First comprehensive study on total contents and hot water extractable fraction of selected elements in 19 medicinal plants from various locations in Nyamira County, Kenya

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Keywords: inductively coupled plasma mass spectrometry, sequential filtration, medicinal plants, trace elements, hot water extraction

Abstract

A large number of medicinal plants is traditionally known in Kenya and used for treatment of various diseases, for example diabetes, where metals are supposed to be involved in pathogenesis and therapy. Therefore, detailed investigation of the concentration of a large number of metals in medicinal plants is required for improved understanding and optimisation of the therapeutic role of metals and also to exclude potentially toxic effects. Our study focused on the determination of 30 selected elements in 19 medicinal plant species each collected from 3 sampling locations in Nyamira County, Kenya. The obtained comprehensive data set showed large variability and multivariate data analysis revealed that the differences in the elemental composition were stronger dependent on the plant species than on the

sampling location. In addition, hot water extractions were performed to mimic the traditional preparation of medicine from the plants. It was found that the mean extraction efficiencies were below 20% except for B, Mg, P, K, Mn, Co, Ni, Cu, Zn, Rb, Mo, Cd and Tl, which are mostly essential elements apart from Cd and Tl. Sequential (ultra)filtration of the extracts was applied as novel approach for molecular size-fractionation of the extracted elemental species. The results indicate more than 50% low molecular weight species (< 3 kDa) for Mg, Mn, Co, Ni and Zn while predominantly larger size-fractions (>3 kDa up to <5 µm) were detected for V, Cu, Al and Fe.

Introduction

Traditional medicinal plants are an important part of health care in Kenya due to the large variety of more than 1200 freely and locally available wildlife plants with known therapeutic effects [1,2]. The high demand due to growing popularity accompanied by increased commercialisation of herbal medicine causes concerns about over-exploitation of the natural plant resources in Kenya [3,4]. Many studies focus on the documentation of the classification, identification and basic traditional knowledge on the preparation and medical application of most commonly used plants. For example, there are many medicinal plants with known anti-diabetic effects which offer the potential to provide affordable health care to the rising number of diabetes patients in Kenya [5]. However, few studies focussed on detailed chemical analysis of the applied medicinal plants. In particular elemental concentrations can be relevant either for therapeutic use in case of deficiency or regarding potentially toxic effects in case of contamination of the plants. More specifically, sufficient evidence was found indicating that metals play an important role in development and treatment of diabetes [6].

Determination of selected metals was reported by Maobe et al. in 8 medicinal plants from Kisii region, Kenya concluding that the observed levels were within the allowed limits of WHO [7], by Adongo et al. in 7 medicinal plants from Chuka community, Kenya, suggesting potential therapeutic effects of Zn, Mg and Fe [8] and by Piero et al. in 5 antidiabetic medicinal plants from Kenya indicating that the

determined levels of some elements with glucose lowering effects are sufficient to potentially contribute to the treatment of diabetes [9]. Oyaro et al. analysed metal contents in 6 medicinal plants from Narok County, Kenya, and also in hot water infusions of these plants [10]. It was found that the percentage extraction efficiencies of the investigated metals in the infusions were very low for Cu and Fe, but much higher for Cr and Co. The determined levels were considered safe for application due to the relatively low solubility in infusions.

All these studies applied atomic absorption spectrometry for elemental detection and thus a maximum of 10 elements was determined due to limited or lacking multi-element capability of this analytical technique. In addition the number of investigated plants was relatively low (5 to 8) due to the use of time-consuming wet ashing procedures. Samples were mainly collected from one location, in some cases from several locations, but finally mixed prior to the analysis and thus resulting in one average metal content per plant species. Variations of metal concentrations for the same plant species collected from different sites were not reported [7-10]. Considering that many medicinal plants are prepared by soaking in water, as decoction or as infusion prior to application, the extraction efficiency is an important parameter to estimate the actual dose of metals during therapy and needs more detailed investigation. Finally, there has been no attempt in the reported studies to characterise the elemental binding forms (species) in the plant extracts regarding their molecular size or structure. However, this information is required in order to better understand the potential role of metals in the therapeutic effect of the medicinal plants. Are certain metal species acting as therapeutic agents or is only the total metal content significant independent of the present elemental species? In analogy this also applies for potentially toxic effects. Considering the availability of the same species of naturally grown medicinal plants in various regions of Kenya, another important aspect is the variability of the elemental contents of the same plant species sampled from different locations.

The present study is the result of a co-operation between the Universities of Chuka and Nairobi, Kenya and the research center in Juelich, Germany. Joint resources allowed access to 19 medicinal plants

from 3 sampling locations in Nyamira County, Kenya and determination of 30 elements using microwave digestion with subsequent quantification by inductively coupled plasma mass spectrometry (ICP-MS). Hot water extractions simulating the traditional preparation were performed for the plants from one location and analysed for the same elements in order to estimate the percentage of the total elemental content which is actually administered to the patient during therapy. Moreover, sequential (ultra-) filtration was applied for selected plant extracts as novel approach to achieve a quick size fractionation of the water soluble elemental species.

Experimental

Sample collection and pre-treatment

Samples from 19 medicinal plants were collected by herbalists in each of the following 3 districts of Nyamira County: Nyamira (NMP), Manga (MMP) and Borabu (BMP). The plants were identified by experts from the National Museum of Kenya, Nairobi. An overview of the investigated plants is given in Table 1 including the number coding for reference throughout this manuscript, e.g. NMP1 refers to *Warburgia ugandensis* from sampling location NMP. The collected plant samples were washed with deionised water to remove soil and other material from the plant surface, then air dried in the shade and pre-ground in a wooden mortar. Then, the samples (except for NMP3 and NMP17) were packed in polyethylene bags and shipped to research center Jülich for further analysis. The plant samples were fine ground using a ball mill with zirconium oxide vessels and balls (Pulverisette 6, Fritsch, Germany). Moisture was determined in duplicate for approximately 0.5 g aliquots of ground plant material at 105°C until constant weight.

Chemicals and reference materials

Nitric acid (65%, suprapure) and hydrogen peroxide (30%, suprapure) were obtained from VWR, Darmstadt Germany. Deionised water was prepared with a Millipore system. Reference materials NIST

1640a (natural water), NIST 1515 (apple leaves) and NIST 1547 (peach leaves) were purchased from LGC standards, Germany.

Microwave digestion

Aliquots of approximately 70 mg of the plant samples were digested in duplicate using 2 mL of nitric acid and 1 mL of hydrogen peroxide in a MARS 5 closed vessel microwave system (CEM, Germany) at 160°C. Complete digestion of the organic matrix was achieved with occasionally slight silicate residues, which was fit for the purpose of this study. The digestion solution was transferred to calibrated polystyrene sample vials and made up to 10 mL with deionised water. Blank digestions and digestions of plant reference materials were processed in the same way.

Hot water extraction

Approximately 150 mg of ground plant material were mixed with 40 mL deionised water in a glass beaker including a glass rod to prevent retardation of boiling and covered by a watch glass. The sample suspension was heated within 6±0.5 min to boiling temperature and kept boiling for 5.5±0.5 min. Temperature was checked using a liquid-in-glass thermometer in a separate beaker containing water only. After cooling to room temperature the mixture was transferred to a polypropylene tube and shaken for 13 h in the dark on a horizontal shaker at 100 motions per minute. Loss of water due to evaporation was compensated by topping up with deionised water to 40 mL. Finally, the plant suspensions were filtered through 0.45 µm syringe filters to obtain clear extracts for elemental analysis.

Sequential (ultra-)filtration

Hot water extractions were prepared for BMP10 and NMP18 as described above (n=3). In addition extractions of the same plant samples were performed by shaking with deionised water at room temperature in the dark for 13 h without any heating (n=3). The obtained raw extracts were first filtered

using a 5 µm syringe filter. An aliquot of the obtained filtrates was then filtered through 0.45 µm syringe filters. An aliquot of this second filtrate was subjected to ultrafiltration through 10 kDa membrane using Amicon filtration units at a speed of 14000 g (Merck-Millipore, Germany). An aliquot of the third filtrate was finally subjected to ultrafiltration through 3 kDa membrane using Amicon filtration units at a speed of 14000 g (Merck-Millipore, Germany). The ultrafiltration units were pre-cleaned by filtration of 0.5% nitric acid and deionised water prior to filtration of the samples following previous work [11]. The filtrates were analysed by ICP-MS as described below. The elemental contents were related to the solid plant material. In addition to the <3 kDa fraction, the size fractions 3 kDa-10 kDa, 10 kDa-0.45 µm and 0.45 µm-5 µm were calculated as difference of the contents in the respective filtrates.

Quantification by ICP-MS

Total contents of 30 elements (B, Mg, Al, P, K, Ca, Ti, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ga, Rb, Sr, Y, Nb, Mo, Cd, Ba, Nd, Sm, Gd, Er, Yb, Tl and Pb) were determined in the digestion solutions of the plant samples using inductively coupled plasma mass spectrometry (Agilent 7500, Agilent Technologies, Japan) with He-collision cell mode. The instrument was equipped with a micromist nebuliser and double pass spray chamber. Quantification was performed by external calibration using Rh as the internal standard. NIST 1640a natural water reference material was analysed for quality control. Recoveries were in a range from 96.4% to 107.7% (22 elements with certified values). In addition plant reference materials were analysed. Moisture corrected recoveries for NIST 1515 and NIST 1547 were in a range from 82% to 116% for B, Mg, Al, K, Ca, V, Mn, Cu, Zn, Sr, Mo, Ba and Pb (mean 98% with standard deviation 8%). Hot water extracts and filtrates obtained from sequential filtration were analysed in the same way.

Results and discussion

Total elemental contents in medicinal plants from three sampling locations

As outlined in the introduction there is limited data available on total elemental contents in medicinal plants from Kenya. More specifically, Maobe et al. determined Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb in 8 plants [7], Adongo et al. reported the concentrations of Zn, Mg and Fe in 7 plants [8], Piero et al. determined Fe, Zn, Pb, Mg, Cr, Cu, Ni, Mn, Mo and Sr in 5 plants [9] and Oyaro et al. analysed the contents of Fe, Cu, Co and Cr in 6 plants [10].

The first goal of our study was to establish a comprehensive overview of the contents of a large number of elements in a substantial number of 19 commonly used medicinal plants from 3 different sampling locations. Initially a screening of 60 elements was done by ICP-MS for selected plant samples. Based on these results 30 elements were chosen for detailed quantification using 3 criteria: i) reliable determination in the plant digests using collision cell technology as verified by analysis of reference materials and obtained precision from replicate digestions. ii) known or supposed therapeutic or toxicological activity. iii) showing at least in part of the plants significant levels well above the limit of detection. In the case of the rare earth elements 5 examples were chosen due to similar profiles of the remaining elements of this group and no reported particular therapeutic or toxicological relevance. Moisture was determined in duplicate in the ground plant samples. Mean and standard deviation calculated from all NMP plants was 9.3% and 1.1%, from the BMP plants 9.3% and 1.0% and from the MMP plants 9.2% and 0.9%. Due to the low variation in moisture across all analysed plants the elemental contents were not corrected for moisture.

A summary of the obtained results is given in Table 2 including the minimum and maximum elemental contents as well as the mean for the areas NMP, BMP and MMP. The range of contents of the same element in the various plants from the same sampling location is often broad with maximum contents approximately 5-fold to 90-fold higher than the minimum contents. In few cases even larger span was observed: 104-fold for Pb in BMP samples, 171-fold for Mn in MMP samples and 191-fold for Nb in NMP samples. Cluster analysis was applied for better visualisation and understanding of this large data

set. Figure 1 shows the resulting tree diagram for all 55 plant samples using single linkage and Euclidian distances. The plants from the same sampling location are quite randomly distributed and not grouped in separate clusters. However, in many cases the same plant species from the 3 different sampling locations are grouped in one cluster or at least in closely related clusters (indicated by circles in Figure 1). This is confirmed by the much higher mean relative standard deviations of the contents (across all 30 elements) of the various plants from the same sampling location (93% for NMP, 103% for BMP and 86% for MMP) compared to the relative standard deviations of the contents (across all elements) of the same plant species from different locations (11% to 52% with one exception at 64%). Consequently, for the investigated sampling locations in 3 districts from Nyamira County it can be concluded that the environmental conditions of the individual locations (soil, water, air pollution/ dust, potential influence from anthropogenic activities like agriculture) have a minor influence on the elemental composition of the medicinal plants. Much more pronounced is the effect of the individual plant species on metal uptake, distribution and storage for therapeutic application. This is partly due to different size of the investigated plants including herbs and trees which reach different soil horizons and thus different sources of metals with their roots. In addition different parts of the plants are sampled for medical use, mainly leaves, but also roots or bark. Regarding the practical application, the results indicate that the same medicinal plant sampled from different locations in Nyamira County has similar elemental composition and thus is expected to provide similar metal-based therapeutic effects. However, the large variation of the elemental contents (Table 2) for different plants from the same location needs to be considered in particular for regular consumption, because the medicinal plants with maximum elemental contents have the potential to substantially increase exposure and thus uptake and body concentrations of trace elements which are present at much lower levels in general food sources. This might support therapy but may also cause toxic effects.

Some of the investigated plants are applied for diabetes treatment. Therefore, Cr and V levels are of special interest, because those trace elements (among others) are known or supposed to support

regulation of blood sugar levels. In case of vanadium a large number of synthetic candidate drugs was prepared and tested for efficient vanadium uptake and therapeutic effects on diabetes [12, 13]. However, relatively high doses are required which may cause adverse effects due to accumulation of vanadium metabolites. In case of Cr it is known that this trace element is required for normal insulin function and Cr deficiency can result in elevated blood glucose levels. Several studies indicated that supplementation with Cr enriched yeast improves regulation of blood glucose levels and shows less toxic effects than single inorganic chromium drugs like Cr-picolinate [14, 15]. An overview of the total Cr and V concentrations in the investigated medicinal plants is shown in Figure 2. The Cr and V levels in most plants are below 1 mg/kg but in the anti-diabetic plants *Bidens pilosa*, *Solanum mauense* and *Clerodendrum myricoides* much higher levels in the range from 2 mg/kg to 6 mg/kg were found. With few exceptions the plants contain nearly the same concentrations of both elements. Compared to phase IIa human clinical trial with uptake of 3 mg vanadium per day in form of a synthetic vanadium containing drug [16] the levels in the medicinal plants seem relatively low because it is not expected to consume half a kilogram or more of herbal medicine per day. However, the speciation of V is critical for oral administration and gastrointestinal resorption. Even much lower vanadium doses may be sufficient for anti-diabetic therapy in case the vanadium complexes naturally present in the medicinal plants are more efficiently taken up. In addition the combined effects of low V and Cr concentrations along with a variety of organic compounds in the plants could be more effective and in particular safer than high doses of single synthetic V or Cr complexes.

Hot water extraction to simulate traditional preparation of herbal medicine

The determined total elemental concentrations are important to estimate maximum intake of metals from herbal medicine. However, depending on the actual process for preparation of the medicine from the collected plants the uptake may be (much) lower. The most frequently applied procedure is boiling and soaking of the plants in water [4]. In our study an aliquot of the ground plants was mixed with

water, shortly boiled and then soaked overnight with shaking followed by filtration (0.45 µm) and elemental analysis by ICP-MS. Using this protocol the water extractable elemental fraction was determined for the plants from Borabu (BMP). The resulting extraction efficiencies were calculated as percentage ratio of the water soluble fraction and the total elemental contents (Table 3). For most elements the water extractable fraction is strongly dependent on the plant species as indicated by about 10-fold difference in minimum and maximum extractable fraction. These results clearly demonstrate that not only the total element contents of the plants are important for estimation of therapeutic or toxic effects but also the actual protocol for preparation of the medicine which is then consumed by the patient may significantly affect metal intake. The dependence of the extraction efficiencies on the plant species is also obvious from a previous study reporting similar results for Fe (<1.5%) and Co (21-41%), but higher values for Cr (13-25%) and much lower values for Cu (concentration in the extracts below LOD) [10]. When comparing the mean extraction efficiencies obtained from the 19 plants less than half of the analysed elements, i.e. B, Mg, P, K, Mn, Co, Ni, Cu, Zn, Rb, Mo, Cd and Tl, exceed 20% using our experimental conditions. Those elements are mostly essential and supposed to support therapy in case of mineral deficiency apart from the toxic elements Cd and Tl. Due to the low total contents of Cd and Tl near the limit of detection the relatively high water solubility seems not critical in these plants, however in case of increased uptake of Cd or Tl from contaminated soil this would be of significant concern. Overall the extractable fraction is strongly dependent on the respective element, for example Al and Pb are detected in low amounts in the aqueous extract which reduces the potentially toxic effects of these metals, while the essential elements Mg, Cu and Zn are extracted in much higher percentage. This is confirmed and visualised by cluster analysis of the extraction efficiencies of 30 elements for the 19 BMP samples (Supplementary information Figure S1). Cr and Tl are special cases with most extracted concentrations below the LOD, the remaining elements with low extraction efficiencies are clustered together in the centre, while the above mentioned elements with higher extraction efficiencies form additional clusters. Therefore, the hot water extraction can also

serve as an efficient way to improve the ratio of beneficial to toxic elemental contents prior to administration of the herbal medicine to the patient.

Size-fractionation of extracted elemental species using sequential filtration

The determination of the total extracted elemental contents improved the estimation of metal intake during therapy with herbal medicine. However, the molecular forms of the extracted elemental species are still largely unknown and therefore detailed understanding of the molecular mechanism of the potential therapeutic or toxic effects of the plant derived elemental species is not available. Hyphenated techniques, that means combining a separation step with subsequent online elemental and molecular detection, have been applied in many studies related to food or nutritional supplements, for example for the identification and quantification of arsenic species in algal extracts [17,18]. Critical aspects of the separation of elemental species for example by liquid chromatographic techniques are the recovery from the column and the stability of the species during separation. In particular for complex mixtures of mostly unknown elemental species, as present in the herbal extracts investigated in this study, development and validation of hyphenated speciation techniques is a big challenge [19]. However, it was shown that ultrafiltration with offline elemental detection is a useful technique for size fractionation of elemental species minimising the problems associated with species stability and recovery. In addition the filtration techniques are often faster and fractions of the size-separated species are obtained without dilution for further analysis with complementary techniques [11]. Therefore, a sequential extraction protocol was established and applied to selected plants extracts.

For this purpose hot water extracts and “cold” water extracts at room temperature were prepared from the samples *Bidens pilosa* (from Borabu, BMP10) and *Tabernaemontana stapfiana* (from Nyamira, NMP18). Average recoveries of the cold extracts compared to the hot extracts were 90% with standard deviation 23% for BMP10 and 100% with standard deviation 21% for NMP18 based on the <5 µm filtrates. Only for few elements the difference in the extracted contents between cold and hot treatment

exceeded 40%: V, Cr and Mo were less soluble in cold water for BMP10 (recoveries 53%, 60% and 38%) while Cu was more soluble in cold water for BMP10 (recovery 161%). This effect was lower or absent for NMP18 extracts. This indicates that the heating step during extraction has for most elements either a beneficial effect by increasing the extracted contents or no significant effect, but improves safety of the medicine due to disinfection of potential microbiological contamination of the water or plant material. The mean relative standard deviations across the 30 monitored elements obtained from triplicate extraction using hot or cold water are 5.0% and 6.6% for BMP10 and slightly higher at 9.0% and 7.0% for NMP18 due to the lower concentrations in this sample.

The hot water extracts were subjected to a 4-step sequential filtration procedure resulting in the following size fractions as described in the experimental section: <3 kDa, 3 kDa-10 kDa, 10 kDa-0.45 µm and 0.45µm-5 µm. Elemental analysis of the fractions clearly showed that the percentage distribution across these fractions was strongly dependent on the respective element. The results for selected elements are summarised in Figure 3. Mg, Mn, Co, Ni, and Zn were mainly (>55%) present as low molecular mass species (< 3kDa) with minor percentage of higher molecular mass species. In case of V, Cu and Y the 3 kDa-10 kDa fraction or the 10 kDa-0.45 µm fraction gain importance at least for BMP10. For Al and Fe the highest percentage is found in the fractions >10 kDa. Cluster analysis of the size fractionation of BMP10 visualises the outstanding distribution for Fe, Cu and V. All rare earth elements show very similar fractionation profile and most remaining elements join the group of predominantly low molecular weight species (Figure S2, Supplementary information). This molecular size fractionation provides initial characterization of the predominant elemental species present in the plant extracts. Further investigations are required for detailed species identification, for example by analysis of the obtained fractions by HPLC separation online with elemental and molecular detection.

Regarding V the concentrations in NMP extracts were below the limit of detection. However, in BMP10 the size fractionation resulted in >60% vanadium species with molecular weight >3 kDa. This result is contrary to the numerous studies focusing on the synthesis of low molecular weight insulin-

mimetic drugs for oral treatment of diabetes. Possibly the plant derived high molecular weight vanadium species are poorly resorbed and not relevant. However, there is also the possibility that these natural forms of vanadium are advantageous for gastro-intestinal uptake and thus could support regulation of blood glucose levels with less toxic side effects compared to current synthetic vanadium containing candidate drugs.

Conclusion

The performed strategy of complementary determination of total elemental contents, extractable elemental fraction and molecular size fractionation of elemental species achieved a comprehensive overview on similarities but also differences in the mineral composition of a broad range of commonly applied medicinal plants from 3 regions of Kenya. This approach substantially supports pharmaceutical as well as toxicological investigations providing complementary data on therapeutic activity, uptake and metabolism of organic as well as metallic compounds in the herbal medicine. Based on the obtained data it will be possible to perform a systematic selection of the most suitable plant species out of the huge variety of available plants in Kenya and other African countries for controlled and safe therapy of diseases with high incident rates, for example diabetes. The detailed characterisation of medicinal plants from Africa opens a perspective to maximise the benefit from locally available low cost medicine and thus releasing pressure from the African health care system. Coordinated studies involving plant research, organic and inorganic mass spectrometry, activity testing and pharmacological studies are required in the future to achieve this goal.

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Table 1 Botanical identification of the collected and analysed medicinal plants

No.	Plant species local Name (Kisii)	Plant family	Parts used	Preparation and form of application
1	*Warburgia ugandensis* (Esoko)	Canellaceae	ash, bark, roots	decoction
2	*Solanum indicum* (Omorobo)	Solanaceae	ash, leaves, roots	decoction, compress from cut leaves
3	*Toddalia asiatica* (Ekenagwa ekiegarori)	Rutaceae	ash, roots	decoction
4	*Erythrina abyssinica* (Omotembe)	Fabaceae	ash, bark, roots	decoction
5	*Senna didymobotrya* (Omobeno)	Fabaceae	ash, leaves	decoction
6	*Veronia auriculifera* (Omosabakwa)	Asteraceae	ash, leaves, roots	decoction
7	*Plectranthus barbatus* (Omoroka)	Lamiaceae	ash, shoots, roots	decoction
8	*Urtica dioica* (Rise)	Urticaceae	leaves	decoction
9	*Croton macrostachyus* (Omosocho)	Euphorbiaceae	ash, leaves, roots	decoction
10	*Bidens pilosa* (Ekemogamogia)	Asteraceae	ash, leaves	decoction
11	*Melia azedarach* (Omwarubaine)	Meliaceae	leaves	decoction (one time daily, one month)
12	*Solanum mauense* (Ekengeta mbori)	Solanaceae	whole plant	decoction (three times daily, for 3 months)
13	*Magnifera indica* (Riembe)	Anacardiaceae	ash, leaves	decoction
14	*Acacia hockii* (Omokonge)	Fabaceae	ash, bark, roots	decoction
15	*Acacia abyssinica* (Omonyenya)	Fabaceae	ash, bark, roots	decoction
16	*Clerodendrum myricoides* (Omonyasese)	Lamiaceae	ash, leaves, roots	decoction
17	*Carissa edulis* (Omonyangatetia)	Apocynaceae	ash, roots	decoction
18	*Tabernaemontana stapfiana* (Omobondo)	Apocynaceae	leaves	decoction (three times daily, for 2 weeks)
19	*Aloe vera* (Omogaka)	Xanthorrhoeaceae	leaves	decoction

Table 2 Overview of the elemental contents in 19 medicinal plants from sampling locations Nyamira (NMP), Borabu (BMP) and Manga (MMP) given as minimum content, maximum content and mean.

	NMP [mg/kg]			BMP [mg/kg]			MMP [mg/kg]		
	min.	**max.**	**mean**	**min.**	**max.**	**mean**	**min.**	**max.**	**mean**
B	10.7	70.8	32.0	9.9	65.7	28.1	9.7	62.9	33.0
Mg	840	6012	2816	442	6219	2501	485	5722	2435
Al	118	8600	1566	237	6040	1435	128	4977	1496
P	304	6451	2456	363	5782	1982	349	5815	2420
K	4801	60822	22992	3042	50397	20263	4692	55704	21515
Ca	5266	42103	16160	3139	39060	15989	2429	36693	15117
Ti	10.1	416	79.3	14.3	297	75.2	7.87	233	71.7
V	0.09	5.32	1.00	0.14	3.68	0.95	0.09	6.00	1.24
Cr	0.05	3.36	0.72	0.05	2.28	0.87	0.08	3.09	0.91
Fe	121	7871	1487	229	5600	1408	136	4675	1489
Mn	18.5	1649	365	58.0	1661	372	17.5	2987	406
Co	0.04	1.63	0.32	0.05	1.24	0.31	0.05	2.07	0.44
Ni	0.40	2.69	1.01	0.25	3.99	1.02	0.21	7.67	1.70
Cu	1.70	16.1	8.11	2.75	14.3	7.93	1.59	28.8	9.82
Zn	4.2	95.7	41.4	5.48	105	41.0	5.21	123	42.8
Ga	0.09	5.92	1.19	0.24	4.39	1.18	0.10	3.47	1.13
Rb	9.8	103	51.6	4.07	93.5	38.1	7.95	122	47.7
Sr	23.4	256	109	22.5	269	111	22.6	269	111
Y	0.29	21.8	4.27	0.68	54.7	5.93	0.27	14.01	3.76
Nb	0.08	15.3	3.06	0.18	11.9	2.90	0.30	9.73	3.01
Mo	0.07	3.65	0.96	0.07	3.44	0.78	0.05	3.35	0.84
Cd	<0.1	0.89	0.26	<0.1	1.00	0.32	<0.08	0.99	0.31
Ba	17.8	148	58.2	15.5	120	55.2	18.9	234	70.9
Nd	0.35	23.4	4.98	0.79	52.9	6.26	0.32	16.7	4.21
Sm	0.05	3.74	0.82	0.15	8.91	1.08	0.06	2.75	0.71
Gd	0.06	4.04	0.84	0.14	9.91	1.12	0.05	2.70	0.68
Er	0.03	1.63	0.38	0.08	3.92	0.52	0.03	1.11	0.35
Yb	0.02	1.13	0.32	0.07	2.53	0.43	0.02	0.78	0.30
Tl	<0.02	0.26	0.08	<0.02	0.23	0.07	<0.01	0.26	0.07
Pb	0.17	5.71	1.32	0.14	14.6	1.71	0.16	4.17	1.28

Table 3 Extraction efficiencies for hot water treatment of 19 medicinal plants from sampling location Borabu (BMP) given as minimum percentage, maximum percentage and mean with standard deviation (SD). Concentrations below the limit of detection (LOD) were not included in calculating the mean and SD (in this case the number of values above the LOD is given in brackets).

	min. [%]	**max. [%]**	**Mean and SD [%]**
B	<2.7	94.9	49.1 ± 22.4 (14)
Mg	14.4	76.5	47.8 ± 14.9
Al	0.19	6.78	1.9 ± 1.6
P	17.4	81.4	49.7 ± 13.6
K	72.0	103.9	84.8 ± 9.3
Ca	2.8	43.0	16.8 ± 13.5
Ti	0.14	3.9	1.3 ± 1.1
V	1.0	30.7	6.8 ± 7.6 (18)
Cr	<8.8	-	- (0)
Fe	0.3	4.2	1.7 ± 1.3
Mn	5.3	61.4	30.0 ± 12.6
Co	18.6	64.0	35.9 ± 12.5
Ni	17.2	103.5	57.0 ± 26.6 (14)
Cu	18.0	61.1	35.9 ± 12.8
Zn	7.2	58.8	28.4 ± 15.3
Ga	<0.3	6.8	3.5 ± 2.1 (12)
Rb	62.5	102.4	80.3 ± 10.9
Sr	2.9	34.3	15.4 ± 10.9
Y	1.3	18.0	5.2 ± 4.1
Nb	<0.3	42.1	11.5 ± 14.0 (7)
Mo	<5.5	85.4	41.8 ± 24.6 (9)
Cd	6.0	52.5	24.5 ± 22.2 (5)
Ba	4.5	16.1	9.2 ± 3.4
Nd	0.4	11.0	4.2 ± 3.0 (18)
Sm	0.8	11.7	4.2 ± 3.2
Gd	1.5	16.9	6.3 ± 4.1 (17)
Er	1.7	17.9	5.1 ± 4.0
Yb	1.5	18.6	5.3 ± 4.4 (18)
Tl	<32	74.4	55.0 ± 16.8 (3)
Pb	1.9	24.4	8.3 ± 7.2

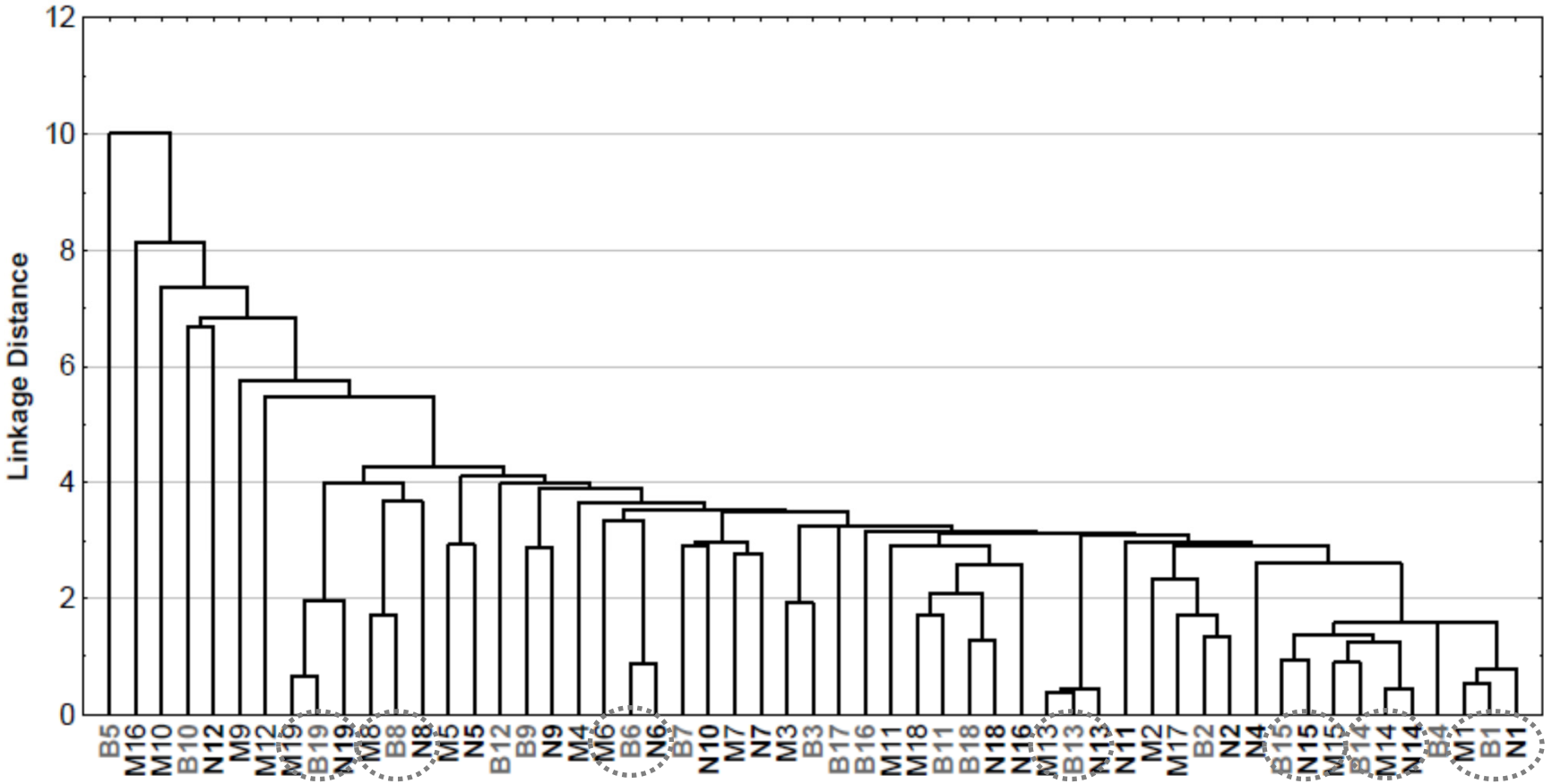
Figure captions

Figure 1 Cluster analysis of the total contents of 30 elements in 19 medicinal plants from locations Nyamira (NMP), Borabu (BMP) and Manga (MMP).

Figure 2 Total contents of vanadium and chromium in plants from three sampling locations in Kenya

Figure 3 Size fractionation of metals in extracts from BMP10 (upper graph) and NMP18 (lower graph) obtained by sequential filtration with subsequent ICP-MS detection (Results for vanadium in NMP18 were below limit of detection).

Figure 1


Linkage Distance
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10
8
6
4
2
0
B5
M16
M10
B10
N12
M9
M12
M19
B19
N19
M8
B8
N8
M5
N5
B12
B9
N9
M4
M6
B6
N6
B7
N10
M7
N7
M3
B3
B17
B16
M11
M18
B11
B18
N18
N16
M13
B13
N13
N11
M2
M17
B2
N2
N4
B15
N15
M15
B14
M14
N14
B4
M1
B1
N1

Figure 2

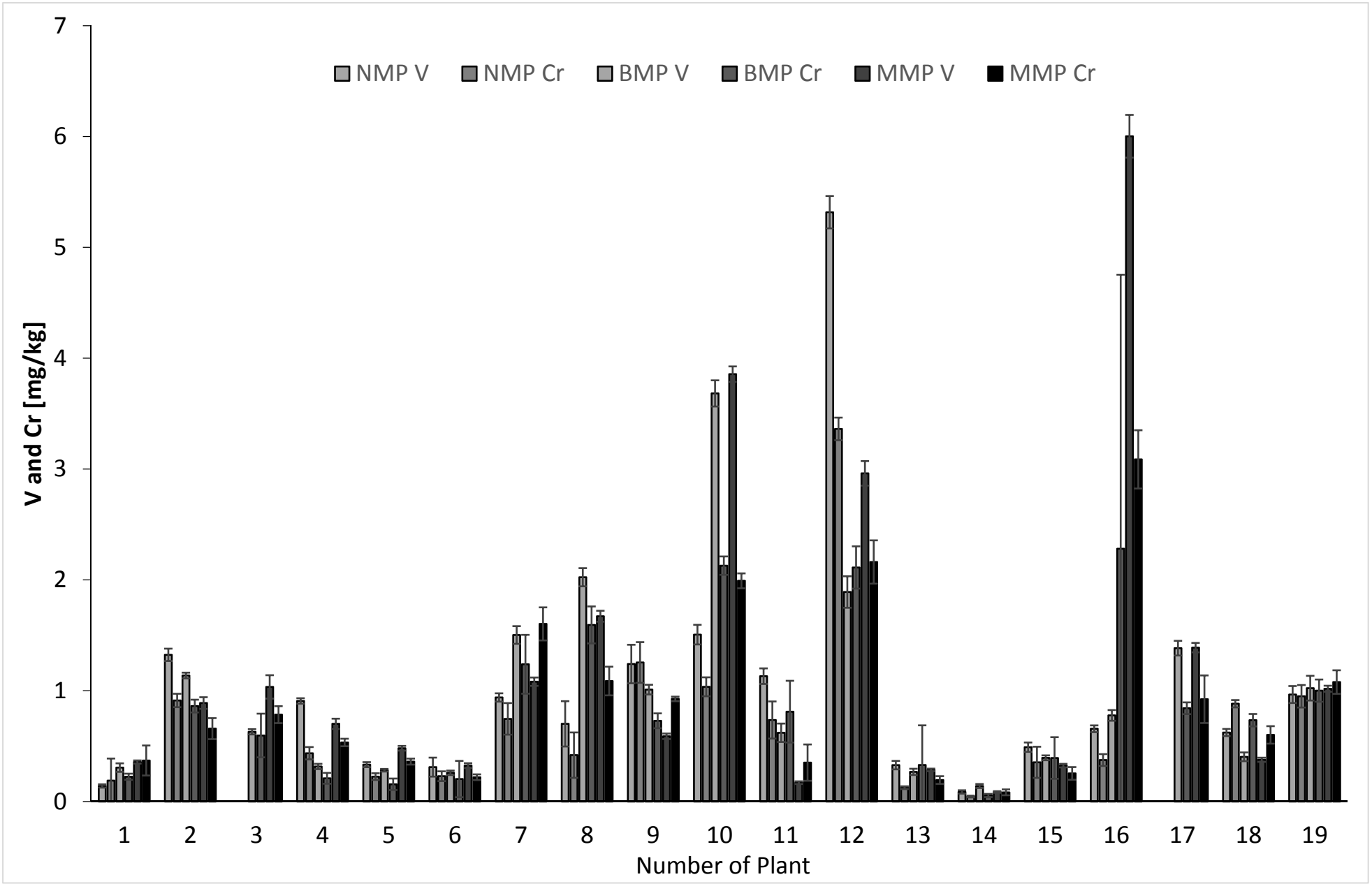
NMP V
NMP Cr
BMP V
BMP Cr
MMP V
MMP Cr
V and Cr [mg/kg]
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Number of Plant

Figure 3 upper part

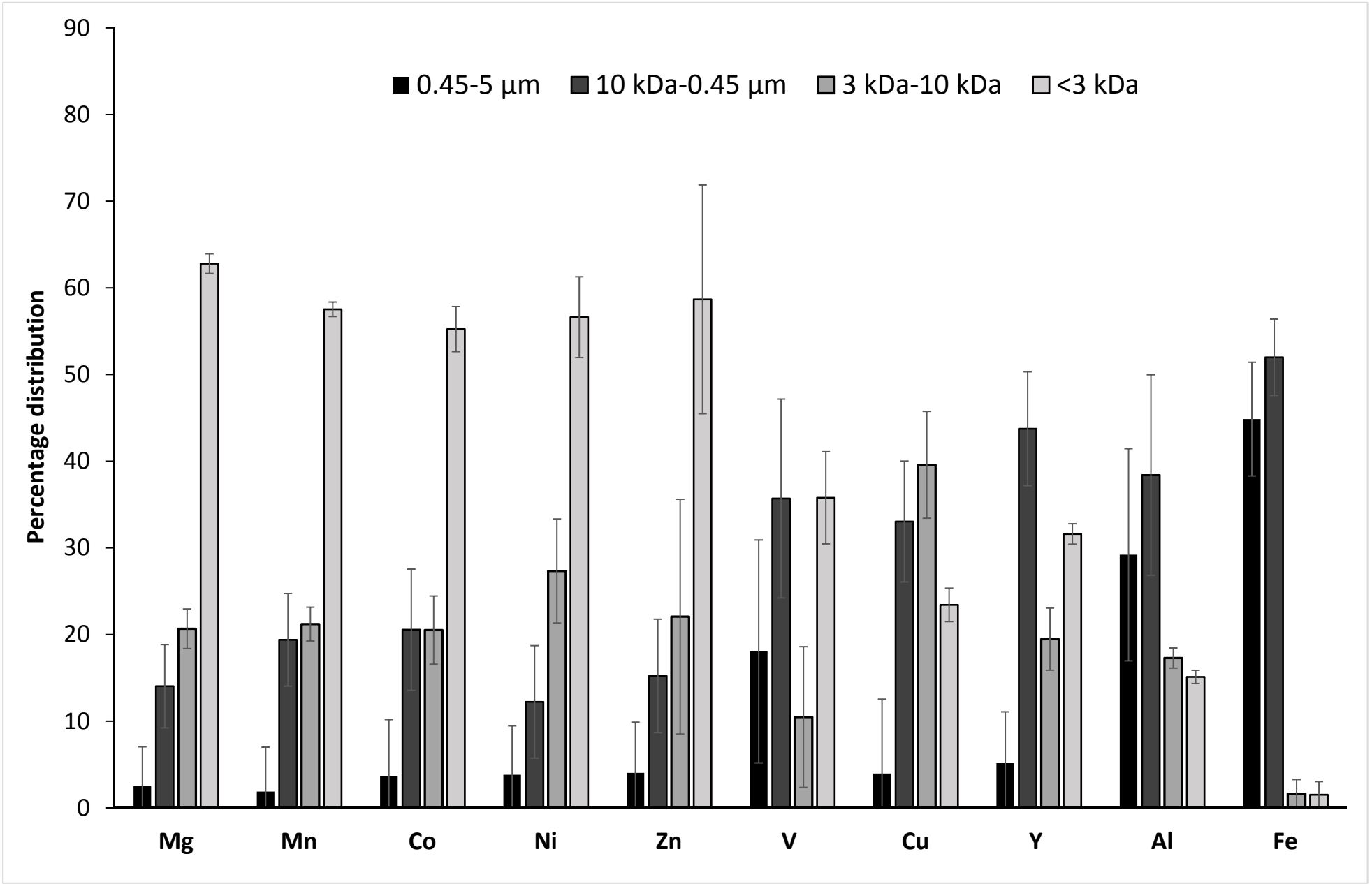

Figure 3 lower part