Determination of potentially toxic heavy metals in traditionally used medicinal plants for HIV/AIDS opportunistic infections in Ngamiland District in Northern Botswana

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A B S T R A C T

The determination of four potentially toxic heavy metals, arsenic, chromium, lead and nickel in twelve plant species used for the treatment of perceived HIV and AIDS-associated opportunistic infections by traditional healers in Ngamiland District in Northern Botswana, a metal mining area, was carried out using atomic absorption spectrometry. The medicinal plants; *Dichrostachys cinerea*, *Maerua angolensis*, *Mimusops zeyheri*, *Albizia anthelmintica*, *Plumbago zeylanica*, *Combretum imberbe*, *Indigofera flavicans*, *Clerodendrum ternatum*, *Solanum panduriforme*, *Capparis tomentosa*, *Terminalia sericea* and *Maytenus senegalensis* contained heavy metals in varying quantities: arsenic 0.19–0.54 $\mu g\,g^{-1}$, chromium 0.15–1.27 $\mu g\,g^{-1}$, lead 0.12–0.23 $\mu g\,g^{-1}$ and nickel 0.09–0.21 $\mu g\,g^{-1}$ of dry weight. Chromium was found to be the most abundant followed by arsenic and lead. Nickel was undetectable in nine plant species. *M. senegalensis* contained the largest amounts of arsenic, chromium and lead. All metals determined were below the WHO permissive maximum levels. The possible maximum weekly intakes of the heavy metals following treatment regimes were insignificant compared to the provisional tolerable weekly intake levels recommended by WHO and the Joint FAO/WHO Expert Committee on Food Additives. This suggests that heavy metal exposure to patients originating from consumption of traditional medicinal plant preparations is within non health-compromising limits.

1. Introduction

Industrialization of the last few hundred years has led to advances in medical sciences and to a better understanding of the causes of infectious diseases as well as those diseases that result from metabolic disorders. Modern drugs manufactured by the multi-national pharmaceutical companies do not in many cases cater for the needs of the majority of poor people that reside in the low and middle income countries of the world [1]. Such drugs are neither readily available nor affordable by the poor. Traditional health care systems and cures based on the use of medicinal plants not only remain a routine treatment because they are the mostly available and are affordable alternatives for many countries but also because their use is ingrained in the culture of the users. The World Health Organisation (WHO) reports that about 80% of the low income countries still consult traditional health care practitioners [2].

Although the benefits derived from traditional medicines are well established [3,4], there are also reported cases of poisoning and toxicity manifestations associated with their use [3–6]. These untoward effects may be due to poisonous constituents, such as alkaloids [7], microbes and their metabolites [8], cardiac glycosides [9], pesticides [10] and metals [5,11].

Health research involving epidemiology and toxicology studies continue to implicate metals as a possible cause of adverse human health effects [5,11] based on the fact that they are actively involved in many biological processes [12]. Metals are non-biodegradable and are cumulative in nature rendering them as persistent pollutants. Several researchers have documented cases in which metal content in plants is related to toxicity. Asian traditional medicines

Abbreviations: AAS, atomic absorption spectrometry; AIDS, acquired immune deficiency syndrome; ARV, antiretroviral; FAO, food and agriculture organisation; HIV, human immunodeficiency virus; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; IOCG, iron oxide copper gold ore deposit; JECFA, joint FAO/WHO expert committee on food additives; PTWI, provisional tolerable weekly intake; WHO, world health organisation.

have been reported to contain high levels of heavy metals such as arsenic, lead and mercury [13,14]. It is worthy to note that the presence of these metals is not always natural. In some Indian medical systems such as Ayurveda and Unani, additions of heavy metals have been an intentional make-up of the remedies [5]. Lead in traditional remedies was determined in 100 products used as aphrodisiacs [15]. Besides Asian medicines, heavy metals have also been detected in herbal remedies in South America [16,17] and Africa [14,18–20]. In South Africa, a case was reported where a 7-month old baby orally ingested a traditional remedy with high levels of manganese and chromium [21]. Though there is a growing interest in heavy metal analysis of medicinal plants and a clear need to monitor its status to avoid health hazards, data remain very scarce.

Studies aiming to determine metals qualitatively and quantitatively in traditional plant remedies are significant to Botswana for several reasons. Firstly, a majority of the population is actively consuming traditional plant medicines for the treatment of several ailments [22]. Secondly, Botswana is a highly mineralized country, whose economy is apart from diamonds dependent on the exportation of copper and nickel. Particularly the North-Western part of Botswana has rich mineral deposits and mining exploration and activities are ongoing with high intensity. Several studies have indicated that the waters and/or sediments in the Okavango Delta do contain several metals including arsenic [23], lead and iron [24], manganese, zinc, copper and nickel [25], sodium, potassium, calcium, magnesium, zinc, copper, manganese, cobalt, iron, chromium, nickel and lead [26]. Thirdly, the profound influence of mining activities on the bioaccumulation of heavy metals in plants has been demonstrated in studies on several plants in Botswana, such as *Blepharis aspera*, *Indigofera melanadenia* and *Tephrosia longipes* which have been identified as accumulators and hyperaccumulators of metals [27–30]. *Helicrysum leptolepis* in Ghantsi, Botswana and *Echolium lugarde* were found to indicate the presence of high amounts of copper [31] which might possibly be the consequence of the Kalahari Copper Belt stretching from Ghantsi to North-Western Botswana. Such studies have also recently been conducted in India [32] illustrating the importance. Though Sawula [25] detected heavy metals (manganese, zinc, copper and nickel) around the Okavango Delta in Northern Botswana which is a major site of considerable plant biodiversity in the country, and Masamba and Mazvimavi [24] reported elevated lead and iron concentrations in water outlets of the Okavango, there are no data on the heavy metal status of medicinal plants in that area.

Fourthly, Botswana is one of the countries in Southern-Africa which is hardest hit by the HIV/AIDS pandemic. All HIV-infected patients in the country who have CD 4 counts less than 250 or have an AIDS-defining illness are eligible for free antiretroviral (ARV) therapy. Nevertheless, like in neighbouring South-Africa and most countries in Africa, many HIV/AIDS patients in Botswana regularly consult traditional healers and are likely to consume medicinal plant preparations [33–35]. It is important therefore to assess whether metals potentially present in traditional medicinal plant remedies would interact with the active ingredients in the ARVs. Previous research has shown that the uptake of drugs, especially of antibacterial drugs, is significantly reduced in the presence of metals ions [36,37].

Lastly, there is currently no national policy to guide the regulation and monitoring of the quality, safety and efficacy of the use of traditional medicines.

In this study, we report the status of potentially toxic heavy metals, arsenic, lead, nickel and chromium in twelve medicinal plant species frequently used by traditional healers to treat opportunistic infections potentially associated with HIV/AIDS in Ngamiland District in North-Western Botswana as determined by atomic absorption spectroscopy after acid digestion.

2. Materials and methods

2.1. The study site and medicinal plant collection

Medicinal plant specimens were collected from Ngamiland District in North-Western Botswana, the third largest district in the country (Fig. 1). Its district capital is Maun with about 43,776 inhabitants. The centre piece of Ngamiland District is the Okavango Delta, the largest inland wetland in the world with considerable biodiversity. Most of the estimated 3000 plant species in Botswana are localized here. Over 95% of people in the Okavango Delta, directly or indirectly depend on natural resources found in the wetland to sustain their livelihoods and many traditional healers are based here. At the same time, Ngamiland District is an area of intensive mineral exploration and mining activities. The mineralization model of Ngamiland is highly likely associated with an iron oxide copper gold (IOCG) ore deposit, also referred to as the Ngamiland IOCG. IOCG type deposits encompass a wide spectrum of ore bodies, often polymetallic and of significant size, which may notably produce iron, copper, gold, silver, uranium, manganese, nickel, platinum group elements, cobalt and rare earth elements, all of which have been identified in the Ngamiland IOCG [38]. Respective medicinal plant samples were collected from three different sites near the village of Makalamabedi (344 residents), situated 56 km south of Maun (Fig. 1) and in an area where many mining prospecting licences have been granted [39]. Plant samples were dried and kept at room temperature until analyzed. All plant specimens were processed according to good botanical practice procedures and were taxonomically authenticated by Mbaki Muzila (Curator of the University of Botswana Herbarium) and kept as voucher specimen in the University of Botswana Herbarium with codes such as KM-Mn08-101.

Medicinal plant uses were established through administering a semi-structured questionnaire to traditional healers asking about traditional treatment regimes in relation to potential HIV/AIDS associated opportunistic infections, whose symptoms form part of the diagnostic criteria for HIV/AIDS [40] and after obtaining informed consent from traditional healers.

2.2. Sample preparation

Respective dried plant parts were coarsely ground using a pestle and mortar and passed through a 1000 μm^2 metal sieve. 1 g of powdered sample was dissolved by acid digestion in a 8 mL-mixture of concentrated HCl and concentrated HNO_3 in a 3:1 ratio. The sample was digested for 3 h and the remaining contents were dissolved in ultra high purity (UHP) water, filtered by gravity and diluted to a final volume of 100 mL.

2.3. Reagents and chemicals

In this study, UHP water (18 mΩ) from a Millipore Water System (Bedford, MA, USA) was used. CP grade hydrochloric acid (HCl) and AR grade nitric acid (HNO_3) were obtained from Rochelle (Johannesburg, South Africa). Metal working standard solutions were prepared by diluting 1000 ppm stock solutions purchased from Rochelle (Johannesburg, South Africa).

2.4. Instrumentation and standards

The atomic absorption spectroscopy (AAS) measurements were performed using an air-acetylene flame atomic absorption spectrometer from Varian (Varian SpectrAA 220FS, Australia). Metal hollow-cathode lamps (Photrons, USA) specific for each of the determined metals were employed as radiation/sources. Working standards of different concentrations as per the instructions of the

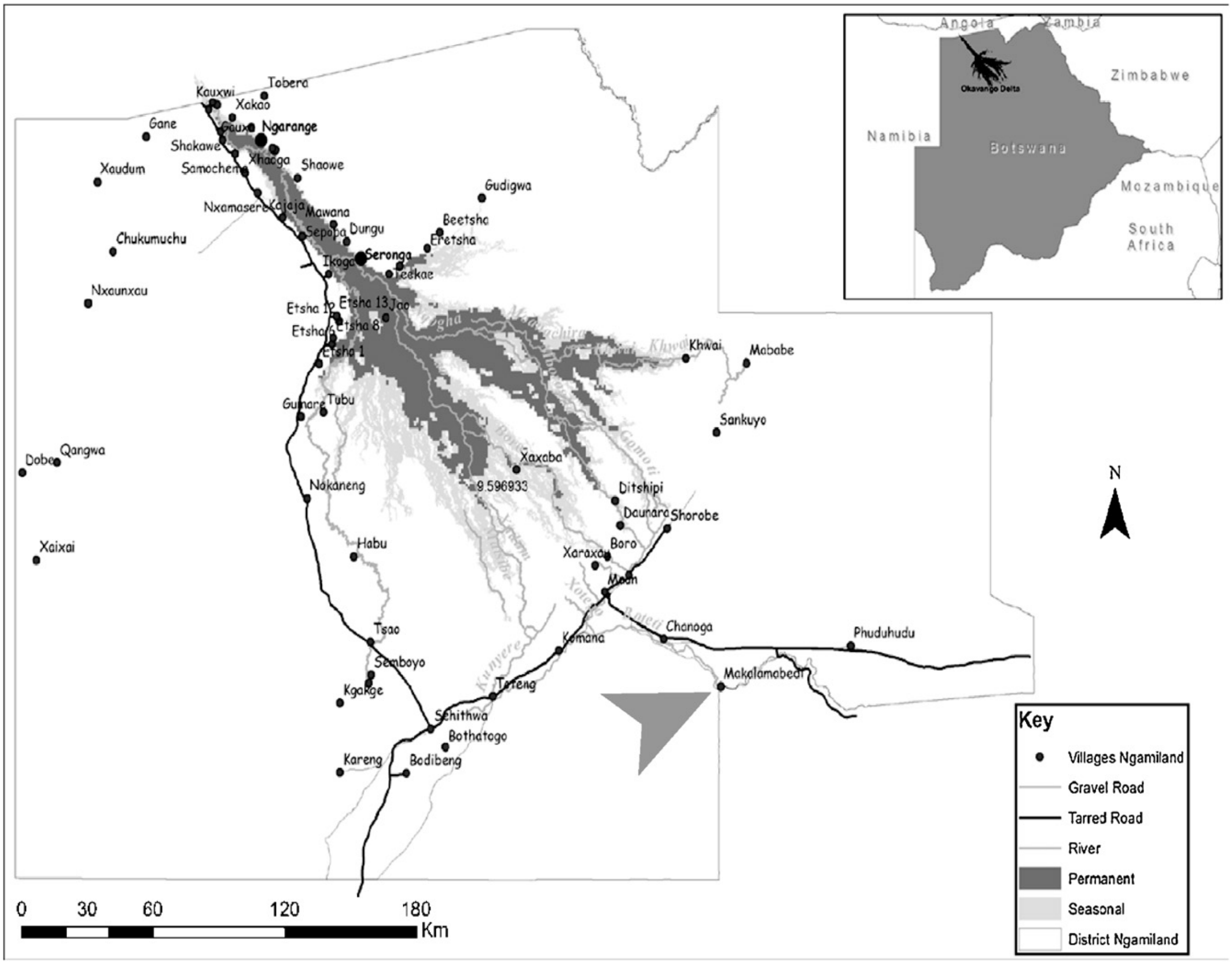

Fig. 1. The map of the study site, the Okavango delta, showing Makalamabedi, the sampling site.

hollow-cathode lamps were prepared to detect each metal concentration. A five-point calibration curve was automatically generated by the instrument. Calibration curves with good linearity, $r^2 > 0.964$, were obtained except for arsenic $r^2 = 0.932$. Analysis of each of the sample solutions was done in triplicate and the results expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Medicinal plants uses in relation to potential HIV/AIDS-related opportunistic infections

Twelve medicinal plants, *Dichrostachys cinerea* (L.) Wight & Arn., *Maerua angolensis* DC, *Mimusops zeyheri* Sond., *Albizia anthelmintica* Brongn., *Plumbago zeylanica* L., *Combretum imberbe* Wawra, *Indigofera flavicans* Baker, *Clerodendrum ternatum* Schinz, *Solanum panduriforme* E. Mey, *Capparis tomentosa* Lam., *Terminalia sericea* Burch. ex DC. and *Maytenus senegalensis* Lam. Exell, were named by traditional healers as useful in the treatment/management of potential HIV/AIDS-related opportunistic infections with symptoms, such as persistent cough, tuberculosis, frequent fevers, diarrhoea, skin rashes and sores, thrush, womb problems and sexually transmitted infections (Table 1). Roots were the most prominent plant part used. The concentrations of arsenic, chromium, lead and nickel were determined in each of the plants using acid digestion for sample preparation followed by atomic absorption spectroscopy.

3.2. Levels of arsenic and chromium in medicinal plants

Arsenic and chromium concentrations were determined in each of the twelve medicinal plant samples using atomic absorption spectroscopy. The results in Table 2 show that arsenic and chromium were present in all plant samples. Arsenic concentrations ranged from 0.19 to 0.54 $\mu g\,g^{-1}$ and the limit of detection was 0.1551 $\mu g\,g^{-1}$. The lowest arsenic level was determined in *M. angolensis* DC (Moretete/Moreketi) and the highest in *M. senegalensis* Lam. Exell (Mothono). Previous studies have shown that plants in different regions of the world contain arsenic levels of similar order of magnitude. For example, the characterization of two medicinal plants in South America revealed plants to contain 0.58 and 0.96 $\mu g\,g^{-1}$ of arsenic [41], Bulgarian herbal tea plant material was determined to contain 0.01–0.22 $\mu g\,g^{-1}$ of arsenic [42] using microwave digestion and inductively coupled plasma mass spectrometry (ICP-MS). Other studies utilizing hydride generation with atomic fluorescence spectrometry have shown higher arsenic concentrations of 6.38 $\mu g\,g^{-1}$ and 2.40 $\mu g\,g^{-1}$ [43] and 20.6 $\mu g\,L^{-1}$ and 35.5 $\mu g\,L^{-1}$ [44] in Chinese herbal medicines. In all medicinal plant samples, the concentration of arsenic did not exceed 1 $mg\,kg^{-1}$ which is the recommended limit for medicinal plants [45].

Table 1
Traditional medicinal plants investigated for heavy metal status and their uses.

Scientific name/family/voucher	Local name	Plant part	Traditional use
Dichrostachys cinerea (L.) Wight & Arn./Fabaceae/KM-Mn08-101	Moselesele	Roots, branches, leaves	Skin rashes, uterus problems, STIs[a]
Maerua angolensis DC/Capparaceae/KM-Mn08-34	Moretete/Moreketi	Roots	Skin rashes, sores, womb cleansing, STIs
Mimusops zeyheri Sond./Sapotaceae/KM-Mn08-102	Mmupudu	Roots	Tuberculosis, weight loss, womb problems, STIs
Albizia anthelminthica Brongn./Fabaceae/KM-Mn (Ser) 08–103	Monoga	Roots	Tuberculosis, persistent cough, STIs
Plumbago zeylanica L./Plumbaginaceae/KM-Mn08-54	Masigomabe	Roots	Skin sores/rash, HIV/AIDS related symptoms/thrush, frequent fevers, persistent cough, STIs
Combretum imberbe Wawra/Combretaceae/KM-Mn08-4	Motswiri	Leaves	Persistent cough, tuberculosis, diarrhoea, weight loss, skin rashes, womb problems
Indigofera flavicans Baker/Fabaceae/KM-Mn08-25	Tshikadithata	Roots	Diarrhoea, HIV/AIDS related symptoms
Clerodendrum ternatum Schinz/Lameaceae/KM-Mn11/104	Legonyana	Roots	Frequent fevers, skin rashes, HIV/AIDS-related symptoms such as thrush, STIs, womb problems
Solanum panduriforme E.Mey/Solanaceae/KM-Mn08-105	Thulathulane	Roots	Persistent cough, womb problems
Capparis tomentosa, Lam./Capparaceae/KM-Mn08-106	Motawana	Roots	Skin rashes
Terminalia sericea Burch.ex DC./Combretaceae/KM-Mn08-107	Mogonono	Roots	Diarrhoea, skin rashes, tuberculosis
Maytenus senegalensis, Lam. Exell/Celastraceae/KM-Mn08-60	Mothono	Roots	Persistent cough, tuberculosis, STIs

[a] Sexually transmitted infection.

Chromium concentrations ranged from 0.15 $\mu g\,g^{-1}$ to 1.27 $\mu g\,g^{-1}$ with a detection limit of 0.0801 $\mu g\,g^{-1}$. *D. cinerea* (L.) Wight & Arn. (Moselesele) contained the lowest concentration whilst *M. senegalensis* Lam. Exell (Mothono) contained the largest concentration of chromium. A study by Gjorgiova et al. [46] of toxic and essential metals in medicinal plants showed chromium levels in the range of 0.57–1.88 $\mu g\,g^{-1}$. These values were in agreement with the determined values in the present study. A study by Canterelli et al. [41] employed the use of inductively coupled plasma optical emission spectrometry (ICP-OES), for the determination of chromium in two medicinal plants. The study determined 4.52 and 3.33 $\mu g\,g^{-1}$ of chromium in the plants, which is higher than values obtained in the present study. Yet another study by Gomez et al. [17] showed analysis of plant material from Argentina by ICP-OES to contain less than 0.04 $\mu g\,g^{-1}$ of chromium. The values determined in the present study were also lower than the WHO and FDA recommended daily intake of chromium for foods and feeds which is set at 120 $\mu g\,g^{-1}$ [45] but not as low as those determined by Gomez et al. [17].

Arsenic and chromium levels determined in the present study only indicate the total metal quantity and not the speciation quantities. Previous studies of arsenic in water from the Okavango Delta showed that As (III), the toxic form of arsenic, constitutes 50–74% of the total amount of arsenic [23]. It is however important to note that metals in plants are organically bound and therefore the toxicity levels of these metals in plants are low [42].

3.3. Levels of lead and nickel in medicinal plants

All plant species contained lead in a concentration range of 0.12–0.23 $\mu g\,g^{-1}$, and a detection limit of 0.03 $\mu g\,g^{-1}$, with the exception of *P. zeylanica* L. (Masimagobe) where no lead was detected (Table 2). Of the plant species in which lead was detected, *A. anthelmintica* Brongn. (Monogo) contained the least amount of lead whereas *C. ternatum* Schinz (Legonyana) and *M. senegalensis* Lam. Exell (Mothono) contained the greatest amount of lead. The WHO maximum permissible levels of lead was set at 10 $\mu g\,g^{-1}$ [45] and the levels obtained for all the plants in the present study were much lower indicating that the plants studied have lead levels well below toxicity levels. Researchers [16,18,19] showed in their studies similar results for lead. A study by Ang [15] determining lead contamination in *Eugenia dyeriana* from different sources using acid digestion followed by AAS showed widely varying concentrations of 2.34–13.20 $\mu g\,g^{-1}$ of lead with 22% of the plants exceeding the limit of 10 $\mu g\,g^{-1}$. Lekouch et al. [14] and Mazzanti et al. [47] also showed in their study varying results of lead in traditional plant derived medicines of 2.2–19.9 $\mu g\,g^{-1}$ and 0.18–8.84 $\mu g\,g^{-1}$, respectively. These values were much higher than those observed in the present study, and suggested that several factors might contribute to the metal concentrations in herbal remedies including location, mode of preparation and different environments.

Nickel was detected in only 3 of the 12 plant species (Table 2); *A. anthelmintica* Brongn. (Monoga) 0.17 ± 0.01 $\mu g\,g^{-1}$, *T. sericea* Burch ex DC. (Mogonono) 0.21 ± 0.02 $\mu g\,g^{-1}$ and *M. senegalensis* Lam. Exell (Mothono) 0.09 ± 0.01 $\mu g\,g^{-1}$. These results may suggest that the nickel content in these plants was below the detection limit of the instrument of 0.055 $\mu g\,g^{-1}$. Gjorgiova et al. [46] also observed metal concentrations lower than the limit of detection. Likewise in a study by Annan et al. [48], of 27 plant samples, nickel was determined in only 8 samples. However, contrary to the present study seven of the eight samples contained levels that were higher than

Table 2
Metal (As, Cr, Pb, Ni) content of plant samples as analyzed by AAS.

Medicinal plant (scientific name)	Plant part	Arsenic (As) ($\mu g\,g^{-1}$)	Chromium (Cr) ($\mu g\,g^{-1}$)	Lead (Pb) ($\mu g\,g^{-1}$)	Nickel (Ni) ($\mu g\,g^{-1}$)
Dichrostachys cinerea	Branches	0.21 ± 0.02	0.15 ± 0.02	0.21 ± 0.01	ND
Maerua angolensis	Roots	0.19 ± 0.02	0.98 ± 0.12	0.21 ± 0.02	ND
Mimusops zeyheri	Roots	0.23 ± 0.01	0.73 ± 0.17	0.20 ± 0.01	ND
Albizia anthelmintica	Roots	0.29 ± 0.01	0.53 ± 0.19	0.12 ± 0.02	0.17 ± 0.01
Plumbago zeylanica	Roots	0.37 ± 0.01	1.22 ± 0.61	ND	ND
Combretum imberbe	Roots/leaves	0.35 ± 0.01	1.16 ± 0.30	0.14 ±± 0.05	ND
Indigofera flavicans	Branches	0.40 ± 0.02	0.94 ± 0.20	0.21 ± 0.03	ND
Clerodendrum ternatum	Roots	0.44 ± 0.02	1.13 ± 0.45	0.23 ± 0.01	ND
Solanum panduriforme	Roots	0.46 ± 0.02	1.02 ± 0.10	0.21 ± 0.02	ND
Capparis tomentosa	Roots	0.50 ± 0.03	1.18 ± 0.44	0.18 ± 0.01	ND
Terminalia sericea	Roots	0.50 ± 0.01	1.12 ± 0.47	0.22 ± 0.02	0.21 ± 0.02
Maytenus senegalensis	Roots	0.54 ± 0.01	1.27 ± 0.80	0.23 ± 0.02	0.09 ± 0.01

ND, not detected.

Table 3
Possible maximum intake of potentially toxic heavy metals by consuming traditional medicinal preparations.

Medicinal plant	Plant part	Dosage and preparation	As		Cr		Ni		Pb	
			Weekly intake (μg)[a]	PTWI[b,c] (μg)	Weekly intake (μg)[a]	PTMI[b,d] (μg)	Weekly intake (μg)[a]	PTMI[b,e] (μg)	Weekly intake (μg)[a]	PTMI[b,f] (μg)
Plumbago zeylanica	Roots	Boil a handful in two cups of water and take 1 cup in the morning and one cup in the evening (Katapo, 2010; pers. comm.[g])	7.7	900	25.3	1260	–	2100	–	1500
Combretum imberbe	Leaves	Boil a handful in two cups of water and take 2 cups day^{-1} (Phaladi, 2008; pers. comm.[g])	7.3	900	24.0	1260	–	2100	2.9	1500
Terminalia sericea	Roots	Grind and boil one tablespoon of powder in water, take 1/2 cup twice/day (Themba, 2009; pers. comm.[g])	10.4	900	23.2	1260	4.4	2100	4.6	1500
Clerodendrum ternatum	Roots	Boil handful with water and drink one cup day^{-1} (Kesiametswe, 2008; pers. comm.[g])	9.1	900	23.4	1260	–	2100	4.8	1500
Albizia anthelmintica	Roots	Take one tablespoon day^{-1} with porridge (Sebetwane, 2009; pers. comm.[g])	8.1	900	14.8	1260	4.8	2100	3.4	1500
Maytenus senegalensis	Roots	Boil tablespoon of powder and drink 3 cups day^{-1} (Chetiso, 2009; pers. comm.[g])	15.1	900	35.6	1260	2.52	2100	6.4	1500

[a] Calculated for an amount of dry leaves of 4 g and an extractability of heavy metals into water infusions of a maximum of 74% [42].
[b] Provisional tolerable weekly intake.
[c] Calculated from 0.015 mg kg^{-1} [54] for 60 kg body weight.
[d] Calculated from daily intake of 0.18 mg for 60 kg body weight [54] multiplied by seven.
[e] Calculated from recommended dietary intake of 300 μg day^{-1} for 60 kg body weight [55] multiplied by seven.
[f] Calculated from 25 μg kg^{-1} [54] for 60 kg body weight.
[g] Mode of preparation according to the information provided by the respective traditional healer and the respective year (personal communication).

the permissible level of 10 μg g^{-1}. Obi et al. [20] also determined high levels of nickel in the samples analyzed.

All plants species were observed to contain heavy metals in low concentrations and in a similar trend of decreasing concentrations: Cr > Pb > As > Ni. This indicates a low variance of heavy metal status in different medicinal plants collected from the same area. This fact might be a consistent reflection of the soil environment. For example, mineralized areas of Ngamiland District are assumed to be covered by thick layers of sand [49] which might prevent bioaccumulation of metals in plants, unless they possess very deep reaching root systems. The specifically observed low levels of nickel may suggest that these plants do not accumulate nickel in considerable amounts, either due to the absence of nickel in the environment or the inability of the investigated medicinal plants to accumulate the metal. *M. senegalensis* (Mothono) consistently showed higher levels of metals than the other medicinal plants indicating that this tree might be a more effective bioaccumulator for metals as compared to the other plants.

3.4. Intake of arsenic, chromium, nickel and lead following traditional medical treatment regimes

Most traditional medicines are prepared from dried plant material, using either dried plant parts directly or ground powder for water infusions or decoctions. Medicinal plant preparations and dosages are summarized exemplary for six medicinal plants in Table 3.

Based on the traditional treatment regime, the possible maximum weekly intake of arsenic, chromium, nickel and lead was calculated assuming the daily consumption of an infusion or decoction prepared from 4 g dried plant material (handful of dried plant material or a tablespoon of ground plant powder) in a volume of water between 125 and 750 mL (1/2 to 3 cups). An extraction efficiency of 74% of heavy metals for water infusions was assumed, which was previously reported as the maximum value in herbal teas of comparable mass of dried material [42]. The results are shown in Table 3. The highest weekly intake was calculated as 15.1 μg arsenic; 35.6 μg chromium; 4.8 μg nickel and 6.4 μg lead. Compared to the provisional tolerable weekly intake (PTWI) values recommended by WHO and the Joint FAO/WHO Expert Committee on Food Additives (JCEFA) [50] of 900 μg per person arsenic, 1260 μg per person chromium, 2100 μg per person nickel and 1500 μg per person lead, the maximum weekly intakes of heavy metals from traditional medicinal plant preparations are significantly lower with 1.7% of PTWI for arsenic, 2.8% of PTWI for chromium, 0.2% of PTWI for nickel and 0.4% of PTWI for lead. It was previously reported that arsenic and lead in herbal infusions were mainly found to be organically bound and not toxic indicating that the actual intake of the toxic forms of metals is even lower. It is therefore reasonable to assume that exposure to potentially toxic heavy metals from traditional plant medicines in Ngamiland District in Botswana does not constitute a health risk.

Though there are no comprehensive recommendations for tolerable heavy metal intake considering an immune compromised health context, the low heavy metal concentrations from traditional plant medicines are unlikely to affect the health of immunocompromised patients considerably. Two examples might illustrate this point. A recent study [51] actually attributed an improvement of insulin resistance, metabolic abnormalities and body composition of HIV-patients to chromium at an intake level of 400 μg day^{-1} in the form of chromium–nicotinate (compare to the maximum daily intake of chromium from traditional medicine of 5 μg reported in the present study).

Contrary to chromium, arsenic is considered to be an immunosuppressant and was recently related to a significant increase in pulmonary tuberculosis mortality in Chile at an exposure of 580 $\mu g L^{-1}$ in drinking water [52]. HIV-infected persons do have an increased susceptibility to tuberculosis. However, the low risk population which served as a control in the above-mentioned study was exposed maximally to 10 $\mu g L^{-1}$ arsenic in drinking water. Assuming the adequate daily intake of 3.7 L day^{-1} drinking water for men and 2.7 L day^{-1} for women [53], the arsenic intake would translate to a weekly dosis of 259 μg and 189 μg, respectively, associated with no increased tuberculosis risk (compared to 15.1 μg estimated weekly intake of arsenic from traditional medicinal preparations reported in this study). It is important to note that the weekly intake is calculated on the assumption that only one plant will be consumed during this period. However, many traditional plant based preparations consist of a mixture of medicinal plants. Therefore the consumption of a cocktail of remedies may increase the weekly consumption to higher levels. But as all medicinal plants tested displayed very low levels of heavy metals even an accumulative concentration is unlikely to exceed the maximal tolerable weekly intake values.

Further studies have to be conducted to determine the influence of growing period, plant part harvested, harvesting season of plants and the metal content of soils on the metal uptake and content of the plant species [42]. Another important area of study to enable assessment of the health implications on ingesting the traditional plant medicinal remedies is to determine the metal content of the processed traditional medicine, as processing may affect the metal content of the remedies. In addition, these evaluations would have to determine the metal content in biological fluids such as urine and plasma to ascertain the absorbed proportions of metals.

4. Conclusions

In this study, twelve different medicinal plants traditionally used for the treatment of opportunistic infections of HIV/AIDS were analyzed for arsenic, chromium, lead and nickel using AAS after acid digestion. In all the plant samples collected from an area with a high level of mineralization the concentrations of metals determined were nevertheless well below the permissible levels recommended by the WHO. This suggests that the plants are safe provided no additional metals are added during the preparation of the remedy/formulation. Additionally, the weekly intake was calculated, based on an estimated extractability value, and found to be much lower than the recommended maximal tolerable weekly intake. Based on this study, it is unlikely that even for HIV/AIDS patients the consumption of these traditional plant based medicines will compromise their health status on the basis of the presence of potentially toxic heavy metals. The study has generated baseline data that can be used to develop other studies in other parts of the country to comprehensively determine the presence and quantities of other heavy metals and trace elements, which can guide policy development.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

[1] D. Taylor, Br. Med. J. 322 (2001) 629–630.
[2] WHO, Traditional Medicine Strategy, 2002–2005, World Health Organisation, Geneva, Switzerland, 2002.
[3] P.A. De Smet, N. Engl. J. Med. 347 (2002) 2046–2056.
[4] L. Rodriguez-Fragoso, J. Reyes-Esparza, S.W. Burchiel, D. Herrera-Ruiz, E. Torres, Toxicol. Appl. Pharmacol. 227 (2008) 125–135.
[5] E. Ernst, Eur. J. Clin. Pharmacol. 57 (2002) 891–896.
[6] H. Kauma, R. Koskela, H. Makisalo, H. Autio-Harmainen, J. Lehtola, K. Hockersted, Scand. J. Gastroenterol. 39 (2004) 1168–1171.
[7] V. Steenkamp, M.J. Stewart, M. Zuckerman, Ther. Drug Monit. 22 (2000) 302–306.
[8] W. Kneifel, E. Czech, B. Kopp, Planta Med. 68 (2002) 5–15.
[9] A. McVann, I. Havlick, P.H. Joubert, F.S.E. Monteagudo, S. Afr. Med. J. 81 (1992) 139–141.
[10] K.S. Leung, K. Chan, C.H. Lu, Phytother. Res. 19 (2005) 514–518.
[11] R.B. Saper, S.N. Kales, J. Paquin, M.J. Burns, D.M. Eisenberg, R.B. Davis, R.S. Phillips, J. Am. Med. Assoc. 292 (2004) 2868–2873.
[12] J.A. Centeno, F.G. Mullick, L. Martinez, N.P. Page, H. Gibb, D. Longfellow, C. Thompson, E.R. Ladich, Environ. Health Perspect. 110 (2002) 883–886.
[13] J.G. Garvey, H. Gary, V.L. Richard, D.H.C. Raymond, Int. J. Environ. Health Res. 11 (2001) 63–71.
[14] N. Lekouch, A. Sedki, A. Nejmeddine, S. Gamon, Sci. Total Environ. 280 (2001) 39–43.
[15] H.H. Ang, Food Chem. Toxicol. 46 (2008) 1969–1975.
[16] E.D. Caldas, L.L. Machado, Food Chem. Toxicol. 42 (2004) 599–603.
[17] M.R. Gomez, S. Cerutti, L.L. Sombra, M.F. Silva, L.D. Martinez, Food Chem. Toxicol. 45 (2007) 1060–1064.
[18] V. Steenkamp, M. von Arb, M.J. Stewart, Forensic Sci. Int. 114 (2000) 89–95.
[19] A.M.O. Ajasa, M.O. Bello, A.O. Ibrahim, I.A. Ogunwande, N.O. Olawore, Food Chem. 85 (2004) 67–71.
[20] E. Obi, D.N. Akunyili, B. Ekpo, O.E. Orisakwe, Sci. Total Environ. 369 (2006) 35–41.
[21] V. Steenkamp, M.J. Stewart, E. Curowska, M. Zuckerman, Forensic Sci. Int. 128 (2002) 123–126.
[22] ACHAP, 2001/2002 Annual Report of the African Comprehensive Partnership for HIV/AIDS. http://www.achap.org/stage/media/ACHAP_docs/Frontline.pdf, 2002 (approached 06.05.06; accessed 27.02.11).
[23] P. Huntsman-Mapila, T. Mapila, M. Letshwenyo, P. Wolski, C. Hemond, Appl. Geochem. 21 (2006) 1376–1391.
[24] W.R.L. Masamba, D. Mazvimavi, Phys. Chem. Earth 33 (2005) 687–694.
[25] G.M. Sawula, Talanta 64 (2004) 80–86.
[26] W.R.L. Masamba, A. Muzila, Botswana Notes Rec. 37 (2005) 218–226.
[27] E.E. Mmatli, H. Malerod, S.R. Wilson, B.M. Abegaz, T. Greibrokk, E. Lundanes, K.E. Malterud, D. Petersen, F. Rise, Anal. Chim. Acta 597 (2007) 24–31.
[28] D. Mogopodi, K. Mosetlha, N. Torto, G. Wibetoe, J. Geochem. Explor. 97 (2008) 21–28.
[29] B.B.M. Nkoane, G. Sawula, G. Wibetoe, W. Lund, J. Geochem. Explor. 86 (2005) 130–142.
[30] D.T. Takuwa, G. Sawula, G. Wibetoe, W. Lund, J. Anal. At. Spectrom. 12 (1997) 849–854.
[31] M.M. Cole, H.D. Le Roex, T. Geol. Surv. S. Afr. 81 (1978) 177–317.
[32] R.S. Maharia, R.K. Dutta, R. Acharya, A.V.R. Reddy, J. Environ. Sci. Health B 45 (2010) 174–181.
[33] D.A. Babb, L. Pemba, P. Seatlanyane, S. Charalambous, G.J. Churchyard, A.D. Grant, Psychol. Health Med. 12 (2007) 314–320.
[34] K. Peltzer, N. Friend-du Preez, S. Ramlagan, H. Fomundam, BMC Public Health 8 (2008) 255–269.
[35] E. Kip, V.J. Ehlers, D.M. van Der Wal, J. Nurs. Scholarsh. 41 (2009) 149–157.
[36] N. Sultana, M.S. Arayne, S. Sharif, Pak. J. Pharm. Sci. 17 (2004) 67–76.
[37] R. Sabri, Interaction studies of clarithromycin, erythromycin and roxithromycin with essential and trace elements, Thesis, University of Karachi, Pakistan, 2004.
[38] Mineweb, http://www.mineweb.com/mineweb/view/mineweb/en/page674?oid=119661&sn=Detail&pid=102055, 2011 (accessed 15.03.11).
[39] Botswana Mineral Investment Promotion, Republic of Botswana, Gaborone, Botswana, 2008.
[40] CDC, Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults, Centres for Disease Control and Prevention, quoted in: Morbidity and Mortality Weekly Report 41 (RR-17), 1993.
[41] M.A. Canterelli, R.G. Pellerano, L.A. Del Vitto, E.J. Marchevsky, J.M. Camina, Phytochem. Anal. 21 (2010) 550–555.
[42] S. Arpadjan, G. Celik, S. Taskeen, S. Gucer, Food Chem. Toxicol. 46 (2008) 2871–2875.
[43] H. Sun, F. Qiao, R. Suo, L. Li, S. Liang, Anal. Chim. Acta 505 (2004) 255–261.
[44] W. Zhang, W. Gan, X. Li, Anal. Chim. Acta 539 (2005) 335–340.
[45] WHO, Monographs on Selected Medicinal Plants, vol. 1, World Health Organisation, Geneva, Switzerland, 1999.

[46] D. Gjorgiova, T. Kadifkova-Panovska, K. Baceva, T. Stafilov, Am. Eurasian J. Toxicol. Sci. 2 (2010) 57–61.
[47] G. Mazzanti, L. Battinelli, C. Daniele, S. Costantini, L. Ciaralli, M.G. Evandri, Food Chem. Toxicol. 46 (2008) 3043–3047.
[48] K. Annan, A.I. Kojo, A. Cindy, A. Samuel, B.M. Tunkumgnen, Pharmacol. Res. 2 (2010) 41–44.
[49] S. Ringrose, P. Huntsman-Mapila, W. Downey, S. Coetzee, M. Fey, C. Vanderpost, B. Vink, T. Kemosidile, D. Kolokose, Geomorphology 101 (2008) 544–557.
[50] JECFA, Joint FAO/WHO Expert Committee on Food Additives. Fifty-third Meeting. Summary and Conclusions, World Health Organisation, Geneva, 1999.
[51] E. Aghdassie, B.M. Arendt, I.E. Salit, S.S. Mohammed, P. Jalili, H. Bondar, J.P. Allard, Curr. HIV Res. 8 (2010) 113–120.
[52] A.H. Smith, G. Marshall, Y. Yuan, J. Liaw, C. Ferreccio, C. Steinmaus, Am. J. Epidemiol. 173 (2011) 414–420.
[53] Panel on Dietary Reference Intakes for Electrolytes and Water, Dietary Reference Intakes for Water, Potassium, Sodium and Sulphate. Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC, USA, 2004.
[54] NSF International, Dietary Supplement-Standard 173: Metal Contaminant Acceptance Levels http://www.nsf.org/business/newsroom/pdf/DS_Metal_Contaminant_Acceptance_Levels.pdf, 2003 (accessed 15.05.11).
[55] WHO, Quality Directive of Potable Water, vol. 197, 2nd edition, World Health Organisation, Geneva, Switzerland, 1994.