DISSERTATION

Transfer of Main and Trace Elements from Soil to Plant with an Emphasis on Trace Element Supply for Biogas Digestion Plants

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Abstract

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Transfer of Main and Trace Elements from Soil to Plant with an Emphasis on Trace Element Supply for Biogas Digestion Plants

In this study trace element uptake by different plants and plant species on different soils was investigated. A special focus was on the uptake of the elements Co, Ni, Mn and Mo, as those were essential elements for biogas production. The research project, in which this study was situated, aimed to provide enough trace elements (TE) by an addition of alternative energy crops as substrate for biogas digestion plants. Maize, which is used as the main (plant) substrate had the disadvantage of a very low trace element content of Co and Ni in particular. This study contributed to the assessment of the most important factors for trace element mobility leading to recommendations for farmers to increase trace element delivery to biogas plants with the plant substrate. Furthermore the aspects: soil parameters (pH, soil type, element concentrations, potentially available elements), the influence of the plant species on element uptake, and the transfer factor (TF) of elements from soil to plant were covered. In this project twelve different variants (plant species, cropping systems) were tested in two main field trials, on a very good quality soil (Garte Nord, Reinshof) and an intermediate quality soil (Sömmerling, Uslar). The sample set was extended with additional plant samples from other projects. A small scale field trial and pot experiments were performed to test the mobility of trace elements on different soil properties. Acidic soil pH conditions led to high mobilities of Cd, Co, Ni, Mn or La, as seen in soil extractions and plant element contents. Plants grown in soil with greater total trace element concentration originating from basalt weathering showed increased content in some plant species as well, for example in Fabaceae (legume) plants. Other plant species (Poaceae; maize, cereals, ryegrass) were almost unaffected. A great influence was detected by the plant species, the different plant structures and uptake mechanisms probably led to a distinct uptake pattern for main and TE. These patterns were visible in the concentration levels, in multivariate analysis on the data after a Principal Component Analysis and in different TF. Greatest concentrations of Co and Ni were found in Fabaceae plants (winter and summer faba bean). For Mn and Mo, ryegrass samples showed the largest enrichment. With the means of soil extractions (mainly ammonium nitrate) and total soil element concentrations it was shown, that correlation trends of plant and soil concentrations were detected mostly for Co and Ni and the plant species winter faba bean and hairy vetch. However, these trends cannot be used for a prediction of the concentration in the plants, because of too high variability within one plant species (namely ryegrass) leading to low correlation coefficients. The plant concentrations were combined with dry mass (DM) yield to obtain element extraction from the field in g/ha, or the delivery to biogas plants (in plant silage). A good annual DM yield and a greater amount of TE can be realized with faba bean (mono or intercropped with triticale) succeeded by maize, or with main crop ryegrass.

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List of Abbreviations

Plant and field crop related abbreviations

A	Amaranth
AM	intercropping of amaranth and maize
AF Mix	Annual flower mixture
Barley	Barley
Cup Pl./ CP	Cup Plant
FB	Faba Bean
FB Su	Faba Bean (summer)
FB Wi	Faba Bean (winter)
FB Wi Tri	intercropping of faba bean and triticale (winter varieties)
Hairy Vetch	Hairy vetch
Hung. vetch	Hungarian vetch
M	Maize
PF Mix	Perennial flower mixture
Rye (Wi)	Rye (winter)
RG	Ryegrass
Tri	Triticale (winter)
Wheat (Wi)	Wheat (winter)
RV	intercropping of rye and vetch
GN	Garte Nord (field trial)
SÖ	Sömmerling (field trial)
DM	Dry matter
FM	Fresh matter

Analytical related abbreviations, methods, units

DF	Dilution Factor
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emmission Spectrometry
KED	Kinetic Energy Discrimination
LOD	Limit of Detection
PCA	Principal Component Analysis
PTFE	Polytetrafluoroethylene
M	molar weight (mol/l)
REE	Rare Earth Elements
SEP	sequential extraction procedure
SinEx	single extraction methods
TF	Transfer factor

Chapter 1

General Introduction

1.1 Setting of the Thesis

This study is part of the joint research project entitled "Trace elements by energy crops – mass fluxes and recommendations for an optimized process biology in biogas plants", funded by the BMEL (German Federal Ministry of Food and Agriculture, Berlin) via the FNR (Agency for Renewable Resources in Gülzow, Germany (FKZ: 22014813, 22019014, 22019114, 22019214). The short project title was "NiCo", pointing to the relevance of cobalt (Co) and nickel (Ni) for the biogas process.

The focus of the project is on trace element requirements in biogas production. A high input of maize substrate without slurry or with only small additions of slurry or manure will lead to trace element deficiencies in biogas plants. A shortage in trace elements during biogas production is often treated with trace element supplements. These are provided for example by biogas companies in the form of powder, powder mixed with peat, or as solutions. The trace elements required are, amongst others, Co, Ni, manganese (Mn) and molybdenum (Mo). This project aims to replace trace element supplements with more diverse plant input substrates. Maize, which is the main input substrate used, has the disadvantage of very low trace element concentrations. This research project will also characterize the minimum trace element thresholds needed for a stable biogas production. Twelve different plant species and variants were tested on two experimental field sites with contrasting soil properties. One site with a very good soil quality (Garte Nord at research station Reinshof) and one with intermediate soil quality (Sömmerling near Uslar). Annual and perennial crops were tested in sole cropping and intercropping. Field crop management was performed by Prof. Dr. Rolf Rauber and PhD student Katharina Hey of the Department of Crops Sciences, Georg-August-University of Göttingen. Field crop parameters such as mineralised nitrogen (N_{min}), physical soil analysis, dry matter (DM) yield, DM content, root density, and biogas yield. Also, social factors like the acceptance of energy crop cultivation by the local population were determined in a survey.

A part of the plant harvest from the site at Reinshof (Garte Nord) was ensiled and transported to project partners in Leipzig (Deutsches Biomasseforschungszentrum, DBFZ) and the Umweltforschungszentrum (UFZ). The DBFZ performs lab-scale biogas digestion studies with semi-continuous fermenters. In the second stage, biogas fermenters on the brink of trace element shortage, were feed with maize and a part of the ensiled plant material from our field trials. Here, it was tested, whether plant silage can deliver enough trace elements for a continued stable biogas production. Additionally, the DBFZ also measures the usual biogas production parameters, such as FOS/TAC values, pH or methane gas production. The UFZ performs microbiological tests and analyzes the microbial communities according to their quantity and activity in the fermenter material of the lab scale biogas plants. One aim in this part of the project was to look for early signs of trace element deficiency, such as shifts in the distribution of microbial strains.

This study was conducted in part by the Geoscience Centre Göttingen (GZG, Department of Sedimentology and Environmental Geology, University Göttingen). This project was led by Dr. Benedikt Sauer and conducted by PhD students Tino Pasold and myself. Within this work, all element analysis of the soil and plant samples were performed by the author. PhD student Tino Pasold of the GZG performed element analysis on the fermenter material from the DBFZ and determined the thresholds for the trace elements. He also performed tests of the bioavailability of trace elements incorporated in the plant silage in the biogas fermenter and tested toxic thresholds for some of the trace elements in small-scale batch tests.

This work contains the element data of the plants from the main field trials and examines the soil-plant transfer. The sample set is extended with samples from former projects, or from other plant research projects (Dr. W. Link, Agronomy; A. Biertümpfel, TLL Jena; M. Fritz, TFZ, Straubing) or self conducted field studies (Bühren) and open-air pot experiments.

Two bachelor Theses were supported and supervised within this body of work. These were:

- *Eignung unterschiedlicher Bodenextraktionsmethoden zur Bestimmung der Pflanzenverfügbarkeit von Cobalt und Nickel* (Suitability of different soil extraction methods to determine the bioavailability of cobalt and nickel) by Markus Simon Willerding-Möllmann at the Faculty of Agronomy, Supervisors: Dr. Steingrobe and W. Fahlbusch, winter term 2014/2015,
- *Spurenelementgehalte verschiedener Getreide-Leguminosen-Gemenge auf zwei bayerischen Standorten und ihre Bedeutung für die Biogasproduktion* (Trace element contents of cereal-legume intercropping grown on two sites in Bavaria and their relevance for biogas production) by Andreas Sorger at the Faculty of Geography and Geosciences, Supervisors: Prof. Dr. H. Ruppert and W. Fahlbusch, summer term 2015.

1.2 Aim of the Study and Approach

In a former research project the soil-plant transfer of heavy metals was tested on contaminated agricultural soil near the Harz mountains and was entitled "Bioenergy Production on Polluted Soils". It was part of the interdisciplinary project "Sustainable Use of Bioenergy: Bridging Climate Protection, Nature Conservation and Society" (Ruppert et al., 2013). The subproject "Bioenergy Production on Polluted Soils" utilized a phytostabilization approach on heavy metal contaminated agricultural fields. Phytostabilization means a cover by field crops to help prevent transportation of heavy metals by wind (Ali et al., 2013). These fields should not be used for the production of food or forage crops, but solely for energy crop production as soil removal or cleaning of the soil was not feasible, or would take thousands of years. Also, a cover by field crops would help prevent transportation of heavy metals by wind. Therefore, several energy crops like sunflowers, sugar beets or cereal crops were cultivated and tested for their heavy metal content. The idea was to use energy crops with low element uptake from the contaminated soils for biogas production. In that way, the fields were covered with plants and the elements returned with the biogas residue to the fields, without entering the food chain.

A very distinct uptake behavior for cadmium (Cd) was observed in plant species like amaranth or sunflower which showed a very high Cd uptake; this was much lower in maize (Sauer and Ruppert, 2013). For the other elements, including Co and Ni, a distinct uptake behavior was observed. Interesting results were also obtained for Mo, where sugar and energy beets showed a small uptake proportionally lower than maize or other cereal crops (Fahlbusch et al., 2013). This result also suggests, that plant replicates should be included in any study, because of the variability in element concentrations. This was demonstrated in the author's Diploma Thesis (Fahlbusch, 2012).

In a pilot project, several energy crops were tested on non-contaminated agricultural soil located close to the biogas plant of Verliehausen near Uslar (Sauer et al., 2014). This preliminary project was entitled "Möglichkeiten einer ausgeglichenen Spurenelementversorgung von Biogasanlagen durch Mischung unterschiedlicher Energiepflanzen" and was funded the German Federal Ministry of Food and Agriculture, BMEL, Berlin via the FNR (Agency for Renewable Resources, Gülzow, FKZ: 22041611. Preliminary findings allude to differences in trace element concentrations in uncontaminated soil for Co and Ni, and also other elements. That led to the actual research project "NiCo" where the most promising energy crops with a potential for high trace element concentrations and high DM yield from the preliminary project were grown on two contrasting soils. These former results suggested, that different plant species would uptake Ni and Co in an individualized manner. Based on these preliminary findings the aim of this present study was to assess the most important factors governing trace element uptake from soil to plant and the characterization of trace element concentrations in the above ground plant samples. Furthermore, the testing of whether trace element uptake can be modelled by simple analysis techniques (sample digestion, soil extraction) to predict the resulting concentrations in the plants was also performed.

From these primary aims, several subgoals were investigated:

- Parameters of trace element determination in plant samples,
- Calculating real uptake of trace elements (subtracting element addition from dust or soil particles),
- Do several plant species have distinctive element uptake and which species' inherent factors may be responsible for this?
- How big is the influence of soil parameters (soil pH, geology, total element contents) on uptake?
- How stable are the element concentrations in (plant) sample replicates grown on the same field?
- Is it possible to predict plant trace element contents?
- What recommendations for action can be derived to achieve a high trace element yield for biogas plants?

The focus in this thesis was on trace elements necessary for biogas production (Co, Ni, Mn, Mo), however a broader element spectrum was taken into consideration including major nutrient elements (K, P, Mg, Ca, S), trace elements and ultra-trace elements.

1.3 Renewable Energy Production with Biogas

The generation of renewable electricity from biogas is a promising resource which can mitigate climate change and decrease dependency on fossil fuels. In Germany, this is mostly achieved by cofermenting of energy crops with animal manure (Scheftelowitz et al., 2015). The biogas produced is usually used to fire combined heat and power plants (CHP). The electricity generated is then fed into the power grid and, in many cases, the heat supplied to local heating networks (Ruppert, 2011).

In Germany, there were about 9300 biogas plants installed with an overall capacity of 4.5 GW (Biogas Fachverband, 2017). This was achieved particularly due to the Renewable Energy Act of 2000 (EEG, "Erneuerbare Energien Gesetz") which promoted electricity production from renewable sources. The EEG has since been constantly revised and in 2004, a bonus for renewable materials (NaWaRo-Bonus) was introduced. Since then, the amount of renewable material utilized as input substrate rose. In the latest revision of the EEG, it was declared that maize input into biogas plants must not exceed 50 mass-% for sites built after 2017 (Bundesministerium für Wirtschaft und Energie, 2017). In 2014, about 52 mass-% of the input substrate were renewable material (plants) and about 43 mass-% from agricultural waste (animal feces and liquid manure). Maize is the most important crop in the group of renewable biomasses (27 %), followed by grass (12 %) and 7 % cereal whole crop silage (Daniel-Gromke et al., 2017). Maize is used predominantly as an input substrate, because of its very high dry matter and methane yield (Herrmann, 2013).

However, some negative aspects should be taken into consideration. Maize is low in trace element content compared to other crops or input substrates such as animal manure or slurry. A low trace element content in biogas fermenters causes instabilities and a low biogas production rate. Cobalt (Co), in particular, is a limiting factor for growth and activity of methanogenic microorganisms in biogas fermenters (Choong et al., 2016; Hinken et al., 2008; Lebuhn et al., 2008; Lindorfer et al., 2012; Pobeheim et al., 2011). Alongside Co and Ni, the elements Mo, Mn, Se and W are considered essential trace elements for biogas microorganisms (Demirel and Scherer, 2011). To avoid negative effects due to low trace element supply by plants, additives are commonly used in more than 3.000 biogas plants in Germany. Whilst this handling stabilizes methane production, environmental, economic and health risks are particular issues (Choong et al., 2016; Kuttner et al., 2015).

1.3.1 Negative Aspects of Maize Cultivation

However, alternate energy crops can contribute greater content of trace elements essential for biogas microorganisms. A substrate input mix of maize with other energy crops such as legumes, amaranth or grass leads to a higher trace element input into biogas digestion plants. Other negative impacts of maize are soil degradation, and decrease of soil organic matter. In a study conducted in South West England 3243 sites of varied crops were investigated to assess their soil structure (Palmer and Smith, 2013). 38% of these sites showed severe to high levels of soil structural degradation. Sites where late harvested crops (such as maize) had been grown were most affected. More than 75 % of the examined maize sites (n = 198) showed severe or high levels of soil structural degradation. When maize is grown in monoculture it may also promote pests like the European corn borer (*Ostrinia nubilalis*; Wührer and Zimmermann, 2007). It is thus advisable to investigate other plant species and cropping systems (e.g. intercropping or double cropping systems). They offer opportunities for broader biodiversity in the field, because in contrast to food and feed production, there are fewer requirements for quality and ingredients. In addition to common energy crops, alternative species including flowering plants can also be cultivated as energy crops. Further aspects of a sustainable bioenergy production also include the social acceptance of energy crop cultivation by the population (Ruppert et al., 2013). In the research project, this aspect is covered by Katharina Hey. She conducted a survey and asked for the impression of alternative energy crop production with amaranth, cup plant or flower mixtures in contrast to maize cultivation.

1.4 Function of Important Trace Elements Within Energy Crops

The trace elements required for biogas production are taken up by the plants. The elements themselves have different functions and mechanisms in plants which may explain the different uptake.

Essential micronutrients for higher plants are B, Cl, Cu, Na Fe, Mn, Mo, Ni and Zn (Taiz and Zeiger, 2010). Co is reported to be beneficial to plants, but toxic in greater amounts (Palit et al., 1994; Marschner, 1995; Hänsch and Mendel, 2009). Toxic effects by large Co doses to the soils result in decreased shoot biomass (Li et al., 2009). Co is favorable for members of the Fabaceae family. Reisenauer (1960) first demonstrated an essential role of Co in legumes for symbiotic nitrogen fixation. However, Co is an essential element for humans and animals as a constituent in Vitamin B12 which is required for energy metabolism and the production of red blood cells. It is produced by microorganisms in ruminant animals when their diet contains adequate levels of Co.

Mn is primarily absorbed by plants as a Mn^{2+} -ion. It becomes more mobile in acidic soils and/or under anaerobic conditions due to its reduction from Mn^{IV+} to Mn^{II+} (Kabata-Pendias, 2011). In plants, Mn is found in a large number of enzymes and is involved in redox processes in II, III, and IV oxidation states. Due to its similar loading and ionic radius, Mn^{2+} can substitute or compete with Mg^{2+} or Ca^{2+}. Mn also plays an important role in photosynthesis, which was first demonstrated in the green algae *chlorella* (Kessler et al., 1957). An insufficient supply of Mn leads to reduced oxygen production and lower chlorophyll content, with leaves turning to a pale green or yellow coloration as demonstrated with *Trifolium subterraneum* (Nable et al., 1984).

Mo is relatively mobile in soils and readily taken up by the plants as molybdate MoO_4^{2-} (Kabata-Pendias, 2011). In contrast to other cationic trace elements such as Co, Fe, Mn and Cu, Mo is less mobile in acidic and more mobile in alkaline soils. Mo is an essential element for higher plants as a component of several enzymes (Hänsch and Mendel, 2009).

Ni is considered essential to higher plants for its role in the enzyme urease, required for the hydrolysis of urea (Dixon et al., 1975) and thus preventing the accumulation of urea to toxic levels (Eskew et al., 1984). Ni is also essential for temperate cereal crops like barley, as they are unable to complete their life cycle in the absence of Ni; supplementation of Ni alleviated deficiency symptoms in maternal plants (Brown et al., 1987).

1.5 Parameters Influencing Elemental Bioavailability

There are several parameters which influence soil-plant uptake. The most important soil parameter affecting the mobility of trace metals is the soil pH. The pH value is the negative logarithmic hydrogen ion activity ($pH = -log_{10}(H^+)$). Soil pH is indicative of geological background and of land use practices. For agricultural purposes, a soil pH between 6 and 7 is preferred. In unlimed grassland the pH is usually between 5 and 6. In forests, typical pH-values are between pH 3 to pH 4 (Alloway, 2013). In acidic soil conditions more H^+-ions are adsorbed to soil particles thus releasing previously adsorbed metals back into the soil solution. The elements Co, Ni and Mn have higher uptake rates by plants at lower soil pH values (Kabata-Pendias, 2004). Mo is less mobile at acidic pH, since it mostly occurs as molybdate ions in oxidizing soil conditions. Its adsorption is greatest at acidic soil pH (Blume et al., 2010).

Most of the trace metals (Co, Ni, Mn, Zn, Cu) are found in soil solution in cationic form. They are adsorbed to soil particles, or bound in an insoluble form, for example as a mineral structure (isomorphous substitution), and are only released by mineral weathering. This means, a different geological background can lead to different element releases.

Other sources of the metals are fertilizers (organic and mineral). The trace elements themselves (Co, Ni, Mn, or Mo) are seldom used in fertilizers, with the exception of manganese. Winter cereal crops are sometimes treated with Mn, when the soil pH is greater than 6.5. There are anthropogenic sources of these elements too. For example, dust transportation from battery recycling stockpiles can lead to very high Co and Ni plant concentrations, because these elements adhere with dust particles to the plant surface. Organic fertilizers (slurry or biogas residue) are not only sources for macro nutrients (P, N, K) but also have a high trace element concentration in the liquid. These are also possible sources for the uptake of trace elements by the plants. For the purpose of this thesis, all plant experiments received biogas residue as an organic fertilizer and thus it can be assumed that all plants received the same planting conditions.

The soil texture –that is the particle size distribution– is important. A soil that is very rich in clay particles will have high total elemental content, but the plant roots cannot access them. Also, the water uptake by the plants is hindered. Another important factor is the soil root architecture, which is plant-species dependent. Monocots develop three to six primary root axes, from which new or nodal roots emerge. This is in contrast to dicots which develop a taproot, a main single root axis, that can reach great depth (Taiz and Zeiger, 2010). The root hairs are most important for the absorption of water and solutes, and lead to a greater root surface area. The root hairs are most active for phosphate absorption (Föhse et al., 1991).

The plant species' inherent uptake mechanisms pose another factor to soil-plant uptake. The plant species have different element acquisition strategies. Some plants can emit root exudates in the form of organic acids to locally lower soil pH and mobilize elements. Some grasses can exude chelate compounds (phytosiderophores) which mobilize Fe (Römheld, 1991). Also, the Fabaceae plants possess the ability to fix nitrogen from the air with the assistance of rhizobia bacteria in the root nodules. The various mechanisms by different plant species can also lead to different element concentrations in the plant tissue. This aspect is adressed by the investigation of 12 different variants in this study. The cultivation system can modify the uptake too. For example, the plant concentrations may be modified when the plants are intercropped with another plant species. Other external factors include humidity and temperature. When there is water shortage, element mobility and transport to the plants is hindered.

1.6 Structure of the Thesis

In Chapter 1 the background and framework of this Thesis are given. An overview of biogas production and trace element requirements are presented. Also, a short summary about the main factors influencing element uptake by plants is provided. Chapter 2 is the *Materials and Methods* section. Here, general techniques utilized frequently throughout this Thesis are described. The sample locations at which either field experiments were conducted, or which soil was taken for pot experiments are shown. The analytical workflow is presented here from the collection of raw data from the analysis device to the fully evaluated concentrations based on dry weight (in mg/kg DM). Finally, the field experiment settings for the main field trials are shown.

Chapter 3 gives recommendations for determining of trace elements with ICP-OES and ICP-MS, with a particular focus on Co. The fit of external reference standards are given in diagrams. Recommendations are given, especially for plant tissue which in most cases have very low concentration. German DIN regulations for ICP-OES measurement are discussed throughout.

Chapter 4 shows how the measured element concentrations are influenced by adhering soil or dust particles. Plants, which are grown in the open air and are subjected to a complete digestion will always be affected by adhering particles. The element data of the plants will be corrected with mathematical functions. Three approaches are presented in this Chapter, in which the part attributed to the soil/ dust is calculated and subtracted from the measured element concentration. For this comprehensive study a large number of samples were considered collected between 2012 and 2016 –at total 1040 plant samples– comprising different species and families. It is shown to what extend the element concentrations change after applying the correction method over a broad range of elements (about 45 elements). Most affected were non-nutrient elements and major elements in the soil (Al, Ti, Sc, REEs, Hf, Ta, Nb, Zr). Major nutrients and most of the trace nutrients (Fe, Mn, Cu) were not changed. Co is affected, and corrected by about 20 %. The second part of this Chapter shows how a very short cutting height can lead to elevated element concentrations in plant tissue and how they are then corrected.

Chapter 5 is a case study on how elevated soil total concentration affects the concentration levels in the plants. The plants were grown in a soil close to the village of Bühren (Dransfeld) and is derived from basalt weathering. Basalts are naturally enriched in Co, Cr, Fe, Mg, Mn, and Ni. A small scale field trial and pot experiments were performed. A detailed study on the plants showed elevated concentrations in Co, Cr, Ni, Mg, and Zn for some plant species compared to the plants grown on the main field trials. Total soil element concentrations from several soil sample locations in this study were given.

Chapter 6 deals with the soil to plant transfer. In this Chapter, all measured elements were considered, with transfer factors per element and a multivariate analysis on a large group of elements. Two transfer factors are introduced, a transfer factor of plant concentrations to soil total concentrations and a factor of plant concentrations to soil extraction data with ammonium nitrate. The results show distinctive patterns, probably resulting from different uptake mechanisms of plant species. The Fabaceae plants always display greater transfer rates. Maize and Poaceae plants (rye, triticale) always had low transfer factors. Biplots, the graphical representations of the results of a Principal Component Analysis, show groups formed by the plant species. The soils (main field trials) do not show this prominent distinguishing feature in the biplots.

The element concentration in plants and the intercroppings from the main field trials are discussed in Chapter 7. The results of the main nutrient elements (K, P, S, Mg and Ca) and of the trace elements Co, Ni, Mn, Mo, Fe, Cu and Zn are presented as plant concentrations. In this part, the concentrations are different for most elements for the different plant species. Ryegrass samples show the most variable concentrations for the elements S, Mg, Ni, Mo, Mn and Fe. The greatest trace element concentrations were detected in plants of faba bean (winter and summer variety), hairy vetch or amaranth. The intercroppings on amaranth/maize, rye/vetch and faba bean/triticale are presented with their yield shares and concentrations. Only faba bean/triticale reached equal shares of DM yield. In the other two intercropping systems, the poaceae plant (maize and rye) equals to more than 90% of DM yield. The resulting concentrations in the intercropping systems were calculated based on yield share. Only for faba bean/triticale elevated concentrations were detected, because of the higher concentration in the faba bean.

The soil extraction method is discussed in detail with respect to bioavailability in Chapter 8. The extraction method with ammonium nitrate is well suited to characterize different soils concerning their readily available pool of metals. The extraction results of the soils are shown, revealing that with a low pH in the soil Cd, Co, Ni, Mn and La are enriched in the soil extraction solution. Figures outlining the plant concentration vs. total soil concentration, or vs. soil extraction are displayed to detect possible correlations. Correlation trends can be observed for amaranth, faba bean and ryegrass for some of the trace elements (Co, Ni, Mn). For Mo, correlation trends were not detected because of variable element concentrations in samples from the main field trials (in particular ryegrass), or no recovery of Mo in the soil extractions.

In Chapter 9 element concentrations and DM yield are considered together. A calculation of total trace element delivery to biogas plants with the harvest, or to say it differently, extraction from the field, was performed. It is shown, how the figures develop for the two main field trials in 2015 and 2016. The total element deliveries are calculated for the whole year, including maize as a follow-up crop. To deliver a high amount of Co in $g/(ha\ a)$, the best choice is either summer faba bean in sole cropping, or winter faba bean-maize, or intercropping of faba bean /triticale-maize. For a high Mo, Mn and Ni harvest, ryegrass is a good option. This Chapter finally shows how addition of alternative energy crops to maize as input substrates can change trace element concentrations in biogas fermenters. Since this Chapter is a submitted manuscript some details are repeated from the Introduction. It was placed here, because it closes the circle towards biogas production.

Chapter 10 is a short summary of the results and gives an outlook.

Chapter 2

Material and Methods

2.1 Sample Collection and Processing

2.1.1 Sampling of Plants and Soil for Trace Element Analysis

All plant samples were collected as whole plants (aboveground plant parts without roots), cut at approximately 7 cm above soil surface.

The soil samples of the main field trials Garte Nord (Reinshof) and Sömmerling were taken in April 2015. The top soil part was taken up to a depth of 30 cm. The samples were air dried and sieved to < 2 mm in grain size. A minimum of 100 g of soil and 500 g of plants were dried at 105°C. The soils were ground in an agate ball mill (Fritsch Pulverisette 5) and the plants cut in a universal cutting mill (Fritsch, Pulverisette 19, Fig. 2.1a). Larger sized plants or mixtures (flower mixtures, maize, cup plant) were dried and cut with a garden shredder, before submitting to the universal cutting mill. The use of the cutting mill is needed to obtain a homogeneous sample with equal small particle size, which is a prerequisite for the total digestion process (Fig. 2.1b). The metal blades in the cutting mill are made from chrome-free (ISO/EN/DIN Code: C60W3) steel to ensure that there is no contamination with metals during the cutting process.

2.1.2 Sample Collection for Yield

The yield in t/ha was evaluated by Katharina Hey for all variants at both locations and is presented as DM yield. All varieties were harvested at the usual time as per normal agricultural practice for the purpose of biogas production. To obtain DM yield all plants from 1 m^2 were cut, except for maize. For maize, all plants from 1.5 x 5 m were cut at about 7 cm above the soil surface. The larger sample area for maize is needed to obtain a representative sample for such tall plants. The other plants were cut near to the soil surface by hand. All plant material was dried at 105°C for 48 h.

2.1.3 Total Digestion and Measurement of Element Composition of Plant and Soil Samples

Aliquots of 150 mg of soil and 700 mg of plant powder were completely digested with a mixture of ultra-pure concentrated HNO_3, $HClO_4$ and HF in closed ultra-clean PTFE vessels (PicoTrace, Bovenden, Acid sample digestion system DAS 30). For soil

(a) (b)

Figure 2.1: Universal cutting mill (Fritsch, Pulverisette 19) (a) and homogeneous sample plant powder after cutting.

samples, a small amount of HCl was added to completely dissolve precipitated aluminum and iron oxide hydroxides. In one digestion series there were 32 positions, about two blind samples and reference standard materials were introduced per digestion series. The total duration for the complete acid digestion was about three days for soil/rock samples and about 5 days for plant samples which included a pressure phase, evaporation and a dissolution phase. The plant samples additionally needed a pre-reaction phase for the oxidation of the organic matter. The final clear digested solutions obtained from the soils were diluted to 100 ml, or 50 ml for plants, before measurement. Additionally, blind solutions without sample material were added to the digestion process to ensure that the digestion process and handling was clean without detectable contamination from the reagents, the digestion procedure or during the measurement process. In the resulting clear sample solutions, 47 elements could be quantified by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES, Agilent 5100 VDV) and - Mass Spectrometry (ICP-MS, Thermo Scientific iCAP Q). The limit of detection (LOD) was calculated by the 3-fold standard deviation of the blind concentrations for each analysis batch. The precision and accuracy of the digestion process and the ICP devices were tested by analyzing several international reference samples and one in-house standard. For plant samples, these reference materials were mainly used: GBW 10052 Tea, NCS DC 73349 (bush leaves), WEPAL- IPE-126 (Maize), WEPAL- IPE- 168 (Sunflower). For soil samples these reference materials were used: Clayey Shale 45 (shale, in-house reference material), JLk-1 (lake sediment) and BB-IGDL (basalt, in-house reference material, Appendix Tables A.3 and A.4).

Generally, the accuracy of the main elements was between 5 and 10%. For the trace elements, typical average accuracies were between 10 and 20%. See Appendix Tables A.7, A.8 and A.9 for the accuracy measurements of all reference materials utilized.

2.1.4 Soil Extraction Procedure with Ammonium Nitrate

Neutral salt solutions are extraction methods representing the readily soluble element fractions in the soil. Soil extractions with NH_4NO_3 follow German DIN protocol 19730 (DIN Deutsches Institut für Normung e. V., 1997). Neutral salt solutions are extraction methods representing the readily soluble element fractions in the soil. 20 g of air-dried soil was shaken with 50 ml of 1 M (mol/l) NH_4NO_3 solution for 2 h. The supernatant was separated by centrifugation for 5 min at 3900 rpm. The supernatant was then filtered through a 0.45 μm membrane with a device fully made of PTFE, to avoid metal contamination. The solutions were stored in PET bottles. For stabilization 1 ml of pure HNO_3 was added to the samples. Until the time of measurement, samples were kept in a refrigerator set to approximately 8°C. The solutions were directly measured by ICP-OES. For every 10 samples one blind sample was introduced. Blind samples are those without soil sample material. Separate limits of detection of the extraction solutions were calculated by a 3-fold standard deviation of the blind samples. The mean concentration of the blind samples were subtracted from the results. The results were then converted from *mg/l* to *mg/kg* via the dilution factor (DF). The dilution factor for the extractions was calculated $Volume/weight = 50\ ml(g)/20g = 2.5$.

2.1.5 Soil Parameters - pH, Soil Type

The grain size distributions were measured with laser diffraction particle size analysis with a Beckman Coulter *LS 13 320 Particle Size Analyzer*. For the soils of the two main field trials, the soil texture was also measured by an external laboratory by hydrometer analysis. The soil pH was measured in 0.01 mol/l $CaCl_2$ according to DIN regulations (DIN Deutsches Institut für Normung e. V., 2005). Prior to pH analysis, the soil was sieved to <2 mm and air-dried. Further soil properties for the main field trials are described in Section 9.2.1.

2.1.6 Data Management, Database and R Tools

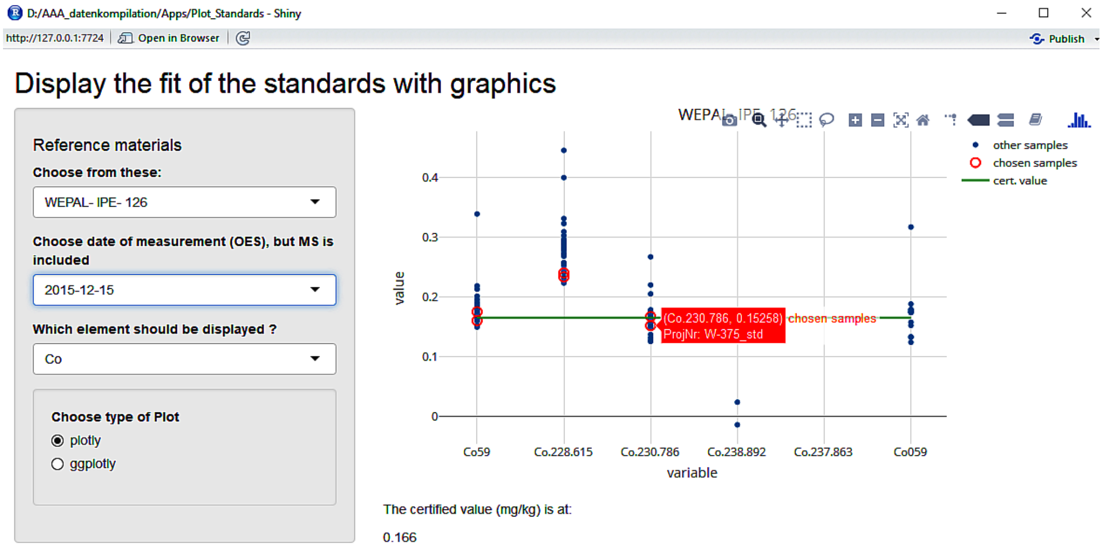
The whole data management pipeline from raw data generated from ICP-OES and ICP-MS, the data processing including the LOD calculations and the concentrations based on dry weight was performed with the software *R* (R Core Team, 2017).

The data was collected in a data compilation in *R*, which includes all types and stages of the data (raw-data, sample informations (sample lists), concentrations of solutions and concentration based on dry-weight). The compilation of this data was managed by members of the working group of Prof. Dr. Ruppert and is organized via a project infrastructure provided by the GWDG Göttingen (`https://projects.gwdg.de/projects`). The data can be accessed by the version control system *git* (`https://git-scm.com/`) directly from approved computers.

The data compilation also includes data from additional research projects and from Bachelor Theses supervised by members of the working group. The data compilation at present comprises of **1318 plant samples, 400 soil samples and 542 fermenter samples (based on dry weight)**. Each of these samples were assessed for approximately 47 elements, and all passed quality tests.

For long-term stability of the sample processing and data evaluation the limits of detection (LODs), the concentrations of the blind samples, the measured concentrations of the standards (reference materials) and tables of their accuracy and precision are stored and updated whenever new data is added to the compilation. The main functions and basic structure of the data compilation were mainly programmed by Solveig Pospiech with my contribution. All necessary functions are found in two *R* packages named "ICPData" (Pospiech and Fahlbusch, 2018a) and "ICPSubfunctions" (Pospiech, 2018). These can be installed from a local source. The database is very valuable as you are able to quickly access a certain sample type (for example, all maize plants) and perform statistical analyses on them. In particular, it is helpful to look at previously analyzed plants of the same species for outlier detection.

To evaluate the accuracy of analyzed concentration compared to published reference standards, the author of this thesis programmed an application (app) which can also be used by the whole working group for quality control. The app helps the user to assess visually the fit of the measured value of a chosen reference material at a certain measurement date, compared to all other available measurements of the same reference material. This provides long term quality control (see example in Fig. 2.2). The app was built with the *R* package "shiny" (Chang et al., 2017). A numerical summary of the accuracy and precision of the concentrations measured in the reference standard materials is also available, and is provided for each digestion analysis batch. At the moment, this app can be accessed from within the data compilation working directory (Apps/Plot_Standards/app.R). The App is included in the electronic Appendix of this Thesis (Appendix C).


D:/AAA_datenkompilation/Apps/Plot_Standards - Shiny

http://127.0.0.1:7724

Display the fit of the standards with graphics

Reference materials

Choose from these: WEPAL- IPE- 126

Choose date of measurement (OES), but MS is included: 2015-12-15

Which element should be displayed ?: Co

Choose type of Plot

● plotly

○ ggplotly

(Co.230.786, 0.15258) chosen samples
ProjNr: W-375_std

The certified value (mg/kg) is at:

0.166

Figure 2.2: Screenshot of the app to check the quality of measurements of reference materials for Co, the date of measurement marked with red circles, blue points mar are all other measurements of the chosen reference material by the working group. Element, date of measurement marked in red and the standard can be chosen with the drop-down menu.

2.2 Workflow of Data Evaluation

Raw data from ICP-MS (intensity) was converted to determine the concentration in solution (mg/l). After that, the raw data of the digestion solutions measured by ICP-OES and ICP-MS are corrected for possible drifts during the analysis. The corresponding sample list with all additional sample information was added (for example, location, date of sampling, type of sample, etc.). The LODs are calculated for the new date of measurement and is saved in the database. The LOD is calculated by

$$LOD = 3 * sd(x_{blind/blank})$$

with $x_{blind/blank}$ being the concentrations of the blind samples. For each sample and element, a unique LOD is calculated according to the dilution factor. The LODs of plant and soil/rock samples by ICP-OES and by ICP-OES are given in Appendix Tables A.5 and A.6. The mean concentration of the blind samples were subtracted. The LOD was then applied to the new data, that means results below the LOD are marked with “-” in front of the value of the corresponding LOD. This handling of data below LOD is favorable, as it preserves "numeric" data class and the exact value of the LOD. The concentrations based on DW (mg/kg) are calculated using the DF :

$$conc_{sample}[mg/kg] = conc_{solution}[mg/l] * DF$$

with $DF = V[ml]/weight[g]$.

with V= volume in *ml, g* after digestion (50 or 100 ml), and weight of powdered sample. The density of the solution was assumed to be 1 (50 ml = 50 g).

In this step, all combined element variables (multiple wavelengths and element masses) and the sample informations result in >260 variables. One unique sample ID ("ProjNr") makes it possible to identify each sample at every step of the data management pipeline. For practical use and data interpretation it is necessary to choose the most reliable element wavelength or atomic mass for every element and sample. For some elements there are ICP-MS and ICP-OES data to choose from. Appendix Tables A.1 and A.2 show the chosen wavelengths or masses per digestion batch. The selection was performed based on element concentrations above the LOD and on the best fit (precision and accuracy) with international and in-house reference materials. This final stage of data and all other steps in between were saved in the database as separate data objects, which can be subsequently exported to other data formats (like *MS Excel* spreadsheets or text formats).

2.3 Element Analysis

This study focused on trace elements essential for biogas production. To get a full picture of element uptake, about 47 element concentrations per sample were analyzed with results from ICP-OES and ICP-MS combined. This comprised of the main nutrient elements (K, Mg, P, S, Ca), minor trace nutrients (Fe, Cu, Zn, Mo), ultra-trace elements and Rare Earth Elements (REEs). Elements which could be measured by both machines with values of good certainty are marked by two colors in Figure 2.3. In general, main elements were determined by ICP-OES. Several trace and ultra trace elements can only be determined by ICP-MS, as with the REEs. Co and Ni results were taken from either ICP-MS or -OES, depending on the quality control evaluation on the day of measurement.

Appendix Tables A.1 and A.2 give the chosen wavelength (ICP-OES) or atomic mass (ICP-MS) per sample digestion batch.

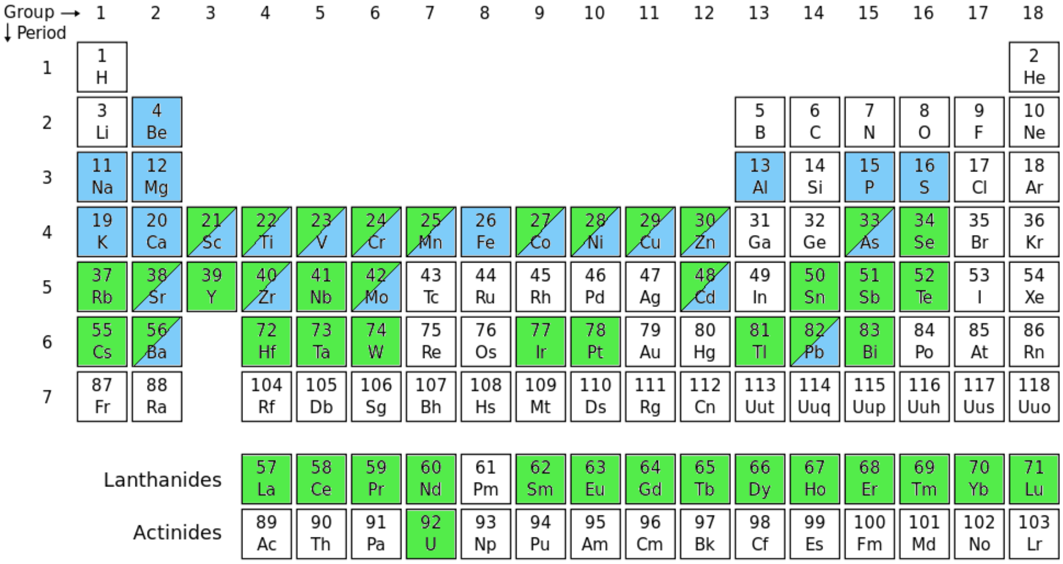


Figure 2.3: Periodic table with measured elements indicated. green = ICP-MS, blue = ICP-OES.

2.4 Conventional and Multivariate Statistics

For univariate data presentation (single element concentrations) of the samples, conventional statistical tools were used. For example the standard deviation of the mean (sd) is used. The problem with all environmental datasets is, that the data is skewed, and conventional statistics can only be applied, if the data is normally distributed. In this study conventional statistics are only used, if there are enough data points (approximately more than 5). In some cases, the median is taken instead. The median is more robust against outliers. In some cases, for example the soil element concentrations of the two main field trials, the sample number was large enough to also calculate the standard error of the mean (SE) by $SE = \sqrt{n}$, with *n= number of samples*.

In multivariate analyses, a data set with several elements are analyzed simultaneously. The data set is transformed and centered via a clr transformation (centered log-ratio transformation). After this, a principal component analysis (PCA) is performed.

This method transforms a set of observations into principal components which are linearly uncorrelated. PCA is usually used to reduce the dimensions/variables. In our case, the elements represent the variables and dimensions. With a PCA it is easier to find general differences between samples. The results are displayed in a covariance biplot, which can be used as an exploratory tool. The biplot was first introduced by Gabriel (1971) and adapted for the use in compositional data by Aitchison (1986). The covariance biplots are used in Chapter 4 and in Chapter 6.4. The links of the rays (representing the variables) in a covariance biplot on clr transformed data are important. If the link between two variables is small (that is, they are close together) they have a quasi-constant log-ratio between them. If the links are very long, with an angle between them of >90°, the log-ratio between them is very wide. If the rays are orthogonal the log-ratio between them is uncorrelated. For multivariate analysis and biplot representation the package "compositions" is used (van den Boogaart et al., 2014).

2.5 The Sample Locations

Figure 2.4 show all sample locations in this study. Table 2.1 gives additional information on the origin of the samples. The two locations marked with green circles are the main field trial sites of the research project, **Garte Nord** a field plot of the research farm Reinshof near Göttingen, and **Sömmerling**, a field plot close to Uslar (Solling region). Verliehausen marks a former field trial site close to Uslar as well, located near the biogas plant of the bioenergy village "Verliehausen". In a preliminary research project several plant species were tested for their trace element content and their potential as trace element rich plant substrates for biogas production ("preNiCo" termed in Table 2.1). Trögen was a soil sample site of a former project of the working group on heavy metal contaminated soils. Trögen was the uncontaminated reference soil and was available for pot experiments. Samples grown in Trögen and Verliehausen soil are also included in Chapter 4. Bühren, Deppoldshausen and Groß Ellershausen are locations of additional plant and soil samples to obtain more information about the element uptake mechanisms. Straubing and Aholfing sites in Bavaria are field trials from the TFZ Straubing (Technologie- und Förderzentrum) where samples of legumes and cereal crops were taken (Bachelor Thesis of A. Sorger) from the research project *Bioenergieträger mit Blühaspekt – Leguminosen-Getreide-Gemenge, short: Legumix* (Eberl and Fritz, 2017). In Dornburg, additional cup plant samples were taken from an ongoing cup plant research project of the TLL ("Thüringer Landesanstalt für Landwirtschaft", Thuringian State Institute for Agriculture, Jena). In Deppoldshausen faba bean and wheat samples were collected belonging to the project Impac^3 (*Novel genotypes for mixed cropping allow for **Imp**roved sustainable land use **AC**ross arable land, grassland and woodland*), by the Centre of Biodiversity and Sustainable Land Use, Georg-August-University (Table 2.1).

Table 2.1: Overview of all sample sites were soil and plant samples were taken. The samples of some sites were part of other research projects and were taken with kind permission.

Location	pot	open field	Project/Inst.	Main topic of interest	State
Garte Nord	**x**	**x**	**NiCo**	**Trace Elements**	**Lower Saxony**
Sömmerling	**x**	**x**	**NiCo**	**Trace Elements**	**Lower Saxony**
Bühren	x	x	own	Trace Elements	Lower Saxony
Gr. Ellersh.		x	own	Trace Elements	Lower Saxony
Lindau	x		own	Trace Elements	Lower Saxony
Deppoldsh.		x	Impac^3	Uptake	Lower Saxony
Aholfing		x	TFZ[a]	Intercropping	Bavaria
Dornburg		x	TLL[b]	Cup plant	Thuringia
Straubing		x	TFZ[a]	Intercropping	Bavaria
Trögen	x		BiS[c]	Trace Elements	Lower Saxony
Verliehausen		x	preNiCo[d]	Trace Element	Lower Saxony

[a] Technology and Support Centre ("Technologie- und Förderzentrum, im Kompetenzzentrum Nachwachsende Rohstoffe"), Straubing Bavaria.

[b] "Thüringer Landesanstalt für Landwirtschaft, Jena", Thuringian State Institute for Agriculture

[c] Pot experiment of uncontaminated reference soil from project: "Bioenergie im Spannungsfeld", Bioenergy production on polluted soils

[d] Samples from a preliminary research project: "Möglichkeiten einer ausgeglichenen Spurenelementversorgung von Biogasanlagen durch Mischungen unterschiedlicher Energiepflanzen"

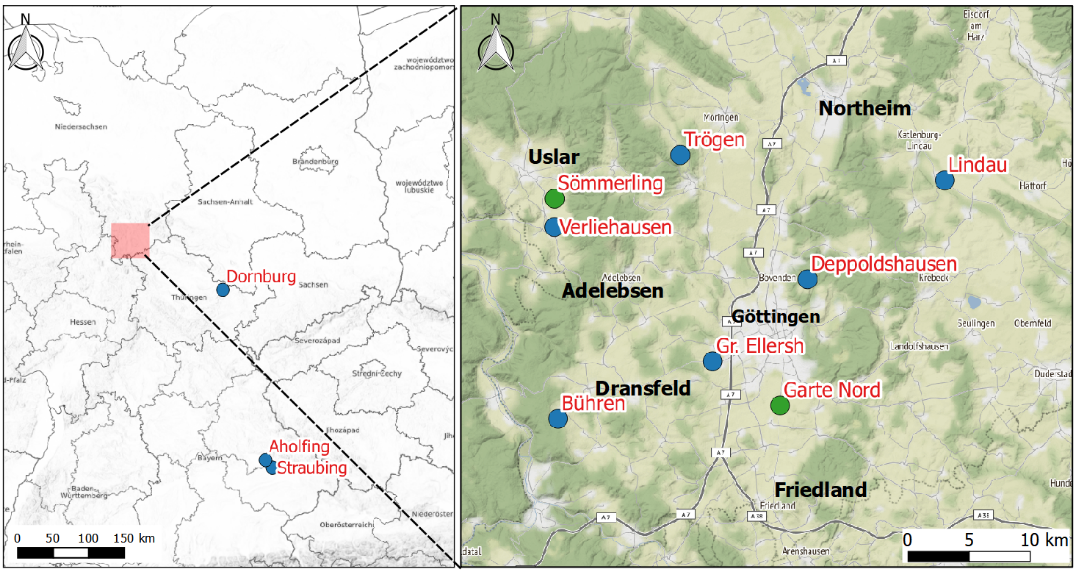
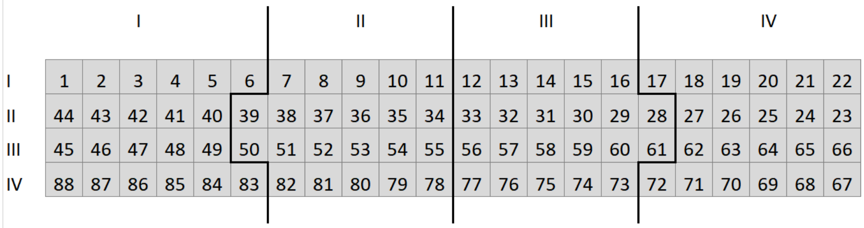


Figure 2.4: All sample sites in Germany (left) and in the vicinity of Göttingen (right), map left: ©OpenStreetMap contributors, openstreetmap.org, licensed as CC BY-SA, map right: Map tiles by Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

2.6 Experimental Setting of Main Field Trials

The field trials consist of 88 plot units each arranged in a semi-latin square design of 4 rows and 4 blocks (Fig. 2.5, roman letters). Each variant was cultivated in 4 replicates, and each was placed once in each row and block unit. Within one block the variants were placed at random (Fig. 2.5). From the two main field trials (Garte Nord and Sömmerling), soil samples of the upper soil samples (up to 30 cm in depth) were taken in separate field plots. Figures 2.6 and 2.7 are aerial photos of the main field trials taken in 2016. 21 soil samples were obtained in Garte Nord and 22 in Sömmerling.



	I						II					III					IV					
I	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
II	44	43	42	41	40	39	38	37	36	35	34	33	32	31	30	29	28	27	26	25	24	23
III	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
IV	88	87	86	85	84	83	82	81	80	79	78	77	76	75	74	73	72	71	70	69	68	67

Figure 2.5: Field plot structure in a semi-latin square design in Garte Nord and Sömmerling. Each variant was placed once in each vertical and horizontal block structure.

Several plant species were tested. Plants were grown in 2014/2015 and 2015/2016, hereafter referred to 2015 and 2016, respectively. They were tested in main/sole and double cropping farming systems. Annual and perennial crops were tested. See Table 2.2 for an overview of plant variants/species and their plant family. Appendix Tables A.19, A.20 and A.21 give an overview about the position of the variants in the field experiment and the DM content at harvest.

Standard crop management practices for fertilization and plant protection were applied (Table 9.2). All crop management of the two main field trials were performed by

Figure 2.6: Aerial photograph of field trials on the site Garte Nord (Reinshof) in August 2016, Image ©2018 GeoBasis-DE/BKG, Google Earth.

Figure 2.7: Aerial photograph of field trials on the site Sömmerling in August 2016, Image ©2018 Digital Globe, Google Earth.

Katharina Hey and members of the Department of Crop Sciences, Faculty of Agronomy (Georg August University, Göttingen). All variants received organic fertilizer (biogas residue, "Gärrest") and mineral fertilizer with the exception of winter and summer faba bean, and faba bean intercropped with triticale. They only received organic fertilizer, since they are able to convert atmospheric nitrogen to ammonia (biological nitrogen fixation). Figures 2.8 show two pictures of the growing winter varieties in June 2015 and 2016 on the field trial Garte Nord. Figure 2.9 show two pictures from the spring time of 2016, and Figure 2.10 shows cup plant and amaranth samples from 2016 on the Sömmerling site.

(a) 03.06.2015

(b) Faba bean, 06.06.2016

(c) Annual flower mixture (15.08.2016)

Figure 2.8: Field trial of Garte Nord: growing winter varieties in 2015 (A) and 2016 (B, C).

(a) Overview of the field trial in Sömmerling, Spring 2016)

(b) Intercropping of faba bean and tricitale (04.05.2016)

(c) Perennial flower mixture (15.08.2016)

Figure 2.9: Field trial in Sömmerling (Uslar).

Table 2.2: Overview of species/variants used in field trials.

Cropping system /variant:	**Abbrev.**	**Plant Family**
Double cropping or intercropping with succeeding maize		
faba bean (*Vicia faba* L.)	FB Wi	Fabaceae
triticale (*Triticosecale Wittm.*)	Tri	Poaceae
intercropping of faba bean/triticale.	FB Wi Tri	Fabac./Poac.
intercropping of winter rye (*Secale cereale* L.) and vetch	RV	Fabac./Poac.
Main Crops		
faba bean summer var. (*Vicia faba* L.)	FB Su	Fabaceae
annual flowering mixture	AF Mix	several
amaranth (*Amaranthus cruentus*)	A	Amaranthaceae
maize (*Zea mays* L.)	M	Poaceae
intercropping of amaranth/maize	AM	Amar./Poac.
Perennial Crops		
perennial ryegrass (*Lolium perenne* L.)	RG	Poaceae
perennial flowering mixture	PF Mix	several
cup plant (*Silphium perfoliatum*)	CP	Asteraceae

(a) Amaranth on site Garte Nord

(b) Cup Plant on site Sömmerling in August

Figure 2.10: Amaranth and Cup plant samples in 2016.

Chapter 3

Recommendations for Determination of Trace Elements in Plant Samples by ICP-OES and ICP-MS

3.1 Introduction

In environmental/ agricultural or provenance studies trace element concentrations in plants often are measured with ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) as these devices have become affordable for many labs. Especially in the agricultural sciences, ICP-OES are used for multiple element quantifications, but is best suited for the analysis of major and minor element constituents in soil or plant samples. For Rare Earth Elements (REEs) or ultra-trace elements determination ICP-MS (Inductively Coupled Plasma Mass Spectrometry) is used predominantly, due to its much lower detection limit. Both machines have the advantage of (almost) simultaneous multi-element analysis.

In Germany, DIN regulation EN ISO 11885 (DIN Deutsches Institut für Normung e. V., 2009) can be consulted for measurement with ICP-OES. It proposes wavelengths for 35 elements in water, sludge and sediment samples. Recommendations for Co are 228.616 and 238.892 nm and 221.648 and 231.604 nm for Ni. The rationale for not including 230.786 nm for Co remains unclear. In the original article by Pickering et al. (1998), it is also listed as an Co-II wavelength with a good intensity. ICP-OES and ICP-MS face different kinds of interferences. ICP-OES can have interspectral interference, where several ions emit overlapping wavelengths. ICP-MS on the other hand, face mass interference when other elements, molecules or double-charged ions have the same mass-to-charge as the element of interest.

For ICP-OES, interspectral interference can be overcome when a different wavelength of the element of interest is chosen with an acceptable intensity. The ICP-OES device software used (Agilent 5100, with *ICP Expert*) has a database incorporated from which recommended wavelengths for every element can be chosen to avoid interference.

The background (baseline) correction used for the ICP-OES was a fitted background correction (FBC) provided by Agilent ICP Expert Software. The detection limits of Co 230.786 and Co 228.616 for plants were alike (0.3 μg/l, Appendix Table A.6). The intensity of Co 228.616 is greater than Co 230.786.

3.1.1 Observations

The following observations mostly concern Co determination in plant samples. For rock or soil samples there are no problems with much greater concentrations. During the measurements of the plant samples the following observations were made:

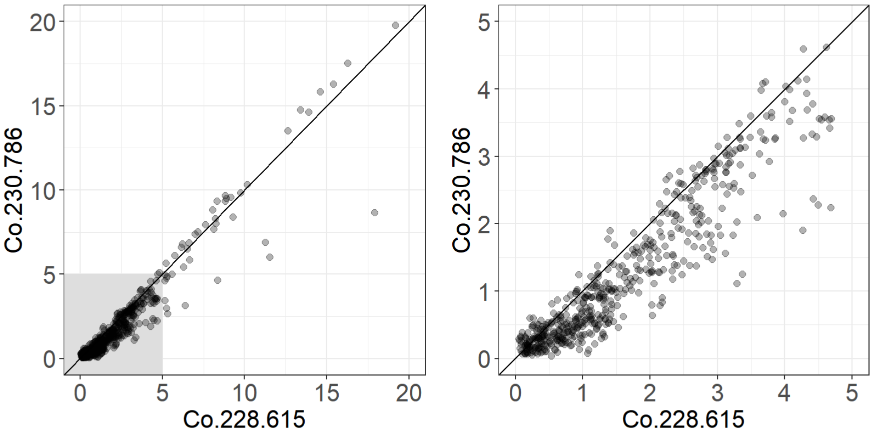
1. For most samples which mainly show small Co concentrations in aboveground plants (1. Quartile to 3. Quartile: 0.019 to 0.115 mg/kg DM)) Co 228.616 showed concentrations greater than, whereas Co 230.786 fits better to, the reference samples. This can be seen in Figure 3.1, where the majority of points below 5 μg/l show a tendency towards greater values for wavelength Co 228.616.

2. Co 238.892 values never correlated with Co concentrations in the reference samples. This wavelength was included during establishment of the measurement routine but later discarded (Fig. 3.3).

3. Co 228.616 showed mostly greater values than the reference, except for standard material GBW Tea (Fig. 3.3 top row).

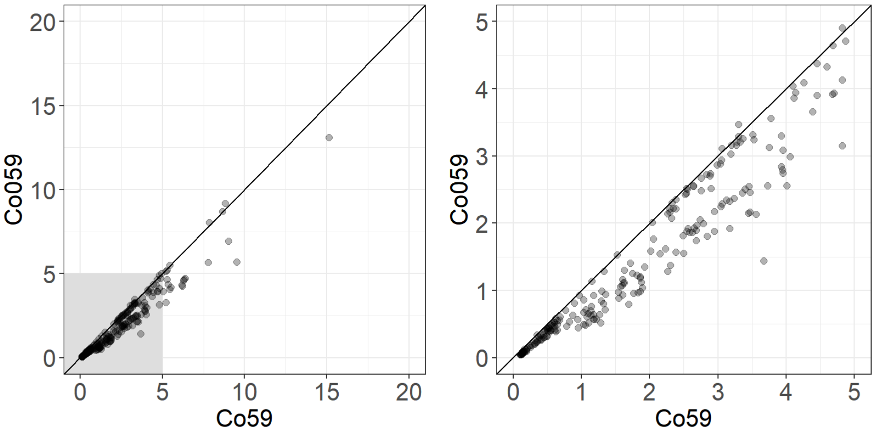
The ICP-MS values mostly agreed with the recommended values for reference samples. A certain deviation in ICP-MS values can be explained by a switch in ICP-MS devices (from Elan Perkin Elmer to Thermo Fisher Icap Q). The newer ICP-MS Icap Q device has a special technique to avoid polyatomic interferences called "KED" (kinetic energy discrimination). In KED mode the collision cell is flooded with an inert gas (helium; He) to facilitate collisions between He and polyatomic species. They collide more often than atomic ions and loose kinetic energy in traversing the cell. Since the introduction of the KED mode, the Co values have improved. These are marked as "Co059" with a leading zero in front of the atomic masses to distinguish Co measured by KED from ordinarily measured Co. Figure 3.2 show Co measured by ICP-MS, Co59 vs. Co059 (KED-mode). For about 50 % of the data points Co59 show values 2-times greater than Co59. In this plot the overall sample number is reduced compared to Figure 3.1 because Co measured with KED mode was only performed since November 2016.

Co show very low absolute concentrations in most plant samples. The reference standards used in this study and most of the commercially available standards have reported greater concentrations. This means a greater uncertainty for the low values obtained in our plants. The lowest cobalt concentration of all reference materials used is 0.12 mg/kg DM in WEPAL-IPE 168 (sunflower), which is already in the range of the 3. Quartile of all our plant samples.



(a) (b)

Figure 3.1: Co 230.786 vs. Co 228.615 solution concentrations in ppb (μg/l) by ICP-OES of 653 plant samples (several species, maturities and plant parts), a) up to 20 μg/l, b) detail from plot (a) (grey area).



(a) (b)

Figure 3.2: Co059 (KED) vs Co59 concentrations in solution by ICP-MS in ppb (μg/l) of 247 plant samples (several species, maturities and plant parts), a) up to 20 μg/l, b) detail from plot (a) grey area.

3.1.2 Accuracy of the Values

The accuracy of the results, especially for small concentrations was checked with international reference standards and by directly comparing ICP-MS and ICP-OES.

For plant samples the four reference standards GBW (Tea), NCS DC 73349 (bush leaves), WEPAL-126 (maize plant) and WEPAL-168 (sunflower) were incorporated in our digestion and measurement series (refer to Appendix Tables A.3 and A.4 for a list of all reference materials and their certified values). Figure 3.3 shows the results for Co from the four reference samples measured 131 times within the working group. In cases of very high values, possible outliers can be identified. These were not part of sample sets of this Thesis. Outliers with very high Ni concentrations can occur during the sample cutting by small metal scraps of the cutting devices. This cannot be completely avoided. Ni wavelengths and atomic masses are in agreement with the reference values (gray line). The masses of Ni 60 and Ni 62 were measured without KED mode, which exhibited the largest variance. For most samples the ICP-OES values agreed the best. In this thesis the Ni values from 4 out of 27 digestion series were taken from ICP-MS measurements. The majority of Ni values were either Ni 216.555 or Ni 231.604 (Appendix Table A.2). Figure 3.4 shows a very good agreement for all four reference standards.

For Mo and Mn, lines and masses from both machines were measured, but optimal results were always gotten by ICP-OES (Figures 3.5 and 3.6). The results obtained by ICP-MS only slightly improved with KED mode and showed a large variance in values. It is possible to adjust method parameters for the ICP-MS to get better results. These efforts were not made because ICP-OES results were available. For these elements ICP-OES can be recommended over ICP-MS if no further adjustment of the ICP-MS had been made.

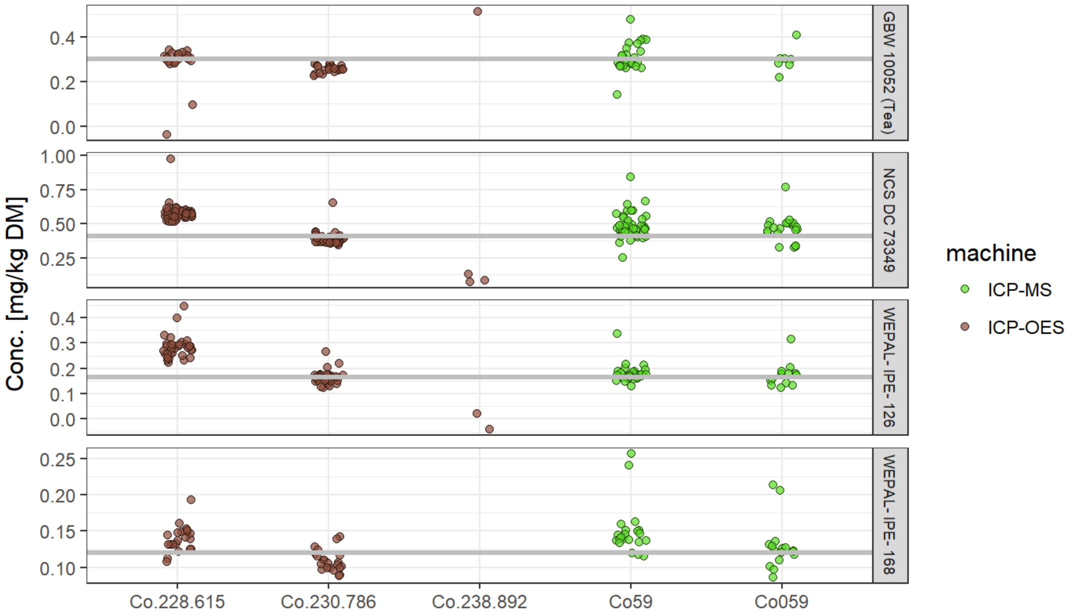


Figure 3.3: Results of Co concentrations in plant reference materials. The lines represent the recommended values.

3.2 Recommendations

External reference samples should be introduced in each measurement batch, or even in each sample digestion batch. The reference materials should correspond to the samples analyzed. If possible, they should feature the same sample matrices. If plant samples are analyzed one should aim for reference standards from the same plant species or plant family. For ICP-OES always several wavelengths per element should be included in the measurement routine, not only based on DIN regulations, but also based on recent literature or provided by the software of the device. Most critical are trace element concentrations close to the limit of detection.

When plant samples are measured true plant replicates (minimum: 3) should be analyzed. Based on these results it can be concluded, that reasonable Ni concentrations can be gained by ICP-OES measurements in most cases.

For elements close to the detection limit which can be measured with ICP-OES and ICP-MS a direct comparison of both machines data is advisable. For cobalt, based on

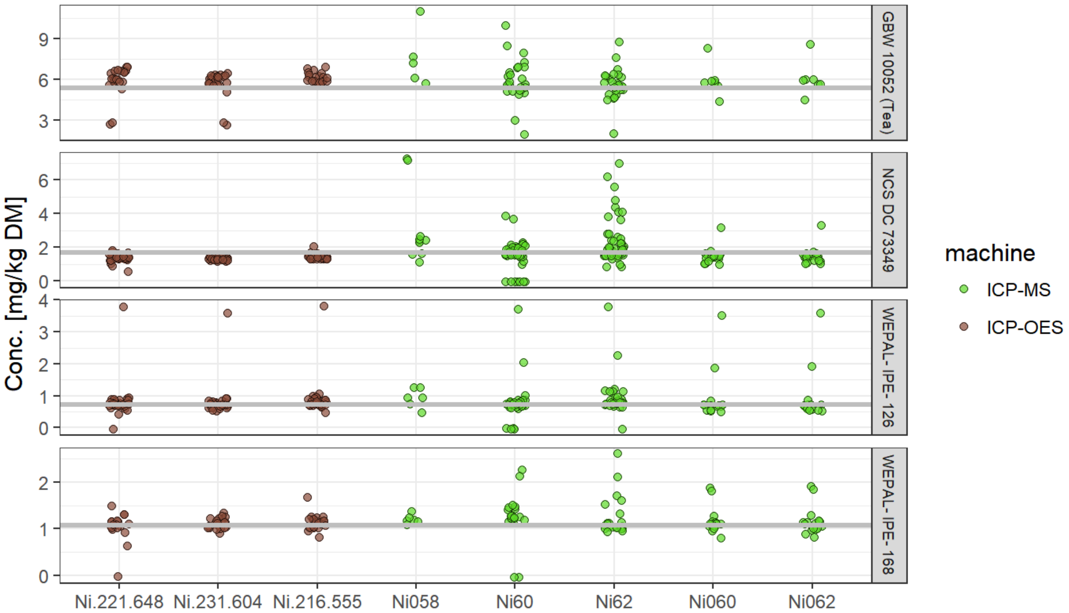


Figure 3.4: Results of Ni concentrations in plant reference materials. The lines represent the recommended values.

all plant samples measured in this study, this holds especially for low plant concentrations of < 0.1 mg/kg in sample or 1.4 ppb in solution.

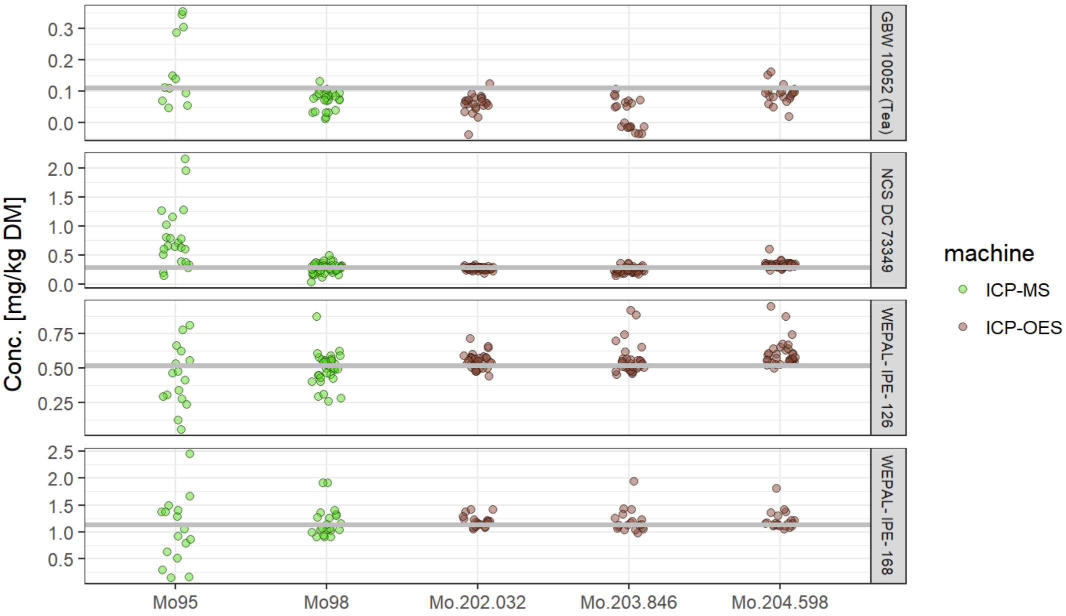


Figure 3.5: Results of Mo concentrations in plant reference materials. The lines represent the recommended values.

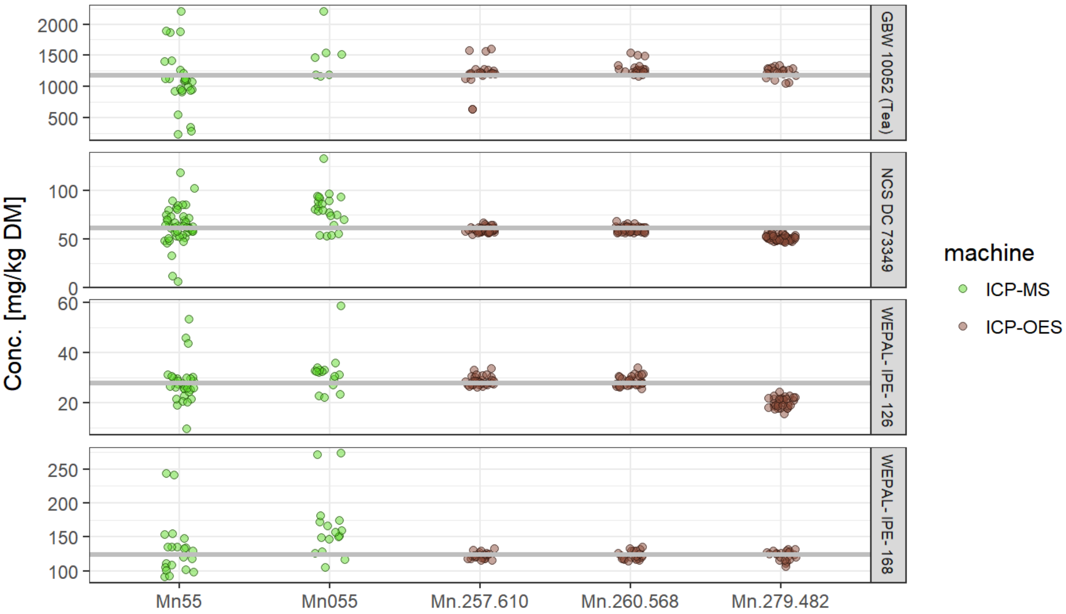


Figure 3.6: Results of Mn concentrations in plant reference materials. The lines represent the recommended values.

Chapter 4

Alteration of Trace Element Concentrations in Plants by Adhering Particles - Methods of Correction

This article is published in Chemosphere (Pospiech et al., 2017).

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Notes: The functions presented here to correct for adhering particles are now accessible with the R package "ratios" via CRAN[1] and can be used for other datasets (Pospiech and Fahlbusch, 2018b);
This is the slightly modified manuscript.

Abstract Trace element concentrations in plants may be influenced by airborne dust or adhering soil particles. Neglecting adhering particles in plant tissue leads to misinterpretation of trace element concentrations in research fields such as phytomining, phytoremediation, bio-monitoring, uptake of micronutrients and provenance studies. In case washing or brushing the samples prior to analysis is insufficient or impossible due to fragile or pre-processed samples mathematical correction should be applied. In this study three methods are presented that permit the subtraction of the influencing adhering particles in order to obtain the element concentrations in plants resulting only from uptake. All mathematical models are based on trace elements with negligible soil to plant transfer. A prerequisite for the correction methods is trace element analytics with good accuracy and high precision, e.g. through complete acid digestion. In a data set of 1040 plant samples grown in open field and pot trials most plants show a small but detectable amount of adhering particles. While concentrations of nutrients are nearly unaffected trace element concentrations such as Al, Cd, Co, Cr,

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[1] The Comprehensive R Archive Network

Fe, Mn, Ni, Pb, REEs, Ti and U may be significantly altered. Different sampling techniques like cutting height can also significantly alter the concentrations measured in the samples.

4.1 Introduction

Exact and reproducible analysis of element concentrations in plant tissue is the basis for many research fields such as environmental, health, phytomining, agricultural or provenance studies. Unfortunately plant samples collected in the field will always contain particles on their tissue surfaces such as airborne dust or soil particles (Chamberlain, 1983; Harrison and Chirgawi, 1989; Faucon et al., 2007). If not removed these particles may introduce a bias to the element concentrations measured in plant samples. The influence of adhering particles on element concentration in plants is negligible for those which have a much greater concentration in the plant tissues compared to the adhering material. This is the case for most main or minor nutrient elements such as P, K, Ca, Mg, S, Mn, B, Mo, Zn or Cu (Arkley et al., 1960; Mitchell, 1960; Bargagli, 1995). However, elements with typically very low concentrations in plant tissue such as Al, Co, Fe, Li, Ni, Ti, Sc, Zr, or REEs, may show significantly altered concentrations measured in the plants due to adhering particles. Mitchell (1960) proposed that elements with concentration ratios of soil to plant above 100 might show biased concentrations in the measured samples.

Reducing the impact of adhering particles on trace element concentration in plants is crucial in order to be able to compare elemental composition of plants, e.g. between sampling periods or slightly different sampling methods (MacNaeidhe, 1995), or for biomonitoring studies. It is also important in studies for plant nutrition to calculate the real uptake of an element by a plant, e.g. for phytoremediation/phytomining (Baker et al., 1994; Fässler et al., 2010; Wiche et al., 2016), or in studies on the trace elements Co, Ni, Mn and Mo which are required by the microorganisms in biogas plants (Sahm, 1981; Demirel and Scherer, 2011).

Most authors recommend washing or carefully rinsing of the plant material to clean the samples prior to analysis. Washing methods were tested by several authors, e.g. ultra-pure water, washing detergents, complexing agents like EDTA, slightly acidic solutions or combinations of these (Husted et al., 2004; Faucon et al., 2007; Cook et al., 2009). The disadvantages of the washing method are (1) particles may remain in leaf axils or on rough foliar (or epidermal) surfaces of the plants (Cook et al., 2009) and (2) elements may get leached from the plant tissue. Washing is sometimes not possible if the plant material was already processed prior to sampling, e.g. by fermentation, drying, cutting or mixing.

Although several authors were aware of the problem of unremovable adhering particles and their bias to trace element concentrations in plant tissue (Mitchell, 1960; Tölgyesi and Nagy, 1990; Markert, 1995; Reimann et al., 2001; Faucon et al., 2007; Anke and Seifert, 2004; Verbruggen et al., 2009; Censi et al., 2017) , only Bargagli (1995) and Sauer and Ruppert (2013) provided a mathematical approach using aluminum or titanium as a reference element to estimate the amount of adhering material and calculate "true" element concentrations in plant tissue.

Based on the model that the analyzed plant material is a mixture of plant tissue and a very minor amount of adhering particles we developed more general methods to calculate a correction term for adhering material. We will present three methods of calculating a correction term and show the effect of these correction terms on the trace element concentrations of plant matter.

4.2 Materials and Methods

4.2.1 Study Sample Set

The sample set consist of plants and corresponding soil samples from different research projects between 2011 and 2015. In all projects a broad element range was measured focusing on trace elements. The plants in these studies comprised forage and energy crops (for biogas production), perennial and catch crops (Table 4.1). In this study the element data of a total of 1040 plant samples of 19 species and at least 10 samples for each species is presented.

The plant and soil samples were retrieved from open arable field trials and from (open) pot trials filled with differently soils. Of the 1040 plant samples 789 plants were from open field (20 locations in southern Lower Saxony and Bavaria, Germany), and 251 from open pot trials (a total of 13 different locations in Lower Saxony). The pots were located close to our department. None of the plant samples were rinsed. The soil types in the field ranged between silt, silt loam and sandy loam with a pH of 5.8 to 7.0 (measured in 0.01 mol/L $CaCl_2$).

Table 4.1: List of plants comprised in the used data set.

Plant Species	Scientific name	Nr. of samples	Nr. of locations
Amaranth	*Amaranthus* spp.	255	12
Barley	*Hordeum vulgare* L.	11	2
Beet (leaves)	*Beta vulgaris* L.	18	7
Buckwheat	*Fagopyrum esculentum*	47	16
Catnip	*Nepeta cataria* L.	14	11
Cup Plant	*Silphium perfoliatum* L.	13	8
Faba Bean	*Vicia faba* L.	59	11
Maize	*Zea mays* L.	151	23
Mustard	*Sinapsis alba* L.	15	12
Oat	*Avena sativa* L.	24	2
Pea	*Pisum sativum* L.	14	3
Quinoa	*Chenopodium quinoa*	50	16
Rye	*Secale cereale* L.	102	20
Ryegrass	*Lolium perenne* L.	28	4
Sorghum	*Sorghum bicolor* L.	13	3
Sunflower	*Helianthus annuus* L.	52	14
Triticale	*Triticosecale* Wittm.	92	19
Vetch	*Vicia villosa* L.	55	6
Wheat	*Triticum aestivum* L.	27	15

4.2.2 Sampling, Sample Preparation and Analysis

The plant samples were cut at ca. 10 cm above soil surface (except from some samples, see section 4.5.4) at the stage of lactic ripeness or end of flowering. The roots are not included in the analysis. About 500 g of soil samples taken in the upper part of the soil (up to 30 cm depth) and are air dried and sieved to < 2 mm in grain size. All samples, soils and plants, were dried at 105°C. A minimum of 100 g of the soils and ca. 500 g of plants were ground in an agate ball mill to avoid metal contamination.

Aliquots of 150 mg of soil respectively 700 mg of plant powder were fully digested with a mixture of ultra pure concentrated HNO_3, $HClO_4$ and HF in closed ultra clean PTFE vessels (PicoTrace, Bovenden, Acid sample digestion system (DAS 30)). In the soil samples a small amount of HCl is added. Soil samples were diluted to 100 ml the plant samples to 50 ml for measurement (see Supplement 1.1. for further remarks on the digestion method). In the resulting clear sample solutions 47 elements could be quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Mass Spectrometry (ICP-MS).

To ensure clean handling and quality of analysis for every 30 samples one to two blank solutions and one or two international reference standards were digested and analyzed together with the samples. For plant samples the following international reference materials were used: GBW 10052 (green tea, Institute of Geophysical and Geochemical Exploration, China), BCR-129 (hay powder, Community Bureau of Reference, Luxembourg), WEPAL-IPE-126 (maize plant, Wageningen University, Netherlands), WEPAL-IPE-168 (sunflower, Wageningen University, Netherlands). For soils the in-house geochemical standard TW-45 (Wissenbach slate, Harz Mountains, Germany) was used. Results were only accepted if concentrations were above the limit of detection (3-fold standard deviation of the blank concentrations for each analysis batch) and if the corresponding reference samples showed concentrations within the certified values. Average precisions for the elements were better than 5% for all main elements and most of the trace elements; for plants the ultra-trace elements Cr, Co, Mo, Sn, and the heavy REE showed average precision between 10% - 20%. All concentration data are given in *mg/kg* dry weight (d.w.).

4.3 Theory

All correction terms are based on the following simplified model: The Plant samples collected from open field trials consist of the pure plant material ($Plant$) and a minor content of deposited solid material from different sources, from now on called adhering particles (AP).
With a digestion method ensuring complete and total dissolution of the sample the analytically determined element concentration, $PlantSample$, contains both components, $Plant$ and AP. Hence the concentrations of $Plant$ can be calculated by subtracting AP from $PlantSample$ (Eq. 4.1).

The most important sources of adhering particles are airborne dust, soil particles from wind or rain splash. In this study the adhering particles are assumed to be mainly soil particles. Especially the lower parts of plants will be strongly affected by splashed soil particles, but this holds also for the higher parts of the plants even when less affected. Hence for the calculation of the correction term we use $AP = Soil$. We are aware that a fractionation of soil particles may occur during transport through the air or that collected atmospheric dust could locally be a better proxy for adhering material, but to simplify our calculations we take into account only data of the soils corresponding to the plants. Air dust collection and analysis would be extremely laborious and time-consuming. The content of adhering particles can be approximated by elements with good analytical precision and accuracy and with negligible uptake by plants, hence concentrations approach zero, but high concentrations in the adhering particles. We call these elements "indicator elements" (El_{ind}). In order to estimate the content of adhering soil (x in Eq. 4.1) to the plant samples we propose three different methods:

Method 1: Using one pre-defined element El_{ind} for calculating the content (x) of AP in *PlantSample* From Elements fulfilling all requirements of El_{ind} one is chosen to calculate x in Eq. 4.4 (Ti, Al, Zr, Sc...). For example using Ti as El_{ind} the content of AP is calculated through $x = PlantSample[Ti]/Soil[Ti]$ and this x is then used in Eq. 4.2. Negative concentrations should be set to zero.

Method 2: Subtracting the smallest possible content of AP (smallest x) For each sample the element with the smallest x of all ratios $x = PlantSample[El]/Soil[El]$ of each sample is taken as El_{ind}, hence every sample is corrected based on a different El_{ind}. With this method only the smallest possible content of AP is subtracted from $PlantSample$ (Eq. 4.2). Typical indicator elements are Ti, Al, Th, Cs, Zr etc., if they can be measured with high sensitivity and reproducibility. With this method there are no negative concentrations.

Method 3: Using the median of several elements with a very small x for calculating the content of AP In order to reduce the uncertainty of the content of adhering particles based on only one element as in method 1 and 2 an average of several x of El_{ind} elements can be calculated.

With Δx being the absolute error of x we suggest to take the median of the x of all elements which values $x - \Delta x$ are smaller than $x_{smallest} + \Delta x_{smallest}$. The value of the median $\tilde{x}$ is then used as x in Eq. 4.2. Negative concentrations should be set to zero. Because statistically the x of all elements, which error overlaps the error of the element with smallest x, are indistinguishable. We also suggest to set all elements contributing to $\tilde{x}$ to zero, because these small values should not be interpreted.

4.4 Calculation

The easiest way to calculate a subtraction of concentrations while taking the mathematical constraints of concentration into account is to use vectors (Aitchison, 1986; Aitchison, 2003; Buccianti et al., 2006; Pawlowsky-Glahn et al., 2015). The composition of each sample can be written as a vector with the concentration of each element as a vector component: $\overline{PlantSample}$, $\overline{Plant}$ and $\overline{AP}$. For easier reading the vector arrow is omitted in the following. For example the concentration of Ca in the adhering particles is written as $AP[Ca]$, for the concentrations of the five elements Ca, Fe, Mg, S and P the notation is $AP[Ca, Fe, Mg, S, P]$. The notation without any $[\,]$, only AP, is short version for $AP[Al, As, ..., Zr]$.

With a variable x for the content of AP in $PlantSample$, $0 < x < 1$, the composition of $PlantSample$ can be expressed as the sum of $Plant$ and AP:

$$PlantSample = Plant * (1 - x) + AP * x \tag{4.1}$$

Resolving equation 4.1 for $Plant$ results in equation 4.2:

$$Plant = \frac{PlantSample - x * AP}{1 - x} \tag{4.2}$$

of which the composition of AP and the content of the adhering particles, expressed as x, are unknown and assumptions have to be made.

Assuming that the concentration of one of the El_{ind} in $Plant$ is zero, $Plant[El_{ind}] = 0$, equation 4.2 can be used for solving x, the content of AP:

$$Plant[El_{ind}] = \frac{PlantSample[El_{ind}] - x * AP[El_{ind}]}{1 - x} = 0 \tag{4.3}$$

$$x = \frac{PlantSample[El_{ind}]}{AP[El_{ind}]} \tag{4.4}$$

Note that for the calculation of x only elements of the group of El_{ind} are allowed. Otherwise the correction term will lead to unrealistic element concentrations in $Plant$.

The absolute error of x, Δx , is calculated through the general formula for error propagation for random and uncorrelated errors of $PlantSample$,with $\Delta PlantSample$ as the absolute error range for analyzed plant values, and AP, ΔAP as the absolute error range of the adhering particles:

$$\begin{aligned} \Delta x &= \frac{\partial x}{\partial PlantSample} * \Delta PlantSample + \frac{\partial x}{\partial AP} * \Delta AP \\ &= \frac{1}{AP} * \Delta PlantSample - \frac{PlantSample}{AP^2} * \Delta AP \end{aligned} \tag{4.5}$$

To determine the absolute error $\Delta PlantSample$ we use the relative standard deviation of more than 30 measurements of the international reference sample GBW10052 as relative error $\delta PlantSample$: $\Delta PlantSample = \delta PlantSample * PlantSample$. To simplify the error calculation $\Delta Plant$ for equation 4.6 we suggest to use in the error-equation as $\Delta \bar{x}$ the standard deviation of $\bar{x}$.

For most results we used Method 3 explained in theory part. We assume that the major source of adhering particles are particles of the soil on which the plants grew ($AP = Soil$). Hence based on equation 4.2 the correction equation is

$$Plant = \frac{PlantSample - \bar{x} * Soil}{1 - \bar{x}} \tag{4.6}$$

If an element has a low concentration in $Soil$ the term $\bar{x} * Soil$ becomes close to zero. With $\bar{x} * Soil \approx 0$ and the term $PlantSample/(1 - \bar{x})$ always $>$ $PlantSample$ the term $\frac{PlantSample - \bar{x} * Soil}{1 - \bar{x}}$ may result for main elements in plants into $Plant > PlantSample$.

For all calculations and graphs we used the free software R (R Core Team, 2017) and the package ggplot (Wickham, 2016).

4.5 Results and Discussion

4.5.1 Discussion of the Methods

Calculating the content of AP via Method 1 The advantage of the method with a pre-defined element is a rather easy calculation because for all samples the same El_{ind} is used. The disadvantage is that an overcorrection may occur for either in the whole data set or in single samples if the chosen El_{ind} has a notable higher $PlantSample/Soil$-ratio than the smallest ratio. That might be the case if due to fractionation of the AP the chemical composition of the adhering particles is not exactly the same as the chemical composition of AP used in the correction term or if the El_{ind} is (unexpectedly) taken up by a plant. This is the case for Al in buckwheat *Fagopyrum esculentum* or in tea *Camellia sinensis* (Zheng et al., 1998; Flaten, 2002; Carr et al., 2003). Even Ti, considered as highly immobile in soils, might be taken up by plants in very small quantities and by some authors is considered as beneficial to in plants (Dumon and Ernst, 1988; Cigler et al., 2010; Haghighi et al., 2012).

Calculating the content of AP via Method 2 Subtracting only the smallest possible content of adhering particles minimizes the risk of overcorrection. Disadvantageous is that the correction depends on one element only (lowest ratio of $PlantSample/Soil$) and not on analytical reasoning. If the chosen element is an (ultra-)trace element with concentrations close to the detection limit with poor reproducibility and high analytical uncertainty, this results into a high uncertainty of the smallest x and that directly determines the uncertainty of the applied correction.

The content of subtracted AP can therefore vary strongly on the set of trace elements considered for the group of El_{ind}. For example plants contain very low concentrations in Hf and therefore it is difficult to determine Hf with high accuracy and precision. If Hf represents the smallest x and Ti second smallest x but considerably higher, the content of AP would be calculated by Hf. If Hf is excluded from the group of El_{ind} due to analytical reasons, the content of AP would be calculated by Ti and would result into a stronger correction. So the calculated content of AP would depend upon if Hf is included to the group of El_{ind} or not.
Additionally, if the El_{ind} has big errors, the calculated "true" concentrations of the elements will also result into big errors because of error propagation.

Calculating the content of AP via Method 3 This method is particularly interesting if several elements of the group of El_{ind} had been analyzed with very small analytical uncertainties. The value of $\bar{x}$ is based on several elements with overlapping error Δx_i. That avoids on the one hand the problem of method 1 that the element might be taken up by the plant and on the other hand avoids the problem of method 2 that it is quite random which element is the element used for correction and that the analytical error might be quite high.
The disadvantage is that the implementation of method 3 on a routine basis is not as simple as for example in method 1, especially if error calculation is included.

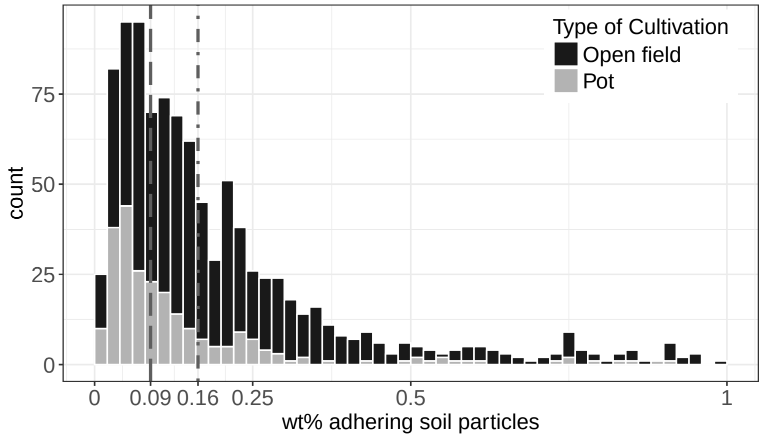

Figure 4.1: Distribution (bin width = 0.02) of wt% of adhering soil particles in 1040 crop samples grown in open field (n = 789) and open pot trials (n = 251). The content of adhering particles calculated via method 3. Right skewed distribution shows that all samples contain at least minor amounts of adhering particles. The median of adhering particles in open field is bigger (dotdash line) than in open pot trials (dashed line) maybe because of severe weather events. The plot is cut at 1 wt%, the maximum value of 6.1 wt% not shown.

4.5.2 Evaluation of the Calculated Content of *AP*

The content of adhering particles in *PlantSample* had been calculated via method 3 for 1040 whole crop samples from fields in Germany. As shown in Fig.4.1 the samples contain about 0.09 wt% of particles in the pot trials and 0.16 wt% in open field trials (median). The minimum content is at zero, that means no measurable content of adhering particles, and the maximum value at 6.1 wt%.

Although the content of adhering material seems to be rather small in comparison to the dry weight mass of the plant samples, it may have a notable impact on many elements. For each sample and for each element the ratio of corrected values to measured values ($Plant/PlantSample$) are calculated, hence for each element there are over 1000 ratios. The distribution of ratios for each element after applying method 3 is displayed in Fig. 4.2. For comparison to the other methods see Supplement 2.

It shows that - as expected - the concentrations of most nutrients, S, P, Ca, K, Mo, Mg, Zn, Cu, and some trace elements with chemical characteristics similar to a nutrient, such as Cd, Sr (similar to Ca) and Ba, Rb (similar to K), are only slightly affected through adhering soil particles, because most of the ratios are very close to 1 (Fig. 4.2).

The strongest impact of adhering particles is on the trace elements Y, Li, La (representative for all REEs), U, Nb, Ta, Al, Ti, Sc, Th, Zr and Hf: The concentrations after the correction are clearly less than 50% of the measured value. These elements belong to the typical "indicator elements" (El_{ind}).

For Mn, Ni, Ba, Cr, Sb, Tl, Co, Na, Cs, Pb and Fe the distribution of the ratios shows that the impact of adhering soil particles varies for every sample: for some the effect is barely measurable, for others the impact is very big. For example the micronutrient Fe shows that for nearly all samples the ratio is significantly smaller than 1, and most sample contain only 64% of the measured concentration after correction. Some samples have even very small concentrations left after correction.

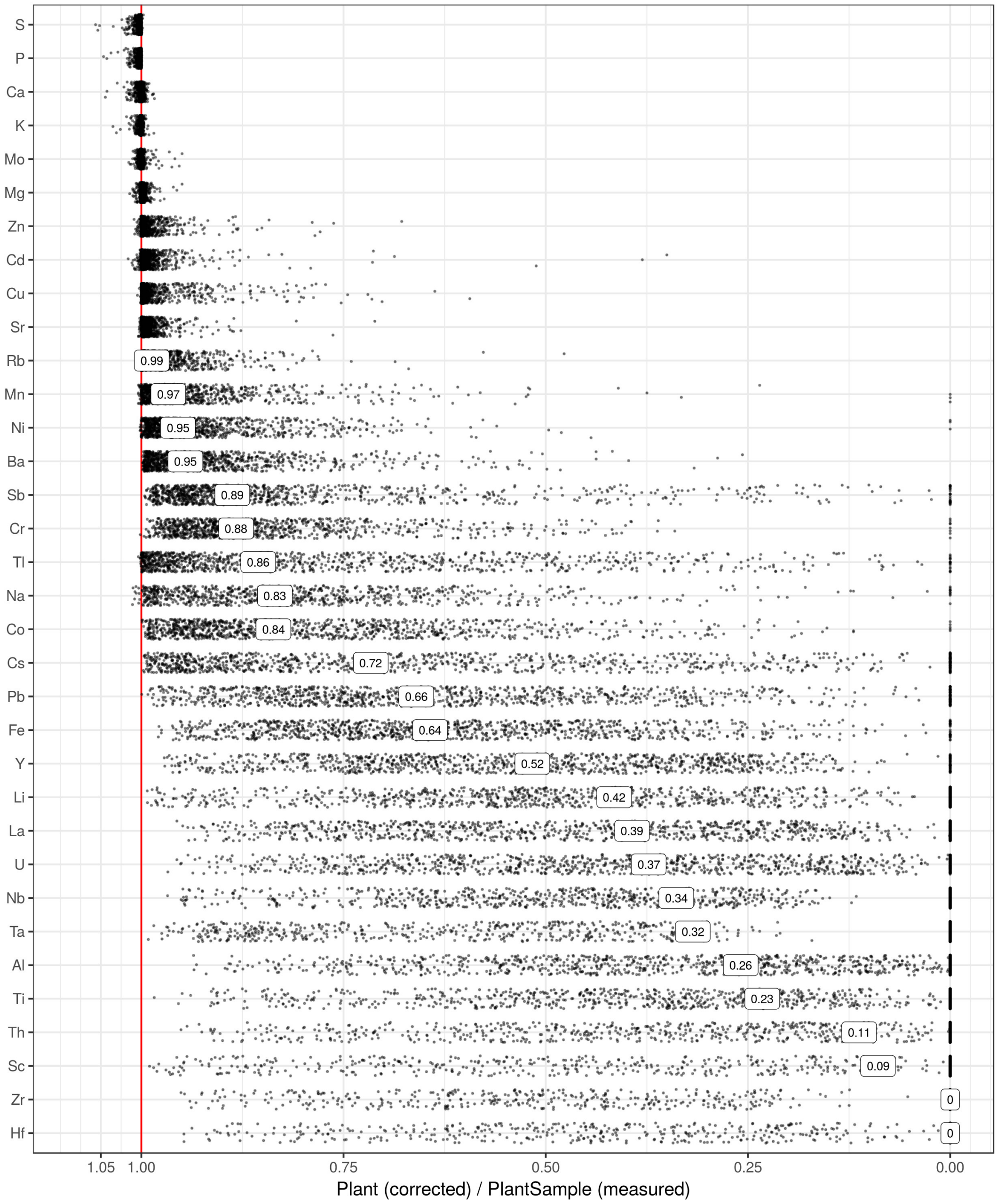

Figure 4.2: Distribution of ratios (1040 samples) of $Plant/PlantSample$ (corrected/uncorrected) for main and trace elements. Corrected values of $Plant$ calculated via Method 3. Each dot in element rows represents one ratio so that in each row there are $\sim$ 1040 dots. The dots jitter vertically to reduce overplotting. The median is plotted as label at the position of the median, and rows are sorted by median. For S to Sr the median label is omitted because the median is close to 1. Main elements may show slightly higher concentrations after applying the correction (see explanantion for Eq. 4.6). Concentrations of elements which contributed to $\bar{x}$ (see explanation method 3) are set to zero to avoid overinterpretation of these small residual values.

In case of essential trace elements for the microorganisms in biogas plants (Co, Ni, Mn, Mo) (Sahm, 1981; Demirel and Scherer, 2011) we see a diverse picture: The correction slightly affects the concentration of Mo, because Mo is a micronutrient for plants and is fairly mobile in the soil solution (Kaiser et al., 2005). It is taken up easily by the plant so that we observe a negligible correction of the measured values (Median = 1.0) only. The median values of Mn and Ni are reduced by 3% (Mn) and 5% (Ni). Co on the other hand shows for most samples a significant reduction of concentration after applying the correction. On average the cobalt concentrations are reduced by 16% (Fig.4.2).

4.5.3 Effect of Correction

Unfortunately there is no direct evidence if an element concentration of a sample is "more correct" after the correction compared to prior to correction. But by looking at the grouping of elements via principal component analysis (PCA) on log-ratios it can be seen that before and after the correction there are significant changes for some elements.

In Figure 4.3 the upper plot represents the correlation and variance of log-ratios of the uncorrected plants (data set $PlantSample$) and the lower plot of the corrected plants (data set $Plant$). For the uncorrected samples ($PlantSample$, Fig. 4.3, upper) the elements Nb, Zr, U, Ti, Al, La, Li, Pb and Sb are lying close together which indicates a correlation between these typical elements of the group El_{ind}. This group of elements forms long links to all nutrient elements, except for Fe and Na, indicating no correlation between the group of El_{ind} and the nutrients. The ratio $PlantSample/Soil$ of the nutrient Fe lies between the ratios $PlantSample/Soil$ of El_{ind} and nutrients and also in Fig. 4.3 in the data set $PlantSample$ the element Fe plots between the two groups. That means that the concentration of Fe is notably influenced by adhering soil particles. A similar situation applies to Na.

For the corrected samples ($Plant$, Fig. 4.3, lower) the grouping of elements is notably reduced and the links between elements are generally shorter. Elements of the group El_{ind} plot in one corner of the plot with Zr and Nb showing a high variation in respect to all other elements. This indicates that the log-ratios between most elements of El_{ind} are still quasi-constant but do not form a distinct group as before. The nutrients Fe and Na have moved towards the nutrient group indicating that their log-ratios are decoupled from the El_{ind}. Additionally, the shorter links between elements indicate that log ratios between all elements are now more constant between all samples. Hence it can be assumed that after applying the correction the mechanisms of element uptake are much better fulfilled as before with adhering particles which form a bias on certain elements.

4.5.4 Influence of Species and Cutting Height

The quantity of adhering soil particles largely depends on the plant structure, the roughness of foliar surface area, and on the plant's height. Crop plants with long blades and small foliar surfaces at lower plant parts, e.g. rye, have significantly less content in adhering particles than crop plants with rough or large foliar surface, e.g. maize, amaranth (see graphical abstract and Supplements).

In order to make the effect of cutting height on the amount of adhering particles visible we compare plant samples cut at 1 cm and 10 cm above ground on a similar soil

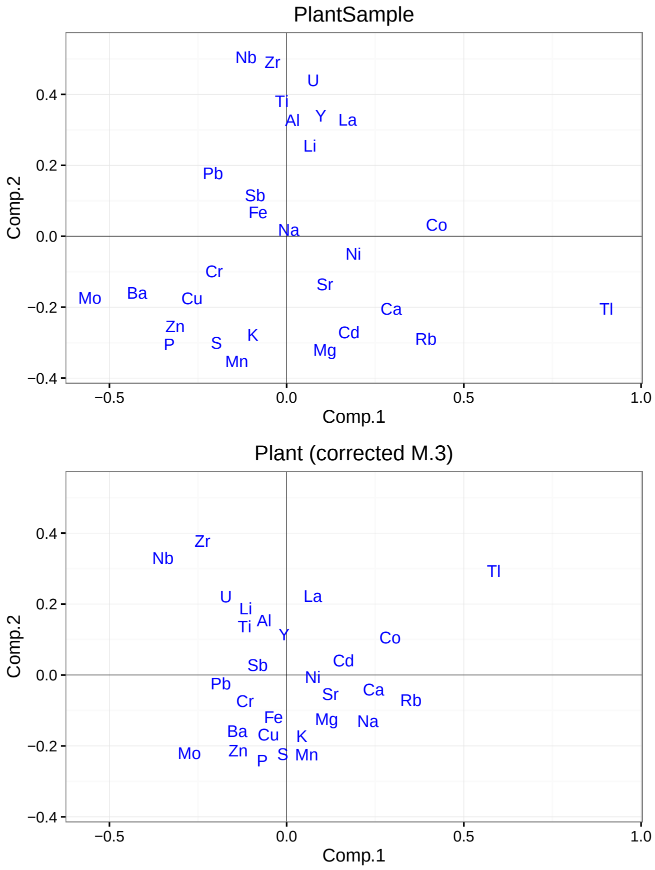

Figure 4.3: Coviariance biplots of *PlantSample* (upper, uncorrected) and *Plant* (lower, corrected) of a principal component analysis of log-ratio transformed (clr) data (712 samples with 29 elements). The proportion of the explained variance is 0.52 for the upper plot and 0.49 for the lower plot. The position of the element labels can be interpreted as follows (van den Boogaart and Tolosana-Delgado, 2013; Greenacre, 2010): the link between two element labels represents the log-ratio between these two elements. A short link indicates that the elements have a quasi-constant log-ratio. An angle of 90°C at the origin means that the log-ratios of the two elements are uncorrelated. Several samples and elements had to be excluded from this examination because of special circumstances (i.e. heavy metal contaminated locations.) The general pattern of the links between elements remains unchanged by this adaption.

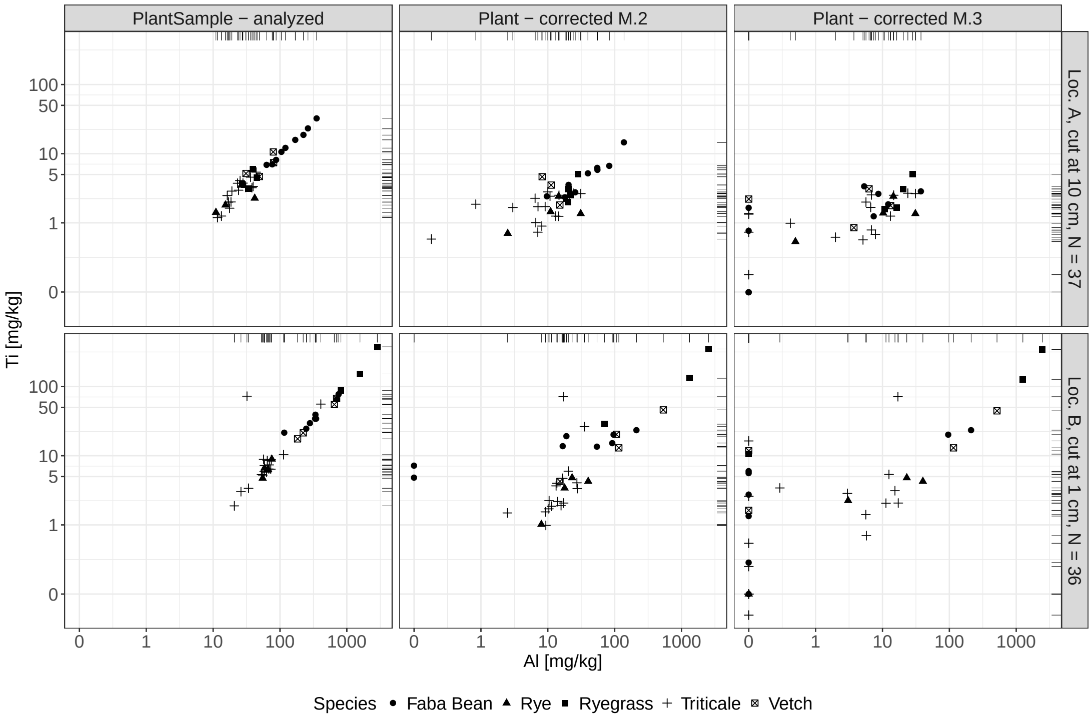

Figure 4.4: Influence of cutting height on the Al and Ti concentrations of different plant species, without correction (Plant - analyzed), corrected by the smallest possible content of adhering soil - Method 2 (Plant - corrected M.2) - and corrected via Method 3 (Plant - corrected M.3) using the median of several very small x for calculating the content of adhering soil.

(Fig. 4.4). At Location A (field trial Sömmerling) plants were cut at 10 cm and at Location B (field trial Garte Nord) the plants were cut at 1 cm and the Locations have similar soil properties and total element concentrations.

In each location five different plant species were analyzed: Faba bean, rye, ryegrass, triticale and vetch. Both data sets had been corrected with method 2 and 3 (Fig. 4.4, Plant-corrected-M.2, and Plant-corrected M.3). In order to show the effect of adhering soil particles and the impact of the two correction methods two typical "indicator elements" (El_{ind}), Al and Ti, are shown (Fig. 4.4).

We assume the concentrations in the analyzed plant material (*PlantSample*) to be biased by adhering soil particles because (1) Al and Ti should show very little to no uptake at pH 6-7 in the soils but nearly all samples show considerable high concentration values (Fig. 4.4, Plant- analyzed), (2) none of the species are known as Al- or Ti-accumulator species and (3) the ratios of Al/Ti of the plants (median 9.8) is very close to the ratio Al/Ti in the soils (median 12.3) and there is no species-dependency which would be expected for elements with different chemical characteristics.

The concentrations prior to correction also show a very huge spread. For samples cut at 10 cm (Location A) Al range from 11 - 350 mg/kg and Ti from 1 - 33 mg/kg. For samples cut close to the ground at 1 cm (Location B) Al range from 21 - 2850 mg/kg and Ti from 2 - 375 mg/kg. Most probably the amount of soil adhering is greater at Location B.

After applying a correction on the plants the concentrations of Ti and Al are notably reduced for most samples and the ratio Al/Ti is smaller (median 3.7 for method 2) and more variable. That indicates that for most samples Al and Ti concentrations after correction are far less biased by adhering particles.

Nevertheless, there are different results for correction method 2 and 3. Correction method 2 is less intense because only the smallest possible amount of AP is removed and this probably results into undercorrection for some samples. For example some samples of faba bean the concentration of Al and Ti calculated via method 2 and cut at 10 cm are elevated (maximum for Al >100 mg/kg).

Correction method 3 shows very good and reliable results for samples with small amount of adhering particles (Location A). But for very high amount of adhering particles, which is the case for the samples cut at 1 cm in Location B, concentrations after correction show a huge spread. More than 15 samples were overcorrected (Al = 0), while some samples show almost no correction (two ryegrass and one rye samples with Al >50 ppm). Only for samples with the lowest Al- and Ti-concentration prior to correction show reasonable Al- and Ti concentrations after correction. Overcorrection can occur if the concentrations of elements of the group of El_{ind} are so high that the absolute error of these elements overlaps with the error of micro-nutrients, hence the algorithm cannot discriminate between El_{ind} and other elements. In this case the median will be calculated by a mixture of El_{ind} and micro-nutrients and $\bar{x}$ will be far too high. On the other hand for samples where the smallest x is very small the error range does not overlap with the error range of El_{ind} with very high concentrations, hence $\bar{x}$ will be calculated by only very few El_{ind} and might lead to undercorrection. For these low cut samples one solution would be to choose a fixed El_{ind} element (Al, Ti) for all samples to overcome these problems.

4.6 Conclusions

We provide three mathematical methods to correct plant samples for adhering particles to obtain real trace element concentrations in plants by using total element concentrations of plant and soil materials in the calculations. The sampling techniques (for example cutting height) can influence the amount of adhering particles to a great extent and the correction method becomes less precise at high adhering particles concentrations. We therefore recommend for research projects on trace elements with a possible bias by adhering material (e.g. Mn, Ni, Ba, Co, Fe, Cs) to adjust the cutting height to at least 10 cm, or correct the analyzed concentration data with one of these three methods.

While adhering soil particles hardly change concentrations of major nutrient elements (P, Mg, K, S, Ca) and some minor nutrient elements (Cu, Zn, Mo) they can have a strong impact on other trace elements in plants, such as Fe, Ni, Co, REE, Al, Ti, Hf, Zr and Th.

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Chapter 5

Element Uptake by Plants at Higher Soil Element Concentrations

5.1 Introduction

The two main field trials contain common trace element concentrations in the soil, especially for Co and Ni. Furthermore, they do not differ to a large extent. To investigate the uptake behavior and bioavailability other locations were investigated which had a higher total element concentration in the soil.

One important location in this study is Bühren, a village close to Dransfeld, about 20 km ‘W of Göttingen. Soil was taken from here for pot experiments and a small scale field trial was established. Results from extractions with diluted ammonium nitrate of soil samples from Bühren and from other sample sites will be incorporated. Some additional extraction methods were tested in the Bachelor Thesis of Markus Willerding-Möllmann who was supervised by the author (Willerding-Möllmann, 2015).

5.1.1 Field Excursion for Magmatic Rocks

Greater total concentrations of the trace elements Co, Ni, Mn and Fe can be expected in certain parent material and their corresponding soils. Magmatic rocks (<52 % SiO_2) are rich in magnesium (Mg) and iron (Fe) because they are major constituents of the mafic minerals in these rocks (mica, amphibole, pyroxene and olivine). Co and Ni can replace Mg and Fe in the mineral lattice by isomorphous substitution, causing higher Co and Ni concentrations in the rocks (Mitchell, 1945). Turekian and Wedepohl (1961) report 48 mg Co kg^{-1} and 130 mg Ni kg^{-1} for basaltic rocks. The highest total concentrations of Co and Ni could be expected on soils derived from ultrabasic rocks. Ultrabasic rocks are rocks with less than 45-weight% of silica, for example dunites or peridotites (Okrusch, 2005). However, these rocktypes are not stable on the earth’s surface and are extremely rare. For this study the best choice was to look for outcrops of the more common basaltic rocks in the vicinity of Göttingen.

The northern-most basalt outcrops in Germany are located in Lower Saxony. On the western shoulder of the Leinetalgraben there are some basalt hills and old basalt quarries. In the vicinity of Göttingen, there the basalt hills: Steinberg (Meensen), Bramburg (Adelebsen), Backenberg (Ossenfeld), Grefenburg (Barterode) and "Hoher Hagen" (Dransfeld). The updoming of the basalt bodies is believed to be associated with the formation of the Leinetalgraben between Miocene and Pliocene ages around 5 Ma, (Wedepohl, 1950). In most cases the basalts lie on top of fossil-free gravel and sands of Tertiary age (Wedepohl, 1950). In the case of the "Hoher Hagen" and some other locations, the basalt stones were quarried. However, there are no more active quarries today.

A field sampling was done in April 2015 to find locations where soils developed from basalt weathering, and are used as farmland. This is a rare situation as most of the basalt bodies and outcrops are covered by forests. The area surrounding the village of Bühren (51.483795N, 9.683211E, Fig. 2.4), close to Dransfeld showed the best conditions. In the village center there are remnants of a basalt quarry and basalt pillars cropping out. The basalt stones were quarried in Bühren until 1969 and the area was recultivated afterwards. Also, other cultural monuments ("Kreuzsteine") made out of basalt can be visited (Fig. 5.1).

Figure 5.1: Basalt pillars in the village center (left) and cultural monument outside of the village of Bühren ("Kreuzsteine", right).

5.2 Experimental Setting

5.2.1 Small Scale Field Trial in Bühren

A small-scale field trial (about 6 m x 2 m) was established SW of the village of Bühren in a corner of one field plot with high Co and Ni concentrations. Triticale, hairy vetch, rye, amaranth, winter and summer faba bean and ryegrass were cultivated between September 2015 and August 2016. Figures 5.2 show pictures from the field plots in 2016 (Table 5.1). All plant element concentrations were corrected for adhering particles with Method 3 from Chapter 4.

Table 5.1: Cultivars, sowing and harvesting dates and dry matter (DM) for the small field trial close to Bühren

Variant	**Cultivar**	**Sowing Date**	**Harvest Date**	DM (%)
Faba bean (Wi)	Nordica	13 Oct 2015	7 June 2016	15.3
Triticale (Wi)	Balu	13 Oct 2015	10 June 2016	28.9
Rye	Conduct	13 Oct 2015	10 June 2016	31.5
Hairy vetch	Welta	13 Oct 2015	10 June 2016	19.5
Rye/vetch	Conduct/Welta	13 Oct 2015	10 June 2016	
Ryegrass	Alligator	5 April 2016	7 June, 17 August 2016	12.9, 40.6
Faba bean (Su)	Fanfare	10 May 2016	2 August 2016	
Amarant	Bärnkrafft	10 May 2016	2 August, 24 2016	20.8
Amarant	Pastewny	10 May 2016	2 August, 24 August 2016	21.5


(a) Winter crops in May 2016. **(b)** Winter crops in June 2016.

Figure 5.2: Small scale field trial close to Bühren.

5.2.2 Additional Samples in Pot Trials

Experimental open air pot experiments were also performed with soil obtained from Bühren. Amaranth and summer faba bean plants were cultivated for the Bachelor Thesis of Markus Willerding-Möllmann. In these small pots, four faba bean plants and four amaranth samples were tested for potential trace element uptake. These pots were relatively small containing about 2.8 kg of soil (Fig. 5.3a). The same cultivars were used as in the main field trials, summer faba bean *Fanfare* and amaranth *Bärnkrafft*, Table 5.2). In the following year 2016 amaranth, winter and summer faba bean, hairy vetch, winter rye, ryegrass and winter triticale were cultivated in larger pots in the open air containing approximately 20 kg of soil (Fig. 5.3b). This sample set consisted of the same plant variants and cultivars as in the main field trials. The soil composition is listed in Table 5.4 with other soil sample locations.

Table 5.2: Cultivars, sowing/harvesting dates and dry matter (DM) for the pot experiments on Bühren soil.

Variant	**Cultivar**	**Sowing Date**	**Harvest Date**	DM (%)
Large pot experiments				
Faba bean (Wi)	Nordica	13 Oct 2015	10 June 2016	16.7
Triticale (Wi)	Balu	13 Oct 2015	10 June 2016	34.7
Rye/vetch	Conduct/Welta	13 Oct 2015	10 June 2016	45.8/31.2
Ryegrass	Alligator	5 April 2016	10 May, 17 August 2016	30, 24.4
Small pot experiments				
Faba bean (Su)	Fanfare	29 May 2015	24 August 2015	20.3
Amarant	Bärnkraft	29 May 2015	10 Sept. 2015	22.3

(a) Small pot experiments, faba bean. **(b)** Pot experiments with larger pots and faba bean plants.

Figure 5.3: Pot experiments with two different pot sizes.

5.2.3 Soil Extraction with NH_4NO_3

In order to assess if a greater amount of elements is accessible to plants, the results of a soil extraction with 1 M NH_4NO_3 (ammonium nitrate) are shown. The procedure is described in Section 2.1.4.

5.3 Results and Discussion

5.3.1 Soil Parameters pH and Soil Texture

The soil pH of the two main field trials Garte Nord and Sömmerling is 6.6 and 6.1, respectively. The pH levels of six out of the ten sites in this study were circum neutral between pH 6 and pH 7 (Table 5.3). The site in Deppoldshausen range highest with 7.3 owing to the soil being derived from limestones with a higher percentage of clay and intermediate soil quality (Soil class 42). The soil at Garte Nord (Reinshof) is classified as a haplic luvisol with very good soil quality (Soil class 90) and at Sömmerling as a cleyic cambisol with average quality (Soil class 47). Dornburg is a site in Thüringia, classified as a loess luvisol, and situated in the Eastern part of the Thuringian basin. Groß Ellershausen is a plot unit located 5 km W of Göttingen (Soil Class 62). Straubing and Aholfing are sites in Bavaria, Straubing has a very good soil quality and is loess derived soil ("Gäuboden") and Aholfing is situated near the Donau floodplains, with an intermediate soil quality. Lindau is situated 20 km NE of Göttingen and is a luvisol, with the soil texture silt loam and intermediate soil quality (Soil class 60). Trögen is situated close to Hardegsen, 15 km N of Göttingen and is classified as a luvisol with intermediate soil quality (Soil class 60). Silt is the main component in all of the investigated soils, ranging between 40 % and 80 % as to be expected from soils in agricultural use. The sites with the highest share of sand are Aholfing, Bühren and Sömmerling, indicative of a lower field capacity.

Table 5.3: Soil pH at all sample sites measured in 0.01 mol/l $CaCl_2$, soil texture measured by laser diffraction analysis. Table is sorted by increasing pH.

Location	pH	clay	silt	sand
Lindau	4.61	5.8	64.8	29.5
Trögen	5.43	5.9	73.7	20.4
Bühren	5.73	5.3	58.0	36.7
Aholfing	6.05	5.5	40.3	54.3
Sömmerling	6.09	6.9	61.8	31.3
Dornburg	6.43			
Straubing	6.46	9.0	80.4	10.6
Garte Nord	6.56	6.2	76.3	17.4
Gr. Ellersh.	7.17			
Deppoldshausen	7.30			

5.3.2 Soil Concentration Data of the Basalt Influenced Soil near Bühren

Figure 5.4 shows 6 locations where soil samples were taken in combination with a geological map. These sample locations follow the basalt body indicated in the geologic map. At each location several samples were obtained (except ID Nr. 3, one sample) and the data averaged. For sample points 3, 4 and 5 the mean values of Co and Ni were below 10 mg/kg and 25 mg/kg, respectively and therefore comparable to the values for the soils of the main field trials Garte Nord and Sömmerling (Table 7.1). In all soil samples around Bühren, larger variations in element concentrations were detected (Table 5.4). Even within one field plot the Co concentrations ranged from 18 to 44 mg/kg, depending on the mixture of soil material with remnants of basalt rock pieces. There was very good correlation for Ni-Fe, Ni-Co and Fe-Co, as expected (Figure 5.5). The scatterplot matrix revealed that the rocks composition was slightly different, with higher concentrations and different element ratios in all selected elements in the plot (Co, Ni, Mn, Mg, Fe and Cr). High pearson correlation coefficients show a stable element ratio, which is reasonable, as these elements behave geochemically similar. For example the ionic radii for Co and Ni are similar with 72 pm and 69 pm, respectively.

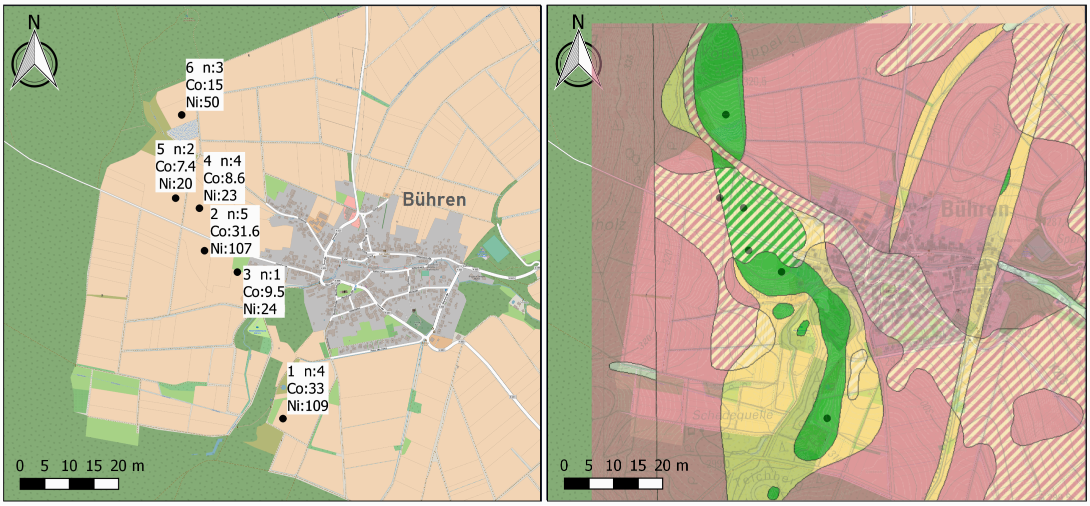

Figure 5.4: Soil sample locations near village Bühren (Dransfeld), left: ID of sample sites, n= number of samples, mean Co and Ni total element concentrations in mg/kg, map: ©OpenStreetMap contributors, openstreetmap.org,licensed as CC BY-SA; right: Overlay of geologic map (GK25), green: Miocene basalt, yellow: Miocene age sandstones, pink: Tertiary aged sandstone from 'Mittlerer Buntsandstein', Geologische Karte 1:25 000. ©Landesamt für Bergbau, Energie und Geologie, LBEG).(NIBIS Kartenserver, 2014).

Table 5.4: Concentrations of selected elements in soil samples close to Bühren for each field ID shown in Fig. 5.4.

ID	Subgroup	Co	Cr	Ni	Mg	Mn	Mo	Fe
		mg/kg						%
R[1]	Rock	39.4	203	205	37 600	1210	2.80	7.67
R[2]	Rock	44.4	211	186	22 100	1110	2.29	8.14
R[2]	Rock	43.1	202	193	31 400	1180	2.64	8.31
1	Soil	33.9	96.5	125	9380	1420	1.44	5.78
	Soil	30.5	82.7	106	6280	1300	1.36	5.27
	Soil	35.4	95.3	123	8870	1580	1.48	6.24
	Soil	32.0	87.7	109	8200	1210	1.20	5.64
2	Soil	37.9	138	139	8230	1130	1.54	5.99
	Soil	38.1	205	135	8250	1160	1.58	6.02
	Soil	27.1	81.9	89.8	8800	1040	1.07	4.64
	Soil	44.0	123	184	7170	1240	1.80	6.90
	Soil	24.8	81.6	85.2	6450	1030	1.04	4.13
	Soil	17.9	47.4	58.3	5510	877	0.84	3.20
3	Soil	9.5	27	20.9	3520	793	0.63	1.84
4	Soil	7.7	24.5	17.2	3880	718	0.60	1.78
	Soil	8.4	25.3	18.8	3510	705	0.64	1.73
	Soil	10.1	27.8	25.5	3480	758	0.67	1.93
	Soil	8.2	22.9	18.4	3800	822	0.68	1.76
5	Soil	7.2	19.9	14.8	3140	813	0.59	1.57
	Soil	7.7	21.4	17	3300	863	0.68	1.69
6	Soil	22.0	62.6	70.1	7610	929	1.23	3.92
	Soil	15.5	38.1	41.4	3940	751	0.88	2.58
	Soil	7.5	25.4	19.9	3020	412	0.52	1.68
Will (BSc)[3]	Soil	24.9	54.5	84.1	7380	1090	0.99	4.32
field trial	Soil	34.2	186	118	10 700	1700	1.51	6.51
pot [4]	Soil	34.7	146	131	8190	1260	1.49	5.98

[1] rock sample from basalt outcrop pillars;
[2] rock samples from field
[3] soil used in bachelor thesis M. Willerding-Möllmann
[4] Bühren soil used in pot trial

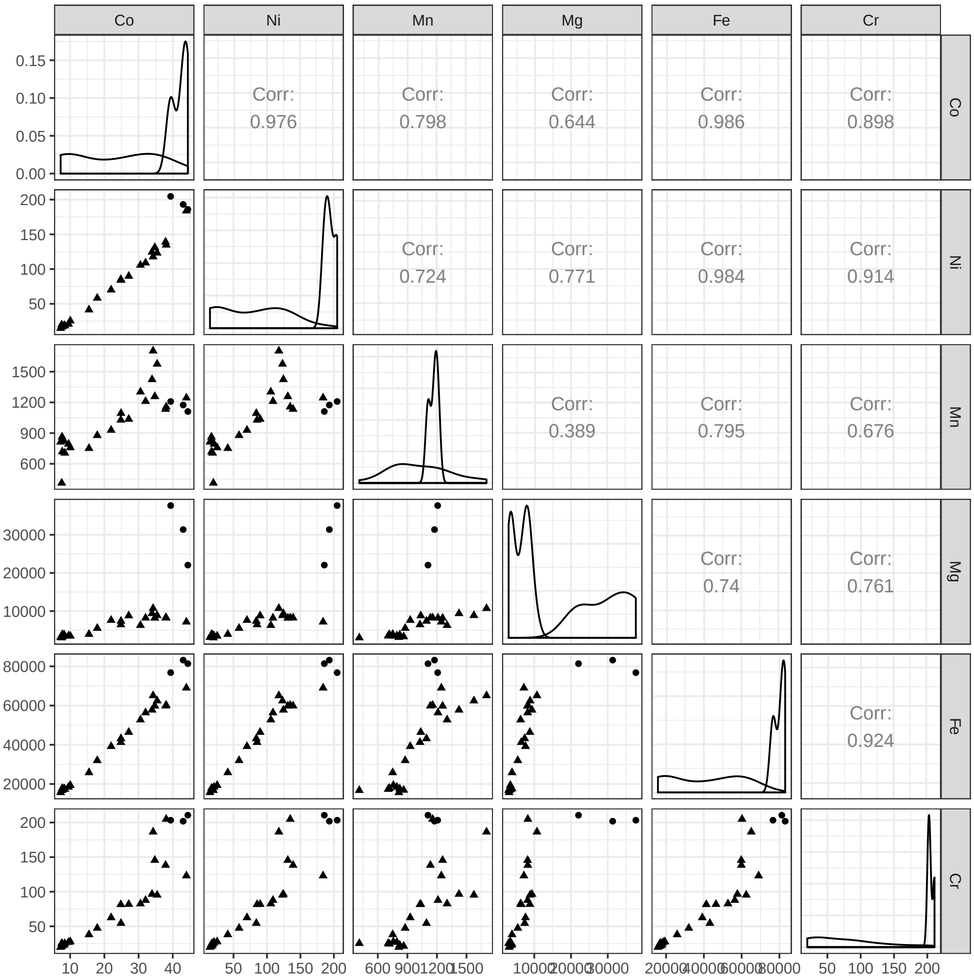

Figure 5.5: Scatterplot matrix of concentrations (mg/kg) in soil samples (triangle) and rocks samples (circles) around Bühren. Upper panel shows pearson correlation coefficient.

5.3.3 Soil Element Concentrations

Table 5.5 contains the mean concentrations for selected trace elements, which are important for biogas production (Co, Mn, Mo, Ni) and plant nutrition (Cu, Fe, Zn). Furthermore, elements indicating adhering dust or which are of environmental concern (Ti, Al, Cd) are shown.

The site in Bühren showed the greatest mean value of Co (23 mg/kg) and Ni (75 mg/kg). The two main field trials Garte Nord and Sömmerling have comparably low Co concentrations of 7 and 8 mg/kg and low Ni concentrations of 16 and 12 mg/kg, respectively. Table 5.5 also lists the concentrations of local loess (Schnetger, 1992), Upper Continental Crust (UCC), (Rudnick and Gao, 2003) and European Soil median concentrations (Reimann et al., 2018). The median European agricultural soil concentrations (GEMAS[1]) are 7.5 mg/kg for Co and 15 mg/kg for Ni (from 2108 samples in total) (Reimann et al., 2018). The trace elements in the main field trials and at most of the other sites have similar values comparable to the loess and the GEMAS soil concentrations. The geochemical situation on site Bühren is unique and the trace elements are better comparable to the values of the UCC because of the basalt component. Mn median concentrations by Reimann et al. (2018) are 445 mg/kg (median) and 701 mg/kg (75th percentile, Q75). Most sample sites do correspond to Q75 of Mn, and only the soils at site Sömmerling have lower values. The mean values of all measured main and trace elements are shown in Appendix Table A.10.

Table 5.5: Mean total concentrations in mg/kg DM for all soil sample locations used in the study in alphabetical order, with additional values for loess, Upper Continental Crust and median European Soil (GEMAS).

Location	State	n	Al	Cd	Co	Cu	Fe	Mn	Mo	Ni	Ti	Zn
Aholfing	BY	6	30 577	0.16	5.2	7.9	13 190	761	0.44	12.7	2904	44
Bühren	LS	23	56 023	0.33	22.6	19.0	39 610	1026	1.06	76.1	8113	93
Deppoldsh.	LS	3	66 070	0.49	13.0	17.8	29 726	917	1.19	29.6	4593	103
Dornburg	Th	6	50 463	0.29	9.9	16.8	23 504	705	0.5	21.9	4909	59
Gr. Ellersh.	LS	1	49 682	0.28	12.6	26.5	28 823	805	0.64	44.0	5243	75
Garte Nord	LS	21	47 245	0.25	7.3	14.0	17 576	703	0.48	16.4	4046	54
Lindau	LS	2	42 866	0.26	6.6	16.4	16 981	723	0.55	15.2	3810	62
Sömmerling	LS	22	41 394	0.24	8.2	7	14 440	479	0.68	11.6	3348	50
Straubing	BY	6	51 117	0.19	11.1	19.1	24 041	942	0.48	27.3	4936	64
Trögen	LS	1	42 190	0.27	7.2	10.7	17 570	814	0.62	14.2	4255	52
UCC[a]			81 500	0.09	17.0	28	39 200	770	1.1	47.0	3800	67
Würm loess[b]			48 200	0.10	10.0	10	21 500	460		25.0	4400	39
GEMAS[c]				0.18	7.5	15		445	0.42	15.0		45

[a] Rudnick and Gao, 2003 (Upper Continental Crust)
[b] Schnetger, 1992
[c] Reimann et al., 2018 (Median of European Soil)

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[1] geochemical mapping of agricultural soil, cooperation project of the Euro-GeoSurveys Geochemistry Expert Group.

5.3.4 Element Data from Plants grown in Bühren Soil

The element concentration of plants grown in Bühren soil (on the small-scale field trial and in pot experiments) were comparable to the median concentrations of plants grown on the main field trials. All plant element concentrations were corrected for adhering particles with Method 3 from Chapter 4. The dotted shapes in Figures 5.6 and 5.7 resemble plants on Bühren soil, either as field samples or pot experiments, and the median of the field trials are marked with yellow triangles. Dark green points mark the plants grown in small pots in 2015.

The plants grown in soil Bühren soil show the greatest element concentrations of Co, Ni and Mn in the aboveground plant tissue (Fig. 5.6). Ryegrass and cereal crops (rye, triticale) have very similar low Co values of 0.01 mg/kg .

The samples from pot and fields trial match very well in their Ni concentrations (yellow and green circles). Amaranth, summer and winter faba bean, hairy vetch and ryegrass plants show greater element concentrations than the same plants grown on the main field trials. No elevated Ni concentrations of the cereal crops (winter rye and winter triticale) grown in Bühren soil were measured. The amaranth, summer and winter faba bean and hairy vetch plants from the small and large pot trials (darkgreen and green circles) contain less Mo than plants in the field, which were even lower than median Mo concentration of both main field trials Garte Nord and Sömmerling.

Another striking observation was that all (small) pot samples from 2015 (dark green circles in the figures) show maximum content of Co and Ni.

For plants grown in Bühren soil it was also worth looking at other elements which are naturally enriched in this particular setting, such are Fe, Mg, Cr and Zn, shown in Fig. 5.7. Greater concentrations of Fe, Mg and Zn in all samples from Bühren were measured relative to those on the two main field trials (Fig. 5.7). Cr content only increased in samples from the field trial (triangles). Faba bean samples from small pots were also elevated in Cr and Zn concentrations. Additional element data can be found in the Appendix (Tables A.16 and A.17).

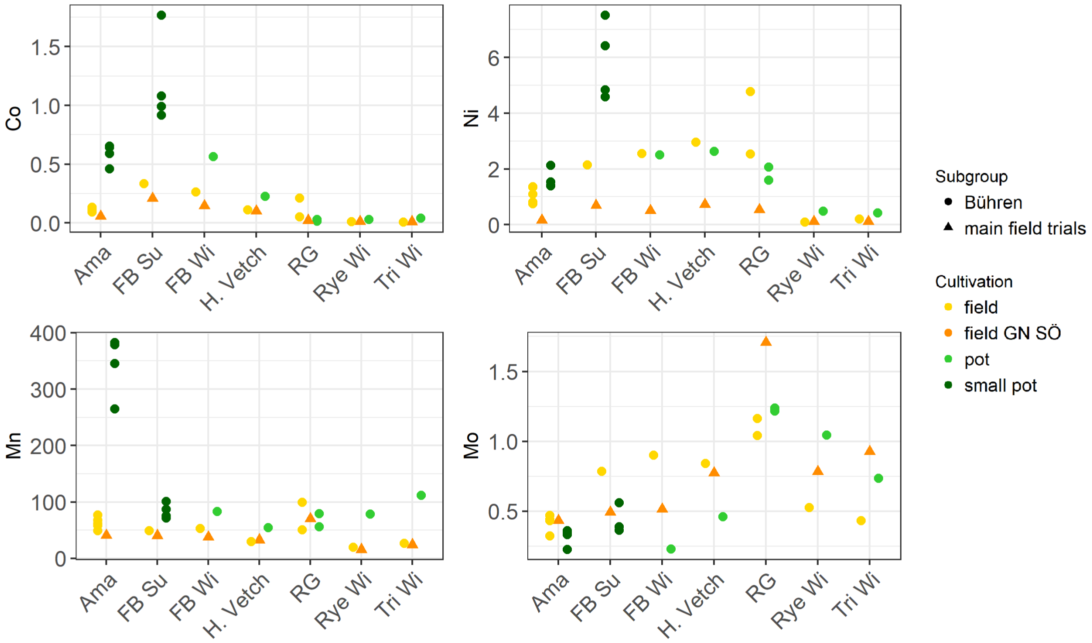


Figure 5.6: Trace element concentrations in mg/kg of Co, Ni, Mn and Mo in whole aboveground plants from Bühren, cultivated in small pots (2015), larger pot trials (2016) and median concentrations (triangle) from both main field trials Garte Nord (GN) and Sömmerling (SÖ).

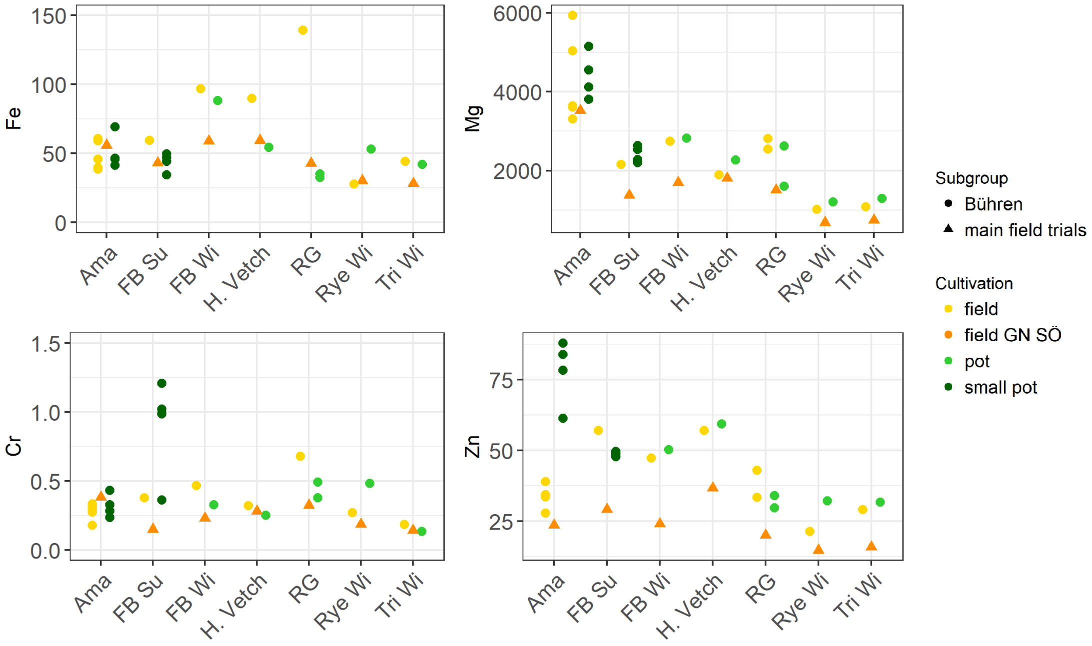


Figure 5.7: Trace element concentrations in mg/kg of Fe, Mg, Cr and Zn in whole aboveground plants from Bühren, cultivated in small pots (2015), larger pot trials (2016) and median concentrations (triangle) from both main field trials Garte Nord (GN) and Sömmerling (SÖ). For Cr and Fe one outlier is excluded (ryegrass).

5.3.5 Extraction Results

All sample site soils were extracted with 1 M NH_4NO_3 according to DIN 19730 (DIN Deutsches Institut für Normung e. V., 1997). This technique was used to determine the potential bioavailability of trace elements to plants. The greatest extraction concentrations for Co, Ni, Fe and Mn were obtained for soil in Lindau, followed by Trögen and Bühren (Fig. 5.8). The extraction rates rose with a lower soil pH. The soil from the site in Lindau with the lowest pH of 4.6, had the highest rates. The extraction results indicated, that the soil obtained from Bühren had a greater bioavailability of these elements than at the two main field trials with lower total soil concentrations of Co, Ni, Fe and Mn.

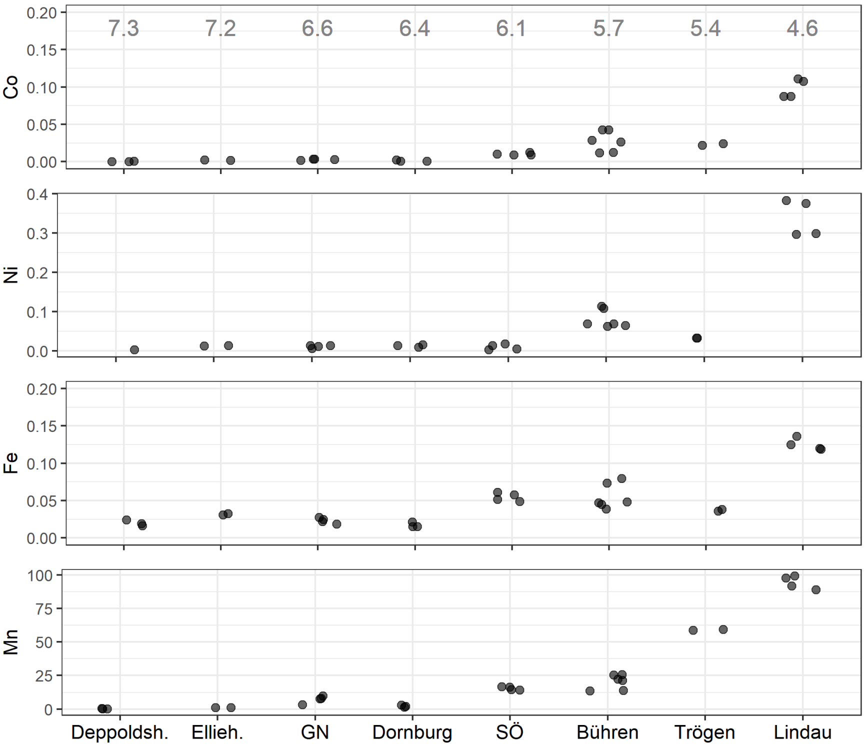


Figure 5.8: Results from soil extractions with NH_4NO_3 in mg/kg DM for Co, Ni, Fe and Mn. Soil pH values are shown in the upper text row. Each data point represent one extraction and the points are horizontally jittered to reduce overplotting.

5.4 Conclusions

Three basic conclusions can be drawn. Firstly, the samples grown in the soil from Bühren show greater concentrations of most samples of Co, Ni, Mo, Mg and Zn in aboveground plant matter. Secondly, the pot samples have greater element concentrations than plants from the main field trials, except for Mo. Thirdly, the small pot samples from 2015 are not comparable to the other pot samples. This was especially the case with amaranth samples from small pots having Mn concentrations about 3 times greater than all other plant species. The faba bean samples from the small pots show maximal values for Co, Ni and Cr. The amaranth and faba bean plants grown in the small pots (dark green points) may have beenn affected by different growing conditions. They grew in small pots so they were most likely to suffer deficiencies resulting in a shorter height. The plants in the second pot experiments of 2016 had much larger pots each containing almost 20 kg of soil. Their height and structure were much better comparable to the samples from open field. Also it may be the case, that the root system could not develop freely in the small pots.

The results from NH_4NO_3 extractions also indicated a potentially higher bioavailability of Co, Ni, Fe and Mn from the soil in Bühren. The extraction concentration results anti-correlated to the soil pH. The main field trials show very small extraction concentrations.

Chapter 6

Influence of Plant Species on Element Uptake

6.1 Element Uptake

As already mentioned, a plant's ability to mobilize elements from the soil is very different between species. In Chapter 5, the influence of the soil was investigated with a detailed look at soils rich in trace elements (for example in Co, Ni, Mn, Fe and Zn).

In this Chapter, the plant samples are characterized in more detail and the whole element spectrum will be taken into account. The concept of transfer factors is introduced to show the variability in plant uptake. Transfer factors are calculated for the different plant species used in the study based on the total soil concentrations and on the concentrations in soil extractions with ammonium nitrate.

Multivariate methods are applied to characterize the plant species with all elements at once. According to the concept of compositional datasets all measured element concentrations in samples (rocks, soils, plants, water) are dependent upon each other. The range of the concentrations (for example, mg/kg = ppm) is limited to 1 000 000. All environmental datasets are compositional by nature. Although, we are measuring only a part of the elements, they are still in a relationship with all other elements in the sample. This is described by Aitchison (1986) in great detail. This field of research was triggered by geochemists in the 1960s who realized spurious correlations in datasets and the problems caused by the skewness of the data. Later on, this concept was renewed by the discovery of the constant sum problem in geochemical data (Rollinson, 1992). A group of biologists recently coined the term *ionome* for the composition of all elements in plants including interelement relationships (Baxter, 2009; Watanabe et al., 2016; Williams and Salt, 2009).

In this study we are able to measure 46 elements with a good precision and accuracy with ICP-MS and ICP-OES. All of these elements sum up to about 2.5 to 7.5 % of total electrolytes, depending on plant species and state of maturity. This number is low because C, O, H and N cannot be measured with the digestion technique used.

6.2 Methods and Data

6.2.1 Plant and Soil Data

In this Chapter the element concentration data of the whole aboveground plants was used. These element concentrations where determined with ICP-OES or ICP-MS after a sample digestion process. The sample digestion was performed with complete digestion method using HNO_3, $HClO_4$ and HF. The procedures are described in Chapter 2.1.3.

The soil extraction concentrations were determined with a neutral salt solution of 1 M NH_4NO_3. The procedure is described in Chapter 2.1.4. This extraction technique was used to determine the "potentially" available pool of elements to the plants. The procedure is discussed in greater detail with respect to other extraction techniques in Chapter 8.

6.2.2 Transfer Factor

The Transfer factor (TF) are a very good instrument to show the plant species impact on a large variety of elements. All elements passing the quality control are included in the following compilations. The TFs are calculated as follows:

$$TF = \frac{conc\ El_{plant}}{conc\ El_{soil}} \tag{6.1}$$

with $conc\ El_{plant}$ concentrations corrected for adhering particles (Method 3) in whole aboveground plant samples, and $conc\ El_{soil}$ as total soil element concentrations in upper soil up to 30 cm in depth. In the first part, the denominator is total soil concentrations to cover all elements in this study. In the second part, the transfer factor is calculated with the extraction results of ammonium nitrate as denominator:

$$TF_{ex} = \frac{conc\ El_{plant}}{conc\ El_{extr.conc.soil}} \tag{6.2}$$

In the literature, the TF is sometimes referred to as the *bioconcentration factor (BCF)* or *translocation factor*. There is no generally accepted definition for TF, and the definition must be checked in each source. It especially must not be confused with the ratio between root element and plant tissue concentration. The definition in this study follows that of "bioconcentration factor" as in Jia et al. (2013). Nevertheless, the total soil as a denominator is a very good tool to characterize single plants and their differences in plant species precisely, because total soil concentrations are based on fully digested samples with high precision and accuracy. Extraction values used as denominators do generally have larger errors, as these methods do not have high reproducibility. Depending on the extraction method used, the number of elements, which can be considered, is smaller. Some elements cannot be measured as the concentrations are below the detection limit. Soil extractions give rise to several problems in element determination (refer to Chapter 8). Despite these problems, one may argue that the total element concentrations in the soil do not represent potentially available elements to plants. With TF_{ex} we therefore analyze the results with soil extractions as denominator and compare these results to the TF based on total soil concentration.

6.2.3 Multivariate Analysis

For multivariate approach covariance biplots a PCA is executed on clr (centered log-ratio) transformed data. Biplots are useful tools to explore datasets qualitatively. There is no quantitative information in these plots because they are built from clr-transformed data. This means that each ray in the biplots (variables) is in relationship with all the other variables. The clr transformation is calculated via

$$clr(x) = ln\left(\frac{x}{g(x)}\right)_{i=1,\dots,D}$$

with $g(x)$ as the geometric mean. D is the number of variables (in this case, elements).

The graphical representation of the results of the PCA is a covariate biplot. Biplots are useful tools to explore the structure of a dataset (Gabriel, 1971). The variables and observations are displayed in a two-dimensional plot. In the covariate biplot the variables are plotted as arrows or rays from the center of the dataset after transformation. That is, rays with a very short link between each other are likely proportional and have a quasi-constant log ratio. If the links between the rays are very long (meaning, the angle in between is more than 90°) the log ratio of the two variables is highly variant. If rays exhibit 90° between them they are likely to be uncorrelated (van den Boogaart and Tolosana-Delgado, 2013).

6.3 Transfer Factors Results

6.3.1 Transfer Factor Based on Total Soil Concentrations

In Figure 6.1 the TF from all samples of the two main field trials (Garte Nord and Sömmerling) are shown ordered by decreasing median with total soil concentration as denominator. Three fields of main nutrients, mobile trace elements and immobile elements are marked based on the TF value only and mostly corresponds to definitions in the literature (Marschner, 1995; Taiz and Zeiger, 2010). The TF over this broad range of elements spans four orders of magnitude and therefore to observe differences in the small TF as well, the data is displayed on a log scale. The first area are TFs > 1, and the second area are marked by TFs > 0.001. The second area termed "trace nutrients and mobile elements" includes all elements considered as micronutrients (Fe, Mn, Zn, Cu, Ni) and elements which are relatively mobile in the soil. Some toxic elements like Cd and As also display good mobility.

In the third area there are elements which are immobile and are only taken up by the plant in very small amounts. Most of these elements are major constituents in the soil (Ti, Al). Also the REEs fall within this range. The group of REEs is represented only by La, to improve the readability of the diagrams.

Differences in transfer factors of plant species

Figure 6.2 shows the TFs from members of the Pocaea family (maize, rye, ryegrass and triticale). For Cu, Zn, As and Fe the median points for these species congregate very close together. In the third area ("immobile elements") the variance is greater, and all species overlap in their 1st to 3rd quartiles. That was expected, as these elements are also mostly affected by the correction for adhering particles (refer to Chapter 4).

Ba, Mo and Na display the greatest variance. The two cereal crops rye and triticale almost overlap perfectly. In Figure 6.3 faba bean (Fabaceae/Leguminosae) and maize are shown. Faba bean plants show a greater TF in almost all elements considered, except for Cd and some immobile elements like Sc, Th, Zr, Ti and Al.

Figure 6.4 revealed distinctive TF patterns of amaranth and cup plant species compared to faba bean and maize. Cup plant has remarkably low TFs of Mo and Na. Amaranth showed high TFs of potentially toxic elements Tl, Cd, and Cs. This holds also for the flower mixtures (annual and perennial, not shown). Cup plant exhibits a remarkably small TF for Mo, Cd and Na. Another possible application of the transfer factors and this kind of visualization is the field of phytoremediation, especially phytoextraction. If plant concentrations corrected for adhering particles are used the real uptake behavior of the plant could be investigated. Of course, then other important factors, like the plant DM yield, need to be included in a following calculation of extractable amount of elements (Sauer and Ruppert, 2013; Sauer et al., 2017).

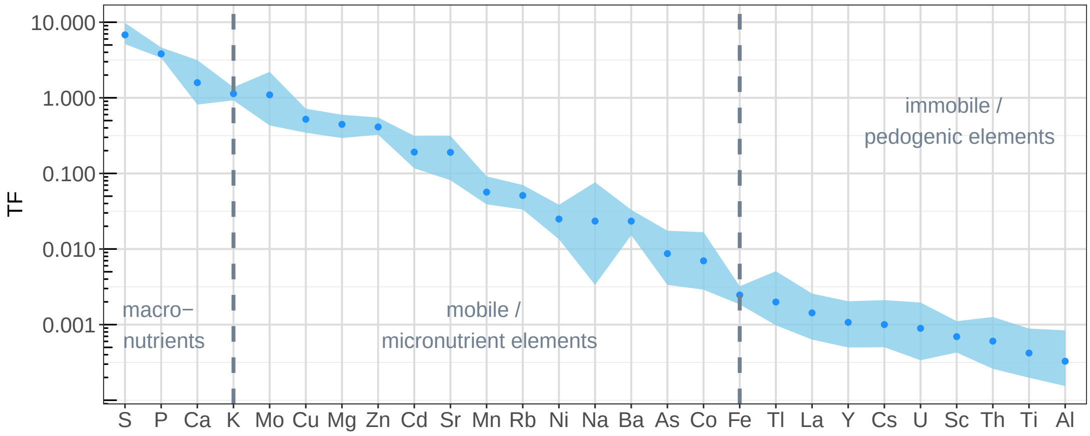


Figure 6.1: Transfer factor of all samples from Garte Nord and Sömmerling (n=228), Element data is ordered by decreasing medians.The blue area represents the area from 1st to 3rd quartile.

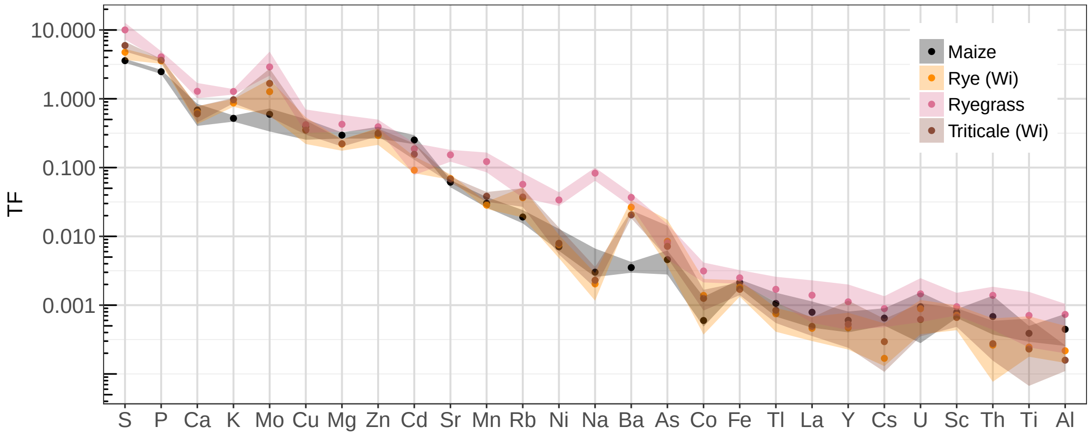


Figure 6.2: Median transfer factor of Poaceae plants, from Garte Nord and Sömmerling with elements ordered by decreasing median TF. The colored area represents the 1st to 3rd quartile area (IQR) of plant species.

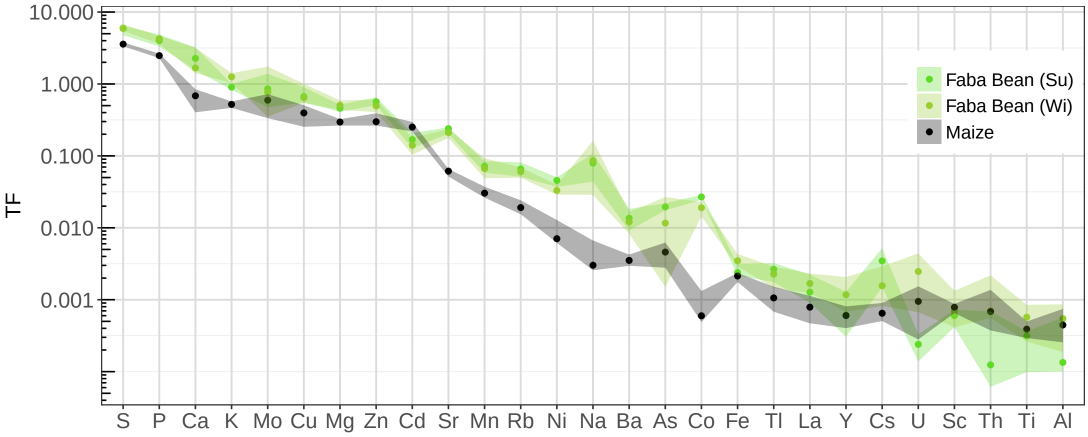


Figure 6.3: Median transfer factor of faba bean plants and maize from Garte Nord and Sömmerling with element ordered by decreasing median TF. The colored area represents the 1st to 3rd quartile area (IQR) of plant species.

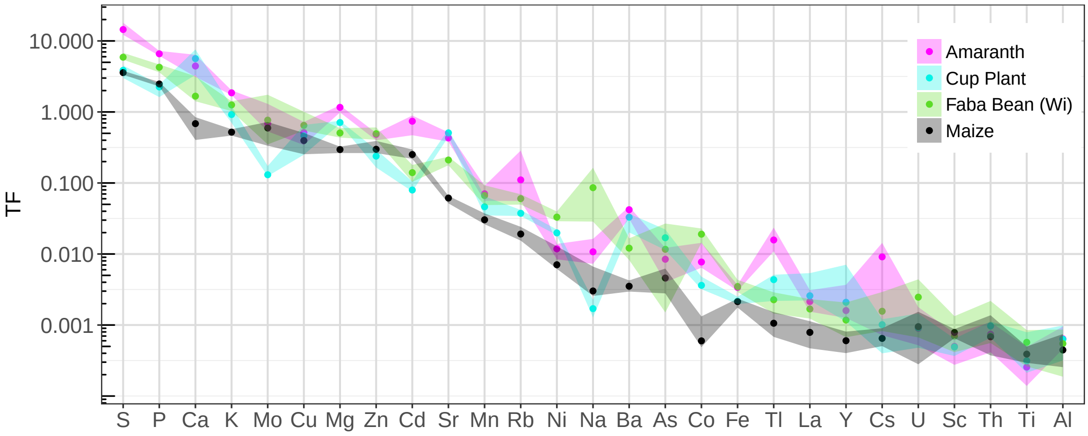


Figure 6.4: Median transfer factor of amaranth, cup plant, faba bean and maize plants, from Garte Nord and Sömmerling with element order by decreasing median TF. The colored area represents the 1st to 3rd quartile area (IQR) of plant species.

6.3.2 Transfer Factor based on Extraction Concentrations

The following diagrams show the TF with the soil extractions with ammonium nitrate as the denominator. In this approach only the elements which could be recovered in the extraction can be investigated (21 elements).

The TFs now result in greater maximum ratios up to a factor of 2000. The order of elements have also changed. The three zones –macro-nutrients, mobile/micronutrients and immobile /pedogenic elements –cannot be defined any more. Elements like Fe, Ti, Al have very large transfer factors (TF_{ex}) based on soil extractions, because their concentration in the extract is very low. This naturally leads to a very high factor of around 1000 for Fe (Fig. 6.5). High TFs between 500 and above might indicate that the plant was able to access other pools in the soil than the pool of "potentially bioavailable" elements, which were targeted with the extraction procedure with ammonium nitrate or similar neutral salt solution extractions.

The spread between the 1st and 3rd quartile is very similar to that in Fig. 6.1. Both diagrams show larger variation in Ti, Na, Co and very small variations in K or Cu. This is not surprising, as these pattern are totally controlled by the concentration in the plants (= numerator in Eq. 6.1 and 6.2).

The TFs of faba bean plants (summer and winter variety) and maize plants are very different (Fig. 6.6). The TF_{ex} for faba bean plants are much greater for Co, Na, Ca, Sr and Mn, than for maize plants. This pattern was similar to the TFs based on total soil concentrations (Fig. 6.3). For cup plant samples, a small TF_{ex} was detected for Mo and Na and a very high TF_{ex} for Ca and Sr (Fig. 6.7). This observation corresponds to Fig. 6.4).

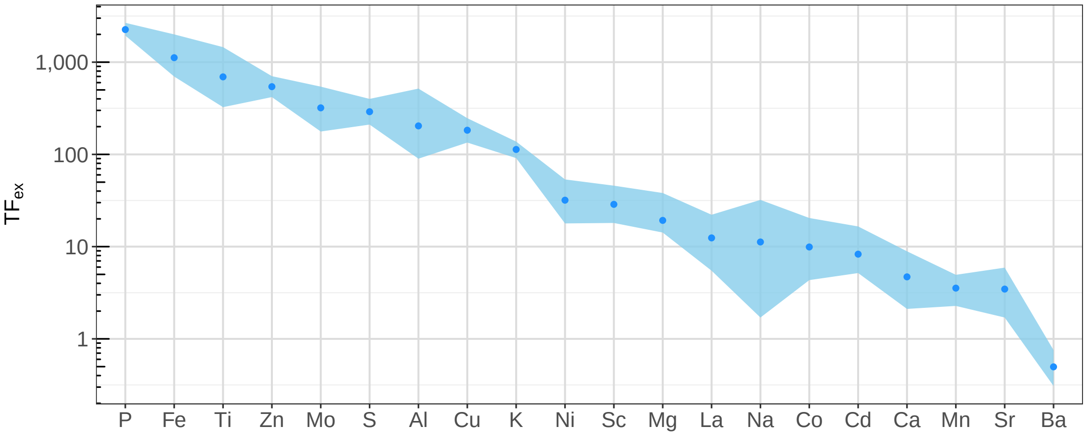


Figure 6.5: Transfer factor (TF_{ex} = plant conc/extr. conc) of all samples from Garte Nord and Sömmerling (n=228), element ordered by decreasing median TF_{ex}, colored area represents the 1st to 3rd quartile area (IQR).

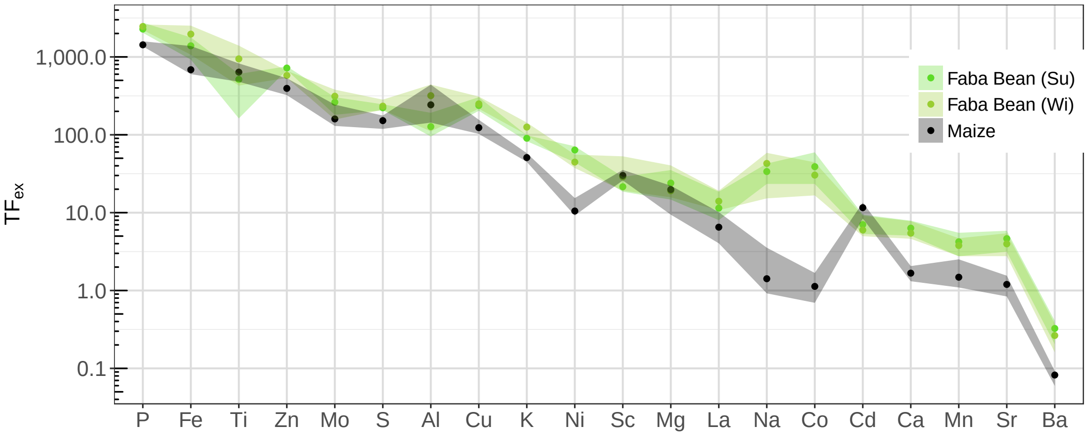


Figure 6.6: Transfer factor (TF_{ex} = plant conc/extr. conc) of faba bean and maize plants from Garte Nord and Sömmerling, element ordered by decreasing median TF_{ex}, colored area represents the 1st to 3rd quartile area (IQR) of plant species.

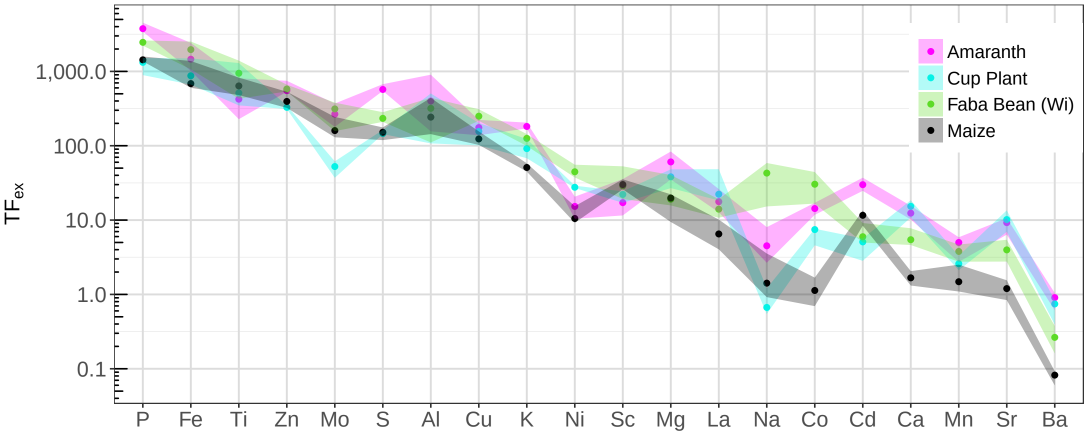


Figure 6.7: Transfer factor (TF_{ex} = plant conc/extr. conc) of amaranth, cup plant, faba bean and maize plants from Garte Nord and Sömmerling, element ordered by decreasing median TF_{ex}, colored area represents the 1st to 3rd quartile area (IQR) of plant species.

6.4 Element Results – Multivariate Approach

The multivariate analysis was applied using the elements S, K, Mg, P, Ca, Cu, Fe, Ni, Sr, Co, Mn and Mo. It is not possible to include all 47 elements in the analysis, these are too many variables. Fig. 6.8 included 218 samples from both main field trials. Species and locations informations were provided by color and shape of the data points. In Fig. 6.9 all samples from this study were included. The locations are not marked as shapes, because these were too many variables. Both Figures are consistent, the rays of the variables (= elements) point in the same direction in each sample species. For Fig. 6.9 the site information cannot be included as there were a different number of samples per site and different sample sets. The color pattern by plant species indicates a unique element uptake pattern per plant species.

The first biplot (Fig. 6.8) also shows the results with respect to the two main field sites Garte Nord and Sömmerling (circle and triangle shapes, respectively). It was observed, that the site location pattern was not predominantly dependent on the pattern by plant species.

For both biplot representations a very short link between Ca and Sr was visible, indicating a quasi- constant log ratio. In the direction of the Ca and Sr ray the cup plant samples are to be found. This indicates a greater uptake of Ca and Sr by this plant species. Indeed, they are members of the Asteracea plant family, which require greater amounts of Ca. Ca and Sr are members of the same main group of the periodic table (alkaline earth metals) and therefore have similar properties. The quasi-constant log-ratio of Ca and Sr can also be confirmed by a narrow Ca to Sr ratio found in all plants of the main field trials. Ca is a main nutrient, and Sr is a trace element. The stable ratio in the plants show that they behave geochemically similar and are probably taken up through the same channels. Cup plants also showed a higher absolute concentration of Ca in aboveground plant matter (refer to Chapter 7, Fig. 7.4).

Co and Ni have a small angle between them but a different length, making the link between them quite large. Therefore, they do not seem to have a quasi constant log-ratio relationship. This holds true for the sample set from the main field trials and for the larger plant data set from different locations. Mo always pointed in a different direction and does not show a log-ratio relationship with any other elements. Co-Ni ratio and ratios between other trace elements of interest are also being discussed in Chapter 7 (Fig. 7.13).

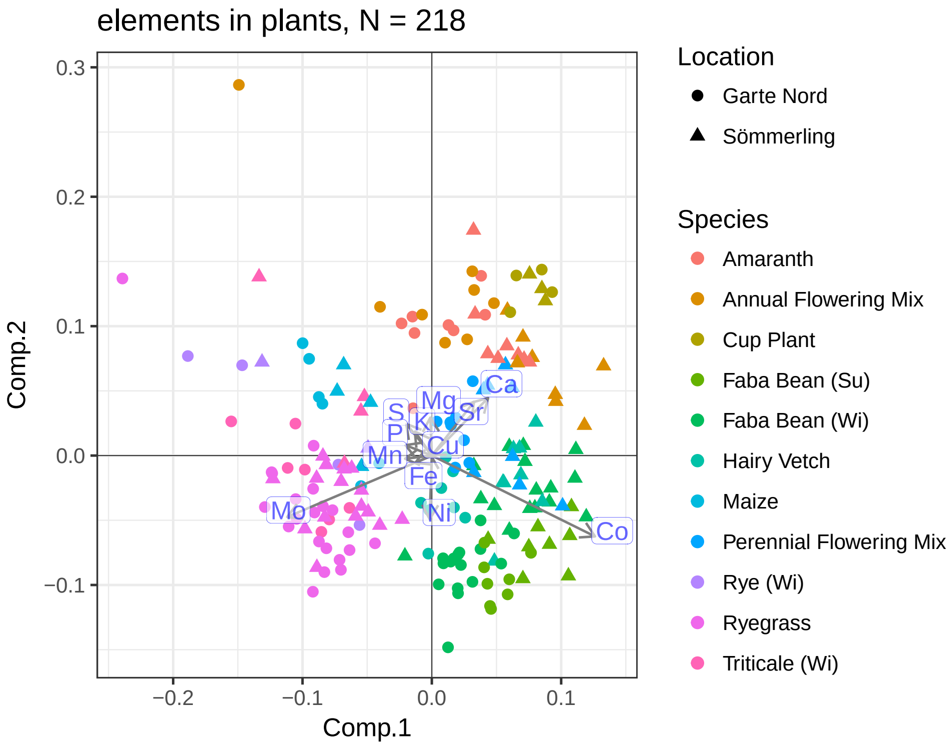


Figure 6.8: Covariance biplot of a PCA on clr transformed plant sample element concentrations of the two main field trials.

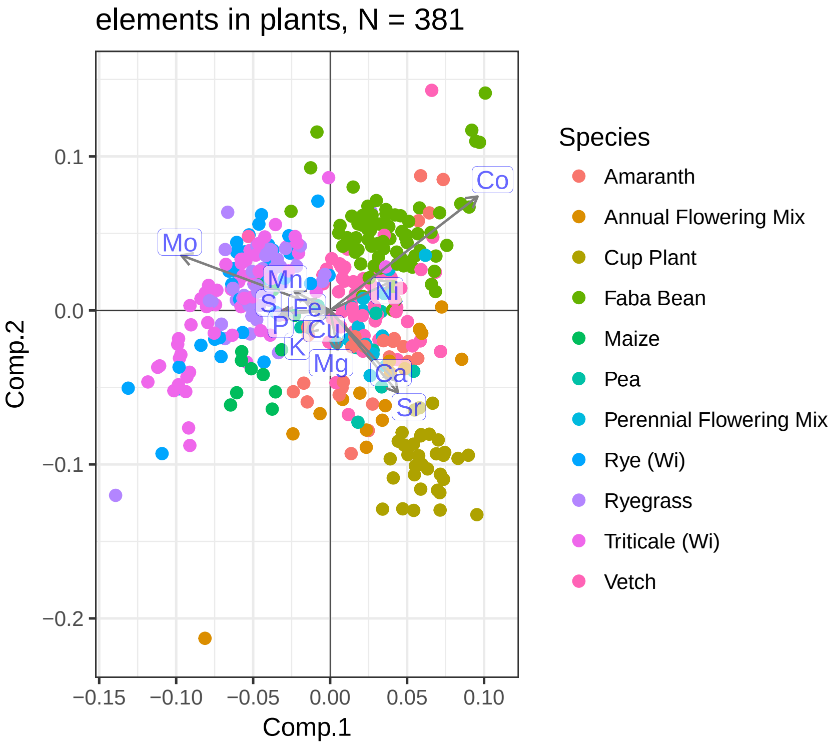


Figure 6.9: Covariance biplot of a PCA on clr transformed plant sample element concentrations of all plant samples in this study, from different locations.

6.5 Discussion

6.5.1 Transfer Factors

Significant differences in TF for the different plant species could be stated for a large range of elements. Also, the variance of the species (Figures 6.3, 6.2 and 6.4) provided an insight into how large the variability of total element concentration of samples from one plant species could be. It can be assumed, that predictions of element concentration in plants can be performed more precisely for elements and plant species with a small variance of TF per species. This was the case for Cu, Zn and Fe.

A disadvantage of the TF approach is that, from these values the amount of uptake cannot be determined, because everything is based on concentrations. For example, the high TF for Cd in plant species of amaranth (Fig. 6.4) do not indicate that the element concentration and the amount taken up by the plant is necessarily high. This depends on the mobile fraction of Cd in the soil and on the DM yield.

The TF based on the soil extractions (TF_{ex}) resulted in ratios of up to 2000. Differences in the TF per plant species were detected, similar to the TF based on total soil concentrations.

6.5.2 Multivariate Results

The covariance biplots are good means to explore the datasets with many components included. The different plant species were plot in distinct regions, and therefore indicate a different uptake pattern for the elements chosen. The site location pattern does not dominate over the pattern by plant species. Ca and Sr showed a quasi-constant log-ratio, with a very small link between them. They also had a stable ratio of Ca to Sr in the plant samples, pointing to simultaneous uptake through the same channels. For Co and Ni, which behave geochemically similar in soil as seen in Chapter 5, do not indicate a stable ratio in the plants. These elements had a long link between them in the compositional biplots.

Chapter 7

Element Concentrations in Plants in Sole Cropping and Intercropping

7.1 Introduction

This chapter sums up all element contents in plants from the two main field trials Garte Nord and Sömmerling. Some plant sample sets from Straubing (Bavaria) and Dornburg (Thüringia) were also included do deepen some aspects. The focus is on the actual element concentrations in the plants. Main nutrient elements (P, K, Mg, S and Ca) and a set of trace elements (Co, Ni, Mn, Mo, Cu, Fe and Zn) are considered. In the first part, the element data is presented per plant species, in the second part the concentrations in the intercropping systems. All plant element data are corrected for adhering soil material with Method 3 from Chapter 4.

In intercropping, two or more plants were placed next to each other during the time of growth. In this study, we performed alley cropping with alternating rows per plant species with legume and non-legume plants. The basic idea of intercropping is derived from sustainable organic farming systems. It is common practice in subsistence agriculture, which leads to greater yields and the risk of crop failure is minimized (Brooker et al., 2015). In Western Europe it is often used in organic farming, where benefits lie in saving of N-fertilizer, weed suppression, or a better usage of soil resources (Willey, 1979). Some intercropped species can also have better stability and are better protected against severe weather conditions (heavy rain, wind). This holds especially true for vetch intercropped with cereals.

In intercropping the two plant species can influence each other, and may result in a greater uptake of elements in one plant component. For example, if the legume plant can convert atmospheric nitrogen to ammonia (NH_3) with biological nitrogen fixation (BNF), H^+ -ions are being excreted into the root zone to balance the cation-anion-uptake. This lowers the soil pH locally and will lead to a greater availability of trace metals such as Fe, Cu, Fi, Co, Mn (Haynes, 1990; Peoples et al., 1995). These metals can then be taken up by the cereal plant companion (sweet grasses) in intercropping, resulting in an increased uptake and plant tissue concentration. The alternate is also possible, that the sweet grasses can release phytosiderophores (Fe mobilizing compounds) for Fe acquisition. These chelate compounds also mobilize other micronutrients such as Cu, Mn, Zn, (Römheld, 1991).

In recent years there was a lot of research on cup plants to test their potential as substrate for biogas production. In the research project, only cup plant samples harvested in 2016 could be included. In the first year they form only plant rosettes. Cup plants can be cultivated as perennial crops. Results from additional cup plant samples (*Silphium perfoliatum*) taken in Dornburg from field trials of the TLL (Jena, Thuringian State Institute for Agriculture) are shown. In Dornburg Cup plants from 6 different geographic origins were tested.

In the context of biogas and energy crop production there are attempts to include intercropped plants in common crop rotations, as these can be harvested and ensiled if both reach maturity for biogas production simultaneously. For example, a research project on legume and non-legume plants was conducted by the TFZ ("Technologie- und Förderzentrum" Straubing, Bavaria) which investigated the field crop parameters and environmental aspects like flowering (Eberl and Fritz, 2017). From this project samples were taken for the Bachelor Thesis of Sorger (2015). Including flowering plants in field cultivation is beneficial against the ongoing decline in insect populations (Hallmann et al., 2017). The companion plants (non-legumes) can benefit from the legume plant. For example N-compounds released by the legume plant in the rhizosphere can be taken up by the non-legume plant (Fustec et al., 2010).

7.2 Materials and Methods

All plants were grown on two field trials, Garte Nord (Reinshof, Göttingen) and Sömmerling (Schoningen, Uslar). These locations are marked on a map in Chapter 2.5. The experimental field setting, the plant sample set and management details are described in Chapter 2.6. Both field trials received fertilizer according to good agricultural practice. All variants received mineral and organic fertilizer (biogas residue), except the winter and summer faba bean, and the intercropping of faba bean with triticale, which received only organic fertilizer. The soil in Garte Nord is classified as haplic luvisol with very good soil quality and the soil in Sömmerling is classified as cleyic cambisol with average soil quality. A more detailed description of the soils can be found in Chapter 9. The plants were harvested at silage maturity suitable for biogas production. The other preparation steps were performed as outlined in Chapter 2.1.1.

To test for statistical significance ANOVA analyses were performed on multiple linear models. The model included the factor of element, location, species and growing year. The analysis was performed with *R* (R Core Team, 2017).

7.2.1 Plants in Sole Cropping and Intercropping

228 plant samples were included –115 in Garte Nord and 113 of site Sömmerling – comprised of 12 variants of one or more plant species. In the first part of this Chapter the samples are examined per species. Each species consist of four samples per location and growing year, except ryegrass with 12 samples, as each replicate was cut three times annually. Triticale and winter faba bean had 8 samples each, including the plants in sole cropping and intercropping. In the second part (Section 7.4.3), the resulting concentrations in the intercroppings AM (amaranth and maize), RV (rye and vetch) and FB Wi Tri (faba bean and triticale) are shown.

Mean values and standard deviations of the concentrations can be found in Appendix Table A.12 and Table A.14. For the relative standard deviations of main nutrient elements see Appendix Table A.13. For trace elements see Table A.15. The focus in this

(a) Amaranth/maize intercropping (AM), August 2016 **(b)** Rye/vetch (RV) intercropping, June 2016

Figure 7.1: Intercroppings of AM and RV, in Garte Nord.

Chapter is on the difference between the plant species. From all plant mixtures, only the flower mixtures (annual and perennial) are included (AF/PF Mix).

The intercroppings AM, FB Wi Tri and RV were included. The two plant species in each intercropping were separated after harvesting. The yield of each plant component was obtained by Katharina Hey and the element analysis was performed according to the other plant samples. The element concentrations resulting in the intercroppings ($c_{Intercrop}$) were calculated based on the yield of each plant component ($yield_A$ and $yield_B$) and their concentration in plant ($conc_A$ and $conc_B$).

$$c_{Intercrop} = \frac{(yield_A * conc_A) + (yield_B * conc_B)}{yield_{total}} \tag{7.1}$$

Figure 7.2: Winter triticale/ faba bean intercropping (FB Wi Tri) in Sömmerling, June 2016.

7.2.2 Data Visualization

The Figures in this subchapter all show concentrations in whole plants without roots in mg/kg per species divided by panels of year and location/growing site to account for the variability in the results. In the diagrams, each data point represents one sample. This style of representation was chosen, because other representations, like boxplots, were unsuitable as there were too few samples per site and year. The points in the diagrams are displayed with a horizontal jitter to reduce overplotting. The plants were grown in 2014/2015 and 2015/2016, hereafter referred to as 2015 and 2016. See Table 2.2 for all plant variants utilized in this study.

7.3 Results and Discussion

7.3.1 Soil Characterization

Table 7.1 shows the results for the elements Cd, Co, Cu, Mn, Mo, Ni and Zn in the two main field trials Garte Nord and Sömmerling as mean values with standard error. The standard error (of the mean) was calculated by $SE = \sqrt{n}$, with number of samples n. The mean values of both field trials were similar, but they do not overlap within the error ranges. There is a tendency towards a greater variance in the values obtained in Sömmerling, as there are higher SE in at least 6 elements (Table 7.1). The soil texture was silt loam (USDA) for both main field trials. The soil at Garte Nord (Reinshof) was evaluated with 90 points (German soil quality system) and the soil at Sömmerling with 47 points; meaning a very high soil quality at Garte Nord.

Table 7.1: Mean ± standard error of the mean (SE) from main field trials Garte Nord (GN n=21) and Sömmerling (SÖ n=22) in mg/kg DM.

Loc	Cd	Co	Mo	Ni	Zn	Cu	Mn
GN	0.25 ± 0.01	7.33 ± 0.09	0.48 ± 0.01	16.38 ± 0.45	53.96 ± 1.25	14 ± 0.2	703 ± 8
SÖ	0.24 ± 0.01	8.24 ± 0.22	0.68 ± 0.02	11.63 ± 0.56	50.17 ± 0.82	7 ± 0.2	479 ± 23

7.4 Results of Main Field Trials of the NiCo Project

7.4.1 Main Nutrient Concentrations

The main nutrient element concentrations considered here (K, P, S, Mg) are in the range of approximately 1000 mg/kg (0.1 %) to 40 000 mg/kg (4 %) in the plant tissues.

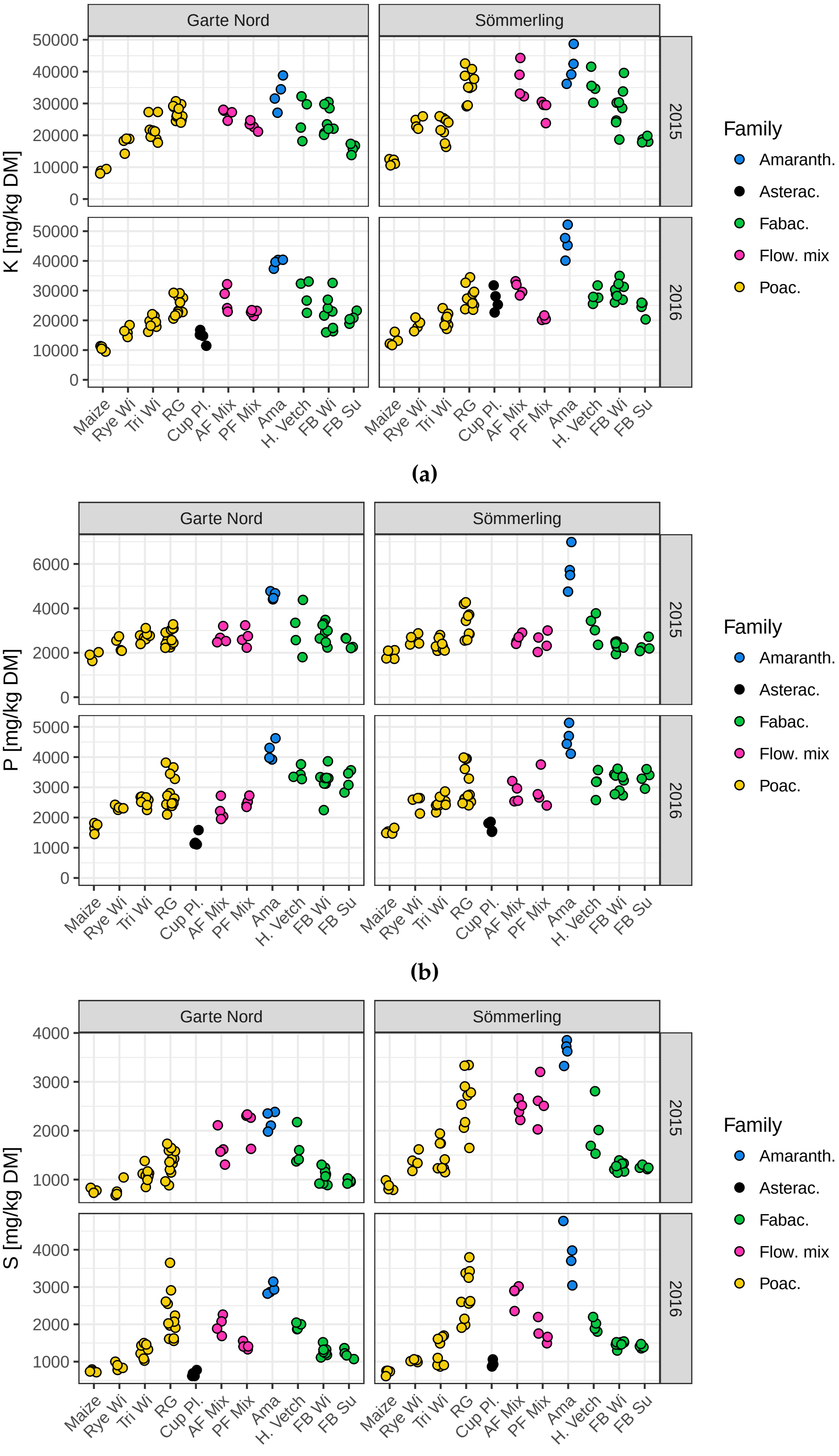
ANOVA analysis with multiple regression showed, that for P, K, Mg, Ca and S there was a significant difference between element concentration and species, location and year for most elements (Table 7.2). That means that the display of data per year and per site is reasonable. All plant element concentrations in this Chapter were corrected for adhering particles with Method 3 as described in Chapter 4. As stated in this Chapter, the correction had no effect on main nutrient elements, and only slightly affected the trace nutrient elements (Chapter 4.2).

Table 7.2: ANOVA p-value results on multiple factor linear model: *lm(element ~Species + Location + Year)*, n.s. = not significant. Significance threshold was set at p = 0.05.

	P	K	Mg	Ca	S
Species	p < .05	p < .05	p < .05	p < .05	p < .05
Location	**n.s.**	p < .05	p < .05	p < .05	p < .05
Year	**n.s.**	p < .05	**n.s.**	p < .05	p < .05

Amaranth plants often have the greatest concentrations of P, S, Mg and Ca. The ryegrass samples display a larger variation in concentration values. This was most evident in Fig. 7.3c. The summer faba bean, maize and cup plant showed a little variation. The concentrations in plants of the Fabaceae family (faba bean, hairy vetch) and flowering mixtures were similar for K, P and Mg. S and Mg concentrations in amaranth were exceeding all other plant variants (for S only site Sömmerling).

For Ca, there was a group with smaller concentrations of <8000 mg/kg (0.8%, Poaceae). Hairy vetch, faba bean (Fabaceae), flower mixtures, cup plant (Asteraceae) and amaranth (Amaranthaceae) had greater concentrations of Ca ranging between 0.8 to 2.5% (Fig. 7.4b). The cup plant samples, were only plants from 2016 were available, show in most cases lower concentrations for K, P, S than the other variants, and higher concentrations of Ca and Mg than the other variants. These results are summarized in Appendix Table A.12. The relative standard deviations of these main nutrient elements ranged between 4 and 25 % (Appendix Table A.13). The concentrations are most likely the results of fertilizer application and physiological plant mechanisms.

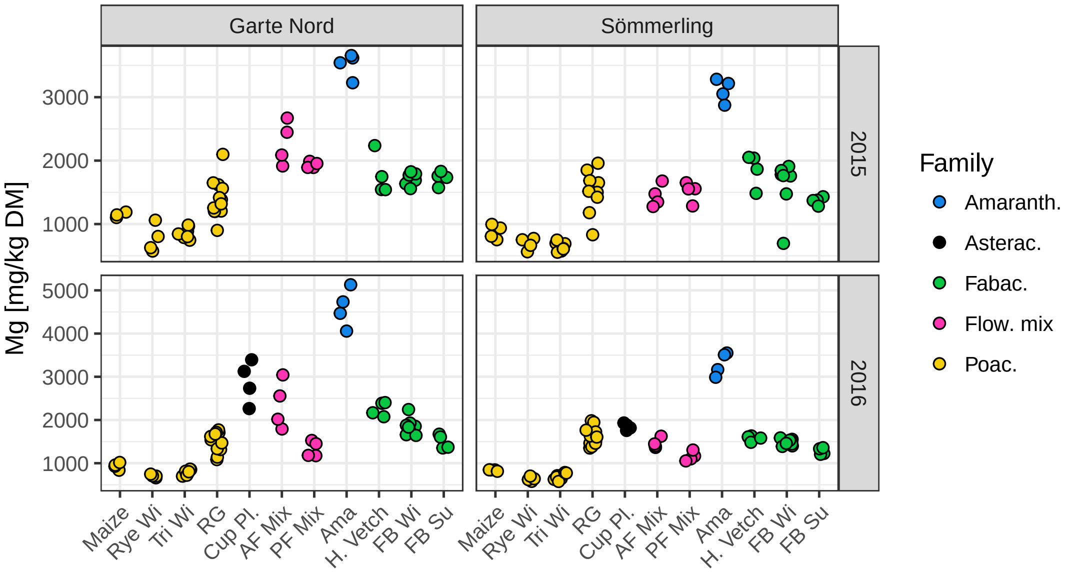


(a)

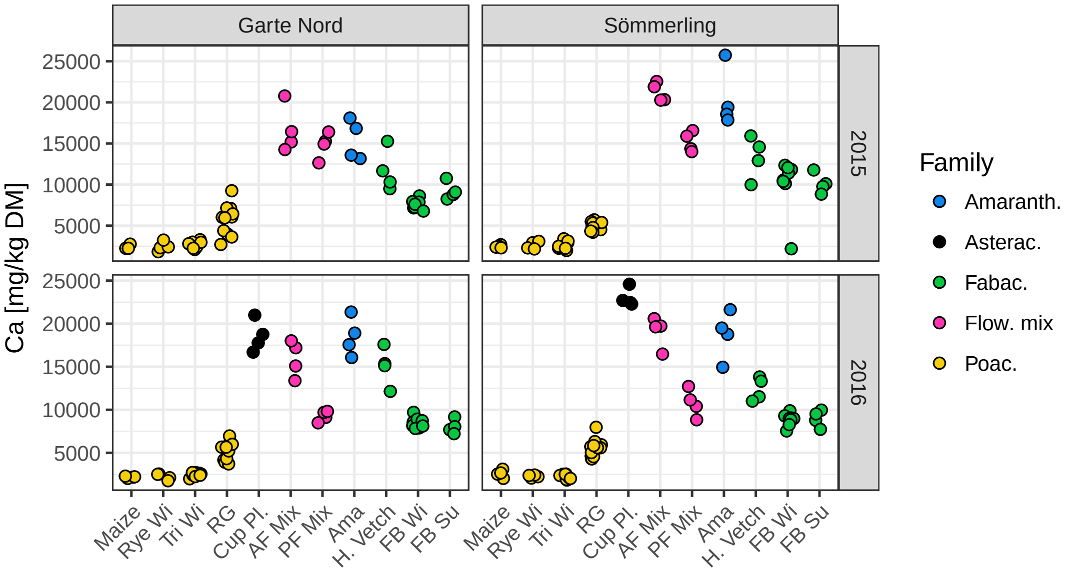
(b)

(c)

Figure 7.3: K (a), P (b) and S (c) in aboveground plant samples grown on the main field trials GN and SÖ, values are in mg/kg.



(a)



(b)

Figure 7.4: Mg (a) and Ca (b) concentrations in aboveground plant samples grown on the main field trials GN and SÖ, values in mg/kg.

7.4.2 Trace Element Concentrations in the Plants

In this Section, four important trace elements for anaerobic digestion (Co, Ni, Mn, Mo) and micronutrients for plants (Fe, Cu, Zn) are presented for the two main field trials during 2015 and 2016. Table 7.3 states significant relationships for most of the combinations of species, location and year.

Table 7.3: ANOVA p-value results on multiple factor linear model: *lm(element ˜Species + Location + Year)*, n.s. = not significant. Significance threshold was set at $p = 0.05$.

	Co	**Ni**	**Mn**	**Mo**	**Fe**	**Zn**	**Cu**
Species	$p < .05$	$p < .05$	$p < .05$	$p < .05$	$p < .05$	$p < .05$	$p < .05$
Location	$p < .05$	$p < .05$	$p < .05$	$p < .05$	$p < .05$	$p < .05$	**n.s.**
Year	$p < .05$	**n.s.**	$p < .05$	$p < .05$	**n.s.**	$p < .05$	$p < .05$

Maize, wheat and triticale contained only a little of Co and Ni (Figures 7.5, 7.6). Members of the Fabaceae plant family (hairy vetch and faba bean) showed the greatest concentrations. The samples grown on site Sömmerling show a greater variability in values than the samples grown on Garte Nord.

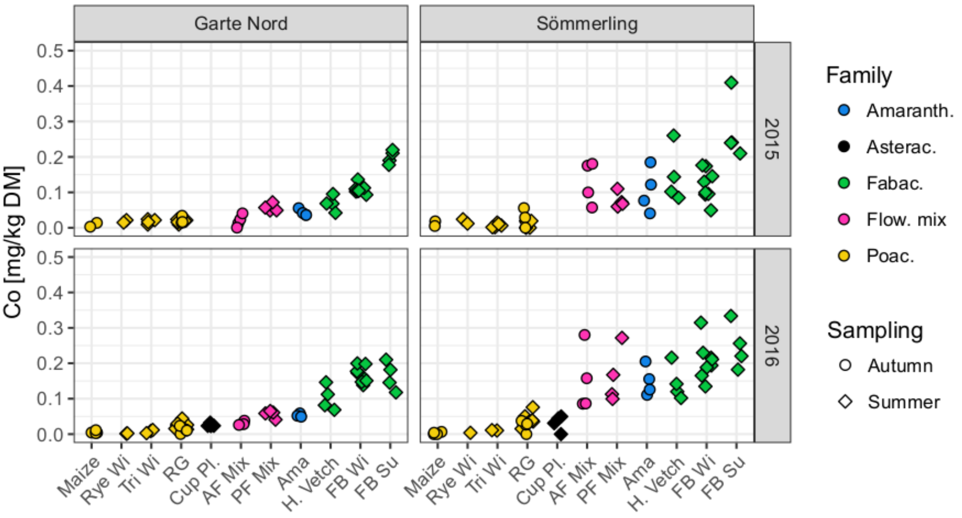


Figure 7.5: Co in mg/kg in aboveground plant samples from GN and SÖ.

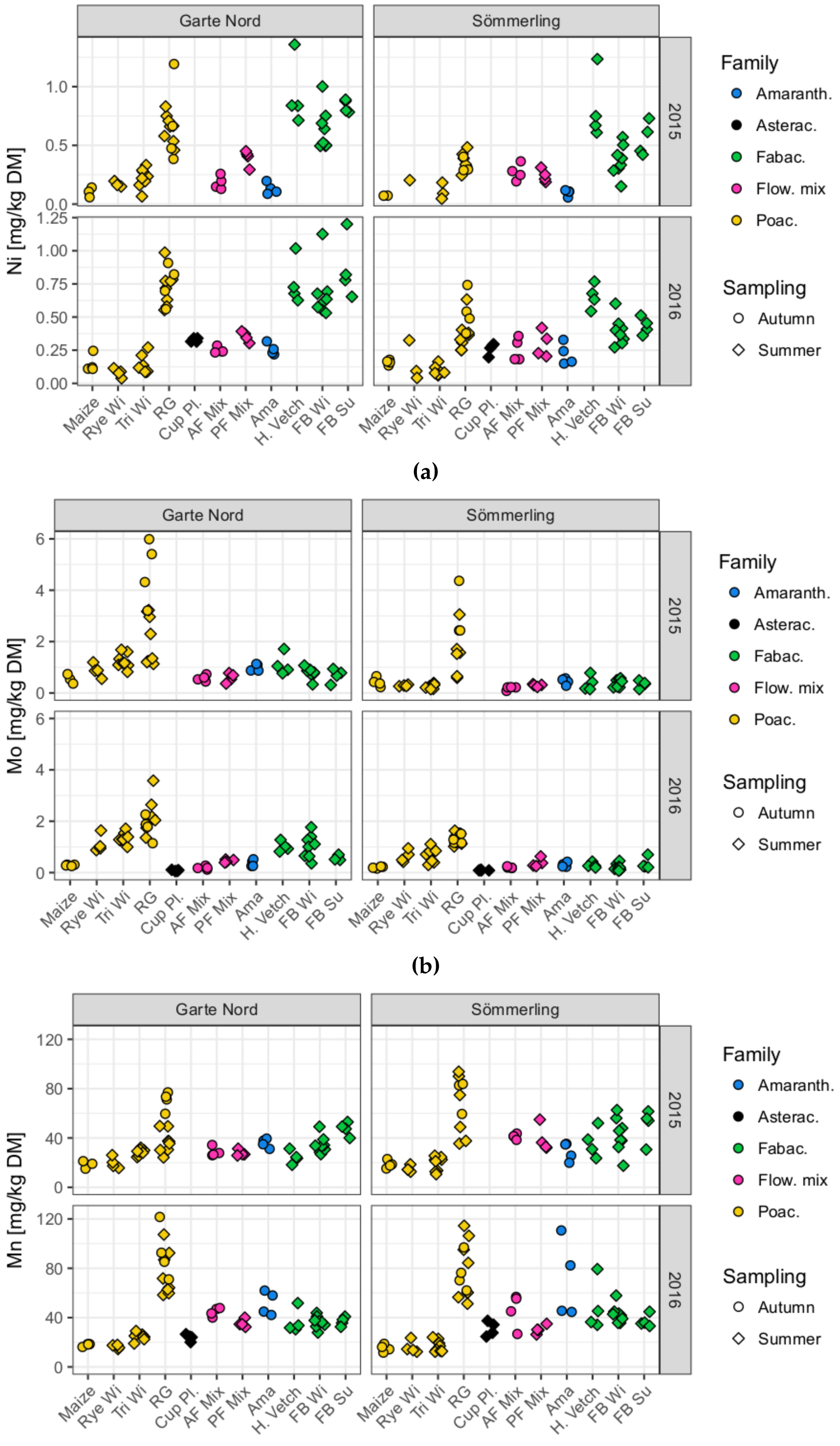
The ryegrass samples, which were sampled in 2 or 3 cuts each year, showed a large variance in concentration for Mn and Mo. This effect was not a result of different sampling dates of ryegrass in the summer and fall of 2015 and 2016, as indicated by the different shapes in Figures 7.5 and 7.6. The ryegrass samples also showed a high absolute and relative standard deviation for Ni, Mn and Mo (Appendix Tables A.14, A.15). Except for ryegrass, all plant species contained relatively equal concentrations of both Mo and Mn. This was also confirmed by earlier experiments on several plant species including sugar beet. It was evaluated, that sugar beet contained very low Mo concentrations; that a large input of sugar beet can possibly lead to a Mo deficiency in biogas plants (Fahlbusch et al., 2013).

There are two separate groups in 2015 for Fe concentrations in winter faba bean. The four samples with greater concentrations are from plot units 20, 44, 56 and 81. In these plot units, faba beans were cultivated as sole crops in 2015. The group with a lower concentration are faba beans in intercroppings with triticale in 2015.

Zn concentrations was at a similar level overall, but with a tendency for greater values in hairy vetch samples. The plants on site Sömmerling showed a greater variance than those grown on site Garte Nord. Also, very high concentrations were measured in winter faba bean in 2015 in Garte Nord between 60 - 106 mg/kg DM. This was true for all plants grown as sole crops and not in mixtures. All the faba beans in mixtures with triticale showed reduced concentrations of Zn between 20 and 30 mg/kg. This effect was only visible in 2015, and in 2016 all winter faba beans ranged between 15 - 25 mg/kg DM.

The concentrations of trace elements in the flowering mixtures (AF Mix and PF Mix) were very stable. This was remarkable as each flower mixture consisted of 15 to 20 different species, which were taken as one sample.

The mean values and standard deviations are displayed in Appendix Table A.14. In Appendix Table A.15 relative standard deviations (sd) are shown. The relative sd are very high for trace elements with low contents. A low relative sd can be observed for Ba, Cu, Sr, Ni, Mn or Zn.

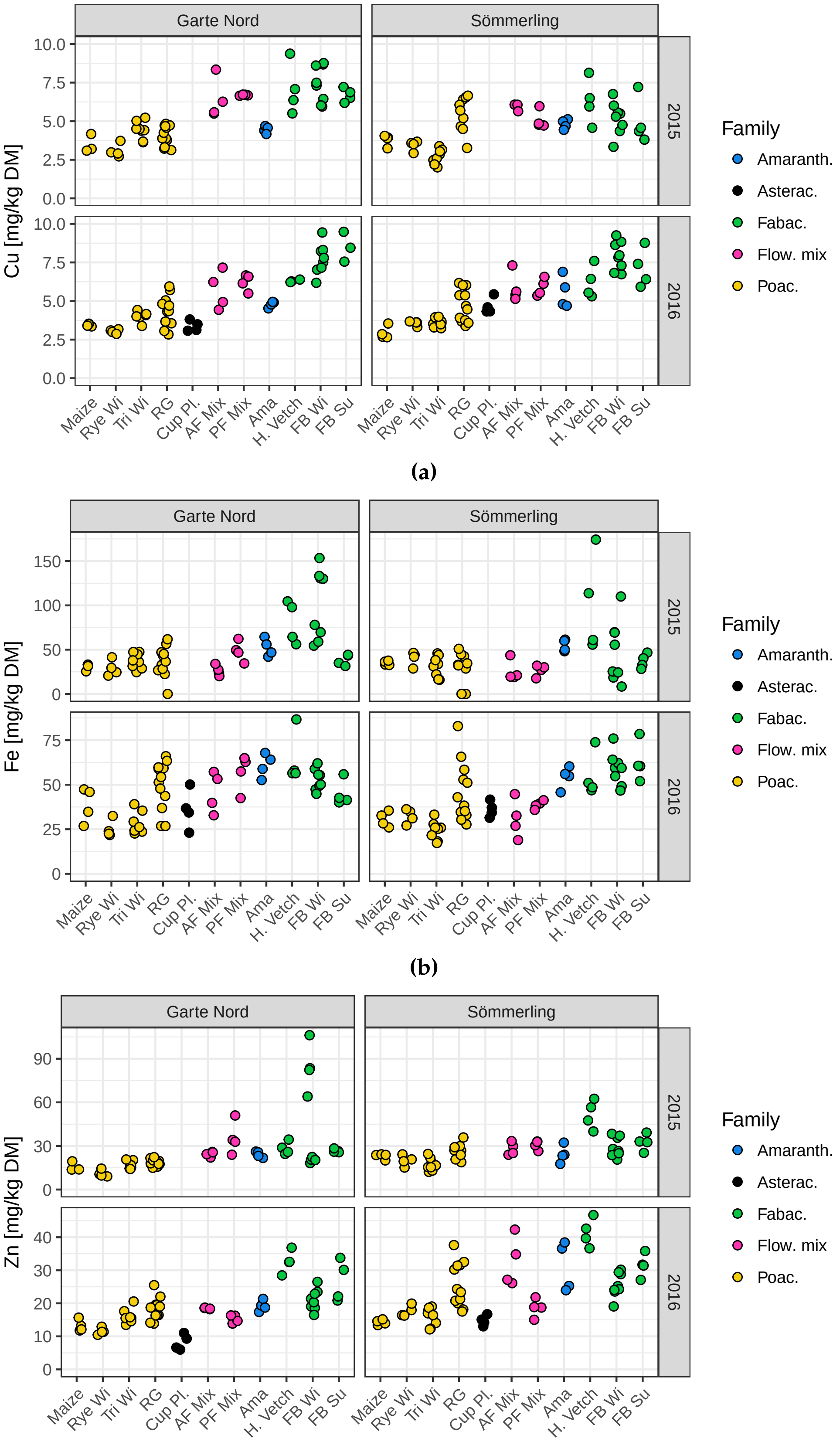


(a)

(b)

(c)

Figure 7.6: Ni (a), Mo (b) and Mn (c) in mg/kg in aboveground plant samples from GN and SÖ.



(a)

(b)

(c)

Figure 7.7: Cu (a), Fe (b) and Zn (c) in mg kg^{-1} in aboveground plant samples from GN and SÖ

7.4.3 Element Concentration in Intercropping

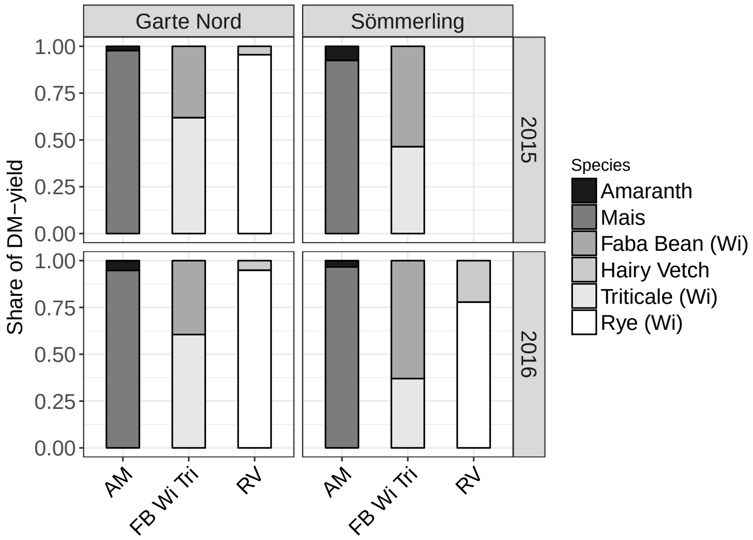
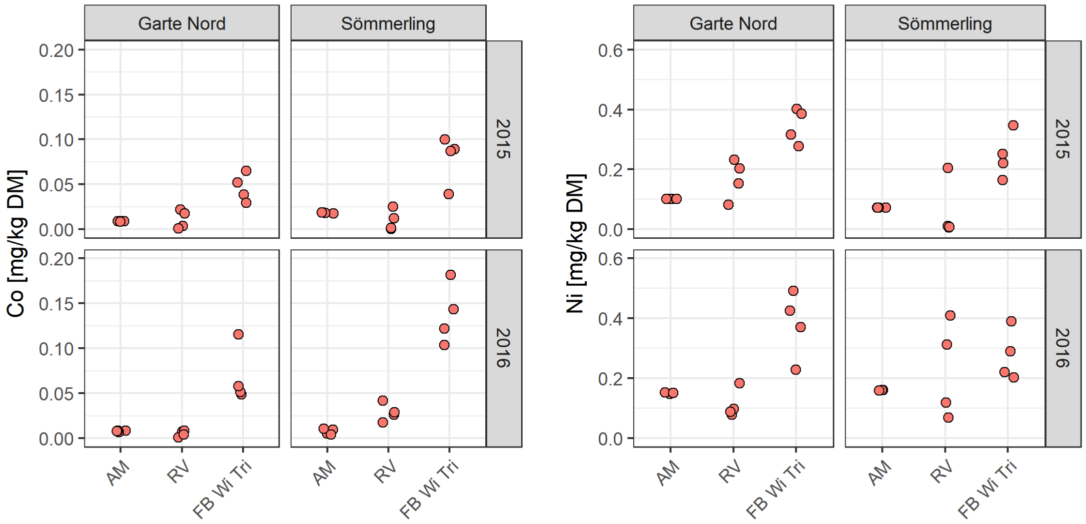


Figure 7.8: Share of the yield in the intercropping amaranth/maize (AM), faba bean/triticale (FB Wi Tri) and rye/vetch (RV). No harvest available of RV in Sömmerling in 2015. Data provided by K. Hey.

The shares of the DM yield were very unequal for RV and AM. The share of vetch in RV and of amaranth in AM was below 5 % (Fig. 7.8). Only FB Wi Tri equally shared DM yield around 50 %. The total DM yield are shown in Chapter 9, Fig. 9.1.

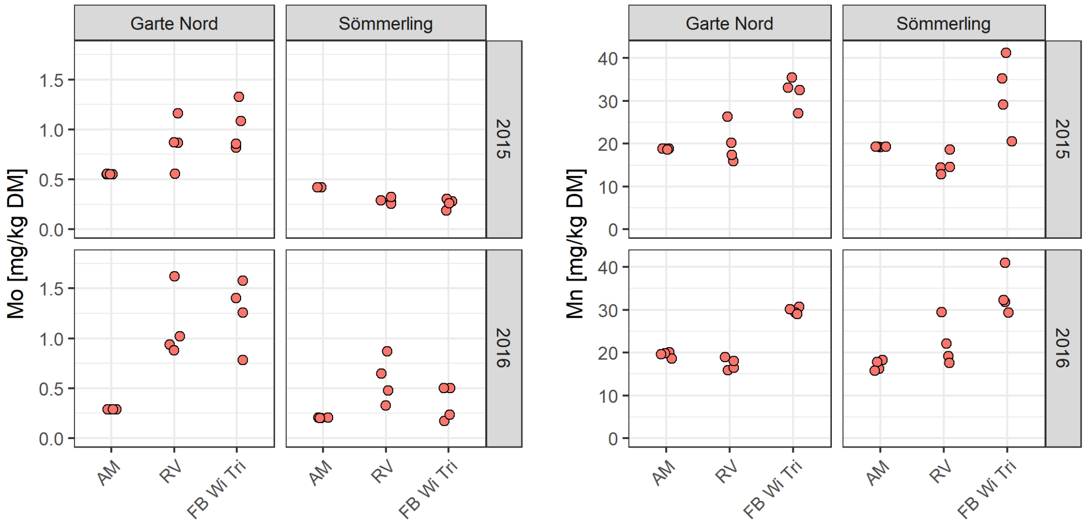
The unequal share of DM yield per component had an effect on the resulting concentration in intercropping. The resulting concentration was caused by the crop with the biggest yield contribution. This was very evident for Co and Ni in the intercropping of AM and RV (Fig. 7.9). Intercropped AM and RV did not have elevated trace element concentrations of Co and Ni due to the very low concentrations in one of the plants (maize or rye). For Mo and Mn the situation was different as all intercropped variants shared the same mean concentration. For Mo though, there were greater concentrations obtained from location Garte Nord (Fig. 7.10a).

For FB Wi Tri it was tested, whether each plant species' trace element concentration changed, if they were grown in intercropping compared to sole/mono cropping. For four trace elements Co, Ni, Mn and Mo there are no indications that the concentrations changed due to the cropping system (Fig. 7.11). The box plots (i.e. the variance of the species concentration) show a similar median concentration per species. It may be the case, that the influence of the companion plant was too low, to led to different trace element concentration. Greater concentrations of Fe and Zn were found in winter faba beans mono cropped in 2015 on site Garte Nord. A reason for the small differences, maybe that the interaction between the plant companions was greater in marginal soils, where plants have to face element deficiencies, but that was not the case here. It is likely that the plants did influence each other, but not to an extent that it became measurable in the trace element concentration. We chose these four elements to test, as these are essential trace elements for biogas production. If there was a greater uptake in one plant component resulting in a greater element concentration, it would have been a very good argument for the use of intercropping in energy plant production.



(a) Co **(b)** Ni

Figure 7.9: Concentrations in mg/kg DM intercroppings: amaranth/maize (AM) and rye/vetch (RV) and winter triticale/faba bean (FB Wi Tri). From 2015 and 2016, plants grown on two main field trials GN and SÖ.



(a) Mo **(b)** Mn

Figure 7.10: Concentrations in mg/kg DM, in intercroppings: amaranth/maize (AM) and rye/vetch (RV) and winter triticale/faba bean (FB Wi Tri). From 2015 and 2016, plants grown on two main field trials GN and SÖ.

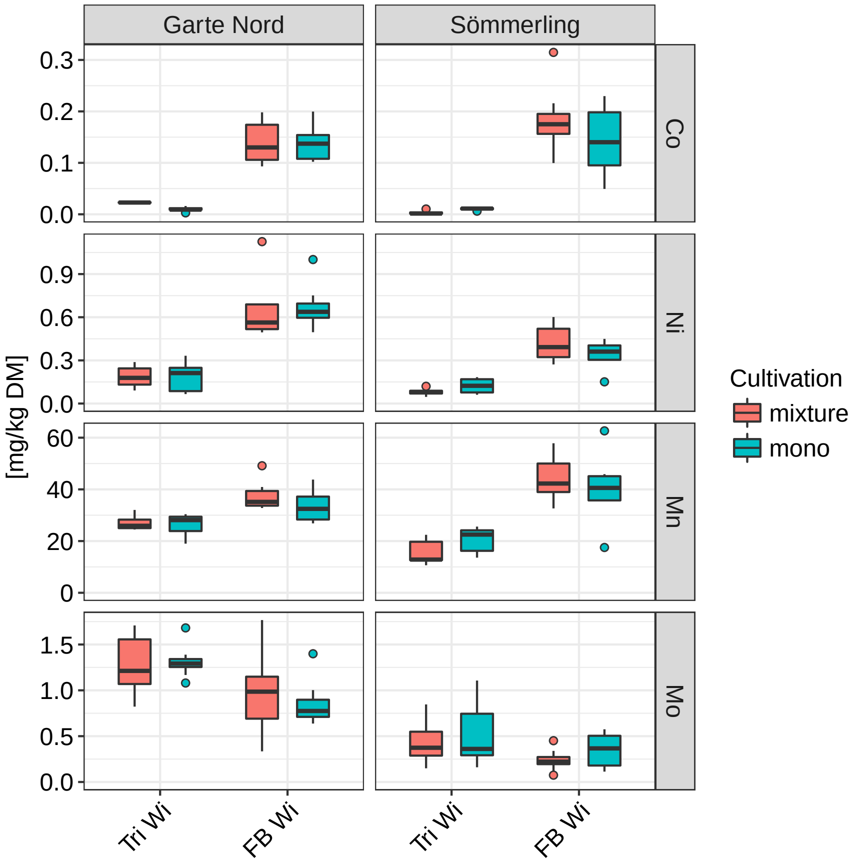


Figure 7.11: Concentrations in mg/kg DM of Co, Ni, Mn and Mo in winter triticale (Tri Wi) and winter faba bean (FB Wi) in 2015 and 2016 on site Garte Nord and Sömmerling. Cultivated in intercropping or sole/mono cropping.

Supplement: Influence of Geographic Origin in Cup Plant Samples in Thuringia

The sample set could be extended with 28 cup plant samples which were collected on 19 August 2016 as whole aboveground plants. These were part of a field trial of the TLL. The TLL tested 6 different geographic sources of cup plant seeds: 1 (USA), 2 (Northern Germany), 3 ("Benko", GDR, German Democratic Republic), 4 (Russia), 5 (Northern Europe) und 6 (Ukraine). The total soil concentrations of the soil in Dornburg is listed in Table 5.5. The soil is a classified luvisol with loess.

No significant differences in trace element concentration of Co, Ni, Mn and Mo could be detected. The concentration ranges of each geographic source were overlapping (Fig: 7.12).

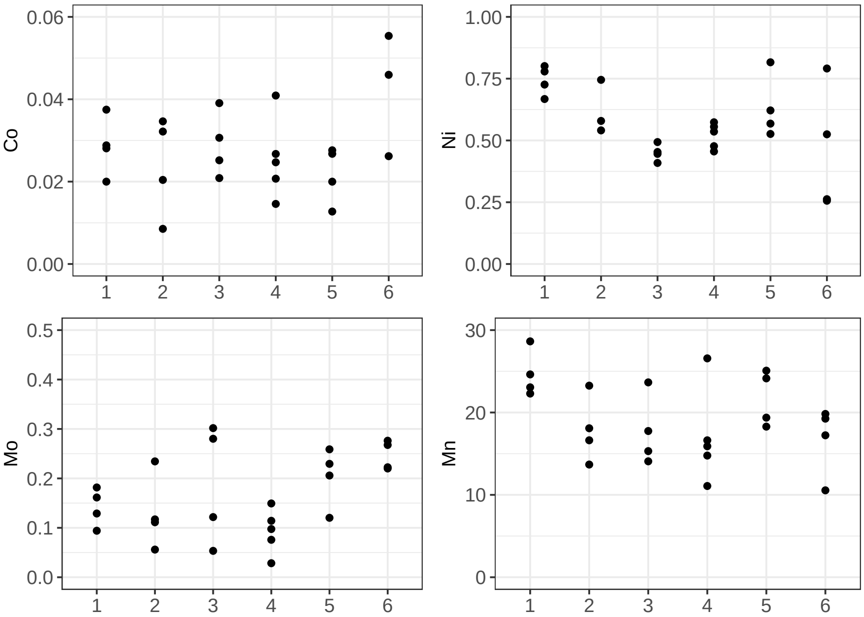


Figure 7.12: Concentrations of trace elements in cup plant samples from Dornburg in 2016 on site Dornburg (TLL, Jena). Cultivated in mono cropping. x-axis shows different origin of seeds: 1 (USA), 2 (Northern Germany), 3 ("Benko" GDR, German Democratic Republic), 4 (Russia), 5 (Northern Europe) und 6 (Ukraine).

Supplement: Intercropped Plants on Sites in Bavaria

The concentrations of plant companions in intercropping vs. in mono cropping were also compared to plants on sites in Bavaria (Aholfing and Straubing). Plants were collected from the project *"Bioenergieträger mit Blühaspekt – Leguminosen-Getreide-Gemenge", short: Legumix* from field trials of the TFZ Straubing, "Technologie- und Förderzentrum" (Eberl and Fritz, 2017). Sample set includes samples collected by A. Sorger for his Bachelor Thesis and by myself in 2015 (additional samples) totaling 122 samples. Some plant species tested were the same in this project (hairy vetch, rye, triticale). Additionally, the legume hungarian vetch (*Vicia pannonica*) and pea (*Pisum sativum*) were cultivated in Bavaria. The concentrations of Co and Ni in hungarian vetch and hairy vetch were alike, but with a range slightly greater than hairy vetch from the main field trials (Table 7.4). For a larger number of elements refer to Appendix Table A.18. Within the standard deviation range, there were also no differences between hairy and hungarian vetch detected. The Poaceae plants (rye and triticale) also showed the lowest concentrations of Co and Ni. In Bavaria, additional legume plants (pea) were collected. These showed similar concentrations for Ni like vetch, but smaller concentration of Co (0.03 mg/kg DM).

Table 7.4: Co, Cu, Mn, Mo and Ni element concentrations corrected for adhering particles from samples obtained in Bavaria, in mg/kg DM, mean ± standard deviation, n= number of samples.

Species	Site	n	Co	Cu	Mn	Mo	Ni
Hairy Vetch	Aholf	13	0.057 ± 0.021	5.28 ± 0.66	36.0 ± 6.1	0.99 ± 0.31	1.01 ± 0.42
Hairy Vetch	Straub	14	0.060 ± 0.018	7.78 ± 1.22	29.8 ± 4.8	0.48 ± 0.23	1.43 ± 0.35
Hung. vetch	Aholf	6	0.058 ± 0.016	4.49 ± 1.26	43.8 ± 14.2	0.53 ± 0.20	0.97 ± 0.13
Hung. vetch	Straub	5	0.034 ± 0.021	6.80 ± 0.52	27.5 ± 3.1	0.21 ± 0.09	0.93 ± 0.37
Pea	Aholf	5	0.028 ± 0.006	4.41 ± 0.28	51.3 ± 9.2	0.75 ± 0.35	1.01 ± 0.14
Pea	Straub	6	0.028 ± 0.013	6.60 ± 0.22	36.0 ± 7.0	0.17 ± 0.10	1.34 ± 0.46
Rye (Wi)	Aholf	17	0.025 ± 0.012	3.65 ± 0.69	29.5 ± 8.9	0.99 ± 0.23	0.21 ± 0.19
Rye (Wi)	Straub	16	0.012 ± 0.008	4.71 ± 0.61	25.5 ± 2.8	0.52 ± 0.11	0.19 ± 0.15
Triticale (Wi)	Aholf	17	0.016 ± 0.010	3.51 ± 0.46	46.7 ± 5.4	1.00 ± 0.18	0.23 ± 0.25
Triticale (Wi)	Straub	16	0.013 ± 0.009	4.48 ± 0.64	52.6 ± 8.4	0.59 ± 0.16	0.24 ± 0.21

Sorger (2015) also calculated the concentrations in the intercropping of rye/(hairy) vetch (RV) for 10, 30 and 50 % proportion of vetch resulting in 0.025, 0.038 and 0.05 mg Co/kg DM, respectively. These figures are comparable our resulting Co concentrations in RV on our main field trials (Fig. 7.9a).

The influence of the cropping system was examined on winter rye and winter triticale plants. Like in the main field trials no significant differences in Co, Ni, Mn or Mo could be detected, suggesting that the plants in mono- or intercropping showed similar concentrations.

7.4.4 Scatterplot Matrix for Element Relationships

The element interactions can be shown via a element scatterplot of a selection of trace elements. In Chapter 5 there were several stable element ratios detected in the soils of Bühren. Co and Ni showed a stable ratio over the soil samples in Bühren. The diagrams in this Chapter showed also plant species indicative element concentrations levels. In Fig. 7.13 patterns formed by the plant families were detected. The color code was set to plant families because of two many variables for plant species. Data is transformed to log scale, because differences are then easier to detect, and an effect of the different scale of the element values and the skewness of the data can be avoided. There were no stable ratios between the elements. There was a tendency towards a linear relationship between Co and Ni and between Co-Cu, or Zn-Cu, but no narrow trends. Obviously, the variance of the trace element concentrations was too great. Interestingly, cup plant samples (Asteraceae, olivegreen circles) showed different ratios, than all other samples (Zn, Mn, or Mo). This was not apparent in the mono concentration diagram in Section 7.4.2.

7.5 Conclusions

The data set from the two main field trials, presented in this Chapter was unique. Field crop research was often limited to one plant species or the combination of two

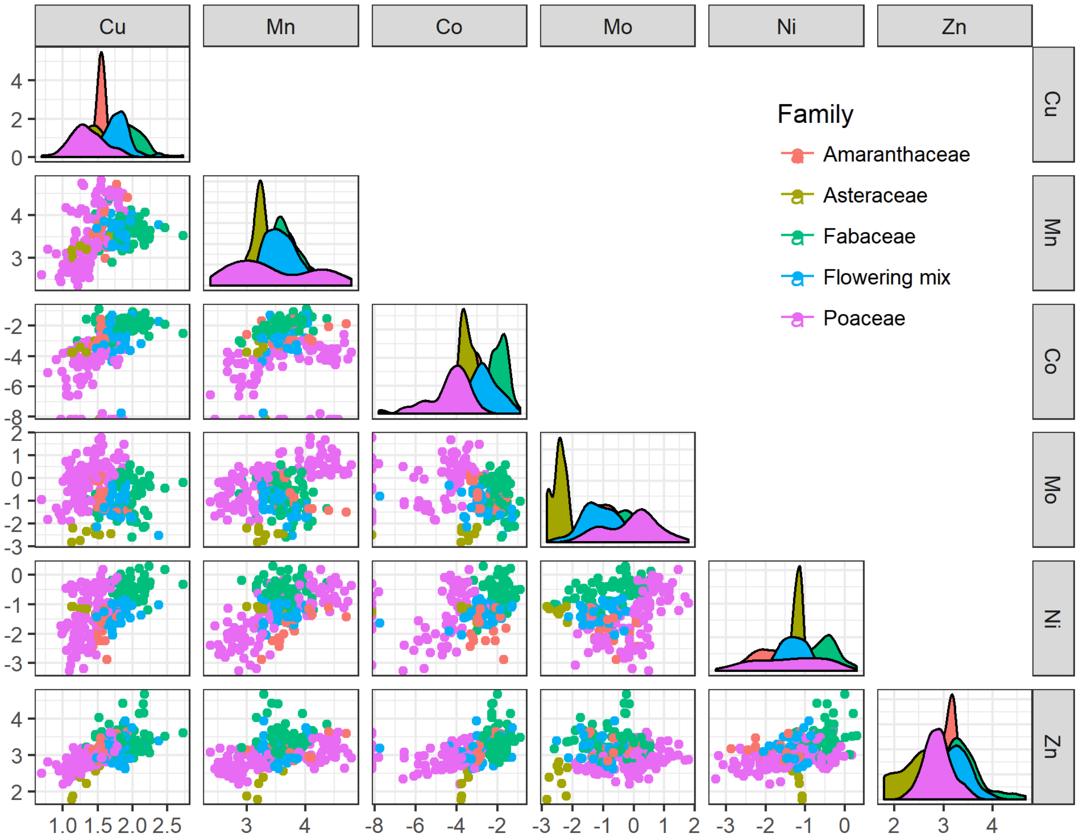


Figure 7.13: Scatterplot matrix of plant sample concentrations of Co, Mn, Cu, Mo, Ni, Zn of all plant samples from GN and SOE (Data in log scale). Diagonal row shows density distributions of the plant families. Data was converted to log scale, because of the skewness.

or three plant species in intercropping. In this study, twelve different plant variants were analyzed, either as sole crops or in intercropping. Furthermore, the plants were grown on two contrasting soils, one very fertile soil, the other of intermediate quality. Additionally, the plants were planted in two successive years.

For most of the elements shown here, the plant samples belonging to the same families (Poaceae, Fabaceae) showed similar concentrations. This may be explained by similar plant physiologies and the same uptake mechanisms.

The concentration in ryegrass was variable for the trace elements Ni, Mo, Mn and Fe. The results showed, that greater Co and Ni concentrations could be gained by Fabaceae plants (faba bean, vetch), whilst greater concentrations of Mn and Mo could be gained by ryegrass. Of course, the quantity of elements harvested also depends on the DM yield of each species. The next step was, to calculate absolute amounts of elements removed/extracted from the field or the absolute amount of element in the harvest. In Chapter 9, the two factors of element concentration and DM yield are combined to achieve this goal. With the element amounts quantities, recommendations for enhanced element delivery to biogas fermenters will be made.

The resulting concentration of intercropped AM, RV and FB Wi Tri plants in the study was calculated. The most important factor in the resulting concentration in the intercropping was the element concentration of the plant component with the highest yield. For AM and RV the concentrations of Co, Ni, Mo and Mn were very similar to those found in maize or in triticale, respectively. Maize and triticale contributed approximately 90 - 98% of the total DM yield. Only in FB Wi Tri the two plant components reached equal yield shares, ultimately leading to elevated element concentrations, due to greater concentration in faba bean.

There were no influences in intercropped plants compared to mono cropped plants detected with respect to changed trace element concentration. No difference in trace element concentration in the intercropped species (winter faba bean or triticale) could be detected compared to the plants in sole cropping. These results were also confirmed for rye and triticale plants in two Bavarian field trials.

Chapter 8

Prediction of Concentrations in Plants - Bioavailability

8.1 Introduction

The following Chapter deals with the bioavailability of elements to plants with a special focus on the trace elements required for biogas production like Co, Ni, Mn and Mo and other trace nutrients (Cu, Zn, Fe). The bioavailability was investigated by analyzing the element concentration in plant tissue and the total and extractable element concentrations in soil. The aim was to assess if a soil extraction procedure could help to predict the element concentration in plant tissues. Furthermore, it should be easy to use.

8.1.1 Soil Extraction Methods and their Boundary Conditions

There are numerous soil extraction techniques proposed in the literature which aim to characterize the "plant available" or "phytoavailable", pool of metals in soils. In the case of toxic heavy metals their risk to enter the food chain is of major concern.

There is a general distinction between single extraction methods (SinEx) and sequential extraction procedures (SEP). SinEx uses only one (or a mixture) of reagents and are therefore easy to apply. SEP use several reagents in several steps to assess different associations and pools of elements in the soil (Zeien and Brümmer, 1989; Tessier et al., 1979; Rauret et al., 1999). The soil sample is treated with the different reagents one after another. In each step the supernatant is recovered for analysis and the remaining solid sample is treated with the next reagent. For example, Tessier et al. (1979) defines 5 fractions such as easily exchangeable, bound to carbonates, bound to Fe and Mn oxide-hydroxides, bound to organic matter and bound to the residue (mostly silicates). This is commonly known as the "Tessier" protocol. Most SEPs follow more or less this concept. The Bureau of Reference (BCR) procedure (now known as "The Standards, Measurements and Testing Programme" of the European Commission) is similar to that of Tessier, but exchangeable and carbonate bound fraction steps are combined (Rauret et al., 1999). The BCR procedure was modified by a group of European experts to tackle the problems of poor reproducibility of their results (Rauret et al., 1999). In general, SEPs aimed for the most mobile element fractions in step one and in the subsequent steps, for fractions with lower availabilities and mobilities.

Zimmerman and Weindorf (2010) reviewed three different SEPs in detail (Short, Galán and one from the Geological Society of Canada). For Germany, the SEP method by

Zeien and Brümmer (1989) comprises 7 fractions to assess the association of heavy metals in soils. The difference to Tessier's fractions is that the first fraction by Tessier is divided into two (mobile fraction with 1 M NH_4NO_3 and a easily soluble fraction with 1 M NHAOc). This SEP was developed by soil scientists. The most commonly used SEP today is the Tessier protocol.

All SEPs face several disadvantages and pitfalls. The reaction time and temperature of each reagent step must be kept identical for each sample. Longer reaction times can cause higher extraction rates. The separation of the sample and the supernatant, mostly performed by centrifugation, is also not easy to perform. External factors, like temperature or the ratio of solute to sample will also affect results. Sample preparation can alter extraction rates, for example, if soil samples are dried or ground before analysis. The extraction reagents are not able to extract all elements in a specific fraction and may also attack elements associated with other fractions. In natural environments, these fractions are not strictly separated from each other. Elements from one fraction can be readsorbed by other fractions, thus leading to less metals extracted in previous fractions and higher extraction rates in later fractions. Shan and Chen (1993) observed this phenomenon during extractions on a model soil.

For these reasons and owing to the high effort, SEPs were not applied in this study. The long duration and the various reagents these methods utilize also not applied in common agricultural analyses.

In terms of macronutrients, extraction methods are used predominantly to adjust the amount of fertilizer (Mg, K, Ca, P) to arable land. For example, available Mg in soils is determined via a method by Schachtschabel (1954) with $CaCl_2$ in Germany. Available P is determined by calcium-acetate lactate (CAL; Schüller, 1969) or by the double-lactate method (DL; VDLUFA, 2012). The determination of the availability of trace nutrients with soil extraction methods is more complicated. There are some extraction methods that specifically target heavy metals. Other procedures use complexing agents such as EDTA or DTPA. For example, the CAT method (VDLUFA, 2004) uses $CaCl_2$ and DTPA to determine the soil nutrient status of Fe, B, Cu, Mn, Mo and Zn. Also the main nutrients N, P, K, and Mg can be determined by the same protocol.

However, for the trace elements Co and Ni there is no recommended extraction scheme. Another problem that arises is that their concentrations are generally about 1 to 3 orders of magnitude lower than that of the major nutrient elements, meaning that they are more difficult to determine.

The SinEx methods face similar problems to the SEPs. They are also not capable to simulate the plant's mechanisms for nutrient uptake. However, the advantage lies in its simplicity and ease of operation. Feng et al. (2005) state that all extraction methods fail to account for rhizosphere mechanisms and therefore propose a labile rhizosphere soil solution fraction. To assess this fraction the rhizoshpere soil is extracted with a mixture of organic acids (acetic, lactic, citric, malic and formic acids). The authors' achieved high correlation between Cr, Cu, Zn and Cd of this rhizosphere fraction eluate and the roots. For the shoots, only good correlations for Cd and Cr were found. Since we are aiming to predict the concentrations in the aboveground plant tissue, the organic extraction method is not useful here. Presumably this approach was not able to sufficiently characterize the highly dynamic rhizosphere zone.

The most crucial fact is that plants from different species also have different physiological mechanisms for nutrient acquisition and have different nutrient needs. For example, Poaceae have developed a special strategy for Fe acquisition. They release

Fe-mobilizing compounds (phytosiderophores) and have developed a special uptake system for ferrated phytosiderophores in apical root zones (Römheld, 1991). The phytosiderophores also mobilize other micronutrients like Cu, Mn and Zn but these are not preferentially being taken up (Römheld, 1991). Another mechanism which can alter the trace nutrient uptake is the excretion of H^+ -ions by Fabaceae plants. These plants are capable of biological nitrogen fixation (BNF) thus leading to an imbalance in the uptake of cations and anions. That is due to the absence of ${NO_3}^-$ which is usually the predominant anion absorbed. To maintain electroneutrality at the root soil surface the plant is actively excreting H^+ -ions which can lead to an decrease in pH in the rhizosphere (Haynes, 1990; Israel and Jackson, 1982). This drop in pH can lead to an enhanced mobility of trace metals (Cu, Co, Ni, Mn, Zn).

8.1.2 Prediction Methods for Soil to Plant Transfer

One idea of this work was to explore if there is one extraction method which could be used to predict phytoavailability, especially those of the trace elements necessary for biogas production: Co, Ni, Mn, and Mo. It turned out that one of the most important factors for uptake cannot be modelled: the impact of the plant species. In Chapter 6 and 7 the results are described in detail. Even at the two main field trials Garte Nord and Sömmerling, which represent soils in the moderate climate region of Western Europe, there were large differences in the plant tissue concentrations.

As described above, the different soil extraction techniques are not perfect and face a lots of boundary conditions. Several extraction methods were also investigated within the Bachelor Thesis of M. Willerding-Möllmann (Willerding-Möllmann, 2015). Methods tested included: CAT, $CaCl_2$, EDTA, NH_4NO_3 and a complete digestion of soils. Six soils were tested obtained from Garte Nord, Sömmerling, Bühren, Düshorn, Wiese and Baerwinkel. Düshorn was a sand dominated soil; Wiese and Baerwinkel were soils contaminated with heavy metals and were situated in floodplains of the river Innerste near Hildesheim.

One simple soil extraction method was however tested on different soils, with different total element concentrations. This was an unbuffered salt-solution single extraction method with ammonium-nitrate (NH_4NO_3), described by DIN Deutsches Institut für Normung e. V. (1997) and corresponding to the method for the exchangeable element fraction in the Tessier protocol. This is perhaps best suited to the variety of soils and plant species investigated as it does not change the soil pH during extraction. A drop in pH during the extraction process will automatically lead to greater extraction rates of trace elements.

The metals are extracted mainly from soil surfaces by replacing them with the ammonium ion. The formation of colloids and metal-organic complexes is suppressed due to the high ionic strength of NH_4NO_3. Colloids and metal-organic complexes are hardly taken up by plants (Gryschko et al., 2005).

Several authors agree, that the salt extraction methods are probably best suited for a variety of (trace) elements (Schöning and Brümmer, 2008; Menzies et al., 2007).

8.2 Methods

8.2.1 Soils and Setting

Several soils were included. Table 8.1 lists the soils with pH and type, where available. Lindau, Trögen were pot experiments only; Bühren were pot and open field experiments. The total element concentrations are listed in Table 5.5. The lowest pH was measured in the soil from Lindau, which was probably caused by poor field management. Straubing and Aholfing were sample locations from the TFZ (Technologie- und Förderzentrum Straubing). These samples were obtained from a field experiment on legumes and cereal crop mixtures ("Legumix"; Eberl and Fritz, 2017). The site at Bühren contained soil from basalt weathering with high total element concentrations of Co, Ni, Mn, Mg and Fe. Deppoldshausen samples (faba bean) were obtained with kind permission of Dr. W. Link from field trials of the *Impac*^{3} research project.

Table 8.1: Soils used in this study, with pH measured with 0.01 mol/l $CaCl_2$ and soil type measured by laser diffraction analysis. First column indicates whether an extraction was performed.

Extr.	Location	Loc. abbrev.	Field exp.	pH	clay	silt	sand
x	Lindau	Lindau		4.61	5.74	64.80	29.50
x	Trögen	Trögen		5.43	5.88	73.70	20.40
x	Buehren	Bühr		5.73	5.28	58.00	36.70
	Aholfing	Aholf	x	6.05	5.47	40.30	54.30
x	Sömmerling	SÖ	x	6.09	6.91	61.80	31.30
	Straubing	Straub	x	6.46	8.99	80.40	10.60
x	Garte Nord	GN	x	6.56	6.24	76.30	17.40
x	Groß Ellerhs.	Ellieh	x	7.18			
x	Deppoldshausen	Dep	x	7.30			

8.2.2 Plant Sample Set

In these experiments five plant species were tested: amaranth, winter faba bean, hairy vetch, ryegrass and triticale (Table 8.2). All plant samples from all locations investigated were included in this study if there were also soil data available. The plant-soil pairs however, may be different for each location. It was not possible to perform analyses on all sample locations or in pot experiments. For some of the field grown plants, several individual plants were sampled. This was not always possible for plants grown in pots.

Table 8.2: Sample set with the number of plant species (n) in this study

Plant species	n
Amaranth	26
Faba Bean (Wi)	51
Hairy Vetch	46
Ryegrass	51
Triticale (Wi)	101

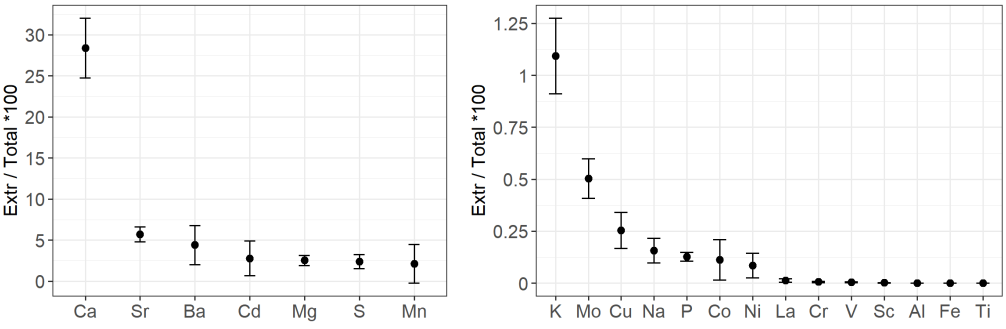
8.2.3 Soil Extraction with Ammonium Nitrate

The procedure to obtain soil extraction concentrations is described in Section 2.1.4.

8.3 Results

8.3.1 Recovery Rates of the Soil Extractions

In the extraction samples with ammonium nitrate 21 elements could be measured (out of 46 elements from total soil element concentrations). It was not possible to measure: As, Ce, Cs, Dy, Er, Eu, Hf, Ho, Li, Lu, Nb, Nd, Pb, Rb, Sb, Sm, Ta, Tb, Th, Tl, Tm, U, Y, Yb and Zn. These elements mostly belong to the group of REEs, or did not represent trace nutrient elements for plants, with the exception of Zn. Zn could be measured in some of the soil extraction samples, the majority of the samples showed values below the LOD. La was the only REE element which could be recovered. Figure 8.1 shows median recovery rates of all soil extractions and total concentration values.



(a) **(b)**

Figure 8.1: Median recovery rates: elements with higher (a) and with intermediate and lower rates (b). Rates are concentration of extractions/total soil concentration in %. Error bars are the Interquartile Range (IQR) of the medians of all sample locations.

Most of the main nutrient elements showed high recovery rates of concentration in soil extractions vs. total concentration in soils. About 25 % of Ca could be recovered from the soil extractions; for Sr and Ba about 5 %. The extraction method was also very effective for Cd (3 %) (Fig. 8.1a). For Cd, Ba and Mn the error bar (= IQR) show a high variability in the extraction rates. This was probably caused by the different soil properties in the various locations. Co and Ni also showed large error bars, but the recovery rates were similar to P (about 0.13%). That indicates that ammonium nitrate may be useful in describing the availability of these elements to the plants. The elements La, Cr, V, Sc, Al, Fe and Ti showed the smallest recovery rates. They generally had very small extraction concentrations leading to the smallest recovery rates (extr/total conc. soil). Most remarkably was that Fe was part of this group, although it represents a minor nutrient element for plants.

Figure 8.2a shows that the soil locations Lindau and Trögen have the highest recovery rates in soil extractions with ammonium nitrate. These two locations also had the lowest soil pH values of 4.6 and 5.4, promoting the high recovery rates in some of the elements (Co, Ni and Mn). Mo in the soil extractions could not be measured for some locations (Trögen, Bühren, Deppoldshausen and Lindau) and is missing therefore.

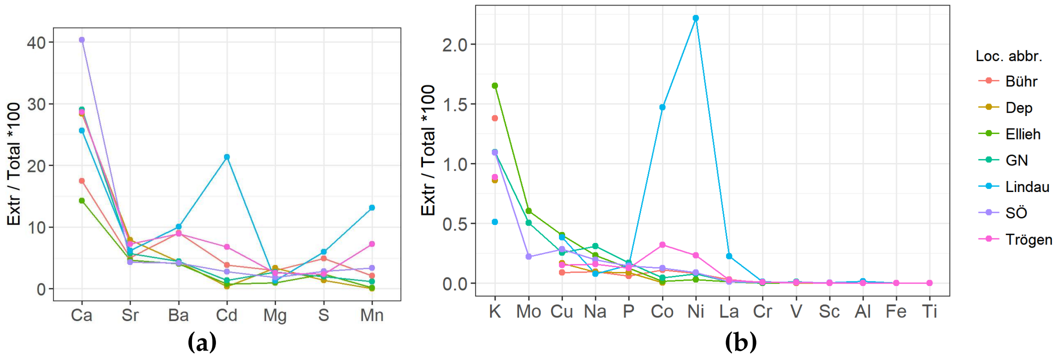


Figure 8.2: Median recovery rates per soil location: elements with higher (a) and with intermediate and lower rates (b). Rates are concentration of extraction/total soil concentration in %. Soil sample locations are abbreviated (see Table 8.1).

8.3.2 Concentration in Plants vs. Total Element Concentration in Soils

Figures 8.3 to 8.6 show the concentrations of the whole aboveground plant on the y-axis vs. the corresponding soil concentration on the x-axis. The y-axis show median concentration in the soils. The Figures are divided into panels according to plant species. These are: amaranth, winter faba bean, hairy vetch, ryegrass and winter triticale. Every point represents one plant sample. There are varying sample numbers per location. The circles indicate pot or field grown plant samples. The pots used were large pots containing approximately 20 kg of soil and were placed in the open air. The only exception were the pots with amaranth (light blue triangles on soil from Bühren) which were grown in small pots with 2.8 kg of soil.

The Figures can be used easily to check if a correlation between plant concentration and soil (total or extracted) is detectable or not. If a trend for a correlation is visible, and if there are enough data points, a regression line including the equation and the coefficient of determination (R^2) is added.

The Poaceae plants (ryegrass and triticale) showed no special enrichment of Co in the plant tissue with increasing soil concentration. The greatest plant concentration was found in the sample from Lindau with the lowest soil pH of 4.6 (green triangles; Fig. 8.3). Faba bean, amaranth and ryegrass plants did exhibit a greater plant concentration of Ni with higher soil concentrations (Fig. 8.4), but this weak trend was mostly caused by plants from Bühren alone. Intermediate Ni soil concentrations were lacking (between 40 and 60 mg/kg).

Mn showed no correlation between plant and total soil concentration (Fig. 8.5). The plant levels were very similar and ranged below 100 mg/kg. Only the pot experiments from Bühren (light blue triangles), Trögen (orange triangles) and Lindau (green triangles) showed elevated Mn concentrations in plant tissue. Trögen and Lindau had the lowest soil pH-values.

There was no correlation pattern for Mo in plants vs. Mo in the soil (Fig. 8.6). The highly variable Mo concentrations in plants from Garte Nord (yellow) and Sömmerling (blue) were striking, but was most evident for ryegrass samples (see also Chapter 7). These highly variable concentrations in the plants of the two main field trials (Garte Nord and Sömmerling) prevented a correlation between plant and soil concentrations.

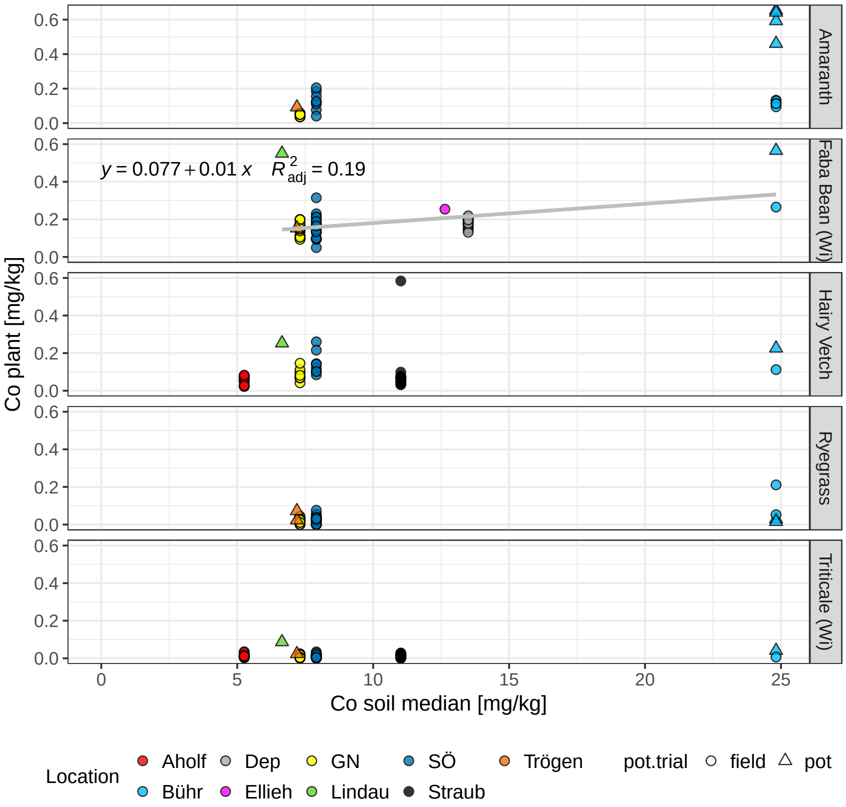


Figure 8.3: Co in plants vs. in soil, sample locations are abbreviated (Table 8.1).

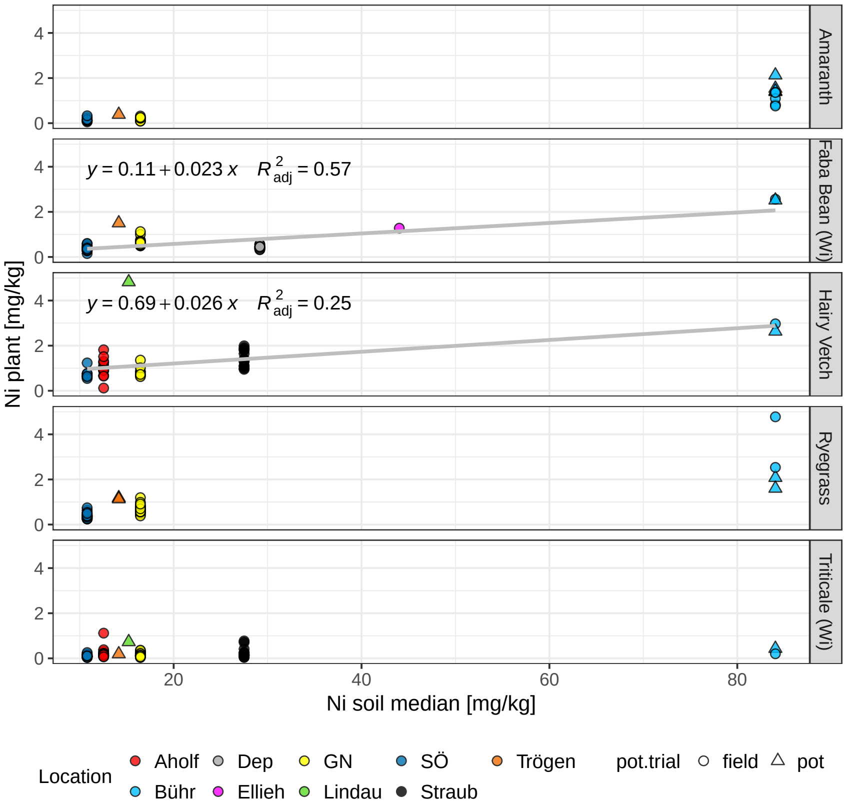


Figure 8.4: Ni in plants vs. in soil, sample locations are abbreviated (Table 8.1).

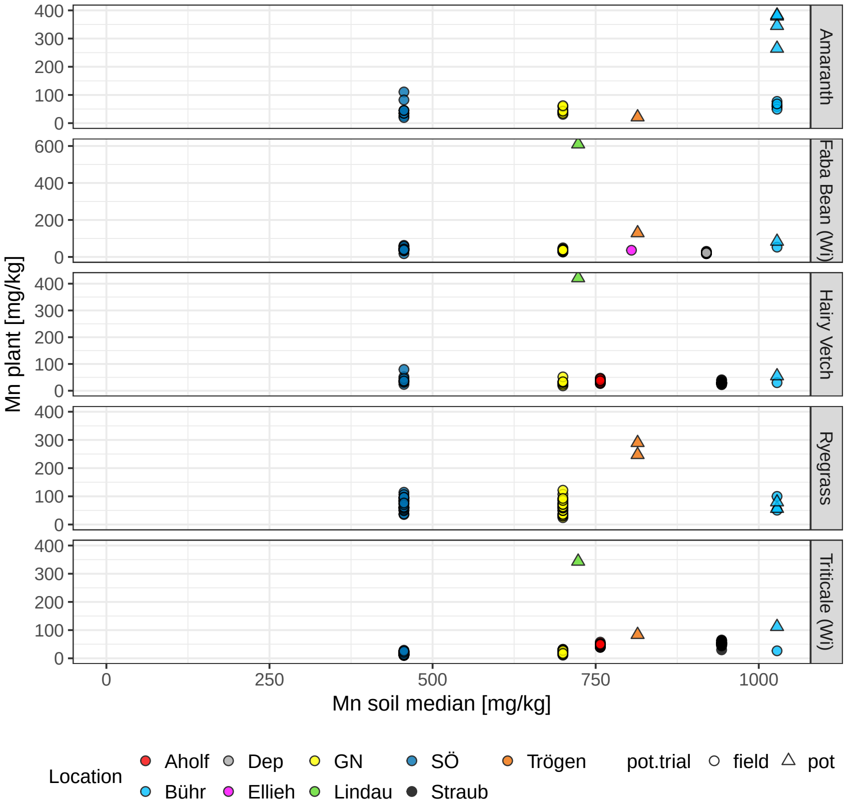


Figure 8.5: Mn in plants vs. concentration in soil, sample locations are abbreviated (Table 8.1).

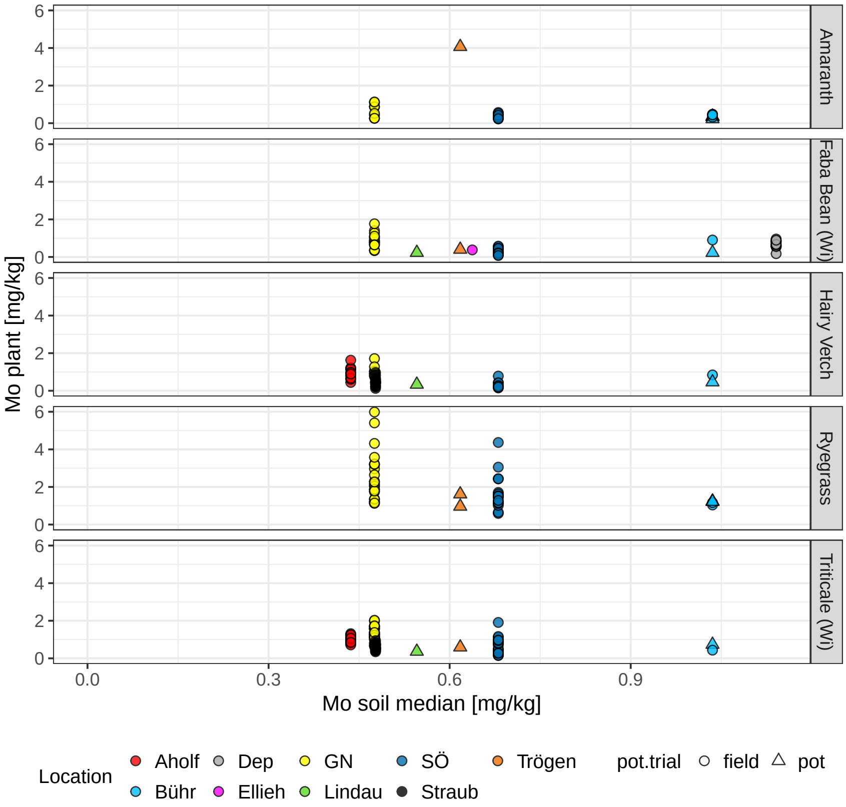


Figure 8.6: Mo in plants vs. concentration in soil, sample locations are abbreviated (Table 8.1).

8.3.3 Concentration in Plants vs. Soil Extraction with Ammonium Nitrate

In the following two Figures of Co and Ni the samples from Lindau (triticale and faba bean, green triangles) were particularly interesting. The low soil pH likely caused the greatest soil extraction rates and plant concentrations (Figures 8.7 and 8.8). Second greatest plant concentrations were measured for plants grown on soil obtained from Bühren. A correlation was hard to detect for Co. The plants on soil from Sömmerling had a high variability in plant concentrations (dark blue). Bakkaus et al. (2008) also stated a high correlation for greater plant concentration of Co with decreasing pH, but 6 out of 8 soils in this study had been subjected to atmospheric deposition of anthropogenic Co and had high total Co soil concentration of > 30 mg/kg. Furthermore, their plants (*Triticum aestivum* L.) only grew for 46 days.

There is a trend for linear correlation for Ni with faba bean, ryegrass and amaranth. The concentration in triticale cannot be modelled with ammonium nitrate as the plant concentrations are small, even for the sample from Lindau (light green).

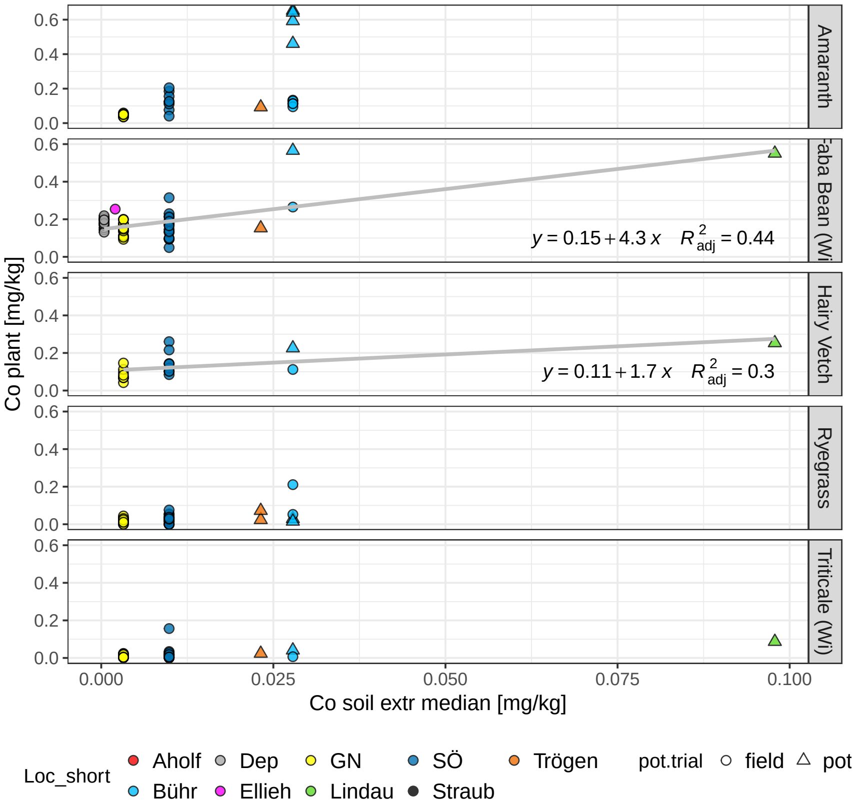


Figure 8.7: Co concentration in plants vs. extracted with NH_4NO_3 in soil, sample locations are abbreviated (Table 8.1).

For Mn also the sites with the lowest pH (Lindau and Trögen) showed the greatest plant concentrations. For ryegrass, faba bean and triticale one could argue a linear correlation exists. This was possibly due to enhanced mobility of Mn in soils with pH lower than 7. Soils with intermediate Mn extraction concentrations were lacking (between 25 and 80 mg/kg).

There was the issue of low extraction rates of Mn from the soils. Most values were below the LOD. Therefore, no figure is presented. Only for soils from Sömmerling, Garte Nord and Groß Ellershausen soil extraction concentrations could be measured.

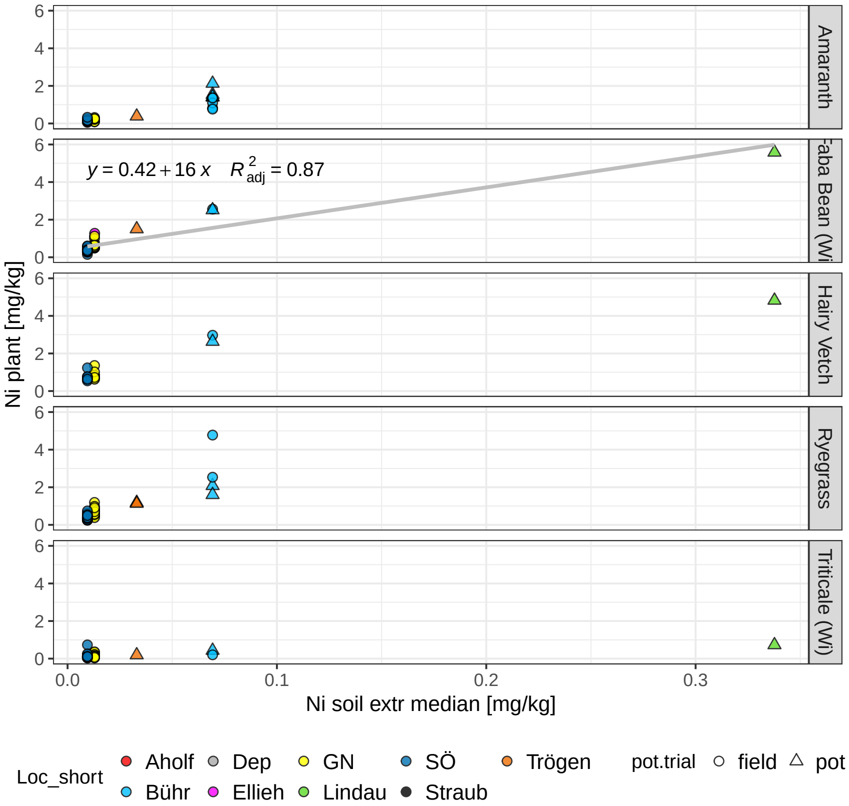


Figure 8.8: Ni concentration in plants vs. extracted with NH_4NO_3 in soil, sample locations are abbreviated (Table 8.1).

Greater Mo concentrations in the locations with lower pH was not be expected due to the lower mobility of Mo in acidic conditions.

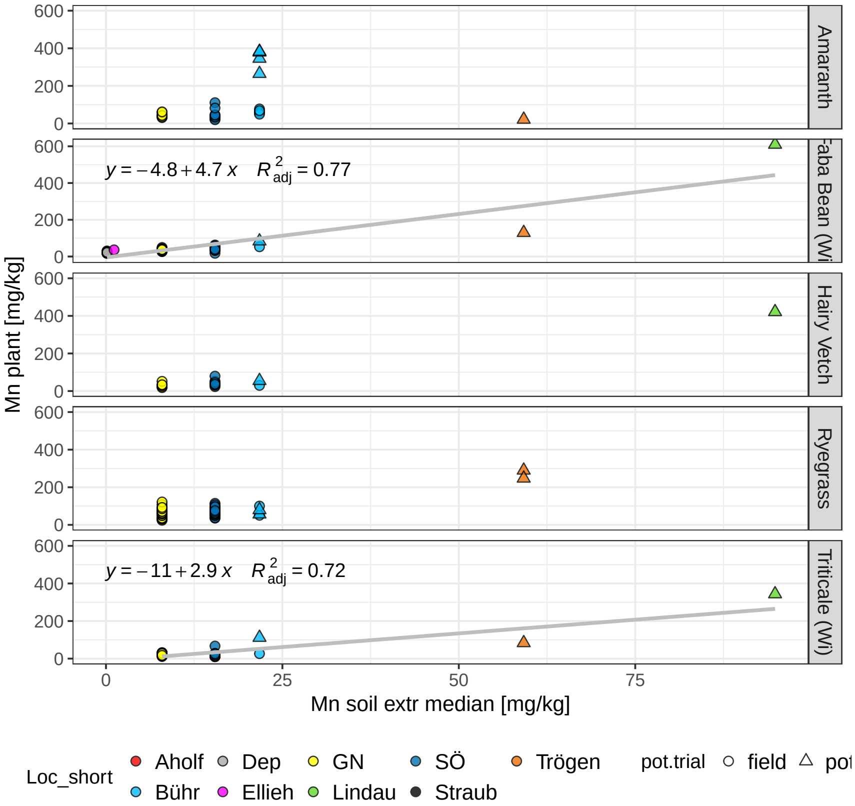


Figure 8.9: Mn concentration in plants vs. extracted with NH_4NO_3 in soil, sample locations are abbreviated (Table 8.1).

8.3.4 Summary of the Conclusions Obtained in Bachelor Thesis

M. Willerding-Möllmann contributed to this Thesis with a Bachelor Thesis in which additional extraction methods were tested. Two plant species, amaranth and summer faba bean were tested in pot experiments which grew for 8 weeks. Faba bean always showed greater enrichment of Co and Ni than amaranth. The correlation trends with total digestion of the soil resulted in good correlations. For Co CAT extraction showed also good results. The extractions with NH_4NO_3 showed a very good correlation for Nickel, for Co it was a good correlation if one soil sample was excluded (Düshorn). Düshorn was a sandy soil with very low total trace element concentration.

8.4 Discussion

The recovery rates of the concentrations in soil extractions vs. the total element concentrations were very different for each element. The main nutrient elements had rates of approximately 20 % (Ca) to 3 - 5 %. Cd was a very mobile element that also had about 3 % recovery rate. The trace elements Co, Ni and Mn showed intermediate rates. The rates from each soil sample location revealed that with acidic soil the rates were increased about 10-fold (soil location in Lindau with pH 4.6). Figures outlining plant concentration vs. total soil concentration showed a linear tendency for Ni and Co, especially for the plant species: winter faba bean, amaranth, ryegrass and hairy vetch.

For triticale plants there was no trend observed, except for Mn results based on soil extractions. This was not unsurprising given the total concentration in the plants was very low for Co and Ni (Chapter 7). Also, low TF for triticale plants in Chapter 6 were detected. The results of plant concentration vs. soil extraction concentration revealed that the plants in low pH soils, in most cases, had elevated concentrations, except for triticale (Co, Ni) and for hairy vetch (Ni). A correlation trend was detected for winter faba bean and triticale (both Mn).

The extraction method was not applicable for molybdenum. The concentrations in the soil solutions were mostly below the LOD. No correlation was also detectable for Mo in plants vs. total concentration. The plants did not have elevated concentrations in the tissue with higher soil concentration. Furthermore, for ryegrass in particular, the plant samples showed large variability in plant concentrations. Only on Garte Nord and Sömmerling there were large sample numbers that could detect this effect. This might apply to the other samples and leads to the recommendation that an equal number of plants should be analyzed to account for the variance.

Chapter 9

Trace Element Delivery for Biogas Production Enhanced by Alternative Energy Crops: Results from two-year Field Trials

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Notes: This is a slightly modified manuscript of the version submitted. In this Chapter the field trial **Garte Nord** is called **Reinshof**. For the manuscript it was referred to the research farm and not to the plot unit as it was done throughout the thesis.

Abstract

Introduction Energy crop production for biogas still relies mainly on maize, but the co-digestion of alternative energy crops (legumes, amaranth, ryegrass, flower mixtures) with maize can have several advantages. First, a greater biodiversity in the fields, second, an enrichment of essential trace elements in biogas substrates (cobalt, nickel, manganese and molybdenum) and third, less use of artificial trace element additives.

Methods In two randomized field trials, 12 different variants of field crops in sole, double and intercropping were tested over a two year period. Dry matter yield, trace element content of the crops and soil parameters like soil texture, pH and soil element concentration were determined. The trace element concentrations in biogas plants resulting from input mixtures of energy crops (legumes, amaranth, faba bean and ryegrass) and maize are calculated.

Results High dry matter yields were obtained for ryegrass, maize, winter faba bean-maize, intercropping winter faba bean/triticale-maize and intercropping rye/vetch-maize. The double croppings with maize reached highest total yields (ca. 30 t DM

ha^{-1}). Total element deliveries from the harvest reveals large differences between the variants and the trace elements. Cobalt is provided most by summer faba bean-maize and intercropping of winter faba bean/triticale-maize. Ryegrass can deliver the greatest amounts of Manganese and Molybdenum to biogas plants.

When these energy crops are added to conventional maize input for biogas production, the trace element concentration in the fermenter can be raised significantly, e.g. 0.03 g Co t^{-1} FM) can be attained compared to 0.003 g t^{-1} with maize silage input only. Sufficient Co can be provided by addition of manure to the input mixture.

Conclusions Alternative energy crops in combination with maize ensure a good dry matter yield per year and provide significantly more trace elements. However, these substrate mixtures alone do not provide enough trace elements, particularly Co. However, enough Co can be supplied by a small addition of manure.

9.1 Introduction

Renewable electricity production from biogas is a promising renewable energy form which can mitigate climate change and decrease the dependence on fossil fuels. In Germany, there are 9300 biogas plants with a total installed electric output of 4.5 GW (Biogas Fachverband, 2017).

Energy crop production for farmers offers many advantages, e.g. more stable delivery contracts for biogas substrates leading to stabilization of the revenues, the production of organic fertilizer in the form of biogas residue and a larger biodiversity on the fields (Deutscher Bauernverband, 2018).

In Germany, maize is the most commonly used energy crop with 72 %, followed by grass (12 %) and 7 % of cereal whole crop silage (Daniel-Gromke et al., 2017). Maize is used because of its very high dry matter and methane yield, and can be easily stored as silage for biogas fermenters. There are some negative traits about long term maize cultivation, because it leads to soil degradation and decrease in soil organic matter. A 27-year field experiment showed, that soil organic carbon decreased by 30 % for continuous maize growth, compared to only 3 % for a permanent grass cover (Vertés and Mary, 2007). Palmer and Smith (2013) stated high to severe levels of soil structural degradation on sites where late harvested crops such as maize had been grown. When maize is grown in monoculture it may also promote pests like the European corn borer (*Ostrinia nubilalis*, Labatte and Got, 1991).

9.1.1 Trace Element Needs of Biogas Fermenters and Thresholds

Low trace element contents in biogas fermenters cause instabilities and low biogas production rates. Cobalt (Co) in particular is a limiting factor for growth and activity of methanogenic microorganisms (Lebuhn et al., 2008; Hinken et al., 2008; Pobeheim et al., 2011; Lindorfer et al., 2012; Choong et al., 2016). Alongside Co, nickel (Ni), molybdenum (Mo), manganese (Mn), selenium (Se) and tungsten (W) are essential trace elements for biogas microorganisms (Demirel and Scherer, 2011). Some authors give minimum thresholds for trace elements in biogas fermenters which should be surpassed to guarantee a stable biogas production. For Co, for example Sauer (2010) evaluated 0.07 mg kg^{-1} FM and (Pobeheim et al., 2011) 0.05 mg kg^{-1} FM. These thresholds depend on different fermenter parameters like the organic loading rate (OLR), the hydraulic retention time (HRT) and the biocenosis.

To avoid negative effects due to too low trace element supply there are two ways to conquer deficiencies. The elements can be supplied by addition of manure which has a high TE content or by addition of trace element additives. The application of high amounts of liquid manure however has the disadvantage that it mainly consists of water (about 2 % DM) occupying space in the biogas fermenter without contributing to the biogas yield (Weiland, 2006).

Trace element additives are commonly used in more than 3.000 biogas plants in Germany. This handling often stabilizes biogas production (Choong et al., 2016; Kuttner et al., 2015) but bears environmental and health risks for operators and is expensive. The natural element cycle ist disturbed with potential toxic (heavy) metals (Co, Ni, Mn, Mo) as these elements become enriched in the biogas residue used as an organic fertilizer (He et al., 2005). Furthermore, the majority of Co production is located in the Democratic Republic of Congo, where labor conditions are questionable. A recently published report state human right abuses by child labor and health problems of the workers (Amnesty International, 2016). For all these reasons, the application of element additives should be minimized. This study uses the approach of providing these trace elements with alternative energy crops to avoid or at least minimize the use of trace element additives.

9.1.2 Physiological Function of Trace Elements and Uptake

Different plant species have differing element uptake mechanisms and tissue structures resulting in different trace element concentrations in the plant matter. The trace elements Ni, Mn and Mo are considered essential to higher plants but are needed only in small amounts Kabata-Pendias, 2004. Cobalt is reported as beneficial to plants (Palit et al., 1994; Marschner, 1995; Hänsch and Mendel, 2009) especially for Leguminosae, since (Reisenauer, 1960) demonstrated the essential role of Co in biological nitrogen fixation (BNF).

Mn in plants is part of a large number of enzymes and is involved in redox processes in oxidation states II, III, and IV. It plays an important role in photosynthesis, first demonstrated for green algae *chlorella* (Kessler et al., 1957). Mo is an essential element for plants as well and is part of several enzymes (Arnon and Stout, 1939; Kaiser et al., 2005; Hänsch and Mendel, 2009). The greatest concentrations of Mo were found in Leguminosae where it is mainly located between the leaf veins (Shkolnik, 1984). Ni belongs to the enzyme urease needed for the hydrolysis of urea Dixon et al., 1975. Ni-deficiency leads to leaflet tip necrosis caused by urea (Eskew et al., 1984). All these trace elements can also be toxic to plants, but such high soil concentrations necessary will not be reached except in areas of non-ferrous mineral deposits or contaminated areas. In fact it is more likely that deficiencies occur as partially stated for the European agricultural soil (Reimann et al., 2018). Co, Ni and Mn do have in common a higher uptake by plants at lower soil pH. On the other hand, Mo is less mobile in acidic soil conditions (Kabata-Pendias, 2004).

9.1.3 Aims of the Study

This study shows results of an interdisciplinary project which combines agronomic, (geo-)chemical and microbiological methods for optimized and more sustainable biogas production with energy crops. Several alternative energy crops (e.g. legumes, amaranth or flowering plants) were tested in comparison to traditional energy crops (e.g. maize, ryegrass) in different cropping systems. This study comprises the first part of the project results: the soil properties, the element composition (TE), dry matter yield of the energy crops and the combined results as flux of element (g ha^{-1} a^{-1}). The trace element concentrations provided here are valuable because there is very limited data on Co, Mn, Mo and Ni concentrations in whole plants available, especially for Co. These results together are the basis for hypothetical calculations of resulting element concentrations in biogas plants that arise from different input mixtures. This data is also required in the second stage of the project where the energy crop mixtures are tested in lab-scale biogas plants. The aims of the study are to give arguments for a broader energy crop as input into biogas plants.

This paper will provide:

- trace element concentrations (Co, Mn, Mo and Ni) of whole plants for a large variety of energy crops
- a new argument for a larger biodiversity in energy crop production
- raise transparency and awareness of the use (and misuse) of trace element additives for biogas production.

9.2 Methods

9.2.1 Soil Properties

Two year field trials were conducted at two contrasting sites in southern Lower Saxony, Germany. At the research farm Reinshof (plot Garte Nord) in the Leine valley (N 5°29′23.41", E 9°56′11.95", altitude: 164 m) and at Sömmerling in the Solling region (N 51°38′26.37", E 9°40′15.46", altitude: 251 m). The soil at the fertile site Reinshof is classified as a haplic luvisol and at the marginal site Sömmerling as a cleyic cambisol. Available field capacity in the rooting depth at Reinshof and Sömmerling is 257 mm and 124 mm, respectively. Long term annual mean temperature is 9.2°C at Reinshof and 9.0°C at Sömmerling and the long term annual precipitation 651 mm and 836 mm, respectively (Deutscher Wetterdienst (German Metereological Service), DWD, 2017).

The pH of the soil (0.01 mol l^{-1} $CaCl_2$ (DIN ISO 10390:2005, 2005)) is 6.8 at Reinshof and 6.4 at Sömmerling. The parent material of the Reinshof soil are fluviatile sediments derived from late quaternary loess and that of the Sömmerling are triassic sand- and siltstones with an addition of some loess. Further important topsoil parameters are shown in Table 9.1. The extractable amounts of phosphorous (P) and potassium (K) were measured in calcium acetate lactate solution (CAL) and magnesium (Mg) measured in $CaCl_2$ (Thun and Hoffmann, 2012). The soil particle size was analyzed by hydrometer analysis (DIN ISO 11277:2002-08, 2002). The experimental design was a randomized Semi-Latin square with four replications. The area of each plot was 9 m x 7.5 m (67.5 m^2) at Reinshof and 4.5 m x 7.5 m (33.75 m^2) at Sömmerling.

Table 9.1: Characteristics of topsoil (0-30 cm) ± SE (standard error of the mean).

Parameter		Reinshof	Sömmerling
P	mg 100g^{-1} (CAL)	15.3 ± 1.1	11.8 ± 2.3
K		9.5 ± 0.7	20.8 ± 1.2
Mg	mg 100g^{-1} (CaCl$_{2}$)	11.8 ± 0.3	7.1 ± 0.2
Humus	%	1.9 ± 0.1	1.9 ± 0.05
Sand	%	12.7 ± 0.5	33.5 ± 0.4
Silt		69.4 ± 0.3	49.8 ± 0.1
Clay		17.9 ± 0.2	16.7 ± 0.4
Al	% (total)	4.7 ± 0.07	4.1 ± 0.09
Fe		1.8 ± 0.03	1.4 ± 0.04
Ti		0.4 ± 0.002	0.3 ± 0.004
Mn	mg kg^{-1} (total)	704 ± 8	479 ± 23
Cd		0.45 ± 0.02	0.37 ± 0.02
Co		7.1 ± 0.1	8.1 ± 0.2
Cu		14.0 ± 0.2	7.0 ± 0.2
Mo		0.7 ± 0.1	0.96 ± 0.09
Ni		16.5 ± 0.6	11.8 ± 0.6
Zn		54 ± 1	50 ± 1

Reinshof: plot Garte Nord

9.2.2 Crop Species, Cropping Systems and Management

The plants were grown in 2014/2015 and 2015/2016, hereafter referred to as 2015 and 2016. Various farming systems were performed. The double cropping system is defined as a crop rotation with a first crop in winter and a succeeding second crop in summer. As winter crops winter faba bean, winter triticale, an intercropping of winter faba bean and winter triticale and an intercropping of rye and hairy vetch were grown. Maize was used as summer crop in this double croppings system. In contrast to this, the summer main crops summer faba bean, annual flower mixture (12 species), amaranth, maize and the intercropping of amaranth and maize were grown after bare fallow over winter. Ryegrass, perennial flower mixture (25 species) and cup plant were grown as permanent crops for duration of the trial.

Cup plant could only be harvested once in 2016, as in planting year 2015 it formed only leaf rosettes. In the two years of field trials different maize cultivars as second crops were used, because the cultivar used in 2015 did not reach full maturity. Standard crop management practices for fertilization and plant protection were applied. Nitrogen (N) fertilizer was applied relating to the default nominal value of N (Zorn et al., 2007). For unknown crops, the amount of N fertilizer was determined by recommendations of breeders and other study results. In any case, the amount of mineral N in the soil (Nmin) was taken into account and subtracted from the nominal value. The fertilizer was applied as a combination of organic and mineral fertilizer. See Table 9.2 for additional cultivation parameters.

The previous crop at Reinshof was winter wheat in both years. At Sömmerling it was rapeseed in the first year and winter wheat in the second year. After the previous crop, the soil was plowed in the first and reduced cultivated in the second year. Seed-bed cultivation took place before sowing in autumn and spring, depending on variant.

The winter crops as well as ryegrass were sown after stubble cultivation and ploughing in the first year. In the second year only a reduced tillage after the previous crop was performed. The perennial crops in the first year and the summer main crops in both years were grown after seed-bed cultivation in spring. The seeds of the partners of the intercropping variants were sown in alternating rows.

9.2.3 Sampling and Analysis of Yield and Dry Matter

The sampling and analysis of dry matter yield at Reinshof and Sömmerling were performed between June and October 2015 and between June and November 2016 (Table 9.2). All variants except ryegrass were harvested once a year. Due to problems in establishing ryegrass at Sömmerling in autumn 2014, the results are only shown for three cuts of ryegrass in 2016. At Reinshof, there were three cuts in both years. To determine dry matter yield a sample of 1 m x 1 m was harvested for all variants except for maize. Plants were cut close to soil surface by hand. For maize a sample of 1.5 m x 1 m cut at approximately 7 cm above soil surface was harvested. The plant material was dried at 105°C for 48 h. All variants were harvested at silage maturity, which is suitable for use in biogas production. Unfortunately, on site Reinshof in 2016 maize as second crop was lost in the variants triticale, intercropping of faba bean/triticale and rye/vetch. Therefore, the yield of this second maize was estimated from the plots of second maize of the variant winter faba bean.

9.2.4 Sampling for Trace Element Analysis

All plant samples for trace element analysis were collected as whole plants (aboveground plant parts without roots) on the harvest dates listed in Table 9.2. The soil samples were taken up to a depth of 30 cm in April 2015. The samples were air dried and sieved to $<$ 2 mm in grain size. A minimum of 100 g of the soils and 500 g of the plant fresh matter were dried at 105°C. The soils were ground in an agate ball mill (Fritsch Pulverisette 5) to $<0.063\ \mu$m and the plants were cut in an universal cutting mill (Fritsch Pulverisette 19) to $<$ 0.5 mm. Aliquots of 150 mg of soil and 700 mg of plant powder were completely digested with a mixture of ultra-pure concentrated HNO_3, $HClO_4$ and HF in closed ultra-clean PTFE vessels (PicoTrace, Bovenden, Acid sample digestion system, DAS 30). For the soil samples, a small amount of HCl was added to completely dissolve precipitated aluminum and iron oxide hydroxides. The soil solutions were diluted to 100 ml, the plant solutions to 50 ml before measurement. In addition, blank solutions without sample material were added in the digestion process to ensure that the handling was clean without detectable contamination from the reagents, the digestion and the measurement process. In the resulting clear sample solutions, 47 elements were quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Agilent 5100 VDV) and mass spectrometry (ICP-MS, Thermo Scientific iCAP Q). The limit of detection was calculated by the 3-fold standard deviation of the blank concentrations for each analysis batch. The precision and accuracy of the digestion process and the ICP devices were tested by analyzing several international reference samples and one in-house standard. The accuracy describes the deviation between measured and reference value. In general, for the main elements the accuracy was between 5 and 10 %. For the trace elements, typical average accuracies were between 10 and 20 %.

Table 9.2: Cultivars of the tested variants and cultivation dates at the main field trials Reinshof and Sömmerling in both years of trial (2015 and 2016)

Variant	**Cultivar**	**Seed**	**Sowing** Reinshof	**Harvest** Reinshof	**Sowing** Sömmerling	**Harvest** Sömmerling
Per. flower mix. (PF Mix)	BG 70	10 kg ha^{-1}	08-May-15	01-Sep-15 15-Aug-16	12-May-15	24-Aug-15 18-Aug-16
Ryegrass (RG)	Alligator	40 kg ha^{-1}	01-Oct-14	3 cuts 2015 3 cuts 2016	25-Mar-15	3 cuts 2016
Cup plant (CP)	Chresten.	4 pl m^{-2}	19-May-15	31-Aug-16	20-May-15	25-Aug-16
Ann. flower mix. (AF Mix)	BG 80	10 kg ha^{-1}	08-May-15 12-May-16	05-Oct-15 11-Oct-16	12-May-15 17-May-16	06-Oct-15 04-Oct-16
Su faba bean (FB Su)	Fanfare	40 s m^{-2}	09-Apr-15 16-Mar-16	15-Jul-15 12-Jul-16	25-Mar-15 17-Mar-16	16-Jul-15 13-Jul-16
Amaranth (A)	Bärnkrafft	40 s m^{-2}	18-May-15 10-May-16	05-Oct-15 11-Oct-16	12-May-15 17-May-16	06-Oct-15 04-Oct-16
Maize-main (M)	Amadeo	25 s m^{-2}	11-May-15 10-May-16	29-Sep-15 12-Sep-16	12-May-15 11-May-16	30-Sep-15 19-Sep-16
Amar./maize (AM)	Bärnkrafft/ Amadeo	78 s m^{-2} 10 s m^{-2}	11-May-15 10-May-16	29-Sep-15 12-Sep-16	12-May-15 11-May-16	30-Sep-15 19-Sep-16
Wi faba bean (FB Wi)	Nordica	25 s m^{-2}	01-Oct-14 06-Oct-15	02-Jun-15 06-Jun-16	06-Oct-14 13-Oct-15	09-Jun-15 13-Jun-16
Wi triticale (Tri)	Balu	375 s m^{-2}	01-Oct-14 03-Oct-15	02-Jun-15 06-Jun-16	06-Oct-14 13-Oct-15	09-Jun-15 13-Jun-16
Faba bean/trit (FB Wi Tri)	Nordica/ Balu	25 s m^{-2} 281 s m^{-2}	01-Oct-14 06-Oct-15	02-Jun-15 06-Jun-16	06-Oct-14 13-Oct-15	09-Jun-15 13-Jun-16
Rye/vetch (RV)	Conduct/ Welta	120 kg ha^{-1}	01-Oct-14 03-Oct-15	02-Jun-15 06-Jun-16	13-Oct-15	13-Jun-16
Maize (second) (M)	Simpatico Cathy (Re)/ P7326 (Sö)	9 s m^{-2}	08-Jun-15 10-Jun-16	21-Oct-15 27-Oct-16	17-Jun-15 21-Jun-16	05-Nov-15 31-Oct-16

s = seeds, pl = plants, Re = Reinshof, Sö = Sömmerling

9.2.5 Correction for Adhering Particles

A total digestion process of the plant samples was performed. To access physiological concentrations resulting only from plant uptake the values were corrected for adhering dust and soil particles. For plants grown on open field a small amount of adhering soil or dust particles cannot be avoided. Severe soil "contamination" was washed off before further sample preparation. The concentrations were corrected with the median transfer factor (TF = conc. in plant/conc. in soil) of several elements with a very small translocation into the plant. For a detailed description refer to Method 3 in Pospiech et al. (2017)[1]. All element plant concentrations in this work were corrected this way. This was most important for Co and Ni. The concentrations of Mn and Mo remain mostly unchanged by the correction, owing to their larger TF.

9.2.6 Statistical analysis

Statistical tests were performed to test, whether the difference in yield or the differences in element delivery were significantly different. The statistical method used was a linear mixed effects model to account for the design and the treatment effect (variant). Effects for year and site were assumed to be fixed. Blocks were treated as random. The statistical analysis was done with *R*, Version: 3.4.2 (R Core Team, 2017) and the packages *lme4* (Bates et al., 2015) and *multcomp* (Hothorn et al., 2008). The graphical representations with the package *ggplot2* (Wickham, 2016). The significance level was set at 5 %, and if significant results were found, Tukey's HSD test (honestly significant difference) was performed to obtain pairwise mean comparisons.

Table 9.3: Results of the p-values of the fixed effects in linear model for yield and for element delivery (Co, Mn, Mo, Ni), n.s. = not significant, significance level is 0.05

	Yield	Co	Mn	Mo	Ni
Variant	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Year	< 0.0001	0.0470	< 0.0001	0.0011	n.s.
Site	< 0.0001	0.0028	< 0.0001	< 0.0001	< 0.0001
Variant × Year	0.0039	n.s.	< 0.0001	n.s.	< 0.0001
Variant × Site	< 0.0001	0.0019	n.s.	< 0.0001	0.0051

9.3 Results and Discussion

9.3.1 Dry Matter Yield

Significant effects of variant, year, site and significant interactions of variant × year and variant × site were detected for yield (Table 9.3). Therefore, pairwise mean comparisons of crop species were conducted separately for each year and each site (Fig. 9.1).

The crops at site Reinshof generally reached greater yields than the crops at Sömmerling. This was most likely the result of the better soil texture, soil quality and available field capacity of the soil at the site Reinshof. The greatest yields at both sites and years were reached for the double cropping system of triticale-maize[2], intercropping faba

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[1] described in Chapter 4

[2] "-maize" = succeeding maize in summer, this term is used throughout this Chapter

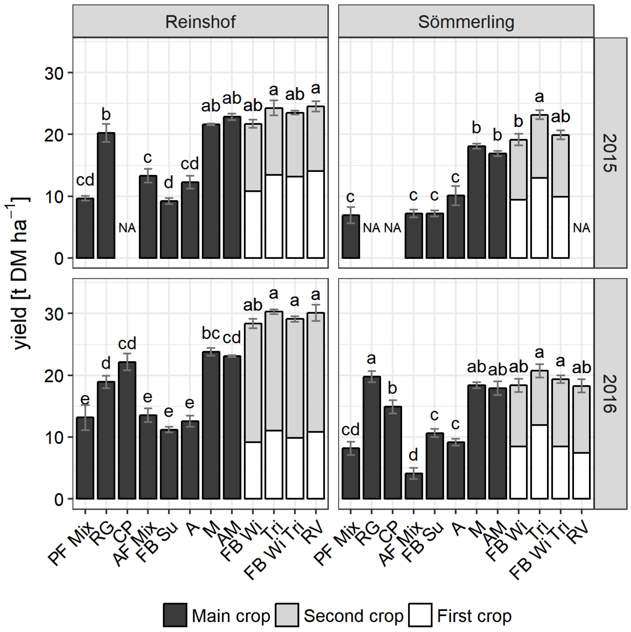

Figure 9.1: yield in t DM ha^{-1} of different energy crops and cropping systems. Maize as succeeding second crop (stacked bars). Different letters indicate significant differences between the variants from Tukey's HSD Test. Error bars = standard errors (SE) of cumulative yield. NA = not available, PF Mix= perenn. flow. mix, RG= ryegrass, CP= cup plant, AF mix= annual flow. mix, A= amaranth, M= maize, AM= amar./maize, FB Wi= wi faba bean, Tri= triticale, FB Wi Tri= wi faba bean/triticale, RV=rye/vetch

bean/triticale-maize and intercropping rye/vetch-maize. In 2015, about 25 t DM ha^{-1} and in 2016 about 30 t DM ha^{-1} were harvested for these variants at site Reinshof. First crops contributed considerably to the total dry matter yield with percentages between 49 % (winter faba bean) and 57 % (rye/vetch) in 2015 and 32 % (winter faba bean) and 57 % (triticale) in 2016. This is consistent with Graß et al. (2013) who also states high dry matter yields in double cropping systems. Like in this study, they found highest yields in variants with cereal based first crops.

Maize as sole crop and the intercropping of amaranth/maize reached high dry matter yields with 22 to 24 t DM ha^{-1} at Reinshof, and slightly smaller yields on site Sömmerling. Though the proportion of amaranth in this intercropping was very small (about 2 % amaranth, 98 % maize, data not shown), because maize suppressed amaranth during growth in this kind of sowing method.

The differences in yield between the variants of the double cropping system and maize were mostly insignificant, apart from Reinshof in 2016. In 2015, only triticale-maize at Sömmerling had significantly greater yield than maize as main crop. In 2016, the double cropping of triticale-maize, intercropping faba bean/triticale-maize and the intercropping rye/vetch-maize at Reinshof, had significantly greater yields than maize as main crop. Ryegrass also reached good results in both years on both sites (20-22 t DM ha^{-1}). Interestingly, at Sömmerling ryegrass showed greater yield figures even if it is not statistically significant greater than maize and a comparable yield to the highest yielding variants at this site. As in this case, in 2015 at site Reinshof the yield of ryegrass was not significantly different with that of maize. The flowering mixtures (annual and perennial) could not compete with the high yielding crops and achieved only less than half of the maximum yields at each site and year. A slight yield increase from the first to the second year could be observed for the perennial flower mixture. In recent years, perennial wild plant mixtures for biogas use gained increasing attention in Germany because of their numerous ecological benefits such as permanent soil coverage, enhanced biodiversity or habitat for various species. Dry matter yields between 3 and 23 t DM ha^{-1} depending on the kind of wild plant mixture, year of use as a permanent crop and site were reported in the literature (Cossel and Lewandowski, 2016). With a maximum yield of 13 t DM ha $^{-1}$ at Reinshof in the second year the yield potential of a wild plant mixture was most probably not reached, but may increase in later years of cultivation.

At Reinshof cup plant achieved a high dry matter yield of about 22 t DM ha^{-1} and did not differ significantly from the yield of main crop maize. This level of yield could not be achieved by cup plant in Sömmerling; on this site the plants showed signs of water deficiency. A greater drought-related above-ground dry matter reduction for cup plant than for other crops was also observed by Schoo et al. (2017). Yield figures comparable to maize could only be attained at sites with a good water supply. Under Bavarian cultivation conditions about 16 to 22 t DM ha^{-1} were measured for several years (Hartmann and Lunenberg, 2016).

9.3.2 Trace Element Concentrations in the Plants

The trace element concentrations of the plant variants are shown in Table 9.4. The main result was that the crops mainly used as substrates for biogas production in the field trial: maize and triticale, showed the lowest concentrations of Co, Ni and Mn.

Different element concentrations of the plants between both field trial sites were detected, showing an influence of the different soil properties on element uptake. Also, the statistic test on influence of the site for the delivery rates state a significant effect of the field site. However, the order of element concentrations between the variants was similar on each site, showing that the plant species itself did have a great influence on element accumulation from soil to plant. The greatest Co concentrations of 0.19 mg kg^{-1} DM (Reinshof) and 0.27 mg kg^{-1} DM (Sömmerling) were analyzed in the legumes summer faba bean, and in winter faba bean (0.13 to 0.15 mg kg^{-1} DM) (Table 9.4). The greatest Ni concentrations were also found in faba bean plants: 0.93 mg kg^{-1} Ni (Reinshof) and 0.53 mg kg^{-1} (Sömmerling) for summer variety and 0.67 mg kg^{-1} (Reinshof) and 0.35 mg kg^{-1} (Sömmerling) for winter variety of faba bean. These findings correspond to literature which also report that Mo is essential for Leguminosae Arnon and Stout, 1939; Kaiser et al., 2005. The greatest concentrations of Mn were analyzed in ryegrass (64.5 mg kg^{-1} (Reinshof) and 77.6 mg kg^{-1} (Sömmerling). Second highest ranged amaranth samples with 47 mg Mn kg^{-1} and summer faba bean with 44 mg Mn kg^{-1} (average of both sites).

Most plant variants belonging to the Poaceae family (maize, rye, triticale) reveal small Co and Ni trace element concentrations of about 0.01 mg Co kg^{-1} DM and about 0.1 mg Ni kg^{-1} DM at both sites (Table 9.4). Co and Ni concentrations were particularly low in maize, triticale and in the intercroppings rye/vetch and amaranth/maize ranging between 0.008 and 0.01 mg/kg for Co and 0.08 and 0.17 mg/kg DM for Ni. Ryegrass plants show the highest Co and Ni concentrations from all plants of the sweet grass family (Poaceae) with 0.02 (Reinshof) and 0.034 mg Co kg^{-1} (Sömmerling) and 0.71 (Reinshof) and 0.42 mg Ni kg^{-1} (Sömmerling).

The concentrations of Co, Ni and Mn in intercropping rye/vetch only range slightly above triticale or maize, Table 9.4), although vetch plants alone did have elevated Ni and Co - concentrations (Data not shown). This was because vetch only reached less than 5 % of total DM yield of the intercropping (data not shown). The same was true for amaranth/maize where amaranth had less than 5 % of total DM yield. Only the intercropping faba bean/triticale had almost equal yield contributions of both plants, resulting also in medium concentrations between both plants as sole crops (especially for Co). The flowering mixtures (annual and perennial) also show elevated trace elements, for example 0.059 mg Co kg^{-1} (Reinshof) and 0.36 mg Ni kg^{-1} (Sömmerling). Although these consisted of several plant species they show a narrow standard deviation.

Table 9.4: Element contents in mg kg^{-1} in above ground plant biomass, values represent means ± sd (standard deviation) from samples of 2015 and 2016, Table sorted by site and decreasing cobalt concentration

Variant	n	Co	Mn	Mo	Ni
Reinshof					
Faba Bean (Su)	8	0.188 ± 0.037	42.6 ± 7.1	0.64 ± 0.19	0.93 ± 0.20
Faba Bean (Wi)	8	0.127 ± 0.029	33.2 ± 5.8	0.86 ± 0.26	0.67 ± 0.16
PF Mix	8	0.059 ± 0.009	31.7 ± 4.5	0.52 ± 0.15	0.36 ± 0.07
Amaranth	8	0.051 ± 0.007	44.5 ± 10.2	0.68 ± 0.36	0.17 ± 0.05
Faba bean/trit	8	0.050 ± 0.018	30.6 ± 2.5	1.14 ± 0.30	0.35 ± 0.09
Cup Plant	4	0.028 ± 0.006	25.1 ± 2.8	0.08 ± 0.02	0.33 ± 0.03
AF Mix	8	0.027 ± 0.012	37.5 ± 8.1	0.39 ± 0.21	0.22 ± 0.05
Ryegrass	24	0.020 ± 0.012	64.5 ± 25.6	2.50 ± 1.30	0.71 ± 0.18
Amaranth/maize	8	0.011 ± 0.002	19.4 ± 0.5	0.43 ± 0.15	0.14 ± 0.01
Rye/vetch	8	0.010 ± 0.007	18.6 ± 3.3	1.00 ± 0.30	0.13 ± 0.07
Triticale (Wi)	8	0.010 ± 0.004	26.3 ± 3.9	1.33 ± 0.18	0.17 ± 0.11
Maize	7	0.009 ± 0.005	18.2 ± 1.6	0.40 ± 0.18	0.14 ± 0.04
Sömmerling					
Faba Bean (Su)	8	0.269 ± 0.074	44.3 ± 11.6	0.35 ± 0.18	0.53 ± 0.14
Faba Bean (Wi)	8	0.148 ± 0.065	40.1 ± 12.3	0.36 ± 0.17	0.35 ± 0.10
AF Mix	8	0.143 ± 0.073	43.6 ± 9.6	0.18 ± 0.05	0.27 ± 0.08
Amaranth	8	0.130 ± 0.052	50.3 ± 30.6	0.38 ± 0.12	0.17 ± 0.09
PF Mix	8	0.126 ± 0.067	35.0 ± 9.1	0.33 ± 0.10	0.26 ± 0.07
Faba bean/trit	8	0.111 ± 0.041	32.5 ± 6.8	0.31 ± 0.13	0.27 ± 0.07
Cup Plant	4	0.044 ± 0.035	32.9 ± 6.1	0.09 ± 0.02	0.28 ± 0.05
Ryegrass	12	0.034 ± 0.019	77.6 ± 21.0	1.30 ± 0.20	0.42 ± 0.13
Rye/vetch	4	0.031 ± 0.012	22.1 ± 5.3	0.58 ± 0.23	0.23 ± 0.16
Amaranth/maize	8	0.014 ± 0.007	18.3 ± 1.5	0.32 ± 0.12	0.09 ± 0.01
Triticale (Wi)	8	0.011 ± 0.003	20.4 ± 4.8	0.52 ± 0.34	0.09 ± 0.06
Maize	8	0.008 ± 0.007	16.9 ± 3.3	0.32 ± 0.17	0.08 ± 0.02

PF Mix = perennial flower mixture, AF Mix = annual flower mixture

9.3.3 Element Delivery from Harvest

The amount of trace element (TE) per variant is called element delivery, as the focus is on the potential to deliver TE to biogas plants. However, the approach is the same for element amounts extracted from the soil. As well as for yield, significant effects of variant, year, site and the interaction of variant × year and variant × site were detected for the element deliveries of Co, Mn, Mo and Ni (Table 9.3). The amount of TE harvest of Co, Ni, Mo and Mn was calculated for main crops ryegrass, cup plant, amaranth, maize and summer faba bean, as well as for the variants of the double cropping systems: winter faba bean-maize, intercropping winter faba bean/triticale-maize, and triticale-maize. The total TE delivery/extraction (TE_{extr}) is calculated via

$$TE_{extr}\ [g\,ha^{-1}] = y\ [t\,ha^{-1}] * conc\ [g\ t^{-1}]$$

with yield (y) and element concentration (*conc*) in the crop.

In Fig. 9.2 TE delivery is shown as bar charts. There are large variations in total amount of TE delivery, corresponding to the concentration of the elements in the plants and their DM yields. The total amount increases in the sequence Co<Ni<Mo<Mn. For Co and Ni only small total amounts were harvested in the range of 0.5 and 3 g ha^{-1} a^{-1}. The largest amount of Co could be realized by sole cropping of summer faba beans (about 2 g ha^{-1} a^{-1}) or double cropping of winter faba bean-maize (1.5 g ha^{-1} a^{-1}) and the intercropping of winter faba bean/triticale-maize (1.25 g ha^{-1} a^{-1}). The first crops contribute about 90 - 95 % of total Co delivery and maize as second crop only about 5 - 10 %.

Amaranth results vary between the sites: 1.1 (2015) and 1.35 g Co ha^{-1} (2016) at Sömmerling, compared to 0.6 g Co ha^{-1} at site Reinshof in both years of cultivation. Absolute Mn delivery rates are by far the greatest for all four elements. The pattern resemble that of Mo with highest rates for ryegrass (1.5 kg Mn ha^{-1} a^{-1}) at Sömmerling. The other variants show similar trends for the years according to the results of Tukey's test. Ryegrass had the greatest delivery rate of Mo (40 to 60 g Mo ha^{-1} a^{-1}, Reinshof). The greatest delivery rates of Ni were by ryegrass and winter faba bean-maize and summer faba bean (11-14 g Ni ha^{-1} a^{-1}) at site Reinshof; at site Sömmerling only rates smaller than 7.5 g Ni ha^{-1} a^{-1} could be achieved.

9.3.4 Calculated TE Concentrations by Applying Substrate Mixtures in Biogas Fermenters

Based on element concentrations of energy crops evaluated in the field trials, hypothetical trace element concentrations in biogas fermenters can be calculated for substrate mixtures. For the calculations we assume an average sized mesophilic biogas plant of 500 kW (electric) and a daily fresh weight input of 20 t of silage. The hydraulic retention time (HRT) is assumed to be 60 days. Each plant substrate faces volume loss according to the conversion of carbon to biogas. An input of 1 t maize (FM) substrate results in only 0.76 t of biogas residue after biogas production. This can be expressed in a mass reduction or degradation factor (F) of 0.76. Liquid manure only has a 2 % mass reduction, giving an mass reduction factor of 98 % (Reinhold et al., 2006). For the calculations the following reduction factors (F) for the substrates were used: maize: 0.76, faba bean and amaranth: 0.8, ryegrass: 0.75 and manure: 0.98 (compiled from Möller et al., 2010; Reinhold et al., 2006; Reinhold, 2005).

Each input substrate is combined with the corresponding mass reduction factor to calculate the mass resulting in the biogas residue. A mixture of 40 % maize (8 t) 60 % faba bean (12 t) results in 15.68 t after conversion in the biogas residue. To be able to calculate concentrations in biogas fermenters the concentrations need to be converted to absolute input masses (gram per day) and then divided by the reduced input mass to calculate the concentrations in gram per ton based on fresh matter. For a 40 % maize (M) and 60 % faba bean (FB) input mixture the equation for Co concentration in the fermenter (Co_{ferm}) is:

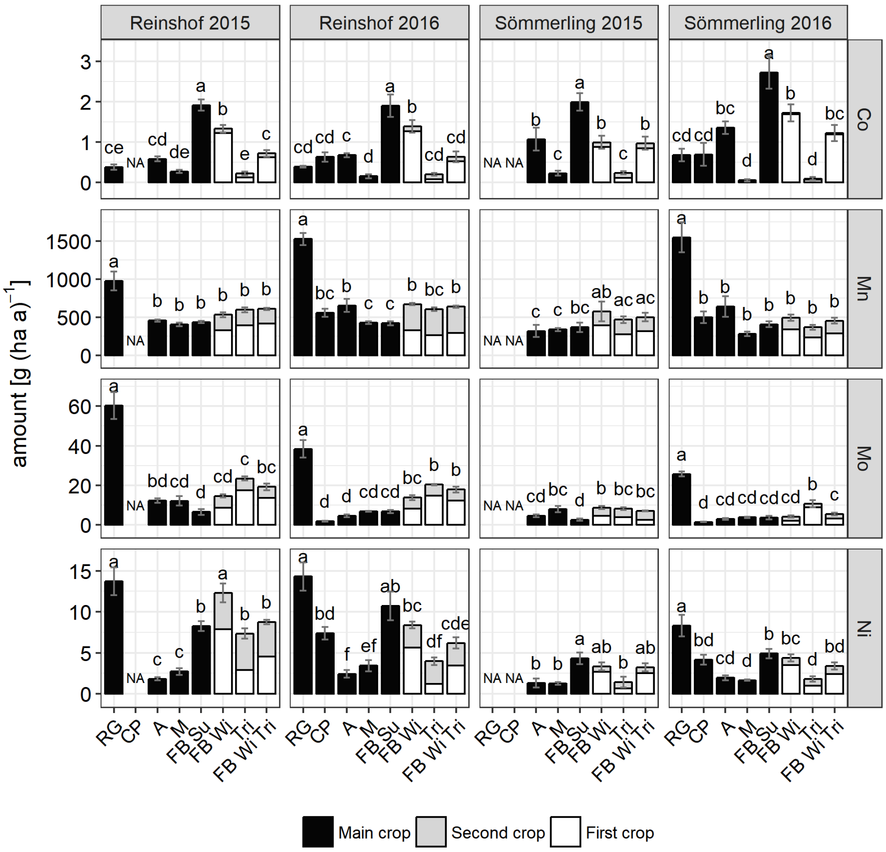

Figure 9.2: Mean element deliveries in g ha^{-1} a^{-1} for selected variants of all 4 repetitions on Reinshof and Sömmerling for both years, Second crop= maize as succeeding crop, errorbars = standard errors (SE), different letters indicate significant differences between the variants from Tukey's HSD Test, NA = not available, RG = ryegrass, CP = cup plant, A = amaranth, M = maize, FB Wi = wi faba bean, Tri = triticale, FB Wi Tri = wi faba bean/triticale

$$
\begin{aligned}
Co_{ferm} &= \frac{conc(M) * input\ [t] * F1 + conc(FB) * input\ [t] * F2}{15.68\ t} \\
&= \frac{0.00266\ g\ t^{-1} FM * 8\ t * 0.76 + 0.050 g\ t^{-1} FM * 12\ t * 0.80}{15.68\ t} \\
&= \frac{0.498\ g}{15.68\ t} \\
&= 0.032\ g\ t^{-1}
\end{aligned}
$$

The other mixtures and TE were calculated in the same way. Note that the plant substrate concentrations were converted to g t^{-1} based on fresh weight (FM). The calculation of trace element concentration in biogas plants follows the principles in Reinhold et al. (2006) in which a calculation of main nutrient element concentration in fermentation residues is described. This principle also holds for trace elements as all concentrations of the remaining elements are being enriched in the biogas residue.

The mixtures were calculated using the mean concentrations of summer faba bean, ryegrass and amaranth of both locations. Liquid manure is assumed to contain 8 % DM and the following element concentrations: Co: 2.25, Ni: 9.1, Mn: 434, Mo: 4.3 mg kg $^{-1}$ DM (from own data). Because Co is often the most limiting (from total element concentrations in the plants), the arguments are given in respect to Co threshold values only.

The first mixture shows the resulting concentrations in a biogas plant for a pure maize input (Table 9.5). Mixtures 1-3 consist only of plants, mixture 4 contains 25 % of liquid manure. The input proportions were calculated based on a daily total fresh matter input of 20 t. The pure maize input results in poor Co concentrations of only 0.003 mg kg^{-1} FM. This is less than 5 % of the Co threshold of 0.07 mg kg^{-1} FM. Other authors also report critical shortage of elements (especially Co) in pure maize-fed biogas fermenters Lebuhn et al., 2008; Hinken et al., 2008; Pobeheim et al., 2011 which are usually stabilized by trace element additives. An input mixture of 40 % maize and 60 % faba bean (Mix 3) resulted in 0.032 mg kg^{-1} FM giving the greatest Co content in plant based mixtures. This was almost half the threshold of 0.07 mg kg^{-1} FM (Table 9.5, Mix 1-3). With a small input of liquid manure (25 % based on FM) plus maize and faba bean, the Co threshold can be surpassed with 0.073 mg kg^{-1} FM (Table 9.5, mix 4). The calculated concentrations in a biogas fermenter show that with the addition of alternative energy crops (faba bean, amaranth, ryegrass) a significant portion of Co and other trace elements can be provided. These conclusions are based on the premise that trace elements in the plants are at least as available as trace elements from element additives. However, this aspect is difficult to analyze, as the fermenter contains an anaerobic, reducing environment with numerous possible ligands and binding partners for the elements. All of the regarded trace elements (except Mn) do also have a high affinity for sulfur, and may also form sulfides but this also applies for elements in trace element additives.

Table 9.5: Calculated concentrations in biogas plants/fermenters in g t^{-1} = mg kg^{-1} FM, of four different substrate input mixtures given in percent and a 100 % maize input (Ref.), based on fresh weight input. Calculated with a total fresh weight input of 20 t per day

Mix	Substrates	Co	Mn	Mo	Ni
		g t^{-1} FM			
Ref.	M 100 %	0.003	6.2	0.13	0.06
1	M 35 %, FB 35 %, A 30 %	0.025	8.8	0.12	0.09
2	M 37.5 %, FB 37.5 %, RG 25 %	0.024	11.2	0.24	0.14
3	M 40 %, FB 60 %	0.032	8.2	0.12	0.12
4	M 35 %, FB 40 %, man. 25 %	0.073	15.9	0.18	0.30

man. = manure, FM = Fresh matter

9.4 Conclusions

A promising outcome of this study was that faba bean, amaranth and ryegrass have a much greater concentration of essential trace elements for biogas production than maize, triticale or winter rye intercropped with vetch. Especially Co deficiency in biogas plants is likely caused by the low input of Co by the plant substrates. Our field studies showed that faba bean (sole and intercropped with triticale) in a double cropping system with maize can deliver a high DM yield per year and can provide a significant input of Co, Ni and Mn to biogas plants. Furthermore, ryegrass is also a good supplier of Ni, Mo, and Mn. A input substrate mixture of faba bean, ryegrass and maize together with a small addition of liquid manure may provide sufficient Co for biogas plants. Flowering mixtures contain elevated trace element concentrations but their DM-yields are too small.

The results of this study reveal the potential of providing trace elements to biogas production in a natural way, without trace element additives. Promoting alternative energy crops will enrich the biodiversity on the field and improve soil quality. This is a very good chance to minimize the use of artificial element additives as they disturb the natural element cycle by remaining in the biogas residue which is reapplied to the fields as fertilizer. In this way this study is unique in the field of trace element research in biogas production. The majority of studies aim on improving trace element additives and do not focus on natural inputs by plants and small additions of animal manure.

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Chapter 10

Final Remarks

10.1 Final Conclusions and Summary

Element uptake with an emphasis on trace elements from the soil by different plants was investigated. The goal was to identify plants, plant species or plant cropping systems with greater uptake of the elements Co, Ni, Mn and Mo in particular. This was a subproject of the joint research project "NiCo: Trace elements by energy crops – mass fluxes and recommendations for an optimized process biology in biogas plants" carried out by the GZG, Department of Sedimentology and Environmental Geology, University of Göttingen, with contribution by the Faculty of Agronomy, Department of Crop Sciences in Göttingen, the DBFZ ("Deutsches Biomasseforschungszentrum") and the UFZ ("Umweltforschungszentrum"), the last two affiliations located in Leipzig, Germany.

The project was about treating trace element deficiencies in biogas plants, resulting from a high input rate of maize silage, with an addition of alternative energy crops. In many biogas plants in Germany (approximately 3000) trace element shortage is handled with addition of artificial trace element supplements. These supplements have a high concentration of trace metals, but the absolute concentration values are usually unknown to the customer (biogas plant operator). This is problematic, as these supplements pose a health risk to human life. These metals are returned to the fields with the biogas residue and might enrich the soil with potentially toxic metals. Also, the needed trace element thresholds or the trace element concentrations in the fermenter are also unknown to the biogas plant operators. The research project strives to provide better transparency to this aspect by publishing minimum threshold concentrations of trace metals in biogas fermenters. This is part of a subproject performed by PhD-student Tino Pasold of the GZG.

In this thesis, the potential of alternative energy plants to supply trace elements to biogas plants in a natural way was investigated and the soil plant transfer characterized. The aspects: soil parameters (pH, soil type, element concentrations, potentially available elements), the influence of the plant species on element uptake, element contents in the plant tissue and the transfer of elements from soil to plant are covered.

The challenges of trace element determination, in particular for Co were investigated. It was shown, that the majority of the plant samples (n=492, full mature plants) contained very low plant tissue concentrations of less than 0.1 mg Co/kg. It was stated, that Co and the trace element concentrations obtained by ICP-OES should be checked with a second analysis method (for examples ICP-MS), when available. The use of reference standard materials with Co values lower than 0.2 ppm (mg/kg) in plant samples is highly encouraged.

It was pointed out, that clean samples without dust/soil particles are important to characterize the plants' uptake. It was shown, that plants grown in open field trials were vulnerable to adhering soil particles. From 1040 plant samples about 20 % (median) of the Co measured was originating from soil particles (when a complete sample digestion process was performed). This was favored here for various reasons. To overcome this uncertainty, ratios of soil-borne elements in the soil should be checked in plant samples to detect soil particle influence, for example the ratio of Al to Ti. Three different methods were shown, to correct the element concentrations measured and to obtain concentrations close to the real plant uptake (physiological concentrations).

It was investigated, whether a higher element uptake results when plants were grown on a soil derived from basalt (close to the village of Bühren, Dransfeld, Lower Saxony). The soil was rich in Fe, Mg, Co, Cr and Ni. Plants grown on a small scale field trial and plants grown in pots were investigated. For many plants elevated concentrations of Co, Ni, Mn, Fe and Zn were detected. The cereal crops (winter rye and winter triticale) showed no enrichment in Co and Ni on Bühren soil. It could be recommended to use large size pots for pot experiments or field trials, to allow the root system to develop freely.

A negative correlation between the concentration of trace metals Co, Ni, Mn and Fe in the soil extractions with ammonium nitrate and the soil pH was detected.

The most important outcome was that the plant species did have a distinctive element uptake, not only for the main nutrients, but also for a large range of elements. The different plant species did show different element concentrations and also diverse TF from soil to plant. The largest differences were often obtained for plant species belonging to different plant families. Lowest trace element contents of Co and Ni were analyzed in Poaceae plants (maize, rye, triticale), and the greatest in Fabaceae plants (hairy vetch, summer and winter faba bean).

The pot and field experiments on soils with soil pH ranging from 4.6 to 7.3 and the soil extractions with ammonium nitrate showed an increased mobility of trace metals (Co, Ni, Mn, Cd and La) at low pH-values. This was an expected result, and this was assumed in the literature for these elements; but there is limited data published on Co and Ni content in plants. These elements are only favorable elements, but not essential in plant nutrition. Although the higher uptake at lower soil pH is an interesting scientific result, it cannot be used as a recommendation of action for farmers to increase their trace element uptake. As the soil pH is low, the DM yield will also be negatively affected.

Concluding with the results of the plant concentrations with the soil parameters, it can be stated that the soil pH is probably the most important factor governing uptake of Co, Ni, Mn and Fe. A high total concentration of these trace metals in the soil can attribute to a greater element concentration in the plants, as was observed for the plants grown on Bühren soil. The differences in uptake of the plant species was also mostly due to different plant families. On Bühren soil the Poaceae plants (triticale, rye) featured in most cases no elevated plant concentrations. In conclusion the factors concerning trace element uptake can be put in order according to their influence:

Soil pH (if < 6.5) < **Total Content (Soil)** < **Plant Species**.

The factor *Soil pH* only takes effect at acidic conditions. The factor *Total Content (Soil)* is determined by the geologic background. After these factors the *Plant Species* is most important. If the first two factors don't change the greatest effect will have the plant species on trace element concentration in the plant.

A prediction of Co trace element concentration in the plant sample resulting only from soil analysis or soil extraction is difficult. For Co or Ni there was a linear trend towards greater plant tissue concentration with greater total soil, or greater concentration in the soil extractions with NH_4NO_3. This held true only for some plant species (winter faba bean or hairy vetch).

The concentrations in the plants were also combined with the DM yield to obtain total element removal from the fields and trace element delivery to biogas plants (Chapter 9). The plants which have the highest element concentrations, did also result in the largest element deliveries in the harvest material. That was because the DM yield of maize and for example faba bean differed by a factor of about two, whereas the Co and Ni concentrations of maize and faba bean differed by a factor of 10 and more.

This led to recommendations for farmers:
Mono grown summer faba bean or double croppings of winter faba bean (sole or intercropped with triticale) followed by maize did result in a good DM yield and in an increased trace element harvest, especially for Co and Ni. Ryegrass is a good crop to increase the amount of Mn, Mo and also Ni in the harvest.

With these recommendations additional environmental benefits will result, for example a greater biodiversity in the fields, without minimizing the farmers' profit. It is unlikely that farmers take care of the trace element content in the harvest, while other parameters, like DM yield are much more (economically) important. One drawback is that faba bean can only be used in crop rotation every 5 years, because it is self-incompatible.

The trends of trace element delivery amounts were the same for the two main field sites with a high quality soil (Garte Nord from research farm Reinshof) and Sömmerling (near Uslar) with average quality soil. For common agricultural soils in western Europe without special geological backgrounds similar trends can be expected. It was pointed out, that with the calculated trace element concentration in the biogas fermenter resulting from a hypothetical input mixture, it is unlikely that a pure plant mixture can deliver all trace element needs. A small addition of liquid manure is needed to guarantee sufficient supply of Co.

10.2 Comments on Phytoremediation

This study was about element uptake of a broad range of elements, including those with economic interest like Co, Ni or REEs. The working methods of researchers in the field of phytoremediation and in this study were similar. As a conclusion from all Chapters in this thesis (with emphasis on Chapter 4), important recommendations for the field of phytoremediation can be derived. For most of the metals of economic interest, it is important to make sure to perform a complete sample digestion, followed by a correction of adhering particles. In Chapter 4, it was pointed out, that the cutting height and also the plant height are crucial factors; small heights and low cutting heights can lead to a higher proportion of adhering soil particles. This soil proportion can alter the trace element concentrations and will feign high element uptake. When calculating metal extraction (phytoextraction) the plant yield should also be taken into consideration. Only then, it is possible to calculate the real time frame to "clean" the soil, which is rarely possible (Sauer and Ruppert, 2013; Sauer et al., 2017).

10.3 Critical Remarks and Outlook

In this study, the element data was corrected for adhering particles and dust, to calculate the element uptake of the different plant species. However, if the energy crops are ensiled and used as biogas substrate the adhering soil particles would contribute to a greater trace element supply. The biggest effect would probably have Co, which was corrected the most (from the trace elements essential for biogas production). For Mn, Mo and Ni this effect was negligible. Until now, research is lacking to identify whether these fine soil particles would be accessible to the microbes in the fermenter.

The challenging measurement of Co with the very small concentration levels in the upper plant harvest implies, that an overall reliable prediction model based on one easy applicable soil extraction method may not be realized. In order to implement a prediction method, a higher sample number, equal sample numbers per plant species, and more soil sample locations with increasing total or available element concentrations are needed to increase the reliability of the correlation.

One approach concerning the Fabaceae plants can pose the implementation of the N-fixation rate. The excretion of H^+ -ions into the rhizosphere should increase with increasing N-fixation rate of the legume. To test this, a special experimental setting is needed, which could not be performed here. For example, with the application of N^{15} marked fertilizer, and a non-legume reference plant the N sources of the plants and the percentage of atmospheric N in the plant tissue can be calculated. Several plant samples with different N-fixation rates are needed to correlate the rate to the element content of Ni, Co, Mn or Fe.

However, this research was a valuable contribution to the field of plant nutrition over a broad element range, and to trace element supply for biogas production. From an ecological point of view, a higher diversity of plant substrates and less use of artificial trace element supplements for biogas plants will help to close element cycles. This avoids the risk of accumulating potentially toxic elements originating from the element supplements, when the biogas residue is returned to the fields. With the overall results of other parts of the research project, for example the field parameters of Katharina Hey and the bioavailability and threshold experiments of Tino Pasold, a more complete picture can be drawn of this highly complex domain.

Appendix A

Additional Data and Tables

A.1 Data Evaluation

Table A.1: Element selection: ICP-OES wavelengths in nm (6 digits), measured in axial mode,"rad" = measured in radial viewing mode, ICP-MS used when atomic mass is given (1 to 3 digits), leading zeros = KED mode.

Digestion	Type	Series	Al	As	Ba	Ca	Cd	Ce	Co	Cr	Cs	Cu	Dy	Er	Eu	Fe	Hf	Ho	K	La	Li	Lu	Mg	Mn	Mo	Na	Nb
2015-05-08	R	w1	396.152	75	233.527	422.673.rad	114	140	230.786	53	133	327.395	163	166	151	234.350	178	165	766.491.rad	139	670.783	175	279.078	257.610	98	568.821	93
2015-05-22	R	w2	396.152	777	233.527	422.673.rad	114	140	230.786	205.560	133	327.395	163	166	151	259.940	178	165	766.491.rad	139	670.783	175	279.078	257.610	98	568.821	93
2015-07-29	R	w5	308.215	777	233.527	422.673	114	140	230.786	53	133	324.754	163	166	151	259.940	178	165	766.491.rad	139	7	175	279.078	257.610	98	568.821	93
2016-11-22	R	w20	396.152	075	233.527	317.933	114	140	230.786	052	133	065	164	166	151	238.204	178	165	766.491.rad	333.749	670.783	175	279.078.rad	257.610	98	568.821	93
2015-07-17	Pl	w4	396.152	777	37	315.887.rad	214.439	140	230.786	267.716	133	327.395	163	166	153	234.350	178	165	766.491.rad	139	7	175	285.213	260.568	98	588.995.rad	93
2015-07-18	Pl	w3	237.312	777	455.403	315.887.rad	228.802	140	230.786	267.716	133	327.395	163	166	153	234.350	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995.rad	93
2015-08-29	Pl	w6	237.312	75	37	315.887.rad	214.439	140	230.786	267.716	133	327.395	163	166	153	234.350	178	165	766.491.rad	139	7	175	285.213	260.568	202.032	330.237	93
2015-09-04	Pl	w7	237.312	75	455.403.rad	315.887.rad	228.802	140	230.786	267.716	133	327.395	163	166	151	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	23	93
2015-09-10	Pl	w8	237.312	777	37	315.887.rad	114	140	230.786	267.716	133	327.395	163	166	151	234.350	178	165	766.491.rad	139	7	175	285.213	260.568	202.032	23	93
2015-09-21	Pl	w9	237.312	777	35	315.887.rad	228.802	140	230.786	267.716	133	327.395	163	166	151	259.940	178	165	766.491.rad	139	7	175	279.553.rad	260.568	98	589.592.rad	93
2015-09-25	Pl	w10	237.312	777	455.403.rad	315.887.rad	228.802	140	230.786	267.716	133	327.395	163	166	151	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	202.032	588.995.rad	93
2015-11-13	Pl	w11	237.312	777	493.408.rad	315.887.rad	228.802	140	230.786	205.560	133	327.395	163	166	153	259.940	178	165	766.491.rad	139	7	175	285.213	257.610	203.846	589.592	93
2015-11-27	Pl	w12	237.312	777	37	315.887.rad	228.802	140	230.786	53	133	327.395	163	166	153	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995	93
2016-01-11	Pl	w13	237.312	777	455.403.rad	315.887.rad	228.802	140	230.786	267.716	133	327.395	163	166	153	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995.rad	93
2016-02-01	Pl	w14	237.312	777	455.403	315.887.rad	228.802	140	59	267.716	133	327.395	163	166	153	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995.rad	93
2016-08-02	Pl	w15	237.312	777	455.403.rad	315.887.rad	228.802	140	59	267.716	133	327.395	163	166	153	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995.rad	93
2016-08-12	Pl	w16	237.312	75	455.403.rad	315.887.rad	228.802	140	230.786	267.716	133	327.395	163	166	151	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995.rad	93
2016-09-28	Pl	w17	308.215	188.980	455.403.rad	315.887.rad	228.802	140	230.786	205.560	133	327.395	163	166	151	259.940	178	165	766.491.rad	139	7	175	285.213	260.568	204.598	588.995.rad	93
2016-11-04	Pl	w18	237.312	075	455.403.rad	315.887.rad	228.802	140	059	267.716	133	327.395	163	166	153	259.940	178	165	766.491.rad	408.671		175	285.213	260.568	204.598	588.995.rad	93
2016-11-14	Pl	w19	237.312	075	455.403.rad	315.887.rad	228.802	140	059	052	133	327.395	163	166	153	259.940	178	165	766.491.rad	408.671	670.783		285.213	260.568	204.598	588.995.rad	93
2016-12-07	Pl	w21	237.312	75	455.403	315.887.rad	228.802	446.021	230.786	267.716	133	327.395	163	166	153	259.940	180	165	766.491.rad	408.671	670.783	175	285.213	260.568	98	588.995.rad	93
2017-01-10	Pl	w22	237.312	075	493.408.rad	315.887.rad	228.802	446.021	059	267.716	133	327.395	163	166	151	259.940	178	165	766.491.rad	408.671	670.783	175	285.213	260.568	204.598	588.995.rad	93
2017-01-20	Pl	w23	237.312	75	455.403.rad	315.887.rad	228.802	446.021	230.786	052	133	327.395	163	166	151	259.940	178	165	766.491.rad	408.671	670.783	175	285.213	260.568	204.598	588.995.rad	93
2017-02-11	Pl	w24	237.312	075	35	315.887.rad	228.802	140	230.786	53	133	327.395	163	166	153	259.940	178	165	766.491.rad	139	670.783	175	285.213	260.568	204.598	588.995.rad	93
2017-02-24	Pl	w25	237.312	188.980	455.403	315.887.rad	228.802	140	Co059	267.716	133	327.395	163	166	153	259.940	178	165	766.491.rad	408.671	670.783	175	285.213	260.568	202.032	588.995.rad	93
2017-03-11	Pl	w26	237.312	188.980	35	315.887.rad	228.802	140	Co059	267.716	133	327.395	163	166	153	238.204	178	165	766.491.rad	408.671	670.783	175	285.213	260.568	204.598	588.995.rad	93
2017-03-24	Pl	w27	237.312	188.980	455.403.rad	315.887.rad	228.802	140	Co059	267.716	133	327.395	163	166	153	259.940	178	165	766.491.rad	408.671	670.783	175	285.213	260.568	204.598	588.995.rad	93
2015-08-28	ext	w28	396.152		55.403	317.933.rad	241.439		230.786	267.716		324.754				259.940			766.491.rad	408.671			279.078.rad	260.568	202.032	588.995.rad	
2015-08-28	ext	w29	396.152		55.403	317.933.rad	241.439		230.786	267.716		324.754				259.940			766.491.rad	408.671			279.078.rad	260.568	202.032	588.995.rad	

Table A.2: Element selection: ICP-OES wavelengths in nm (6 digits), measured in axial mode,"rad" = measured in radial viewing mode, ICP-MS used when atomic mass is given (1 to 3 digits), leading zeros = KED mode.

Digestion	Type	Series	Nd	Ni	P	Pb	Rb	S	Sb	Sc	Se	Sm	Sn	Sr	Ta	Tb	Th	Ti	Tl	Tm	U	V	Y	Yb	Zn	Zr
2015-05-08	R	w1	146	216.555	214.914	220.353	85	182.562	121	335.372	77	152	120	460.733	181	159	232	336.122	205	169	238	92.401	89	172	213.857	343.823
2015-05-22	R	w2	143	231.604	214.914	220.353	85	182.562	121	335.372	77	152	120	460.733	181	159	232	334.941	205	169	238	92.401	89	172	213.857	343.823
2015-07-29	R	w5	146	216.555	213.618	220.353	85	181.972	121	335.372	77	152	120	460.733	181	159	232	334.941	205	169	238	92.401	89	172	213.857	343.823
2016-11-22	R	w20	146	062	214.914	220.353	85	181.972	121	335.372	078	152	120	460.733	181	159	232	334.941	205	169	238	92.401	89	172	066	343.823
2015-07-17	Pl	w4	146	231.604	178.222	220.353	85	182.562	121	361.383	77	147	118	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-07-18	Pl	w3	146	62	178.222	208	85	182.562	121	361.383	77	152	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-08-29	Pl	w6	146	231.604	178.222	217.000	85	182.562	121	361.383	77	147	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-09-04	Pl	w7	146	231.604	178.222	217.000	85	182.562	121	361.383	77	147	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-09-10	Pl	w8	146	231.604	178.222	217.000	85	182.562	121	361.383	77	147	118	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-09-21	Pl	w9	146	231.604	178.222	217.000	85	182.562	121	361.383	77	147	118	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-09-25	Pl	w10	146	231.604	178.222	220.353	85	182.562	121	361.383	77	152	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	172	213.857	90
2015-11-13	Pl	w11	146	231.604	178.222	208	85	182.562	121	361.383	77	147	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2015-11-27	Pl	w12	146	231.604	178.222	208	85	182.562	121	361.383	77	147	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	172	213.857	90
2016-01-11	Pl	w13	146	231.604	178.222	208	85	182.562	121	361.383	77	147	118	421.552.rad	181	159	232	368.520	205	169	238	51	89	174	213.857	90
2016-02-01	Pl	w14	146	231.604	178.222	208	85	182.562	121	361.383	77	147	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2016-08-02	Pl	w15	146	231.604	178.222	208	85	182.562	121	361.383	77	147	118	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2016-08-12	Pl	w16	143	231.604	178.222	208	85	182.562	121	361.383	77	147	118	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2016-09-28	Pl	w17	146	231.604	178.222	217.000	85	182.562	121	361.383	77	147	120	421.552.rad	181	159	232	336.122	205	169	238	51	89	174	213.857	90
2016-11-04	Pl	w18	146	060	178.222	207	85	182.562	121	361.383	078	152	120	421.552.rad	181	159	232	368.520	205	169	238	92.401	89	174	213.857	327.307
2016-11-14	Pl	w19	146	231.604	178.222	220.353	85	182.562	121	361.383	078	147	118	421.552.rad	181	159	232	368.520	205	169	238	292.401	89	174	213.857	343.823
2016-12-07	Pl	w21	146	231.604	178.222	07	85	182.562	121	361.383	077	147	118	421.552.rad	181	159	232	368.520	205	169	238	292.401	89	174	213.857	90
2017-01-10	Pl	w22	146	060	178.222	220.353	85	182.562	121	361.383	078	152	118	421.552.rad	181	159	232	336.122	205	169	238	292.401	89	174	213.857	90
2017-01-20	Pl	w23	146	216.555	178.222	220.353	85	182.562	121	361.383	077	147	118	421.552.rad	181	159	232	368.520	205	169	238	292.401	89	172	213.857	90
2017-02-11	Pl	w24	146	231.604	178.222	220.353	85	182.562	121	361.383	078	147	118	421.552.rad	181	159	232	368.520	205	169	238	292.401	89	174	213.857	90
2017-02-24	Pl	w25	146	221.648	178.222	207	85	182.562	121	361.383	078	147	118	421.552.rad	181	159	232	368.520	205	169	238	292.401	89	174	213.857	90
2017-03-11	Pl	w26	146	231.604	178.222	217.000	85	182.562	121	361.383	077	147	118	421.552.rad	181	159	232	368.520	205	169	238	292.401	89	174	213.857	90
2017-03-24	Pl	w27	146	221.648	178.222	207	85	182.562	121	361.383	078	147	118	421.552.rad	181	159	232	336.122	205	169	238	292.401	89	174	213.857	90
2015-08-28	ext	w28		231.604	187.222			181.972		361.383				407.771.rad				368.520				311.837			213.857	
2015-08-28	ext	w29		231.604	187.222			181.972		361.383				407.771.rad				368.520				311.837			213.857	

Table A.3: Certified values of rock type reference materials in mg/kg, bold face numbers indicate non-certified (informational) values.

Element	**JLk- 1** **Lake sedim.**	**MESS-3** **Marine sedim.**	**TW 45** **Clayey Shale** (in-house std)	**TW 59** **Clayey Shale** (in-house std)	**BB-IGDL** **Basalt** (in-house std)
Al	88500	85900	81500	81500	71400
As	26.8	21.2	8.5	8.5	
Ba	574		380	380	668
B	**15.4**				
Be	3.3	2.3	3	3	
Bi	**2.1**		0.27	0.27	
Ca	4900	14700	25400	25400	59200
Cd	**0.57**	0.24	0.23	0.23	0.092
Ce	88		81	81	82.7
Co	18	14.4	18	18	56.9
Cr	69	105	105	105	151
Cs	10.9		7.8	7.8	0.955
Cu	63	33.9	35	35	51.6
Dy	6.6		5.5	5.5	5.4
Er	3.6		3.1	3.1	2.39
Eu	1.27		1.2	1.2	2.77
Fe	48500	43400	45000	45000	78500
Ga	21.4		21	21	22.5
Gd	6		5.9	5.9	7.81
Ge	**2.2**				
Hf	3.8		4.7	4.7	5.64
Ho	1.06		1.1	1.1	
K	23300	**26000**	30300	30300	14400
La	40.6		40	40	46
Li	52	74	48	48	11.2
Lu	0.57		0.44	0.44	0.25
Mg	10500	**16000**	23500	23500	48200
Mn	2060	324	550	550	1290
Mo	2.2	2.8	0.48	0.48	1.71
Na	7800	**16000**	6600	6600	26200
Nb	15.8		16	16	71.8
Nd	35.7		38	38	46.1
Ni	35	46.9	72	72	188
P	910	**1200**	393	393	2300
Pb	43.7	21.1	38	38	4.86
Pr	8.5		9.3	9.3	11.9
Pt	**0.0014**				
Rb	147		155	155	48.8
S	1050	**1900**	4000	4000	
Sb	1.68	1.02	1	1	0.119
Sc	15.9		14	14	16.9
Se	0.64	0.72	0.7	0.7	
Sm	7.9		6.9	6.9	8.83
Sn	**5.7**	2.5	3.3	3.3	1.95
Sr	68	129	90	90	956
Ta	1.57		2	2	3.97
Tb	1.23		0.85	0.85	0.997
Te	**113**				
Th	19.5		13	13	7.28
Ti	4000	4400	4300	4300	14600
Tl	1.17	0.9	0.77	0.77	0.041
Tm	0.53		0.5	0.5	0.288
U	3.8	**4**	3.3	3.3	1.76
V	117	243	105	105	179
W	4		2	2	0.839
Y	40		31	31	24.8
Yb	4		2.9	2.9	1.87
Zn	152	159	115	115	136
Zr	137		180	180	187

Table A.4: Certified values of plant type reference materials in mg/kg, bold face numbers indicate non-certified (informational) values.

Element	**BCR- 129 hay powder**	**GBW 10052 tea**	**NCS DC 73349 bush leaves**	**WEPAL- IPE- 126 maize plant**	**WEPAL- IPE- 168 sunflower**
Al	112		2000	**700**	**68**
As		0.27	1.25	0.35	0.069
Ba		41	18	2.4	9.2
B		14.1	38	7.1	30.4
Be		0.025	0.051	**0.022**	**0.0051**
Bi		0.04	0.023	**0.0058**	**0.004**
Ca	6400	12100	16800	3640	11000
Cd		0.076	**0.38**	0.132	0.45
Ce		0.81	2.2		
Co	0.12	0.3	0.41	0.166	0.12
Cr	2.5	0.92	2.6	1.11	**0.56**
Cs		0.58	0.27	**0.185**	**0.013**
Cu	10	24	6.6	4.4	8.1
Dy		0.065	**0.13**		
Er		0.037			
Eu		0.022	0.039		
Fe	114	322	1070	530	88
Ga					**0.058**
Gd		0.076	**0.19**		
Ge		0.015			
Hf			**0.15**		
Ho		0.013	**0.033**		
K	33800	15500	9200	18800	27500
La		0.54	1.25		
Li		0.52	2.6	**0.54**	**0.33**
Lu		0.0062	**0.011**		
Mg	1450	2200	4800	1770	2000
Mn	72	1170	61	27.9	124
Mo	1	0.11	0.28	0.52	1.13
Na	3490	100	19600	**179**	388
Nb		**0.05**			
Nd		0.35	**1**		
Ni		5.4	1.7	0.72	1.07
P	2360	2800	1000	1800	3640
Pb		1.6	47	0.96	0.67
Pr		0.093	**0.24**		
Rb	50	89	4.5	**21.4**	**20.5**
S	3160	**4200**	7300	1030	1850
Sb		**0.052**	0.095	**0.015**	**0.03**
Sc	0.017	**0.07**	0.32		
Se	0.025	0.1	0.12	**0.035**	0.23
Sm		0.066	0.19		
Sn		**0.17**	**0.27**	**0.08**	**0.068**
Sr	22.1	36	246	13.2	28.4
Tb		0.0114	0.025		
Th		0.079	0.36		
Ti	3.8	**21**	95	**24.9**	**3.3**
Tl		0.057			
Tm		0.0059			
U		0.047	**0.12**		
V		0.6	**2.4**	1.02	**0.093**
W			**0.06**		
Y		0.52	0.68		
Yb		0.038	0.063		
Zn	32.1	35	55	30.4	148
Zr					

A.1.1 Limits of Detection

Table A.5: Limits of detection for ICP-MS and soil/rocks and plant samples, for Elan Perkin Elmer and iCapQ (since 12.2016). Leading zeros in atomic masses mark KED mode.

	$\mu g/l$				mg/kg			
	plants	**rock/soil**	**plants**	**rock/soil**	**plants**	**rock/soil**	**plants**	**rock/soil**
Machine	**Elan.PerkinElmer**		**iCAPQ**		**Elan.PerkinElmer**		**iCAPQ**	
DF					70	600	70	600
As075			0.009	0.004			0.0006	0.0026
As75	0.465	1.027	0.214	4.237	0.0326	0.6161	0.0150	2.5420
As777	0.173	0.596			0.0121	0.3575	0.0000	0.0000
Ba135	0.384	0.456	0.155	0.342	0.0269	0.2735	0.0109	0.2053
Ba137	0.370	0.190	0.149	0.368	0.0259	0.1138	0.0104	0.2207
Bi209	0.002	0.004	0.001	0.001	0.0001	0.0026	0.0001	0.0008
Cd0114			0.003	0.005			0.0002	0.0027
Cd111	0.008	0.003	0.005	0.007	0.0005	0.0021	0.0004	0.0043
Cd114	0.013	0.004	0.002	0.007	0.0009	0.0025	0.0002	0.0042
Ce140	0.014	0.009	0.006	0.010	0.0010	0.0055	0.0004	0.0060
Co059			0.016	0.005			0.0011	0.0030
Co59	0.030	0.008	0.015	0.004	0.0021	0.0049	0.0011	0.0023
Cr052			0.919	0.481			0.0643	0.2885
Cr053			0.940	1.143			0.0658	0.6858
Cs133	0.005	0.003	0.001	0.003	0.0003	0.0019	0.0001	0.0020
Dy163	0.001	0.001	3.4E-4	4.4E-4	0.0001	0.0006	2.4E-5	0.0003
Er166	0.001	0.002	9.4E-5	3.3E-4	0.0001	0.0009	6.6E-6	0.0002
Eu151	0.001	0.001	2.0E-4	2.3E-5	4.3E-5	0.0009	1.4E-4	1.4E-5
Hf178	0.012	0.020	0.003	0.002	0.0009	0.0119	0.0002	0.0012
Ho165	0.0004	0.0003	0.0001	0.0001	0.00003	0.0002	4.2E-6	0.0001
La139	0.010	0.010	0.003	0.003	0.0007	0.0062	0.0002	0.0020
Li7	0.045	0.130	0.009	0.027	0.0032	0.0779	0.0007	0.0163
Lu175	0.0002	0.0003	4.2E-5	6.4E-5	1.4E-5	0.0002	3.0E-6	0.00004
Mo98	0.042	0.023	0.049	0.012	0.0029	0.0138	0.0034	0.0073
Na23	22.99	8.462			1.6088	5.0773		
Nb93	0.021	0.020	0.004	0.056	0.0014	0.0122	0.0003	0.0335
Nd146	0.007	0.027	0.002	0.001	0.0005	0.0161	0.0002	0.0009
Ni060			0.255	0.153			0.0178	0.0918
Ni062			0.270	0.133			0.0189	0.0800
Ni60	0.532	0.179	0.274	0.144	0.0372	0.1072	0.0192	0.0864
Ni62	0.831	0.187	2.751	1.561	0.0582	0.1125	0.1925	0.9364
Pb206			0.109	0.050			0.0076	0.0303
Pb207			0.112	0.053			0.0078	0.0320
Pb208	0.198	0.148	0.115	0.054	0.0139	0.0886	0.0081	0.0321
Rb85	0.082	0.024	0.078	0.023	0.0058	0.0145	0.0055	0.0138
Sb121	0.006	0.004	0.005	0.006	0.0004	0.0026	0.0003	0.0038
Sm147	0.002	0.003	3.0E-4	1.8E-4	0.0002	0.0021	2.1E-5	0.0001
Sm152	0.003	0.008	4.5E-4	0.001	0.0002	0.0051	3.2E-5	0.0003
Sn0118			0.380	0.246			0.0266	0.1478
Sn118	0.213	0.054	0.371	0.263	0.0149	0.0324	0.0260	0.1576
Sn120	0.343	0.055	0.377	0.256	0.0240	0.0331	0.0264	0.1537
Sr88	0.392	0.160	0.115	0.520	0.0274	0.0959	0.0081	0.3119
Ta181	0.005	0.007	0.000	0.003	0.0003	0.0044	3.5E-5	0.0020
Tb159	0.0002	0.0003	9.7E-5	6.7E-5	1.4E-5	0.0002	6.8E-6	4.0E-5
Th232	0.005	0.008	0.002	0.002	0.0003	0.0046	0.0001	0.0012
Ti049			1.378	0.945			0.0965	0.5670
Ti47	0.800	1.133			0.0560	0.6796		
Ti49	1.556	2.096	2.107	0.260	0.1090	1.2575	0.1475	0.1559
Tl205	0.002	0.002	2.6E-4	0.001	0.0001	0.0011	1.8E-5	0.0007
Tm169	0.0003	0.0003	4.5E-5		5.1E-5	0.0002	1.8E-5	7.3E-4
U238	0.001	0.001	0.001	0.002	0.0001	0.0008	4.8E-5	0.0010
V051			0.073	0.585			0.0051	0.3513
V51	5.082	6.956	2.448	63.18	0.356	4.174	0.171	37.906
Y89	0.008	0.031	0.001	0.002	0.0006	0.0189	0.0001	0.0010
Yb172	0.001	0.005	0.0002	0.0002	0.0001	0.0028	0.00001	0.0001
Yb174	0.002	0.003	0.0001	0.0004	0.0001	0.0017	0.00001	0.0003
Zn066			2.671	0.950			0.1869	0.5700
Zn66	2.805	0.788	2.400	1.029	0.1963	0.4727	0.1680	0.6175
Zr90	0.289	0.120	0.116	0.064	0.0202	0.0719	0.0081	0.0387

Table A.6: Limits of detection for ICP-OES, for Optima Perkin Elmer and Agilent 5100 (used since 04.2015).

	mg/l				mg/kg			
	plants	**rock/soil**	**plants**	**rock/soil**	**plants**	**rock/soil**	**plants**	**rock/soil**
Machine	**Optima Perkin Elmer**		**Agilent 5100**		**Optima Perkin Elmer**		**Agilent 5100**	
VF					70	600	70	600
Al.237.312			0.01470	0.00425			1.029	2.55
Al.396.152	0.02573	0.03275	0.00940	0.00592	1.801	19.651	0.658	3.55
As.188.980			0.00199	0.00043			0.139	0.26
Ba.233.527		0.00106		0.00002		0.638		0.02
Ba.455.403	0.00081	0.00089	0.00018	0.00001	0.057	0.537	0.013	0.01
Ba.455.403.rad			0.00025	0.00001			0.017	0.01
Ba.493.408	0.00512		0.00018	0.00005	0.359		0.013	0.03
Ca.315.887		0.02515		0.00486		15.092	0.000	2.92
Ca.315.887.rad			0.03684	0.01199			2.578	7.19
Ca.317.933	0.23123	0.02716	0.02967	0.00428	16.186	16.296	2.077	2.57
Ca.317.933.rad			0.03078	0.00735			2.154	4.41
Ca.422.673	0.20906	0.01593		0.00820	14.634	9.561	0.000	4.92
Ca.422.673.rad			0.02422	0.01143			1.696	6.86
Cd.214.439	0.00050	0.00065	0.00014	0.00010	0.035	0.391	0.010	0.06
Cd.228.802	0.00091	0.00096	0.00014	0.00007	0.064	0.576	0.010	0.05
Ce.446.021			0.00048	0.00054			0.033	0.32
Co.228.615	0.00175	0.00289	0.00030	0.00035	0.123	1.732	0.021	0.21
Co.230.786	0.00176	0.00100	0.00028	0.00033	0.123	0.599	0.020	0.20
Co.238.892	0.00030	0.00035	0.00083	0.00037	0.021	0.210	0.058	0.22
Cr.205.560		0.00454	0.00111	0.00042		2.722	0.078	0.25
Cr.267.716	0.00299	0.00162	0.00111	0.00034	0.209	0.971	0.078	0.20
Cu.324.754	0.00165	0.00373	0.00060	0.00006	0.116	2.237	0.042	0.03
Cu.327.395	0.00122	0.00197	0.00058	0.00011	0.086	1.183	0.041	0.06
Fe.234.350			0.01659	0.00784			1.161	4.70
Fe.259.940	0.01253	0.01675	0.00502	0.00709	0.877	10.052	0.352	4.25
K.766.491.rad			0.33453	0.07092			23.417	42.55
La.333.749			0.00003	0.00012			0.002	0.07
La.408.671		0.00223	0.00015	0.00005		1.336	0.011	0.03
Li.670.783		0.00088	0.00017	0.00009		0.525	0.012	0.05
Mg.279.078	0.02841	0.00845	0.00513	0.00201	1.989	5.071	0.359	1.20
Mg.279.078.rad			0.01707	0.01997	0.000	0.000	1.195	11.98
Mg.279.553.rad			0.00531	0.00081	0.000	0.000	0.372	0.46
Mg.285.213		0.00770	0.00458	0.00052	0.000	4.618	0.321	0.31
Mn.257.610	0.00251	0.00054	0.00064	0.00012	0.175	0.322	0.045	0.07
Mn.260.568			0.00078	0.00022			0.054	0.13
Mo.202.032	0.00264	0.00279	0.00044	0.00034	0.185	1.674	0.031	0.20
Mo.203.846			0.00063	0.00033			0.044	0.20
Mo.204.598			0.00051	0.00030			0.036	0.18
Na.588.995	0.10999	0.38247	0.00412	0.00115	7.699	229.480	0.288	0.70
Na.588.995.rad			0.12123	0.09931			8.486	59.59
Na.589.592	0.04759	0.03050	0.00475	0.00145	3.332	18.298	0.332	0.87
Nb.309.417		0.00010	0.00026	0.00010		0.057	0.019	0.06
Ni.216.555			0.00097	0.00072			0.068	0.43
Ni.221.648	0.00545	0.00600	0.00094	0.00049	0.382	3.602	0.065	0.29
Ni.231.604	0.00210	0.00203	0.00102	0.00047	0.147	1.219	0.072	0.28
P.178.222	0.03275	0.02814	0.02207	0.00388	2.293	16.884	1.545	2.32
P.214.914	0.03399	0.03450	0.01685	0.00488	2.379	20.703	1.179	2.93
Pb.217.000		0.00645	0.00353	0.00429	0.000	3.868	0.247	2.57
Pb.220.353	0.00995	0.00841	0.00144	0.00066	0.697	5.043	0.101	0.39
S.180.669	0.04619	0.03192	0.01108	0.01793	3.233	19.151	0.776	10.76
S.181.972	0.07115	0.07918	0.01754	0.00259	4.981	47.510	1.228	1.55
S.182.562			0.01955	0.02339			1.369	14.03
Sc.335.372			0.00006	0.00001			0.004	0.01
Sc.361.383	0.00013	0.00008	0.00003	0.00000	0.009	0.046	0.002	0.01
Se.196.026			0.00293	0.00126			0.205	0.76
Sn.189.925		0.00897	0.00154	0.00073		5.381	0.108	0.44
Sr.407.771	0.00146	0.00008	0.00012	0.00002	0.102	0.050	0.008	0.01
Sr.407.771.rad			0.00009	0.00003			0.006	0.02
Sr.460.733		0.00233		0.00014		1.396		0.08
Ti.334.941	0.00121	0.00191	0.00073	0.00053	0.085	1.145	0.051	0.32
Ti.336.122	0.00163	0.00229	0.00069	0.00037	0.114	1.372	0.048	0.22
Ti.368.520		0.00223	0.00081	0.00043		1.341	0.056	0.26
V.292.401		0.00072	0.00016	0.00003		0.431	0.011	0.02
Zn.213.857	0.00376	0.00279	0.00206	0.00026	0.263	1.671	0.144	0.16
Zr.343.823		0.00270	0.00082	0.00053		1.621	0.058	0.32

A.1.2 Reference Value Results - Accuracy

Table A.7: Accuracy of measured rock reference standards in percent deviation from the reported value. Elements sorted alphabetically, these were used for the rock and soil samples, lake sed. = lake sediment, Cl. Sh. = clayey shale (TW-45, in-house std),empty cells = no reported conc. for the standard.

	W-631 BB-IGDL basalt	**W-160 Cl. Sh. 45 shale**	**W-32 Cl. Sh. 45 shale**	**W-64 Cl. Sh. 45 shale**	**W-647 Cl. Sh. 45 shale**	**W-154 JlK-1 lake sed.**	**W-16 JLK-1 lake sed.**	**W-48 JLK-1 lake sed.**
Al	0.4	0.5	1.3	1.7	0.3	3.0	3.0	3.3
As		11.8	27.5	6.2	9.4	13.9	23.4	3.5
Ba	8.1	5.9	2.2	1.7	4.1	0.5	2.1	2.8
Bi		6.9	9.7	6.9	10.6	45.7	47.1	49.9
Ca	2.4	2.5	0.4	0.0	4.4	7.2	0.3	0.3
Cd	2.7	29.7	19.6	33.1	19.2	13.8	12.1	18.1
Ce	15.4	2.1	0.7	2.7	6.4	0.9	1.6	2.2
Co	25.4	2.4	2.0	1.1	3.4	4.8	9.3	7.9
Cr	18.5	29.3	63.2	50.9	51.1	63.2	65.1	1.8
Cs	9.4	3.9	4.2	8.5	4.7	21.3	17.7	14.4
Cu	28.9	1.5	3.8	9.7	4.2	1.5	4.8	4.4
Dy	10.6	5.5	7.6	7.3	1.4	13.5	8.7	10.9
Er	16.5	13.4	11.4	13.4	3.4	10.9	9.3	11.7
Eu	8.1	10.6	7.0	5.8	2.8	2.9	0.4	2.3
Fe	2.3	0.1	0.9	0.3	4.4	0.7	0.3	0.0
Gd	13.9	6.9	2.3	1.7	1.1	22.0	22.3	28.5
Hf	26.6	8.0	11.3	9.8	20.7	2.6	4.3	6.2
Ho		9.1	6.0	7.3	2.8	36.0	35.9	39.0
K	3.4	1.4	1.5	1.9	2.8	2.5	1.2	2.4
La	4.5	1.9	2.9	2.0	3.8	3.0	1.7	2.0
Li	13.6	0.8	5.6	6.5	5.0	4.3	3.4	4.3
Lu	12.1	11.5	2.6	4.5	1.4	2.4	0.2	4.1
Mg	0.3	4.5	3.8	3.2	2.9	0.6	0.2	0.8
Mn		2.3	4.3	3.0	1.8	0.4	2.2	0.3
Mo	1.4	42.5	15.6	16.5	29.7	22.9	10.5	13.1
Na	11.1	8.2	8.1	6.6	6.5	8.2	5.9	5.8
Nb	38.1	5.2	8.0	8.7	14.3	4.5	3.7	3.7
Nd	0.4	9.8	8.0	10.0	9.3	1.0	1.3	3.5
Ni	4.3	0.3	0.1	4.3	6.9	17.2	18.7	5.7
P	1.1	7.3	2.5	1.5	0.7	4.1	6.2	7.3
Pb	9.5	10.0	2.1	0.0	8.1	4.4	13.2	11.5
Pr	2.8	4.1		2.1	19.5	18.3		16.7
Rb	3.8	2.0	1.1	2.0	2.5	1.2	0.5	2.2
S		7.0	15.2	15.5	14.1	5.3	13.0	11.5
Sb	18.3	4.8	6.3	12.8	10.6	13.6	14.6	17.1
Sc	2.5	1.7	8.4	9.0	0.9	1.8	3.7	4.1
Sm	9.1	7.5	3.6	4.0	0.2	2.7	2.0	1.2
Sn	6.7	11.7	6.6	11.7	14.6	4.8	4.7	2.4
Sr	0.1	1.5	0.7	0.3	0.6	4.1	4.3	4.3
Ta	97.6	39.4	45.1	46.0	36.9	7.8	16.2	22.6
Tb	12.3	7.5	0.1	1.0	2.5	10.0	7.1	3.7
Th	17.7	1.7	1.0	6.6	8.9	2.0	2.5	5.0
Ti	4.8	2.6	1.4	1.6	2.2	1.2	2.1	2.1
Tl	6.3	0.6	3.3	1.4	4.2	20.5	21.7	20.9
Tm	8.7	17.0	13.4	16.0	9.2	13.1	8.9	13.8
U	15.3	1.3	0.5	4.9	1.0	6.6	4.1	8.2
Y	0.7	7.1	0.9	3.5	0.5	8.3	8.0	10.3
Yb	12.3	3.6	0.4	2.1	7.4	5.0	1.0	1.7
Zn	17.9	2.7	5.3	4.5	6.6	1.5	1.0	0.1
Zr	0.6	24.8	24.0	22.5	21.7	14.7	14.0	13.4

Table A.8: Accuracy of measured plant reference standards in percent deviation from the reported value. Elements sorted alphabetically, from Al to Mn, GBW= tea leaves, bush l. = bush leaves (NCS DC73349), maize= WEPAL-IPE-126, sunfl.= WEPAL-IPE-168 (sunflower), empty cells = no reported conc. for the standard.

ProjNr	Type	Al	As	Ba	Bi	Ca	Cd	Ce	Co	Cr	Cs	Cu	Dy	Er	Eu	Fe	Gd	Hf	Ho	K	La	Li	Lu	Mg	Mn
W-128	GBW		10.3	2.1	15.7	6.9	6.3	5.4	14.5	10.0	3.7	0.0	7.9	5.7	49.0	2.8	19.3		5.6	0.1	10.1	7.4	15.4	6.5	6.3
W-319	GBW		0.8	5.2	9.6	3.0	0.1	12.5	13.8	20.7	15.9	1.8	7.1	11.4	28.0	4.7	5.3		10.8	0.4	4.3	24.7	2.0	9.2	9.1
W-391	GBW		11.5	0.3	40.9	1.0	8.4	6.7	10.4	54.5	1.6	1.3	12.1	13.5	26.1	2.5	0.5		14.2	0.5	0.4	17.7	0.8	6.3	14.0
W-96	GBW		6.6	5.8	22.6	8.1	6.4	19.2	15.0	28.4	2.7	0.3	1.0	8.5	48.2	0.3	6.3		6.8	0.2	8.6	9.2	13.0	6.7	6.4
W-192	bush l.	0.2	8.9	0.2	30.7	0.9	84.3	0.9	6.2	26.4	4.0	10.9	15.9		11.1	7.1	6.2	42.5	15.0	0.3	8.3	7.0	11.0	8.6	2.7
W-224	bush l.	0.3	10.3	9.1	34.6	2.2	81.2	3.2	5.9	21.4	5.5	12.5	0.0		11.4	10.5	10.5	35.5	18.9	4.2	12.7	14.9	10.1	9.9	4.3
W-256	bush l.	0.7	11.6	2.1	32.0	0.3	78.0	0.9	0.4	25.8	13.0	9.6	2.5		7.8	5.7	14.9	43.0	19.9	2.0	8.7	12.5	9.4	8.3	2.7
W-287	bush l.	0.1	14.6	0.1	33.5	4.1	78.6	2.2	11.3	23.5	4.0	12.8	1.1		13.5	13.3	9.8	34.0	13.9	3.0	13.1	6.9	9.1	4.1	4.0
W-359	bush l.	0.8	4.3	10.3	42.4	3.0	75.9	1.1	4.4	20.2	11.9	14.1	21.1		1.7	16.4	5.9	36.2	5.7	1.8	10.2	12.6	3.8	9.4	2.8
W-423	bush l.	0.2	9.8	2.8	37.5	1.9	81.7	42.1	4.5	16.1	1.1	12.5	26.8		2.3	13.3	0.5	38.6	3.5	5.8	26.7	26.0	3.5	8.1	0.2
W-455	bush l.	1.3	4.0	0.0	36.2	3.9	87.1	33.6	5.6	34.7	1.5	14.5	27.8		0.0	15.1	4.7	33.4	2.0	3.8	19.7	19.6	5.4	9.8	2.2
W-487	bush l.	3.3	3.6	6.4	36.3	3.6	84.8	1.6	9.9	34.1	12.1	10.7	10.6		15.6	12.0	14.7	37.8	16.5	8.2	12.7	5.5	3.1	5.7	5.4
W-519	bush l.	1.9	31.0	7.5	36.4	2.8	86.9	1.2	8.6	17.3	8.7	9.9	9.1		11.4	11.4	12.2	37.0	14.3	6.7	12.8	1.9	4.4	6.5	5.3
W-551	bush l.	5.3	6.5	8.5	48.9	4.6	78.4	21.8	7.3	29.6	25.9	14.2	9.8		26.8	15.1	31.1	43.8	29.5	1.5	30.4	13.1	19.9	10.5	7.6
W-583	bush l.	3.6	11.6	0.3	43.6	2.2	83.8	14.6	9.3	25.9	4.8	10.0	32.1		0.7	3.5	15.4	80.2	1.7	8.2	16.7		16.6	5.1	5.1
W-615	bush l.	2.8	6.7	3.7	49.0	0.7	83.6	10.3	11.4	1.8	11.1	11.2	9.0		16.2	2.8	8.0	47.3	17.6	6.3	5.2	0.1		6.6	5.3
W-679	bush l.	2.4	5.6	11.2	45.1	2.1	94.8	16.0	4.5	15.9	22.7	11.5	5.5		19.3	6.6	16.6	30.6	10.0	5.4	7.6	3.6	13.6	7.2	3.8
W-711	bush l.	1.6	22.8	5.5	47.1	1.7	89.2	8.7	14.6	15.2	24.6	12.7	1.0		24.7	5.4	23.0	38.7	15.9	4.3	15.7	2.3	18.1	6.1	6.2
W-743	bush l.	4.5	9.5	3.4	41.9	3.0	103.0	14.8	8.7	4.0	17.4	7.1	2.0		22.1	2.1	24.6	34.2	12.3	10.2	11.0	3.3	16.1	2.5	2.2
W-775	bush l.	1.2	32.1	0.8	44.4	0.5	94.2	26.7	1.7	12.2	6.8	11.5	14.6		33.9	5.3	36.8	30.6	8.7	5.6	4.9	3.2	28.4	8.2	6.6
W-807	bush l.	2.5	12.5	10.0	44.6	0.1	83.6	30.3	18.4	24.5	7.6	13.1	16.9		35.8	7.3	39.7	27.7	11.5	4.5	18.0	3.9	30.5	6.8	8.0
W-839	bush l.	2.3	6.6	0.1	46.2	0.3	88.2	28.7	20.6	24.6	5.7	12.7	16.6		34.4	6.1	38.3	31.5	9.5	3.6	17.6	1.3	29.9	7.6	7.5
W-871	bush l.	2.6	0.9	6.0	42.3	0.7	85.8	27.1	17.3	21.5	3.0	12.0	12.0		32.0	7.1	36.2	37.2	5.4	4.9	17.4	0.1	25.7	7.3	7.0
W-343	maize	20.3	6.5	115.1	6.5	2.9	6.7		1.1	62.4	12.1	4.5				5.2				7.3		46.7		5.9	1.1
W-375	maize	3.0	3.2	88.7	25.5	2.8	2.9		8.1	8.6	10.7	6.6				7.8				2.9		32.7		2.9	8.3
W-439	maize	19.1	5.2	89.0	12.0	3.3	5.0		7.3	60.0	1.2	10.9				6.3				3.6		71.2		3.5	2.5
W-471	maize	4.4	6.6	61.5	13.6	1.1	3.2		22.8	36.9	13.7	5.7				0.7				4.5		17.6		0.7	3.6
W-663	maize	23.3	4.7	119.7	13.8	4.8	4.3		0.7	54.3	24.4	6.3				10.2				3.5		33.4		2.2	0.7
W-727	maize	12.0	12.7	95.7	22.1	0.4	9.3		4.6	52.8	23.9	2.3				3.5				1.9		23.4		1.5	3.5
W-791	maize	20.4	22.5	90.3	9.7	4.5	8.4		19.3	21.8	2.3	4.0				13.5				4.8		36.1		2.6	0.8
W-823	maize	35.2	12.1	143.0	12.1	1.0	2.3		19.8	33.5	3.9	6.3				15.3				3.7		41.5		0.7	2.4
W-870	maize	22.5	11.3	108.7	20.2	2.0	0.7		24.9	34.4	5.8	8.0				7.1				4.5		30.6		2.6	6.4
W-95	maize	14.6	0.3	102.1	9.9	7.0	4.9		8.8	14.5	8.0	4.0				17.9				2.0		35.2		0.5	4.6
W-503	sunfl.	2.1	224.1	5.6	48.8	2.2	4.1		3.6	17.0	1.2	3.6				5.6				2.6		4.4		3.2	5.4
W-535	sunfl.	0.1	121.6	2.4	47.5	3.0	6.7		15.1	20.6	1.8	7.0				6.4				2.4		14.6		4.2	2.3
W-567	sunfl.	0.1	13.4	0.4	39.5	2.4	3.5		19.7	3.8	10.5	4.4				7.4				36.4				2.7	0.9
W-599	sunfl.	2.1	1.9	1.5	49.5	0.9	1.8		2.2	3.5	3.7	5.4				5.5				2.1		2.1		2.6	3.3
W-695	sunfl.	7.0	27.7	3.0	49.1	1.2	0.6		1.1	15.5	18.2	4.7				8.1				2.9		4.4		3.2	5.0
W-759	sunfl.	2.5	22.3	3.9	54.8	2.9	6.3		2.3	40.8	7.1	2.3				4.5				0.3		9.3		0.3	0.7
W-855	sunfl.	5.5	38.9	7.8	60.2	1.3	4.6		27.6	29.8	3.3	8.3				9.4				6.6		3.5		4.8	7.2

Table A.9: Accuracy of measured plant reference standards in percent deviation from the reported value. Elements sorted alphabetically, from Mo to Zn, GBW= tea leaves, bush l. = bush leaves (NCS DC73349), maize= WEPAL-IPE-126, sunfl.= WEPAL-IPE-168 (sunflower), empty cells = no reported conc. for the standard.

ProjNr	Type	Mo	Na	Nb	Nd	Ni	P	Pb	Pr	Rb	S	Sb	Sc	Sm	Sn	Sr	Tb	Th	Ti	Tl	Tm	U	Y	Yb	Zn
W-128	GBW	29.5	4.3	65.8	9.6	5.7	2.7	3.2	4.8	66.0	1.4	6.5	9.0	6.2	19.8	13.1	22.5	12.9	19.4	21.8	6.8	2.8	7.0	9.2	2.3
W-319	GBW	39.3	2.6	133.0	11.6	16.4	7.5	8.0	8.6	19.5	3.1	20.6	18.3	7.8	58.8	10.1	9.4	7.0	28.6	3.8	2.1	24.9	32.3	6.0	5.4
W-391	GBW	11.7	28.0	138.8	2.8	14.6	2.7	1.6	5.1	38.7	2.7	14.9	24.1	8.4	55.3	8.4	0.4	14.7	55.9	12.4	9.6	30.5	35.4	10.4	2.6
W-96	GBW	54.6	2.4	83.3	12.3	0.6	2.2	13.0	18.0	66.3	1.3	15.6	10.5	8.7	20.8	12.4	16.4	21.4	26.0	20.4	12.3	4.7	10.9	6.4	3.7
W-192	bush l.	1.5	3.9		0.8	23.3	2.6	0.3	10.7	7.1	5.1	34.0	7.0	3.8	115.8	2.2	5.0	1.1	11.1			12.6	23.9	15.6	2.5
W-224	bush l.	19.3	9.0		7.8	19.5	9.0	16.3	4.3	7.0	6.2	3.6	12.5	7.3	85.8	1.6	7.9	1.3	9.1			10.4	19.1	5.1	6.1
W-256	bush l.	2.2	3.3		0.5	17.6	2.7	4.8	11.6	12.4	5.1	13.5	6.2	3.1	121.3	1.1	3.9	3.5	9.7			10.8	14.3	5.6	1.9
W-287	bush l.	4.2	35.9		4.5	22.5	8.9	10.8	5.7	6.6	6.2	6.6	11.6	0.5	399.0	3.7	5.0	4.4	9.6			10.9	22.3	11.8	6.4
W-359	bush l.	4.3			1.1	20.0	8.4	40.8	7.3	17.4	7.8	2.4	9.9	0.9	108.7	1.6	9.3	5.7	8.6			17.5	29.9	25.6	3.5
W-423	bush l.	15.5	0.0		8.7	24.6	8.6	29.7	20.0	10.1	5.1	18.4	11.2	10.1	102.6	2.2	7.6	45.2	5.2			21.8	32.7	31.3	5.3
W-455	bush l.	11.0	1.6		2.2	25.6	9.9	28.8	13.3	11.5	6.1	11.4	12.2	3.9	80.8	0.7	9.7	39.3	4.5			19.9	34.0	31.1	6.8
W-487	bush l.	7.4	2.0		5.4	25.6	7.5	118.4	5.7	20.5	4.5	12.0	6.4	3.8	113.7	6.6	6.8	16.6	10.8			11.5	21.6	15.6	3.7
W-519	bush l.	8.2	0.6		7.2	23.4	6.6	118.7	5.8	17.5	4.2	4.6	7.3	3.3	144.3	5.7	5.9	0.3	3.9			8.3	19.3	14.6	4.2
W-551	bush l.	12.1	5.5		25.0	23.1	10.7	0.8	16.4	28.0	6.7	3.7	11.1	19.9	27.0	0.2	23.5	14.7	7.2			6.3	2.1	1.3	8.4
W-583	bush l.	9.5	0.7		6.3	12.6	7.1	4.1	17.2	13.5	0.6	12.3	7.6	10.9	40.8	3.8	12.2	4.2	8.7			3.6	59.1	33.9	3.9
W-615	bush l.	22.8	1.4		0.2	15.9	7.6	17.7	11.3	0.4	2.3	10.6	8.6	6.5	59.1	2.4	2.6	1.4	8.6			0.6	34.6	8.0	3.7
W-679	bush l.	1.4	10.6		25.4	24.4	8.7	27.8	40.4	18.0	4.1	14.2	10.4	5.5	45.7	3.8	30.5	50.9	3.4			48.1	36.6	6.7	5.4
W-711	bush l.	16.9	0.9		9.7	12.2	7.1	15.2	25.4	14.9	2.3	9.3	10.0	12.0	32.5	2.7	20.4	35.7	21.8			44.5	29.7	2.4	5.9
W-743	bush l.	16.8	6.3		15.3	10.3	2.3	19.4	31.6	0.8	2.9	5.5	7.7	10.9	22.2	6.6	22.6	43.6	9.2			50.7	34.2	3.6	0.6
W-775	bush l.	21.4	1.4		0.1	26.4	7.5	14.7	15.2	39.4	2.9	25.6	9.1	26.7	12.9	3.9	6.4	45.2	6.2			55.6	19.5	25.5	5.3
W-807	bush l.	0.9	1.6		5.0	17.8	8.8	12.5		37.1	2.4	29.7	11.3	30.3	55.9	0.5	3.4	36.3	6.1			58.2	18.9	20.3	6.2
W-839	bush l.	24.1	0.3		3.2	27.0	8.9	9.7	12.1	35.4	3.0	28.0	11.6	30.1	22.0	0.2	2.5	35.6	5.1			50.3	20.9	23.5	7.2
W-871	bush l.	11.0	0.9		1.5	15.7	8.9	7.2	13.9	33.9	2.7	18.4	11.1	26.8	13.9	0.8	8.5	32.6	23.4			50.2	21.0	26.5	5.9
W-343	maize	2.2	33.2			1.3	3.1	1.1		7.4	5.1	144.9			55.9	1.3			142.5						0.6
W-375	maize	16.8	40.4			0.9	0.7	1.4		1.9	2.2	134.4			13.6	2.4			100.6						0.6
W-439	maize	9.4	42.6			6.8	3.3	8.9		0.2	9.0	147.8			25.0	2.9			120.4						6.4
W-471	maize	4.3	23.9			14.3	0.1	0.1		9.1	8.9	142.4			31.8	0.5			95.7						6.9
W-663	maize	10.0	42.4			3.2	3.4	2.6		15.4	7.3	85.2			6.2	2.9			126.7						6.4
W-727	maize	9.2	44.2			5.4	2.3	6.1		0.4	3.2	125.2			19.4	4.3			116.9						4.9
W-791	maize	6.3	33.0			0.4	1.8	40.6		27.0	4.8	80.2			32.8	4.9			126.6						4.8
W-823	maize	12.2	48.9			1.9	0.9	70.1		32.4	5.0	84.9			10.3	2.4			153.0						5.0
W-870	maize	5.0	32.6			3.9	3.2	29.7		29.3	7.3	75.6			80.4	0.6			138.5						7.5
W-95	maize	1.6	42.6			2.9	0.7	0.5		4.0	5.8	158.1			16.5	2.6			124.2						6.4
W-503	sunfl.	1.8	1.8			6.1	3.7	10.2		9.5	2.8	30.2			22.6	5.3			24.2						5.9
W-535	sunfl.	1.2	1.7			0.3	4.8	125.9		13.4	5.8	29.0			35.0	4.7			12.0						0.3
W-567	sunfl.	1.7	0.1			4.3	2.0	2.8		22.7	2.7	38.1			106.3	1.5			12.8						9.6
W-599	sunfl.	0.3	1.6			5.7	3.5	8.3		6.8	3.6	13.6			27.9	2.6			37.3						6.9
W-695	sunfl.	2.7	0.7			3.8	3.9	16.4		13.7	4.6	4.0			31.9	5.0			28.1						4.4
W-759	sunfl.	2.9	4.1			3.9	0.5	11.7		28.1	0.5	7.3			54.2	1.5			36.3						10.7
W-855	sunfl.	0.9	9.9			2.4	5.9	14.0		30.6	6.2	17.8			10.6	7.8			21.2						1.1

A.2 Soil

A.2.1 Soil Element Concentrations

Table A.10: Means of total concentrations in soils (mg/kg DM). From all sites in this study with upper continental crust, local loess and European soil median (GEMAS). Elements in alphabetical order, LS = Lower saxony.

	GN	**SÖ**	**Bühren**	**Ellieh.**	**Lindau**	**Depp.**	**Trögen**	**UCC**[1]	**W. loess**[2]	**GEMAS**[3]
State	LS	LS	LS	LS	LS	LS	LS			
samples	21	22	23	1	2	3	1			
Al	47245	41394	56023	49682	42866	66070	42189	81500	48200	
As	7.6	6.1	5.2	8.7	7.7	13.8	7	4.8	0	5.5
Ba	456	440	497	407	488	393	445	630	410	62
Ca	5545	3125	11685	17703	3652	15332	4039	25700	5000	
Cd	0.25	0.24	0.33	0.28	0.26	0.49	0.27	0.09	0.1	0.18
Ce	73	51	65	76	65	87	66	63	85	28
Co	7.3	8.2	22.6	12.6	6.6	13	7.2	17	10	7.5
Cr	26	21	75	120	28	69	31	92	77	20
Cs	3.5	4.8	1.8	3.2	3.3	7	2.8	4.9	4	
Cu	14	7	19	26.5	16.4	17.8	10.7	28	10	15
Dy	4.2	3	3.8	4.5	4	4.7	3.8	3.9	6.3	
Er	2.3	1.6	1.9	2.5	2.2	2.6	2.1			
Eu	0.97	0.8	1.32	1.2	0.95	1.15	0.87	1	1.4	
Fe	17576	14440	39610	28823	16981	29726	17569	39200	21500	
Hf	9.9	7.1	8.2	8.3	7.9	6.7	8.4	5.3	15	
Ho	0.83	0.59	0.72	0.89	0.78	0.92	0.76	0.83	1.2	
K	21272	20796	16238	21089	20273	32455	20017	23200	19100	
La	39	25	32	35	30	40	29	31	36	14
Li	30	20	18	26	24	101	23	24	37	11
Lu	0.39	0.27	0.3	0.4	0.35	0.41	0.36	0.31	0.55	
Mg	3865	2434	6201	8530	3059	15739	3697	15000	2400	
Mn	703	479	1026	805	723	917	814	770	460	445
Mo	0.48	0.68	1.06	0.64	0.55	1.19	0.62	1.1		0.42
Na	6517	3132	10635	7310	7018	4578	5840	24300	9600	
Nb	28.8	13.2	30.5	25.3	19.1	30.4	18.2	12		
Nd	29	22	27	35	29	37	29	27	35	
Ni	16.4	11.6	76.1	44	15.2	29.6	14.2	47	25	15
P	742	732	1294	936	906	825	700	650		
Pb	28	27	30	32	47	46	30	17	12	16
Rb	107	101	67	90	100	149	87	84	87	
S	243	233	318	309	212	314	237	620	59	
Sb	0.76	0.95	0.69	0.92	1.02	1.1	0.76	0.4		0.23
Sc	6.7	5.2	9.6	8.8	5.8	9.8	6.1	14	9	
Sm	5.4	4.2	5.2	6	5.1	6.2	5	4.7	7.4	
Sr	97	79	214	154	87	89	84	320	180	
Ta	1.28	0.94	1.49	1.29	0.87	0.97	1.01	0.9	1.3	
Tb	0.68	0.5	0.64	0.74	0.65	0.76	0.62	0.7		
Th	13.4	8.4	8.4	11.2	10.7	14.7	10.5	11	12	
Ti	4046	3348	8113	5243	3810	4593	4255	3800	4400	
Tl	0.53	0.58	0.37	0.45	0.51	0.81	0.47	0.9	0.3	0.12
Tm	0.36	0.25	0.29	0.39	0.34	0.4	0.34	0.3		
U	2.9	2.41	2.61	2.46	2.69	3.96	2.7	2.7	3.8	0.77
V	61	45	102	78	48	78	60	97	59	25
Y	24.3	17	20.9	26.7	23.7	28.6	22.4	21	30	
Yb	2.55	1.75	2	2.75	2.37	2.8	2.43	2	3.9	
Zn	54	50	93	75	62	103	52	67	39	45

[1] Upper Continental Crust (Rudnick and Gao, 2003)

[2] local loess, Würm loess (Schnetger, 1992)

[3] Median European Soil (Reimann et al., 2018)

A.2.2 Soil Extraction Data (Ammonium Nitrate)

Table A.11: Concentrations in mg/kg in soil extractions with ammonium nitrate. No value = below LOD (limit of detection). LOD in last row (LOD in the solid sample (mg/kg)).

ProjNr	Series	Location	pH	Al	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	La	Mg	Mn	Mo	Na	Ni	P	S	Sc	Sr	Ti	V	Zn
W-876	w28	Bühr	5.7	0.15	45.9	1780	0.011	0.027	0.0030	0.017	0.048	224	0.009	164	21.3		6.78	0.065	0.90	7.9	0.00014	6.54	0.0029	0.0038	0.07
W-877	w28	Bühr	5.7	0.16	46.0	1780	0.012	0.029	0.0032	0.018	0.047	225	0.009	165	22.3		6.76	0.070	0.93	8.07	0.00018	6.57	0.0027	0.0034	0.08
W-893	w29	Bühr	5.7	0.14	38.1	2210	0.009	0.012	0.0028	0.018	0.074	160	0.010	189	13.5		9.79	0.063	0.75	16.1	0.00024	10.40	0.0044	0.0020	0.04
W-894	w29	Bühr	5.7	0.16	39.4	2180	0.010	0.013	0.0026	0.017	0.080	162	0.010	192	14.0		9.86	0.069	0.76	16.0	0.00021	10.70	0.0047	0.0030	0.05
W-900	w29	Bühr	5.7	0.14	52.6	2130	0.013	0.043	0.0019	0.021	0.039	286	0.014	250	25.8		11.5	0.114	0.72	38.4	0.00018	9.63	0.0023	0.0027	0.11
W-901	w29	Bühr	5.7	0.14	53.9	2180	0.012	0.043	0.0020	0.017	0.045	289	0.014	251	25.6		11.6	0.108	0.74	37.9	0.00016	9.80	0.0026	0.0031	0.10
W-885	w28	Dep	7.3	0.08	18.2	2680	0.002	0.001		0.024	0.019	284	0.006	348	0.4		4.46	0.003	0.69	4.34	0.00011	6.91	0.0034	0.0018	
W-886	w28	Dep	7.3	0.05	17.7	2490	0.002			0.030	0.017	258	0.005	422	0.1		4.37		0.55	3.42		5.81	0.0035	0.0016	
W-887	w28	Dep	7.3	0.06	19.3	2390	0.002	0.001		0.033	0.024	361	0.005	569	0.1	0.002	4.56		0.69	3.93	0.00011	6.83	0.0035	0.0017	
W-882	w28	Dobu	6.4	0.05	27.9	2100	0.003	0.001		0.030	0.016	206	0.004	143	2.1		12.4	0.014	1.01	8.47	0.00014	19.00	0.0028	0.0023	
W-883	w28	Dobu	6.4	0.08	24.6	2070	0.003	0.002		0.036	0.022	224	0.005	141	3.1		6.55	0.016	1.50	9.41		19.20	0.0032	0.0030	
W-884	w28	Dobu	6.4	0.04	26.1	2110	0.002	0.001		0.029	0.016	301	0.004	151	1.5	0.003	5.93	0.009	1.21	11.0		16.60	0.0030	0.0021	
W-902	w29	Ellieh	7.2	0.06	16.4	2520	0.002	0.002		0.106	0.032	347	0.005	80	1.2	0.005	16.9	0.014	1.19	7.2	0.00007	7.17	0.0040	0.0035	
W-903	w29	Ellieh	7.2	0.05	16.7	2540	0.002	0.002		0.107	0.031	350	0.005	81	1.1	0.003	17.1	0.013	1.18	7.52	0.00010	7.20	0.0038	0.0034	
W-872	w28	GN	6.6	0.05	20.1	1620	0.003	0.004		0.038	0.028	235	0.004	105	7.7	0.0025	20.2	0.012	1.24	5.21	0.00012	5.43	0.0028	0.0077	
W-873	w28	GN	6.6	0.05	20.2	1630	0.004	0.003		0.039	0.025	235	0.004	105	8.2	0.0022	20.2	0.014	1.25	5.25	0.00024	5.44	0.0021	0.0083	
W-878	w28	GN	6.6	0.05	14.4	1520	0.002	0.002	0.0022	0.034	0.022	142	0.003	78	3.2	0.0033	9.81	0.006	1.83	2.42	0.00013	4.41	0.0022	0.0091	
W-879	w28	GN	6.6	0.05	38.3	2060	0.005	0.003	0.0010	0.023	0.019	277	0.007	161	10.0		22.4	0.014	0.87	3.95	0.00009	9.61	0.0027	0.0044	
W-892	w29	Lindau	4.6	8.15	50.8	940	0.061	0.111	0.0039	0.060	0.137	92	0.082	39	99.3		4.3	0.383	1.29	12.1	0.00032	5.47	0.0041	0.0022	3.40
W-895	w29	Lindau	4.6	8.04	50.0	928	0.059	0.108	0.0043	0.066	0.125	90	0.080	38	97.8		4.25	0.376	1.28	11.9	0.00033	5.40	0.0041	0.0019	3.34
W-896	w29	Lindau	4.6	4.46	48.3	930	0.049	0.088	0.0035	0.061	0.119	116	0.055	42	89.2		6.54	0.297	1.62	13.6	0.00025	5.26	0.0039	0.0023	2.79
W-897	w29	Lindau	4.6	4.61	48.1	946	0.050	0.088	0.0035	0.064	0.120	116	0.056	42	91.8		6.41	0.299	1.46	13.3	0.00028	5.41	0.0039	0.0018	2.83
W-874	w28	SÖ	6.1	0.12	18.3	1250	0.007	0.009	0.0012	0.017	0.061	218	0.003	40	14.6	0.0021	7.32	0.005	1.26	6.73	0.00017	3.45	0.0022	0.0045	
W-875	w28	SÖ	6.1	0.11	18.0	1260	0.006	0.009	0.0012	0.019	0.058	217	0.003	40	14.2		7.23	0.003	1.23	6.74	0.00013	3.46	0.0023	0.0045	
W-888	w28	SÖ	6.1	0.14	19.2	1080	0.007	0.010	0.0011	0.019	0.049	186	0.003	32	16.6		4.28	0.014	0.87	6.37	0.00018	2.91	0.0019	0.0023	
W-889	w28	SÖ	6.1	0.14	18.1	1270	0.008	0.013	0.0010	0.032	0.052	222	0.003	40	16.3		4.06	0.019	0.92	6.13	0.00014	3.35	0.0016	0.0029	0.04
W-898	w29	Trögen	5.4	0.24	39.5	1150	0.018	0.022	0.0025	0.016	0.036	176	0.008	93	59.0		9.11	0.033	0.87	5.54	0.00009	5.99	0.0024	0.0031	0.27
W-899	w29	Trögen	5.4	0.25	39.9	1160	0.019	0.024	0.0031	0.016	0.039	179	0.007	95	59.4		9.22	0.033	0.90	5.69	0.00018	6.11	0.0024	0.0029	0.28
LOD				*0.008*	*0.005*	*0.7*	*0.0005*	*0.0004*	*0.0009*	*0.008*	*0.006*	*0.5*	*0.0002*	*0.05*	*0.01*	*0.0021*	*0.12*	*0.002*	*0.02*	*0.05*	*6.2E-05*	*0.007*	*0.0002*	*0.0003*	*0.01*

A.3 Plants

A.3.1 Main Nutrient Concentrations in Plants of Main Field Trials

Table A.12: Mean and standard deviation of main nutrients of whole aboveground plant samples in % (g/100g) from sites Garte Nord and Sömmerling, samples from 2015 and 2016.

Species	Ca	Ca sd	K	K sd	Mg	Mg sd	P	P sd	S	S sd
Garte Nord										
Ama	1.69	0.27	3.62	0.48	0.405	0.066	0.439	0.031	0.257	0.042
AF Mix	1.63	0.24	2.70	0.30	0.232	0.043	0.247	0.041	0.181	0.032
Cup Pl.	1.86	0.18	1.45	0.22	0.288	0.049	0.125	0.023	0.067	0.008
FB Su	0.86	0.11	1.84	0.31	0.161	0.018	0.284	0.050	0.109	0.015
FB Wi	0.80	0.07	2.34	0.50	0.178	0.016	0.306	0.045	0.118	0.017
Hairy Vetch	1.34	0.29	2.71	0.56	0.201	0.036	0.324	0.077	0.179	0.030
Maize	0.23	0.02	0.98	0.12	0.103	0.013	0.175	0.019	0.076	0.004
PF Mix	1.20	0.32	2.29	0.12	0.163	0.034	0.260	0.031	0.178	0.044
Rye Wi	0.23	0.05	1.69	0.20	0.074	0.015	0.235	0.022	0.084	0.014
RG	0.55	0.15	2.60	0.29	0.144	0.027	0.277	0.047	0.179	0.064
Tri Wi	0.26	0.03	2.06	0.31	0.082	0.008	0.263	0.021	0.120	0.019
Sömmerling										
Ama	1.95	0.31	4.40	0.54	0.320	0.024	0.517	0.091	0.375	0.051
AF Mix	2.02	0.18	3.40	0.52	0.145	0.014	0.272	0.028	0.262	0.030
Cup Pl.	2.30	0.11	2.70	0.39	0.185	0.008	0.169	0.017	0.095	0.008
FB Su	0.96	0.12	2.13	0.35	0.132	0.008	0.281	0.059	0.133	0.009
FB Wi	0.95	0.24	2.93	0.49	0.156	0.029	0.275	0.051	0.137	0.013
Hairy Vetch	1.29	0.20	3.18	0.52	0.172	0.023	0.314	0.048	0.199	0.039
Maize	0.25	0.03	1.25	0.17	0.086	0.008	0.173	0.026	0.079	0.011
PF Mix	1.30	0.27	2.46	0.45	0.133	0.023	0.270	0.052	0.218	0.057
Rye Wi	0.25	0.04	2.12	0.34	0.066	0.008	0.255	0.023	0.120	0.022
RG	0.53	0.09	3.10	0.59	0.157	0.027	0.319	0.065	0.268	0.058
Tri Wi	0.24	0.04	2.12	0.31	0.067	0.007	0.242	0.024	0.137	0.034

FB Wi/FB Su = winter/summer faba bean, Ama = amaranth, AF/PF Mix = Annual/Perennial flower mixture, RG = ryegrass, Tri Wi = winter triticale

Table A.13: Relative standard deviations (sd) in %, from absolute *mean* and *sd* in Table A.12.

Species	Ca	K	Mg	P	S
Garte Nord					
Amaranth	16	13	16	7	16
AF Mix	15	11	19	17	18
Cup Plant	10	15	17	18	11
Faba Bean (Su)	13	17	11	18	14
Faba Bean (Wi)	9	21	9	15	14
Hairy Vetch	21	21	18	24	17
Maize	10	12	12	11	5
PF Mix	26	5	21	12	25
Rye (Wi)	20	12	20	9	16
Ryegrass	26	11	19	17	36
Triticale (Wi)	14	15	9	8	16
Sömmerling					
Amaranth	16	12	7	18	14
AF Mix	9	15	10	10	11
Cup Plant	5	14	4	10	8
Faba Bean (Su)	12	16	6	21	7
Faba Bean (Wi)	25	17	18	19	10
Hairy Vetch	15	16	14	15	20
Maize	12	14	9	15	14
PF Mix	21	18	17	19	26
Rye (Wi)	15	16	12	9	19
Ryegrass	17	19	17	20	22
Triticale (Wi)	18	15	11	10	25

AF/PF Mix = Annual/Perennial flower mixture

A.3.2 Trace Element Concentrations in Plants of Main Field Trials

Table A.14: Mean and standard deviation (sd) of trace elements corrected for adhering soil particles of whole aboveground plant samples in mg/kg from sites Garte Nord and Sömmerling, samples from 2015 and 2016.

Species	Ba	Ba sd	Co	Co sd	Cu	Cu sd	Fe	Fe sd	La	La sd	Mn	Mn sd	Mo	Mo sd	Ni	Ni sd	Rb	Rb sd	Sr	Sr sd	Zn	Zn sd
Garte Nord																						
Ama	14.4	5.2	0.048	0.009	4.62	0.26	57	9	0.08	0.02	44	11	0.68	0.36	0.19	0.08	32.5	11.4	39.9	10.4	21.7	3.2
AF Mix	8.4	2.9	0.025	0.013	6.05	1.25	36	13	0.07	0.04	37	9	0.38	0.23	0.22	0.05	8.5	2.6	36.8	5.1	21.5	3.4
Cup Pl.	9.9	4.6	0.026	0.005	3.38	0.34	36	11	0.08	0.01	24	3	0.08	0.02	0.33	0.01	4.3	0.7	42.3	3.6	8.2	2.4
FB Su	5.3	2.5	0.182	0.035	8.00	1.86	42	7	0.06	0.04	42	7	0.63	0.19	0.85	0.16	7.9	2.2	21.0	3.4	26.7	4.2
FB Wi	4.2	1.6	0.139	0.036	7.56	1.07	77	37	0.08	0.08	35	6	0.90	0.37	0.66	0.18	6.2	1.3	18.1	4.0	36.6	29.4
H Vetch	11.2	6.7	0.085	0.032	7.81	3.23	73	20	0.11	0.05	31	10	1.06	0.31	0.85	0.24	8.6	2.9	33.0	7.8	30.5	4.3
Maize	1.3	0.5	0.007	0.005	3.47	0.35	35	9	0.03	0.02	18	2	0.40	0.18	0.13	0.06	2.2	0.8	5.3	1.2	14.3	2.6
PF Mix	10.7	5.0	0.057	0.010	6.45	0.43	53	11	0.09	0.04	32	5	0.52	0.14	0.37	0.06	6.8	2.0	31.6	6.5	25.4	13.1
Rye Wi	11.0	2.7	0.010	0.010	3.06	0.30	27	7	0.02	0.01	18	4	1.00	0.32	0.12	0.05	4.6	1.9	6.4	1.1	11.2	1.7
RG	17.1	5.9	0.019	0.010	4.24	0.82	43	16	0.04	0.03	65	25	2.50	1.30	0.70	0.18	8.5	4.6	15.7	4.3	18.6	2.7
Tri Wi	8.4	1.8	0.013	0.008	4.22	0.48	33	9	0.02	0.01	27	3	1.29	0.25	0.19	0.09	5.2	1.3	7.0	0.8	16.5	2.3
Sömmerling																						
Ama	22.3	7.9	0.128	0.054	5.19	0.82	55	6	0.07	0.04	50	31	0.38	0.13	0.16	0.09	5.5	0.8	39.1	9.0	27.7	7.3
AF Mix	15.6	1.3	0.140	0.073	6.50	1.84	28	11	0.33	0.13	44	10	0.19	0.05	0.26	0.07	4.6	2.1	39.6	3.3	30.3	6.2
Cup Pl.	16.2	1.8	0.031	0.022	4.67	0.52	36	4	0.15	0.07	31	6	0.09	0.01	0.26	0.04	3.8	0.6	44.9	1.9	14.8	1.5
FB Su	7.3	2.0	0.262	0.074	6.06	1.73	50	17	0.04	0.03	44	12	0.35	0.18	0.49	0.12	6.2	1.1	19.4	1.9	32.0	4.4
FB Wi	7.1	2.1	0.164	0.065	6.56	1.70	53	25	0.05	0.04	43	11	0.29	0.16	0.38	0.11	6.7	2.2	17.4	4.1	27.5	5.6
H Vetch	16.2	7.4	0.146	0.061	6.25	1.18	78	45	0.06	0.04	43	17	0.33	0.21	0.74	0.21	8.1	2.6	27.5	3.9	46.5	8.9
Maize	1.7	0.3	0.006	0.007	3.36	0.58	33	4	0.02	0.01	17	3	0.31	0.17	0.13	0.05	2.1	0.6	5.0	0.7	18.6	4.8
PF Mix	13.5	3.2	0.120	0.070	5.48	0.69	33	8	0.12	0.06	35	9	0.34	0.13	0.27	0.08	3.5	0.7	27.1	4.2	24.7	7.0
Rye Wi	16.1	4.9	0.013	0.010	3.49	0.26	36	7	0.02	0.01	15	4	0.46	0.24	0.17	0.13	3.5	1.9	6.1	0.9	18.8	2.9
RG	15.9	4.3	0.026	0.020	5.02	1.13	39	19	0.06	0.05	73	22	1.62	0.85	0.39	0.13	5.2	2.3	11.6	2.3	26.1	5.9
Tri Wi	10.5	1.7	0.007	0.006	3.15	0.59	28	9	0.01	0.01	18	5	0.48	0.29	0.10	0.04	3.1	1.4	5.2	0.9	16.6	3.5

FB Wi/FB Su = winter/summer faba bean, Ama = amaranth, AF/PF Mix = Annual/Perennial flower mixture, RG = ryegrass, Tri Wi = winter triticale

Table A.15: Relative standard deviation (sd) in % from absolute *mean* and *sd* in Table A.14

Species	Ba	Co	Cu	Fe	La	Mn	Mo	Ni	Rb	Sr	Zn
Garte Nord											
Amaranth	36	18	6	16	32	25	52	41	35	26	15
AF Mix	35	52	21	37	51	25	59	25	31	14	16
Cup Plant	46	18	10	31	18	11	30	4	16	9	29
Faba Bean (Su)	47	19	23	17	57	17	30	19	28	16	16
Faba Bean (Wi)	38	26	14	48	109	17	41	27	21	22	80
Hairy Vetch	60	38	41	28	46	33	29	28	34	24	14
Maize	41	73	10	25	56	11	44	45	36	22	18
PF Mix	46	18	7	21	43	16	28	15	29	21	52
Rye (Wi)	25	99	10	26	73	20	32	46	42	18	16
Ryegrass	35	53	19	37	86	39	52	26	54	27	15
Triticale (Wi)	21	56	11	26	69	13	19	46	25	11	14
Sömmerling											
Amaranth	35	42	16	11	61	62	34	55	15	23	26
AF Mix	8	52	28	38	40	22	26	28	46	8	20
Cup Plant	11	71	11	12	48	19	16	17	17	4	10
Faba Bean (Su)	27	28	28	33	76	27	51	25	18	10	14
Faba Bean (Wi)	29	40	26	47	72	25	55	30	33	24	20
Hairy Vetch	46	42	19	57	56	40	62	29	32	14	19
Maize	19	119	17	12	60	20	53	36	28	13	26
PF Mix	24	58	13	24	49	25	37	30	21	16	28
Rye (Wi)	31	77	7	20	65	24	53	76	54	15	16
Ryegrass	27	79	22	48	80	30	53	32	44	20	23
Triticale (Wi)	16	79	19	34	72	29	61	46	46	18	21

AF/PF Mix = Annual/Perennial flower mixture

A.3.3 Element Concentrations of Plant Samples from Additional Sites

Table A.16: Element concentrations (Al to Mo) corrected for adhering particles (method 3) of (whole aboveground) plants in mg/kg DM. All samples grown on soil of Bühren, Cultiv. = Cultivation (field or pot experiments).

Spec .	Cultiv.	Al	As	Ba	Ca	Cd	Ce	Co	Cr	Cs	Cu	Dy	Er	Eu	Fe	Hf	Ho	K	La	Li	Lu	Mg	Mn	Mo
Ama	field	7	0.126	21.8	18200	0.24	0.04	0.13	0.28	0.021	4.7	2.2E-03	2.5E-03	2.9E-04	40	8.8E-03	9.2E-04	54200	0.160		1.5E-04	3650	62	0.44
Ama	field	20		30.9	24600	0.36	0.09	0.13	0.18	0.033	5.5	5.0E-03	5.1E-03	6.8E-04	61	1.8E-04	1.9E-03	50600	0.256		2.9E-04	5940	77	0.47
Ama	field	18	0.099	20.9	17800	0.33	0.05	0.11	0.34	0.019	4.0	2.4E-03	2.3E-03	4.0E-04	46	1.5E-03	8.9E-04	42400	0.164	0.007	7.5E-05	3620	58	0.32
Ama	field	15	0.099	17.0	14000	0.20	0.06	0.09	0.30	0.027	3.5	2.9E-03	2.6E-03	4.7E-04	39		9.6E-04	32200	0.153	0.005	8.8E-05	3310	49	0.47
Ama	field	22	0.163	33.0	21000	0.32	0.09	0.11	0.33	0.029	5.0	3.6E-03	3.3E-03	5.7E-04	59	2.0E-03	1.3E-03	41100	0.227	0.007	1.0E-04	5050	68	0.43
FB Su	field	16	0.012	9.1	10100	0.09	0.05	0.34	0.38	0.020	7.9	9.2E-04	1.3E-03		60		5.1E-04	20700	0.113	0.009		2160	49	0.79
FB Wi	field	77	0.086	8.8	9030	0.11		0.27	0.47	0.020	7.4	4.1E-03	1.8E-03	8.4E-04	97	3.1E-03	8.4E-04	29400	0.131	0.064	1.4E-04	2750	53	0.90
H. Vetch	field	17	0.152	13.3	11100	0.02	0.00	0.11	0.32	0.008	6.1	5.0E-03	1.8E-03	5.3E-04	90		1.0E-03	25900	0.250	0.002	5.8E-06	1900	30	0.84
Rye Wi	field	5	0.004	14.9	3060	0.09	0.01	0.01	0.27	0.003	3.2	1.1E-04	2.4E-04		28	1.8E-04	8.1E-05	17800	0.024	0.012		1030	20	0.53
RG	field	282	0.095	23.8	7710	0.14	0.17	0.21	2.69	0.012	10.7	5.0E-04	2.1E-04	2.5E-03	454		9.5E-04	50900	0.027	0.018		2810	51	1.04
RG	field	94	0.093	21.6	9230	0.04		0.05	0.68	0.010	6.7	1.6E-03	1.4E-03		139	1.2E-03	7.8E-04	40800	0.070	0.025	7.7E-06	2550	100	1.16
Tri Wi	field	29	0.060	12.2	2600	0.13		0.01	0.19	0.005	3.3	5.4E-04	1.7E-04		45	6.2E-06	1.5E-04	20300	0.020	0.007		1080	27	0.43
FB Wi	pot	18	0.070	34.6	8280	0.09	0.02	0.57	0.33	0.012	8.7	4.4E-03	2.0E-03		88	2.0E-03	9.7E-04	36800	0.190	0.015	1.4E-04	2820	84	0.23
H. Vetch	pot	16	0.127	76.5	14100	0.05	0.10	0.23	0.26	0.011	6.3	1.4E-02	5.5E-03		55	2.6E-04	2.8E-03	31100	0.424	0.031	3.5E-04	2280	55	0.46
Rye Wi	pot	10	0.006	30.3	2840	0.12	0.02	0.03	0.48	0.001	4.6	6.4E-04	4.4E-04		53	1.2E-03	1.8E-04	18400	0.018	0.019	3.6E-05	1210	79	1.04
RG	pot	10	0.047	45.4	4290	0.06		0.03	0.49	0.002	4.4	3.4E-03	1.2E-03		33	2.8E-03	6.3E-04	27500	0.092	0.008	1.4E-04	1600	57	1.24
RG	pot	141	0.236	64.7	6810	0.08	0.18	0.02	0.38	0.033	7.6	7.5E-03	1.2E-02		35	2.3E-02	3.8E-03	26300	0.105	0.244	8.6E-04	2630	80	1.22
Tri Wi	pot	6	0.056	30.9	3210	0.13		0.04	0.14	0.005	4.6	5.2E-04	2.1E-04		42	1.6E-04	1.1E-04	18700	0.041	0.046	2.8E-05	1300	112	0.74
Ama	small pot	33	0.030	36.6	11700	1.35	0.03	0.46	0.29	0.309	8.4	4.6E-03	2.3E-03	2.8E-03	46		1.0E-03	27000	0.102	0.074	1.9E-04	4560	346	0.35
Ama	small pot	59	0.017	33.2	9610	1.07	0.02	0.65	0.44	0.263	9.8	2.1E-03	1.7E-03	2.3E-03	69		7.2E-04	21700	0.081	0.068	1.3E-04	3820	379	0.36
Ama	small pot	67	0.036	33.9	12500	1.34	0.04	0.59	0.33	0.397	9.3	4.4E-03	2.2E-03	2.7E-03	42	4.6E-04	1.5E-03	21900	0.100	0.065	3.6E-04	5160	383	0.33
Ama	small pot	30		23.7	8010	0.94	0.03	0.64	0.24	0.255	8.1	2.9E-03	1.9E-03	2.0E-03	47		6.8E-04	21000	0.075	0.104	2.2E-04	4120	265	0.23
FB Su	small pot		0.017	21.0	8540	0.15	0.07	1.08	1.02	0.029	5.7	4.7E-03	2.1E-03	1.9E-03	44		1.0E-03	34300	0.144	0.231	2.2E-04	2540	76	0.56
FB Su	small pot		0.028	22.9	8250	0.16	0.06	0.99	1.21	0.037	5.2	4.1E-03	2.9E-03	1.7E-03	35		1.1E-03	31800	0.124	0.226	2.5E-04	2290	88	0.39
FB Su	small pot	19	0.030	28.0	7040	0.16	0.11	1.77	0.37	0.025	5.4	5.7E-03	3.1E-03	2.7E-03	50		1.2E-03	33400	0.187	0.079	3.1E-04	2640	101	0.56
FB Su	small pot	20	0.004	18.6	7510	0.12	0.06	0.92	0.99	0.027	5.4	3.5E-03	2.2E-03	1.8E-03	47		1.1E-03	31800	0.102	0.196	3.9E-04	2210	72	0.37

Ama = amaranth, FB Su/Wi = winter/summer faba bean, H. Vetch= Hairy vetch, rye wi = winter rye, RG = ryegrass

Table A.17: Continued table: Element concentrations (Na to Zn) corrected for adhering particles (method 3) of (whole aboveground) plants in mg/kg DM. All samples grown on soil of Bühren (field or pot experiments).

Spec .	Cultiv.	Na	Nb	Nd	Ni	P	Pb	Rb	S	Sb	Sc	Sm	Sr	Ta	Tb	Th	Ti	Tl	Tm	U	Y	Yb	Zn
Ama	field	6	0.000	0.029	0.82	3570	0.17	23.4	2290	5.0E-03	1.4E-03	1.7E-03	66.3	6.0E-04	8.6E-04	5.8E-03		0.013	1.4E-04	6.8E-03	0.046	2.2E-03	34
Ama	field	23	0.006	0.062	1.36	4180	0.65	36.2	2410	6.9E-03		5.1E-03	91.4	9.4E-04	1.6E-03	6.4E-03		0.033	3.6E-04	6.4E-03	0.087	3.8E-03	34
Ama	field	19	0.005	0.038	1.09	3000	0.16	16.5	2070	7.8E-03	6.1E-04	2.4E-03	72.8	1.3E-03	8.9E-04	2.5E-03		0.018	1.0E-04	2.3E-03	0.044	1.5E-03	39
Ama	field	11	0.005	0.039	0.76	2890	0.15	20.5	1460	7.2E-03	1.6E-03	2.9E-03	51.2	9.5E-04	9.3E-04	4.6E-03		0.013	1.5E-04	3.2E-03	0.042	1.7E-03	28
Ama	field	19	0.004	0.060	1.36	3740	0.16	25.0	2380	7.2E-03	6.7E-04	4.8E-03	80.1	9.7E-04	1.3E-03	5.5E-03		0.020	1.8E-04	3.5E-03	0.064	2.0E-03	35
FB Su	field	194	0.013	0.028	2.15	3050		13.4	1310	6.9E-03	6.1E-03	1.8E-03	34.7	1.3E-03	5.4E-04	2.0E-03	2.6	0.003	3.1E-05	4.6E-03	0.027	7.9E-04	57
FB Wi	field	314	0.060	0.062	2.56	3040	0.02	31.6	1650	7.2E-03	1.3E-02	6.6E-03	34.3	2.8E-03	1.3E-03	1.2E-02	10.6	0.011	2.0E-04	3.9E-03	0.037	1.0E-03	47
H. Vetch	field	12	0.019	0.087	2.97	2650	0.05	19.6	1780	5.7E-03	3.2E-03	7.5E-03	48.8	6.1E-04	1.9E-03	2.0E-03	1.2	0.007	1.5E-04	7.5E-04	0.060	4.7E-04	57
Rye Wi	field	6	0.001	0.008	0.09	2070		9.5	1290	3.1E-03	2.6E-03	5.1E-04	14.3	2.8E-04	1.2E-04	2.4E-03		0.001		5.9E-04	0.004	1.5E-04	21
RG	field	487	0.396	0.087	4.78	4160	0.10	51.0	3030	7.0E-03	3.7E-02		33.5	1.5E-02	3.4E-03	2.6E-02	90.0	0.008		1.2E-02	0.040		43
RG	field	327	0.092	0.037	2.53	3220	0.28	32.5	2170	1.3E-02	1.2E-02	2.0E-03	26.8	3.6E-03	1.3E-03	2.0E-02	15.7	0.003	1.5E-04	1.5E-02	0.035	5.9E-04	34
Tri Wi	field	13	0.026	0.010	0.20	2240	0.11	17.4	1960	3.3E-03	5.2E-03	7.8E-04	10.2	8.8E-04	3.2E-04	2.1E-03	3.6	0.001	1.2E-05	1.1E-03	0.006		29
FB Wi	pot	133	0.012	0.073	2.52	3660	0.10	10.6	1680	1.5E-02	6.4E-03	7.5E-03	30.6	6.4E-04	1.4E-03	3.7E-03	2.3	0.004	2.1E-04	1.2E-03	0.053	1.1E-03	50
H. Vetch	pot	11	0.010	0.185	2.63	2720	0.21	11.9	1690	4.2E-02	4.0E-03	2.0E-02	55.5	6.2E-04	3.8E-03	5.6E-03		0.020	5.8E-04	1.8E-03	0.150	2.7E-03	59
Rye Wi	pot		0.004	0.009	0.50	3300		1.8	1900	6.7E-03	3.7E-03	1.1E-03	10.8	3.7E-04	1.8E-04	2.1E-03	0.9	0.001	3.8E-05	6.9E-04	0.006	3.8E-04	32
RG	pot	88		0.066	2.08	3190	0.15	3.6	1870	1.3E-02	3.5E-03	8.4E-03	15.8	9.0E-05	1.1E-03	2.4E-02		0.001	1.6E-04	5.2E-03	0.022	9.2E-04	30
RG	pot	235	0.028	0.088	1.60	3770	3.19	4.0	2960	7.4E-02	1.1E-02	5.1E-03	31.5	6.4E-03	2.9E-03	5.7E-02		0.006	8.1E-04	2.4E-02	0.104	1.2E-02	34
Tri Wi	pot	3	0.003	0.010	0.43	2650	0.08	2.8	3190	9.0E-03	2.1E-03	9.4E-04	11.9	3.5E-04	2.0E-04	2.4E-03		0.002	2.7E-05	5.6E-04	0.006	1.4E-04	32
Ama	small pot	72		0.032	1.39	6410	0.68	115.0	3220	2.7E-02	3.9E-03	5.4E-03	30.7	2.7E-04	8.1E-04	6.1E-04		0.041	2.7E-04	2.4E-03	0.047	1.5E-03	78
Ama	small pot	60		0.024	1.54	6260	0.28	86.1	3240	2.8E-02	4.5E-04	2.8E-03	23.4	1.8E-04	4.2E-04	1.1E-03		0.025	1.8E-04	1.6E-03	0.037	1.5E-03	88
Ama	small pot	60		0.038	1.42	6690	0.45	112.0	3620	2.9E-02	4.2E-03	6.4E-03	32.5		1.2E-03	2.4E-03		0.037	3.9E-04	2.0E-03	0.059	2.0E-03	84
Ama	small pot	47		0.025	2.13	6020	0.48	81.4	3020	2.5E-02	1.3E-03	3.7E-03	20.8	6.1E-05	5.1E-04	1.8E-03		0.026	1.3E-04	1.6E-03	0.039	1.8E-03	62
FB Su	small pot	205		0.049	6.42	2600	0.67	13.0	1940	4.4E-02	6.6E-04	6.9E-03	26.6	6.5E-04	9.3E-04	2.9E-03		0.008	2.4E-04	2.7E-03	0.046	1.7E-03	50
FB Su	small pot	160		0.041	4.84	2100	1.03	9.8	1970	5.8E-02	6.1E-04	6.9E-03	26.0	1.2E-03	9.3E-04	2.6E-03	4.7	0.005	3.9E-04	2.7E-03	0.047	2.5E-03	50
FB Su	small pot	168		0.077	7.52	2300	0.90	10.5	2090	3.2E-02	6.2E-03	1.3E-02	26.6	3.2E-04	1.7E-03	6.4E-03	0.6	0.004	3.1E-04	2.6E-03	0.059	2.2E-03	49
FB Su	small pot	151		0.034	4.60	2160	0.73	8.5	1780	4.3E-02	2.6E-03	7.0E-03	23.4	1.6E-03	8.3E-04	4.8E-03		0.005	4.3E-04	2.6E-03	0.038	2.2E-03	48

Ama = amaranth, FB Su/Wi = winter/summer faba bean, H. Vetch= Hairy vetch, rye wi = winter rye, RG = ryegrass

Table A.18: Mean plant element concentrations in mg/kg DM per plant species, corrected for adhering particles (M3), from samples from Aholfing and Straubing, collected in 2015, n = number of samples, for REEs only La is included.

	Hairy vetch Aholf	**Hairy vetch Straub**	**Hung. vetch Aholf**	**Hung. vetch Straub**	**Pea Aholf**	**Pea Straub**	**Rye Aholf**	**Rye Straub**	**Triticale Aholf**	**Triticale Straub**
Al	14	17	34	9	14	14	13	17	15	14
As	0.023	0.010	0.053	0.018	0.062	0.017	0.022	0.009	0.021	0.007
Ba	9.8	7.4	9.8	5.6	8.6	8.0	18.0	16.4	15.1	13.2
Ca	9000	11900	10700	11700	11100	10900	2690	2450	3130	2630
Cd	0.019	0.019	0.035	0.023	0.033	0.033	0.027	0.032	0.030	0.033
Co	0.057	0.060	0.058	0.034	0.028	0.028	0.025	0.012	0.016	0.013
Cr	0.357	0.346	0.408	0.295	0.125	0.253	0.249	0.296	0.220	0.317
Cs	0.014	0.020	0.023	0.034	0.012	0.021	0.003	0.007	0.004	0.003
Cu	5.28	7.78	4.49	6.8	4.41	6.6	3.65	4.71	3.51	4.48
Fe	56.8	53.3	63.8	40.2	55.2	44.3	36.4	34.2	38.1	31.9
K	23500	20800	22500	17600	16000	13800	15700	14600	16000	15000
La	0.18	0.07	0.15	0.08	0.09	0.08	0.02	0.03	0.03	0.02
Li	0.018	0.017	0.024	0.008	0.011	0.044	0.034	0.042	0.019	0.015
Mg	1840	2080	2170	2230	2110	2060	993	959	1150	1080
Mn	36.0	29.8	43.8	27.5	51.3	36.0	29.5	25.5	46.7	52.6
Mo	0.99	0.48	0.53	0.21	0.75	0.17	0.99	0.52	1.00	0.59
Na	12.1	28.5	12.9	10.3	22.3	54.6	9.98	20.5	14.5	29
Nb	0.004	0.008	0.007	0.003	0.002	0.003	0.008	0.004	0.005	0.006
Ni	1.01	1.43	0.967	0.926	1.01	1.34	0.209	0.194	0.23	0.241
P	3230	2820	2590	2510	2820	2500	2750	2560	2860	2540
Pb	0.12	0.10	0.25	0.09	0.06	0.09	0.10	0.05	0.08	0.05
Rb	7.8	6.0	6.4	5.8	5.9	4.8	3.5	3.7	3.4	3.8
S	1830	1800	1650	1570	1530	1660	1520	1240	1940	1600
Sb	0.0114	0.0164	0.0139	0.0163	0.0062	0.0085	0.0062	0.0064	0.0086	0.0076
Sc	0.0035	0.0022	0.0060	0.0012	0.0046	0.0035	0.0043	0.0037	0.0056	0.0029
Sr	16.2	18.3	17.9	16.9	17.4	16.5	5.88	5.31	6.37	4.93
Ti	0.60	0.95	2.03	0.38	0.44	0.99	0.99	1.13	1.05	1.15
Tl	0.0015	0.0041	0.0020	0.0047	0.0073	0.0099	0.0003	0.0003	0.0007	0.0005
Y	0.15	0.05	0.12	0.04	0.05	0.04	0.02	0.02	0.04	0.02
Zn	38.1	30.1	25.2	22.5	34.5	23.6	21.3	19.5	26.2	21.2
n	*13*	*14*	*6*	*5*	*5*	*6*	*17*	*16*	*17*	*16*

A.3.4 Field Experiment Details

Table A.19: Crop plan Garte Nord for both years of trial. Plot indicates plot number in experimental field site. Growing season is indicated.

Plot	Var.	Variants	Plot	Var.	Variants
1	M 15	M 15	45	DW 14/15	Ryegrass 14/15
2	SIL	Cup plant	46	einj Blüh 16	AF Mix 16
3	A 16	A 16	47	A/M 15	A/M 15
4	WAB/WT 15/16	Fb (Wi)/Trit. (Wi) 15/16	48	WAB 15/16	Faba b (Wi) 15/16
5	SAB 15	Faba b (Su) 15	49	WT 15/16	Trit. (Wi) 15/16
6	SAB 16	Faba b (Su) 16	50	SIL	Cup plant
7	WAB/WT 14/15	Fb (Wi)/Trit. (Wi)14/15	51	SAB 15	Faba b (Su) 15
8	WT 15/16	Trit. (Wi) 15/16	52	einj Blüh 15	AF Mix 15
9	WT 14/15	Trit. (Wi) 14/15	53	M 15	M 15
10	WAB 15/16	Faba b (Wi) 15/16	54	A 15	A 15
11	einj Blüh 16	AF Mix 16	55	SAB 16	Faba b (Su) 16
12	A/M 15	A/M 15	56	WAB 14/15	Faba b (Wi) 14/15
13	einj Blüh 15	AF Mix 15	57	mehr Blüh	PF Mix
14	WiR 15/16	Rye/vetch 15/16	58	WiR 14/15	Rye/vetch 14/15
15	M 16	M 16	59	WT 14/15	Trit. (Wi) 14/15
16	A 15	A 15	60	A/M 16	A/M 16
17	WiR 14/15	Rye/vetch 14/15	61	DW 14/16	Ryegrass 14/16
18	mehr Blüh	PF Mix	62	M 16	M 16
19	DW 14/15	Ryegrass 14/15	63	WAB/WT 14/15	Fb (Wi)/Trit. (Wi) 14/15
20	WAB 14/15	Faba b (Wi) 14/15	64	A 16	A 16
21	A/M 16	A/M 16	65	WiR 15/16	Rye/vetch 15/16
22	DW 14/16	Ryegrass 14/16	66	WAB/WT 15/16	Fb (Wi)/Trit. (Wi)15/16
23	SAB 16	Faba b (Su) 16	67	SIL	Cup plant
24	WT 14/15	Trit. (Wi) 14/15	68	A/M 15	A/M 15
25	WAB 15/16	Faba b (Wi) 15/16	69	SAB 15	Faba b (Su) 15
26	WT 15/16	Trit. (Wi) 15/16	70	einj Blüh 16	AF Mix 16
27	M 15	M 15	71	A 15	A 15
28	WAB/WT 15/16	Fb (Wi)/Trit. (Wi) 15/16	72	einj Blüh 15	AF Mix 15
29	A 16	A 16	73	WT 15/16	Trit. (Wi) 15/16
30	einj Blüh 16	AF Mix 16	74	SAB 16	Faba b (Su) 16
31	SIL	Cup plant	75	DW 14/15	Ryegrass 14/15
32	WAB/WT 14/15	Fb (Wi)/Trit. (Wi) 14/15	76	M 15	M 15
33	SAB 15	Faba b (Su) 15	77	WAB 15/16	Faba b (Wi) 15/16
34	mehr Blüh	PF Mix	78	M 16	M 16
35	A/M 15	A/M 15	79	A 16	A 16
36	DW 14/16	Ryegrass 14/16	80	WiR 14/15	Rye/vetch 14/15
37	A/M 16	A/M 16	81	WAB 14/15	Faba b (Wi) 14/15
38	WiR 15/16	Rye/vetch 15/16	82	WAB/WT 15/16	Fb (Wi)/Trit. (Wi) 15/16
39	DW 14/15	Ryegrass 14/15	83	A/M 16	A/M 16
40	WiR 14/15	Rye/vetch 14/15	84	DW 14/16	Ryegrass 14/16
41	einj Blüh 15	AF Mix 15	85	WAB/WT 14/15	Fb (Wi)/Trit. (Wi) 14/15
42	M 16	M 16	86	mehr Blüh	PF Mix
43	A 15	A 15	87	WiR 15/16	Rye/vetch 15/16
44	WAB 14/15	Faba b (Wi) 14/15	88	WT 14/15	Trit. (Wi) 14/15

Table A.20: Crop plan Sömmerling for both years of trial. Plot indicates plot number in experimental field site. Growing season is indicated.

Plot	Var.	Variants	Plot	Var.	Variants
1	A/M 15	A/M 15	45	A 16	A 16
2	WT 15/16	Trit. (WI) 15/16	46	SIL	Cup plant
3	DW 14/15	Ryegrass 14/15	47	M 15	M 15
4	WAB 15/16	Faba b (Wi) 15/16	48	SAB 16	Faba b (SU) 16
5	einj Blüh 16	AF Mix 16	49	SAB 15	Faba b (SU) 15
6	M 15	M 15	50	WAB/WT 15/16	Fb (Wi)/Trit. (Wi) 15/16
7	SAB 16	Faba b (SU) 16	51	WAB/WT 14/15	Fb (Wi)/Trit. (Wi) 14/15
8	einj Blüh 15	AF Mix 15	52	WT 15/16	Trit. (WI) 15/16
9	SIL	Cup plant	53	einj Blüh 16	AF Mix 16
10	A 15	A 15	54	WT 14/15	Trit. (WI) 14/15
11	SAB 15	Faba b (SU) 15	55	WAB 15/16	Faba b (Wi) 15/16
12	DW 14/16	Ryegrass 14/16	56	WiR 15/16	Rye/vetch 15/16
13	WT 14/15	Trit. (WI) 14/15	57	einj Blüh 15	AF Mix 15
14	mehr Blüh	PF Mix	58	A 15	A 15
15	WiR 14/15	Rye/vetch 14/15	59	M 16	M 16
16	A/M 16	A/M 16	60	A/M 15	A/M 15
17	WAB 14/15	Faba b (Wi) 14/15	61	DW 14/15	Ryegrass 14/15
18	A 16	A 16	62	WiR 14/15	Rye/vetch 14/15
19	WiR 15/16	Rye/vetch 15/16	63	mehr Blüh	PF Mix
20	M 16	M 16	64	WAB 14/15	Faba b (Wi) 14/15
21	WAB/WT 14/15	Fb (Wi)/Trit. (Wi)14/15	65	DW 14/16	Ryegrass 14/16
22	WAB/WT 15/16	Fb (Wi)/Trit. (Wi)15/16	66	A/M 16	A/M 16
23	A 15	A 15	67	WT 14/15	Trit. (WI) 14/15
24	einj Blüh 16	AF Mix 16	68	WAB 15/16	Faba b (Wi) 15/16
25	SAB 15	Faba b (SU) 15	69	SAB 16	Faba b (SU) 16
26	A/M 15	A/M 15	70	M 15	M 15
27	SIL	Cup plant	71	WT 15/16	Trit. (WI) 15/16
28	einj Blüh 15	AF Mix 15	72	WAB/WT 15/16	Fb (Wi)/Trit. (Wi) 15/16
29	DW 14/15	Ryegrass 14/15	73	einj Blüh 16	AF Mix 16
30	WT 15/16	Trit. (WI) 15/16	74	SIL	Cup plant
31	SAB 16	Faba b (SU) 16	75	A 16	A 16
32	WAB 15/16	Faba b (Wi) 15/16	76	WAB/WT 14/15	Fb (Wi)/Trit. (Wi) 14/15
33	M 15	M 15	77	SAB 15	Faba b (SU) 15
34	M 16	M 16	78	A/M 16	A/M 16
35	WAB 14/15	Faba b (Wi) 14/15	79	mehr Blüh	PF Mix
36	WiR 14/15	Rye/vetch 14/15	80	A/M 15	A/M 15
37	A 16	A 16	81	DW 14/16	Ryegrass 14/16
38	WAB/WT 15/16	Fb (Wi)/Trit. (Wi) 15/16	82	WiR 15/16	Rye/vetch 15/16
39	A/M 16	A/M 16	83	DW 14/15	Ryegrass 14/15
40	WiR 15/16	Rye/vetch 15/16	84	WiR 14/15	Rye/vetch 14/15
41	mehr Blüh	PF Mix	85	einj Blüh 15	AF Mix 15
42	WAB/WT 14/15	Fb (Wi)/Trit. (Wi) 14/15	86	WAB 14/15	Faba b (Wi) 14/15
43	DW 14/16	Ryegrass 14/16	87	A 15	A 15
44	WT 14/15	Trit. (WI) 14/15	88	M 16	M 16

Table A.21: DM content of plant variants of the two main field trials. Data collected by Katharina Hey.

Variant	Cultivar	DM (%)	
		Reinshof, Garte Nord	Sömmerling
Rye/ vetch	Conduct, Welta	25.6	
		28.4	24.9
Faba bean (Wi)	Nordica	13.9	12.6
		13.3	12.9
Triticale (Wi)	Balu	23.9	28
		26.9	25
Faba bean/ triticale	Nordica, Balu	20.1	20.8
		22	19.3
Faba bean (Su)	Fanfare	16.8	16.5
		18.1	18.8
AF Mix	BG 80	23.6	18.9
		21.2	25.8
Amaranth	Bärnkrafft	19.8	23.5
		22.8	24.9
Maize (main)	Amadeo	36.7	32.9
		34.4	40.4
Amaranth/ maize	Bärnkrafft, Amadeo	35.3	31.5
		33	41.4
Maize (second)	Simpatico	21.6	22.5
	Cathy (GN), Pion7326 SÖ)	33.3	29.7
Ryegrass	Alligator	22.7*	17.7*
		25.5*	26.9*
PF Mix	BG 70	24.5	22
		31.8	42.3
Cup plant	Chrestensen	21.5	35.4

* mean of annual cuts.

A.4 Supplement to Chapter 4

This Chapter is a published article in Chemosphere. This is a part of the supplement data to this article.

A.4.1 Discussion and Comparison of the Correction Methods

Evaluation of Method 1

The correction with a fixed indicator element (Ti used here) results in a stronger correction than method 2 and method 3 (A), Figure S A.1. In that case we will set all Ti values to 0. Ta, Al, Th, Sc, Zr and Hf are also strongly diminished, the median of corrected to uncorrected is almost 0 for these elements.

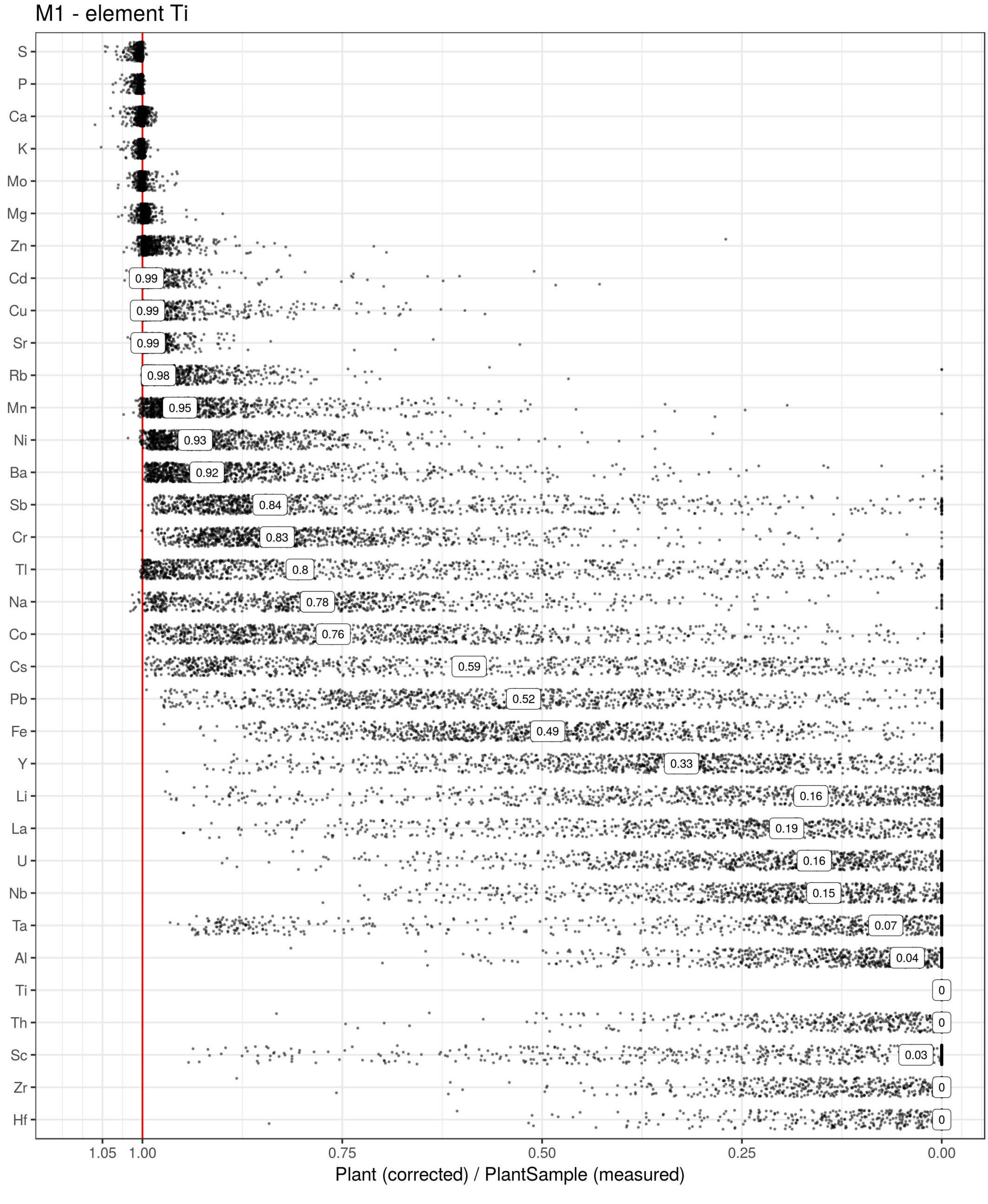

Figure A.1: Distribution of ratios (1040 samples) of *Plant*/*PlantSample* (corrected/uncorrected) for main and trace elements. Corrected values of *Plant* calculated via Method 1, with indicator element Titanium. The elements are sorted as in Figure 2 of the publication.

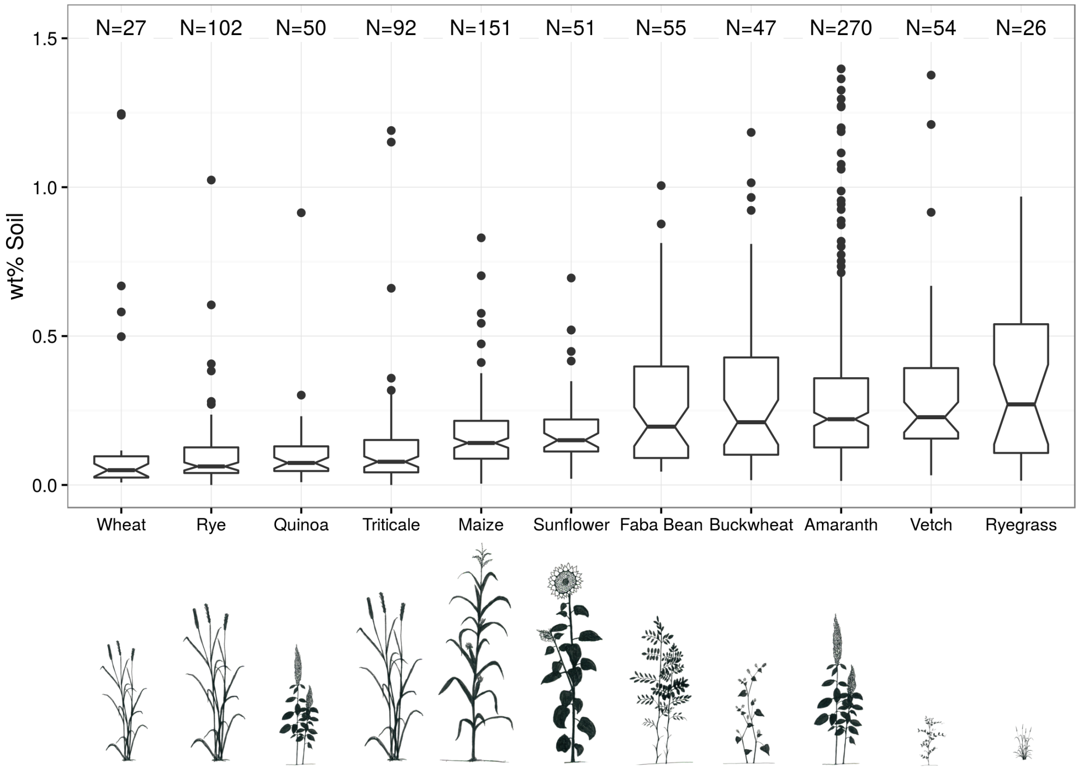

Figure A.2: Wt-% of adhering soil particles on plants by species grown in field trials - sorted by median of wt-% of adhering particles (x). N = number of samples per species.

A.4.2 Influence by Plant Species

The quantity of adhering soil particles largely depends on the type of the plant surface structure, the roughness of foliar surface area, and on the plant's height. The median of the content of AP, x derived via method 3, was calculated for each species with more than 25 samples. In Figure S A.2 the distribution of adhering particles is shown per species in notched boxplots. For each species the samples are derived from minimum 4 different locations from north and south Germany. Crop plants with long blades and small foliar surfaces at lower plant parts (wheat (0.05), rye (0.06), triticale (0.08)) have significantly less adhering particles than crop plants with rough or large foliar surface (maize (0.14), amaranth (0.22)). Plants with large foliar surface close to the ground show the largest amount of adhering soil particles: ryegrass (0.27) and vetch (0.23). Quinoa (0.07), and Faba Bean (0.20) loose their lower leaves before harvest and therefore only the stem is exposed to sputtering soil. Hence the plant has significant less adhering soil.

Appendix B

Curriculum vitae

Wiebke Fahlbusch

Date of Birth: 01.04.1985
Place of Birth: Duderstadt

Scientific education

06/2004	Abitur (Gymnasium Corvinianum Northeim)
2004-2005:	Study of Physics (Georg-August-Universität Göttingen)
2005-2012:	Study of Geosciences (Georg-August-Universität Göttingen)
Bachelor thesis 2009:	(Structural Geology) Strukturgeologische Analyse von Klüften und Mineralgängen im Götemargranit und seinen Rahmengesteinen (supervisors: Dr. A. Vollbrecht, Prof. B. T. Hansen)
Mapping thesis 2011:	Geologische Kartierung von frühproterozoischen Plutoniten in der Umgebung von Hummelstad, Västervik Region (SE-Schweden) (supervisors: Dr. A. Vollbrecht, Prof. B. T. Hansen)
Diploma thesis 2012:	(Geochemistry) Sortenspezifischer Transfer von Spurenelementen in Energiepflanzen auf kontaminiertem Boden bei Harlingerode (Niedersachsen) (supervisors: Prof. Dr. H. Ruppert, Dr. B. Sauer)
07/2012-07/2017	Doctoral study, Dep. Sedimentology/Environmental Geology

Working experience

2012-2014:	**Research assistant**, research project: Handlungsempfehlungen für eine optimierte Prozessbiologie in Biogasanlagen
2012-2017:	**Research assistant**, research project: Möglichkeiten einer ausgeglichenen Spurenelementversorgung von Biogasanlagen durch Mischungen unterschiedlicher Energiepflanzen und Bioenergetische Nutzungskonzepte für kontaminierte landwirtschaftliche Standorte (NiCo), and research project: Bioenergie im Spannungsfeld: Bioenergetische Nutzung von kontaminierten Standorten
2009-2012:	**student assistant** research project: Bioenergie im Spannungsfeld: Bioenergetische Nutzung von kontaminierten Standorten (H. Ruppert)
05-08/2008:	**student assistant** Department for Applied Geology
02/2008-03-2009	**Trainee** at LBEG, Landesamt für Bergbau, Energie und Geologie, Hannover.

Publications (articles and abstracts)

- **Fahlbusch, W.**; Sauer, B.; Ruppert, H.: *Molybdän in Biogasanlagen: Mangelsituation durch Rübensubstrat*, Kassel, KTBL/FNR Biogaskongress, 26./27. September 2013 (poster)
- **Fahlbusch, W.**; Sauer, B.; Ruppert H.: *Energy crop production on mining and smelting impacted arable land - A non-phytoremediation approach*, Florence, Goldschmidt, 2013 (oral presentation)
- Eberl, V.; **Fahlbusch, W.**; Fritz, M.; Sauer, B. (2014): Screening und Selektion von Amarantsorten und –linien als spurenelementreiches Biogassubstrat. Berichte aus dem TFZ, Straubing, (37), pp 1-116
- Sauer, B; **Fahlbusch, W**; Ruppert, H. (2017): Bioenergetische Nutzungskonzepte für kontaminierte Standorte. In: *Bioenergie im Spannungsfeld*. Ed. by Ruppert, H and Ibendorf, J. Göttingen: University Press, pp 333-353. ISBN: 978-3-86395-164-1
- Pospiech, S.; **Fahlbusch, W.**; Sauer, B.; Pasold, T.; Ruppert, H. (2017): Alteration of trace element concentrations in plants by adhering particles - Methods of correction. Chemosphere 182, S. 501-508 **(dual first authorship)**
- **Fahlbusch, W.**; Hey, K.; Sauer, B.; Ruppert, H.: *Können vielfältigere Pflanzenmischungen den Spurenelementmangel in Biogasanlagen bei hohem Maisinput beheben?*, KTBL/FNR Biogaskongress, Bayreuth, 10./11. September 2017 (poster)
- **Fahlbusch, W.**; Pospiech, S.; Sauer, B; Pasold, T; Ruppert, H.: *Trace elements in plants - correction methods for adhering particles to get real plant uptake*, Paris, Goldschmidt, 2017 (poster)

Teaching

- Workshops in 2016 and 2017: Introduction to *R* and *R* Studio. (2x 2h), Seminar for Sedimentology and Environmental Geology.
- Lecture and seminar: **Using R for geochemical analysis**, working with ggplot2, plotly and rmarkdown. 3 day course.
- Referee of 3 bachelor theses in environmental geochemistry:
  - A. Sorger: *Spurenelementgehalte verschiedener Getreide-Leguminosen-Gemenge auf zwei bayerischen Standorten und ihre Bedeutung für die Biogasproduktion* (Trace element contents of cereal-legume intercropping grown on two sites in Bavaria and their relevance for biogas production), supervisors: Ruppert, Fahlbusch, summer term 2015
  - M. Simon Willerding-Möllmann: *Eignung unterschiedlicher Bodenextraktionsmethoden zur Bestimmung der Pflanzenverfügbarkeit von Cobalt und Nickel* (Suitability of different soil extraction methods to determine the bioavailybility of cobalt and nickel), Faculty of Agronomy, supervisors: Steingrobe, Fahlbusch, summer term 2015
  - J. Popp: *Contamination in the Tsumeb mining area (Namibia) – a comparison of ICP-OES and –MS with portable XRF-results*, supervisors: Ruppert, Fahlbusch, summer term 2017.

Appendix C

Data on CD-ROM

Additional data is provided on CD-ROM.

C.1 Element data of all soil and rock samples from ICP-OES and ICP-MS

The most appropiate wavelength or atomic mass is chosen. All data in mg/kg.

C.2 Element Data

All plant element data in mg/kg DM. Data combined from ICP-MS and ICP-OES. The most appropiate wavelength or atomic mass was chosen as listed in Tables A.1 and A.2. The MS Excel file contains:

- "plants_uncorr-ppm": Uncorrected element data from plant samples, that means, not corrected for adhering particles.

- "plants_corrected-M3-ppm": Corrected data for adhering particles, corrected with Method 3.

- "Ref_Standards_measured_values": All measured concentrations of the international and in-house reference standard materials, measured in this study. Data combined from ICP-MS and ICP-OES. The most appropiate wavelength or atomic mass was chosen. Rock and plant reference materials.

C.3 Application: Fit of the Reference Standard Materials

The Folder named: "App_plot_Standards" contains the App to check the fit of the Standards as described in Section 2.1.6. All Standard materials measured in the working group of Prof. Ruppert between September 2015 and February 2018 are included. The App can be accessed with *R*. Open "app.r" and either click "RunAPP" or type: runApp("PATH") in the console. The package "shiny" is required (Chang et al., 2017).

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