Open Journal of Soil Science, 2023, 13, 474-489
https://www.scirp.org/journal/ojss
ISSN Online: 2162-5379
ISSN Print: 2162-5360

Assessment of the Level of Metal(loid)s Pollution and Bioactive Compounds Screening of Anthill Soil

Graça K. Kandanda, Festus S. Shafodino, Simeon I. Ambuga, Lamech M. Mwapagha*

Department of Biology, Chemistry and Physics, Faculty of Health, Natural Resources and Applied Sciences, Namibia University of Science and Technology, Windhoek, Namibia
Email: *lmwapa@gmail.com

How to cite this paper: Kandanda, G.K., Shafodino, F.S., Ambuga, S.I. and Mwapagha, L.M. (2023) Assessment of the Level of Metal(loid)s Pollution and Bioactive Compounds Screening of Anthill Soil. *Open Journal of Soil Science*, **13**, 474-489.
https://doi.org/10.4236/ojss.2023.1311022

Received: October 11, 2023
Accepted: November 11, 2023
Published: November 14, 2023

Abstract

The anthill soil is used by hypertensive elderly and teenagers from Oshikoto region (Namibia) and many of them testified stabilization of their blood pressure to normal after consuming the anthill soil-derived aqueous extracts. This study therefore investigated and/or assessed the physicochemical parameters, the contents of some metal(loid)s (and their associated potential health risks) and the qualitative composition of bioactive compounds of this anthill soil. The homogenous soil sample collected from various anthill soils in the Oshikoto region was used to obtain the measurements of physiochemical parameters. The elemental contents were determined (using an Inductively Coupled Plasma Optical Emission Spectrophotometer) after acid digestion in accordance with the EPA method 350B and their potential health risk assessments were performed. Methanol, aqueous methanol, and aqueous-based extracts were generated via maceration extraction process prior to the screening of bioactive compounds using standard diagnostic assays. The oxidation reduction potential (164.4 ± 16.6 mV) was the only physicochemical parameter whose value was within the World Health Organization limits for drinking water whereas, total dissolved solids (23 ± 5.5 mg/L), electrical conductivity (44 ± 10.1 uS/cm) and pH (5.35 ± 0.33) were out of specifications. Phenolic compounds, flavonoids, terpenoids, and cardiac glycosides were present in anthill soil (with respect to the extractants used) to which its anti-hypertensive properties can be attributed in addition to some of the studied mineral components. With respect to the pH, TDS and EC, and the contents of most metal(loid)s in relation to their health risk assessment values, the results suggest that aqueous extracts derived from this anthill soil can be deemed unsuitable for human consumption.

Keywords

Anthill Soil, Physicochemical Parameters, Metal(loid)s Contamination, Antihypertensive, Bioactive Compounds

1. Introduction

Heavy metals are those elements with density greater than five and constitute more than 35% of the elements in the periodic table [1]. Although many metals or metalloids are essential for life, they can be harmful to humans, animals, plants, and microorganisms at toxic levels. The presence of heavy metals and/or metalloids in soil is mainly attributed to anthropogenic activities such as industrial mining, and agricultural operations as well as natural weathering of metal-rich parent materials. Soil has its own capacity to immobilize introduced chemicals, and overtime these toxicants accumulate in it. When heavy metals/metalloids are present in soils, they can inhibit the biodegradation of organic contaminants and subsequently, pose great risks and hazards to biota [2] [3] [4]. The level of toxicity is quite dependent on the type of metal/metalloid, its biological role and the type of organisms that are exposed to it [5].

Since soil science became part of the realm of modern science [6], it has been explored for various purposes. While much progress has been made in understanding many parts of soil science, the specific characteristics, functions, and ecological roles of anthill soil remain an area of active investigation. Anthill soil, also known as ant mound soil or formicary soil, is the unique material meticulously excavated and modified by ants to create their subterranean colonies [7]. These tiny and complex structures are not simply the result of Ant activity, they also act as dynamic hubs of ecological activity that have an impact on the local soil composition, nutrient cycling, and even plant growth. Due to its makeup, anthill soil can have unique qualities and potential uses in agriculture [8] and traditional medicine [9].

Recent advancements in the field of bioactive compounds and soil chemistry have guided man to discover effective pharmaceuticals to treat various human and plant diseases [10] [11]. Soil has been utilized as a source of medicine and has been proven to contain various bioactive compounds which originate from plant materials and eventually get transferred into the soil. Apart from studies of substances derived from plants, literature also describes various studies about bioactive compounds originating from insects, especially ants [10] [12]-[16]. Additionally, various studies have established the association between ants and their hill soil with gram-positive actinomycetes, which are known to produce various bioactive compounds of diverse clinical effects and important human medicine applications [17] [18] [19] [20].

In the current study, the aqueous extracts of the soil analyzed is often traditionally used in the Oshikoto region as a hypertension reliever. However, while extensive research has explored heavy metal contamination in soils, the unique role of anthills in accumulating and potentially posing health risks to those exposed to them remains underexplored. Additionally, despite its traditional usage as an antihypertensive agent, there is a lack of scientific data to support the antihypertensive properties of this anthill soil or unknown knowledge regarding its health risks. Therefore, this study seeks to bridge this critical knowledge gap by establishing the elemental composition and assessing the potential human health risks that are due to exposure to trace elements present in anthill soil, as well as establish bioactive compounds present in this anthill soil.

2. Materials and Methods

2.1. Materials

Sample collection.

Soil samples from the outer parts of anthill soils in the Northern side of Namibia (Oshikoto region, located at 18.4153˚S and 16.9123˚E, as illustrated in Figure 1) were collected at randomly selected sites where villagers have been collecting soil for their use and the obtained soil portions were mixed to form a homogenous anthill soil sample. The sampling technique used was based on indigenous knowledge with the aid of the native people within the community. The sample was put in a sterile plastic zip bag and transported to the Namibia University of Science and Technology laboratories for processing and subsequent analysis.

2.2. Methods

1) Measurement of anthill soil physicochemical parameters

A concentrated muddy paste was created from the original homogenous anthill soil using distilled water in a 100 ml beaker prior to the measurement of pH, electrical conductivity (EC), total dissolved solid (TDS) and oxidation reduction

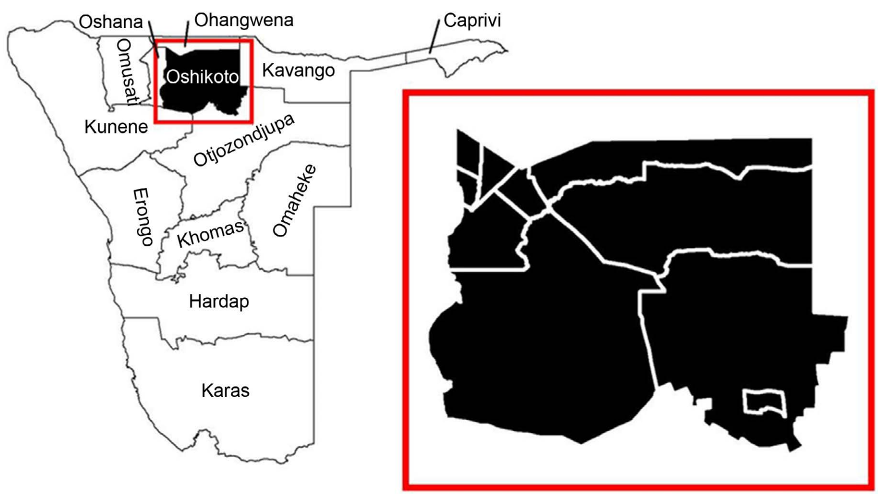

Figure 1. The location of Oshikoto region (where anthill soil sample was collected) on the Namibian map (Source: Thomson, 2018).

potential (ORP) using a portable meter (HI9811–5, Hanna instrument) [21].

2) ICP-OES analysis

The standard Environmental Protection Agency (EPA) method (3050B) was used to obtain the digestate from the anthill soil with slight modifications prior to analysis using an ICP-OES [22]. Approximately 300 mg of the soil sample was weighed and mixed with 10 ml of 1:1 nitric acid in a beaker covered with a watch glass. The sample was then heated at 95˚C ± 5˚C and refluxed for about 10 - 15 minutes, ensuring not to let the solution boil. Then the sample was allowed to cool and 5 ml of concentrated nitric acid was added and refluxed for 30 minutes. The solution was then heated at 95˚C ± 5˚C for 2 hours, allowed to cool, and 2 ml of water and 3 ml of 30% hydrogen peroxide was added to it and mixed, while covered with a watch glass and heated until effervescence subsided and the vessel was cooled again. Hydrogen peroxide (30%) was added in 1 ml aliquots while warming until effervescence was minimal and the sample appeared unchanged, ensuring not to exceed 10 ml of hydrogen peroxide (30%). The sample was heated again for 2 hours at 95˚C ± 5˚C until 5 ml was left in the vessel. The vessel with the sample solution was again allowed to cool before adding 10 ml of concentrated hydrochloric acid and refluxing at 95˚C ± 5˚C for 15 minutes. Finally, the sample was filtered through a filter paper (Double Rings 99 mm) and stored at 4˚C for later analysis. This ICP-OES analysis was done in triplicates and final mean elemental contents were reported after taking into account the dilution factors.

3) Health risk assessment

Humans are exposed to heavy metals via several pathways. However, given that people (adults and/or teenagers) consume the aqueous extracts of this anthill soil, they are exposed to the metals and/or metalloids in the soil via ingestion route [23]. Therefore, this study focused on assessing the exposure based on the ingestion pathway in adults.

The chronic daily intake (CDI) of heavy metals (mg/l) to which consumers are exposed and the reference dose (RfD) is the daily dosage that enable an individual to sustain this level of exposure over a long period of time without experiencing any harmful effects [24]. The values for CDI via drinking water were calculated using the modified Equation (1) [25].

$$\mathrm{CDI} = \mathrm{C}\dot{x}\left(\frac{\mathrm{DI}}{\mathrm{BW}}\right) \tag{1}$$

where daily intake (DI) was assumed to be 2 L, body weight (BW) is 70 kg for adults, and the mean concentration (C) of elements in samples was taken in mg/kg.

The health risk index (HRI) for most of the detected elements was calculated according to Equation (2), using the CDI and the oral RfD [26] [27] values for these elements. The HRI was set by the Agency for Toxic Substances and Disease Registry (ATSDR) or the Environmental Protection Agency (EPA).

$$\mathrm{HRI} = \mathrm{CDI}/\mathrm{RfD} \tag{2}$$

The population's non-carcinogenic response to heavy metals is the consequence of adding up all the HRIs caused by individual heavy metals and according to the United States Environmental Protection Agency, the sum of HRIs can be referred as Hazard Index (HI) [28]. The HI value was also calculated and discussed.

4) Maceration extraction and qualitative bioactive compounds screening

In an attempt to understand the principle behind the anthill soil serving as an antihypertensive agent, bioactive compound screening was conducted. The different extracts were obtained via cold maceration method, with solvents of increasing polarity, namely, aqueous, aqueous methanol, and pure methanol as outlined by Shafodino *et al.* [29], with minor modifications. An amount of 25 g of the soil sample was macerated with 100 ml of each solvent for 6 hours at room temperature on a shaker at 120 rpm. The extracts were then filtered using a filter paper (Double Rings 99 mm), to separate it from the solid residues. All filtrates were concentrated under reduced pressure using the rotary evaporator at 54˚C - 90˚C to obtain the final extracts (*i.e.*, methanol, aqueous methanol, and aqueous, respectively). The extracts were stored at 4˚C until further analysis. The extracts obtained through cold maceration were subjected to bioactive compounds screening using standard methods that are summarized below [29] [30].

Test for flavonoids: A few drops of 10% of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution were added to 2 ml of each extract. An intense green color indicated indicated a positive result.

Test for cardiac glycosides: To 2 ml of the extract, 2 ml of glacial acetic acid and 1 drop of 5% ferric chloride hexahydrate solution were added, followed by the addition of 0.5 ml of concentrated sulfuric acid by the side of the test tube. A greenish brown ring formation indicated a positive result.

Test for phenolic compounds: An amount of 2 ml of distilled water and a few drops of 10% ferric chloride were added to 1 ml of each extract, the formation of a green coloration indicated a positive result.

Test for terpenoids: An amount of 5 ml of the concentrated sulfuric acid were added along with 2 ml chloroform to 1 ml of the extract. The appearance of a brown ring indicated a positive result.

2.3. Statistical Analysis

All analyses (*i.e.*, physicochemical parameters and ICP-OES) were done in triplicates and the results are presented as mean ± standard deviation ($\overline{x}$ ± SD). The graphical illustration was designed using the GraphPad prism version 5 software, and the health risk assessments calculations were done using the Microsoft Office's standard Excel program.

3. Results and Discussion

Electrical Conductivity, pH, Total Dissolved Solids and Oxidation-Reduction Potential measurements.

The electrical conductivity (EC), hydrogen potential (pH), total dissolved solids (TDS) and oxidation reduction potential (ORP) of anthill soil were measured, and the results are presented in **Table 1**. Since the main exposure route to this soil is via drinking water as mentioned earlier, the WHO acceptable range values for these four parameters in drinking water [31] are presented along with the above-mentioned data. The values for EC, pH, TDS and ORP were, 44 ± 10.1 uS/cm, 5.35 ± 0.33, 23 ± 5.5 mg/L, and 164.4 ± 16.6 mV, respectively.

Electrical conductivity and total dissolved solids are indicators of the concentration of dissolved ions in water. While they don't directly influence pH, they can indirectly affect it by altering the water's ion composition. For instance, high TDS levels may indicate the presence of alkaline or acidic ions, which can influence pH, should either of them be dominant in the solution. Furthermore, formic acid is secreted by formicine ants as a chemical defense mechanism and it has a low pH, therefore, it could be a constituent of the anthill soil that can account for the observed low pH [32] [33]. Similarly, while the oxidation reduction potential of a solution towards other substances does not directly change pH, it can reflect the presence of oxidizing or reducing agents that may influence the pH indirectly, therefore, a negative ORP value is generally considered ideal for drinking water. In the current study, except for the ORP, the measurements of the remaining three physicochemical parameters of the anthill soil fall below the acceptable range values set by the WHO for drinking water. The observed electrical conductivity value indicates low nutrient amount in the soil, while the total dissolved solids value indicates low salinity levels in the soil [34], which can in turn be attributable to the low EC observed. These values are quite unusual for anthill soil when compared to other studies [8] [35], that reported high concentrations of organic nutrients in anthill soil used for agricultural applications. However, considering that the nutrient accumulation and biochemical properties of anthill soils is affected by their location, properties of the surrounding soil, as well the ants' selectivity of materials to construct the anthills [36], these can account for the observed variations. The observed average pH indicates that the anthill soil is acidic, which can increase the mobility and bioavailability of heavy metals due to proton competition and decreased negative binding sites, resulting in heavy metal poisoning or toxicity to those that consume its aqueous extracts. Symptoms associated with heavy metals poisoning due to acidic water consumption include: nausea and vomiting, diarrhea, suppression of immune

Table 1. Physicochemical parameters values and WHO required values for drinking water.

Parameters	Mean ± SD	WHO permissible limit for drinking water
EC	44 ± 10.1	200 - 400 uS/cm
pH	5.35 ± 0.33	6 - 8
TDS	23 ± 5.5	500 - 1000 mg/L
ORP	164.4 ± 16.6	−50 - 200 mV

system, organ damage, and shortness of breath [37]. Although the oxidation-reduction potential values observed in the current study is within WHO limits, it indicates contamination by pollutants in soil which poses potential health risks to humans, according to the EPA guidelines [38]. The changes in the chemistry of the aqueous soil phase tend to profoundly influence the solubility, bioavailability, and mobility of heavy metals in the soil.

1) The elemental concentration in the anthill soil.

To determine the elemental composition of the anthill soil presented in **Table 2** and **Figure 2**, the ICP-OES was used.

Table 2. The mean elemental concentrations in the anthill soil (mg/kg) and the WHO permissible limits of the elements in drinking water (mg/l).

Elements	Concentration in anthill soil (mg/kg)	WHO permissible limits in drinking water (mg/l)
Na	396.3 ± 42.2	200
Fe	201.7 ± 82.8	0.3
Ca	109.3 ± 15.2	75
Mg	80.5 ± 24.0	50
Cr	12.6 ± 1.2	0.05
Ni	7.1 ± 0.3	0.07
B	3.9 ± 1.3	2.4
Zn	3.5 ± 1.4	3
Mn	1.8 ± 0.8	0.4
Cu	1.3 ± 0.1	2
Pb	1.3 ± 0.2	0.01
Ag	1.2 ± 1.7	0.1
Au	0.2 ± 0.035	-
Se	Nd	0.02
V	Nd	-

Note: (−) Not available, Nd: Not detected.

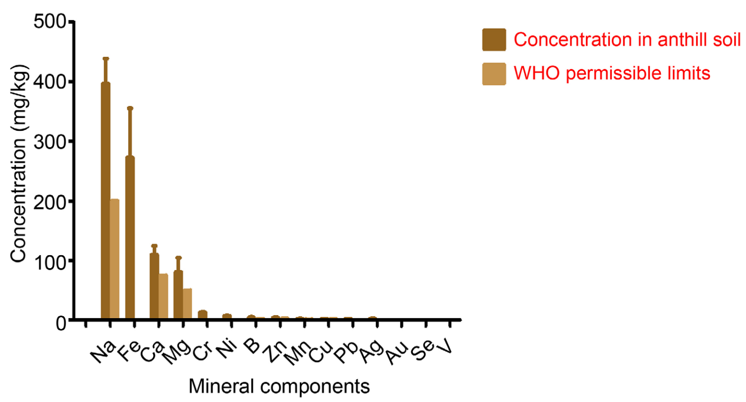

Figure 2. The concentrations of the mineral components that are present in anthill soil. The mean elemental concentrations in the anthill soil were high compared to the WHO maximum permissible limit values for drinking water except for Cu.

The mean concentrations of Na, Fe, Ca, Mg, Cr, Ni, B, Zn, Mn, Cu, Pb, Ag, and Au are as shown in **Table 2** whereas, Se and V were not detected. Considering that the exposure route of the heavy metals (*i.e.*, Cr, Ni, Zn, Mn, Cu, Pb, and Ag) present in this anthill soil is via ingestion of its aqueous extracts, the WHO permissible limits for the detected elements for drinking water is also presented along with the above-mentioned data.

The elemental concentrations determined by the ICP-OES analysis exceeded the standard permissible limits set by the WHO for drinking water [39]-[46] (except for Cu). Similarly, a study conducted by Osobamiro *et al*. and Mohod and Dhote [5] [47], also recorded low levels of Cu in soil samples and drinking water as compared to the maximum WHO permissible limits.

The mineral composition trend shows that Na > Fe > Ca > Mg > Cr > Ni > B > Zn > Mn > Cu > Pb > Ag > Au > Se = V. The concentration of Na was the highest, followed by Fe, Ca, Mg and Cr. These results align with those presented by Glaser *et al*. [48] that also detected very high levels of Na in termitaria. This can be due to the fact that these elements are part of the essential major elements that naturally occur in high concentrations in soil, since they are highly required as nutrients by plants.

Given that this soil is from an undisturbed land mass, high concentrations of some heavy metals detected could be of natural sources such as weathering, dew, atmospheric dust storms and pedogenesis. Weathering and pedogenesis are the primary sources of heavy metals and/or metalloids especially when the parent material contains high level of heavy metals and dissolution of mineral ores release heavy metals contained in their structure into the environment [1]. Boron was the only metalloid present in the anthill soil whereas, vanadium (V) and selenium (Se) were not detected in the anthill soil sample as mentioned earlier, indicating that their concentrations in the soil sample could be lower than the detection limit of the ICP-OES instrument used [49].

2) The chronic daily intake and health risk indices of the elements.

The oral reference dose (RfD) values for most of the elements (except for Ca, Mg, Ag, Au, Se, and V) and the calculated chronic daily intake (CDI) and health risk indices (HRI) values of the detected elements in the anthill soil are shown in **Table 3**. The HRI values were interpreted as follows: values < 1 = no health risk, and > 1 = health risk [28].

The health risk assessment in this study indicated that the HRI values of Fe, Cr, Ni, Pb and Cu were all > 1, indicating health risks, and non-carcinogenic adverse effects of concern due to consumption of these metals [50]. These high HRI values are as a result of high CDI values as compared to the reference dose values for the above-mentioned elements. These results concur with the study published by Hussain *et al*. [25] and Obeka and Numbere [27], which also reported HRI values > 1 for Cr and Ni in drinking water from one of their study locations.

The HRI values for B, Zn, and Mn were all < 1, therefore, no health risk and

Table 3. The Chronic daily intake via drinking water (CDI; mg/kg/day), Oral reference dose (mg/kg/day) and Health risk indices (HRI) values of the trace elements in the anthill soil.

Elements	Chronic daily intake (mg/kg/day)	USEPA Oral reference dose (mg/kg/day)	Health risk indices
Na	11.24	-	-
Fe	5.76	0.007	822.86
Ca	3.12	-	-
Mg	2.29	-	-
Cr	0.36	0.002	180
Ni	0.20	0.02	10
B	0.11	0.2	0.55
Zn	0.1	0.3	0.33
Mn	0.051	0.014	0.36
Cu	0.051	0.001	51
Pb	0.037	0.004	9.25
Ag	0.034	-	-
Au	0.00057	-	-
Se	-	-	-
V	-	-	-

Note: (-) Not available/Not Applicable.

non-carcinogenic adverse effect of concern was found from these elements. However, the calculated cumulative HI value was 1023.35, which exceeds the threshold value of 1. Implying possible concern for potential non-carcinogenic effects posed by all these elements present in the anthill soil [50].

Although some detected elements are essential for human health in trace amounts, based on the reported high HRI and HI values, they can pose human health risks over time due to long-term exposure via ingestion of these elements through the aqueous extracts. These deleterious effects include but are not limited to, kidney damage, blood damage, stunned mental faculty, birth deformities, liver damage, coma, allergies, hyper pigmentation, skin cancer, neurological disorders, cardiovascular diseases and even death [24] [47].

Furthermore, it is well established that some metals such as lead (Pb) and cadmium (Cd) do not serve any significant biological function in our bodies. Exposure to even minute contents of these metals and relatively high concentrations (including those that can contribute to improved human health such as iron and manganese especially in the case of normalizing blood pressure) can be associated with adverse effects in blood pressure levels [51].

3) Bioactive compound screening.

Three solvents were used to extract bioactive constituents from anthill soil, and these chosen solvents cover a wide range of the polarity chart, enabling various solvation powers to dissolve bioactive constituents of varying chemical nature [52]. The bioactive compound screening showed different results in the different extracts of the soil as shown in **Table 4**. Cardiac glycosides and phenolic compounds were present in the aqueous methanol and methanol extracts, while the other tested compounds were absent in these extracts. In the aqueous extracts, flavonoids and terpenoids were the only bioactive compounds that were detected.

The bioactive compounds screening results revealed that the anthill soil contained cardiac glycosides, phenolic compounds, flavonoids and terpenoids. These results align to the notion that bioactive compounds can enter the soil environment as exudates from various plant parts such as roots, plants particulate matter or leachates. In the soil they can exist as free or dissolved, absorbed or reversibly bound by clay minerals or chelated complexes with metals [53].

The results of this study concur with the work published by Bai *et al.* [54] which reported the presence of various terpenoids in soil samples. Lannuci *et al.* [55] also reported the presence of phenolic compounds in the rhizosphere soil of wild oat. Terpenoids, flavonoids and phenolic compounds are recognized to have a wide range of antimicrobial property against various germs, pharmacological properties such as antioxidant and anti-cancer. Moreover, they function as neuropharmacological agents [29] [56]. According to a study by Grossman and Kirch [57], cardiac glycosides are known to have antihypertensive properties, given that a particular cardiac glycoside drug (digoxin) demonstrated the ability to lower diastolic blood pressure and heart rate in both normotensive and hypertensive people overnight. This cardiac glycoside is also known to enhance cardiac contractility and induce apoptosis of tumor cells [58]. The presence of the previously stated pharmaceuticals in the anthill soil suggests its potential use as an antihypertensive agent. Furthermore, these findings warrant further large-scale investigations on anthill soil samples from other geographical areas

Table 4. Bioactive screening results of aqueous methanol, methanol, and aqueous extracts of the anthill soil.

Compound	Absent (−) and Present (+)			Observation
	Aqueous methanol extract	Methanol extract	Aqueous extract	
Cardiac glycosides	+	+	−	Greenish brown ring
Phenolics compounds	+	+	−	Green coloration
Flavonoids	−	−	+	Intense green
Terpenoids	−	−	+	Brown ring

Note: + = Present; − = Absent.

to explore the potential this anthill soil in the treatment of hypertension or other illnesses.

4. Conclusions

Bioactive compounds screening revealed four major bioactive compounds namely, cardiac glycosides, flavonoids, terpenoids and phenolic compounds, which can account for its antihypertensive properties (and other medicinal value) in addition to co-occurring mineral components such as iron and manganese that are linked with normalizing blood pressure. However, the concentrations of the elements revealed by the ICP-OES analysis exceeded the standard permissible limits set by the WHO for drinking water, except for Cu. The health risk assessment indices such as reference dose values, health risk index and hazard index indicated a cumulative effect of concern posed by these detected elements. The hazard index for all the elements highly exceeded the threshold value of 1. This calls for concern for adults who are exposed to these elements via ingestion since there is possibility of accumulation of these elements in the body due to its long-term consumption. This can have various harmful effects on human health as stated earlier. Additionally, the soil's measured chemical parameters/quality indicators values fell way below the WHO acceptable standard values, with exception to ORP. Thus, deeming the soil's aqueous extracts unsuitable for consumption. Therefore, it can be strongly recommended that the consumption of this anthill soil's aqueous extracts should be ceased.

Whilst the current study provides data concerning the elemental and bioactive compounds composition and physicochemical properties of the anthill soil, it had some limitations which are worth mentioning. Firstly, only a homogeneous soil sample was collected from several anthills in an attempt to generate reliable information on its bioactive compounds and elemental contents in relation to toxicity due to heavy metals contamination. Secondly, seasonality during sampling and impact of anthropogenic activities (*i.e.*, farming) were not considered. However, it is worth noting that the anthill soil used was collected from undisturbed land mass that has never been exposed to anthropogenic activities that are primary polluters of the natural environment.

If this anthill soil is to be repurposed or explored to serve as an antihypertensive agent, future studies should focus on establishing a reasonable dosage (with appropriate contents of required mineral components and/or adjusted to an acceptable pH, TDS, and EC) and its level of toxicity, as well as conduct seasonal investigations (encompassing anthill soil samples from other geographical areas) to contribute to the knowledge base of this anthill soil.

Acknowledgements

We would like to thank the Namibia University of Science and Technology for providing infrastructural support. We also thank Dr. Julien M. Lusilao for his assistance with analyzing the ICP-OES data.

Declaration of Competing Interest

The authors declare no conflict of interest.

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