

Evaluation of heavy metal content of two Senegalese varieties of *Combretum micranthum* leaves and their decoctions

ABSTRACT

Leaves of two species of *Combretum micranthum* produced in the central (I) and southern (II) regions of Senegal and their decoctions in ultrapure water (II) and (III) were analyzed for 12 elements (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Ag, Cd, Pb, Bi) by ICP-OES. The concentrations of heavy metals ($\mu\text{g/g}$) are in the ranges 2.55 – 84.19 for Cr, 5.21 – 379.72 for Mn, 0.00 – 317.80 for Fe, 0.08 – 1.08 for Co, 1.54 – 8.88 for Ni, 1.75 – 8.08 for Cu, 0.91 – 9.70 for Zn, 0.00 – 0.16 for Cd, 0.46 – 0.84 for Pb. As, Ag and Bi are not detected in any of the samples by ICP-OES analysis. For all among all the samples, manganese content was had the highest content and represented 46.85% for I-, 64.18% for II-, 86.88% for II and 46.27% for III-, of the total heavy metal concentrations. The results showed that the leaves of both varieties of *Combretum micranthum* and decoctions made from these leaves contain heavy metal levels lower than the values recommended by the WHO for consumed medicinal plants.

Comment [a1]: ICP-OES

Comment [a2]: Below detectable limits

Comment [a3]: ICP-OES

Keywords: *Combretum micranthum*, heavy metal, extraction, decoction, ICP-OES,

1. INTRODUCTION

Combretum micranthum also known as *Kinkéliba* is a plant widely distributed in West Africa, particularly in Senegal [1-3]. It is an indicator of poor soils that is resistant to drought and fire. It is found throughout Senegal and is particularly common in Casamance and in the region of Thiès, around the ponds of the Sahel. It is a medicinal plant widely used in the treatment of various diseases [4-6]. *Combretum micranthum* leaves are known for their beneficial effects on health, particularly in improving digestion and managing diabetes [7,8]. The leaves are used in the care provided for many conditions, including kidney disease [9,10]. In Casamance, the population uses the leaves to prevent malaria by chewing. *Combretum micranthum* is also used in the treatment of respiratory diseases such as bronchitis and cough, malaria, hepatobiliary diseases as an adjunctive medicine [11-14]. The leaf decoction is particularly used for its antihypertensive effects [15]. *Combretum micranthum* leaves are very popular within the Senegalese population. They are used in decoction especially at breakfast. Medicinal plants are often flagged because of their heavy metal content. Increasing urbanization and industrialization have accentuated the exposure of plants and soils to heavy metals such as chromium, nickel, lead, cadmium, mercury and arsenic. Due to their cumulative lightness, heavy metals are a source of significant risks to human health when consumed, even in very small quantities. This poses a health problem. Indeed, medicinal plants absorb heavy metals and when detected in contaminated areas, the heavy metals present may exceed the recommended limits. It is in this context that we decided to carry out the qualitative and quantitative assessment of heavy metal contents in the leaves of the two varieties of *Combretum micranthum*, the decoction of whose leaves is consumed by all the Senegalese people.

2. MATERIALS AND METHODS

2.1 Instrumentation

All experiments were carried out using a Perkin Avio 220 Max ICP Optical Emission Spectrometer with double-monochromator (Perkin Elmer, Waltham, USA). Pure Argon was used as plasma.

2.2 Reagents and standards

All solutions were prepared with distilled-deionized water (18MΩcm, Milli-Q, Millipore, Bedford, MA, USA). Hydrogen peroxide (H₂O₂ 30%) and nitric acid (HNO₃ 65%), from Sigma-Aldrich France, were used in the procedure of digestion of the samples. The analytical solutions were prepared from standard solution dilutions 1000 μg mL⁻¹ of As, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Ag, Cd, Pb, Bi (Aldrich, France). All materials used were decontaminated in nitric acid solution 10% v/v by 24 h.

2.3 Extraction of heavy metals from *Combretum micranthum* leaves

Two varieties of *Combretum micranthum* were purchased from the markets of Dakar: a variety from central Senegal (I) and a variety from Casamance in the south Senegal (II). The leaves are dried in the shade for two days before completing the drying in an oven at 50°C for two days. The leaves are then finely ground using a blender. For each sample, 5 g of leaves are mixed with 10 mL of a 1:1 mixture of ethanol and glycerol before being calcined at 500°C for 24 hours. After cooling, the ashes are collected and then treated with concentrated HNO₃ to dissolve the different metal ions present in the matrix. The suspensions were filtered into a graduated flask before making up to 100 mL with ultrapure water.

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2.4 *Combretum micranthum* leaves decoctions

For the decoction of both *Combretum micranthum* varieties, 2.5 g of leaves are suspended in 100 mL of ultrapure water and heated at reflux for 30 minutes. The suspension is cooled, filtered and made up to 100 mL with ultrapure water to give samples II (variety I) and III (variety II).

Comment [a5]: Please add reference of the decoctions procedure

3. RESULTS AND DISCUSSION

3.1 General study

The massive application of fertilizers and pesticides in plantations and near major roads and highways, have led to persistent pollution of the air and soil by chemicals and heavy metals. Plants that often assimilate chemical compounds found in soils and air can be victims of this pollution. The high levels of heavy metal contamination exceed the limits set by WHO indicating that plant samples are contaminated and unfit for consumption. Some of these heavy metals are non-essential and soluble elements in biological media that they end up poisoning depending on their bioaccumulations. In this work, we study samples of *Combretum micranthum* taken next to major roads (sample I) and in the middle of the forest in the absence of industrial pollution (sample II). ICP-OES analysis of raw leaves and leaves decoctions showed the presence of different metals in the plant with notable differences (Table 1). The analysis of the two varieties of *Combretum micranthum* shows a great difference in the presence of the different heavy metals and their quantities. For the variety produced in central Senegal (I), the concentration of heavy metals evolves-detected in the following order: Mn > Fe > Cr > Zn > Ni > Cu > Co > Pb > Cd (Table 1). For the variety produced in Casamance in southern Senegal (II), we observe the absence of certain metals found in variety I. The concentration of metals evolves in the following order: Mn > Zn > Cr > Ni > Cu > Pb > Co (Table 1). In variety I, the total quantity of heavy metals is 810.45 μg/g, while variety II gives a total quantity of 163.96 μg/g. The decoctions give a total quantity of heavy metals of 74.59 μg/g and 12.04 μg/g for II and III, respectively. The yields of the extraction of metals ions from leaves is low as shown in Table 1. The

elements Mn, Co, Ni and Zn are best extracted from variety I with a maximum yield of 37% for cobalt, while the elements Cr and Cu are best extracted from variety II with a maximum yield of 24.95% for Cu.

Table 1. Concentration of heavy metal in *Combretum micranthum* leaf and their water decoctions.

	I	II	Yield (%) ^a	II	III	Yield (%) ^a
Cr	84.19±0.02	2.93±0.01	3.48	16.17±0.01	2.55±0.02	15.77
Mn	379.72±0.01	64.81±0.03	24.96	105.23±0.0	5.21±0.01	4.95
Fe	317.80±0.03	< DL		< DL	< DL	-
Co	1.08±0.01	0.40±0.02	37.00	0.32±0.03	0.08±0.01	25.16
Ni	8.88±0.01	2.57±0.01	28.93	16.17±0.02	1.54±0.02	9.52
Cu	8.08±0.02	0.99±0.02	12.25	7.01±0.02	1.75±0.04	24.95
Zn	9.70±0.02	2.89±0.04	29.78	18.60±0.03	0.91±0.03	4.89
As	< DL ^b	< DL	< DL	< DL	< DL	-
Ag	< DL	< DL	< DL	< DL	< DL	-
Bi	< DL	< DL	< DL	< DL	< DL	-
Cd	0.16±0.01	< DL	< DL	< DL	< DL	-
Pb	0.84±0.01	< DL	-	0.46±0.002	< DL	-

^aYield of heavy metal extraction, ^bDetection Limit

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3.2 Levels of heavy metals in *Combretum micranthum*

Chromium. Chromium is a micronutrient involved in the regulation of glucose metabolism by enhancing insulin action [16], helping in the regulation of nucleic acids and lipoproteins. Chromium deficiency induces impaired glucose metabolism [17], while chromium excess leads to renal, hepatic or pulmonary damage [18]. The WHO permitted limit for chromium in medicinal plants is 2000 µg/g [19]. In the present study, the chromium concentrations in the two varieties of *Combretum micranthum* studied are well below the WHO authorized limit for medicinal plants and are 84.19 µg/g and 16.17 µg/g, respectively for I and II. The results obtained after decoction of the leaves with ultrapure water show an extraction yield of 3.48% for II and 15.77% for III. These yields indicate that chromium is well retained by the organic molecules contained in the plant. This corresponds to concentrations of 2.93 µg/g for (II) and 2.55 µg/g (III) which are largely below the WHO authorized limit [19]. These values do not give rise to safety concerns for human consumption.

Manganese. Manganese is an essential element involved in biological functions such as the regulation of glucose and lipid metabolism in humans. Its involvement in oxidative stress is well known. Excess manganese has negative effects on metabolism by enhancing the production of reactive oxygen species (ROS) [20]. Manganese is the most prevalent heavy metal in both varieties and its concentration represents 46.85% of the total heavy metal concentration in variety I, while it is 64.18% for variety II. The concentration of manganese in variety I is 379.72 µg/g and, it is 105.23 µg/g for variety II. These manganese concentrations in *Combretum micranthum* are within the range reported for several medicinal plants used in the pharmacopoeia. *Millefolli herba* has a concentration of 380 µg/g, *Hyperici herba* gives a value of 440 µg/g [21], *Tussilago farfara* gives a manganese concentration of 106.7 µg/g [22], while *Gentiana lutea L.* gives 112.37 µg/g [23]. The Mn values [379.72 µg/g (I) and 105.23 µg/g (II)] in these two varieties of *Combretum micranthum* is not dangerous for consumption. The extraction yields after decoction with ultrapure water are 24.96% (II) and 4.95% (III) which corresponds to concentrations of 64.81 µg/g (II) and 5.21 µg/g (III). In these two decoctions manganese is the most present heavy metal its concentration represents 86.88% of the total concentration of heavy metals of II decoction, while it is 43.27% for the III decoction.

Iron. The functioning of the human body requires iron, which is a micronutrient that plays a fundamental role in several areas such as the supply of oxygen or the production of energy. It is an essential element for the control of immunity. An excess of iron causes various disorders. Nausea, vomiting, and previous dizziness can occur in the event of massive ingestion of iron. A negative effect is then observed for the digestive system and the cardiovascular system [24]. Excess iron can also lead to the formation of free radicals that can cause irreversible damage to cells [25]. We studied two samples of *Combretum micranthum* of which variety I has an iron concentration of 317.80 µg/g while variety II does not contain any. WHO has not set any limits for iron in medicinal plants. Although our study shows a large difference in iron concentrations in the two varieties of *Combretum micranthum*. The results are lower than those reported in the literature for medicinal plants [26]. After decoction of variety I in ultrapure water, no iron is found in the decoction liquid. Therefore, we believe that iron is strongly sequestered in variety I by organic molecules.

Cobalt. Cobalt is a microelement that is not very present in medicinal plants but is essential for certain biological actions in the human body at low doses. It is involved in the formation of vitamin B12. In high concentrations, its action can induce visual impairment, cardiomyopathy and other serious conditions [27]. In our study, variety I gives a cobalt concentration of 1.08 µg/g while variety II gives a value of 0.32 µg/g. The WHO/FAO [28] permissible limit for cobalt in medicinal plants is 3.50 µg/g and the cobalt concentrations in both varieties of *Combretum micranthum* are below this limit. These values are lower than those found for other medicinal plants whose concentrations range from 3.41 µg/g to 11.26 µg/g [29]. After extraction the yield values are 37% (Ii) and 25.16% (Iii), corresponding to 0.40 µg/g and 0.08 µg/g concentrations, respectively. These values remain below the WHO recommended value.

Nickel. The essentiality of nickel in higher organisms is not clearly established for humans [30]. However, this element may have a physiological function in higher organisms. Given the natural abundance of nickel and constant exposure to this element, there is no natural nickel deficiency. Since the WHO has not established any standards, some authors have estimated the daily dietary intake of nickel between 70 µg and 400 µg [31,32]. Nickel is highly toxic to humans and animals and can affect the cardiovascular system, cause respiratory problems, impair kidney function, or cause dermatitis [31,33,34]. The nickel concentration is twice as high in variety II (16.17 µg/g) than in variety I (8.88 µg/g). These values do not present any particular danger. For this parameter, the decoction of *Combretum leaves micranthum* is eligible for consumption with concentrations of 2.57 µg/g (Ii) and 1.54 µg/g (Iii) with extraction yields of 28.93% and 9.52%, respectively. It appears that nickel is better extracted from variety I than from variety II. This implies a difference in the organic composition of the two varieties.

Copper. Copper is an essential element that plays an important role in many biological processes. It is present in many enzymes that play fundamental roles in health and biological development [35,36]. Despite its very important role, excess copper can cause significant tissue damage and lead to irreversible injuries [37]. Both samples have similar copper concentrations (8.08 µg/g) for I and (7.01 µg/g) for II. These values are lower than the value limit of 20 µg/g, defined by WHO /FAO [38]. These concentrations are low compared to values reported for other medicinal plants and do not constitute a health concern for the consumption of *Combretum micranthum* [39]. Consequently, the extraction yields of Ii (12.25%) and Iii (24.95%) do not alter the quality of the decoctions.

Zinc. Zinc is a trace element essential for the proper functioning of the biological system. It is present in metalloenzymes [40] and is involved in DNA synthesis [41], and in the functioning of the thyroid gland [42]. According to the WHO, the permitted limit of Zn in medicinal plants is 50 µg/g. The results revealed that the zinc concentration of 9.70 µg/g and 18.60 µg/g in I and II, respectively are lower than the WHO permitted limit and that *Combretum micranthum* can be safely consumed. Although zinc is an essential element for humans, it can cause significant damage beyond the permitted limits [43]. Therefore, the extraction yields of Ii (29.78%) and Iii (4.89%) do not alter the quality of the decoctions.

Cadmium. Cadmium is a non-essential and toxic element that is toxic to the kidney and can accumulate over a long period of time. Since it is slowly eliminated, it can cause irreversible damage to kidney and liver functions [44]. At high concentrations, cadmium produces severe effects on the liver, vascular and immune systems [45]. Cadmium is detected only in variety I, which is harvested at the roadside with a concentration of 0.16 µg/g. It is completely absent from variety II which grows in the forest, far from road pollution and agricultural inputs. The concentration value found for I is lower than the WHO limit value of 300 µg/g for medicinal plants [46]. Cadmium is not extracted by ultrapure water of variety I. Neither decoction is contaminated with cadmium.

Lead. Lead is a non-essential element for life that is highly toxic to the nervous system and kidneys. High concentrations of lead above the permitted limits in medicinal plants have been reported in several studies [47,48]. Lead is known to be one of the most toxic environmental pollutants. It can complex with various biomolecules and affect their functioning, have a negative effect on the nervous system, kidneys, heart and develop gastrointestinal diseases [49,50]. Fetal and childhood exposures to lead cause cognitive and learning disabilities [51]. In our samples, lead is present at low concentrations (0.84 µg/g) in I and (0.46 µg/g) in II. The value limit defined by FAO/WHO [46] of 10 µg/g for medicinal plants is significantly higher than the values found in the two varieties of *Combretum micranthum*. As with cadmium, lead is not extracted by ultrapure water and both decoctions are not contaminated with lead.

3.3 Intake of heavy metal

Combretum micranthum bag contains 5 g of plant material. Weekly intakes of heavy metals through consumption of *Combretum micranthum* decoction were calculated assuming a daily consumption of three bags of herbal material decocted in a total of 600 mL of water (Table 2). Tolerable Maximum Weekly Intake (TMWI) (70 Kg body weight) is calculated using the method described in the literature [52]. The results are presented in Table 2. The maximum intake reached 272.2 µg per week of manganese for consumers of variety I and 21.88 µg per week for users of variety II. The maximum intake of chromium and zinc are, respectively, 12.31 and 12.14 µg per week for variety I and 10.71 and 3.82 µg per week for variety II. The weekly doses of cobalt are not significant (1.68 and 0.34 µg per week for varieties I and II, respectively). Nickel and copper have maximum weekly intakes of 10.69 and 4.16 µg for variety I and 6.47 and 7.35 µg per week for variety II. Comparing the provisional tolerable weekly intakes of FAO/WHO and other organizations [53-55] and the doses found in the study, it can be concluded that the consumption of decoctions of the two varieties of *Combretum micranthum* will not affect human health.

Table 2. Possible maximum intake of heavy metal through the consumption of *Combretum micranthum* decoction.

Sample	I	II
Cr (µg/week) ^a	12.31	10.71
% of TMWI	3.74	3.25
Mn (µg/week) ^a	272.2	21.88
% of TMWI	1.30	0.10
Co (µg/week) ^a	1.68	0.34
% of TMWI	8.00	1.62
Ni (µg/week) ^a	10.79	6.47
% of TMWI	2.06	1.23
Cu (µg/week) ^a	4.16	7.35
% of TMWI	0.04	0.07
Zn (µg/week) ^a	12.14	3.82
% of TMWI	0.009	0.003

^aCalculated using the level in each decoction (Table 1) and assuming an average daily consumption of 0.6 L of *Combretum micranthum* decoction from three bags (5 g).

4. CONCLUSION

This study determined the contamination of the two varieties of *Combretum micranthum* consumed by the population in Senegal. The presence of heavy metals Cr, Mn, Fe, Co, Ni, Cu, Cd, Pb and the absence of As, Ag and Bi are noted. The results revealed that the heavy metal contents studied in this plant used as a medicinal plant or in decoction for drink are lower than the authorized limits (WHO and other organizations) for medicinal plants consumed. The results also showed that manganese represents the largest part of all the heavy metals present. These values of heavy metal concentrations, both in the leaves and in the decoctions, seem to indicate that these leaves and decoctions can be consumed safely. However, it is still necessary to constantly monitor this plant sold on the market as a medicinal plant or for use as a drink to ensure that the safety of users for human consumption.

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