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MEDFLUX: Effects of Mercuric Chloride and Protease Inhibitor on Degradation of Particulate Organic Matter

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Introduction – Mercuric chloride (HG) can stop microbial activity by reacting with protein sulfhydryl groups, thus denaturing most enzymes irreversibly. In laboratory and field studies, HG has often been used to preserve marine organic matter in decomposition experiments or sediment trap deployments. However, it is not exactly known how HG affects the quantity and composition of organic matter. Protease inhibitors (PI) can be used to prevent hydrolysis of proteins by blocking active sites of proteases. The effectiveness of PI in inhibiting exoenzymatic activities during degradation of marine particles has not been well studied. The objective of this study was to investigate the effects of HG and PI on the quantity and composition of particulate organic matter in a one-month degradation experiment.

Materials and Methods

- A marine diatom, *Thalassiosira pseudonana* 3H, was used to simulate particulate organic matter.
- The diatom culture was split into 7 aliquots. No preservative was added to two control aliquots; $HgCl_2$ (180 μM) was added to two aliquots; 0.5 ml PI cocktails (Sigma P8465) was added to two other aliquots; both $HgCl_2$ (180 μM) and 0.5 ml PI were added to one aliquot.
- One of each HG-treated (HG-0), PI-treated (PI-0) and non-treated control (Ctrl-0) sample was filtered immediately. The remaining samples were incubated at 9°C in the dark for 33 d.
- POC and PN, amino acids, fatty acids, sterols and Chl-*a* were measured in samples taken at various times.
- Principal components analysis (PCA) was applied to the compositional data of all treatments.

Results and discussion

1. Quantitative changes – POC, particulate amino acids (PAA), and fatty acids were all extensively degraded in control and PI-treated samples after 33 d; about 50% of POC and PAA and 85% of fatty acids were lost. In contrast, only 10-20% were lost in HG-treated samples (Fig. 1a). Based on the measurement of amino acids in the dissolved phase, we attributed the materials lost in HG-treated samples to dissolution. However, certain fatty acids including 16:3, 18:3 and 18:4 were lost significantly in HG-treated samples, probably due to either preferable dissolution or oxidation. A typical example of fatty acids (16:3) is shown in Fig. 1b.

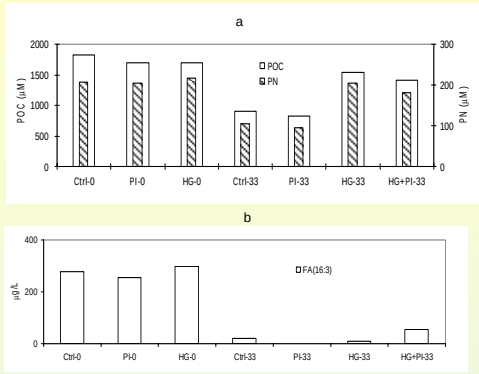

Fig. 1 Concentrations of (a) POC and PN, and (b) 16:3 fatty acids among different treatments; 0 and 33 represent 0 and 33 days, respectively.

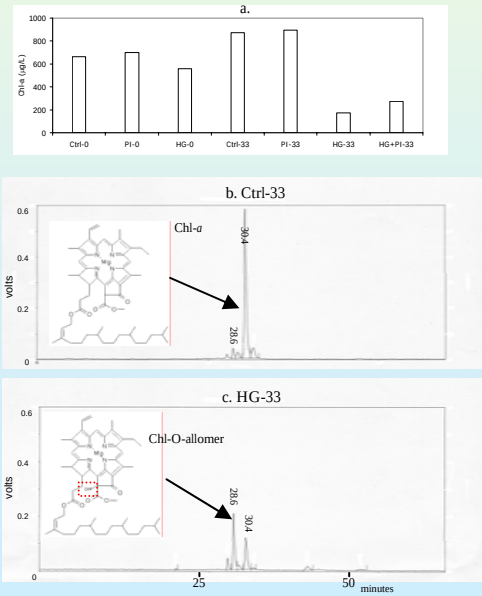

Fig. 2 Chl-*a* concentrations in different treatments (a) and chromatograms of Chl-*a* in control (b) and HG-treated (c) samples after 33 d.

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2. Allomerization of Chl-*a* – Chl-*a* concentrations in Ctrl- and PI-treated samples increased 20% after 33 days compared to time-zero samples, suggesting that degradation released some bound Chl-*a* from Chl-protein complexes, and the bound Chl-*a* otherwise could not be extracted by pure acetone. However, in HG-treated samples, Chl-*a* decreased by a factor of 4, while an "unknown" peak was greatly enhanced. Using HPLC-ToF-MS, we assigned this "unknown" peak as Chl-O-allomer. Allomerization in sinking particles from sediment traps (allomer/Chl-*a*: 0.33) was not as extensive as in this fresh diatom culture (allmoer/Chl-*a*: 1.32). Sinking particles contained degraded diatom material; perhaps much of the Chl-*a* in the degraded diatoms had already been released from the Chl-protein complex before sinking, resulting in a lower allomer/Chl-*a* ratio. Sediment trap particles would include mixtures of phytoplankton; Chl-*a* in these plankton might allomerize differently than in the *Thalassiosira pseudonana* 3H culture we used.

3. Compositional change – PCA was applied to the compositional data of all treatments (Fig. 3). The samples were clearly separated into 3 groups: 3 time-zero samples, 2 biologically degraded samples along the X axis, and 2 HG-treated samples along the Y axis. Group 1 was enriched in POC and PN, LYS, and GLU amino acids, 14:0, 16:1, 16:3 fatty acids and 24-methylcholesterol; group 2 was enriched with PHE, GABA, HIS, LEU and ILE, 15:0, 18:1ω7, 18:0, 18:1ω9 and 22:6 fatty acids, and 24-ethylcholesterols. Along the y-axis (PC2), group 3 was mostly enriched in ARG, TYR, THR, and 16:0 and 14:0 fatty acids, and Chl-O-allomer.

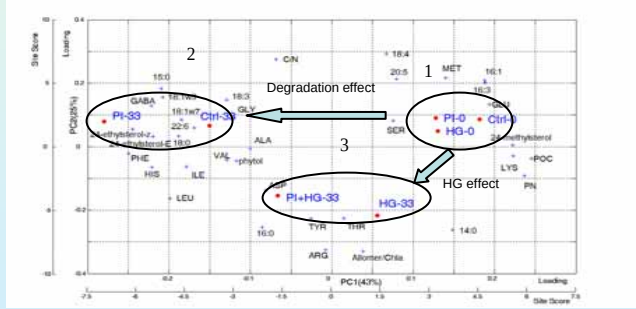

Fig. 3 PCA on compositional data of all treatments.

Conclusion – In general, particulate organic matter (POM) from a diatom culture was preserved well by HG, but not PI. However, HG had both quantitative and compositional effects on the diatom organic matter. Quantitatively, about 10-20% of POM was lost in a month due to dissolution. Compositionally, arginine, tyrosine and threonine were slightly enriched, and 16:3, 18:3 and 18:4 fatty acids were significantly lost. More importantly, Chl-*a* was rapidly allomerized into Chl-O-allomer in the diatom matter. Therefore, care is needed in studies using HG as a preservative.