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Concentration of Toxic Heavy Metals and Phytochemicals in a Medicinal Plant (*Asclepias fruticosa*) Collected Around Mining Areas in Brits, Pretoria

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ABSTRACT

The use of African traditional medicine in rural and peri-urban areas is common due to its affordability and accessibility. The study aimed to determine the levels of toxic heavy metals in the medicinal plant (*Asclepias fruticosa*) samples collected around three mining areas in Brits using ICP-OES. The phytochemical screening analysis was done to indicate the absence or presence of different phytochemicals in the medicinal plant. The results of the qualitative phytochemical analysis indicated the presence of flavonoids, alkaloids, proteins, and carbohydrates in *Asclepias fruticosa* collected from all the mining areas. The results of the heavy metals showed that the mean highest concentration for all the heavy metals was recorded for Mn from the leaves of the medicinal plants. The trend in the heavy metals accumulation was roots > leaves > stems from all the sites, and the differences were significant ($p < 0.05$). The range of heavy metals in the plant was in the range Mn (12.33 ± 2.31-85.33 ± 51.07 $\mu g.g^{-1}$), Zn (10.67 ± 0.58-60.33 ± 0.56 $\mu g.g^{-1}$), Cr (3.43 ± 0.06 -34.90 ± 0.10 $\mu g.g^{-1}$), Cu (8.67 ± 0.12-18.8 ± 1.57 $\mu g.g^{-1}$), Ni (5.67 ± 0.12-23.23 ± 1.7 $\mu g.g^{-1}$) and Pb (0.53 ± 0.013-1.59 ± 0.15 $\mu g.g^{-1}$). The values of the heavy metals Cr, Zn, and Ni in the plant exceeded the recommended limits set by WHO for human consumption. Heavy metals in the medicinal plant were accumulated in the roots and not translocated to the stems and leaves. It is therefore recommended that communities staying around the mines should be discouraged from picking and using medicinal plants growing around the mines and should be educated on the safety of medicinal plants growing around the mines.

INTRODUCTION

The consumption of medicinal plants has been practiced since ancient times (Brima 2017) by all populations worldwide (Niaz et al. 2013). Medicinal plants have been widely used for treating, preventing, and managing diseases and most of the world's population depends on them for health benefits. About 65-80% of the world's population in marginal communities is dependent on traditional medicines and supplements as a form of primary healthcare for the treatment of several diseases in some rural communities where there is no availability or access to western medicine or where western medicine is not affordable (Dghaim et al. 2015, Kulhari et al. 2013, Kostić et al. 2011, Ekor, 2014, Annan et al. 2013, Mabona & Vuuren 2013, Olowoyo et al. 2012, Mulaudzi et al. 2017). Even though there has been an overwhelming introduction of medicinal plants in South Africa, there has not been any scientific validation of their efficacy and toxicity (Mtunzi et al. 2012).

Medicinal plants are preferable over synthetic remedies because they are believed to be natural, more effective, affordable, and original (Mulaudzi et al. 2017, Kulhari et al. 2013, Ayaz et al. 2014). However, traditional medicines may be toxic due to contaminants such as heavy metals, chemical toxins, pesticides, and microbes, which might be present in the soil, water, and air (Lajayer et al. 2017). In addition, the geography and the geochemical characteristics of the soil, the storage and transport conditions, harvesting and handling can also affect the quality and properties of traditional medicines (Aliyu et al. 2008, Dghaim et al. 2015).

According to Olowoyo et al. (2012), heavy metals are considered among medicinal plants' primary contaminants or pollutants. Most are toxic, even at low concentrations (Fahimirad & Hatami 2017). Heavy metals are assimilated into medicinal plants due to the presence of residues and the high prevalence of heavy metals in the environment (Fahimirad & Hatami 2017). The accumulation of heavy metals by medicinal plants is due to the extraction of metals soluble from the sediments, soil, and air, which are contaminated (Sarma et al. 2011). The primary source of heavy metals has been linked to environmental pollution, which includes emissions from traffic, municipal wastes, pesticides and fertilizers, sewage sludge, industrial and mining activities (Fatima et al. 2014, Fahimirad & Hatami 2017, Kulhari et al. 2013, Rai et al. 2019, Asati et al. 2015). Pegadogenesis and weathering of parent rocks are also responsible for the non-anthropogenic origin of heavy metals resulting in their occurrence in soils (Street 2013).

Heavy metals are non-biologically degradable and have specific gravity of more than 5 $g.cm^3$ and atomic weights between 63.5–200.6 $g.mol^{-1}$ (Kulhari et al. 2013). Examples of common heavy metals are lead (Pb), copper (Cu), chromium (Cr), zinc (Zn), mercury (Hg), aluminum (Al), manganese (Mn), and cadmium (Fahimirad & Hatami 2017). Plants require certain heavy metals for uptake and growth. They can take in trace elements and heavy metals into their tissues because they can withstand toxic ions that are potentially toxic in the environment (Shirin et al. 2009, Street 2013). The concentrations and accumulation of the heavy metals vary from plant to plant found in the exact location (Niaz et al. 2013). Contamination of the environment results mainly from the toxicity, non-biodegradability, persistence, and bioaccumulative nature of the heavy metals, making them major hazardous chemicals in the environment that pose potential risks to the environment and human health (Zulkafflee et al. 2022, Malikula et al. 2021).

The majority of the curative effects of traditional medicines are a result of the presence of a minimal amount of trace metals (Shirin et al. 2009) which include elements such as Cobalt (Co), Nickel (Ni), magnesium (Mg), iron (Fe), iodine (I) and copper (Cu) which plants take up through the roots, foliar adsorption and deposition into the leaves (Kulhari et al. 2013, Nema et al. 2012). However, the levels of heavy metals in traditional medicines which are grown or collected from polluted or contaminated places are usually above the limits which are safe for human consumption resulting in traditional medicines being a crucial source of heavy metals through ingestion and consumption (Sarma et al. 2011, Glavač et al. 2017). Incidents of metal poisoning linked to the consumption of traditional medicines in South Africa are popular with magnesium (Mg), chromium (Cr), and arsenic (As) which are the most often implicated metals causing morbidity, poisoning, and mortality (Okem 2014).

Phytochemicals are bioactive compounds derived from plants that are non-nutritional and promote healing and health effects such as anti-inflammation, anti-hypertension, antioxidant, antibacterial, pain relief, and therapeutic effects (Khan et al. 2019, Khaleel 2018, Mahmood et al. 2019). Plants exposed to stress due to heavy metals exhibit different responses towards the phytochemicals, such as in the composition and the effectiveness of the medicinal plants (Street 2013, Okem 2014). Some plants can even show deviations in the production of phytochemicals with the increased levels of heavy metals contamination in medicinal plants leading to a decrease in the production of phytochemicals (Lajayer et al. 2017). Accumulating heavy metals in medicinal plants can also cause the production of phytochemicals in most species of plants (Nasim & Dhir 2010).

A synonym for *Asclepias fruticosa* is *Gomphocarpus fruticosus,* and its common English name is milkwood. The different parts of the plant are used medicinally for other purposes in various African countries. The infusion from the roots and leaves or the powder made from the leaves and roots dissolved in water is used medicinally to induce vomiting (https://uses.plantnet-project.org/en/Gomphocarpus_fruticosus; PROTA). The decoction of the plant roots and leaves can also be used to treat abdominal pains, gonorrhea, general body pain, infertility, malaria, diabetes, liver problems, asthma, diuretic, and nerve pain. The crushed leaves or dried leaves can be drunk as tea, used for skin cancer, or applied to sores, while the plant's latex is used for toothache treatment (https://uses.plantnet-project.org/en/Gomphocarpus_fruticosus; PROTA). The decoction made from the seed is used as a medicine for the treatment of cough, or the cooked roots of the plant are eaten as a vegetable (https://uses.plantnet-project.org/en/Gomphocarpus_fruticosus; PROTA).

The demand and consumption of traditional medicines and herbal products are growing globally. However, with an increase in the introduction of new developments in the market, there is also increasing recognition of concerns on the health issues of the public and their safety with regards to the consumption of traditional medicines (Ekor 2014, Nasim & Dhir 2010, Kostić et al. 2011). The consumption of medicinal plants collected around soils polluted by heavy metals must be carefully monitored and managed to reduce the levels of heavy metals in medicinal plants to protect the public from the harmful and adverse effects of the heavy metals (Nianz et al. 2013,0 Fahimirad & Hatami 2017). Hence the aim of the study was to determine the levels of heavy metals and phytochemicals in the medicinal plant *Asclepias fruticosa* growing around mining areas close to residential areas.

MATERIALS AND METHODS

Collection of Plant Samples

The medicinal plant, *Asclepias fruticosa,* known commonly in English as milkweed, was collected from around the three mining areas in Brits, in the North-West Province at a radius of about 50m from the mining areas (Olowoyo et al. 2015) and authenticated by a botanist in the Department of Biology at Sefako Makgatho Health Sciences University in the north of Pretoria in South Africa. The collected plant samples were washed thoroughly with distilled water to remove superficial dust, separated into roots, leaves, and stems, dried in an oven, and then pulverized into a fine powder with a mortar and pestle. The pulverized plant samples were then stored in sample bags until they were analyzed.

Analysis of Heavy Metals

The collected plant samples were analyzed for the heavy metals, lead (Pb), copper (Cu), zinc (Zn), manganese (Mn), nickel (Ni), iron (Fe), and chromium (Cr) with the aid of the ICP-OES (Perkin Elmer ICP-OES Optima. 4300 DV (USA). Before analysis, 0.5 g of the dried plant samples were digested by mixing with 10.0 mL of nitric acid (HNO3,65% Merck supra pure) and 3.0 mL of HClO4 (65% Merck supra pure). The resulting mixture was then heated up to 150°C using an oven for two hours and brought to a volume of 10.0 mL with deionized water. For quality assurance, plant samples were analyzed in triplicate. The same procedure was performed twice with a blank without plant samples with the digest of CRM042-050 containing the heavy metals to quantify any related contamination by the reagents. The subsequent solutions were analyzed for heavy metals with the aid of ICP-OES.

The translocation factors (TF) from the roots to stems and leaves to determine where the heavy metals accumulated within the different plant parts were calculated as flows: TF = conc. in the stems /conc. in the roots and TF = conc. in the leaves/conc. in the roots (Kutty & Al-Mahaqeri 2016). TF > 1 indicates that the different heavy metals were being translocated from the roots to the upper parts and that the medicinal plant is a hyperaccumulator, and TF < 1 suggests that the heavy metals were remaining in the roots and not being translocated and that the plant is not a hyperaccumulator (Kutty & Al-Mahaqeri 2016).

The mean concentrations of the heavy metals were presented as mean ± standard deviation. The tables and graphs were used to represent the results. For statistical analysis, the Analysis of Variance (ANOVA) was used to determine if there was any significant difference between the concentrations of the trace metals in the plant samples collected from the three different mines.

Qualitative Determination of Phytochemicals

The phytochemical screening analysis was done to indicate the absence or presence of different phytochemicals (tannins, saponins, alkaloids, and flavonoids) using standard methods as described with slight modifications (Yadav & Agarwala 2011).

The method of McGaw & Eloff (2008) was used for the plant extraction with minor modifications using Methanol, ethyl acetate, hexane, and distilled water. To obtain the plant extracts, 30.0ml of each solvent was added to 3.0 g of plant material and shaken for 30 minutes. The solution was then filtered using the Whitman No. 1 filter paper, and the filtered plant extracts were analyzed to identify the chemical constituents.

Determination of Tannins

About 0.5 ml of the extract solution was added to 1 mL of distilled water in a test tube to test for tannins. 2 to 3 drops of diluted ferric chloride solution were added until brownish green or blue-black color was observed

Determination of Flavonoids

For the presence of flavonoids, a few drops of 1% NH_3 were added to the extract solution in a test tube. The yellow coloration observed indicated the presence of flavonoids.

Determination of Saponins

About 2 mL of the extract solution was mixed with 2 mL of distilled water in a test tube and shaken thoroughly. The formation of stable foam indicated the presence of saponins.

Determination of Alkaloids

About 1 ml of 1% HCI was added to 3 mL of the extract solution in a test tube and treated with a few drops of Meyer's reagent. A formation of a creamy precipitate indicated the presence of alkaloids

RESULTS AND DISCUSSION

The results in Table 1 show that from all the heavy metals investigated, the highest mean concentration recorded for all heavy metals in the roots, stems, and leaves was Mn, followed by Zn and then Cr, while the lowest mean concentration was for Pb. The concentration of Mn in the leaves, stems, and roots ranged between 12.33 ± 2.31 $\mu g.g^{-1}$ and 85.33 ± 51.07 $\mu g.g^{-1}$. The trend of Mn accumulation in *Asclepias fruticosa* from around the mining areas was: roots > leaves > stems. As the World Health Organisation (WHO) recommended, the permissible limit of Mn in medicinal plants is 200 $mg.kg^{-1}$ (Niaz et al. 2013). The Mn content in the roots, stems, and leaves was within the permissible limit recommended by WHO. Even though Mn plays and essential role in the production of proteins in the human body and is crucial in reproduction, it has been associated with neurotoxicity and reduced neural activation (Barahona et al. 2022).

Zn in the medicinal plant's roots, stems, and leaves from the mining areas ranged from 10.67 ± 0.58 $\mu g.g^{-1}$ to 60.33 ± 0.56 $\mu g.g^{-1}$ (Table 1). The maximum content limit of Zn (to 60.33 ± 0.56 μg.g^{-1}) was above the permissible limit of 50mg/kg in medicinal plants as recommended by the WHO (Niaz et al. 2013). Zn in the human body is crucial for the functioning of the nerves, the production of white and red blood cells, vitamin stimulation, and fertility in males (Mtunzi et al. 2012). In contrast, excess Zn causes growth retardation and impairment in reproduction (Kamunda et al. 2016). The results of Mn and Zn in the present study agree with those in Maharia et al. (2010), where the Mn and Zn in the medicinal plants collected from around the mines ranged from 36.4 mg/kg to 69.3 mg/kg and 24.9mg/kg to 49.9mg/kg respectively.

The ranges of Cu and Cr concentrations in the roots, stems, and leaves of *Asclepias fruticosa,* as shown in Table 1, were 8.67 ± 0.12 μg.g^{-1} - 18.8 ± 1.57 μg.g^{-1} and 3.43 ± 0.06 μg.g^{-1} - 34.90 ± 0.10 μg.g^{-1} respectively. The highest Cu content in the medicinal plant collected around the mines was in the roots followed by the leaves, while the stems showed the lowest concentration. The trend for the Cr content was roots > stems > leaves. The concentration levels of Cu were significantly different ($p<0.05$). The upper limit of Cu was above the permissible limit of 15mg/kg in medicinal plants, as recommended by WHO for Cu (Olowoyo et al. 2012). The maximum value of 34.90 ± 0.10μg/g for Cr was also higher than the value of 1.5mg/kg for Cr as recommended by the United States Environmental Protection Agency (Nianz et al. 2013).

The results of Cu concentrations in the current study are comparable with those in the medicinal plants which were collected from polluted areas in Nianz et al. (2013), which ranged between 3.12 mg.kg^{-1} and 21.40 mg.kg^{-1} while those of Cr are not comparable with those in the same study which ranged from 0.17 mg.kg^{-1} to 8.00 mg.kg^{-1}. Cu in humans is an essential cofactor for an enzyme that is crucial for the cross-linking of collagen. It also prevents the osteoclast's activity even though it excessively decreases lipids' metabolism (Skrajnowska et al. 2022). Cr in excess can contribute to skin allergies, bronchial asthma, cancer, problems in reproduction and development, and may even lead to death (Shekhawat et al. 2015).

From all the heavy metals determined in *Asclepias fruticosa* collected around the mines, the lowest values were for Pb, which ranged between 0.53 ± 0.013 μg.g^{-1} and 1.59 ± 0.15 μg.g^{-1} followed by Ni, which ranged from 5.67 ± 0.12 μg.g^{-1} to 23.23 ± 1.7 μg.g^{-1}. The highest Pb and Ni concentrations were in the roots, followed by the stems, while the leaves showed the lowest values of Pb and Ni contents. All the measured values were below the recommended limit of 10mg/kg for Pb, while the upper limit of Ni was above the recommended permissible limit of 10 mg.kg^{-1} (Annan et al. 2013, Okem 2014).

The results of Pb in the present study are not in agreement with those in Nawab et al. (2015), which were found in medicinal plants collected from around mine-affected areas and ranged between 3.66 mg.kg^{-1} and 11.6 mg.kg^{-1} in the shoots while in the shoots Pb was in the 2.00 mg.kg^{-1} – 7.00 mg.kg^{-1}. In Kutty & Al-Mahaqeri (2016), the highest value of 9.79 ± 0.13 μg/g^{-1} was determined in a medicinal plant around abandoned mining areas compared to the highest value of 1.59 ± 0.15 μg/g^{-1} in the current study. The results of Ni in the present study did not compare with those in Maharia et al. (2010), where the levels of the medicinal plants collected from around the Copper mines ranged between 3.09mg/kg and 9.01mg/kg. High levels of Pb in humans can result in abnormal hemoglobin levels and size in red blood cells (Sithole et al. 2016) and cause health problems such as neurological disorders, anemia, hyperactivity, and changes in enzymes (Nawab et al. 2015) while elevated levels Ni can cause blindness, increased levels of cholesterol in the serum and sugar in the blood (Mugivhisa & Olowoyo 2017).

Table 2 shows that all the heavy metals were not translocated from the roots to the stems and leaves of

Table 1: The heavy metal concentration in the roots & stems of *Asclepias fruticosa* harvested from around the three mining areas in Brits.

Mine	Plant part	Heavy metals concentration [μg.g^{-1}]					
		Cr	Mn	Ni	Cu	Zn	Pb
Mine 1	Roots	25.67 ± 0.31	44.33 ± 0.58	12.47 ± 0.06	17.73 ± 0.06	59.00 ± 0.00	0.83 ± 0.00
	Stems	11.87 ± 0.23	36.67 ± 0.58	8.93 ± 0.05	11.97 ± 0.21	22.33 ± 0.58	0.81 ± 0.00
	Leaves	4.10 ± 0.10	52.33 ± 2.31	5.67 ± 0.12	12.33 ± 0.15	28.67 ± 0.57	0.53 ± 0.013
Mine 2	Roots	27.53 ± 2.58	85.33 ± 51.07	23.23 ± 1.7	18.8 ± 1.57	51.00 ± 4.58	1.59 ± 0.15
	Stems	3.43 ± 0.06	12.33 ± 0.58	8.07 ± 0.21	8.67 ± 0.12	10.67 ± 0.58	1.00 ± 0.00
	Leaves	17.27 ± 6.87	39.67 ± 0.587	9.97 ± 0.15	9.7 ± 0.100	19.33 ± 0.58	0.78 ± 0.06
Mine 3	Roots	34.90 ± 0.10	77.67 ± 0.58	10.57 ± 0.06	16.03 ± 0.40	39.33 ± 1.16	1.00 ± 0.00
	Stems	33.30 ± 0.17	64.00 ± 0.00	8.87 ± 0.12	11.90 ± 0.17	60.33 ± 0.56	0.59 ± 0.01
	Leaves	26.63 ± 0,93	75.33 ± 2.52	8.7 ± 0.20	10.47 ± 0.40	23.33 ± 0.58	0.55 ± 0.02

Table 2: Translocation factor (TF) values for the stems and leaves in *Asclepias fruticosa* collected from the different mining areas.

Heavy metal	Mine 1		Mine 2		Mine 3	
	Stems	Leaves	Stems	Leaves	Stems	Leaves
Cr	0.46	0.16	0.12	0.63	0.95	0.76
Mn	0.83	1.18	0.14	0.46	0.82	0.97
Ni	0.72	0.45	0.35	0.43	0.84	0.82
Cu	0.68	0.70	0.46	0.52	0.74	0.65
Zn	0.38	0.49	0.21	0.38	1.53	0.59
Pb	0.98	0.64	0.63	0.49	0.59	0.55

Table 3. Phytochemical screening of *Asclepias fruticosa* collected from the mines.

Phytochemical	Mine 1				Mine 2				Mine 3			
	Ethyl acetate	Hex-ane	Methanol	Distilled water	Ethyl acetate	Hex-ane	Metha-nol	Distilled water	Ethyl acetate	Hex-ane	Metha-nol	Distilled water
Tannins	+	-	-	+	+	-	-	+	+	-	-	+
Saponins	-	+	+	+	-	-	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	+	-	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	-	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+

Asclepias fruticosa (TF < 1) except for Mn and Zn, which were translocated from the roots to the leaves and stems and hyperaccumulated (TF > 1) by the medicinal plant which was collected around mine one and mine two respectively.

The results of the qualitative phytochemical analysis, as shown in Table 3, indicated the presence of flavonoids, alkaloids, proteins, and carbohydrates in *Asclepias fruticosa* collected from all the mining areas in all the fractions of the solvents except for Methanol and distilled water. The tannins were only in Ethyl acetate and Methanol extracts, whereas saponins were not present in ethyl acetate extract

CONCLUSION

The phytochemicals flavonoids, alkaloids, proteins, and carbohydrates were present in the medicinal plant. The upper limits of the majority of the heavy metals (Zn, Cu, Cr, and Ni) in the *Asclepias fruticosa* were above the allowable limits as set by WHO and the United States Environmental Protection Agency for medicinal plants and edible plants and exceeded the safe and recommended limits for human consumption except for Mn and Pb. The heavy metals were mostly accumulated in the roots of the medicinal plant and not taken to the aerial parts. Recommendations are that communities should be discouraged from using medicinal plants growing around mines, and communities should be educated on the dangers of consuming medicinal plants growing around mines. More similar studies can be done on other medicinal plants growing around the mines or other polluted areas to determine the levels of the heavy metals in the medicinal plants and regular monitoring of the heavy metals in medicinal plants should be encouraged in all communities making use of such plants for health purposes.

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