Title: Revisiting herbage sample collection and preparation procedures to minimise risks of trace element contamination

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Abstract

A renewed interest in trace elements (TE), as micronutrients as well as potentially toxic elements, and new options for multi-element analysis has led to an increased number of scientists engaging in TE studies. Accreditation, certification and quality control of TE analyses often applies only to the last step in the sample chain when prepared samples are sent to the laboratory for digestion/extraction and subsequent analysis. However, all stages of the chain from initial sampling to final analysis require an understanding of the specific challenges involved in TE studies and an awareness of the contamination risks as well as approaches to limit these. Contamination can potentially be introduced during all stages of handling and preparation of plant samples, e.g. through dust and the materials that make up the different work surfaces, tools and containers used. Milling devices originally used during preparation of two sets of archived herbage samples were tested to indicate the degree of contamination that can arise from milling. For example, some of the milling devices tested showed effects on several TE concentrations while also increasing the variability between samples. A titanium knife mill which was included for comparison gave the best results, showing no measurable contamination by TE of primary interest, while it allowed a high throughput of samples. To enhance the quality of data on TE in bulky plant material such as herbage and to ensure future usability of newly archived samples, we suggest that field handbooks and sample preparation protocols (where needed) are revised to include

precautions against TE contamination in all handling steps. This will ensure reliable data on concentrations of micronutrients and potential toxic TE in plant material.

Keywords: micronutrient, plant sample, sample drying, sample milling, sample storage

Introduction

Over recent years, there has been a renewed interest in trace elements (TE) from various perspectives including: agronomic requirements, feed/food quality, and the environmental impacts of potentially toxic elements (e.g. Alloway, 2008; Stein, 2010; Cooper et al., 2011; Tidemann-Andersen et al., 2011). Technical advances in analytical equipment and preparation procedures have opened up new possibilities for including comprehensive multi-element analyses of soil and plant samples. Such analyses are frequently carried out on newly collected samples, but there is also interest in re-analysing archived samples, e.g. samples from surveys and monitoring studies as well as long-term field experiments. These samples may have been originally collected for a specific purpose, but they now have a key role in the study of time trends for a range of elements or to pursue new research questions beyond the scope of the original sampling programme. This change in emphasis has led to an increase in the number of researchers involved in TE studies. In the past, specialists with their own rigorous procedures and analytical equipment determined TE in studies specifically designed to secure high quality data. More recently, however, it is less common to find the same people responsible for the whole chain from sampling through to analysis. The new generation of scientists often have a primary focus other than TE per se and may not be familiar with practical aspects relating to TE research, especially contamination risks.

77 Trace element analyses of plant material pose specific demands with regard to sampling,
78 sample preparation and pre-treatment. There are various potential sources of contamination
79 which include soil and the equipment used for the different processing stages. Various aspects
80 of uncertainties and errors along the whole sampling, sample preparation and analysis
81 sequence were discussed during a workshop on 'Improvements of trace element in plant
82 matrices' held in Brussels in May 1994 (Quevauviller, 1995).

83 If samples from studies that were originally designed and undertaken with a different focus
84 are to be reused for contemporary TE studies, the potential risks for TE contamination must
85 be evaluated and the consequences this might pose for archived material assessed. It is
86 therefore appropriate to revisit some of the issues associated with such TE studies,
87 particularly for the benefit of researchers who are relatively new to the research subject. This
88 is supported by the fact that out of the ten most recent papers on micronutrients or TE in
89 herbage found during a search of Web of Knowledge only one paper clearly stated
90 precautions against contamination (Smith et al., 2009), whereas in the remaining nine papers
91 either no reference was made to this or the described methods indicate that contamination was
92 likely.

93 The overall aim of this paper is to provide an overview of risk of contamination from sources
94 associated with herbage sample collection and preparation. The overview is based on a
95 literature review complemented by examples from our own laboratories to demonstrate the
96 issues. Literature searches of peer-reviewed publications, and other sources such as
97 conference publications, reports and field protocols were thus undertaken with keywords that
98 included, but were not limited to, sample collection, sample storage, sample preservation,
99 sample preparation, milling and TE contamination. References were reviewed with a special
100 focus on herbage samples.

Sample handling and preparation of herbage samples to avoid TE contamination

Published scientific literature was generally focused on the individual steps in the sample collection or preparation chain and also included other aspects of each step beyond risks of TE contamination, e.g. procedures to ascertain collection of representative samples (Table 1). Notable exceptions were a special issue reporting on the 1994 workshop 'Improvements of trace element in plant matrices' (STOTEN, 1995), and two publications from the early 1970's (Scott et al., 1971; Scott and Ure, 1972). Protocols for sample collection and preparation for use by field staff was generally found in 'grey' literature. Sample collection and preparation protocols to minimise TE contamination have, for example, been published for a range of grain and tuber crops and for plantains and bananas (Stangoulis and Sison, 2008), but other protocols do not always include considerations of TE (e.g. Försökshandboken, 2009). Sample handling procedures to prevent accidental contamination are also mentioned as an important aspect when implementing the EC Directive concerning the performance of sampling and analysis for the official control of different substances (including some TE) in foodstuffs (European Commission, 2007), although few practical directions are given.

The 1994 workshop on the state of the art of TE determinations in plant matrices summarised the most crucial aspects of plant material sampling, preparation, pre-treatment and detection (Quevauviller, 1995). However, the discussion covered all possible types of plant matrices, and as a result conclusions and recommendations were very general, pointing out the need for adjustment of procedures in relation to the aims and objectives of each individual study.

Mixed species herbage samples involve special challenges during collection and sample preparation as the major part of the above-ground plant material is collected, potentially giving rise to highly heterogeneous samples. The heterogeneity of herbage materials emphasises the importance of extracting a representative sample both at the time of collection and also subsequent preparation stages, together with the need for herbage sample homogenisation. In the following text, the recommendations and conclusions from the 1994 workshop (STOTEN, 1995) will hence be revisited and developed specifically for herbage and with the aim of illustrating the need for overall quality assurance in TE studies of herbage and other bulky crops.

Trace element studies demand rigorous protocols to avoid contamination during sample collection and preparation. Dust evolving from soil and plant material and other incidental sources constitutes a potential contamination risk and obviously calls for a high standard of hygienic maintenance of rooms and equipment used during sample preparation. It follows that work areas and equipment used for plant material processing should be kept separate from those used for soil processing. Work facilities should also be designed to give a minimum and predictable level of contamination, e.g. by the use of impermeable surfaces (Hamilton, 1995). Equipment should be stored in closed containers when not in use to protect it and the test materials from dust (Stangoulis and Sison, 2008). Samples may also become contaminated from the surfaces of containers and tools (e.g. metals, paints, tanned leather, rubber) (Lockman, 1980; Fleming et al., 1986; Stangoulis and Sison, 2008). Tools, containers and procedures used throughout the various stages should therefore be chosen with care. Further potential TE contamination sources during different stages of the sample chain are transfers from metal structures and from skin-care products via hands (Stangoulis and Sison, 2008).

146 **Sample collection**

147 Factors such as sample collection strategy, plant species identification, and collection of
148 consistent proportions between plant parts is of importance for acquiring representative
149 samples (e.g. Ernst, 1995; Wagner, 1995) and avoiding erroneous and highly variable results.
150 For herbage sample collection, a standardised cutting height some distance above the soil
151 surface not only decreases variability in sample composition but also decreases the risk of soil
152 contamination. Risks of contamination by soil and dust during growth or sampling have been
153 recognised for decades and recommendations issued to minimise it; including avoiding
154 sampling after high winds, heavy rains and prolonged drought, and waiting to sample until at
155 least two weeks after grazing (Scott et al., 1971). Soil or dust contamination is obviously most
156 critical for elements where concentrations are much higher than the corresponding plant
157 concentrations: most notably cobalt (Co), chromium (Cr) and iron (Fe), but also copper (Cu),
158 zinc (Zn) and boron (B) (Fleming et al., 1986; Wyttenbach and Tobler, 1998). As part of
159 quality assurance procedures, indicators of soil contamination (e.g. aluminium (Al), Fe,
160 titanium (Ti) or scandium (Sc)) should thus be observed (Scott et al., 1971; Bargagli, 1995;
161 Wyttenbach and Tobler, 2002; Elias et al., 2008; Cook et al., 2009).

162 Procedures for counteracting sample contamination by soil and dust through picking,
163 brushing, and washing of samples have been developed (Porter, 1986; Markert, 1992; Aboal
164 et al., 2008; Elias et al., 2008) and can, to some extent, counteract differences over the year in
165 the magnitude of contamination by dust. Apart from this, it has been shown that variation
166 between repeated samplings may be decreased by sampling under similar weather conditions,
167 as well as using similar storage times and storage conditions before sample cleaning
168 (Fernández et al., 2010). Washing of plant material may lead to losses of TE from inside the
169 cells though, the magnitude increasing if unfavourable ratios between solvent and plant
170 material or long washing times are applied (Markert, 1992; Rossini Oliva and Raitio, 2003). A

summary of different washing techniques and recommendations for when to apply them is given by Rossini Oliva and Raitio (2003). However, cleaning of samples is not always recommended. For example, where the aim is to study the contribution of atmospheric derived 'contamination' or actual intake by livestock, then a direct analysis of unwashed material would be required. Sampling of lodged herbage should be avoided, though, if at all possible.

Plant concentrations of TE are influenced by soil factors, hydrological conditions, plant species, phenological stage and plant part, and ley/pasture management (Mayland and Sneva, 1983; Anke et al., 1994; Belesky et al., 2000; Fystro and Bakken, 2005; Sinclair and Edwards, 2008; Roche et al., 2009). Hence it is important to use the same sampling protocol on every occasion and, unless corresponding soil samples are collected, at least notes of the soil and hydrological conditions, farm management and signs of herbivory and pathogen infestation, should be taken. Examples of such protocols are given by Ernst (1995) and Hamilton (1995) and may be adapted to suit herbage sampling.

Sample drying and storage

Herbage is generally bulky and heterogenous and large samples are needed to attain a representative sample. Hence the freeze-drying procedures recommended by Hamilton (1995) for preparing plant material prior to TE analysis are generally only applicable when large capacity freeze driers are available. Instead forced ventilation drying ovens are frequently used. It is important that driers (and surroundings) are cleaned thoroughly before use and that separate driers are used for soil and plant material. Also sample bags (e.g. perforated plastic bags) should be clean.

If herbage samples are stored prior to further preparation or stored after milling, containers for storage should similarly be clean (e.g. new or acid washed) and samples stored in a dry and clean environment. The composition of storage containers is also important. Glass containers work well in many ways, but may contaminate samples with B from the glass or other elements from the closures. Some TE are further used in colouring of e.g. plastics and are also found as likely traces from the manufacturing process (Waheed et al., in press) and may be released into the samples. Details on drying and storage of samples are given by Lockman (1980), Houba et al. (1995), Quevauviller (1995) and Stangoulis and Sison (2008).

Sample milling

Creation of a representative subsample is a crucial step in all analytical work and the homogenisation frequently needed for this can be the most risky step with regard to contamination, in particular if the plant material contains mineral particles which are likely to abrade grinding equipment (Hamilton, 1995). To avoid the risk of contamination from the mill, samples of e.g. grains in some laboratories are not milled but digested as whole grain (e.g. Öborn et al., 1995; Wångstrand et al., 2007). Where whole grains are used it is important to ensure representativeness by using larger sample weights and digestion volumes compared with standard procedures. For bulky samples of heterogeneous material such as whole herbage samples, however, it is not possible to avoid particle size reduction by milling or grinding prior to homogenisation and extraction of a representative subsample. A number of mill types made from materials low in TE (Hamilton, 1995; Markert, 1995) are used for grinding small sample sizes, but larger samples still present difficulties as many agate and ceramic mortar mills are suitable only for smaller sample sizes. Mills generally used for the preparation of larger, fibrous samples of varying hardness are cutting mills and hammer mills.

These are most often made from steel with TE as major constituents or as minor components and thus likely to introduce these elements into the samples through wear. Use of reference materials is of little help in the quality control of this step as these are generally already milled (or otherwise fine powder) and thus will not be milled or ground in the laboratory along with the material to be analysed. Reference materials consequently constitute only a quality control for onward steps in the analysis and not for all stages during sample preparation.

The European Commission (2007) regulation for the methods of sampling and analysis for the official control of the levels of some TE in foodstuffs, states that the analyst should ensure that samples do not become contaminated during sample preparation. According to their recommendations devices should be of inert materials such as polypropylene or polytetrafluoroethylene, but high quality stainless steel is (surprisingly) permitted for cutting edges. However, Cubadda et al. (2001) tested a range of milling devices (glass and porcelain mortars, and four steel mills) and revealed significant contamination by all the tested devices with one or several TE. Statistical differences with respect to the control were thus detected for ten TE (Al, cadmium (Cd), Co, Cr, Cu, Fe, manganese (Mn), molybdenum (Mo), nickel (Ni), and lead (Pb)). The contamination was found to be higher with hard durum wheat than when softer wheat was milled, indicating that the scope of the contamination risk may differ depending on the hardness of the material to be milled. On the other hand, Sager and Mittendorfer (1997) did not find any significant difference between continuously and discontinuously operating milling devices, nor did they find any significant differences in the efficiency of different cleaning methods (washing, blowing, brushing and discarding of the first milled portion).

A practical test of different cutting and milling devices for preparation of herbage samples

241 Materials and methods

242 Mills that had been used during preparation of potentially useful archived samples available in
243 our institutions (SLU, SAC) were tested in two experiments comprising a) a steel hammer
244 mill, a steel hammer mill followed by ball mill, and a Ti knife mill using a plexi-glass knife as
245 a control (Test 1), and b) a steel cutting mill using plastic scissors as a control (Test 2) (Table
246 2). The plant material used for Test 1 was mature mixed hay consisting of perennial ryegrass
247 (*Lolium perenne* L.), timothy (*Phleum pratense* L.) and white clover (*Trifolium repens* L.),
248 and for Test 2 timothy harvested at the emerging ear stage. For each experiment, the plant
249 material was split into equivalent weight subsamples which were randomised with five
250 replicates being processed by each cutting or milling device.

251 Digestion of the plant material was carried out according to the procedures developed and
252 routinely used in the laboratory of the Department of Soil and Environment, Swedish
253 University of Agricultural Sciences.

254 Day 1: One gram plant sample was weighed into acid-washed Tecator glass tubes (Höganäs,
255 Sweden). Ten ml conc. (15.6 M) HNO_3 (Merck suprapure) was added and the sample, covered
256 with a glass pear, incubated in the Tecator blocks at 30 ˚C for 9.5 h, followed by 100 ˚C for 1
257 h, and 135 ˚C for 1.5 h.

258 Day 2: When cooled to approx. 70 ˚C, the tubes were removed from the Tecator blocks, and
259 another 5 ml conc. HNO_3 was added, where-after incubation was resumed at 135 ˚C for
260 another 2.5 h.

261 Filter papers (Munktell 00H, Ø185 mm) were washed twice with 10% (1.56 M) HNO_3. The
262 digests were diluted to a total volume of 100 ml with ultrapure water (maximum 0.055µS cm^{-1})
263 and then filtered directly into acid washed plastic bottles.

264 The digests were analysed for Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn by inductively
265 coupled plasma mass spectrometry (Elan 6100 ICP-MS; Perkin Elmer SCIEX instruments).
266 Dry matter content in the plant material was determined and metal concentrations expressed
267 in mg kg^{-1} plant material dry weight (dw).

268 Certified reference material (NIST Wheat Flour, National Institute of Standards and
269 Technology, Gaitersburg, MD, USA) was included in all batches. There were no values for Cr
270 or Ni concentrations provided with the certified reference material and therefore the in-house
271 average of the NIST material was used as test values for these two elements. Detection and
272 analytical limits were calculated from the composition of 10 blanks with the detection limit
273 set to 3×standard deviation and analytical limits to 10×standard deviation for each element.

274 Differences due to the milling devices were evaluated through ANOVA using JPM 8.0.1
275 software (SAS, Cary, NC, USA) using ln transformed data to get a normal distribution of
276 residuals where appropriate (Zn in Test 1, and Cr in Test 2). Where ANOVA indicated
277 significant differences ($P<0.05$) between means, the effects of individual devices were tested
278 by Tukey's HSD.

279 Results and conclusions

280 In the first of the current tests of mills the pattern of contamination relative to controls for 10
281 elements fell into three broad groups. In the group which included Cd, Co, Cu, Fe, Mn and
282 Mo, the mills tested generally showed a difference of <20% from the control (Tables 3, 4, Fig.
283 1a). Nickel and Zn approximately doubled in samples milled in the hammer mill and/or ball
284 mill, and an increasing, massive, contamination of Cr and Pb was caused by the hammer mill
285 and subsequent ball mill (Table 3, Fig. 1b,c). An increase in variability of Pb and Zn (Fig. 1b)
286 was apparent in samples that had been hammer milled, and this was accentuated by the ball

mill that also increased the Cr concentrations (Fig. 1c). This was not the case with the other elements, or was only expressed as a trend, presumably because the contamination that arouse during milling contributed less to the total concentrations in the analysed plant material, and that the inherent variability within the original material was large. On the other hand with the Ti mill, there was no significant difference in element concentrations or variability as compared to the control. Titanium is a very hard, strong and corrosion resistant metal and thus suitable for construction of cutting and milling devices. However, it can also include some impurities; an example of the TE composition (21 elements) in Ti used to construct cutting blades and bearings for processing other biologically derived materials showed that it contained Fe 130, Sn 100, Cu 24 and Cr 4 mg kg^{-1} as impurities (Shand et al., 1983).
In the second test there were no significant differences between the Cd, Co and Pb in the steel milled samples compared with the control samples whereas the steel mill significantly increased concentrations of all other elements (Table 4, Fig. 1a-c). The variability, however, was not affected by milling with the steel mill, except for Cr (Fig. 1a-c).

The test of mills used for archived samples demonstrated contamination with a range of TE. For some elements the milling introduced an error much greater than that suggested by Markert (1995), indicating that the samples could only be used for studies on a few of the tested elements. Furthermore, two of the mills increased the variability of some element concentrations, contrary to the objective of milling, which is carried out to increase homogeneity and reduce variability in samples. On the other hand, the Ti knife mill did not significantly contaminate the processed plant material with any of the focus elements (Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, and Zn), at least not detectable with the analytical protocol and sensitivity of the instruments used. One drawback of using a Ti cutting mill for sample preparation is that the Ti concentration cannot be used as indicator of soil contamination of the plant samples but other elements such as Al or Sc may be used instead (see above).

Reanalysis of archived samples to answer new research questions

The use of historical data and sample archives potentially has great value in improving our understanding of TE dynamics, e.g. in different ecosystems and the food chain. However, an appreciation of the potential contamination issues surrounding TE studies, some of which are outlined in the present paper, for each set of data or archived sample will be of key importance in reducing the risk of data misinterpretation and inaccuracy in calculations. In order to assess the data or sample quality, there is a need to know what equipment and procedures were used during sample collection and preparation and these must subsequently be tested for potential contamination. Some elements are more likely to be introduced via contamination and the prospect of using existing samples from earlier studies may be limited. Other elements, as suggested by this study, may less often be introduced via contamination. Research questions concerning these elements may well benefit from investigating the large amounts of samples stored in archives at different institutions.

Concluding remarks and recommendations

There is a wealth of archived material that can potentially be used for TE studies. These include samples from field experiments, surveys and environmental monitoring programmes where research funds have been invested in maintaining experiments and collecting and archiving samples, and for which other data are already available. If uncontaminated, such samples can be used for contemporary TE studies, potentially providing added value. To enable this, the general consciousness about the risks of TE contamination in archived samples needs to be raised among non-specialists. One step towards reaching quality

assurance throughout the entire chain is to incorporate precautions against TE contamination into the general protocols for e.g. field experiment maintenance and sampling, and environmental monitoring. To our knowledge, such protocols either contain insufficient information on TE issues, or none at all (e.g. Försökshandboken, 2009). Thus quality assurance with respect to TE often depends on the personal interest of individuals engaged in research, advisory services or environmental monitoring. Considering the recent increase in interest in TE, from nutritional as well as toxicological and environmental perspectives, it is timely to raise these issues, and e.g. introduce a comprehensive approach to sample collection and preparation that allows for complementary TE analysis of future archived samples. However, this cannot be the responsibility of the individual non-TE specialists alone but needs to be a joint effort of TE specialists and non-TE specialists along the entire sample chain within the fields of research, advisory service and environmental monitoring.

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463

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468 Table 1. Potential sources of sample contamination and otherwise erroneous data in the collection-analysis chain of herbage samples. Bold lettering indicates

469 potential contamination sources, normal lettering other sources of error. Literature references for contamination sources are given below.

Should be given in Field handbooks, Standard Operating Procedures, ISO etc.						Included in Accreditation Schemes	
Sampling		Sample preparation		Storage	Sub-sampling for analysis	Extraction/ digestion	Analysis
Sub-sample from field or plot (sampling design)	Cutting, handling and transportation	Drying & sub-sampling from bulk sample	Milling				
Area No of samples Sampling pattern (grid, random etc)	**Soil**[1,2,3, 4, 5, 6, 7] **Equipment/ surfaces**[4, 8, 9] **Weather conditions**[10] Phenological stage Part of plant	**Washing/cleaning**[9] **Surfaces/ containers**[4,11] Subsampling method Temperature (**freeze**[12], dry etc)	**Device or technique; glass, metal, porcelain; cutting, grinding**[4,12,13,14,15,16,17] **Surfaces**[4] Type of plant material **Cleaning procedures**[16]	**Container**[4,11] Chemical & biological effects of unsuitable storage conditions Size/quality separation	Subsampling method (affecting size/quality separation) **Surfaces/ devices**	**Chemical agents** **Water** **Vessels** **Chance contamination** Digestion Lack of GLP – blanks etc Lack of reference samples (cross-lab tests etc)	Working below detection/analytical limits Instability in analytical performance (e.g. quality/purity of gas and chemicals, electricity, temperature, humidity etc). Analysing and reporting elements not planned for in previous stage

- **Dust**[4]
- **Skin care products on bare hands**[4]

Human variation

[1] Bargagli (1995); [2] Calder and Voss (1957); [3] Cook et al. (2009); [4] Stangoulis & Sison (2008); [5] Wolterbeek (1995); [6] Wyttenbach and Tobler (1998); [7] Wyttenbach and Tobler (2002); [8] Fleming et al. (1986); [9] Lockman (1980); [10] Fernández et al. (2010); [11] Waheed et al. (in press); [12] Hamilton (1995); [13] Allan et al. (1999); [14] Cubadda et al. (2001); [15] Markert (1995); [16] Sager and Mittendorfer (1997); [17] Santos et al. (2008).

473 Table 2. Milling/cutting devices tested in the two experiments and the plant material used for

474 the tests.

Experiment	Device	Type	Device material	Plant material
1	Glen Creston Stanmore (bench top, swing tooth hammers)	Hammer mill	Stainless steel	Mature herbage from mixed stand[a]
1	Glen Creston Stanmore (bench top, swing tooth hammers) + Retsch Mixer Mill MM200	Hammer mill + Ball mill	Stainless steel + Stainless steel	
1	Retsch Grindomix GM 200	Knife mill	Ti knives, plastic bowl	
1	Plexi-glass knife, dept workshop	Knife	Plexi-glass	
2	Retch 2000	Cutting mill	Stainless steel	Vegetative timothy
2	Plastic scissors, Kärnan AB	Scissors	Polystyrene resin	

475 [a] Perennial ryegrass, timothy and white clover.

476 Table 3. Test of hammer mill, hammer mill+ball mill, Ti knife mill, with plexi-glass knife as
477 control and mature mixed herbage as test material (n=5); concentrations of elements after
478 sample cutting or milling with the respective devices. Numbers in a column that are followed
479 by a different letter are significantly different.

Device	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
					mg kg^{-1}					
Plexi-glass	0.0070	0.0176	0.008a*	1.96	19.5a	61.0	1.52	0.131a	0.076a	7.95a
Hammer	0.0055	0.0174	0.172b	2.20	22.6ab	65.4	1.64	0.198b	0.127b	14.4b
Hammer+ball	0.0068	0.0166	0.586c	2.16	26.7b	62.9	1.67	0.231b	0.217b	14.3b
Ti knife mill	0.0069	0.0166	0.008a	2.13	18.6a	58.1	1.50	0.151a	0.069a	8.83a
p value	*ns*	*ns*	*<0.0001*	*ns*	*0.0022*	*ns*	*ns*	*<0.0001*	*<0.0001*	*<0.0001*

480 *Two samples below the detection limit.

481

Table 4. Test of steel knife mill with plastic scissors as control and young timothy herbage as test material (n=5); concentrations of elements after sample cutting or milling with the respective devices.

Device	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
					mg kg^{-1}					
Plastic	0.0078*	0.0204	0.060*	4.060	33.6	33.0	1.09	0.664	0.063	15.4
Steel knife mill	0.0071*	0.0226	0.414	4.478	36.8	36.9	1.41	0.812	0.063	16.3
p value	*ns*	*ns*	*0.0006*	*0.0017*	*0.0114*	*0.0269*	*0.0012*	*0.0020*	*ns*	*0.0336*

*Several samples below the detection limit.

Figure captions

Fig.1. Relative concentrations of a) Cu, b) Zn, and c) Cr of two herbage materials after sample milling/cutting, expressed as a percentage of the average concentration in control samples cut by plexi-glass knife (left) or plastic scissors (right).

Fig. 1a

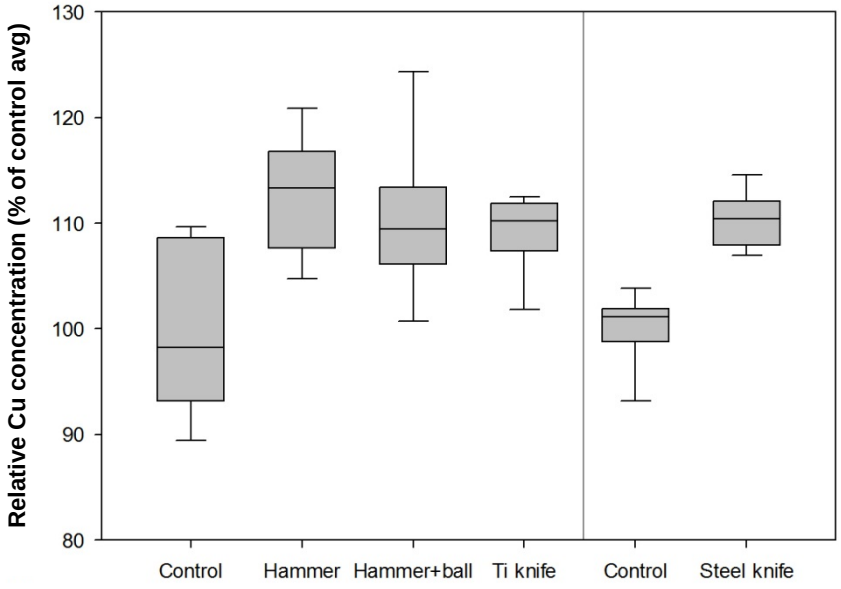

Relative Cu concentration (% of control avg)
130
120
110
100
90
80
Control
Hammer
Hammer+ball
Ti knife
Control
Steel knife

Fig. 1b

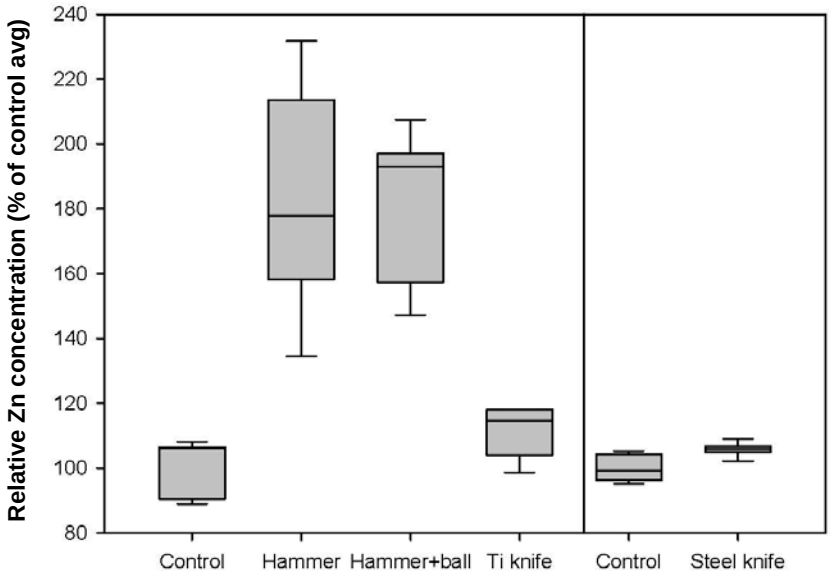
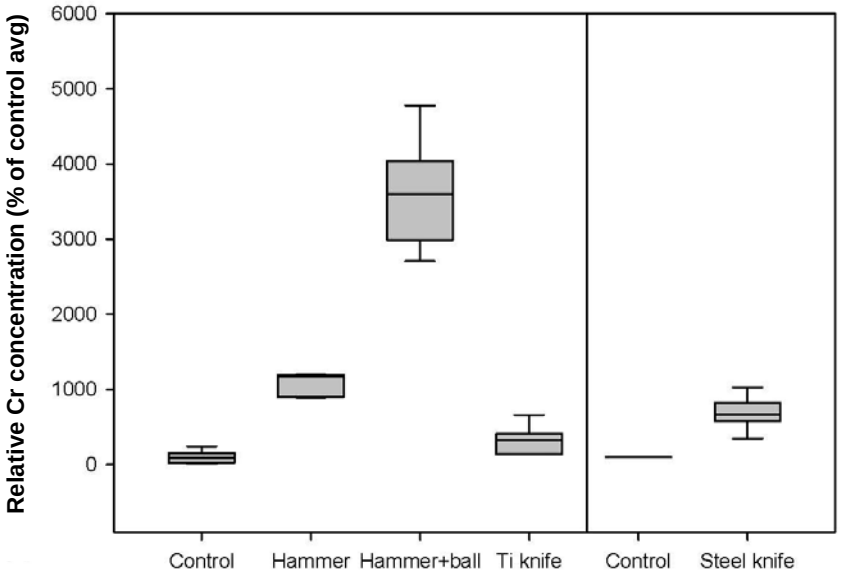

Relative Zn concentration (% of control avg)
240
220
200
180
160
140
120
100
80
Control
Hammer
Hammer+ball
Ti knife
Control
Steel knife

Fig. 1c


Relative Cr concentration (% of control avg)
6000
5000
4000
3000
2000
1000
0
Control
Hammer
Hammer+ball
Ti knife
Control
Steel knife