



Biogeochemical fate of emerging Anthropogenic pollutants in the sedimentary Record

Elisa Costanzi

DiSVA, Laboratory of Stratigraphy, Sedimentology and Paleoecology

Tutor: Prof. Anna Sabbatini

Objective

The purpose of the project is to investigate the impact of littered smoked CBs and its associated toxicant, particularly nicotine on benthic foraminifera by evaluating the effect they cause on their shells, which leave a trace over time, considering them as an index of anthropogenic pollution on the marine environment.

Acute toxicity test on selected benthic foraminiferal species

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Cigarette butts, a threat for marine environments: Lessons from benthic foraminifera (Protista)

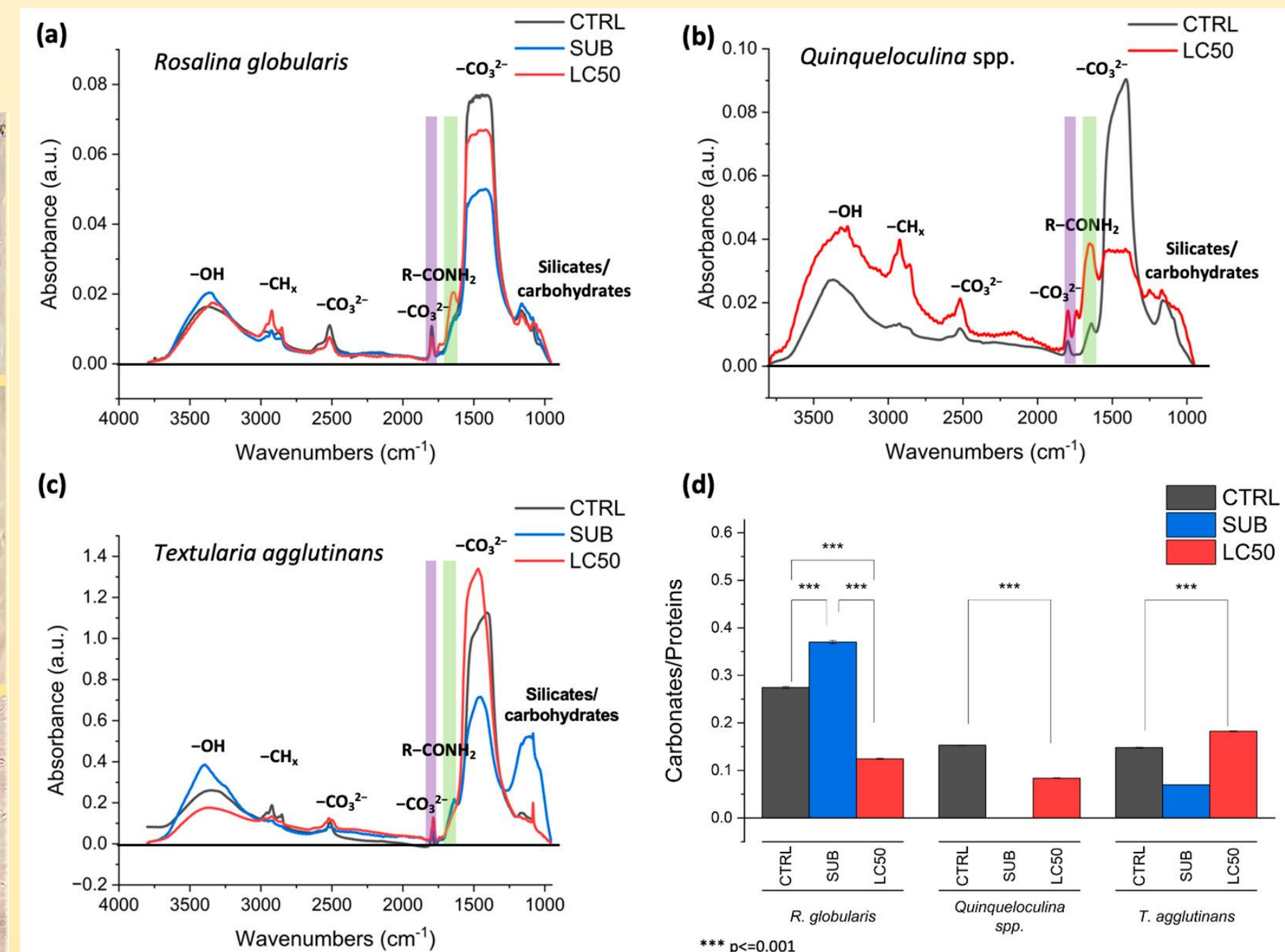
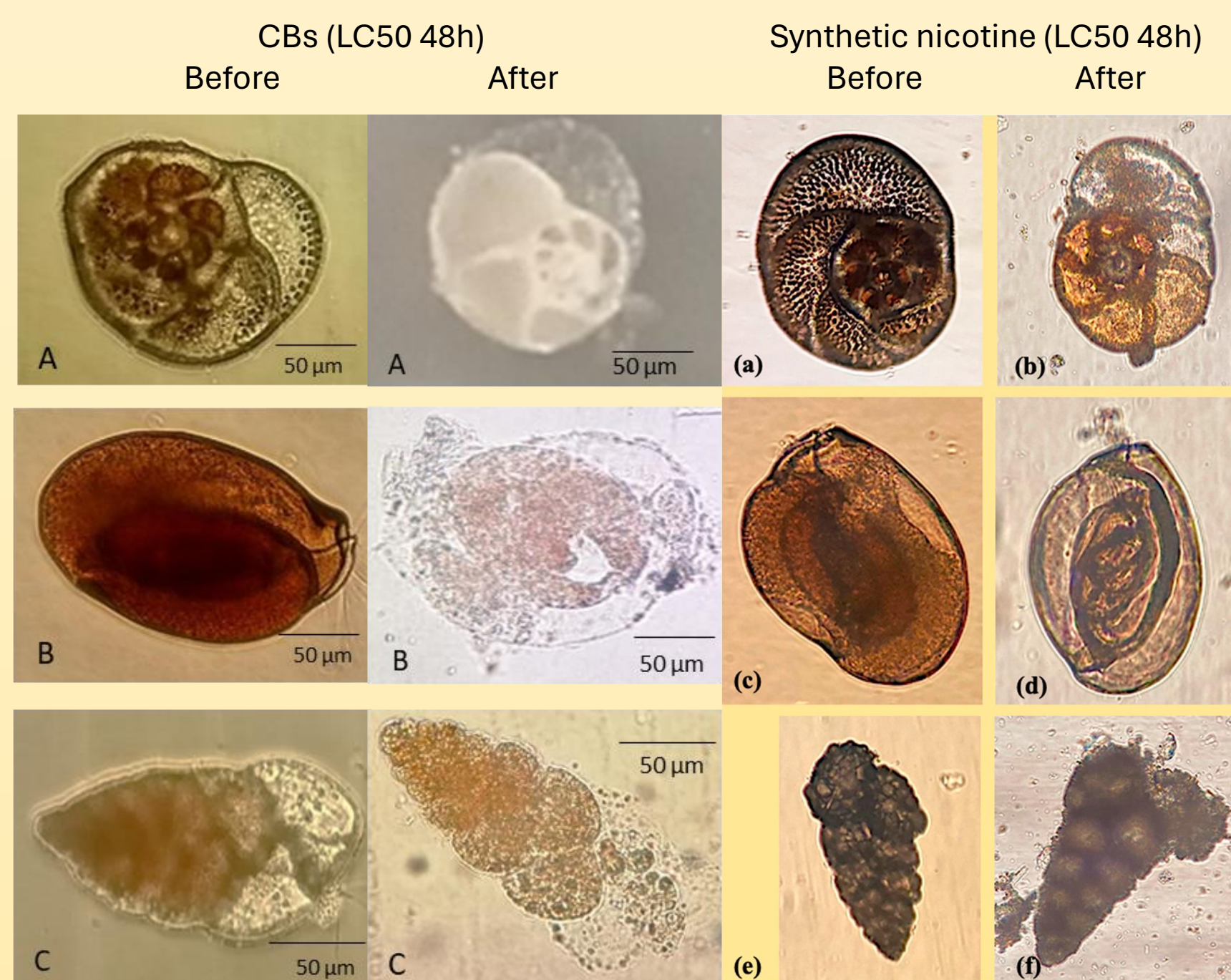
Francesca Caridi¹, Anna Sabbatini², Giovanni Birarda³, Elisa Costanzi^{1,3}, Giovanni De Giudici⁴, Roberta Galeazzi⁵, Daniela Medas⁶, Giovanna Mobbili³, Massimo Ricciutelli⁵, Maria Letizia Ruello⁶, Lisa Vaccari⁷, Alessandra Negri⁸

These findings indicate the potential toxicity of cigarette butt leachate, which is associated with a reduction in pH and the release of toxic substances, particularly nicotine.

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Article
Response of Foraminifera to Anthropogenic Nicotine Pollution of Cigarette Butts: An Experimental Approach

Anna Sabbatini^{1,4}, Francesca Caridi¹, Giovanni Birarda², Elisa Costanzi^{1,3}, Adolfo Amici³, Giovanna Mobbili³, Carla Buosi⁴, Giovanni De Giudici⁴, Daniela Medas⁴ and Alessandra Negri¹



Chronic toxicity test on benthic foraminiferal fauna: mesocosm approach

Synthetic nicotine LC50

5 cm sediment cores
340 ml unfiltered seawater

Taxonomic composition

Total benthic foraminifera

Average living depth

Sediment cores
Setting up mesocosms in sediment cores allowed us to assess, besides the density, biodiversity and taxonomic composition of foraminifera in relation to the presence of the pollutant, also their vertical distribution.

Figure 1. *Psammophaga zirconia* (e.g. TMO)
Figure 2. *Eggerelloides advenum* (e.g. TPA)
Figure 3. *Ammonia parkinsoniana* (e.g. TPC)

Areal mesocosms
Areal mesocosms were set up at the aquarium infrastructure (DiSVA, UnivPM).

Individuals were isolated for structural analysis (Sissi, HR-TEM) from each sample.

Data in process

Name of the time series	T0	T1	T2	T3	T4
Duration of the experiment (days)	0	30	45	60	75
Name of the samples	T0	T1-ctrl T1-R1 T1-R2 T1-R3	T2-ctrl T2-R1 T2-R2 T2-R3	T3-ctrl T3-R1 T3-R2 T3-R3	T4-ctrl T4-R1 T4-R2 T4-R3
Method	T0, T-CTRL, T-R1, T-R2 CTG; T-R3 RB				

Pollutants (nicotine-DEHP) detection in marine water and sediment (in collaboration with DiSCO)

A protocol for nicotine extraction from seawater samples was developed: (1) sample filtration, (2) extraction, concentration and purification of nicotine by SPE-C18, followed by (3) HPLC analysis.

Synthetic nicotine LC50 SUB-LET

Eight mesocosms were established at the Aquarium Infrastructure (DiSVA, UnivPM) to assess the higher affinity of nicotine for seawater or sediment (plastic vs. glass). HPLC analysis of the aqueous matrix revealed that in the presence of sediment, regardless of the container material used, the residual nicotine concentration in the water column was less than one-third of the amount introduced.

Accordingly, an additional protocol for extracting nicotine from sediment was devised, incorporating soxhlet extraction, which was then added to the previously established protocol.

The results of the HPLC analysis confirmed the higher affinity of nicotine for sediment.

Biomineralization studies

200 Frozen Forams + Liquid nitrogen

Mechanical crushing

Total proteins (T)

Supernatant Cytoplasmic proteins (S)

Pellet Membrane proteins (P)

SDS-PAGE

Western blot

Annexins are a family of proteins that is able to bind the plasma membrane in the presence of calcium ions and is involved in mineralization processes.

Western blot

Enzyme-conjugated Secondary Antibody

Enzyme Substrate

Primary Antibody

Target Protein

Membrane Containing Transferred Protein

Detection Signal (colorimetric or chemiluminescent)

Anx13 36 kDa

Given that nicotine is responsible for the decalcification of the foraminifera shell, and that the process of biomineralization takes place under strict control of the cell, the aim is (1) to identify a protein (molecular target) involved in biomineralization and (2) to test its sensitivity / resistance / response to the presence/concentration of the pollutant (plan for the future).