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Determination of total and bioavailable heavy and trace metals in South African commercial herbal concoctions using ICP-OES

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Abstract

In South Africa, the use of commercial herbal concoctions is becoming very popular. However, due to lack of Good Manufacturing Practices there is a possibility of heavy metal contamination in these commercial herbal concoctions. The aim of this investigation was to screen for heavy metal and trace elements in commercial herbal concoctions used in traditional medicine to treat various kinds of ailments in South Africa. This is the first investigation in which metal concentrations in commercial herbal concoctions was measured. Fourteen herbal concoctions were obtained from traditional herbal (*muthi*) shops and were subjected to analysis for total and bioavailable metal concentrations using Inductively Coupled Plasma-Optical Emission Spectrophotometry (ICP-OES). Of all the concoctions tested only *Vusa umzimba* and *Sejeso herbal mixture* contained high amounts of Cd (2.2 and 0.6 mg/kg) respectively, exceeding the safety limit of 0.3 mg/kg (WHO, 1998). *Vusa umzimba* also exhibited high amounts of bioavailable Cd (1.2 mg/kg) thereby making it unsafe for human consumption. Surprisingly very high amounts of Hg were detected in *Ingwe muthi mixture* and *Sejeso herbal mixture* (14.9 and 12.3 mg/kg) respectively. These by far exceeds the weekly allowance limit of 2 µg/kg given by WHO/UN committee (2007). Relatively high amounts of Pb were found in *Sejeso herbal mixture*. The levels of bioavailable metals detected in this study raise concerns, not only for consumer safety, but also the quality of herbal concoctions available as part of the traditional medicinal practice in South Africa.

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Keywords: Bioavailable metals; Heavy metals; Herbal concoctions; Traditional medicine

1. Introduction

South Africa is at the point of incorporating traditional medicine into the National Healthcare System (ATMSA, 2008). However, national surveillance to check for quality, safety, Good Manufacturing Practices (GMP) and standardization of phytomedicinal products is lacking (Diederichs et al., 2006). This has led to the situation that the South African traditional medicine industry is largely unregulated.

It is estimated that more than 20 000 tonnes of medicinal plant materials are traded annually in South Africa, which amounts to revenue generation of R 2.9 billion per year (Mander,

1998). Trade of indigenous medicinal plants contribute to both the formal and informal economy, particularly in the emerging phytomedicinal market (Makunga et al., 2008). One of the recent innovations in South African traditional medicine is the manufacturing, packaging and selling of herbal concoctions over the counter. This ranges from general tonics and herbal teas, to snuff and herbal cigarettes (Ndhlala et al., 2009). Herbal concoctions are mixtures of medicinal plants or plant parts that are used in treating specific ailments. Herbal concoctions are prepared using different methods, which vary from simple brewing processes to more complex techniques that use alcohol and other organic solvents to extract plant compounds (Pujol, 1990).

Lack of standardization and contamination with toxic heavy metals, inadvertent and deliberate substitution of plant materials

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Table 1
Information on commercial herbal concoctions commonly found in Pietermaritzburg, KwaZulu-Natal.

Product name Trade name Batch number	Medicinal uses	Ingredients (as listed on the label)	Directions of use Packaging Shelf life
<i>Umzimba omubi</i>	Used to treat wounds, skin rashes, fungal infections and boils.	NL	Not on label. 500 ml
<i>Umuthi wekukhwehlehle ne zilonda</i>	Used as a cough mixture, to treat chest infections and difficulty in breathing.	NL	Not on label. 500 ml
<i>Mvusa ukunzi</i>	A 'man tonic' for increasing sexual prowess and can be used as an energizer.	NL	Not on label. 500 ml
<i>Imbiza ephuzwato</i>	A detoxifying and energizing tonic used to increase sexual prowess, relieve constipation, reduce stress, reduce high blood pressure, clear skin conditions, boost energy, boost vitality, helps to prevent arthritis, kidney problems and relieve general body pains.	Contains 21 medicinal plants. The plants are listed in Ndhlala et al. (2011a)	1/4 cup in the morning after meals twice a week. 1 l
<i>Umpatisa inkosi</i>	An 'adult tonic' used for increasing sexual prowess, as an energiser also used to treat sexually transmitted diseases (STDs), to stop menstrual pains, increase appetite, treat high blood pressure and fight arthritis.	NL	Not on label. 500 ml
<i>Ingwe muthi mixture</i> Ingwe AMM 0011	A traditional African mixture for chest infections, STDs, arthritis, heart burn, relieving constipation and increasing sexual prowess.	NL	3 tablespoons every morning. 500 ml <i>6 months</i>
<i>Ibhubezi</i>	Used for wounds, fungal infections, STDs, treatment of influenza, to reverse impotence, clean the body system and stimulate blood production.	NL	1/4 cup twice a week. Not for children under 14 years of age and pregnant women. Shake well before use. 500 ml <i>6 months</i>
<i>Supreme one hundred</i> BODicare 05020207	Used for nervous disorders, skin conditions, stimulates blood production, boosts sexual performance, treats back pains, fights influenza and strengthens the body.	NL	1/4 cup every night before sleeping after meals. Not for children under 14 years of age and pregnant women. 500 ml <i>6 months</i>
<i>Sejeso herbal mixture</i> Ingwe AMM 005	Used to relieve heartburn, constipation, stomach ache, stomach cramps and indigestion.	NL	1/4 cup three times a day after meals. Not to be taken by children and pregnant women. 500 ml <i>6 months</i>
<i>Stameta</i> BODicare® 04020207	Used for nervous disorders, skin conditions, boosts sexual performance, poor blood quality, high blood pressure. Chest, lung and kidney infections. Fever and flu. Heart problems, back pain, persistent tiredness. Menstrual pain, cleans out bile, bleeding gums, body sores. Strengthens bones and boosts the immune system.	<i>Hypoxis rooperi</i> , <i>Mentha piperital</i> , <i>Pimpinella anisum</i> , <i>Aloe</i> (unspecified). Fortified with multivitamins (unspecified), calcium, magnesium, potassium, phosphorus and iron	1/2 or 1/4 cup three or four times a week. Not for children under 14 years of age and pregnant women. Drink water after using <i>Stameta</i> TM . 500 ml
<i>Lion izifozoneke</i> Ingwe AMM 003	Used for chest infections, STDs, arthritis, heart burn, relieving constipation and increasing sexual prowess.	NL	1/4 cup three times a day after meals. Not to be taken by children and pregnant women. 500 ml <i>6 months</i>
<i>African potato extract- South</i>	Used to boost the immune system and treatment of HIV/AIDS symptoms.	NL	1 cup daily 1 l

Table 1 (continued)

Product name Trade name Batch number	Medicinal uses	Ingredients (as listed on the label)	Directions of use Packaging Shelf life
<i>Africa's miracle herb</i> 6008306000506			
<i>Ingwe Kidney and bladder mixture</i> Ingwe 6008658001664	Used to treat Kidney and bladder infections	<i>Bulbine natalensis</i> bulbs, <i>Eucomis autumnalis</i> bulbs, <i>Sena alexandrina</i> pods and leaves	1/2 a cup 3 to 4 times a week. Not to be taken by children and pregnant women. 500 ml 6 months
<i>Vusa umzimba</i> Ingwe AMM 0016	Used to treat wounds, rashes, fungal infections, boils and chest infections, stop menstrual pains, increase stamina and fight against influenza virus.	NL	4 tablespoons twice a day. Not for children under 14 years of age and pregnant women. 500 ml 6 months

Information in the table was obtained through informal surveys from herbal shops in Pietermaritzburg in order to document the commonly sold herbal preparations. Some of the information was taken from Ndhlala et al. (2009, 2010, 2011a, 2011b).

NL—not listed on the packaging.

1 cup is equivalent to 250 ml.

and adulteration with pharmaceuticals are causes of great concern in the phytomedicinal industry (Drew and Myers, 1997). Inadequate methods of manufacturing, and unhygienic processing and packing are the major sources of microbial contamination in herbal concoctions. The poor quality of utensils, usually containing aluminum or copper that are used in the manufacturing processes could be a major source of metal contamination in herbal concoctions.

Heavy metal poisoning from lead, mercury and cadmium has been repeatedly associated with traditional medicines (Ernst, 2004). Between 1991 and 1995, the Johannesburg Forensic Database revealed 206 cases of death in which traditional remedies were either implicated to be the cause of death or were found to be present in cases of poisoning with unknown substances (Stewart et al., 1999). In 2008, there was a case of 13 members of a family reported to have died after consuming a herbal concoction with unknown substances in their home near Port Shepstone on the KwaZulu-Natal south coast (IOL news, 2008). Malangu and Ogunbanjo (2009) pointed out that of 423 cases of acute poisoning leading to admissions in eight hospitals in South Africa, traditional medicines were responsible for 2.4%. Hence, there is an urgent need for quality assurance, safety and standardization of commercial herbal concoctions used in South African traditional medicine.

Table 2

Instrumental conditions for metal determination by ICP-OES.

Viewing geometry	Axial	RF power (kW)	1.0
Plasma flow (L/min)	15.0	Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75	Replicate read time (s)	9.0
<i>Analytical wavelength (nm)</i>			
Al	237.312	Fe	238.204
Cd	226.502	Hg	194.164
Cr	267.716	Mn	259.372
Cu	324.754	Ni	231.604
		Pb	220.353
		Zn	213.857

The aim of this study was to screen for heavy and trace metal concentrations in 14 herbal concoctions commonly used in KwaZulu-Natal as a quality check to ensure their safe use for treating various kinds of ailments.

2. Materials and methods

2.1. Sample preparation for total metal analysis

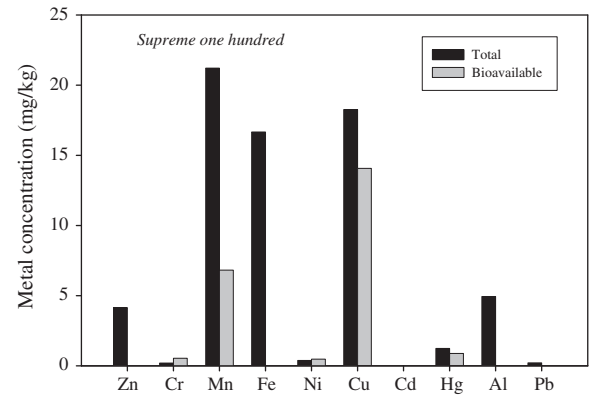
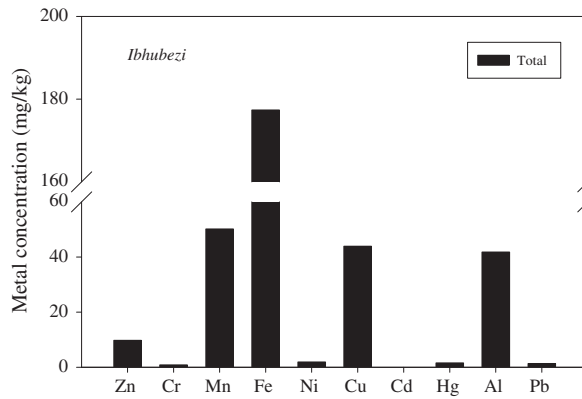
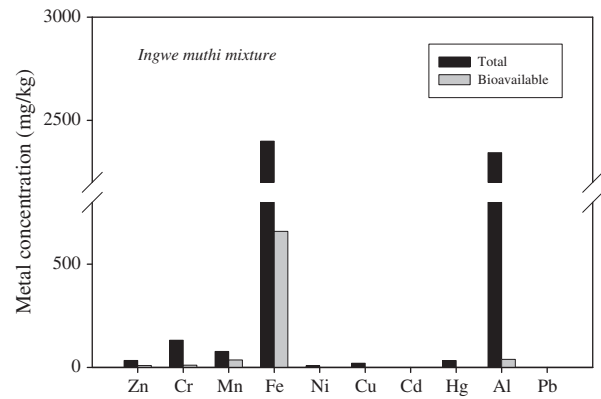
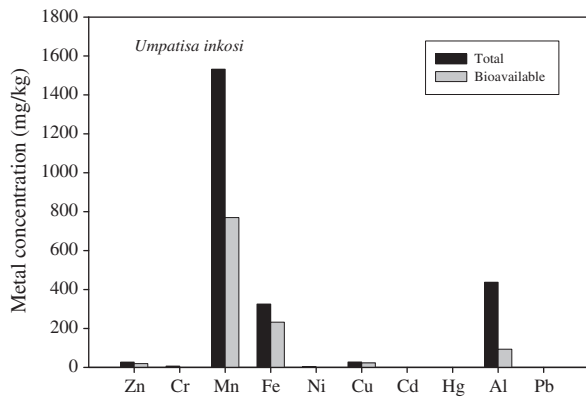
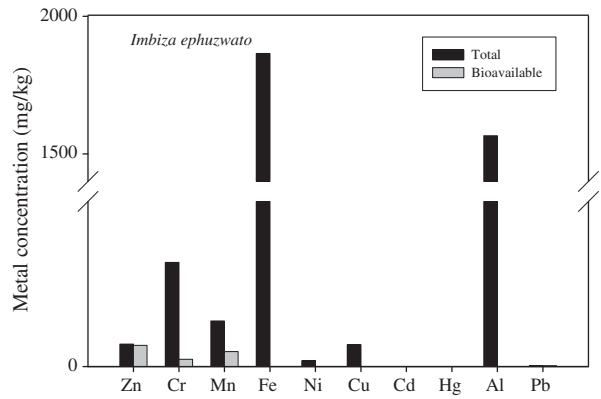
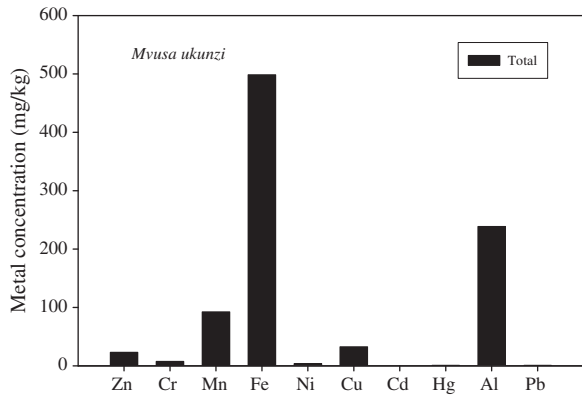
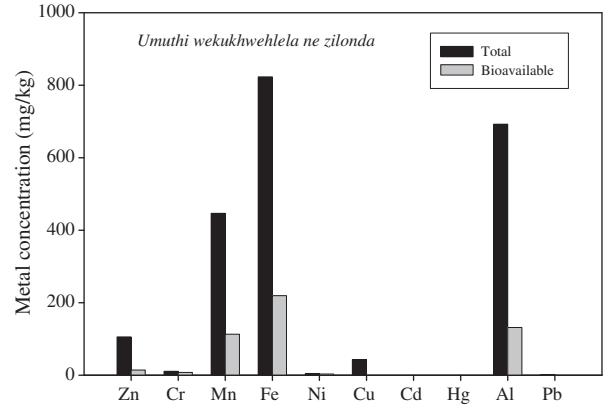
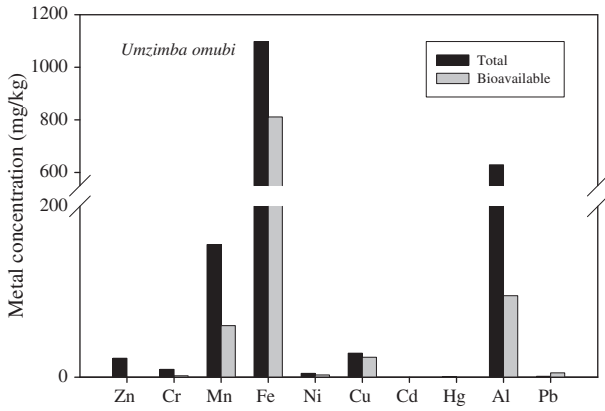
Fourteen commercial herbal concoctions used in traditional medicine were obtained from herbal (*muthi*) shops in Pietermaritzburg, KwaZulu-Natal province of South Africa. For detailed information on the use and therapeutic dosage of these concoctions refer to Table 1. Of the 14 samples, 13 were in liquid form and 250 mL of each of the herbal concoctions were freeze-dried. The freeze-dried samples were stored in airtight containers ready for digestion using open-vessel acid digestion procedures.

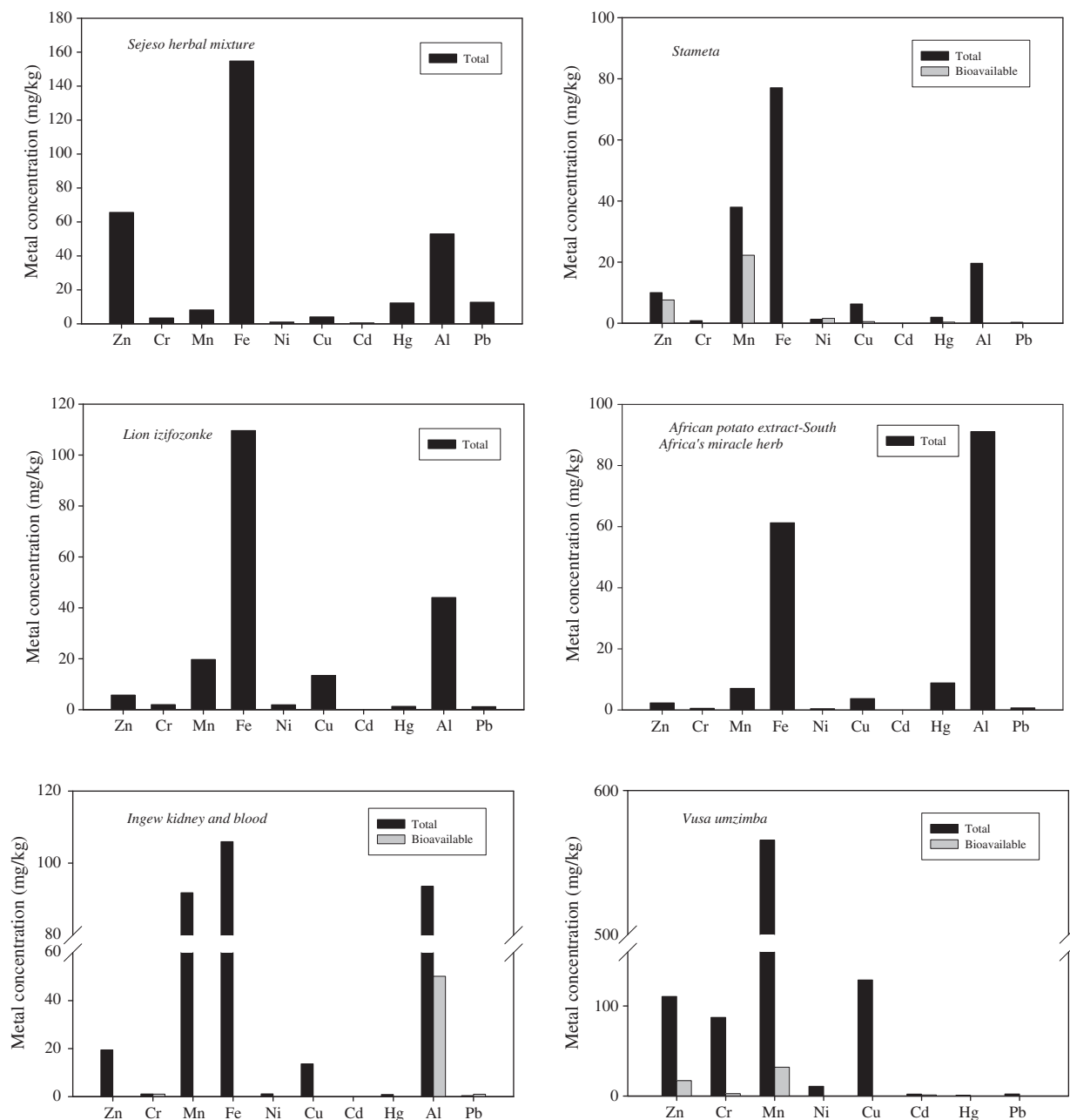
2.1.1. Sample preparation for bioavailability analysis

Of the 13 concoctions that were in liquid form, 9 were filtered through a Millipore filter (0.22 µm). The filtrate was stored in high density polyethylene bottles. The residues were dried in quartz crucibles and were stored in air-tight plastic bags until analysis.

2.2. Open-vessel acid digestion of solid samples

The dried samples (0.5 g) together with the one obtained as a solid, were placed in borosilicate glass digestion tubes. To each of the tubes, 10 mL of HNO₃-HCl-H₂O₂ (8:1:1, v/v/v) were added and the tubes were then placed on a heating block with the temperature set to increase to 120 °C for about 3 h or until the solutions were completely digested. After the digestion was completed the clear solutions were transferred to 50 mL volumetric flasks and made to volume with ultrapure (UP) water. The dilute samples were stored in high density polyethylene bottles until analysis. The samples were analyzed in duplicate. A





Figs. 1–14. Concentration of total and bioavailable metals in commercial herbal concoctions used to treat various kinds of ailments in KwaZulu-Natal Province of South Africa.

blank solution that was composed of 10 mL of HNO_3 - HCl - H_2O_2 (8:1:1, v/v/v) was included.

2.2.1. Digestion of liquid filtrate

An open-vessel acid digestion procedure was used to digest the liquid filtrate samples. A 50 mL aliquot of each filtrate was digested in a beaker by adding 10 mL of HNO_3 (55% v/v) and 3 mL H_2O_2 . The beaker was then covered with a watch glass. The temperature of the heating block was set to 120 °C for a period of 1 h until complete digestion. After the digestion the

clear solutions were transferred to 50 mL volumetric flasks and made to volume with UP water. The digested samples were stored in high density polyethylene bottles until analysis.

2.3. In vitro bioavailability measurements

2.3.1. Stomach-phase extraction

An in vitro dissolution test was carried out using a standard procedure for stomach-phase extraction (SPE) as described by Ryu et al. (2010) with modifications. A 0.4 M glycine solution at

pH 1.5 (adjusted with concentrated HCl) was used as the extraction fluid to determine the amount of bioavailable metals in the residue obtained after the filtration of the herbal concoctions. Each of the residues and the other samples obtained in powdered form were treated with the extraction fluid using an ultrasonic water bath at 37 °C to mimic human body temperature for 1 h. After the digestion was completed the solutions were filtered through a Millipore filter (0.22 µm) and the clear solutions were transferred to 50 mL volumetric flasks and made to volume with UP water and then stored in high density polyethylene bottles until analysis.

2.4. Standard solutions

Standard solutions were prepared from 1000 mg/L stock solutions (BDH Spectrosol®, Fluka Chemika®). The concentrations of the standards for calibration were in the range of 0.0125–0.5 mg/L for Cd, Cr, Cu, Ni and Pb. For Mn and Zn the range was 0.05–5 mg/L, for Sn it was 0.5–50 mg/L, for Fe it was 0.1–10 mg/L, for Hg it was 0.02–2 mg/L and for Al 0.2–2 mg/L. The standards were prepared in 100 mL volumetric flasks with 20 mL of HNO₃ and made to volume with UP water.

2.5. Elemental analysis using ICP-OES

Elemental analysis was performed using Inductively Coupled Plasma-Optical Emission Spectrophotometry ICP-OES (Varian 720-ES, Varian Inc, Palo Alto, CA, USA). ICP-OES provides a multi-elemental analysis and supports a broad linear calibration range (Standard Operation Procedure, 2005). ICP-OES is a powerful tool for the determination of metals in a variety of different sample matrices (Hou and Jones, 2000). The instrumental working conditions were as shown in Table 2.

3. Results and discussion

The results of total and bioavailable metal concentrations in commercial herbal concoctions used in treating various kinds of ailments in KwaZulu-Natal Province of South Africa are presented in Figs. 1–14. All values of elemental concentrations reported in this study were above the detection limit as given in Section 2.4 and data that fell below the detection limits were not reported. The analysis showed that only *Sejeso herbal mixture* and *Ingwe muthi mixture* contained high amounts of Pb (12.7 and 14.9 mg/kg respectively). This amount is above the permissible limit of 10 mg/kg (WHO, 1998). This is not so high to cause alarm for human consumption. However, there is still a need to control the amount of Pb in this product to avoid cumulative toxicity. Lead (Pb) poisoning has been reported to have several clinical manifestations in the nervous, haematic, renal, gastrointestinal, cardiovascular, musculoskeletal and endocrine systems (Ibrahim and Latif, 2002). Lead poisoning is known to elicit insidious conditions that can result in developmental delays, behavioral disorders and irreversible brain damage (Hanchette, 2008).

Sejeso herbal mixture had high levels of Cd (0.6 mg/kg). The highest concentration of Cd (2.2 mg/kg) was detected in *Vusa umzimba* exceeding the permissible limit of 0.3 mg/kg (WHO, 1998). The bioavailable Cd from *Vusa umzimba* was 1.20 mg/kg, making this concoction unsafe for human consumption. The absorption and distribution of Cd is usually influenced by low intake of Zn and Fe (Yang and Lee, 2009), with the high levels of Zn and Fe in *Sejeso herbal mixture* and *Vusa umzimba* there is likelihood that the absorption of Cd from these concoctions will be low. However, caution should be exercised in the application of these concoctions since most toxicity is dose depended. Cadmium is known to exert toxic effects on the kidneys, the skeletal system and the respiratory system and is classified as a human carcinogen (WHO, 2010). Cadmium has a negative effect on enzymatic systems of cells with its ability to substitute for other metal ions (mainly Zn²⁺, Cu²⁺ and Ca²⁺) in metalloenzymes and has a strong affinity for biological structures containing sulfhydryl (–SH) groups, e.g. proteins, enzymes and nucleic acids (Jacobson and Turner, 1980; Stohs and Bagchi, 1995).

Except for *Imbiza ephuzwato* that had very low amounts of Hg, all the other samples tested exhibited relatively high concentrations of Hg above the weekly allowance limit of 2 µg/kg given by WHO/UN (2007). The highest amounts of Hg were detected in *Sejeso herbal mixture* and *Ingwe muthi mixture* (12.3 and 14.9 mg/kg respectively). According to WHO report food contaminated with more than 0.5 mg/kg of Hg is not permitted for human consumption (Zahir et al., 2005). The high amount of heavy metals in some of the evaluated concoctions could be from accidental contamination (from the environment) or deliberate adulteration with metals due to traditional belief that some specially treated metals such as Hg have therapeutic properties (Ernst, 2004). Toxicity of Hg causes several chronic neurodegenerative disorders, it also plays pivotal roles in Parkinson's, and Alzheimer's diseases, impaired hearing and visual changes (Wilkinson and Waring, 2002). Steenkamp et al. (2006) reported that metal toxicity from the use of South African medicinal plants is not of great concern, but chances of contamination were not ruled out. In the present study, some of the investigated concoctions exhibited high amounts of metal elements which could have resulted from contamination or adulteration with heavy metals/metal salts at different stages of production.

The source and quality of raw material used is very important in guaranteeing the quality and safety of herbal concoctions. The source of contamination of heavy metals in some of the herbal concoctions might be from the hyper-accumulative effects in plant species for specific metals. Heavy metals have been a regular and sometimes deliberate constituent of traditional Asian/Ayurvedic medicine (Ernst, 2002b). The trend of toxicity of Pb, As and Hg in Ayurvedic medicine has been reviewed by Saper et al. (2004) and Ernst (2002a). The detection of high amounts of Cd, Pb and Hg in some of the evaluated herbal concoctions is not surprising, due to the fact that African traditional medicine is becoming gradually blended with other cultural and medicinal practices such as Hinduism and Ayurvedic medicine (Ndhlala et al., 2011a). For all the evaluated concoctions, the recommended therapeutic dose

written on the labels is between 30 and 250 mL two to three times per day for a period of three to five days. Most of the metals that are less bioavailable could become toxic due to extremely high levels of the applied dose and accumulative effects. A substance is defined as bioavailable if there is a possibility of it being absorbed by a living organism when in contact with it or when it is ingested (Yang and Lee, 2009). All the samples analyzed are taken orally and several factors can influence the absorption of the elemental species such as their solubility in gastric fluids, the physical and chemical state of the elements and time of ingestion (with or without a meal) (WHO, 2006).

The concentrations of essential metals such as Fe, Zn, Ni and Cr were generally found to be high, above the recommended daily allowances of 18 mg/day for Fe, and 15 mg/day for Zn as advised by the National Research Council (Hina et al., 2011). Almost all the concoctions have relatively high levels of bioavailable metals such as Fe, Zn, Cr and Al. Extremely high amounts of bioavailable Mn (769.6 and 112.9 mg/kg) were found in *Umpatisa inkosi* and *Umuthi wekukhwehlela ne zilonda* respectively which could be toxic. Apart from *Umzimba omubi*, *Umpatisa inkosi* and *Supreme one hundred* that had relatively high amounts of bioavailable Cu (23.3, 23.1 and 14.1 mg/kg respectively) all the other samples contained low amounts of bioavailable Cu. Essential elements are known to have several beneficial properties, however, when taken in amounts above the recommended allowable range, they can be detrimental to health.

In the present study there are differences in the values of the total and bioavailable metal concentrations in all the evaluated concoctions. These differences could be as a result of matrix effects of the herbal medicine which can substantially decrease the amount of bioavailable metals. Hence, determining the total amount of metal elements in herbal products is not sufficient in assessing the safety of herbal medicine, bioavailability screening is very important. A simple open-vessel digestion used in this study was sufficient to give clearly digested material to measure the total metal contents. An *in vitro* dissolution test was used to quantify bioavailability using a simulated gastric fluid without enzyme. This gives more realistic assessments of the toxic potential of metals in herbal medicine than those based on total metal content (Yang and Lee, 2009).

4. Conclusions

High levels of Cd and Hg were detected in *Ingwe muthi mixture*, *Sejeso herbal mixture* and *Vusa umzimba*, exceeding the WHO safety limits. Other potentially toxic metals such as Pb were also detected in high levels in *Sejeso herbal mixture*. However, only *Umpatisa inkosi* did not contain high levels of Cd, Hg and Pb. There is clearly an urgent need to investigate bioavailable metal contents in all South African commercial herbal concoctions to guard against intake of potential toxic metals when using these products. Development of accurate analytical methods which can reliably profile the composition, including quantitative analyses of heavy metals, pesticides and herbicides as well as microbial contaminations are needed in standardization of all South African commercial herbal concoctions. These will help in setting up a National Monograph that

will have detailed information on the contents of all herbal concoctions. Proper monitoring and control of starting materials and finished products of all commercial herbal concoctions and batch–batch reproducibility is essential. This will help to closely monitor practitioners and formulators of traditional medicine, so that medicinal irregularities and unethical practices will be reduced. Stringent adherence to GMP by all firms involved in manufacturing, packing and selling of commercial herbal concoctions is needed urgently to protect consumer safety and the quality assurance of all herbal concoctions available as part of traditional medicinal practice in South Africa.

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