

A bioindicator is an organism or biological response that reveals the presence of the pollutants by the occurrence of typical symptoms or measurable responses and is therefore more qualitative.

The advantage of application of foraminifera over other chemical and biological techniques for pollution monitoring can be summarized on the following points:

1. Their tremendous taxonomic diversity gives them the potential for diverse biological responses to various pollutants.

2. Due to relatively small size and great population diversity, statistically significant sample sizes can be collected quickly and relatively inexpensively for either faunal assemblage or for experimental studies, with minimal environmental impact.
3. Their short reproductive cycle and rapid growth make their community structure responsive to quick environmental changes.
4. Some species can be readily maintained in culture, so laboratory protocols can be established to determine responses of selected taxa to pollutants of concern.
5. They have biological defence mechanisms which protect them against unfavourable environmental factors, thus providing detectable biological evidence of the effects of pollution.

Further, foraminifera have an edge over the rest of the pollution monitoring techniques as the adverse effects of pollution could be incorporated into the agglutinated and calcareous tests; shells have an excellent preservation potential, thus providing concrete evidence for the presence and duration of existence of pollutants in an affected area and in the sedimentary records.

1.4 Citizen Science

As it was said previously, this work in addition to dealing with a toxicological evaluation of synthetic nicotine on foraminifera, has also dealt with a second aspect: Citizen Science.

The field of Citizen Science is growing at an incredible speed. Thousands of citizen science projects are now underway around the world, engaging millions of individuals in the scientific discovery process (Bonney et al., 2016).

Citizen Science is defined in different ways as it has multiple origins and derives from different concepts. The Oxford English Dictionary in 2014 defined it as "scientific activity conducted by members of the independent public in collaboration with or under the direction of professionals and scientific institutions".

The definition provided by the Oxford English Dictionary describes a Citizen Science having in mind and referring to those projects in which the participants collect and data and observations, often over large geographical areas or over long periods of time, or those in which people, working online, help to process unimaginable amounts of data such as in the classification of images or in the transcription of texts. By the study of Bonney et al. (2016), this first definition overlooks the fact that Citizen Science embraces projects in which participants volunteer in roles beyond data collection and analysis; projects in which individuals work not only in teams but also by on their own, with or without the collaboration of scientists; projects focused on humans rather than ecology; projects that emphasize issues raised not by scientists but by communities; and certainly, more types of participatory science that have yet to be imagined.

Figure 9. Factors of success in Citizen Science projects.

Some researchers believe that citizen science has emerged as a distinct field of investigation (e.g., Jordan et al., 2015).

By examining, critiquing, and sharing the results of a variety of Citizen Science initiatives, can delve into the foundations and assumptions of Citizen Science and critically analyze its practice and results. Such explorations can examine methods, approaches, benefits, costs, impacts and challenges of Citizen Science and will help us better understand the role that Citizen Science can play in environmental sciences, public health, physics, biochemistry, development. of the community, in social justice, democracy and more.

Some practical projects have already been activated by various non-profit organizations to promote initiatives that include citizens in the well-being of beaches and the sea.

For example, some Italian non-profit organizations as *Marevivo, Legambiente* and *Reef check*, thanks to the involvement of citizens, implement waste cleaning programs in various beaches in Italy. Once collected, the waste is sorted and then disposed of according to local regulations. All cleaning activities are made possible thanks to the support of citizens, local institutions and associations, and partnerships with national and international companies and in some cases, activities are made in collaboration with scientific partners (Figure 10).

Figure 10. *Campaign organized by marevivo against the abandonment of cigarette butts* (2009).

"Let's take care of our beaches because what we give to the sea comes back" (*Marevivo*).

Marevivo organizes campaigns and initiatives like "Small gestures big crimes" (Figure 11). It is a national awareness campaign against the abandonment of CBs in the environment in collaboration with British American Tobacco Italy, the patronage of the Ministry of

Ecological Transition together with the ANCI - National Association of Italian Municipalities. This campaign aims to raise awareness among citizens on the risks associated with littering, i.e., the deliberate or involuntary abandonment of small-sized waste into the environment.

Figure 11. Advertising to sponsor the day "small gestures big crimes" – *Marevivo* (2021).

Another project "Blue activities" was created with the aim informing and raising awareness among citizens, especially younger ones, on the importance of protecting the sea and their living environment. Through educational courses aimed at schools and initiatives that aim to stimulate active participation and direct involvement of the entire local community.

Legambiente promoted the activity "Clean up the world" (largest environmental volunteer event in the world); this initiative invites citizens of all ages, associations, municipal administrations to clean up streets, squares and city parks, but also beaches and riverbanks from abandoned waste. An active citizenship path built over the years to defend the environment and strengthen the sense of community.

With this initiative the parks, gardens, streets, squares, rivers and beaches of many cities around the world are freed from waste. Brought to Italy in 1993 by *Legambiente*, which took on the role of organizing committee, it is present throughout the country thanks to the tireless work of over 1,000 groups of "environmental volunteers" who organize the initiative at a local level (mainly collaborating with associations, companies and city administrations).

Legambiente also wants to involve the youngest, promoting in all schools an activity of observation and classification of waste abandoned in the area in front of the school building.

A real citizen science action that provides for the involvement and active participation of students, teachers, parents.

The results of the survey can also be used by the school to ask the Municipality or the Town Hall to guarantee the decorum of public spaces.

Reef Check is an international non-profit organization leading Citizen scientist to promote stewardship of sustainable reef communities worldwide. In particular, it is an organization dedicated to the conservation of tropical coral reefs and temperate kelp forests around the globe.

Reef Check's EMBARC program was launched in 2018 to expand the citizen science work mission to educate young people from some of the most disadvantaged communities in California. This interactive marine education program gives students a chance to become marine biologists for the day and a first-hand experience of the ocean environment.

Reef Check rains and organizes teams of local volunteer citizen scientist divers. People collect data on reef health and assess climate change impacts on their reefs. Their work produces reliable information used by marine resource managers, scientists, and policymakers to make science-based ocean management and conservation decisions.

This onlus promotes public education about reefs and the ocean. Their goal is to develop a team of ocean ambassadors with the skills and knowledge to make a tangible difference in marine conservation in their local communities.

Also *Reef Check* develops ecologically and economically sustainable solutions for reef conservation and restoration.

Finally, the aim of these associations is to promote conscious and eco-sustainable behaviors for the management and care of one's living environment, with particular attention to beaches, the sea and its ecosystem.

1.5 Smartphone app development

In a world almost completely globalized as the one in which we grew up and live, it is necessary to find the best way to communicate with everyone. Now the use of smartphones is a fact and that is why the teams decided to combine the world of the laboratory and the help that citizens themselves can offer.

It was, therefore, quite natural to develop the idea of going beyond the laboratory desk and involving not only ourselves - which is taken for granted as experimental scientists - but also our friends and the wider community of sensitive people.

In particular, one of the tools used by our team is the development of a new app with the aim of raising awareness of the environment.

The same associations involved in Citizen Science activities (paragraph 1.4) have begun to promote the use of mobile apps to improve the link between the scientific community and that of citizens to collaborate reciprocally in safeguarding the environment and the sea.

For example, *Marevivo* developed a smartphone app: "Clean swell" (Figure 12) to improve the Citizen-Researcher relationship; the app has the double aim: 1) increase the citizens awareness on their impact on the environment and 2) to provide data that will be converted in scientific results by researchers.

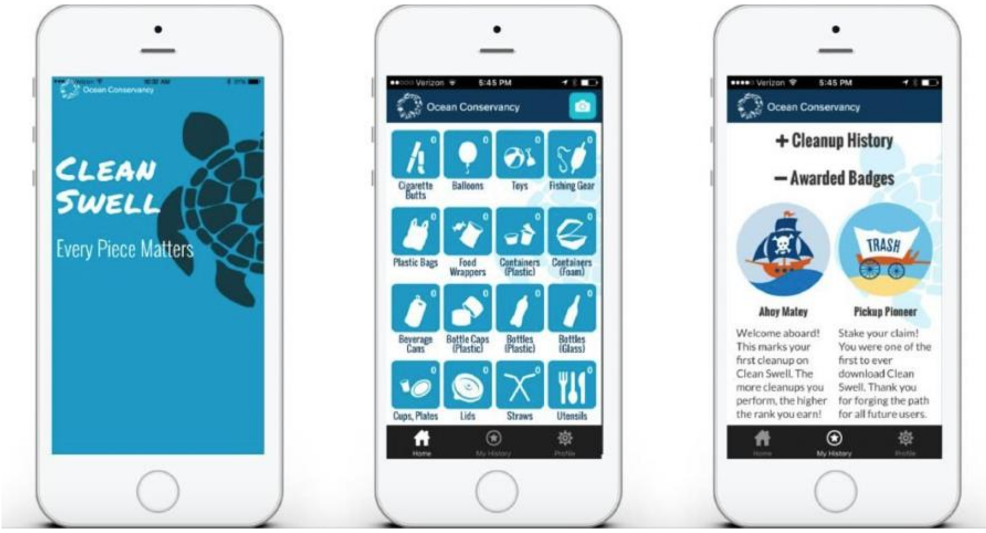


Figure 12. Clean swell app.

With this app people can start to collect trash in every single sea or beach around the world. The user to collect the waste just takes a picture, puts it in the right category of waste (Figure 12) and loads it. Automatically this photo will be inserted in Ocean Conservancy's global ocean trash database. These data deliver a global snapshot of ocean trash.

Another app developed to map litter is "Litterati – Clean the earth" (Figure 13). Users are asked to take and upload photos of litter which are then tagged and sorted into a huge machine learning database that is in turn used to influence the behavior of companies and governments alike. Users are rewarded for loading and throwing out trash with rewards, badges and financial incentives in good time.

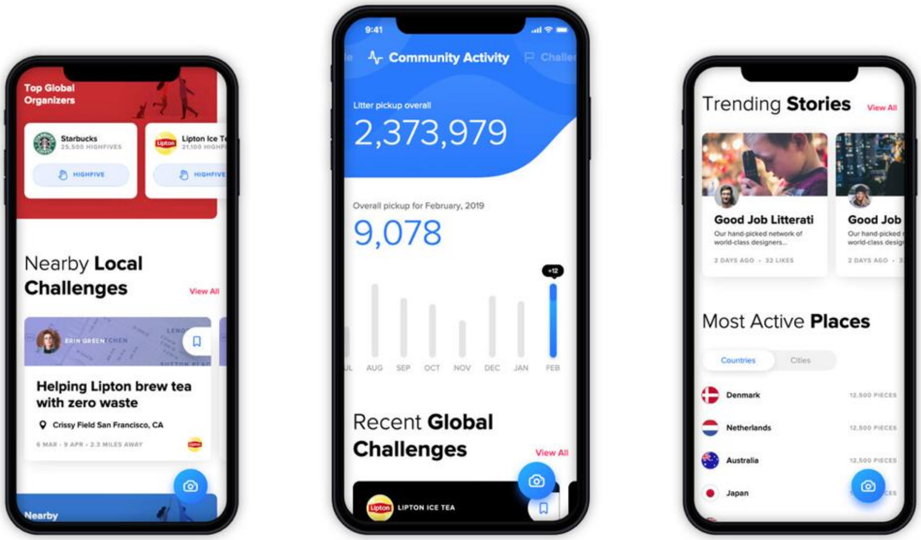


Figure 13. “Litterati-Clean the earth” app.

Given the previous examples and the use of apps to monitor the amount of waste, including marine litter, the future of this technology can be useful for improving the quantity and quality of data provided by volunteer citizens. These numbers could potentially be used appropriately by the scientific community and translated into scientific data as the distribution of the main pollutants in the marine system and the effects of these concentrations dispersed in the sea.

To this purpose, in this degree thesis, an innovative approach was taken into consideration: a smartphone app able increasing awareness in the citizens about the CBs litter and their associated toxicants, and at the same time, providing the scientific community with important data to quantify the problem. To create this app an active collaboration was established with colleagues from the

Polytechnic University of Marche and in particular computer engineering and automation researchers from the Department of Information Engineering.
By downloading on the personal smartphone, and using the app, the citizen becomes an active part of the project and a direct contributor to the scientific research.

The purpose of the application is to provide, in the future, to the scientific community, quantitative data on the number of CBs found on the ground, whether on the street, in public parks or on the beaches. These data, as well as further information that will be described later, are intended to have an estimate of the levels of toxicants that are released into the sea from the CBs.
The amount of nicotine released to the environment and that ultimately reach the sea could be estimated, for the first time, and compared with the data from previous studies (Huerta-Fontela et al., 2008).

Thanks to the important characteristics that foraminifera have, the scientific team realized how it is increasingly necessary to collaborate between disciplines, the laboratory and the science dissemination, thus integrating the various areas and creating a connection.
In the scientific world Researchers need a lot of data and that's why the team thought to involve citizens to get them.

In order to evaluate the impact that man has on these organisms, the project described below will be possible only thanks to the large collection of data carried out by citizens.

The team came up with the idea of trying their hand at this world of science outreach by including the world of the lab in everyday life.

Citizens will help the scientific community in obtaining data that then in this case will have the purpose of quantifying the concentration of nicotine in the sea. the collection of data is important because you can go and test the exact amounts of nicotine on foraminifera and see what is the impact.

2. Materials and Methods: foraminifera

2.1 foraminifera cultures

Except for Caridi et al.'s work (2020), studies on foraminifera phylum investigating the toxicity of CBs have not been performed yet.

Starting from this paper, the first aim of the present study is to evaluate the biological effects of synthetic nicotine on three selected species of benthic foraminifera (*Rosalina globularis*, *Quinqueloculina* spp., *Textularia agglutinans*), with a particular focus on their vitality and possible changes in the biocalcification rates.

Rosalina globularis (Figure 14) is a perforate calcareous species which precipitates $CaCO_3$ to build its shell; their shells are usually made of low Mg calcite and this group is perforated with numerous microscopic pores that are found on most of the surface area of the shells (Erez, 2003). *Rosalina globularis* is a common symbiont foraminiferal species used as a model in ecotoxicological studies (Schintu et al., 2015). *Quinqueloculina* spp. (Figure 15). Miliolids precipitate their needle-shaped calcite crystals (usually high Mg calcite) within intracellular vacuoles (Caridi et al., 2020). *Textularia agglutinans* (Figure 16) is an agglutinated foraminifera that allocates particles of different type and size in a sufficient quantity to create a new case gluing them with a biomineralized calcium carbonate cement (Sen Gupta, 1999), although there are studies showing that not all agglutinated species have $CaCO_3$ in their cement (e. g. van Dijk et al., 2017). These cultured genera allow to compare three different foraminiferal groups related to different shell structure (perforate and agglutinated) and test building mechanism.

Viability and possible change in shell calcification rate were checked respectively through acute toxicity tests, by replacing CBs leachate as used in Caridi et al. (2020) to evaluate the effects of exposure to this emerging pollutant with a synthetic nicotine stock solution.

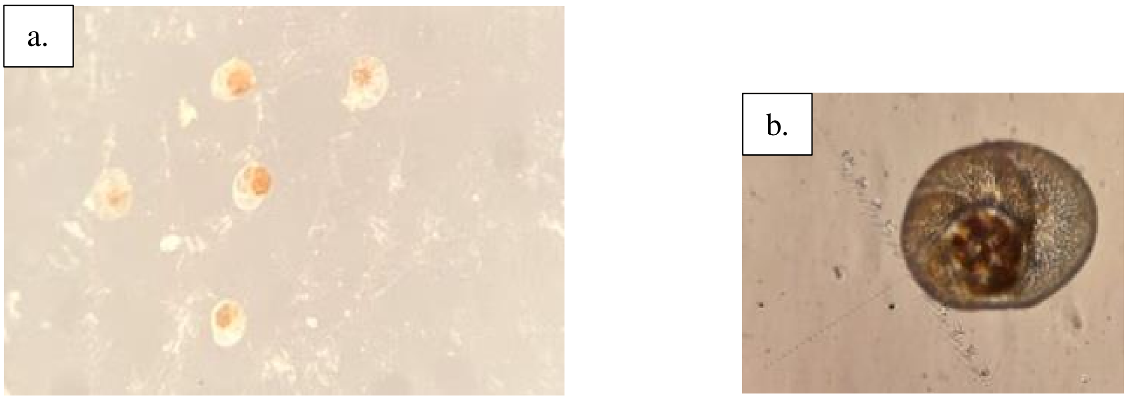


Figure 14. a: *Rosalina globularis* under the microscope. b: *Rosalina globularis* under optical microscope SEM.

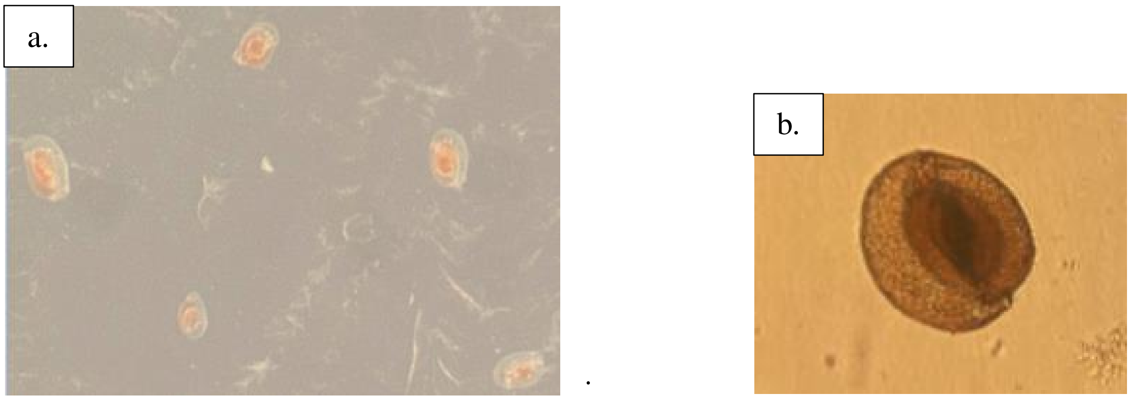


Figure 15. a: *Quinqueloculina* spp. under the microscope. b: *Quinqueloculina* spp. under optical microscope SEM.

Figure 16. a: *Textularia agglutinans*. under the microscope. b: *Textularia agglutinans* under optical microscope SEM.

2.2 Preparation of stock solution

The Stock solution for the acute toxicity test (LC50-48h) was prepared by adding 10 ml of a 35% synthetic nicotine solution (Nicotine bemisulfate salt, Sigma Aldrich) to 90 ml of artificial seawater to obtain a synthetic nicotine concentration of 35g/ L (1:10 dilution).

The artificial seawater was made adding 2 L of deionized water and 75 g of Instant Ocean® and after an overnight stabilization, yield a pH range between 8.0 and 8.4 and salinity range of 35-37ppt.

2.3 Foraminiferal LC50 test

From the previous experiment conducted by Caridi et al., 2020, GC-MS (gas chromatography-mass spectrometry) analysis allowed us to show that the leachate made from CBs (32 cigarettes/ L) contains an amount of unsmoked nicotine equal to 62.5mg/ L.

Then, considering the number of butts that correspond to the lethal and sublethal dose of each species (see Caridi et al., 2020), this amount was converted into synthetic nicotine (Table 6).

	Rosalina globularis	***Textularia agglutinans***	***Quinqueloculina* spp.**
LC50	3,72 mg/ L	10,98 mg/ L	14,11 mg/ L
SUBLETHAL	1,96 mg/ L	3,91 mg/ L	3,91 mg/ L

Table 6. Nicotine amounts relative to lethal and sublethal concentrations for the three foraminiferal species.

For each concentration, the amount of stock solution was calculated for a volume of 40 ml:

From the value of nicotine using the appropriate formula for calculating a dilution ($C_1V_1 = C_2V_2$), where C_1 and C_2 represent the respective concentrations of the starting and ending solutions, and V_1 and V_2 represent their volumes, the exact volumes of synthetic nicotine stock solution are calculated to be tested in the acute test were calculated considering a V_2 of 40 ml (Table 7).

$C_1V_1=C_2V_2$ —> $V_1=C_2V_2/C_1$

V1 (sublethal R.) = (0,00196g/ L x 40 ml)/(35g/ L) = 0,00224 ml—> 2,24 µl

V1 (sublethal Q.T.) = (0,00392g/ L x 40 ml)/(35g/ L) = 0,0046 ml—>4,46 µl

V1 (LC50 R.) = (0,00372g/ L x 40 ml)/(35g/ L) = 0,0042 ml—>4,2 µl

V1 (LC50 Q.) = (0,01411g/L x 40 ml)/(35g/ L) = 0,016 ml—>16 µl

V1(LC50 T.) = (0,01098g/L x 40 ml)/(35g/ L) = 0,0124 ml—>12,5 µl

	Textularia agglutinans	***Rosalina globularia***	***Quinqueloculina* spp.**
CONTROL	0 µl	0 µl	0 µl
SUBLETHAL	4,46 µl	2,24 µl	4,46 µl
LETHAL	12,5 µl	4,2 µl	16 µl

Table 7. Quantity of synthetic nicotine for a solution of 40 ml.

As in the paper by Caridi et al. (2020), the ecotoxicological test was carried out by using 3 replicates with 5 individuals for each concentration (lethal and sublethal) plus the control.

The foraminiferal specimens belonging to the 3 genera, previously living-checked (based on the coloration of foraminifera and pseudopodial activity) were divided into groups of 5 for control, lethal and sublethal concentrations and placed in special 40 ml jars with the stock solution and artificial sea water.

After 48h they were rinsed with clean seawater for three times to remove residual of nicotine solution. Then, they were put in glass Petri dishes with 15 ml filtered seawater and 0.2 µl (0.3 µM) of fluorescent probe Cell Tracker TM Green CMFDA (CTG) at 37 PSU salinity for 30 min.

Soon after, the foraminifera were rinsed again with clean seawater to remove any residual CTG and checked for cell activity using a binocular fluorescence microscope. The foraminifera were then allowed to recover for 72 hours in clean seawater, following the protocols developed by Ross and Hallock (2012).

After 72 hours, each individual's color and activity were visually assessed to identify possible quiescent organisms.

Previous works (Ross and Hallock, 2016; Ross, 2012) has reported that foraminifera can survive a stressful condition by entering a low-activity dormant state. This life strategy could affect the experiment results by causing underrated survivors, so CTG was used to overcome this problem (Caridi et al., 2020).

Fundamental parameters such as pH, temperature and salinity were measured at T_0 (first day of test start) and at T_f (at the end of the test) in order to identify significant variations.

The number of live individuals was used to create the viability chart.

3. Materials and Methods: app development

Development of the app was only possible with the help of colleagues in Information Engineering.

The name given to the app is **CliccalaCicca**, a name that represents the purpose of the app, which is to take pictures of CBs.

The app is developed in Flutter (https://flutter.dev/), an open-source framework created by Google for the creation of native interfaces for iOS and Android.

The programming language used in Flutter is called Dart (https://dart.dev/).

This framework allows cross-platform development because it allows you to program the code once and then from that get an Android app and an iOS app.

for development the team use Android Studio, an IDE (Integrated Development Environment) created by Google and JetBrains.

The database is built on the Firebase platform (https://firebase.google.com/).

For the design of mockups of interfaces, engeneer used Framer (https://framer.com/).

4. Results: foraminifera

The seawater quality parameters as temperature (T), salinity (S), and pH are measured during the experiment and at the end (see the Table 8-9-10).

R. globularis		T (°C)		S (PSU)		pH	
		0 h	48 h	0 h	48 h	0 h	48 h
CTRL		22	22,6	35	36	8,34	8,26
sublethal	R1	22	22,6	35	36	8,32	8,24
	R2	22	22,6	35	36	8,34	8,3
	R3	22	22,6	35	36	8,33	8,23
LC50	R1	22	22,6	35	36	8,32	8,23
	R2	22	22,6	35	36	8,32	8,27
	R3	22	22,6	35	36	8,33	8,23

Table 8. Parameters measured at T_0 and T_f for R*osalina globularis*.

***Quinqueloculina* spp.**		T (°C)		S (PSU)		pH	
		0 h	48 h	0 h	48 h	0 h	48 h
CTRL		23	22,6	35	36	8,34	8,27
sublethal	R1	23	22,6	35	36	8,32	8,24
	R2	23	22,6	35	36	8,33	8,25
	R3	23	22,6	35	36	8,34	8,23
LC50	R1	23	22,6	35	36	8,29	8,23
	R2	23	22,6	35	36	8,31	8,28
	R3	23	22,6	35	36	8,33	8,24

Table 9. Parameters measured at T_0 and T_f for *Quinqueloculina* spp.

T. agglutinans		T (°C)		S (PSU)		pH	
		0 h	48 h	0 h	48 h	0 h	48 h
CTRL		22	22,6	36	36	8,36	8,29
sublethal	R1	22	22,6	35	36	8,34	8,25
	R2	22	22,6	35	36	8,33	8,31
	R3	22	22,6	35	36	8,34	8,25
LC50	R1	22	22,6	35	36	8,33	8,24
	R2	22	22,6	35	36	8,32	8,25
	R3	22	22,6	35	36	8,33	8,24

Table 10. parameters measured at T_0 and T_f for *Textularia agglutinans*.

The temperature of the culture medium did not change in the two measurements made for the 3 species considered for the experiment. The pH, as expected, has slightly decreased and the salinity increased from 35 psu to 36 psu (Table 8-9-10).

In the case of the control of *Rosalina globularis* after 48h, the death of two individuals is noted not due to the administration of synthetic nicotine (since the control is not subjected to pollution) but probably due to natural causes and a previous fragility (Table 11).

Rosalina globularis decrease up to 20% of vitality both in the case of the sublethal and lethal concentration.

In the case of *Quinqueloculina* spp. group, from a total of 100% of living individuals in the control, it dropped to 86% of vitality in the sublethal concentration and arrived at 66% of vitality in the lethal concentration.

Finally, for *Textularia agglutinans*, vitality varies from a total of 100% in the control to 34% with the sublethal concentration and 20% for the lethal concentration (Table 12).

		Rosalina globularis	Quinqueloculina spp.	Textularia agglutinans
		48h	48h	48h
CTR		3	5	5
Sublethal	R1	1	3	1
	R2	1	5	2
	R3	1	5	2
LC50	R1	0	4	1
	R2	1	2	1
	R3	2	4	1

Table 11. Numbers of alive individuals after 48h.

% VIABILITY			
	R. globularis	***Quinqueloculina* spp.**	***T. agglutinans***
Control	60%	100%	100%
Sublethal	20%	86%	34%
LC50	20%	66%	20%

Table 12. Percentage of viability of foraminifera.

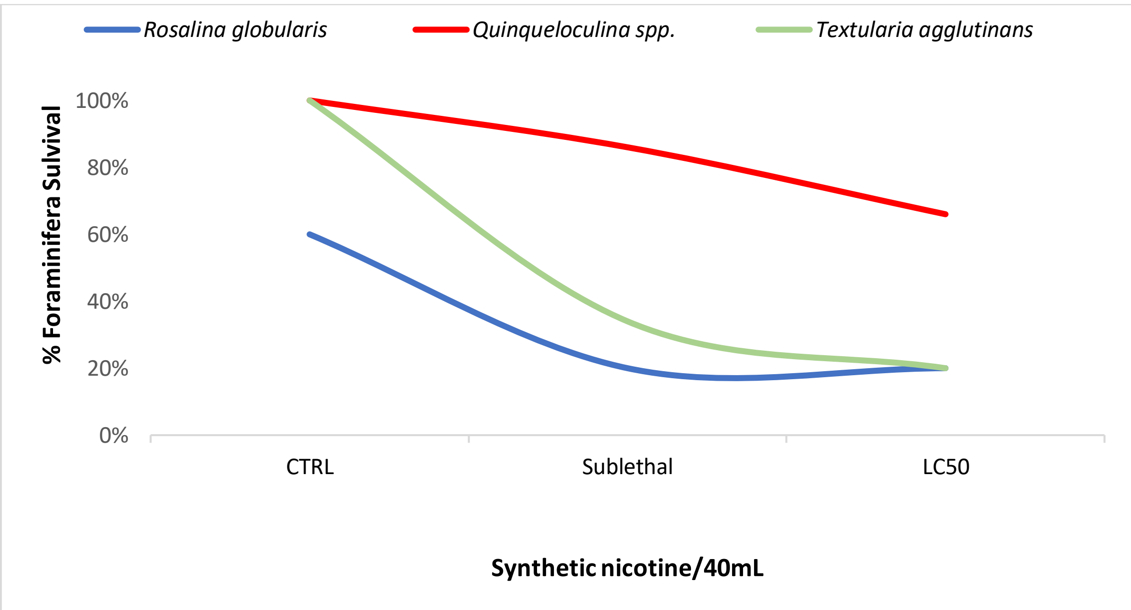


Figure 17. Graph of the % of foraminifera viability: *Rosalina globularis* (blue), *Quinqueloculina* spp. (red) and *Textularia agglutinans* (green)

5. Results: App development

After an initial consultation phase on significant and measurable input coming from users and the goal to achieve, the team focused on the most important information that is necessary for the purpose of the work.

For the graphic part, the scientific Team was inspired by the app described above like “Litterati – Clean the earth” and “Clean swell” (paragraph 1.5).

These images show all the steps that can be developed in the app.

5.1 If I am considered as a user not yet registered

As the first screen (Figure 18) there is the logo and a slogan that intrigues the user and encourages him to continue. a second screen implies registration by the user (Figure 19).

Figure 18. Stage 1: home screen with logo and app name.

Figure 19. Stage 2**:** registration phase throught various platforms (Facebook or Google) or the possibility of login if already registered.

Initially the app will present various screens dedicated to the purpose and objectives of the app to correctly inform the user who has downloaded it. These passages may also not be read by the user, and are therefore optional, so you can skip the page (Figure 20).

Figure 20. Stage 3: information and purpose of the app.

These three screens below are mandatory, and the user must give his consent in order to continue (Figure 21).

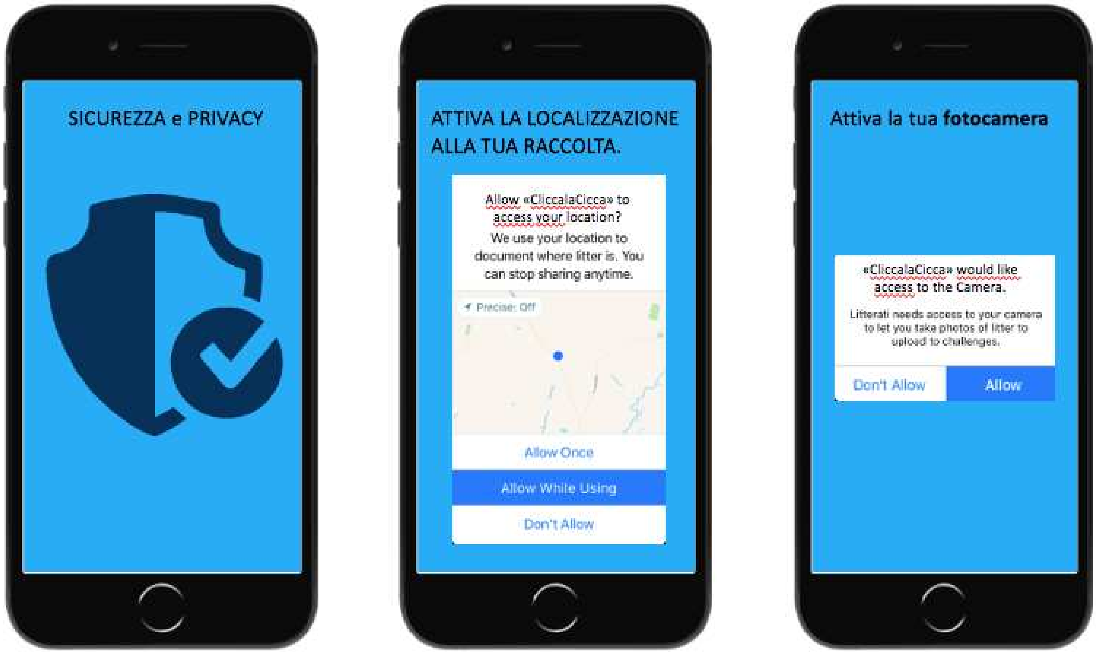


Figure 21. Stage 4: consent to privacy, location and camera.

After the request for access to the camera, the screen will open directly to be able to take the photo of the CBs that are found on the ground. In this phase, the area to be taken will be indicated by a rectangle on the screen so that you can subsequently identify the m^2 to which the photo corresponds (considering that the CBs has an approximate size. Some preliminary information is given in order to be able to shoot at best (Figure 22).

By pressing the appropriate button of the camera, you can take and send the photo to some operators who continue the counting work (Figure 23).

Figure 22. Stage 5: information to position the phone before shooting.

Figure 23. Stage 6: camera login screen.

Immediately after submitting the photo, the user is prompted to a short questionnaire to help us with the scientific purpose (Figure 24).

This questionnaire, not mandatory, it is statistically important for the scientific team: from the questionnaire it’s obtained a more or less subjective distribution of CBs in the area where the citizen took the photo. In addition to the distribution the first question, that is a pop-up, (point one) is asked only the first time the citizen sends a photo. The citizen is asked whether he is a smoker or not, the question is important to identify that part of the population most sensitive to the problem.

The answers are selected from a drop-down menu:

1. If the user is a smoker, in order to understand the sensitivity of the smoker or not towards the problem.
2. The approximate number of butts.
3. The context in which they were found, whether urban, wooded, fluvial or on the beach.
4. The density of the distribution of butts with a specific question: does the photo mirror the distribution of the whole area you are in? Random accumulation or produced by bodies proposed to clean.

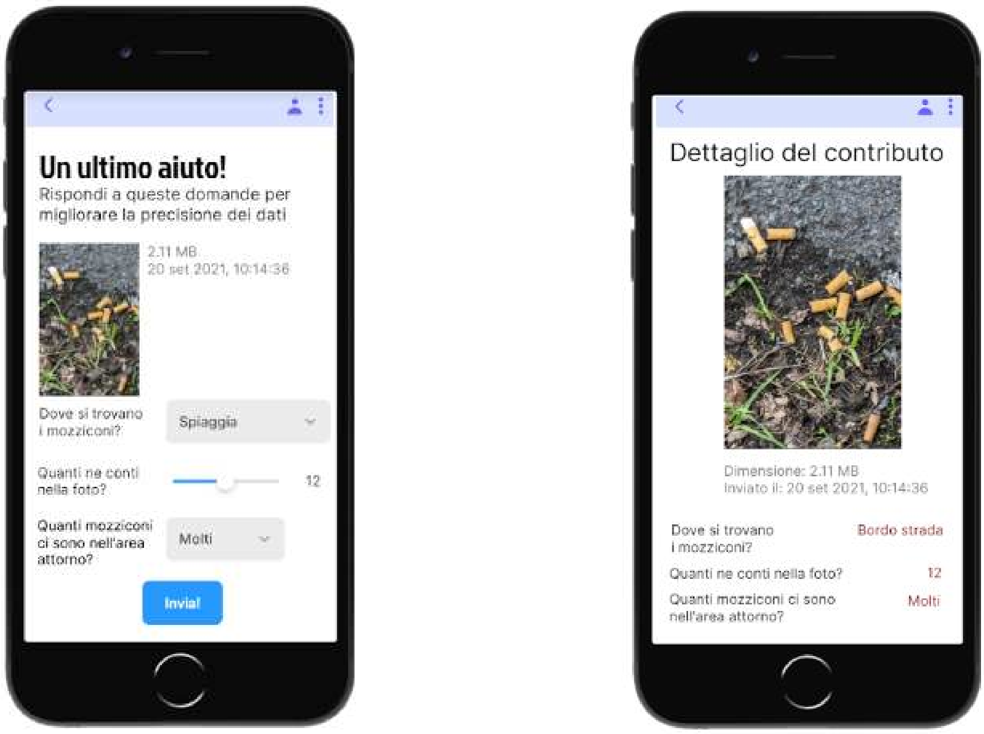


Figure 24. Stage 7: questionnaire.

5.2Registered user

In this case the registration steps required for privacy, location and camera, but also all the purposes of the app are skipped.

The home screen (Figure 25) will in fact be the following: a map of Italy will appear, and the user will also be able to see the contributions to the project of the individual regions: by clicking on the region concerned, the user may obtain the relevant data.

Figure 25. Stage 8: map of Italy.

Obviously from every page the user has access to the camera.

By clicking the 3 dots at the top right, personal contributions may be viewed with the photo, the day and the relative hour in which the contribution has been posted (Figure 26).

Contributions can also be displayed in the form of a map (Figure 27).

Figure 26. Stage 9: your contributions.

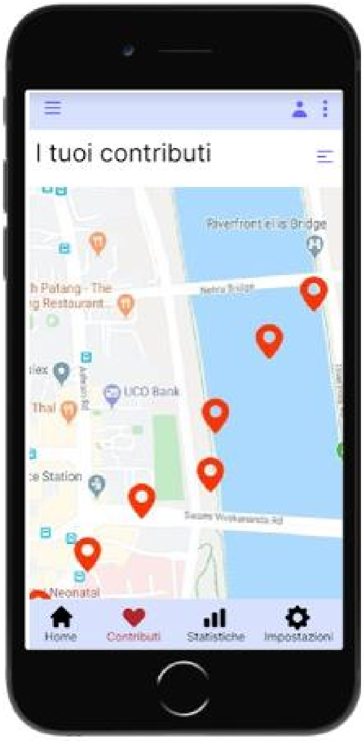


Figure 27. Stage 10: your map contribution.

General statistics are also visible (Figure 28).

Figure 28. Stage 11: general statistics.

Pop-up is sent when the user uploads a photo and possible updates of the number of records sent by other users. This Pop-up is important to motivate and incentivize the user to continue using the app (Figure 29).

Figure 29. Stage 12: Pop-up

After these first phases the operator can in fact click from the appropriate button present in the screens and thus return to display from the stage 8 (Figure 25) of the app.

5.3 Metadata useful for Researchers

Figure 30. Stage 6: camera login screen.

Figure 31. Photo of cigarette butts.

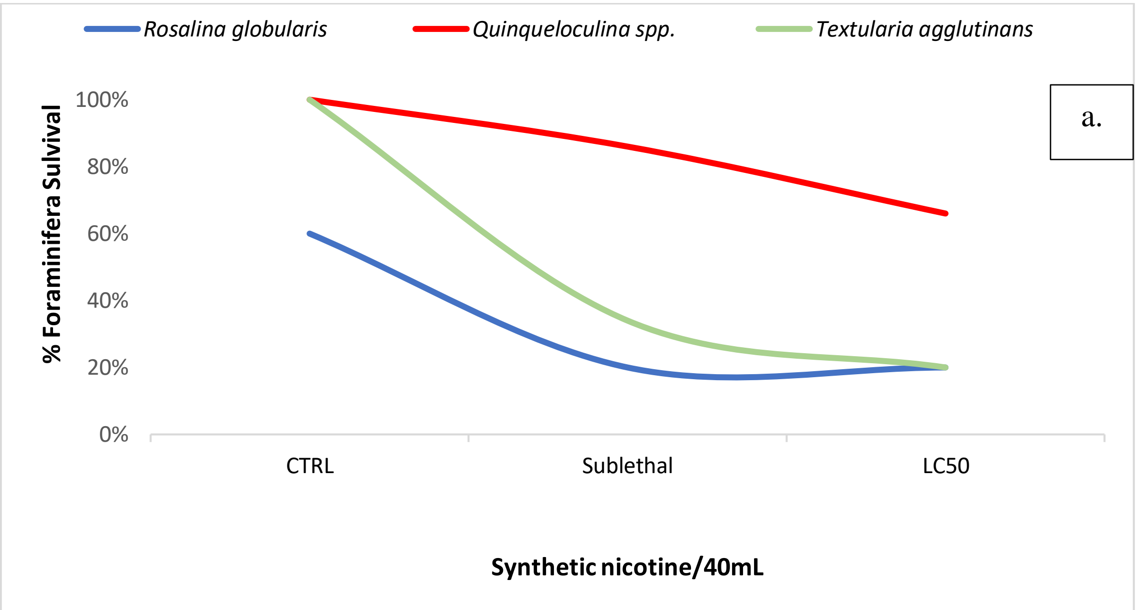
After this screen (Figure 30) where the user takes the picture (Figure 31) it's possible to see some metadata like GPS position, time, and data (Figure 32).

Metadata are then analyzed by the researchers which will extract the information and will be able to identify the areas more or less impacted by human.

Figure 32. Metadata useful for Researchers

6. Discussion: foraminifera

In this study the variations of the parameters are measured. Temperature, salinity and pH have not varied excessively, there was a slight decrease in pH. The data obtained by this degree thesis do not reflect the study previously done by Caridi et al., 2020 in terms of species-specific response to the toxicant represented in this case by synthetic nicotine leachate (Figure 33).



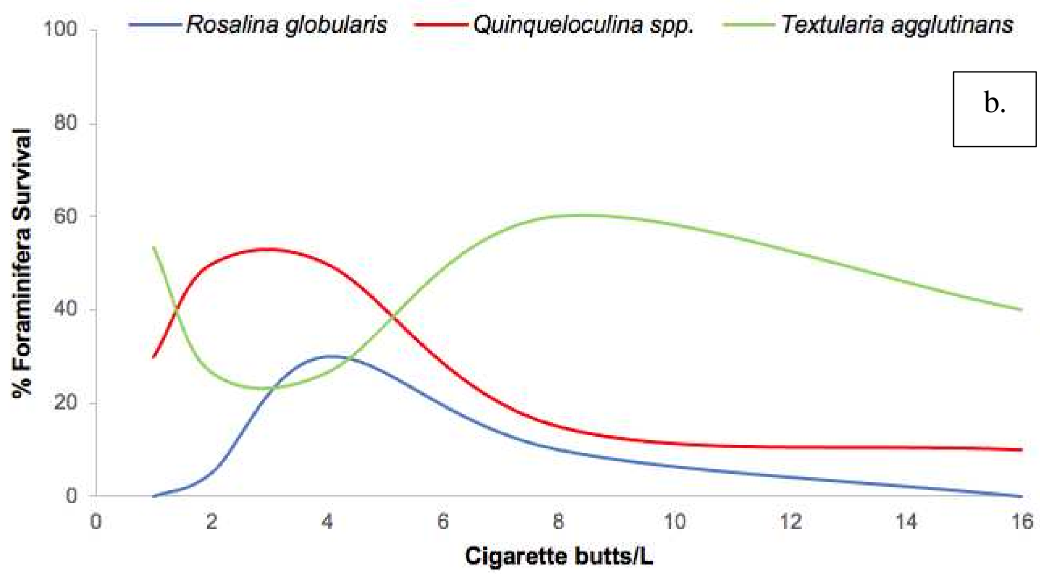


Figure 33. Comparison between the vitality of the two experiments.

a) Viability in this work; synthetic nicotine concentration vs percentage of survived foraminifera for the three studied species: *Rosalina globularis* (blue), *Quinqueloculina* spp. (red) and *Textularia agglutinans* (green).

b) Work of Caridi et al. The cigarette butts leachate concentration vs percentage of survived foraminifera for the three studied species: *Rosalina globularis* (blue), *Quinqueloculina* spp. (red) and *Textularia agglutinans* (green).

In this work *Quinqueloculina* spp. individuals have a major resistance in the current experience in comparison with the CBs leachate experiment while *Textularia agglutinans* together with *R. globularis,* do not tolerate the pollutant starting from a sublethal concentration of 4,46 µl/40 ml (1.96 mg/ L) of synthetic nicotine for *Textularia* and 2,24 µl/40 ml (3.91 mg/ L) for *Rosalina* respectively.

From the data described above it can be seen how the calcareous perforate species *R. globularis* results as the most fragile organism in culture as it starts with a viability of 60% and then decreases to 20% (see Table 13). At the same time *Quinqueloculina* spp. is a most resistant taxonomic group.

Conversely, the data obtained by Caridi et al. (2020) show that the foraminiferal vitality curve of both calcareous foraminifera *R. globularis* and *Quinqueloculina* spp., perforate and imperforate respectively, is similar.

Caridi et al. (2020) observed also a biomineralization problem on shells that it is not evident for the current experiment. In particular, starting from 4 CBs/ L a decalcification shell effect is recorded that does not correspond to individual death. Instead, starting from 8 CBs/ L the death of individuals for both calcareous genera is observed and in the case of *R. globularis*, at the highest concentration (16 CBs/ L), the curve reaches zero. The agglutinated species shows a different trend compared to the calcareous ones. In fact, at the highest CBs concentrations (16 CBs/ L) *T. agglutinans* shows partially dissolved test of the last chambers probably due to the consequent dissolution of the calcitic cement, while cytoplasm seems still well organized and it fills the rest of the shell (Caridi et al., 2020).

Quinqueloculina spp. is the most resistant species. In some papers, miliolids are cited as the most resistant to environmental stresses also represented by impact from heavy metals (Nardelli et al., 2013; Bergamin et al., 2003; Romano et al., 2003). Results strongly suggested that zinc, even at high concentrations, does not cause test deformities. (Nardelli et al., 2013). Some foraminiferal species such as *Quinqueloculina parvul*a, *Haynesinagermanica,* and *Milionella subrotunda* have been shown to be tolerant to Mn, Pb, and Zn (pollution-tolerant species) (Bergamin et al., 2003; Romano et al., 2003).

In fact, from a study of Romano et al. (2008), the percentages of abnormal foraminifera shells (Foraminiferal Abnormal Index, FAI), not exceeding the 5.5% overall; this value, therefore, does not appear particularly high considering the high contamination levels measured in the study area (Bagnoli industrial area).

As a matter of fact, for both ecotoxicological experiments (Caridi et al., 2020 and present study) the response is always species-specific and the genus *Rosalina* which biomineralizes by secreting calcium carbonate is the most sensitive to CBs toxicants and nicotine (Uthicke et al., 2013; Fujita et al., 2011; Kuroyanagi et al., 2009).

Birarda et al. 2021 show that DEHP (bis-(2-ethylhexyl) phthalate), a common plastic additive can be incorporated in the cytoplasm of calcareous foraminifera, *R. globularis*, grown in cultures. FTIR data confirm that there are noticeable biochemical differences in the shell and the cytoplasm depending on the exposure of the cell organism to microplastics and bio-accessible plasticizers.

In addition, the present work confirms the foraminifera show a strong resistance to the CBs contaminants including nicotine, because no total death of individuals is observed (Table 13 and Figure 20). We thus obtain data comparable to those obtained by Caridi et al. (2020) with leachate.

Finally, it's possible to conclude that data obtained in this degree thesis are comparable to those obtained by Caridi et al. (2020), which reported as foraminifera survive at higher concentrations of CBs than other taxa (Table 13). Some works done by Register et al. (2000) on *Daphnia magna* and Warne et al. (2002) on *Ceriodaphnia* cf. *dubia* and *Vibrio fisheri* show that foraminifera are much more resistant organisms as they show a higher EC50 for the three different species.

	R. globularis Concentration of cigarette butts / L	*Quinqueloculina* spp. Concentration of cigarette butts / L	*T. agglutinans* Concentration of cigarette butts / L	*D. magna* Concentration of cigarette butts / L	*C. cf. dubia* Concentration of cigarette butts / L	*V. fisheri* Concentration of cigarette butts / L
LC50-EC50	1.9	7.2	5.6	1.0 - 2.0	0.125 - 0.25	0.3 - 2-7

Table 13. LC50 and EC50 for three different species of foraminifera from the study of Caridi et al. (2020) and EC50 values for other organisms are given for comparison: *Daphnia magna, Ceriodaphnia* cf. *dubia* and *Vibrio fisheri* (Register et al., 2000 and Warne et al., 2002).

7. Discussion: App development

The app was developed to create a collaborative link between users, considered as citizen, and the scientific community. **CliccalaCicca** is an app specifically designed to monitor the number of CBs in various urban, rural contexts ans beaches.

The team and I thought about a project very intuitive and therefore suitable for everyone, from the youngest to the oldest. Also, possibility of being available both on Apple and Android will allow a greater diffusion.

Being able to see the contributions of other people will allow individual users to be able to compare and then be more participatory. All data will be collected (photos with their location and time, number of butts and density) and organized by researchers within a database. In the database the scientific community can, in the future, get a map of the most polluted areas of Italy and determine the potential flows of nicotine that arrive at the sea from urban runoff effluents and beaches. These data could then be used as the harmful concentration to be tested on organisms *in vivo* and *in vitro* studies.

In fact, nicotine data in marine aquatic habitats are still limited (Roder Green et al., 2014; Huerta-Fontela et al., 2008). Wright et al. (2015) conclude that the concentration of nicotine in the aquatic environment is variable; up to 32 µg/ L in effluent and 11.400 µg/ L in urban run-off It was recently estimated that one smoked cigarette filter could contaminate 1000 L of water at a concentration exceeding the predicted no effect concentration (24 µg/ L) (Roder Green et al., 2014). In comparison, reported urban run-off concentrations are comparable to the effective concentration of nicotine in the study of Caridi et al. (2020) (15.63 mg/ L for 8 CBs / L and 7.82 mg/ L for 4 CBs / L). These estimates confirm that the marine species in proximity to urbanized areas are at risk of nicotine exposure via run-off contaminated with smoked CBs and their leachates.

Owing to the ubiquitous nature and magnitude of cigarette butts discharged into the environment, the data elaborated from the app will help to determine whether cigarette butt waste can exert ecotoxic effects when in aquatic environments.

The app development project was inspired by other existing litter apps. These apps use the help that citizens provide to collect large amounts of data. **CliccalaCicca** was inspired by previous Citizen Science apps such as "Clean swell" and "Litterati - Clean the earth" for the graphics.

This app prototype can be considered different from other similar ones already available. It has the characteristic of having an important scientific purpose for future studies on how the substances that impact on organisms are the direct or indirect result of an abandonment of waste (the butt in this case) in the environment, and at the same time, of raising awareness among citizens about the problem. Is an app that focuses on a single "emerging" contaminant as opposed to other described apps which include various types of pollutants and generic waste.

Figure 34. Poster for the presentation of SHARPER 2021.

In order to evaluate the possible app imperfections but also to get feedback from the population, the latter was previewed through a prototype at the European Reaserchers' Night – SHARPER (SHAring Researchers' Passion for Engaging Responsiveness).

"SHARPER project was born to respond to the need to enhance the figure of researchers and their role in society. To achieve this goal, the main idea of the promoters of Sharper has been, since 2013, to interpret the night as an opportunity to share with the general public the passions that animate researchers in their work, discovering that these passions are common to everyone”.

During this event it was possible to show the app and understand the degree of interest from citizens (Figure 35).

Figure 35. Presentation of the **CliccalaCicca** prototype app brought to SHARPER.

It was also possible to increase the impact of scientific dissemination thanks to the creation of some posters explaining well the problem of CBs in order to capture the attention of less aware citizens (Figure 36).

Thanks to people feedback, scientific team can understand that the theme of the sea pollution and in particular that caused by CBs is at the center of attention.

Obviously, when the app will start to be used by a larger number of users, researchers will be able to actively confront with these users asking them what the possible improvements of the app in order could be to make it more intuitive and effective.

Figure 36. Posters created by our research group for SHARPER.

8. Conclusions

As can be seen from Tables 8-9-10 in paragraph 4 (Results: foraminifera) it can be seen that there is a slight decrease in the pH of the solution in which the organisms are found. From these results it can be deduced that this "emerging" source of pollution (CBs) could contribute to the already existing problem of acidification and therefore interfere with biocalcifying organisms such as foraminifera.

The harmful effect of synthetic nicotine on the 3 species of foraminifera is established; in particular, the percentage of survival is low and species-specific for both lethal and sublethal concentration of the toxicant. As observed in Caridi et al., 2020 the pH lowering could affect the foraminiferal shells and promote their dissolution.

These preliminary data lead to suppose that the environment and its biocalcifying inhabitants could undergo the same effects.

Therefore, the CBs are not only defined as a physical pollutant, because it can be ingested by animals, but also as a chemical one due to the toxicants present in them. As exposed above, these chemicals that potentially arrive at the coastal areas and the relative shallow-water may lead to a drastic lowering of the viability of organisms.

Data of this degree thesis confirm that CBs and the relative nicotine toxicant impact marine organisms and this frame is a direct consequence of the indiscriminate abandonment of the CBs waste in the environment. In order to improve our knowledge of the number of CBs accumulated in the environment and so on the nicotine concentration dispersed in the coastal sea water I contributed to develop the app **CliccalaCicca**. Nowadays the smartphone is one of the most powerful tools for socializing information/data and that is why it was taken into consideration as a center for dissemination of scientific information.

In these initial phases of **CliccalaCicca** development, a scientific committee will take care of the precise count of the butts that are photographed as they are fundamental to have real quantitative data that can be used. Obviously, the team also rely on citizens for estimation by asking them (stage 6 - Figure 23) to give an approximate number of litters. This step becomes crucial for the researcher when a large amount of data must be analyzed.

The app development project was inspired by other existing litter apps. These apps use the help that citizens provide to collect large amounts of data. **CliccalaCicca** was inspired by previous Citizen Science apps such as "Clean swell" and "Litterati - Clean the earth" for the graphics. But this app prototype can be considered different because it can help the scientific community to better understand how toxicants impacting on marine organisms are the direct or indirect result of a waste abandonment (the butt in this case) in the environment; moreover, the **CliccalaCicca** will raise awareness among citizens about the problem.

I expect that the app **CliccalaCicca** became an important scientific purpose for future studies, and at the same time, to raise citizens' awareness on the problem.

Figure 37. Possible pathways for human health risks (Novotny et al., 2014)

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