Assessment of bio-availability of Cd, Pb, As and Tl via Gastric/Intestinal Digestion in Plants Collected From Different Kenyan Regions

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Research Article

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Abstract

In this study, total and bio-available levels of cadmium (Cd), Lead (Pb), arsenic (As) and Thallium (Tl) in 19 Kenya plants from two study sites (Manga and Borabu) were evaluated. ICP-MS technique was used to determine total Cd, Pb, As and Tl contents, while ultra-filtration and physiological extraction tests evaluated bio-accessibility of these elements. The plants had low concentrations; Cd (0.27±0.02 and 0.26±0.02), As (0.32±0.02 and 0.48±0.04), Tl (0.07± 0.02 and 0.06±0.01) and Pb (1.23±0.11 and 1.16±0.10) mg/kg in Manga and Borabu sites. However, sequential ultra-filtration showed more than 70% high molecular weight species (>10 kDa) predominated with Pb, Cd and Tl. The physiological based tests showed Cd, Pb, As and Tl extracted enzymatically were significantly higher (0.45±0.11, 0.46±0.12, 0.37±0.10 and 0.81±0.19μg/g) than aquatically extracted (0.32±0.07, 0.34±0.11, 0.26±0.08 and 0.50±0.27) μg/g, ($p<0.05$). Low bio-accessibility levels of these elements in medicinal plants justifies their use therapeutically.

1. Introduction

Plants have been used in the treatment and management of various diseases and conditions. The information on the use of plants has been based on traditional folk perpetuated along generations. Most of the conventional medicines are very expensive for most rural folk and this has led to the upsurge use of herbal plants with no proven scientific efficacy on their curative powers or safety. Herbs are assumed to be safe when used, though many unsafe side effects have been reported (Clemens and Ma 2016; Zeng et al. 2016; Adie and Adekunle 2017). The side effects reported include allergic reactions, contaminations, interactions with drugs and other herbs, altered food consumption, altered body and organ weights, visible pathological changes and altered enzyme levels (Subramanian et al. 2012; Ogunbanjo et al. 2016; Zeng et al. 2016).

A number of herbs and plants used in Africa by the traditional healers are believed to be effective (Annan et al. 2010; Ahmad et al. 2013; Ogunbanjo et al. 2016; Adie and Adekunle 2017). Some of them have been reported to be effective in treatment and management of diseases associated with the liver, circulatory and respiratory systems (Jena 2012; Korfali et al. 2013), even though few of them have been shown to be potentially toxic and carcinogenic (Arpadjan et al. 2008; Jayawardene et al. 2010; Mirosławski and Paukszto 2018). The most vulnerable organs to the toxins in the plants are the liver and kidney which are involved in the metabolism and excretion of chemical compounds such as those present in contaminated plants (Koch et al. 2011). The efficacy and safety of the plants must be evaluated before their uptake into in the human body to avoid toxic effects (Randelovic et al. 2008; Zeng et al. 2016; Abbas et al. 2018; Zhou et al. 2018).

Incidences of heavy metals toxicity of Cd, Pb, As and Tl have been reported in many parts of the world (Annan et al. 2010; Jayawardene et al. 2010; Koch et al. 2011; Pizarro et al. 2016; Zeng et al. 2016; Zhou et al. 2018). Cd, Pb, As and Tl are present in different amounts in water and certain foods such as leafy vegetables, potatoes, grains and seeds (Zeng et al. 2016; Yang et al. 2016; Ha Nguyen et al. 2018). Large populations in different parts of the world have been reported to be exposed to high levels of As in their drinking water and are displaying various clinico-pathological conditions such as cardiovascular and peripheral vascular disease, developmental anomalies, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic disorders and carcinoma (Arpadjan et al. 2008; Khan 2013; Ogunbanjo et al. 2016). Exposure to high lead levels causes abdominal pains, constipation and loss of appetite, nausea, vomiting, insomnia, headache, irritability, dizziness and lead encephalopathy (Mogwasi et al. 2013). Exposure to cadmium in high levels can result in flu-like symptoms (chills, fever, and muscle pain) and can damage the lungs while chronic exposure for a long period of time can result in kidney, bone and lung disease (Karbowska 2016). Ingested large amounts of thallium over a short period in man results in vomiting, diarrhea, temporary hair loss, and affects the nervous system, lungs, heart, liver, kidneys and may result in death (Rahimzadeh et al. 2017). This necessitates thorough toxicity assessment of plant products in order to ensure safety to their users.

The WHO, (2017) recommended that medicinal plants need to be checked for the presence of toxic elements and it regulates the maximum permissible limits for Pb, As and Cd in herbal medicine to be 10.0mg/kg, 0.4 mg/kg, and 0.3 mg/kg respectively. This requirement is not observed in the third world countries where the use of plants is neither controlled nor regulated by quality assurance parameters (Annan et al. 2010; Khan 2013; Ogunbanjo et al. 2016; Adie and Adekunle 2017). Though the determination of the total content of the heavy metal in the plants give an indication of the levels of toxicity much more information can be obtained by measuring the bioavailability and bio accessibility (Arpadjan et al. 2008; Intawongse and Dean 2008; Musa Özcan et al. 2008; Yemane et al. 2008; Jayawardene et al. 2010; Lv et al. 2013; Zhu et al. 2013; Okem et al. 2014; Čadková et al. 2015; Parviz et al.

2015; Bolan et al. 2016; Pizarro et al. 2016; Yang et al. 2016; Nischwitz et al. 2017; Mogwasi et al. 2018; Zhou et al. 2018; Mogwasi et al. 2019).

In this study, results of assessment of heavy metal concentration in plants from two sub-counties of Kenya are presented. We focused on the level of Cd, Pb, As and Tl in commonly used Kenyan plants from two sampling locations in Kenya. The heavy metal levels were determined by inductive coupled plasma mass spectrometry (ICP-MS). Physiologically based extraction tests were used to estimate bioavailability while sequential ultra-filtration by size fractionation of Cd, Pb, As and Tl into molecular mass species determined the amount of the elements bio accessed by the human body from the plants thus determining the long-time therapy risk poisoning to users. In Borabu sub-county the farmers applied agricultural additives frequently while in Manga sub-county additives application on farms was limited. The plants used in the two study areas absorbed different amounts of As, Pb, Cd and Tl which were availed to the human body when the plants were consumed forming the basis for the plant selection in the study.

2. Experimental

2.1 Study sites

The study sites were selected from Borabu and Manga sub-counties of Nyamira County, which lies between latitudes $0^{o}30'$ and $0^{o}45'$ South and longitudes 34^{o} $45'$ and 35^{o} $00'$ East, with altitude range of between 1,250 m and 2,100 m above sea level (Kiende Judy Inoti 2012), (Figure 1). Nyamira County is divided into two major agro-ecological zones. The highland zone covers 82 per cent while the upper midland zone covers the remaining (Kiende Judy Inoti 2012). The low zones comprise of swampy, wetlands and valley bottoms while the upper zones are dominated by the hills. The major type of soil is the red volcanic (Nitosols) which is deep, fertile and well drained accounting 75 per cent and the remaining which clay is found in valley bottoms and swampy areas. The two sub-counties have bimodal pattern of annual rainfall that is well distributed, reliable and adequate for the growth of a wide range of plants. The annual rainfall ranges between 1200 mm-2100 mm per annum. The long and short rain seasons start from April to August and September to December respectively, with three months of dry spell separating them. The tree cover in the county is mainly agro-forestry with only a small fraction of natural forests round the hilltops with no gazetted forest area. A total of ten sampling sites in each sub-county were selected and three experienced herbalists at each site were randomly selected and recruited for the study. The selected areas for medicinal plant harvest were located in the neighborhood of agricultural activities.

2.2 Recruitment of herbalists and Sampling of plant materials

The details on herbalist recruitment in Manga and Borabu sub-counties were as described in our previous works (Nischwitz et al. 2017; Mogwasi et al. 2018; Mogwasi et al. 2019). Based on the herbalist information he most commonly used herbal plants and the protocols used in their preparations were established. Ten herbalists from each study region were recruited and requested to supply one kilogram of a dry plant sample for each species. The plant materials were collected between February 2019 and April 2019. The plant materials collected were botanically identified (at the University of Nairobi herbarium and the voucher plant samples kept), washed with deionized water to remove soil and other adsorbed material, placed in paper bags, separately air-dried under shade and ground using a different pestle and wooden mortar for each species to avoid exogenous contamination.

Care was taken during the collection and the storage of the plant samples to avoid contamination by placing each ground samples in well labeled and sealed paper envelopes and kept at 4^{o}C until analysis. The medicinal plants used in the study were *Warburgia gandensis, Toddalia asiatica*, *Erythrina abyssinica, Senna didymobotrya, Veronia auriculifera, Croton macrostachyus, Melia azedarach, Magnifera indica, Acacia abyssinica, Tabernae montanastapfiana, Acacia hockii, aloe Aloe vera, Carissa eludes Plecaranthus babatus, Urtica dioica, Bidens pilosa,Solanum indicum, Solanum mauense* and *Clerodendrum myricoides*.

2.3 Reagents, chemicals and reference materials

Analytical grade reagents were used in the study. The calibration standards were prepared from Certipur stock solutions (Merck, Darmstadt, Germany) in 3% nitric acid. Deionized water used in the study was prepared using a Millipore system. The reagents in the enzymatic digestion were pepsin, sodium malate, sodium citrate, lactic acid, acetic acid, bile salts and pancreatin. The reference materials were NIST 1647 (peach leaves) and NIST 1640 (natural water) purchased from LGC standards, Germany.

2.4 Determination of total content of Cd, Pb, As and Tlby ICP-MS

The total concentrations of Cd, Pb, As and Tl in the plant samples were determined by closed vessel microwave digestion with subsequent analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) according to Nischwitz et al. (2017). Before microwave digestion medicinal plants were fine ground using a ball mill of zirconium oxide vessels and balls (pulverisette 6, Fritsch, Germany). Aliquots of 50 mg of the ground plant samples were digested in triplicate using 2 mL of 15.8 M nitric acid (suprapur, Merck, Germany) and 1 mL of 6.29 M hydrogen peroxide (suprapur, Merck, Germany) in a MARS 5 closed vessel microwave system (CEM, Germany) at 160°C. The complete digestion of the organic matrix in the plant was achieved with occasional addition of silicate residues. The mineralized solution was transferred to calibrated polystyrene sample vials and made up to 10 mL with deionized water. Blanks and plant reference materials were processed in a similar way. The levels of Pb, Cd, As and Tl in the plant sample digests were determined using an ICP-MS Agilent 7500 quadrupole ICP-MS with collision cell in He-Mode (Agilent Technologies, Japan). A micromist nebulizer with double pass spray chamber was used during the analysis. The He-flow rate of the collision cell was 4 mL/min, the sample uptake rate was 400 µL/min while using an argon (nebulizer gas) flow rate of 0.98 L/min. The mean and standard deviation from triplicate digestion and measurement were calculated (n=3) (the ICP-MS software determined the amount of each element in each sample in triplicates). NIST 1647 peach plant reference material was analyzed for quality control.

2.5 Enzymatic determination of toxic elements in medicinal plants

The in vitro gastrointestinal digestion method used in the study was based on Navarro et al. (2008) and Mogwasi *et al.*(2019). 0.3g of accurately weighed plant material was placed in a 50 mL polypropylene tube and treated with 30 mL of gastric solution (1.25g of pepsin, 0.5g of sodium malate, 0.5g of sodium citrate, 420µL of 11.3M lactic acid and 500µL of 17.4M acetic acid, made up to 1L with deionized water and the pH adjusted to 2.5 with 11.65M hydrochloric acid). The mixture was shaken at 100 g in a thermostatic bath maintained at 37^{o}C for one hour. The mixture was then centrifuged at 3000 g for 10 minutes and 5 mL aliquot was removed, filtered through 0.45µm micro filter and replaced with the original gastric acid solution to retain the original amount of solution.

The small intestinal digestion conditions were created by adding 52.5 mg bile salts and 15 mg of pancreatin into the same sample tube and saturated sodium bicarbonate solution was added to adjust the pH to 7.0. The mixture maintained at 37^{o}C in a thermostatic bath was shaken at 100 g for 2 hours and then a second 5 ml aliquot was removed and filtered. The remaining sample solution was centrifuged at 3000 g for 10 minutes, filtered and the residue was retained for acid microwave digestion and ICP-MS analysis. The extracted samples were stored at 4^{o}C and analyzed within 24 hours with ICP-MS. Gastric, intestinal and water plant extracts were performed in every batch and all the plant samples were extracted and analyzed in triplicates and were diluted to 1:10 w/w before analysis. The bio accessible toxic metal contents in plant extracts (gastric and intestinal fractions) were determined by ICP-MS and the daily intake of the element (the actual oral dose of the element) was determined by adopting Bolan *et al.*(2016) equation.

DI = DD x W x MC

Where DI is the daily intake of the element, DD is the daily dose of the medicinal plant, W is the medicinal plant weight taken in daily and MC is the medicinal plant element content (mg/kg).The *in vitro* determination of Cd, As, Tl and Pb in the medicinal plants to simulate the human digestion in six medicinal plants from Manga study area was based on two step enzymatic extractions procedure according to Navarro (Navarro et al. 2008) and Mogwasi et al.(Mogwasi et al. 2019) The amount of As, Cd, Tl and Pb extracted in each phase was divided by the total amount of the element extracted (extracted by acid digestion) to give the elemental extraction percentage (%).

2.6 Sequential ultra-filtration for determination of Cd, Pb, As and Tl in medicinal plants

The modified Mogwasi et al. (2018) sequential ultra-filtration method was used to determine bio accessibility of Cd, Pb, As and Tl from the plants. Approximately 140 mg of ground plant material was mixed with 40 mL deionised hot water in a polypropylene tube and shaken for 13 hours in the dark using a horizontal shaker at 100 motions per minute (n=3). Water lost due to evaporation was compensated by topping up with deionised water to 40 mL. The extracts obtained were first filtered using a 5 µm syringe filter. An aliquot of 15 mL of the obtained filtrates was then filtered through 0.45 µm syringe filters. An aliquot of 10 mL of the second filtrate was subjected to ultra-filtration through 10 kDa membrane using a micon filtration units at a speed of 4000 g (Merck-Millipore, Germany) and finally an aliquot of 5 mL of the third filtrate was subjected to ultra-filtration through 3 kDa membrane using a micon filtration units at a speed of 4000 g (Merck-Millipore, Germany). The water extracts and filtrates obtained from sequential filtration were analysed using ICP-MS. NIST 1640 natural water reference material and 1647 peach plant reference material were analysed for quality control.

3. Results And Discussion

3.1 Total content of Cd, Pb, As and Tl in the medicinal plants

The mean total concentrations of Cd, Pb, As and Tl in the plant species collected from the two study regions (Manga and Borabu Sub-counties) are presented in Table 1. Most plants from Manga sub county had As concentrations of between 0.11±0.06 to 0.5±0.03mg/kg. *C. myricoides* and *S. didymobotrya* plant species had concentration of 0.6±0.1mg/kg and 0.9±0.07mg/kg respectively, which were higher while *W. ugandensis* and *E. abyssinica* (0.08±0.01mg/kg) were below the range stated.

Table 1
Botanical names, common names, local names and parts of the Medicinal plants used in the study

Botanical Name	**Common Name**	**Local Names (Ekegusii)**	**Part of the plant used**
*Warburgiaugandensis*Sprague.	Ugandan greenheart	Esoko	Stem bark
*Urticadioica*L.	Stinging nettle California Nettle, Slender Nettle, Tall Nettle	Rise	Leaves
*Solanumindicum*L.	Bush tomato, Indian night shade, Poison berry	Omorobo	Roots
*Solanummauense*Bitter	*nightshade*, Purple *nightshade*, Small flower *nightshade*, American black night shade	Ekengentambori	Leaves
*Clerodendrummyricoides*Vatke	Blue-flowered tinderwood, Ugandense', Blue Glory Bower, Butterfly Bush	Omonyasese	Root bark
*Toddaliaasiatica*Lam.	orange climber, Forest pepper, Wild orange tree	Ekenagwaekiegarori	Roots
*Erythrinaabyssinica*Lam.	Red-hot-poker, Coral tree, Lucky-bean tree	Omotembe	Stem bark
Senna didymobotrya(Fresen)Irwin &Barneby.	African senna, Popcorn senna, Candelabra tree and Peanut butter cassia	Omobeno	Leaves
*Vernoniaauriculifera*Hiern.	Bitterleaf	Omosabakwa	Leaves
*Croton macrostachyus*Del.	Broad-leaved Croton	Omosocho	Leaves
Melia azedarach L.	Persian lilac, Chinaberry, Bead tree, Syringa, white cedar, mwarubaini (Kiswahili)	Omwarubaine	Leaves
*Magniferaindica*L.	Mango	Riembe	Leaves
*Acacia abyssinica*Benth.	Umbrella thorn, Flat-top *acacia*, Nyanga flat-top	Omonyenye	Stem bark
*Tabernaemontanastapfiana*Britten.	Soccerball fruit	Omobondo	Leaves
*Acacia hockii*De wild.	Shittim wood	Omokonge	Stem bark
Aloe Vera Miller.	Chinese Aloe, Indian Aloe, True Aloe, Barbados Aloe, Burn Aloe, First Aid Plant.	Omogaka	Leaves
Carissa edulis (Forssk) Vahl.	Simple-spinednum-num, Climbing num-num, Small num-num	Omonyangatetia	Roots
Plectranthus barbatus Andr.	Big leave *Plectranthus*, Indian coleus and Forskohlii	Omoroka	Leaves
*Bidenspilosa*L.	Black jack,Beggarticks, cobbler's pegs and Spanish needle	Ekemogamogia	Leaves

Twelve plants from Borabu sub-county had mean arsenic concentrations ranging from 0.2±0.01 to 0.62±0.07 mg/kg, with *S. didymobotrya* (2.1±0.2mg/kg) and *B. pilosa* (0.94±0.07mg/kg) having concentration above and only five plant species had means below this range. The five plant species with As concentrations of <0.2 mg/kg from both study regions were *W. ugandensis*, *V. auriculifera*, *M. indica, A. abyssinica* and *A. hockii*. The plants could be releasing low amounts of arsenic to the body of the plant users. The mean content of As in the various plants from the same sampling region was often broad with maximum concentrations approximately 11-fold in Manga sub-county and 30-fold in Borabu sub-county higher than the minimum concentration. 57.9 % of Borabu sub-county plants had slightly high mean levels of arsenic than those from Manga sub-county. *S. didymobotrya, C. myricoides, S. mauense, C. edulis, B. pilosa* and *U. dioica* from sub-counties had high levels of As. These plant species can be used for phyto remediation in cases of high arsenic contaminated soils. The levels of arsenic in most plants from Borabu sub-county were as a result of using animal manure, agrochemicals or inorganic fertilizers in the areas the plants were collected. Our findings for As are similar to those in different flora of less than 0.01 to 0.4 mg/kg reported by different authors (Karanja et al. 2012; Zhu et al. 2013). Tadesse et al. (2019) reported As levels in different plants collected from Awash river basin in Ethiopia as ranging from not detected to 0.009 mg/kg. The values reported by Tadesse et al. (2019) were lower than our findings, due to the fact that they collected their plant samples from natural environment. The maximum allowable limit of As in consumed food by a person is 0.2 mg/kg per day (World Health Organisation 2017). The presence of As in plants even in trace amounts is a primary risk to food safety and human health given that As is classified as a toxin (World Health Organisation 2017). Exposure to As may lead to hyper pigmentation, keratosis, carcinogenesis, cardiovascular diseases fetal loss, premature delivery and long term loss of memory (Arpadjan et al. 2008; Zeng et al. 2016; Adie and Adekunle 2017).

The concentration of cadmium in Manga sub-county plants ranged from <0.08 to 0.98±0.03 mg/kg. *C. macrostachyus* had the highest concentration (0.98±0.03mg/kg) while *S. indicum* had the lowest concentrations (<0.08mg/kg) (Table 1). Cadmium concentration in Borabu sub-county for plants ranged from <0.08 to 0.98±0.06mg/kg with the highest in *T. stapfiana* (0.98±0.06 mg/kg) and the lowest in *A. vera* (<0.0 9mg/kg). 53.6% of Borabu sub-county plants had high levels of cadmium than those from Manga sub-county. 68.4% of plants from both sub-counties had mean levels of cadmium of below the permissible limit (World Health Organisation 2017) and six plants, *V. auriculifera*, *C. macrostachyus, M. azedarach*, *T. stapfiana, B. pilosa* and *C. myricoides* had levels above the permissible limit (World Health Organisation 2017). Five plants from both sub-counties with low levels of Cd were *A. vera*, *M. indica, A. hockii, S. indicum*, and *E. abyssinica.*

The range of cadmium in plants from the same sampling region was often broad with maximum and minimum concentrations approximately 12-fold in Manga sub-county and 11-fold in Borabu sub-county. Our findings are similar to those reported for vegetation and indicated that the vegetation absorbed Cd from contaminated soil and water (Khattak and Khattak 2011; Khan 2013; Ting et al. 2013; Zhu et al. 2013; Filipiak-Szok et al. 2015; Ababneh 2017).

The lead levels in Manga sub-county plants ranged from 0.16±0.02to 4.2±0.2mg/kg with the highest and lowest in *U.dioica* and *A.hockii* respectively (Table 1). All the plants had mean lead levels of below the permissible limit (World Health Organisation 2017). Borabu sub-county plants had lead concentrations ranging between 0.15±0.01 to4.5±0.4 mg/kg with the highest and lowest in *B. pilosa* and *A. hockii* respectively. Five plants from both regions which had low levels of lead were *W. ugandensis, T. stapfiana, A. hockii, M. indica* and *E. abyssinica*. 57.9% of Manga sub-county plants had high mean levels of lead than those from Borabu sub-county (Table 1). The mean lead range in the plants from the same sampling region was often broad with maximum and minimum concentrations approximately of 27-fold in Manga and 30-fold in Borabu sub-county. The results of lead reported in the present study are similar to those reported by others in different vegetation (Harris et al. 2011; Khattak and Khattak 2011; Tokalioğlu 2012; Korfali et al. 2013; Onwordi et al. 2015; Ferdosi and Farooq 2017; Tadesse et al. 2019). Some of the vegetation could be growing on contaminated soils or on effluents from industries or in areas with vehicular fumes which raised the levels of lead in the vegetation(Emongor 2007; Nabulo et al. 2008). Onyango et al. (Onyango et al. 2008) reported that an adult Kenyan consumes about 200g of kale vegetables daily, which translated to ingestion of 0.078 mg to 0.612 mg of lead. On the other hand, Ababneh, (Ababneh 2017) reported the mean levels of lead of 10.4 mg/kg in herbs purchased from general stores in Irbid city, Jordan which were higher than those reported in the present study. He further found 40% of the analyzed samples contained more than 10 mg/kg of lead levels. Our results on the other hand are contrary to those reported by Inoti, (Kiende Judy Inoti 2012) in vegetables grown in Thika town, Kenya. This is because most urban vegetables were grown in soils contaminated with heavy metals while the plants in the present study were mainly collected from rural areas accounting for the low lead concentrations.

The mean levels of thallium in plants from both regions ranged <0.01 mg/kg to 0.26±0.01mg/kg with 84.2% having mean levels of less than 0.1mg/kg (Table 1). *T. asiatica* had the highest mean thallium content and *T. stapfiana* the lowest. 94.7% of the plants had mean thallium levels of below 0.1 mg/kg. *T. asiatica* had the highest mean level of thallium of 0.23±0.01 mg/kg while *E. abyssinica*, *V. auriculifera*, *M. indica*, *A. abyssinica* and *T. stapfiana had* the lowest mean of <.02 mg/kg (Table 1). The mean contents range of the thallium in the plants from the same sampling region was often broad with maximum and minimum concentrations of approximately 26-fold in Manga and 12-fold in Borabu. The maximum permissible limits for thallium is set at 0.1 mg mL^{-1} (Karbowska 2016), meaning that plants in the present study had low amounts and are safe to be used.

In this work, four toxic elements; lead, arsenic, thallium and cadmium were quantified in the dry materials of plants with average values close to or slightly above their permissible limits of 10 mg/kg, 0.3 mg/kg, 0.3mg/kg and 0.1 mg/kg for Pb, Cd, As and Tl respectively (Karbowska 2016). It was found that none of the analyzed samples contained more than 10 mg/kg of lead, 31.6% of the analyzed samples in both study regions contained more than 0.3 mg/kg of cadmium, while 36.8% of the plants from Manga and 57.9% from Borabu sub-county contained more than 0.3mg/kg of arsenic. 10% of the plants from the study regions had Cd and As levels above their permissible limits. The major inputs of Cd, Pb and As to agricultural soils are atmospheric deposition, sewage sludge, animal manures, agrochemicals and inorganic fertilizers (Emongor 2007). Most plants from both sub-counties were collected from around farms where different amounts of farm inputs were applied. The different element absorption abilities of the plant species, accounts for the differences in the levels of the elements among the plants from the same study region. The ages of the plants used explain the intra plant species differences of Cd, Pb, As and Tl. Indeed, the determination of total concentration of the toxic elements in the dry matter gives an indication of the toxicity of the plant, but it is not sufficient in assessing the safety of the plant products because the consumers use most of the plants in the form of infusion in hot water or decoctions as directed by the herbalist. Therefore, the quantities of these elements in the infusions should be determined to give the clear picture of the amount of each element bio availed to the body of the user. Since some plants are taken with other food substance in solid form, more in-depth information of availability of the elements in the plants can be obtained by carrying out in vitro studies to simulate the digestion process through the alimentary canal.

The detection limit for ICP-MS instrument used was 1×10^{-19} g. ICP-MS results were backed up with analysis of plant reference material NIST 1647 (peach leaves) which had recoveries of 95% for Cd, 99% for As, 89% for Tl and 93% for Pb and 5.7% moisture content. This indicates that the digestion and quantification procedures gave valid and reliable results. The few outliers for Pb and As were not included and these were likely due to in homogeneities of the plant samples used for ICP-MS analysis. The separate aliquots of pre-ground material which were used could have led to mass loss due to the open digestion process and the moisture in the plant material.

3.2 Physiologically based extractions of As, Cd, Tl and Pb from the medicinal plants

The lowest percentage gastric phase extracted element was in lead from *S. mauense* with 0.02% while Arsenic from *S. indicum* was the highest with 18%. The lowest intestinal phase extracted element was lead from *P. barbatus* and *S. mauense* with 0.06% and the highest was arsenic from *S. indicum* with 45% (Table 2). The lowest total enzymatically extracted element was lead from *P. barbatus* with 0.08% and the highest was arsenic from *S. indicum* with 63% while the lowest aquatically extracted element was lead from *U. dioica* with 0.01% and the highest was arsenic from *S. mauense* with 22% (Figure 2 and Figure 3, Supplementary Figure S3 and Figure S4). The extraction efficiency for each enzymatic phase and aquatic differed from one element to the other and among the plants. The total amount of Cd, Pb, Tl and As extracted by water was statistically significant from the enzymatically extracted. The amount of each element extracted in the gastric phase was not statistically significant from that extracted in the intestinal phase ($p<0.05$) (Supplementary Table S2). There was a positive correlation between the amounts of the elements extracted in the gastric and the intestinal phases with the highest correlation being in Lead ($r = 0.931$) and the lowest in arsenic ($r = 0.103$). Similar trend was observed when the total enzymatic extraction was compared with aquatically extracted

Table 2
The mean total levels plus standard deviation (mg/kg) of Cd, Pb, Tl and As in medicinal plants from Manga and Borabu Study areas, Kenya

Botanical Name	Arsenic (As) (mg/kg)		Cadmium (Cd) (mg/kg)		Thallium (Tl) (mg/kg)		Lead (Pb) (mg/kg)	
	MMP	BMP	MMP	BMP	MMP	BMP	MMP	BMP
W. ugandensis	0.08±0.01	0.07±0.02	0.13±0.03	0.13±0.03	0.034±0.010	0.027±0.007	0.43±0.02	0.37±0.06
S. indicum	0.11±0.06	0.50±0.04	<0.08	<0.10	0.055±0.009	0.047±0.004	1.00±0.10	1.25±0.05
T. asiatica	0.27±0.02	0.50±0.05	0.17±0.02	0.13±0.01	0.260±0.010	0.230±0.010	1.40±0.10	1.25±0.05
E.abyssinica	0.08±0.01	0.50±0.02	<0.09	<0.10	0.115±0.008	<0.02	0.17±0.04	0.27±0.03
S. didymobotrya	0.90±0.07	2.10±0.20	0.16±0.05	0.20±0.05	0.029±0.009	0.030±0.004	0.65±0.03	0.39±0.03
V.auriculifera	0.40±0.03	<0.20	0.34±0.05	0.22±0.06	0.026±0.003	<0.02	0.73±0.03	0.40±0.10
P. barbatus	0.30±0.02	0.35±0.07	0.16±0.05	0.16±0.04	0.031±0.004	0.053±0.005	1.00±0.04	1.62±0.10
U.dioica	0.30±0.02	0.62±0.07	0.10±0.02	0.09±0.02	0.085±0.005	0.094±0.007	4.20±0.20	2.69±0.09
C. macrostachyus	0.30±0.02	0.45±0.05	0.98±0.03	0.50±0.04	0.027±0.003	0.030±0.002	2.20±0.10	0.81±0.08
B. pilosa	0.39±0.08	0.94±0.07	0.51±0.04	0.33±0.04	0.089±0.006	0.180±0.030	2.76±0.10	4.50±0.40
M. azedarach	0.20±0.02	0.30±0.01	0.40±0.02	0.70±0.10	0.104±0.007	0.029±0.003	0.24±0.02	1.70±0.30
S. mauense	0.50±0.03	0.5±0.02	0.24±0.02	0.42±0.04	0.142±0.007	0.100±0.008	2.90±0.10	1.76±0.10
M.indica	0.35±0.07	<0.20	0.08±0.02	<0.10	0.017±0.002	<0.02	0.25±0.02	0.21±0.05
A.hockii	0.24±0.07	<0.20	0.09±0.04	<0.10	0.028±0.002	0.025±0.003	0.16±0.02	0.15±0.01
A.abyssinica	0.24±0.05	<0.20	0.14±0.02	<0.10	0.022±0.006	<0.02	0.34±0.02	0.72±0.09
C.myricoides	0.60±0.10	0.40±0.03	0.47±0.05	0.16±0.04	0.077±0.005	0.041±0.005	1.72±0.09	0.64±0.04
C.edulis	0.39±0.07	0.51±0.08	0.27±0.03	0.32±0.05	0.061±0.007	0.065±0.006	1.30±0.10	1.36±0.04
T.stapfiana	0.10±0.05	0.20±0.01	0.53±0.04	0.98±0.06	<0.01	<0.02	0.14±0.03	0.22±0.03
A. Vera	0.28±0.07	0.32±0.08	0.12±0.02	<0.09	0.046±0.005	0.038±0.004	1.75±0.05	1.80±0.10
R		**0.731**		**0.647**		**0.789**		**0.713**
t-test		**0.163**		**0.441**		**0.162**		**0.370**
BMP- Borabu Medicinal Plant; MMP- Manga Medicinal Plant								

Table 3
Speciation, total enzymatic and daily intake of As, Cd, Tl and Pb from Manga medicinal plants

Medicinal Plant	Toxic Element	5-0-45µm (µg/kg)	0.45-10kDa (µg/kg)	10kDa-3kDa (µg/kg)	<3kDa (µg/kg)	Total enzymatic extraction (µg/kg)	Total water extraction (µg/kg)	Daily intake (µg/day)
S. indicum	As	0.126	0.016	0.031	0.081	0.697	0.247	0.014
	Cd	0.049	0.101	0.106	0.157	1.956	0.493	0.039
	Tl	0.075	0.377	0.251	0.017	5.865	0.629	0.117
	Pb	0.124	0.289	0.000	0.000	3.712	0.307	0.074
P. barbatus	As	0.225	0.018	0.059	0.065	0.578	0.347	0.012
	Cd	0.400	0.135	0.000	0.000	1.556	0.588	0.031
	Tl	0.233	0.479	0.036	0.011	2.024	0.794	0.040
	Pb	0.096	0.584	0.000	0.000	0.829	0.486	0.017
U.dioica	As	0.046	0.096	0.014	0.104	0.617	0.231	0.012
	Cd	0.099	0.086	0.017	0.008	2.350	0.288	0.047
	Tl	0.487	0.398	0.133	0.006	7.442	0.128	0.149
	Pb	0.045	0.321	0.150	0.000	7.410	0.504	0.148
B. pilosa	As	0.108	0.066	0.018	0.087	0.461	0.264	0.001
	Cd	0.014	0.322	0.232	0.009	3.914	0.624	0.078
	Tl	0.077	0.579	0.033	0.161	8.632	0.310	0.173
	Pb	0.123	0.373	0.024	0.000	6.517	0.561	0.130
S. mauense	As	0.112	0.119	0.013	0.121	0.688	0.331	0.014
	Cd	0.023	0.149	0.080	0.172	1.921	0.350	0.038
	Tl	0.143	0.978	0.003	0.081	7.950	1.055	0.159
	Pb	0.153	0.322	0.000	0.000	2.447	0.491	0.049
C. myricoides	As	0.062	0.101	0.016	0.280	0.904	0.243	0.018
	Cd	0.030	0.173	0.029	0.112	3.132	0.379	0.063
	Tl	0.171	0.453	0.011	0.000	5.521	0.544	0.110
	Pb	0.123	0.321	0.082	0.000	1.994	0.342	0.040
C. edulis	As	0.210	0.185	0.061	0.088	0.841	0.188	0.017
	Cd	0.019	0.260	0.072	0.065	1.632	0.308	0.033
	Tl	0.143	0.372	0.013	0.000	3.562	0.228	0.071
	Pb	0.084	0.121	0.000	0.000	1.844	0.202	0.037
A. Vera	As	0.087	0.096	0.017	0.081	1.064	0.134	0.021
	Cd	0.229	0.169	0.067	0.090	1.302	0.431	0.026
	Tl	0.453	0.313	0.010	0.000	2.214	0.083	0.044
	Pb	0.072	0.212	0.102	0.045	1.453	0.343	0.029

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The amount of Cd, As, Pb and Tl infused from the investigated plants ranged from 0.288-0.624, 0.134-0.347, 0.202-0.561, and 0.083-1.055 μg/kg respectively (Figure 4, Figure 5 and Supplementary Figure S3, Figure S4). The levels of the elements reported in the infusions of the plants are similar to those reported by de Oliveira et al. (de Oliveira et al. 2018) for As, Cd and Pb of not detected to 1.25, not detected to 0.61 and not detected to 5.3μgL^{-1} and 0-60%, 0-14% and 0-80% respectively released traditional and herbal teas. The physiologically based extractions assessed the bio accessibility of an element which dissolved during the digestion process and was availed for absorption into the human body. The amount of element extracted in the intestinal phase and the gastric phase differed from one element to the other and among the plant species. The As levels in the plant infusions were low with little transfer from the plants to infusion. Szymczycha-Madeja et al. (Szymczycha-Madeja et al. 2012)reported that the As levels in the infusions was low as compared to the total elemental concentration in the plant. They also reported that 29-88% of As was in the inorganic species form. de Oliveira et al. (de Oliveira et al. 2018) reported also that Cd concentrations in tea infusions ranged from 0.13-0.61μgL^{-1} with 5-21% being solubilized. Zhou et al. (Zhou et al. 2018) reported the average bio-accessibilities of Pb, As, Hg and Cd to be 40.11, 64.46, 18.91, and 81.14%, respectively from *C. sinensis.* 8.69% of the total As was the in inorganic form which constituted 0.56 ± 0.16 and 0.29 ± 0.06 mg $kg^{-1}$of As (III) and As (V) respectively. The sum of the gastrointestinal solutions was 2.00–2.73% of the total and the hazard bio-accessibility quotient target values for Pb, As, Cd and Hg were 0.0040, 0.5334, 0.0020 and 0.0005 which were much higher than those reported in the present study (Figure 3, Figure 4 and Figure 5).

The result of the enzymatic breakdown of the plant as it passes down the human alimentary canal was used to calculate the daily element intake amounts. If a consumer took 200 mL infusion prepared from 2 g of the powdered plant sample twice a day, as recommended by the herbalist and by using the mean extraction efficiency (%) of Pb, As, Tl and Cd, the estimated daily intake were calculated to be 0.027-0.148 μg/day, 0.001-0.021 μg/day, 0.04-0.159 μg/day and 0.026-0.078 μg/day respectively for the investigated plants. Our results are similar to those reported by Mirosławski and Paukszto, (Mirosławski and Paukszto 2018) for Cd and Pb in Chamomile blossom and Peppermint leaves of 0.07, 0.16 and 0.08, 0.08 μg/day respectively. Zhang et al. (Zhang et al. 2018) reported intake of lead from young tea leaves of less than 1.23×10^{-4} mg/kg /day and that of As of about 100 folds lower than the value of 2.67×10^{-4} mg/kg /day reported by Nkansah et al. (Nkansah et al. 2016) These values were far below the tolerable daily intake of Pb (0.3 mg/day) and Cd (1.5 ug/day). The mature tea leaves had higher estimated daily intake than the young leaves for Pb, Cd, Hg and As implying that drinking mature tea infusions could increase the source of heavy metals to the human body. The estimated daily intake values in that study were below the recommended daily intake values for the four elements suggesting that the consumption young tea leaves did not pose a health risk to human beings (Sarma et al. 2011; Nkansah et al. 2016; Zhang et al. 2018). This means that using the young leaves of plants (those plants in which the leaves are used for therapeutic purposes) to make the infusion could further increase the safety.

3.3 Ultra filtration of As, Cd, Tl and Pb in the medicinal plants

The results for As, Cd, Tl and Pb are summarized in Figure 2, Figure 3 and Supplementary Figure S1 and Figure S2. Cd, Tl and Pb existed mainly (>70) in high molecular mass species with minor percentage of lower molecular mass species. All molecular mass species of Arsenic were present in the plant species investigated. Since As, Cd, Tl and Pb existed in the high molecular species, lower amounts of the elements diffused into the blood stream across the villi in the ileum. This means that the amount of the As, Cd, Tl and Pb absorbed could be lower than that bio-available from the plants. This increases the plant safety as low amounts of As, Cd, Tl and Pb elements are absorbed. The results are contrary to those we reported for Zn, Mn, Cr and Cu in the sequential extraction as novel approach to compare 12 medicinal plants from Kenya regarding their potential to release chromium, manganese, copper and zinc (Mogwasi et al. 2018). The low molecular mass species enhanced the absorption of the therapeutic elements while the existence of the toxic elements in large molecular weight species means less amounts are absorbed, lowering their toxicity.

Plants investigated in the present study had minimal potential of releasing the toxic elements and can safely be used for nutrient supplementation and therapeutically. However, the content of Pb, Tl, As and Cd must be monitored frequently to avoid the accumulation of these toxic elements.

The determination of total elemental content of Cd, As, Hg, Tl and Pb in the plants improves the estimation of the toxicity estimates during the therapy with the said plants. The molecular forms of the extracted elemental species are largely not known and a detailed understanding of the molecular mechanism of the potential toxic effects of the plant derived elemental species should be unveiled. Ultra-filtration with offline elemental detection has been shown to be effective in the identification of the elemental molecular size species in a given plant (Nischwitz et al. 2017). Therefore, sequential ultra-filtration was applied to selected plants from Manga sub-county extracts. Most plants were usually infused in hot water for at least one hour before consumption. The procedure was used to extract cadmium, lead, thallium and arsenic in the plants which were then fractionated into different molecular mass species. The hot water extracts of the eight plants were subjected to a 4-step sequential filtration procedure resulting in the following size fractions as described in the experimental section: <3 kDa, 3-10 kDa, 10 kDa-0.45 μm and 0.45-5 μm to estimate the toxic metal uptake from the plant. Elemental analysis of the fractions showed that the mass distribution across the mass fractions dependent on the element and the plant species.

4. Conclusion

The strategy of total element, physiologically based extraction tests and molecular size fractionation of elemental species achieved a comprehensive determination of the total content, bio accessibility and bioavailability of Cd, As, Tl and Pb to the human body from the commonly used plants from two regions in Kenya. The authorities can use the data generated in regulating the use of the medicinal plants to ensure safe consumption and to take appropriate measures to avoid using plants grown in areas with high contamination. The approach substantially supports toxicological investigations to provide complementary information on uptake and metabolism of toxic elements such as Cd, As, Tl and Pb from the plants. Based on the data collected it is possible to select the safest plant species out of the many available plants in Kenya and other third world countries for the safe management of diseases. This approach opens a perspective to maximize the benefit from locally available low cost plant product thus lowering the health care systems pressure for the third world countries such as Kenya. However, the safest age of the plants, the organic ligands of Cd, As, Tl and Pb in the plants needs to be determined for detailed organometallic compound determination using HPLC-MS/MS. In vivo bio availability and bio accessibility studies of Cd, As, Tl and Pb from the plants using laboratory animals need to be done to understand the toxicity of these elements in detail.

Declarations

Conflict of interest

The authors declare no conflict of interest whatsoever

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Data availability statement

The data presented in the paper cannot be used in any form without the authors’ permission.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Authors' contributions

Richard Mogwasi: Carried out the analysis and authored the paper.

Zachary Moronga Getenga: Designed the research and edited the manuscript.

Kennedy Olale: Carried out the research and edited the manuscript

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Figures

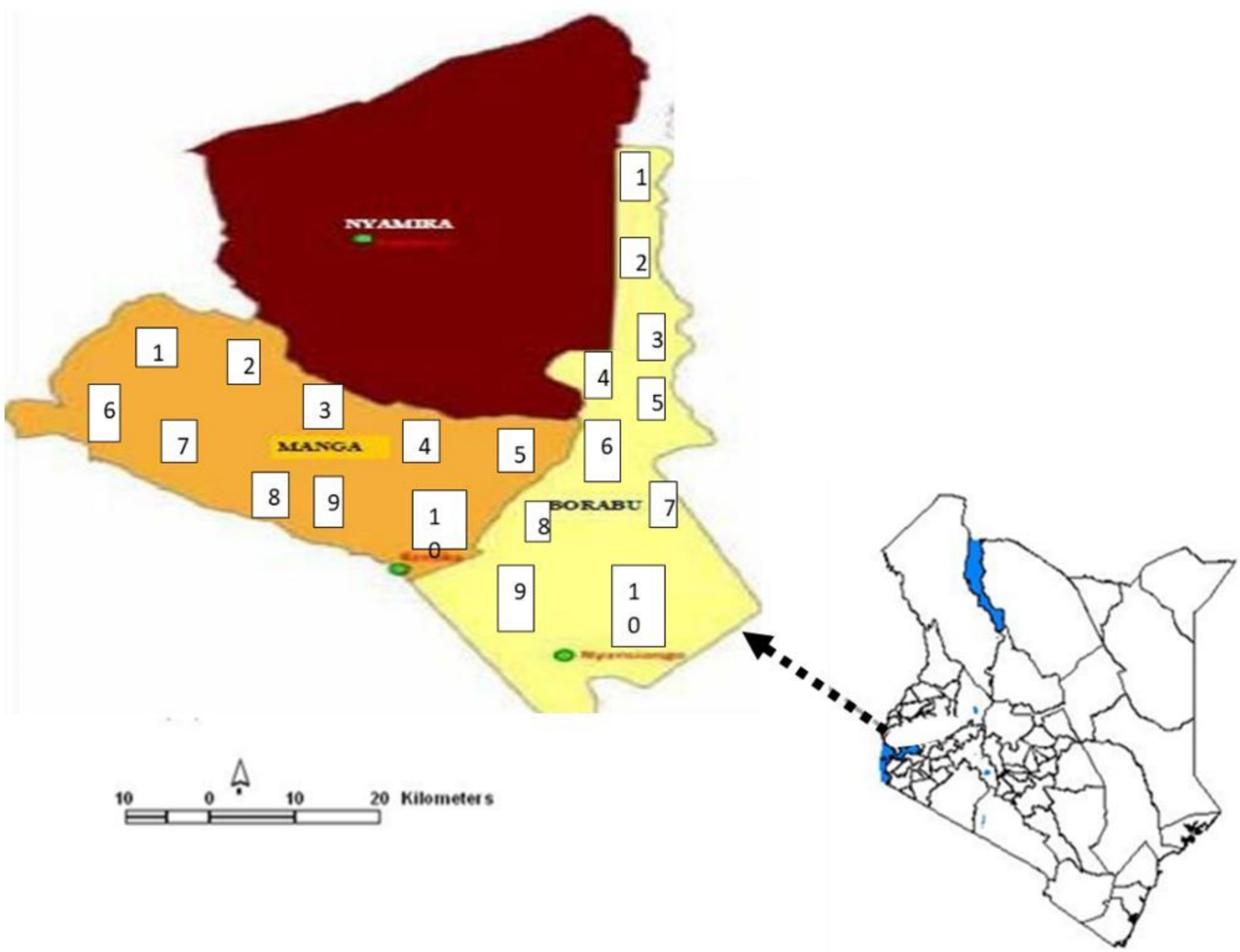

Figure 1

Study area showing sampling points in Manga and Borabu sub counties

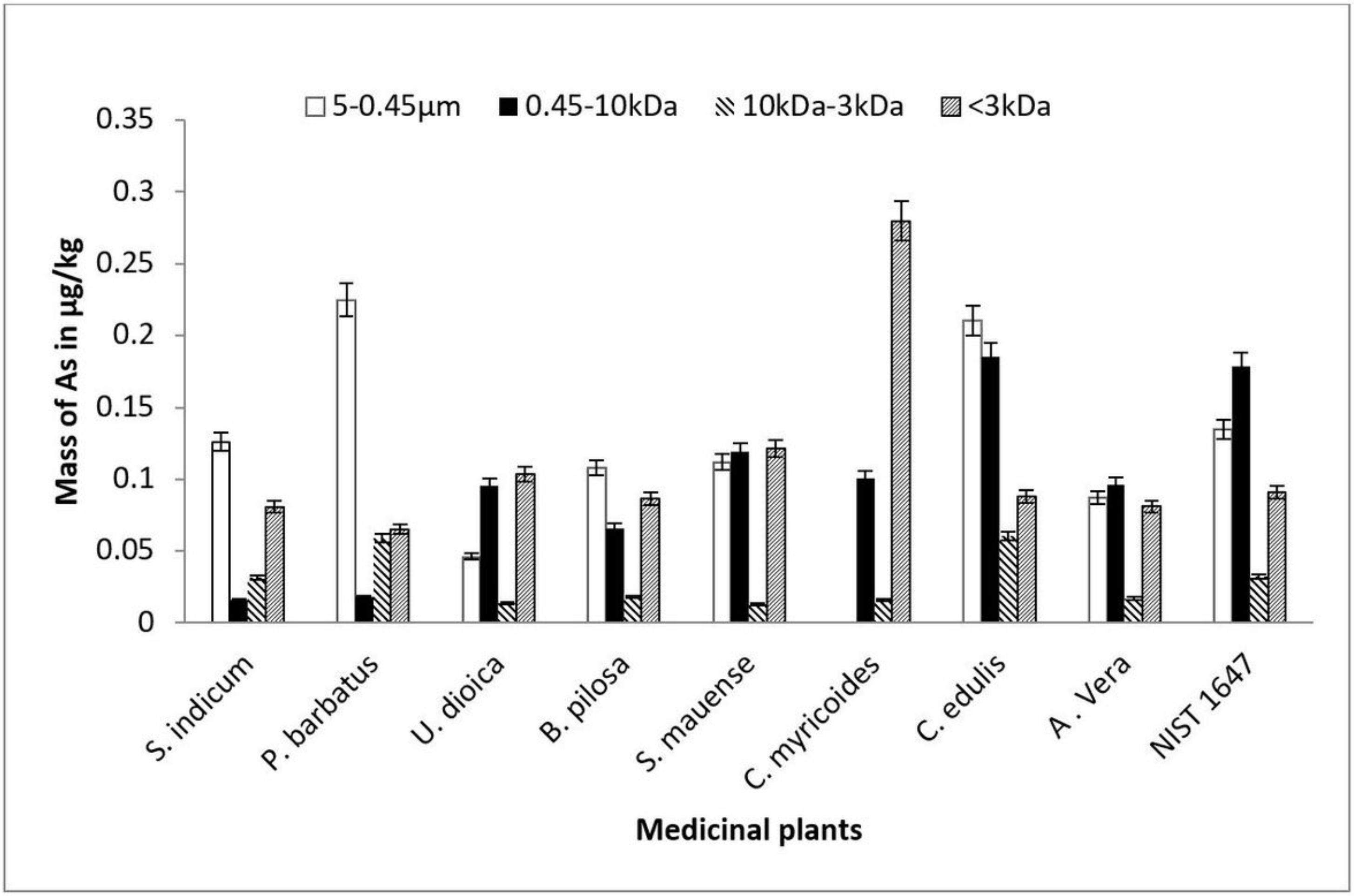

Figure 2

Size fractionation of maximum mass of As in extracts from 8 plants obtained by sequential filtration with subsequent ICP-MS detection

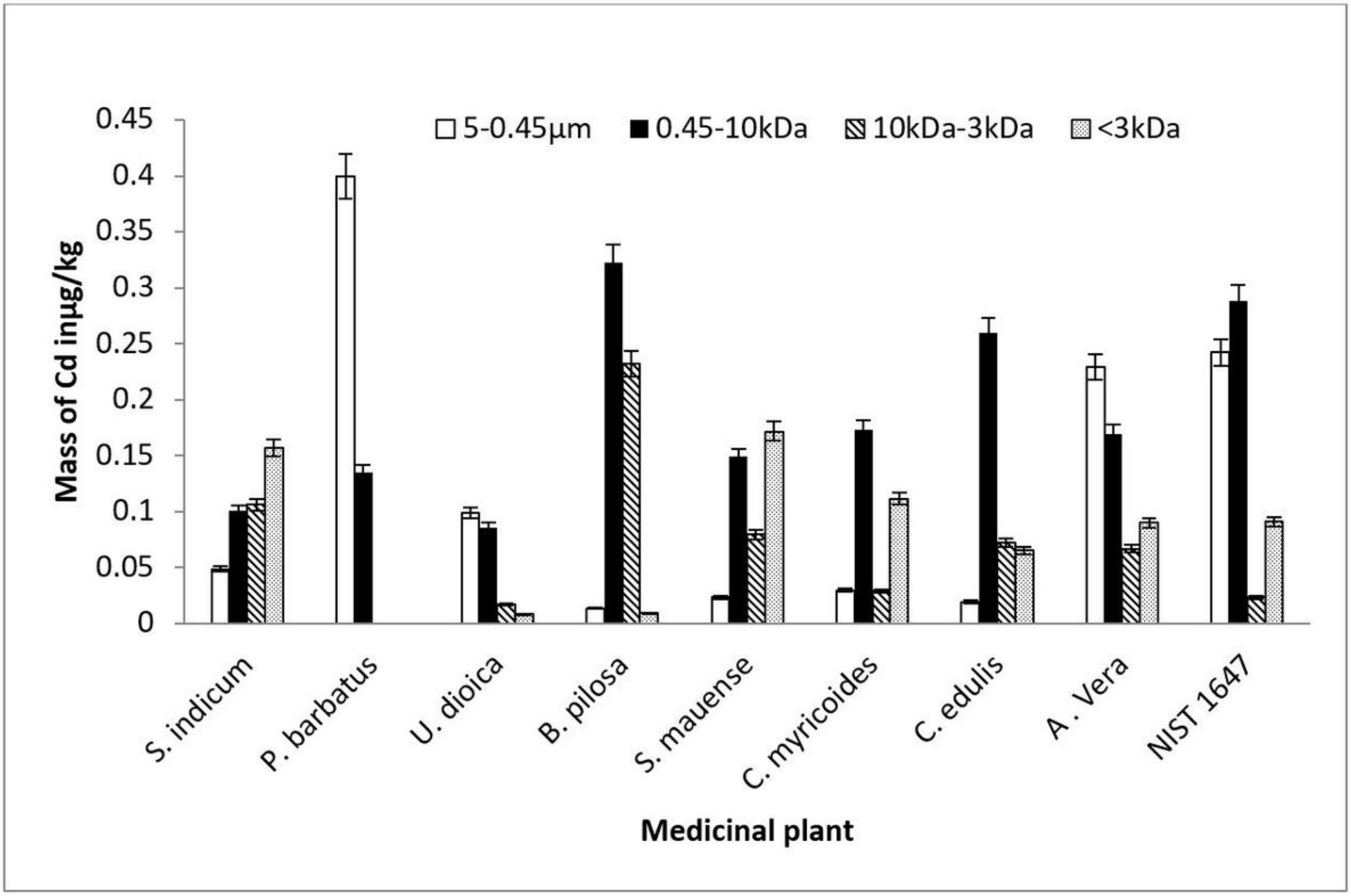

Figure 3

Size fractionation of maximum mass of Cd in extracts from 8 plants obtained by sequential filtration with subsequent ICP-MS detection

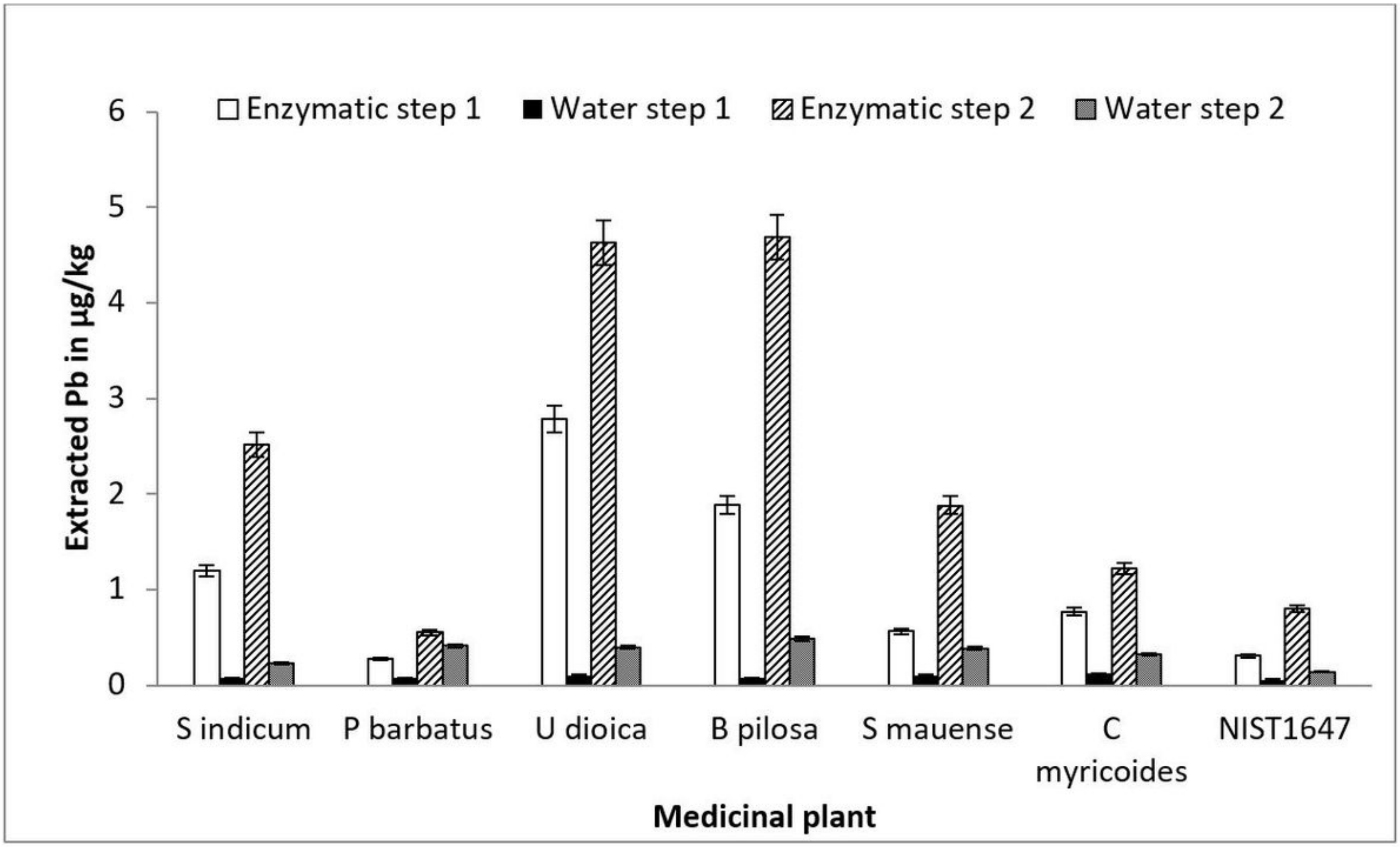

Figure 4

Comparison of 2-step of maximum mass of aqueous and enzymatic extraction for medicinal plants Pb in selected medicinal plants

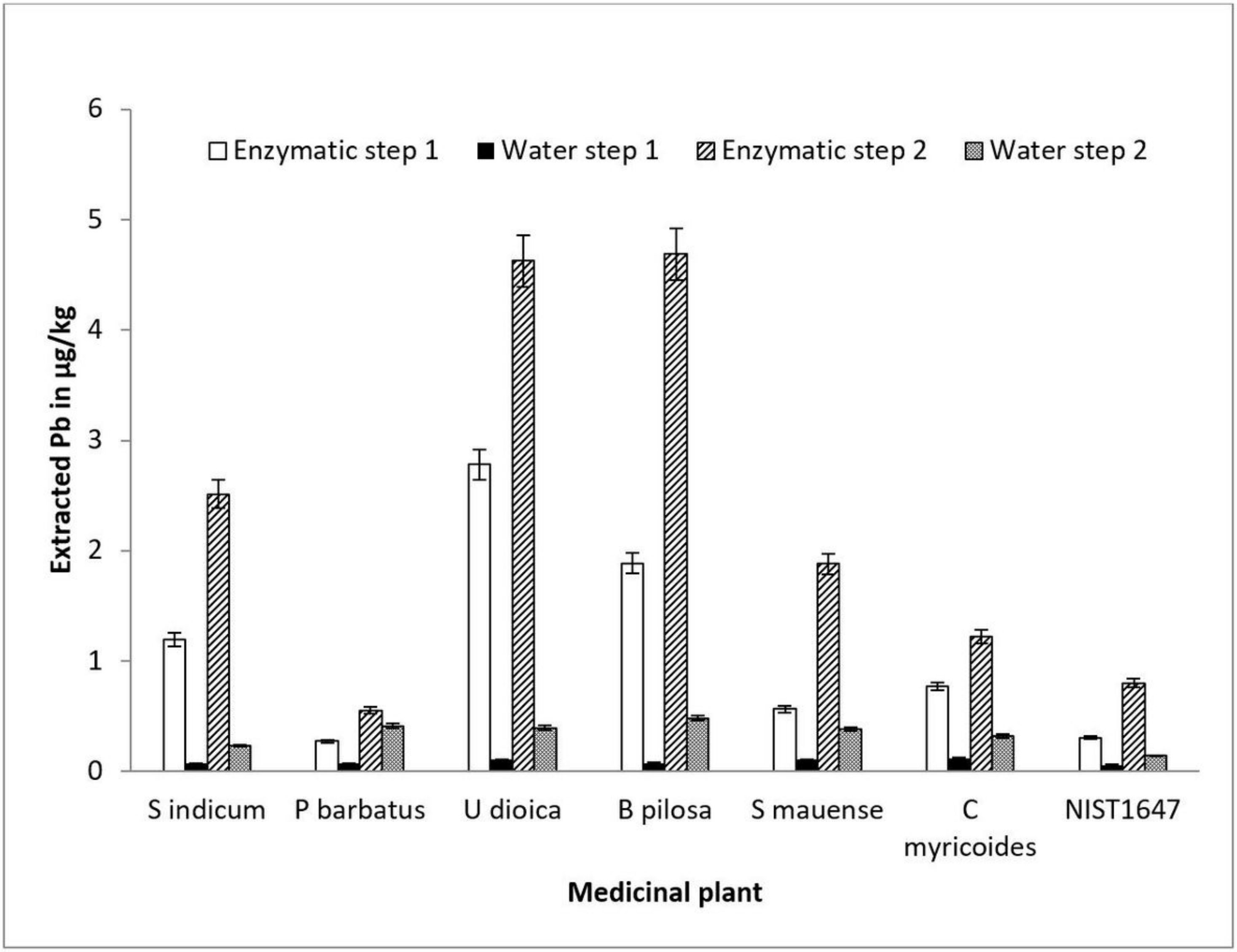

Figure 5

Comparison of 2-step of maximum mass of aqueous and enzymatic extraction for medicinal plants Pb in

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