Analysis of heavy metals in small insects

In the discussion below, Pb is used as the example heavy metal and can be replaced with the heavy metal you are interested in.

Solutions

Concentrated Pb standard

To a 100 ml volumetric flask add 2.8 ml of high-purity concentrated HNO_3 and 1 ml of 1000 ppm Pb stock standard. Dilute to volume and pour into a clean storage bottle.

Concentrated standard	
Pb	10 ppm
HNO_3	2%
Total volume	100 ml

Diluting solution

Fill a 2000 ml plastic storage bottle with DI water and add 2 ml of concentrated, high-purity HNO_3, and 0.01 ml of 1000 ppm Bi stock solution. Bi is the internal standard. You don't need a volumetric flask. It doesn't matter so much what the bismuth concentration is, just that it is the same for all samples, blanks, and standards.

Diluting solution	
Bi	5 ppb
HNO_3	0.07%
Total volume	2000 ml

Day 1: initial prep

1. Samples should be in plastic 13-15 ml test tubes. We have had very good results with Falcon brand polypropylene and polystyrene disposable centrifuge tubes with blue screw caps. Have four 50 ml Falcon brand centrifuge tubes for two blanks and two standards.
2. Add 0.5 ml of high-purity concentrated HNO_3 to each of the unknown test tubes (5 x 0.5 ml to the blank and standard tubes). Soon afterward, add 0.1 ml of high-purity 30% H_2O_2 (5 x 0.1 ml to blanks and standards). Make sure that all insects are immersed in the solution.
3. Leave overnight at room temperature. The hollow chitin exosleletons largely remain, though they become somewhat swollen and bleached, and the acid turns pale-yellow. Longer digestion times and other, more aggressive extraction techniques do not yield significantly more Pb in solution.

Day 2: final dilutions

Sample type	Add diluting solution, ml	Add concentrated standard solution, ml	Final volume	HNO_3	Pb	Bi
Unknowns (13 ml tubes)	10	-	10.5 ml	3.8%	?	4.76 ppb
Blank (50 ml tube)	5 x 10	-	52.5 ml	3.8%	~0	4.76 ppb
Standard (50 ml tubes)	5 x 10	0.2	52.7 ml	3.8%	38.1 ppb*	4.74 ppb

*Effective concentration taking into account that adding the 0.2 ml of concentrated standard dilutes the internal standard, and so this dilution will be corrected automatically.

Gently tilt the samples back and forth a few times. The samples should be mixed, but you don't want to unnecessarily disaggregate the exoskeletons.

Day 3: vacuum degas

Works most efficiently using the 200-position sample racks. Slightly unscrew the caps so air can exit and re-enter the samples. Put the samples in their rack into the big, gray vacuum chamber. Set the orange vacuum pump up on a cart, connect the long tube from the vacuum chamber, and plug the motor in. Set the valve to pull a vacuum in the chamber, and put the polycarbonate cover over the end.

Use a flashlight to watch the tubes. First, bubbles on the sides expand and rise to the surface. Soon thereafter, the water will start to boil. Boiling can cause sample to overflow, so immediately turn the valve to block the pump tube and let air back into the vacuum chamber.

Degassing has two benefits. It reduces the chance of bubbles forming in the sample tubing during analysis, which cause unstable and low signals. It also deprives many floating insect parts of their bubbles, allowing them to sink, decreasing the probability of tubing blockage.

Day 4: analysis

Analyze the samples. Don't shake them today.

Warnings

Leftover insect parts can clog the sample introduction system, though we have found that serious clogs are relatively rare (once in >1000 samples). To avoid clogging, make sure the autosampler probe tip enters the solution close to the middle of the meniscus, and also that the probe stays a few centimeters above the tube bottoms. After the analysis is done, inspect and replace parts of the sample introduction system where insect parts accumulate, especially where vinyl tubing meets glass or Teflon tubing. If parts are found in the nebulizer or expensive nebulizer tube, these can be back-flushed. If that fails, try concentrated sulfuric acid.

The H_2O_2 is not necessary for putting Pb into solution, but it speeds digestion, reduces the foam-producing characteristics of the final solution, and produces exoskeleton fragments that are more likely to sink by the time analysis starts. Sinking fragments makes clogging of the sample introduction system less likely.

Don't try to be clever and pre-mix HNO_3 and H_2O_2 to reduce pipetting steps. The H_2O_2 quickly starts to decompose into O_2, making pipetting an inaccurate mess. More slowly, the mixture evolves smelly and dangerous NO_2 as well.