

Potential Sources of Zn Contamination in the Analysis of Plant Tissue

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INTRODUCTION

Analysis of plant tissue for Zn by inductively coupled plasma-optical emission spectrophotometry (ICP-OES) is relatively straightforward in most cases. However, the presence of Zn in many materials commonly used on farms and in laboratories means the potential for contamination of tissue samples is high. Because Zn is often low or deficient in the agricultural food chain (Welch and Graham 2004), contamination by external Zn could render experiments inconclusive or falsely suggest increased Zn accumulation. This article describes potential sources of contamination by their location within the analysis chain; (1) sampling, (2) storage, (3) handling and (4) instrumental analysis. Some sources (*e.g.* gloves) can occur in several categories, increasing the risk of errors.

The examples presented have been derived from the past decade of work by Waite Analytical Services in the course of routine nutrient analysis for multiple clients, as well as capacity building projects funded by HarvestPlus, involving on-site visits to numerous laboratories and collaboration with colleagues at Cornell University.

METHODS

ICP-OES analysis was performed with a Spectro CIROS radial ICP spectrometer (Spectro GmbH, Germany). Samples were delivered in 4% v/v HNO₃ solutions. Collection of samples varied depending on the type of material: Fabrics or containers (including paper envelopes) were rinsed with acid for 10-30 seconds and the solution poured into a sample tube. Solid materials were either placed in a vial containing acid or the material was held firmly on top of a tube filled with acid and inverted several times. Plant samples (0.3 g) were digested in 69% HNO₃ heated to 122 °C for 90 min.

RESULTS AND DISCUSSION

Contamination of control treatments by fertilisers was not considered, as was mislabelling of samples. Many of the sources of Zn contamination are well known and are usually avoided (*e.g.* Galvanised steelwork in glasshouses). Other sources require detailed testing to confirm they are responsible for Zn contamination.

- (a) Sources in sampling
 - (i) Grinders and milling machines may be made with Zn alloys (Table 1).
 - (ii) Collection trays, harvester parts *etc* of galvanised iron used to prepare samples may add Zn particulates to the samples.
- (b) Sources in storage
 - (i) Paper envelopes are the most common container for seeds and leaves. Zn from whiteners and other substances used in manufacturing may leach into the sample over prolonged storage: Table 1 shows some envelopes can vary 7-fold in Zn content from acid washes.
 - (ii) Shelving. Galvanised brackets or storage lockers can oxidise and create a high Zn environment that raises the background Zn. Sheds made of galvanised iron used for storage could also be sources of Zn.
 - (iii) Contamination of individual tubes by air-borne dust can be prevented by sealing sample tubes or covering with plastic sheeting.
- (c) Sources in handling
 - (i) Gloves. Many brands use Zn-based powders to prevent sticking to skin. Low-Zn gloves have 10-50 times less Zn than powdered varieties (Table 1).

- (ii) Significant amounts of Zn can be transferred from fingers when sunscreens (up to 10% ZnO, w/v) have been applied (Table 1).
- (d) Sources in analysis
 - (i) Purity of acids and reagents used for sample dissolution should be monitored to avoid adding Zn to samples (Table 1).
 - (ii) Carryover of Zn in tubing *etc* from high Zn treatment samples to low Zn should be minimised by re-ordering samples for analysis (Table 1).

Table 1. Zinc contamination from different materials encountered in the analytical process.

Stage	Material	N samples	Zn (range, mg L ⁻¹)
Sampling	Grain mills	10 brands (\pm milled grain)	0 – 2.5
Storage	Envelopes	3 paper types	0.01 – 0.07
Handling	Gloves	10 brands	0.7 – 40
“	Sunscreen	15 sequential samples	0.2 – 3.5
Analysis	Nitric Acid	4 manufacturers	0.0001 – 0.0010
“	Sample carryover	Individual examples	0 - 8.3 (mg kg ⁻¹)

Multiplier effect of contamination events

The later in the analysis chain a contamination event occurs, the greater the effect: 1 μg Zn in a 1 kg sample of grain is negligible, but in a 4.5 ml sample tube, the Zn concentration of the sample is increased $\sim 18.5 \text{ mg kg}^{-1}$ after accounting for the dilution factor ($25 \text{ ml} \div 4.5 \text{ ml} \div 0.3 \text{ g}$). Considering a 4 mg kg^{-1} increase is sufficient for some species to overcome marginal Zn deficiency (Genc *et al.* 2002), contamination of this magnitude could easily alter experimental findings. Extreme contamination from sunscreens (Table 1) could add 15 μg Zn. Uniform or widespread contamination at early stages (*e.g.* harvesting, storage) is more difficult to detect as the contamination is at a much lower concentration. Because all samples are affected, this reduces the chance of finding any significant treatment differences.

CONCLUSIONS

The most significant contamination of individual samples occurs while handling, while generalised storage contamination can affect all samples simultaneously. Eliminating contamination reduces risk of claiming improvements in Zn uptake by plants when there was none, or failing to detect differences that do exist. The purpose of this paper is to re-emphasise the need for careful sample handling and quality control of all steps of the Zn analysis chain.

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REFERENCES

- Genc Y., McDonald G.K., Graham R.D. (2002) Critical deficiency concentration of zinc in barley genotypes differing in zinc efficiency and its relation to growth responses. *Journal of Plant Nutrition* **25**, 545-560.
- Welch R.M., Graham R.D. (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**, 353-364.