PhD course in Sustainable Development and Climate Change
University School for Advanced Studies IUSS Pavia
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BIogeochemiCal fate of emerging Anthropogenic pollutants in the sedimentary Record

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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.

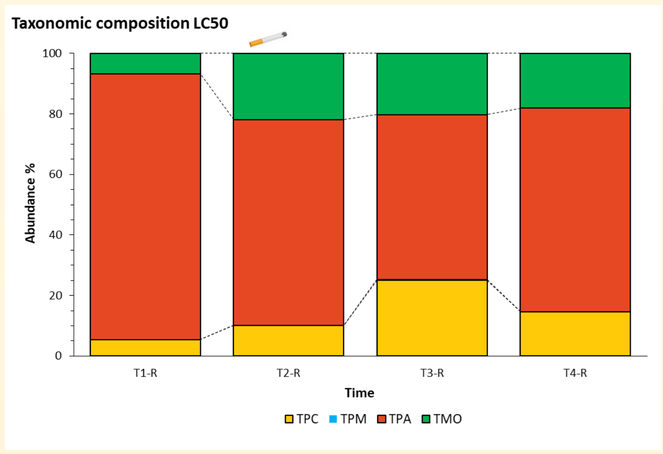
Chronic Toxicity Test on Benthic Foraminiferal Fauna: Mesocosm Approach

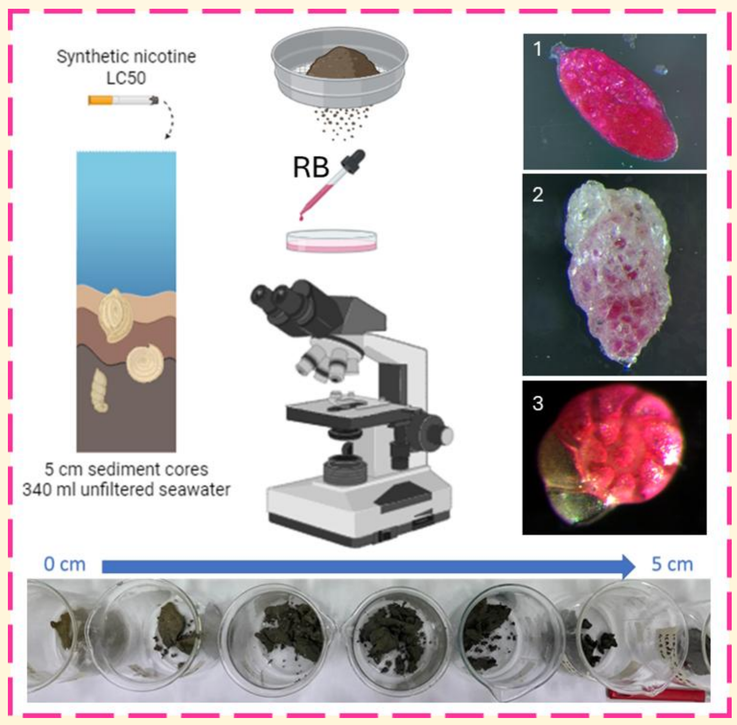
Two experimental approaches were used to assess chronic toxicity: sediment cores treated with Bengal Rose dye and areal mesocosms using Cell Tracker Green. In the sediment cores, lethal concentrations were analyzed vertically over 75 days, focusing on foraminiferal density, biodiversity, and vertical distribution. The areal mesocosms allowed simultaneous testing of both lethal and sublethal concentrations.

Chronic toxicity test on benthic foraminiferal fauna: mesocosm approach

Sediment core

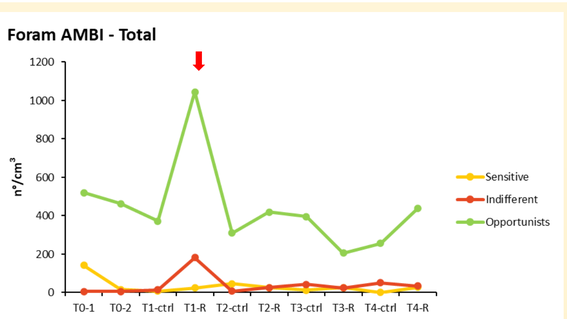
In the sediment cores, agglutinated species were the most abundant. Their density declined until Time 3, followed by a recovery at Time 4, coupled with downward vertical migration. This adaptive response supports the overall increase in total abundance.

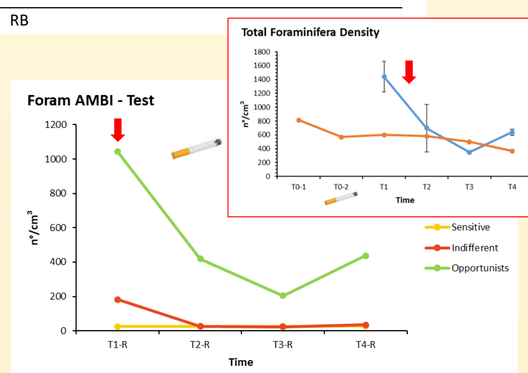




Name of the time series	T0-1	T0-2 + Start	T1	T2	T3	T4
Duration of the experiment (days)	0	45 from T0-1	30 from T0-2	45 from T0-2	60 from T0-2	75 from T0-2
Name of the samples	T0-1	T0-2	T1-ctrl T1-R1 T1-R2	T2-ctrl T2-R1 T2-R2	T3-ctrl T3-R1 T3-R2	T4-ctrl T4-R1 T4-R2
Method			RB			

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





The foram AMBI index, a sensitivity measure, showed a peak in opportunistic species already at Time 1 in contaminated samples—mirroring the trends observed for agglutinates and total foraminifera.

Chronic toxicity test on benthic foraminiferal fauna: mesocosm approach

Areal mesocosm

In areal mesocosms, density trends showed mirror-image patterns between lethal and sublethal concentrations. Calcareous species were dominant but exhibited evident signs of decalcification, with a marked decline toward Time 4.

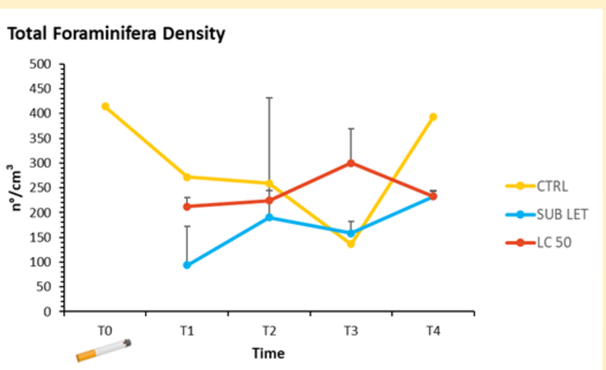
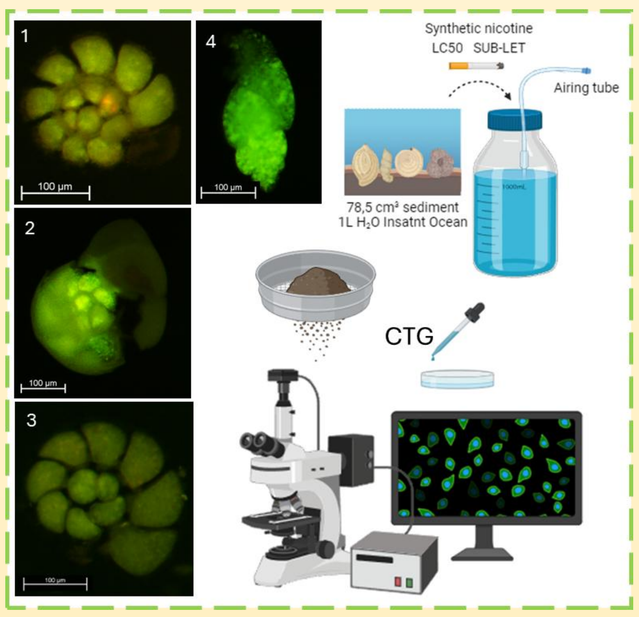


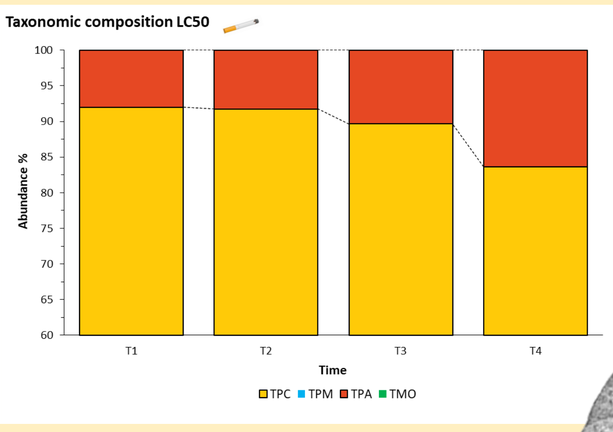
Figure 1, 2, 3. *Ammonia parkinsoniana*
Figure 4. *Bulimina elongata*

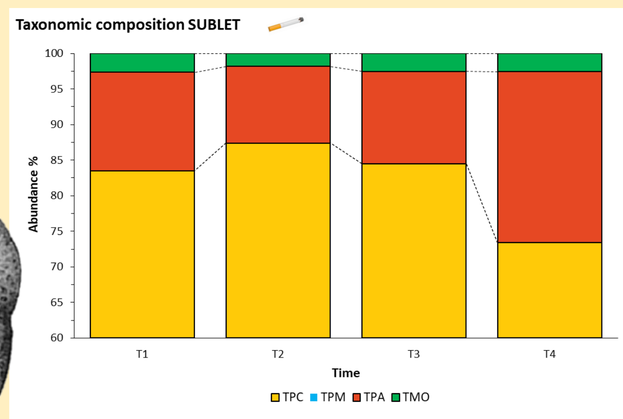


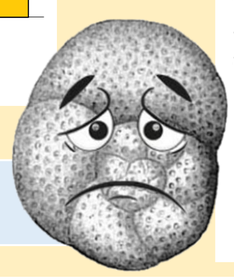
Individuals were isolated for structural analysis (Sissi, HR-TEM)

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				



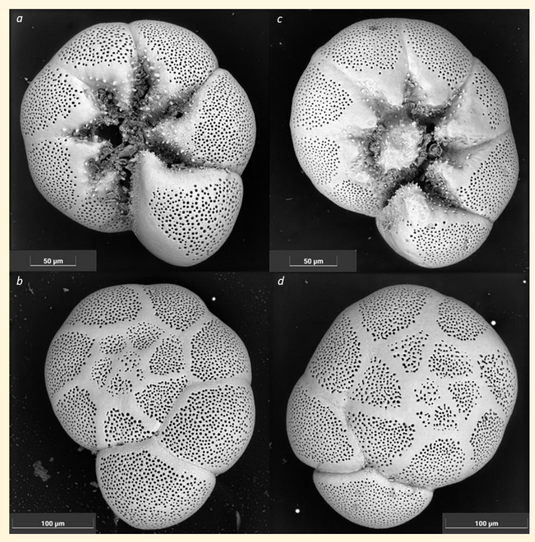




Calcareous foraminifera showed clear signs of decalcification

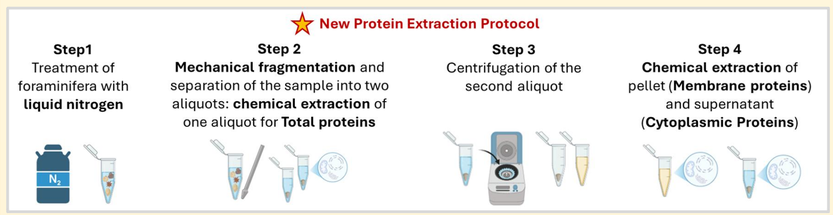
Biomineralization Studies

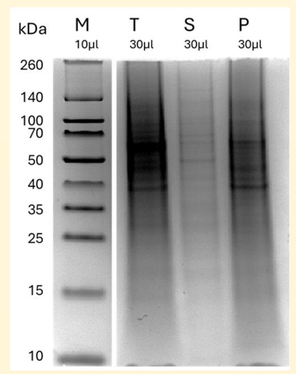
Following evidence of shell decalcification in foraminifera, biomineralization studies were initiated in collaboration with the **CNRS** and the **Natural History Museum** of Paris. Acute toxicity tests revealed that cigarette butts acidify the medium, while synthetic nicotine does not alter pH, suggesting that decalcification is due to acidification but to disruption of the cellular calcification machinery.



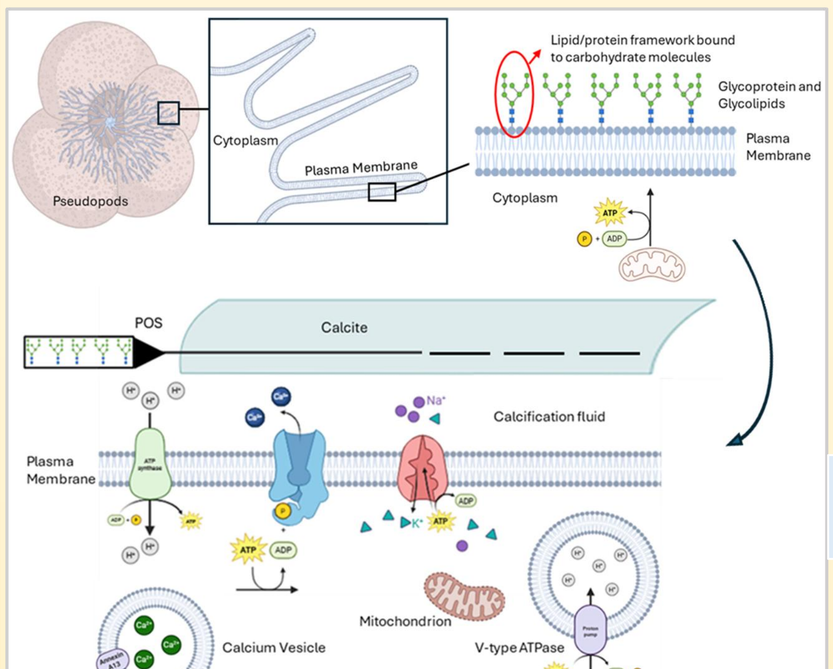
SEM images of Ammonia tepida: *(a)* Ventral, *(b)* Dorsal; and Ammonia parkinsoniana: *(c)* Ventral, *(d)* Dorsal.

To investigate this, we developed a protocol for protein extraction from foraminifera, separating total, cytoplasmic, and membrane protein fractions.



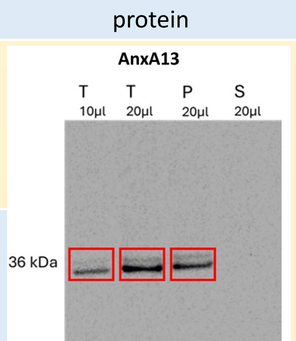


T (Total proteins)
P (Membrane proteins)
S (Cytoplasmic proteins)
M (Marker molecular weight)



Our focus then shifted to the Annexin protein family, known for membrane-binding in the presence of Ca^{2+} and roles in biomineralization.

Western blot
Annexin A13 as membrane protein



Mass spectrometry and **Proteomic** analysis revealed:
- Ion transporters on the plasma membrane
- Clathrin-associated trafficking proteins, indicating vesicle-mediated transport
- An integrated model of Ca^{2+}/H^{+} regulation and intracellular trafficking
- Annexin A13 potentially plays a central role in nucleation.

Ongoing chronic toxicity test will provide insight into the molecular impact of nicotine on shell-forming proteins

Nicotine Detection in Marine Water and Sediment

To better understand the environmental fate of nicotine, we aimed to assess its potential to interact with abiotic marine matrices—specifically seawater and sediment—with a focus on its persistence over time. This evaluation is crucial to determine whether nicotine can become integrated into the sedimentary record, thus representing a long-term contaminant and potential environmental tracer.

Results demonstrated that nicotine was undetectable in seawater samples, likely due to its hydrophobic nature, which promotes adsorption to sediment particles.

Sample collection:
- Shoreline: surface sediment
- Offshore: surface and ~ 20 cm deep samples (10m water column depth).

Preliminary results have shown the **protocol's ability to detect nicotine** in all three samples, also demonstrating its **persistence** in the **deeper sediment layers**