Print

The Indonesian Throughflow: Data Analysis

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Please check back soon for more on the data analysis process...

Sediment Preparation

Samples in the freeze-drier at -41ºC. (A.W. Jacobel)

The first step in the data analysis is to prepare the sediments for analysis.

When sediments are initially removed from the multi cores and placed in plastic bags for storage, the sediment is highly saturated with water. To get an accurate measure of how much our sample weighs (important for later analyses) we must remove the water. To do this we first freeze the samples (to solidify any water) and then freeze-dry them to remove it.

After the water has been removed from the samples they are powdered and a fraction (less than a gram) is bottled for total organic carbon (TOC) and total % nitrogen analyses. Normalization of our data to % TOC will help us minimize the effects of different sedimentation rates on our data and ratios of C/N have the potential to help us discern the sources of our sterols.

The remaining fraction of the powdered sediment is then dissolved with an organic solvent composed of 10% methanol (CH_3OH) and 90% dichloromethane (CH_2Cl_2). While dichloromethane (DCM) is the dominate component of the solution, the use of methanol is important because it is a highly polar molecule and thus aids in the dissolution of the polar lipids (like dissolves like). Both DCM and MeOH are dangerous to human health so I work under the hood and wear goggles and gloves.

Once these steps are complete the sample is ready to be placed in the microwave extractor.

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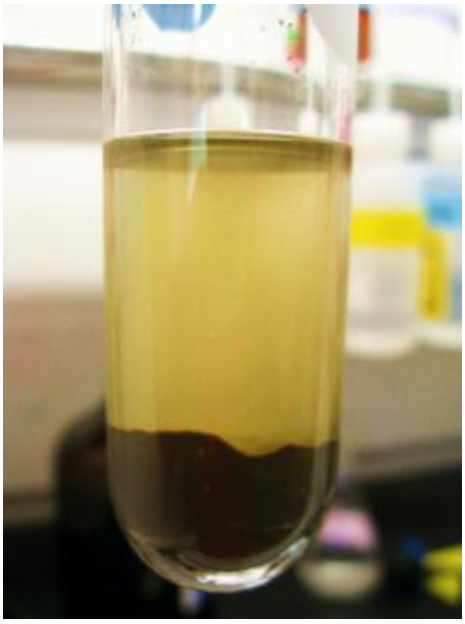
A MARS Xtraction Unit. (A. W. Jacobel)

Total Lipid Extraction

In this step of the sediment analysis, lipids contained in the sample are separated from other compounds and removed for further analysis. This process is accomplished through the use of a microwave extractor, in our case a Mars Xtraction unit with Fiber Optic temperature and pressure controls.

When the samples are removed from the Xtraction unit they contain two distinct layers. On the bottom is a layer of dark-colored sediment and on the top is a light yellow layer of fluid which contains organic solvent and our total lipid extract (TLE). Because some of the TLE might still exist in the sediment, we repeatedly use a centrifuge to spin it out.



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Extracted Sediment (A.W. Jacobel)

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The nitrogen blowdown apparatus and heatblock (inset). (A.W. Jacobel)

Blowdown and Derivatization

After the total lipid extracts have been seperated from the sediment, the next step is to remove the organic solvent from the TLE. This is accomplished by the evaporation of the organic solvent using a nitrogen blowdown system. The vials containing solvent and TLE are placed under needles through which nitrogen gas is blowing. After a short period of time no liquid remains and we can move on.

After blowdown is complete we add 1ml of DCM to the vials so that they are all volumetrically equal. From this volume we take an aliquot of 100 ul and combine it with 50 ul pyridine and 50ul Bis(trimethylsilyl)trifluoroacetamide (BSTFA). This step prepares the samples to be run on the gas chromatograph (GC) and is called derivatizing. The final step in the process is to place the vials in a heat block for 30 min at 70 ºC.

Instrumental Analysis

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The coupled GC-MS. (Thermo Scientific)

Once the samples have undergone derivitization they are ready to be run on the Gas Chromatograph-Mass Spectrometer or GC-MS. In Dr. Eglinton's lab we use a Thermo Finnigan Trace GC Ultra coupled with a Finnigan TEMPUS Time of Flight (TOF) MS (web site).

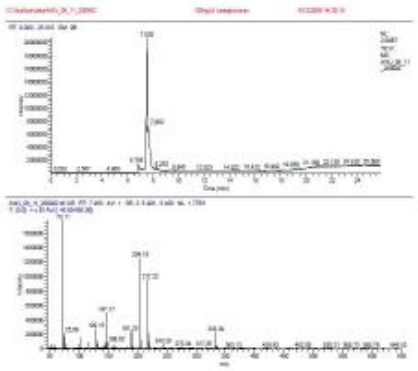
Gas Chromatograph

A GC is an important tool in an organic chemists lab. The GC vaporizes the compound under analysis and brings it into contact with the walls of the column (inside the GC.) This column is coated with different phases of silica which causes the analyte to emerge from the column at different times. When these retention times are compared, we can get an idea of the compound's identity.

Time of Flight Mass Spectrometer

The TOFMS is connected to the GC and carries out a kind of mass spectrometry in which ions are created from the analyte and then accelerated down a tube by an electric field of a known strength. At the end of a tube is a detector, which measures the time an ion takes to travel the length of the tube. From this 'time of flight' the TOFMS calculates particle velocity, which is a function of an ion's mass-to-charge ratio. This value is then compared with known compounds and the identities of the ionized particles determined.

Data Processing



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Analysis Output
Top panel shows a chromatogram for the compound levoglucosan (standard). Bottome panel shows a plot of intensity versus mass-to-charge ratio which can be matched to the known plot for levoglucosan (visual and probability comparisons). (A. W. Jacobel)

Once data has been collected from the GC-MS we use a computer program to create a chromatogram. This is a plot of detector response (y-axis) versus retention time (x-axis). The resulting graph can then be used to identify analytes.

In our case, we are interested not only in qualifying our compounds but also in quantifying them. By calculating the area under the peak of the chromatogram (integration), the concentration of an analyte can be easily determined.

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