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# Laser Ablation-ICP-MS Analysis of Dissected Tissue: A Conservation-Minded Approach to Assessing Contaminant Exposure

BRIAN P. JACKSON,*
WILLIAM A. HOPKINS, AND
JENNIFER BAIONNO

*Savannah River Ecology Laboratory, University of Georgia, P.O. Drawer E, Aiken, South Carolina 29802*

Minimally invasive sampling techniques are an essential ecotoxicological tool for continuous assessment of contaminant exposure and in instances where it is not desirable or practical to sacrifice the animal. In this paper, we report on the application of laser ablation-ICP-MS (LA-ICP-MS) for sampling of minute (∼1 mg, 2−3 mm) tail clips of the banded water snake, *Nerodia fasciata*, as a means to assess contaminant exposure. The snakes were split into three treatments ($n = 8$) and were fed three increasing levels (control, medium, and high) of fish contaminated with As, Se, and Sr for 24 months. LA-ICP-MS concentrations of tail clips for all three elements were significantly correlated with the remaining whole tail concentration determined by homogenization, acid digestion, and ICP-MS analysis. Additionally, LA-ICP-MS concentrations for As and Se in the tail clip were similar to the acid digestion solution analysis values for the whole tail, which suggests that these elements are homogeneously distributed. Strontium concentrations were underestimated by LA-ICP-MS when compared to whole tail concentrations. Statistical analysis showed that LA-ICP-MS tail clip concentrations differed significantly according to dietary treatment. Posterior probability error rates from nonparametric discriminant function analysis indicated that LA-ICP-MS analysis of tail clips was useful for predicting exposure to Se with only a 4% probability of misclassification among treatments. Errors associated with misclassification of As were greater (17%) but this was, in part, related to the low concentrations of As in the tail (<1 ppm for the highest treatment). Taken together, the findings from this study suggest that LA-ICP-MS of microdissected tissue shows promise as a nondestructive technique for conservation-minded ecotoxicological studies.

## Introduction

Ecotoxicological studies of elemental exposure in animals traditionally involve sacrificing individuals from the population prior to quantifying elemental concentrations in the whole body or individual organs. However, in many situations nondestructive sampling techniques (i.e., techniques having no adverse health consequences for the organism) are preferable for assessing exposure. For example, recent evidence of declining reptile populations (*1*) has stimulated demand for toxicological studies while simultaneously placing responsibility on the researcher regarding the appropriateness of sacrificing field-captured animals. Obviously, nondestructive sampling techniques such as analysis of blood samples or tail clips for reptiles are preferable for assessing sublethal exposure to trace elements, provided it can be demonstrated that these procedures provide useful information about contaminant exposure. In a previous study, we showed that analysis of blood and tail are reliable indicators of Se, Sr, and As exposure in snakes (*2*). Sampling techniques used in that study involved acid digestion of a relatively large tail clip followed by ICP-MS analysis of the digested tissue. This approach was problematic because the tail is comprised of multiple tissues, including an inorganic bone matrix in addition to blood, muscle, and skin. The bone matrix results in a high salt concentration in the tissue digestate causing general signal suppression and specific polyatomic interferences by ICP-MS. Additionally, a sample mass of up to 100 mg (dry wt) was necessary to achieve detectable concentrations of target analytes, and this required a relatively large tail clip sample. Hence, this is not a suitable method for repeated monitoring of an individual over time. Thus development of techniques that utilize less tissue mass and produce fewer analytical interferences will be a significant advance to the current methodology.

An ideal nondestructive sampling technique should provide an accurate and representative assessment of contaminant exposure, utilize minimal sample mass, and provide environmentally relevant detection limits and relative freedom from instrumental interferences. The use of LA-ICP-MS for trace element studies in biological media holds great promise. It is a microsampling technique with laser spot sizes as small as 5 $\mu$m and is applicable to spatial analysis within a sample. Most previous biological applications of LA-ICP-MS have focused on spatial analysis of incremental or annular growth structures such as tree rings (*3*), mollusk shells (*4, 5*), and fish otoliths (*6*). In fact, one of the only reports of biological applications of LA-ICP-MS on nonmineralized tissue was the analysis of individual gut sacs of brine shrimp (*7*).

Elemental fractionation during either ablation or sample transport to the ICP-MS remains a problem in achieving truly quantitative data (*8, 9*). In essence this means that, unless standards and samples are completely matrix matched, the ablation and transport characteristics of elements may differ between samples and standards. Thus, laser ablation analysis is often considered semiquantitative. However, a number of different strategies have been employed in an attempt to achieve quantitative analysis by LA-ICP-MS. Two methods used successfully are (i) referencing element responses to an element present at equal concentration (or known concentration) in the standards and samples (*5, 10, 11*) and (ii) exact matrix matching of the standards and samples (*12−14*).

In this study, we examine the feasibility of employing LA-ICP-MS for the direct solid sampling of ∼1-mg tail clip samples taken from banded water snakes (*Nerodia fasciata*) exposed to varying levels of dietary contaminants. We compare concentrations derived from LA-ICP-MS of the tail clip with solution-based analysis of the remainder of the whole tail and investigate the usefulness of LA-ICP-MS analysis of the tail clip as a predictor of prior contaminant exposure.

* Corresponding author e-mail: Jackson@srel.edu; telephone: (803)725-0854; fax: (803)725-3309.

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**TABLE 1. Geometric Mean Trace Element Concentrations ($\mu$g g$^{-1}$) Dry Mass in the Diet of Water Snakes (*Nerodia fasciata*) Used in This Study[a]**

| | dietary exposure concentrations ($\mu$g g$^{-1}$) | | |
|---|---|---|---|
| element | control | medium | high |
| As | 0.16 | 0.69 | 1.28 |
| Se | 0.99 | 11.4 | 22.7 |
| Sr | 128 | 198 | 275 |

[a] Dietary concentrations for the medium treatment represent an estimate of intermediate concentrations because snakes in that treatment received 32 contaminated and 35 uncontaminated meals. Data originally published in ref *15*.

## Materials and Methods

**Animal Exposures.** Snake samples used in this study were taken from a 2-yr study on effects of dietary contamination on snake physiology (*15*). Briefly, snakes were separated into three dietary groups ($n = 8$ snakes/treatment), nominally called control, medium, and high. Over a 2-yr period, control snakes were fed fish collected from a reference site, the high diet group was fed fish collected from a site contaminated with coal fly ash effluent, and the medium group was fed a 50:50 mix of reference/contaminated food (see Table 1 for dietary concentrations). Upon termination of the study, the snakes were sacrificed, and half from each treatment were dissected. The tail sections from these individuals were sampled at this time and were kept at −70° C until their use in this study. Because snakes were only exposed to trace elements via ingested contaminated prey, significant surface contamination of tail clips was not a concern. However, future applications of these techniques in field studies will require cleaning of surficial tissues prior to analysis to ensure that LA-ICP-MS measurements reflect trace element accumulation in tissues rather than surface contamination. A small clip from each of these tail sections was taken for laser ablation analysis; the clipping was generally 2−3 mm long, and the average weight of the clips was 1.18 ± 0.09 mg dry mass. Tail clips were mounted on a glass microscope slide using double-sided adhesive tape with four clippings per slide.

The remainder of each tail (~70 mg) was microwave digested, and the digestate was analyzed by ICP-MS. Analysis of the acid-digested tail samples indicated that only Se, As, and Sr showed significant differences between treatments; hence, only these elements were considered as analytes in the LA-ICP-MS analysis. The standard reference material (SRM) TORT2 (NRC, CNRC, Ottawa, Canada) was included in the digestion procedure ($n = 5$) at an equivalent weight to the samples to assess recovery at this low digest mass. Recoveries of As, Se, and Sr in the SRM at a sample mass of 75 mg were 81%, 85%, and 87%, respectively. Despite the small sample mass used for digestion, Se and Sr concentrations in the tail digests were present at detectable levels (at least 5 times the method detection limit; MDL) in all samples. Arsenic concentrations were lower (<1 $\mu$g g$^{-1}$ for the high treatment), and six samples in the control treatment were below the MDLs. Reproducibility of triplicate digestion and analysis was good with CVs of <10% for As and Se and 15% for Sr.

**Calibration Preparation for LA-ICP-MS Analysis.** Concurrent with the above-described feeding study, another group of snakes were provided with the same dietary treatments for 12 months ($n = 6$ snakes/treatment). Tail samples collected from these animals were processed similarly and used to make solid calibration standards for calibration of the LA-ICP-MS. Individual tail clips from within each dietary treatment were pooled and thoroughly homogenized using a cryo-mill (Spex CertiPrep, Metuchen, NJ). The homogenized sample was split, and a portion was used for acid digestion and ICP-MS analysis in triplicate. The remaining 100 mg for each of the three treatments (control, medium, and high) was pressed ($15 \times 10^5$ Pa for 5 min) into a pellet for use as LA-ICP-MS standards. The elemental concentrations determined from the solution ICP-MS analysis were used in the LA-ICP-MS calibration as the accurate concentration of the corresponding pressed pellet.

**TABLE 2. Instrumental Operating Conditions for LA-ICP-MS Analysis**

| | |
|---|---|
| **Laser Ablation** | |
| operating mode | continuous |
| frequency | 5 Hz |
| laser power | 80% |
| spot size | 200 $\mu$m |
| raster size | 500 × 500 $\mu$m |
| raster spacing | 200 $\mu$m |
| scan speed | 100 $\mu$m/s |
| **ICP-MS** | |
| neb flow | 1.14 L min$^{-1}$ |
| auxiliary flow | 1.2 L min$^{-1}$ |
| plasma flow | 15 L min$^{-1}$ |
| RF power | 1050 W |
| dwell time per amu | 20 ms for Sr, 50 ms for As, Se |
| sweeps | 1 |
| readings per replicate | 22 |
| analysis time | 13.7 s |

**Instrumental Parameters.** LA-ICP-MS analysis was conducted on a UP213 laser ablation system (New Wave, Freemont, CA) interfaced with an Elan 6100 DRC plus (Perkin-Elmer, Shelton, CT). The UP213 employs a frequency quintupled Nd:YAG laser with a resulting laser wavelength of 213 nm. The LA-ICP-MS was optimized prior to analysis for optimum nebulizer gas flow, $X-Y$ torch position, and lens voltage by monitoring the multiple element signals arising from a 0.5 mm × 0.5 mm raster ablation of the pressed pellet of TORT2. Full operation conditions for LA-ICP-MS are given in Table 2.

Standards and samples were ablated using a raster pattern, and the timing/read delays within the laser and ICP-MS software were set such that the ICP-MS collected data from the continuous signal portion of the raster pattern. Each sample was analyzed in triplicate, and the mean was used for statistical analysis. A gas blank was used for the calibration curve (generated by running a raster ablation pattern at 0% power). Analyte responses were ratioed to the $^{13}$C response throughout all analyses. The gas blank was subtracted from standard and sample intensities prior to internal standard correction. In using $^{13}$C as an internal standard, we assume that it is present at equivalent concentration in the tail clips and hence can be used as a measure of mass transport to the ICP-MS.

**Statistical Analyses.** The relationship between ablated tail clip concentrations of individual trace elements were compared to whole tail tissue concentrations from the same individual snakes (determined using solution analysis following tissue digestion) using linear regression analyses. Trace element concentrations from ablated tissue were compared among dietary treatments and between sexes using two-way multivariate analysis of variance (MANOVA). We log-transformed tissue element concentrations before analysis to more closely approximate assumptions of the model. Pillai's trace statistic was used to test the null hypothesis of no treatment or sex effects. For illustrative purposes, we then conducted individual two-way ANOVAs to examine the effect of food treatment and sex on each trace element concentration in ablated tissue.

We used nonparametric discriminant function analysis to determine whether trace element concentrations from

**TABLE 3. Laser Ablation Calibration Parameters for Pressed Pellet Standards from Homogenized Snake Tail Clips**

| | concn range ($\mu$g g$^{-1}$) | slope | $R^2$ |
|---|---|---|---|
| As | 0.06−0.79 | 0.23 | 0.999 |
| Se (82) | 0.20−11.8 | 0.01 | 0.999 |
| Sr (88) | 165−325 | 0.08 | 0.997 |

ablated tissue samples were accurate predictors of previous exposure to trace element-contaminated prey. The nearest neighbor option of the DISCRIM procedure of SAS with three nearest neighbors was used to estimate densities (i.e., estimated distributions) for each dietary treatment (SAS/STAT, 1990). To evaluate the performance of the classification criteria, we used cross-validation procedures. Posterior probability error rate estimates from cross-validation were used as an indication of the accuracy of our classification criteria.

## Results and Discussion

Calibration statistics for LA-ICP-MS analysis of pressed pellet standards are given in Table 3. Precision of replicate analysis of standards was acceptable, with an average intensity RSD of <15%. Similar and higher levels of precision have been reported for biological solid standards with trace element concentrations <20 mg kg$^{-1}$ (*10*). Detection limits based on 3$\sigma$ of the gas blank analysis were 0.01, 0.37, and 0.16 $\mu$g g$^{-1}$ on a solids basis for As, Se, and Sr, respectively. These values are comparable with solution-based detection limits and compare favorably with other reported LA-ICP-MS detection limits for As (*4*) and Sr (*11*).

Concentrations of all three trace elements in ablated tissue were positively correlated with actual whole tail tissue concentration as determined by acid digestion and ICP-MS analysis (Figure 1). Correlations between whole tail and ablated tissue concentrations were stronger for As and Se than for Sr. For As and Se, the relationship between LA-ICP-MS derived concentrations using the tail clip solid standards and solution-based analysis of the homogenized whole tail approached 1:1. This implies that As and Se are fairly homogeneously distributed throughout the tail and that the tail clip standards were appropriate for matrix matching during the ablation analysis. However, LA-ICP-MS concentrations for As within a treatment displayed greater variability than the solution analysis of the whole tails. This may be due to the low levels of As present in the tails and the inherent poor precision of LA-ICP-MS when compared to solution nebulization. Although Sr LA-ICP-MS concentrations in the tail clip were significantly related to the Sr concentration in the whole tail, LA-ICP-MS of the tail clip markedly underestimated the whole tail Sr concentration. One possible explanation for this is that Sr is heterogeneously distributed within the snake tail (likely as a co-precipitant in the $CaCO_3$ matrix of the tail) such that the bulk Sr concentration was not accurately reflected in the minute tail clip taken for LA-ICP-MS analysis. However other explanations, such as C being an unsuitable internal standard for this element, may also explain the inaccurate Sr measurements by LA-ICP-MS.

Despite the fact that LA-ICP-MS concentrations for Sr were not representative of the whole tail concentration, the LA-ICP-MS concentration of As, Se, and Sr among treatments differed significantly from one another ($P < 0.001$, Figure 2). Results of MANOVA indicated that ablated tissue concentrations of trace elements differed according to dietary treatment; as dietary concentrations of trace elements increased, so did the concentrations in ablated snake tail tissue (Table 4). However, there was also a marginal effect ($P = 0.06$) of

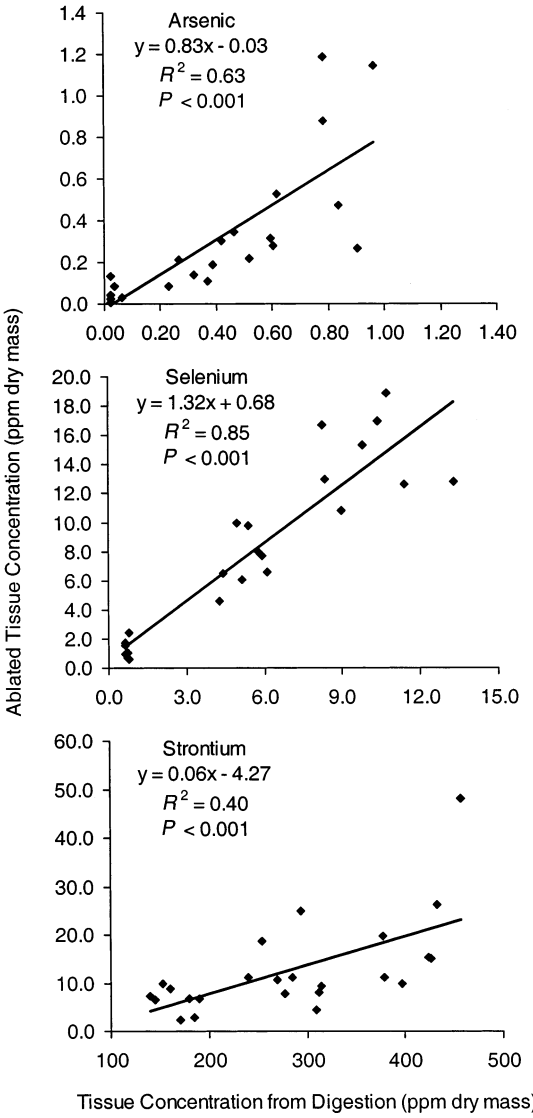


**FIGURE 1. Regression relationships of LA-ICP-MS derived tail clip concentrations based on homogenized tail clip pellet standards with whole tail concentrations derived from acid digestion and solution analysis. Six control snakes had whole tail As concentrations below method detection limits; for statistical and graphing purposes, these values were input as one-half MDL.**

**TABLE 4. Results of Multivariate Analysis of Variance (MANOVA) of Effects of Food Treatment and Sex on Concentration of Three Trace Elements in Snake Tail Tissue**

| source | Pillai's trace | DF | $F$ | $P$ |
|---|---|---|---|---|
| treatment | 1.0152 | 6, 34 | 5.84 | <0.001 |
| sex | 0.3328 | 3, 16 | 2.66 | 0.083 |
| treatment × sex | 0.5718 | 6, 34 | 2.27 | 0.060 |

the interaction between snake sex and dietary treatment. Individual two-way ANOVAs indicated that this marginally significant interaction was due to a significant effect of sex on Se accumulation in snake tail tissue and a significant interaction between sex and treatment affecting Sr accumulation (Table 5). Selenium concentrations were consistently higher in males as compared to females across dietary treatments (Figure 2). Males in the control and intermediate dietary treatments also had higher Sr concentrations than females in the respective treatment, but ablated tissue from females had higher Sr concentrations than tissue

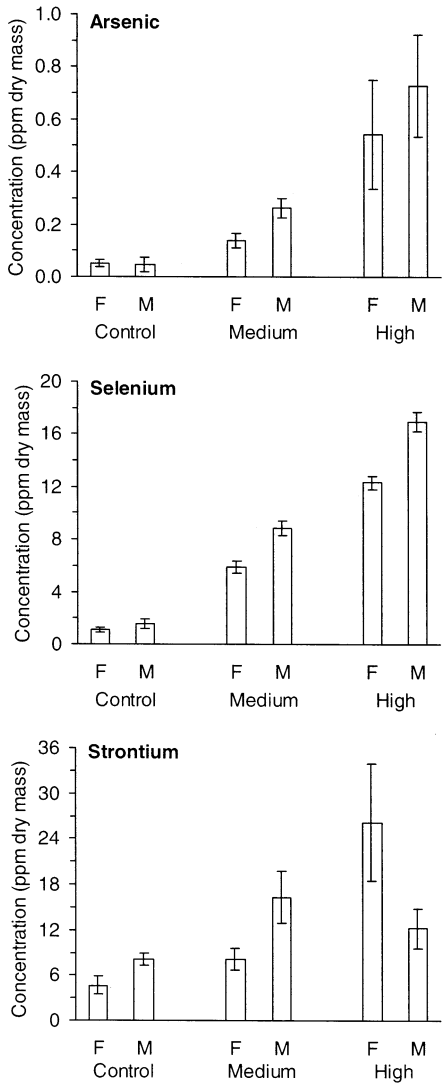


**FIGURE 2. LA-ICP-MS derived concentration means and standard deviations for male and female snake tail clip samples. Snakes had been fed varying levels of contaminated food over 2 yr.**

**TABLE 5. Results of Individual ANOVAs of Effects of Food Treatment and Sex on Element Concentration Determined by LA-ICP-MS in Snake Tail Tissue**

| element | source | DF | MS | *F* | *P* |
|---|---|---|---|---|---|
| As | treatment | 2 | 2.9745 | 27.56 | <0.001 |
| | sex | 1 | 0.0224 | 0.21 | 0.654 |
| | treatment × sex | 2 | 0.1711 | 1.59 | 0.232 |
| | error | 18 | 0.1080 | | |
| Se | treatment | 2 | 2.4729 | 152.33 | <0.001 |
| | sex | 1 | 0.1286 | 7.92 | 0.012 |
| | treatment × sex | 2 | 0.0013 | 0.08 | 0.924 |
| | error | 18 | 0.0162 | | |
| Sr | treatment | 2 | 0.4124 | 11.63 | <0.001 |
| | sex | 1 | 0.0482 | 1.36 | 0.259 |
| | treatment × sex | 2 | 0.2343 | 6.61 | 0.007 |
| | error | 18 | 0.0355 | | |

from males in the high dietary treatment. These findings are consistent with our previous work indicating sex-specific differences in Se and Sr accumulation in different snake organs (*15*).

Posterior probability error rates from nonparametric discriminant function analysis indicated that laser ablation of the tail clip was useful for predicting exposure to Se, with only a 4% chance of misclassification, but not for As or Sr. Cross-validation tests based on Se concentration in ablated tissue revealed that one individual from the high dietary treatment was misclassified as an individual in the medium dietary treatment. Cross-validation tests based on As concentrations in ablated tissue revealed that 5 of 24 individuals were misclassified, but four of these misclassifications occurred between adjacent treatment groups (i.e., between high and medium or between medium and control). The higher frequency of misclassification in the case of As (17%) is likely due to the low concentrations of As; the control concentrations were around the detection limit of the LA-ICP-MS technique while the differences between the lowest As value in the high treatment and highest in the medium treatment were on the order of 4−5 times the detection limit of the technique. For Sr, error rates were unacceptably high (≥25%).

Taken together, the results of our study suggest that LA-ICP-MS of microdissected tissue is a potentially powerful tool for contaminant exposure assessment in animals. The ability to assess contaminant exposure through ablation of a 1-mg tail clip suggests that frequent monitoring through the lifetime of an individual is possible. Additionally, LA-ICP-MS can allow determination of elemental concentration through direct analysis of the solid sample obviating the need for time-consuming digestion procedures that may also introduce contamination artifacts. Using ablated tissue, we were able to detect significant differences in As, Se, and Sr concentrations among dietary treatments. Moreover, in the case of Se, ablated tissue concentrations differed sufficiently among treatments so that they could be used to predict the previous exposure history of animals with very low probability of error. It appears that such techniques may be particularly applicable in future studies focusing on trace elements that are readily incorporated into surficial tissues such as feathers, hair, nails, and skin (e.g., Se, Hg, As) and in cases where elements are homogeneously distributed throughout the tissue matrix. However, our results suggest that, in cases where low-level tissue accumulation occurs (e.g., As in present study), the power of LA-ICP-MS for predicting previous exposure history decreases. In such cases, solution digestion of larger tissue samples may be needed for adequate predictive power (*2*). Future studies that address the efficacy of LA-ICP-MS for tissue analysis of animals collected from the field will be a significant step toward further development of this more conservation-minded approach of contaminant exposure assessment.

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