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Levels of trace metals in five anticancer medicinal plants harvested from soil treated with organic and inorganic fertilizers

Idris O. Raimi[1,*], Liziwe L. Mugivhisa[1], Francis B. Lewu[2], Stephen O. Amoo[3] and Joshua O. Olowoyo[1]

[1]Department of Biology, Sefako Makgatho Health Sciences University, P.O. Box 139, Medunsa 0204; [2]Department of Agriculture, Cape Peninsula University of Technology, Wellington Campus, Wellington 7655, Western Cape; [3]Agricultural Research Council, ARC-Roodeplaat Vegetable and Ornamental Plants, Private Bag X293, Pretoria, 001, South Africa.

ABSTRACT

The use of medicinal plants for the treatment of diseases including cancer in Africa is gaining recognition and wide acceptability. However, some of these plants may bio-accumulate trace metals including heavy metals in their tissues, resulting in potential safety hazards. A greenhouse study was set up to investigate the presence and levels of trace metals in five anticancer medicinal plants (*Withania somnifera*, *Artemisia afra*, *Catharanthus roseus*, *Centella asiatica* and *Taraxacum officinale*) harvested from soil treated with sewage sludge, poultry droppings, cow dung, biosolid and NPK as soil nutrient boosters. ICP-MS was used to determine the trace metal contents in the different parts of the plants upon harvest. The root of *Centella asiatica* harvested from soil treated with cow dung recorded the highest concentration of Mn (2739 ± 26.91 mg/kg) while the least Mn concentration (47.64 ± 0.63 mg/kg) was recorded in the stem of *W. somnifera* harvested from soil treated with cow dung. The leaves of *A. afra* harvested from sewage sludge treatment recorded the lowest concentration of U (0.06 ± 0.03 mg/kg). The values of other trace metals in the different plant parts ranged from 2.01-66.00 mg/kg for Pb, 11.16-499.51 mg/kg for Cu, 7.64-538.36 mg/kg for Cr, 60.15-953.11 mg/kg for Zn, 0.82-123.76 mg/kg for Co, and 17.79-499.51 mg/kg for Ni. The overall concentration of trace metals in the five plants across all the treatments and the control was above the permissible limit set by the World Health Organization (WHO) for safe human consumption. The bioaccumulation factor (BAF) for Zn, Cr and Pb was above 1 in the five plants across all the treatments and the control which showed that the plants are hyper-accumulators for Zn, Cr and Pb.

KEYWORDS: bioaccumulation factor, hyper-accumulators, medicinal plants, trace metals, translocation factor.

INTRODUCTION

Trace metal contamination of medicinal plants including anticancer plants is a disturbing issue considering the growing acceptability of herbal plants in Africa and other parts of the world [1]. Trace metal concentration and abundance in plant parts are relative and largely dependent on the growth medium [2]. Studies on the use of medicinal plants especially in curing diseases such as cancer are on the rise globally, particularly in Africa where there is huge flora diversity [3-5].

The popularity of medicinal plants in recent times has been attributed to their availability, relative cost-effectiveness, robust knowledge from folk

*Corresponding author : rimiidris@yahoo.com

medicine and ultimately the adherent (consumer) perception of no or minimal adverse effects when used [6-8]. Based on the relatively cheaper cost and reported minimal adverse side effects of herbal plants when compared to orthodox medicine, a large population of the developing countries, mostly African nations, patronises herbal medical practitioners for treatment of diseases ranging from the lesser ones to complicated ones such as cancer [9].

However, the assumption that herbal plants have no adverse side effects (or minimal side effects) when used for treatment of diseases may be untrue and mythological in some instances; hence, further probe may be necessary. A significant number of natural products from herbs and shrubs showed adverse side effects on the patients partly due to some pollutants that may inhibit the normal functions of the metabolites in plants [10, 11]. There is a reported degree of interaction between chemotherapeutic drugs of plant origin and pollutants such as trace metals in soil or from the environment [12]. Studies have shown that higher levels of some trace metals such as Pb, Cu, Ni, Cr, Co, U, Zn, Cd in higher plants may be carcinogenic when consumed as herbal plants, damage the central nervous system, cause kidney and liver dysfunction as well as present adverse effects on memory and reproductive system [13, 14]. Investigation of herbal plants including those used as anticancer agents becomes necessary to ascertain the level of trace metal uptake in their tissues.

Different sources have been reported in the literature as the possible origin of trace metal contamination in herbal plants found growing on the terrestrial ecosystem. These sources include soil pollution from irrigation water, atmospheric dust from quarrying activities, industrial emissions and pollution from fertilization (organic or inorganic) [15, 16]. Intensive use of fertilizers is an important contributor to trace metal uptake and accumulation in plants grown on agricultural soils [17]. Toxic trace metals from the above-listed sources may mix with the soil matrix and remain in the soil for a longer period for uptake/transmission into the plants [5]. The persistent status of toxic trace metals in the soil poses danger to the environment and human health especially when medicinal plants are cultivated on such soil [5, 18]. These metals may not only be hazardous for the plants but could be harmful to human health as medicinal plants are part of the food chain [19].

Many medicinal plant regimen and their mixtures have been reported to contain toxic trace metals such as Pb, Zn, Mn, Ni, Cr, Cu, Co, U and pose a huge health risk to humans [20-22]. For instance, Olowoyo *et al.* [23] reported medicinal plants harvested from waste dumpsites with concentrations of toxic trace metals such as Pb, Cr and As above the WHO recommended limit in their tissues. The study further revealed that *D. stramonium* and *Amaranthus spinosus* were bio-accumulators of trace metals. The elemental composition of medicinal plants is often characterised by the geochemical characteristics of the soil, type of fertilizers applied, environmental/climatic factors, extensive agricultural activity, and the potential of the plants to accumulate trace metals [24].

Plants absorb trace metals (both toxic and essential) among other compounds from the soil and accumulate them in their harvestable parts (roots, stems and leaves) for onward passage into the food chain *via* direct human consumption of herbal medicinal recipe or through animal consumption [25]. Plant uptake, accumulation and bioavailability of trace metals in the soil depend largely on its abundance in the soil (weathered rock) and other sources such as atmospheric deposition and fertilizer application sources [16].

Medicinal plants are globally valuable sources of herbal products, and they are disappearing at an alarming rate due to factors such as over-exploitation, climate change and the industrial revolution [26]. Considering the inherent residual knowledge of medicinal plants possessed/acquired by the locals, coupled with the affordable nature of the traditional style of healthcare management in Africa, there is therefore the necessity to consider growing these important medicinal plants in large quantity for sustainable healthcare management as embraced by more than half of the African population. The input of fertilizers (organic and inorganic) as soil nutrient boosters for increased crop yield is well known in developing countries; however, there is a need to investigate their usage regarding their safety in medicinal plants domestically cultivated. The current study intends to investigate among others, the possible effect of using both organic and inorganic materials not only to increase the plant yield but also to determine the ability of these plants to either exclude or bio-accumulate toxic pollutants such as trace metals either from the soil or from the added soil fertilizers (organic and inorganic).

This study is therefore set out to evaluate the concentrations of toxic trace metals in the anticancer medicinal plants cultivated domestically upon addition of both organic and inorganic fertilizers as soil nutrient. The investigated anticancer medicinal plants are *Artemisia afra* Jacq. ex Willd., *Catharanthus roseus* (L.) G. Don., *Withania somnifera* (L.) Dunal, *Centella asiatica* (L.) Urb. and *Taraxacum officinale* F.H. Wigg while the soil nutrient fertilizers from the organic and inorganic sources include sewage sludge, poultry droppings, cow dung, biosolids and NPK. This study was taken up to evaluate the bioaccumulation and translocation of selected toxic trace metals (Pb, Zn, Mn, Ni, Cr, Cu, Co and U) in anticancer medicinal plants grown under greenhouse condition.

MATERIALS AND METHODS

The plants used for this study were harvested from the experimental pots treated with organic (sewage sludge, biosolids, poultry droppings, cow dung) and inorganic fertilizers grown for four months under a greenhouse condition at the Sefako Makgatho Health Sciences University (25°37’8” S and 28°1’22” E). The matured plants were harvested, washed thoroughly in distilled water and separated into leaves, stems and roots, before oven drying at 60 °C to constant weight. The dried plant samples were milled by a mechanical grinder and sieved through a 0.5 mm diameter sieve. The pulverised plant samples (leaves, stems and roots) were then kept in paper sample bags and stored in the desiccators for further investigation.

Soil samples were collected from each pot and stored in polythene bags. Soil samples were air-dried and ground into fine powder for further investigation. Both plant and soil samples were digested following the procedure described by Olowoyo *et al*. (2011) with slight modification. The elemental analyses of the plant and soil samples were performed using an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Model: ICP-MS 7750 Series Agilent).

Digestion of plant samples was performed using 0.5 g of dried plant samples with the addition of 10 ml of HNO_3 (65% Merck supra pure) and 3 ml of $HClO_4$ (65% Merck supra pure). This mixture was heated up to 150 °C (an air oven Scientific series 9000) for 2 h and brought to a volume of 10 ml with deionized water. This procedure was repeated with a blank digest carried out the same way. A similar procedure was followed for the digestion of soil samples. However, with soil samples, 0.5 g of air-dried sample was used with the addition of 12 ml of HNO_3 (65% Merck supra pure) and 5 ml of $HClO_4$ (65% Merck supra pure). For the purpose of quality assurance, the analysis for both plant and soil samples was carried out in triplicate, with the analysis of CRM042-050 that contains metals.

The soil pH was determined by 0.01 M $CaCl_2$ (1:2 soil solution ratio) and in distilled water using a pH meter fitted with a glass electrode (Jenwal Model 3015 digital). Organic matter content was determined using Walkley and Black method as described by [27].

Data generated from the ICP-MS was subjected to analysis of variance (ANOVA) using IBM SPSS 26.0. Furthermore, Bioaccumulation Factor (BAF) and Translocation Factor (TF) were calculated for the trace metals using the formula; BAF = Mean elemental content in plant/Mean elemental content in soil; TF = Mean elemental content in Leaves/ Mean elemental content in Roots [28, 29].

RESULTS

Soil organic matter and pH

The highest organic matter concentration (4.42%) was recorded in the biosolid soil treatment where *T. officinale* was harvested while the least concentration of organic matter (2.94%) was recorded in the sewage sludge-treated soil where *A. afra* was harvested. Organic matter concentration was least in the control soil where *C. asiatica* was harvested. The soil pH in $CaCl_2$ ranged from 5.76 in the NPK-treated soil where *W. somnifera* was harvested to 7.12 in cow dung-treated soil where *A. afra* was harvested. The soil pH in water ranged from 6.53 in the NPK-treated soil where *C. asiatica* was harvested to 7.60 in the biosolid-treated soils where *C. roseus* was harvested.

Trace metal concentrations in the soil

The concentration of Mn ranged from 992.60 ± 9.00 mg/kg - 4597.30 ± 12.20 mg/kg. The highest mean concentrations for all the toxic trace metals in the soil was recorded for Mn in the soil treated with cow dung where *T. officinale* was harvested, followed by Zn. The least concentration of toxic trace metals in soil was recorded for U from the soil treated with cow dung where *C. asiatica* was harvested (Table 1).

The concentration of Zn ranged from 151.83 ± 8.34 mg/kg to 464.68 ± 19.89. The highest concentration of Zn was recorded in soil treated with cow dung where *T. officinale* was harvested while the least concentration of Zn was recorded in soil treated with poultry droppings where *A. afra* was harvested (Table 1).

The concentration of Cu ranged from 67.80 ± 1.64 mg/kg to 369.22 ± 3.81 mg/kg. The highest concentration of Cu was recorded in the soil treated with cow dung where *T. officinale* was harvested while the least Cu concentration was recorded in the soil treated with poultry droppings where *A. afra* was harvested. The concentration of Ni in the soil ranged from 74.80 ± 4.18 mg/kg to 361.80 ± 5.08 mg/kg. The Highest Ni concentration in the soil was recorded from soil treated with cow dung where *T. officinale* was harvested, similar to other trace metals such as Mn, Zn, Cu, Pb, and Co except Cr and U (Table 1).

The concentration of Pb from the soil ranged from 2.75 ± 0.18 mg/kg - 27.37 ± 1.44 mg/kg. The highest mean concentration of Pb was reported in soil treated with cow dung fertilizer where *T. officinale* was harvested while the lowest was reported from soil treated with poultry droppings where *A. afra* was harvested (Table 1). The statistical analysis showed that the differences obtained in the concentration of this metal were significant ($p < 0.05$). Uranium concentration in the soil ranged from 0.33 ± 0.03 mg/kg - 1.87 ± 0.17 mg/kg. The highest mean concentration of U was recorded in the soil treated with NPK where *A. afra* was harvested (Table 1). There was a significant difference ($p < 0.05$) in the mean concentrations of all the toxic trace metals across all the treatments and the control in all the five plants (Table 1). The concentrations of trace metals in the soil for all the five anticancer medicinal plants across all the treatments were in the order Mn > Zn > Cu > Ni > Co > Cr > Pb > U. From the report presented above, the highest concentration for most of the metals in the soil was from soil treated with cow dung.

Trace metal concentrations in the roots, stems and leaves of the five anticancer medicinal plants

The concentration of Mn in the plant ranged from 47.64 ± 0.63 mg/kg to 2739.10 ± 26.91 mg/kg. The highest mean concentrations in all the toxic trace metals in the different plant parts was recorded for Mn found in the root of *C. asiatica* harvested from soil treated with cow dung (Table 2a), followed by Cu whose highest mean concentration was recorded in the root of *C. roseus* harvested from soil treated with sewage sludge (Table 2a). The concentration of Zn in the plant ranged from 60.15 ± 1.32 mg/kg in the stem of *W. somnifera* harvested from cow dung-treated soil to 953.11 ± 7.96 mg/kg in the leaves of *A. afra* harvested from the sewage sludge-treated soil (Table 2b & 2c). The concentration of Zn in the leaves of the five anticancer medicinal plants varied significantly ($p < 0.05$).

The concentration of Cr as recorded in our study ranged from 11.77 ± 1.70 mg/kg in the stem of *A. afra* harvested from cow dung-treated soil to 538.36 ± 0.63 mg/kg in the root of *T. officinale* harvested from poultry dropping-treated soil (Table 2a & 2c).

Ni concentration ranged from 17.24 ± 8.78 mg/kg to 499.51 ± 4.84 mg/kg. The highest concentration of Ni was recorded in the root of *A. afra* harvested from sewage sludge-treated soil while the lowest concentration was recorded in leaves of *W. somnifera* harvested from sewage sludge-treated soil (Table 2a & 2b). The concentration of Ni in the leaves of the five anticancer medicinal plants varied significantly ($p < 0.05$) with some exceptions found in the leaves of all the five anticancer plants harvested from soil treated with sewage sludge and the control (Table 2b). Nickel was, however, not detected in the stems of *A. afra* harvested from soils treated with sewage sludge and control, as well as in the stem of *C. roseus* harvested from poultry dropping-treated soils, and lastly, in the stem of *W. somnifera* harvested in the NPK-treated soils as the concentrations were < 15 mg/kg detection limit set by the ICP-MS in the study (Table 2c).

Table 1. Trace metal concentrations of organic and inorganic-fertilized soils after harvest of five anti-cancer medicinal plants.

	Pb (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Co (mg/kg)	U (mg/kg)
Catharanthus roseus								
Control	15.29 ± 0.27^{c}	216.37 ± 4.48^{b}	214.91 ± 2.71^{b}	2580.43 ± 13.50^{c}	25.84 ± 2.57^{c}	271.06 ± 7.05^{d}	173.66 ± 0.47^{b}	0.48 ± 0.05^{d}
Biosolids	18.85 ± 0.33^{ab}	207.31 ± 8.27^{b}	206.26 ± 2.52^{c}	2804.93 ± 26.80^{b}	45.64 ± 4.47^{a}	381.76 ± 5.33^{b}	158.61 ± 1.73^{c}	0.68 ± 0.10^{bc}
Cow dung	19.85 ± 0.94^{a}	241.56 ± 3.31^{a}	248.29 ± 2.15^{a}	3816.93 ± 26.10^{a}	41.52 ± 5.08^{ab}	321.20 ± 3.61^{c}	202.73 ± 3.25^{a}	0.57 ± 0.04^{cd}
Poultry droppings	12.71 ± 0.82^{d}	115.80 ± 7.20^{c}	137.94 ± 1.48^{e}	1588.23 ± 1.50^{f}	34.91 ± 2.06^{b}	248.51 ± 6.78^{e}	93.84 ± 0.53^{e}	0.52 ± 0.04^{d}
Sewage sludge	17.72 ± 0.44^{b}	122.26 ± 8.47^{c}	187.47 ± 2.17^{d}	2260.73 ± 2.80^{d}	33.77 ± 1.99^{bc}	441.09 ± 14.25^{a}	102.63 ± 1.88^{d}	0.88 ± 0.04^{a}
NPK	15.51 ± 0.27^{c}	123.89 ± 7.65^{c}	124.66 ± 0.87^{f}	1880.63 ± 19.70^{e}	33.01 ± 0.97^{bc}	242.64 ± 0.94^{e}	100.00 ± 1.14^{d}	0.79 ± 0.01^{ab}
Artemisia afra								
Control	16.26 ± 0.73^{b}	152.48 ± 4.36^{b}	165.10 ± 2.36^{b}	2557.30 ± 9.50^{a}	26.66 ± 1.25^{c}	226.07 ± 2.80^{c}	128.44 ± 0.40^{b}	0.62 ± 0.04^{c}
Biosolids	14.58 ± 0.38^{b}	114.60 ± 4.95^{d}	122.89 ± 1.38^{e}	1667.73 ± 9.50^{c}	16.46 ± 2.11^{d}	187.00 ± 2.29^{d}	104.74 ± 0.58^{c}	0.53 ± 0.01^{c}
Cow dung	11.15 ± 0.61^{c}	116.74 ± 2.10^{d}	132.09 ± 1.45^{d}	1399.43 ± 6.30^{d}	29.99 ± 3.34^{c}	192.60 ± 9.44^{d}	85.82 ± 1.32^{d}	0.73 ± 0.08^{c}
Poultry droppings	2.75 ± 0.18^{d}	74.80 ± 4.18^{e}	67.80 ± 1.64^{f}	992.60 ± 9.00^{e}	-6.48 ± 3.05^{e}	151.83 ± 8.34^{e}	76.04 ± 0.66^{e}	0.55 ± 0.09^{c}
Sewage sludge	19.50 ± 1.29^{a}	140.31 ± 1.32^{c}	153.12 ± 2.14^{c}	2005.20 ± 13.40^{b}	39.88 ± 4.18^{b}	298.37 ± 8.26^{a}	103.38 ± 0.46^{c}	1.47 ± 0.06^{b}
NPK	18.51 ± 0.70^{a}	189.95 ± 4.27^{a}	199.97 ± 1.43^{a}	2562.47 ± 16.70^{a}	47.99 ± 0.53^{a}	271.52 ± 9.16^{b}	144.70 ± 0.96^{a}	1.87 ± 0.17^{a}
Taraxacum officinale								
Control	15.73 ± 0.57^{b}	179.27 ± 9.35^{b}	162.44 ± 2.26^{c}	2569.97 ± 2.70^{b}	29.85 ± 1.48^{b}	242.60 ± 2.57^{c}	128.83 ± 0.96^{c}	0.64 ± 0.04^{bc}
Biosolids	9.74 ± 0.54^{c}	108.05 ± 3.93^{d}	108.77 ± 0.59^{e}	1417.70 ± 9.60^{e}	7.69 ± 1.60^{c}	191.88 ± 5.38^{e}	91.92 ± 1.83^{e}	0.41 ± 0.07^{d}
Cow dung	27.37 ± 1.44^{a}	361.80 ± 5.08^{a}	369.22 ± 3.81^{a}	4597.30 ± 12.20^{a}	38.18 ± 2.79^{b}	464.68 ± 19.89^{a}	269.99 ± 1.66^{a}	0.88 ± 0.11^{a}
Poultry droppings	11.67 ± 0.36^{c}	129.01 ± 6.72^{c}	141.39 ± 1.72^{d}	1914.70 ± 2.50^{d}	50.29 ± 2.90^{a}	227.86 ± 3.54^{cd}	100.70 ± 0.87^{d}	0.47 ± 0.01^{cd}
Sewage sludge	13.94 ± 0.30^{b}	75.48 ± 5.22^{e}	110.83 ± 1.16^{e}	1267.53 ± 2.70^{f}	30.96 ± 4.48^{b}	281.26 ± 5.17^{b}	67.86 ± 0.28^{f}	0.63 ± 0.08^{bc}
NPK	15.45 ± 0.89^{b}	187.52 ± 4.99^{b}	192.64 ± 1.32^{b}	2417.00 ± 13.70^{c}	11.78 ± 3.95^{c}	217.12 ± 4.75^{d}	150.73 ± 2.60^{b}	0.75 ± 0.04^{ab}
Withania somnifera								
Control	19.89 ± 0.73^{a}	221.31 ± 6.71^{a}	213.16 ± 1.94^{b}	2895.53 ± 22.00^{b}	41.83 ± 2.81^{b}	423.03 ± 1.09^{a}	183.40 ± 1.75^{a}	0.76 ± 0.02^{b}
Biosolids	11.18 ± 0.17^{c}	183.24 ± 3.05^{b}	128.91 ± 2.62^{e}	1566.90 ± 3.80^{d}	15.57 ± 2.01^{c}	228.34 ± 8.33^{c}	92.68 ± 2.08^{e}	0.39 ± 0.01^{c}

Table 1. continued..

Cow dung	12.07 ± 0.87^{bc}	135.45 ± 1.68^{c}	160.26 ± 1.24^{c}	1420.77 ± 6.70^{e}	20.36 ± 1.37^{c}	222.87 ± 6.39^{c}	109.40 ± 1.02^{b}	0.39 ± 0.07^{c}
Poultry droppings	20.51 ± 0.33^{a}	125.68 ± 8.37^{c}	148.88 ± 2.46^{d}	1624.20 ± 15.20^{c}	18.74 ± 4.79^{c}	225.32 ± 4.55^{c}	104.57 ± 1.40^{c}	0.42 ± 0.01^{c}
Sewage sludge	13.20 ± 0.74^{b}	103.53 ± 2.09^{d}	134.35 ± 3.14^{e}	1661.00 ± 23.50^{c}	56.24 ± 5.01^{a}	222.01 ± 10.69^{c}	98.18 ± 1.02^{d}	0.48 ± 0.06^{c}
NPK	19.81 ± 0.87^{a}	215.78 ± 4.12^{a}	227.43 ± 3.01^{a}	2975.63 ± 15.30^{a}	43.97 ± 3.88^{b}	309.05 ± 4.38^{b}	185.56 ± 1.64^{a}	1.45 ± 0.09^{a}
Centella asiatica								
Control	14.87 ± 0.77^{a}	119.91 ± 4.75^{e}	131.12 ± 2.02^{e}	1920.63 ± 13.20^{b}	33.37 ± 1.28^{b}	211.91 ± 4.76^{c}	100.52 ± 1.57^{d}	0.42 ± 0.07^{cd}
Biosolids	11.37 ± 0.38^{b}	191.94 ± 2.63^{a}	191.78 ± 0.55^{b}	1821.77 ± 6.40^{c}	8.68 ± 2.89^{d}	200.46 ± 9.10^{cd}	133.69 ± 0.27^{b}	0.37 ± 0.07^{cd}
Cow dung	9.54 ± 0.41^{c}	125.57 ± 1.91^{de}	135.34 ± 1.59^{de}	1657.23 ± 9.50^{d}	8.93 ± 3.16^{d}	169.50 ± 8.74^{d}	93.49 ± 0.80^{e}	0.33 ± 0.03^{d}
Poultry droppings	11.63 ± 0.57^{b}	130.32 ± 4.16^{d}	140.37 ± 1.18^{d}	1911.17 ± 1.30^{b}	50.41 ± 0.55^{a}	227.26 ± 2.31^{bc}	100.50 ± 0.67^{d}	0.49 ± 0.01^{c}
Sewage sludge	14.34 ± 0.73^{a}	155.81 ± 1.28^{c}	198.20 ± 3.60^{a}	1814.70 ± 19.30^{c}	17.44 ± 4.31^{c}	370.20 ± 6.63^{a}	129.81 ± 1.78^{c}	0.83 ± 0.02^{b}
NPK	14.32 ± 0.34^{a}	172.76 ± 3.19^{b}	168.61 ± 1.65^{c}	2364.07 ± 20.30^{a}	27.69 ± 4.01^{b}	240.63 ± 3.48^{b}	138.50 ± 1.08^{a}	1.20 ± 0.04^{a}

Note: Different letters in the same column per plant species indicate significant difference at $p < 0.05$.

Table 2a. Concentrations of trace metals in the roots of the anticancer medicinal plants harvested from organic and inorganic-treated soils.

Catharanthus roseus								
	Pb (mg/kg)	**Ni (mg/kg)**	**Cu (mg/kg)**	**Mn (mg/kg)**	**Cr (mg/kg)**	**Zn (mg/kg)**	**Co (mg/kg)**	**U (mg/kg)**
Control	49.17 ± 0.47^{a}	260.98 ± 3.88^{b}	169.57 ± 5.55^{b}	348.60 ± 3.15^{e}	53.69 ± 2.46^{e}	726.86 ± 5.27^{b}	11.63 ± 0.36^{f}	0.25 ± 0.02^{d}
Biosolids	27.10 ± 0.47^{b}	129.39 ± 3.56^{c}	115.99 ± 4.53^{c}	1355.49 ± 10.10^{a}	108.03 ± 2.10^{b}	511.82 ± 4.45^{c}	53.80 ± 0.17^{a}	0.64 ± 0.01^{b}
Cow dung	6.92 ± 0.29^{f}	51.61 ± 0.06^{e}	55.76 ± 4.18^{e}	442.53 ± 1.15^{d}	37.41 ± 0.46^{f}	212.85 ± 2.43^{f}	15.27 ± 0.32^{e}	0.31 ± 0.03^{d}
Poultry droppings	12.52 ± 0.26^{e}	77.57 ± 4.14^{d}	86.88 ± 5.25^{d}	910.22 ± 8.82^{c}	89.67 ± 1.23^{c}	304.24 ± 3.95^{e}	43.40 ± 0.38^{b}	0.50 ± 0.01^{c}
Sewage sludge	21.93 ± 0.29^{c}	302.24 ± 4.62^{a}	1881.00 ± 4.80^{a}	1254.50 ± 6.00^{b}	486.21 ± 6.01^{a}	888.20 ± 2.30^{a}	40.16 ± 1.06^{c}	6.49 ± 0.07^{a}
NPK	14.24 ± 0.14^{d}	49.96 ± 2.95^{e}	75.76 ± 1.23^{d}	893.15 ± 4.42^{c}	74.52 ± 3.04^{d}	435.59 ± 8.22^{d}	31.68 ± 0.38^{d}	0.59 ± 0.04^{bc}
Artemisia afra								
Control	18.83 ± 0.15^{b}	49.00 ± 12.81^{d}	77.04 ± 5.03^{c}	680.56 ± 1.01^{c}	65.06 ± 0.60^{c}	536.56 ± 1.58^{a}	24.37 ± 0.27^{e}	0.86 ± 0.06^{a}

Table 2a continued..

Biosolids	27.32 ± 0.18^a	98.46 ± 1.46^c	47.24 ± 4.16^e	1041 ± 8.61^b	131.46 ± 2.81^a	487.49 ± 2.06^b	44.00 ± 1.76^b	0.64 ± 0.01^c
Cow dung	5.05 ± 0.09^f	43.86 ± 3.29^d	54.96 ± 2.59^d	494.34 ± 6.21^d	28.82 ± 3.10^e	189.19 ± 3.92^e	28.09 ± 0.50^d	0.38 ± 0.03^e
Poultry droppings	13.50 ± 0.21^c	154.67 ± 4.95^b	155.19 ± 6.08^a	1608.61 ± 13.25^a	103.58 ± 0.69^b	327.46 ± 9.30^c	97.61 ± 0.28^a	0.71 ± 0.02^b
Sewage sludge	8.87 ± 0.10^e	499.51 ± 4.84^a	93.33 ± 4.34^b	690.30 ± 0.40^c	36.88 ± 1.56^d	327.80 ± 8.70^c	31.40 ± 0.29^c	0.46 ± 0.03^d
NPK	10.90 ± 0.23^d	19.01 ± 2.15^e	44.41 ± 1.01^e	351.90 ± 5.80^e	27.67 ± 1.86^e	316.04 ± 5.05^d	10.68 ± 0.39^f	0.37 ± 0.02^e
Taraxacum officinale								
Control	37.16 ± 0.34^c	186.42 ± 1.95^b	79.20 ± 4.16^d	1125.40 ± 1.55^d	320.18 ± 0.72^b	526.16 ± 6.90^c	39.61 ± 0.52^d	0.73 ± 0.02^c
Biosolids	10.73 ± 0.19^f	154.38 ± 5.07^c	64.86 ± 2.72^e	844.65 ± 4.65^f	140.72 ± 1.86^e	274.63 ± 6.04^e	37.00 ± 0.78^e	0.27 ± 0.03^e
Cow dung	15.41 ± 0.08^d	150.44 ± 9.85^c	168.97 ± 4.71^c	1699.56 ± 11.08^b	114.57 ± 1.85^f	458.76 ± 1.41^d	83.39 ± 0.51^b	0.76 ± 0.01^c
Poultry droppings	13.71 ± 0.23^e	322.02 ± 3.46^a	72.31 ± 2.56^{de}	986.47 ± 4.46^e	538.36 ± 0.63^a	269.13 ± 4.35^e	37.29 ± 1.10^e	0.60 ± 0.01^d
Sewage sludge	48.27 ± 0.10^b	158.18 ± 2.50^c	239.32 ± 2.90^a	1423.60 ± 7.58^c	198.64 ± 1.73^d	1088.30 ± 14.83^a	55.79 ± 0.29^c	1.51 ± 0.02^b
NPK	66.00 ± 0.30^a	187.38 ± 3.11^b	196.30 ± 5.51^b	2281.22 ± 37.22^a	268.59 ± 3.16^c	916.54 ± 2.46^b	88.45 ± 1.31^a	1.59 ± 0.05^a
Withania somnifera								
Control	18.47 ± 0.24^d	41.31 ± 5.10^e	103.25 ± 2.14^d	824.93 ± 5.55^f	226.44 ± 0.79^a	618.25 ± 13.77^a	20.98 ± 0.05^e	0.66 ± 0.04^c
Biosolids	21.36 ± 0.38^c	188.93 ± 4.90^a	160.28 ± 1.84^a	2118.11 ± 50.25^a	189.92 ± 3.42^c	532.17 ± 7.11^d	88.82 ± 0.53^a	0.79 ± 0.02^b
Cow dung	19.88 ± 0.37^{bc}	98.01 ± 8.53^c	38.37 ± 2.21^b	1564.67 ± 2.00^b	221.19 ± 2.51^b	356.82 ± 8.96^e	51.59 ± 2.18^b	0.37 ± 0.07^a
Poultry droppings	11.81 ± 0.11^e	75.31 ± 2.71^d	74.29 ± 1.70^e	995.71 ± 7.62^e	140.78 ± 1.93^f	260.46 ± 3.51^f	37.57 ± 0.80^d	0.46 ± 0.04^d
Sewage sludge	23.36 ± 0.73^a	116.31 ± 11.65^b	124.30 ± 4.70^c	1295.50 ± 12.50^d	180.47 ± 0.16^d	538.60 ± 7.40^c	36.84 ± 0.33^d	0.85 ± 0.02^b
NPK	22.19 ± 0.29^b	95.64 ± 4.94^c	132.61 ± 3.59^b	1495.52 ± 8.31^c	169.15 ± 2.87^e	576.52 ± 0.73^b	46.22 ± 0.98^c	1.08 ± 0.03^a
Centella asiatica								
Control	29.05 ± 0.16^b	46.55 ± 3.93^d	79.42 ± 3.39^d	776.71 ± 5.14^e	483.63 ± 3.58^a	774.66 ± 9.04^a	18.92 ± 0.08^e	1.12 ± 0.01^a
Biosolids	17.07 ± 0.32^d	195.99 ± 2.85^a	161.21 ± 3.16^b	2327.14 ± 32.51^b	206.51 ± 3.15^b	483.73 ± 5.88^d	103.18 ± 2.33^b	0.71 ± 0.04^c
Cow dung	26.18 ± 0.13^c	203.55 ± 5.12^a	234.99 ± 5.02^a	2739.10 ± 26.91^a	110.61 ± 1.62^d	554.81 ± 6.17^c	123.76 ± 0.81^a	1.22 ± 0.09^a
Poultry droppings	8.28 ± 0.18^e	146.21 ± 1.78^b	72.31 ± 0.38^d	874.23 ± 3.12^d	97.47 ± 2.08^e	186.91 ± 3.64^e	28.44 ± 1.18^d	0.31 ± 0.01^d
Sewage sludge	26.29 ± 0.26^c	96.53 ± 2.33^c	131.70 ± 4.20^c	1627.10 ± 21.80^c	122.29 ± 0.89^c	601.00 ± 14.20^b	51.85 ± 1.03^c	0.89 ± 0.02^b
NPK	29.80 ± 0.28^a	20.34 ± 2.63^e	37.64 ± 0.80^e	341.15 ± 0.93^f	31.92 ± 1.10^f	553.76 ± 4.64^c	5.44 ± 0.17^f	0.17 ± 0.01^e

Note: Different letters in the same column per plant species indicate significant difference at $p < 0.05$.

Table 2b. Concentrations of trace metals in the leaves of the anticancer medicinal plants harvested from organic and inorganic-treated soils.

	Pb (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Co (mg/kg)	U (mg/kg)
Catharanthus roseus								
Control	39.74 ± 0.52^{a}	41.66 ± 3.94^{a}	160.00 ± 4.40^{a}	370.90 ± 3.80^{d}	23.54 ± 1.78^{a}	678.10 ± 15.2^{a}	9.64 ± 0.31^{a}	0.11 ± 0.01^{b}
Biosolids	10.27 ± 0.26^{e}	33.55 ± 2.30^{b}	49.03 ± 4.30^{c}	539.82 ± 2.97^{a}	26.03 ± 2.20^{a}	414.70 ± 2.96^{d}	8.17 ± 6.57^{b}	0.26 ± 0.33^{a}
Cow dung	26.01 ± 0.10^{c}	21.44 ± 0.44^{c}	31.63 ± 0.46^{d}	335.43 ± 2.44^{e}	17.52 ± 0.76^{b}	482.63 ± 0.96^{c}	7.74 ± 0.41^{b}	ND
Poultry droppings	8.42 ± 0.07^{f}	34.58 ± 4.29^{ab}	41.00 ± 1.74^{c}	372.99 ± 3.28^{d}	18.25 ± 4.29^{b}	343.13 ± 4.96^{e}	4.96 ± 3.32^{d}	ND
Sewage sludge	27.34 ± 0.15^{b}	29.16 ± 3.49^{bc}	90.31 ± 4.28^{b}	402.29 ± 1.84^{c}	18.42 ± 1.75^{b}	668.77 ± 3.96^{a}	4.64 ± 6.72^{d}	ND
NPK	21.83 ± 0.19^{d}	31.79 ± 2.70^{b}	31.99 ± 0.18^{d}	421.53 ± 2.59^{b}	23.72 ± 1.23^{a}	533.25 ± 2.96^{b}	6.00 ± 1.29^{c}	ND
Artemisia afra								
Control	37.43 ± 0.22^{b}	61.37 ± 1.94^{a}	92.05 ± 2.10^{b}	921.00 ± 4.26^{a}	123.03 ± 1.08^{a}	936.3 ± 5.16^{a}	28.30 ± 0.33^{a}	0.52 ± 0.07^{b}
Biosolids	12.28 ± 0.20^{e}	29.63 ± 2.41^{d}	54.62 ± 1.63^{c}	533.14 ± 5.80^{d}	22.85 ± 2.47^{d}	567.78 ± 2.96^{d}	6.44 ± 1.66^{e}	ND
Cow dung	51.66 ± 0.10^{a}	37.85 ± 6.00^{c}	54.24 ± 2.23^{c}	514.10 ± 7.68^{d}	22.27 ± 6.95^{d}	868.32 ± 6.96^{b}	12.69 ± 2.86^{d}	0.10 ± 0.05^{c}
Poultry droppings	19.59 ± 0.71^{c}	33.94 ± 5.03^{cd}	64.64 ± 0.83^{c}	643.04 ± 7.90^{c}	43.47 ± 5.10^{b}	662.68 ± 5.96^{c}	13.77 ± 5.23^{cd}	0.71 ± 0.22^{a}
Sewage sludge	12.73 ± 0.06^{e}	50.24 ± 2.11^{b}	114.52 ± 3.20^{a}	744.03 ± 9.66^{b}	36.32 ± 2.30^{c}	953.11 ± 7.96^{a}	14.77 ± 0.21^{c}	0.06 ± 0.03^{c}
NPK	17.59 ± 0.07^{d}	37.63 ± 5.00^{c}	56.62 ± 3.33^{c}	901.95 ± 6.23^{a}	20.37 ± 5.75^{d}	528.63 ± 3.96^{e}	16.20 ± 0.98^{b}	0.07 ± 0.04^{c}
Taraxacum officinale								
Control	27.98 ± 0.28^{c}	61.37 ± 3.65^{b}	79.92 ± 2.48^{a}	697.80 ± 3.80^{bc}	46.93 ± 1.78^{c}	712.20 ± 10.8^{b}	24.75 ± 0.27^{b}	0.25 ± 0.04^{c}
Biosolids	14.27 ± 0.44^{e}	64.62 ± 2.00^{b}	58.56 ± 2.13^{b}	808.00 ± 10.69^{d}	47.30 ± 2.40^{c}	356.46 ± 2.96^{e}	34.55 ± 6.96^{a}	0.44 ± 0.10^{b}
Cow dung	30.80 ± 0.18^{b}	45.58 ± 1.28^{c}	57.72 ± 1.28^{b}	618.31 ± 1.23^{bc}	50.04 ± 8.80^{bc}	730.16 ± 8.96^{b}	23.72 ± 7.97^{bc}	0.18 ± 0.04^{d}
Poultry droppings	23.27 ± 0.55^{d}	110.00 ± 2.06^{a}	52.01 ± 2.23^{c}	709.44 ± 8.31^{b}	145.47 ± 2.41^{a}	616.68 ± 2.96^{c}	22.88 ± 0.96^{c}	0.55 ± 0.91^{ab}
Sewage sludge	33.97 ± 0.62^{a}	58.34 ± 9.60^{c}	83.46 ± 0.81^{a}	690.18 ± 7.78^{c}	33.31 ± 2.65^{d}	803.50 ± 9.96^{a}	33.59 ± 5.37^{a}	0.62 ± 0.07^{a}
NPK	28.83 ± 0.19^{c}	31.40 ± 1.10^{d}	58.05 ± 1.36^{b}	813.01 ± 3.58^{a}	55.37 ± 8.96^{b}	396.25 ± 8.96^{d}	19.56 ± 2.86^{d}	0.19 ± 0.01^{c}
Withania somnifera								
Control	23.36 ± 0.20^{b}	25.02 ± 3.52^{cd}	55.40 ± 3.63^{b}	263.8 ± 3.20^{c}	23.99 ± 0.81^{b}	471.70 ± 3.90^{a}	8.03 ± 0.27^{bc}	0.16 ± 0.07^{b}
Biosolids	10.29 ± 0.06^{d}	35.39 ± 2.98^{b}	85.46 ± 3.69^{a}	299.07 ± 3.32^{d}	18.11 ± 2.49^{a}	407.17 ± 2.96^{b}	17.97 ± 5.86^{a}	0.26 ± 0.07^{b}

Table 2b continued..

Cow dung	19.88 ± 0.37^{c}	38.21 ± 8.53^{a}	38.37 ± 2.21^{c}	253.52 ± 2.00^{d}	18.03 ± 2.51^{de}	356.82 ± 8.96^{c}	7.05 ± 2.18^{c}	0.37 ± 0.07^{a}
Poultry droppings	24.95 ± 0.39^{a}	22.04 ± 9.22^{d}	52.12 ± 5.53^{b}	249.94 ± 3.32^{d}	28.77 ± 9.30^{a}	398.00 ± 9.96^{b}	7.64 ± 2.76^{bc}	0.39 ± 0.06^{a}
Sewage sludge	6.87 ± 0.07^{e}	17.24 ± 8.78^{e}	41.66 ± 2.18^{c}	162.70 ± 0.69^{e}	15.16 ± 0.75^{c}	253.46 ± 8.96^{e}	3.81 ± 2.88^{d}	ND
NPK	10.09 ± 0.23^{d}	30.55 ± 0.41^{c}	41.67 ± 1.27^{c}	342.99 ± 3.67^{a}	24.34 ± 0.68^{b}	284.84 ± 0.96^{d}	8.14 ± 1.32^{b}	ND
Centella asiatica								
Control	21.36 ± 0.11^{a}	73.05 ± 3.97^{a}	82.11 ± 3.76^{b}	930.50 ± 6.95^{b}	148.85 ± 3.44^{a}	714.00 ± 3.96^{a}	32.06 ± 0.84^{b}	0.39 ± 0.03^{a}
Biosolids	20.25 ± 0.29^{b}	78.22 ± 2.67^{a}	97.25 ± 4.94^{a}	923.91 ± 3.86^{b}	32.42 ± 2.91^{d}	621.45 ± 2.96^{b}	47.38 ± 2.98^{a}	0.29 ± 0.12^{b}
Cow dung	17.56 ± 0.26^{c}	42.92 ± 2.33^{c}	68.33 ± 7.25^{c}	510.26 ± 3.07^{e}	35.44 ± 5.40^{d}	548.08 ± 5.96^{c}	17.97 ± 5.86^{d}	0.26 ± 0.07^{b}
Poultry droppings	14.94 ± 0.25^{d}	34.53 ± 9.17^{d}	48.17 ± 2.64^{d}	613.00 ± 9.00^{c}	63.08 ± 9.64^{b}	492.33 ± 9.96^{d}	18.98 ± 1.29^{d}	0.25 ± 0.07^{b}
Sewage sludge	20.46 ± 0.37^{b}	48.96 ± 1.57^{bc}	69.60 ± 1.36^{c}	559.91 ± 1.91^{d}	44.35 ± 4.21^{c}	697.86 ± 4.96^{a}	14.44 ± 9.11^{e}	0.37 ± 0.03^{a}
NPK	12.67 ± 0.16^{e}	55.41 ± 6.70^{b}	56.11 ± 1.98^{d}	1849.62 ± 20.18^{a}	31.93 ± 6.57^{d}	533.28 ± 6.96^{c}	20.74 ± 5.48^{c}	0.17 ± 0.01^{c}

Note: Different letters in the same column per plant species indicate significant difference at $p < 0.05$; ND: Not detected (Ni < 15 mg/kg; U < 0.10 mg/kg).

Table 2c. Concentrations of trace metals in the stem of the anticancer medicinal plants harvested from organic and inorganic-treated soils.

Catharanthus roseus								
	Pb (mg/kg)	**Ni (mg/kg)**	**Cu (mg/kg)**	**Mn (mg/kg)**	**Cr (mg/kg)**	**Zn (mg/kg)**	**Co (mg/kg)**	**U (mg/kg)**
Control	9.45 ± 0.09^{b}	36.19 ± 1.17^{c}	433.91 ± 5.01^{a}	410.89 ± 2.85^{b}	48.65 ± 0.60^{c}	340.49 ± 8.42^{b}	4.01 ± 0.05^{b}	0.15 ± 0.01^{b}
Biosolids	4.19 ± 0.11^{d}	64.17 ± 2.86^{b}	36.16 ± 1.07^{d}	284.00 ± 0.82^{d}	64.13 ± 0.29^{b}	194.34 ± 3.81^{d}	3.40 ± 0.12^{bc}	ND
Cow dung	3.35 ± 0.18^{e}	21.89 ± 1.53^{d}	25.54 ± 0.25^{e}	244.16 ± 0.87^{e}	21.55 ± 1.87^{d}	125.11 ± 5.19^{e}	2.25 ± 0.11^{de}	0.14 ± 0.01^{b}
Poultry droppings	5.68 ± 0.15^{c}	ND	22.48 ± 3.21^{e}	136.33 ± 1.49^{f}	17.52 ± 0.22^{e}	83.58 ± 3.25^{f}	1.54 ± 0.15^{e}	ND
Sewage sludge	2.01 ± 0.01^{f}	18.51 ± 2.97^{de}	42.68 ± 1.81^{c}	359.63 ± 3.04^{c}	22.98 ± 1.26^{d}	220.78 ± 2.38^{c}	2.78 ± 0.06^{cd}	ND
NPK	31.96 ± 0.74^{a}	145.58 ± 2.46^{a}	206.72 ± 5.82^{b}	2447.82 ± 10.35^{a}	206.42 ± 4.33^{a}	700.41 ± 15.58^{a}	91.15 ± 1.15^{a}	1.48 ± 0.06^{a}
Artemisia afra								
Control	9.72 ± 0.16^{d}	ND	51.17 ± 1.48^{b}	154.45 ± 1.15^{c}	18.24 ± 0.75^{d}	384.58 ± 2.61^{b}	3.44 ± 0.18^{c}	0.18 ± 0.02^{a}

Table 2c continued..

Biosolids	1.83 ± 0.12^{f}	23.76 ± 1.69^{a}	10.16 ± 1.22^{d}	48.64 ± 0.63^{e}	7.64 ± 0.50^{f}	61.15 ± 1.32^{e}	0.82 ± 0.14^{d}	ND
Cow dung	11.27 ± 0.26^{b}	37.37 ± 3.26^{a}	12.10 ± 0.45^{d}	107.21 ± 0.30^{d}	11.77 ± 1.70^{e}	146.81 ± 8.99^{d}	4.86 ± 0.09^{b}	ND
Poultry droppings	3.70 ± 0.10^{e}	23.33 ± 1.72^{a}	29.50 ± 2.45^{c}	198.07 ± 0.87^{b}	24.84 ± 0.72^{c}	180.46 ± 2.04^{c}	3.31 ± 0.21^{c}	ND
Sewage sludge	23.60 ± 0.24^{a}	ND	56.81 ± 2.42^{a}	192.95 ± 2.99^{b}	29.89 ± 0.56^{b}	509.31 ± 10.08^{a}	3.75 ± 0.23^{c}	ND
NPK	10.38 ± 0.23^{c}	17.79 ± 2.67^{a}	34.21 ± 1.58^{c}	444.54 ± 6.94^{a}	38.87 ± 0.52^{a}	395.56 ± 3.39^{b}	6.50 ± 0.10^{a}	0.19 ± 0.02^{a}
Withania somnifera								
Control	3.35 ± 0.18^{d}	21.89 ± 1.53^{c}	25.54 ± 0.25^{bc}	244.16 ± 0.87^{a}	21.55 ± 1.87^{c}	125.11 ± 5.19^{e}	2.25 ± 0.11^{d}	0.14 ± 0.01^{a}
Biosolids	9.71 ± 0.03^{c}	24.90 ± 1.66^{bc}	31.42 ± 0.64^{a}	98.79 ± 0.60^{d}	24.87 ± 0.40^{e}	183.54 ± 5.34^{c}	4.29 ± 0.37^{c}	ND
Cow dung	1.83 ± 0.12^{e}	23.76 ± 1.69^{bc}	11.16 ± 1.22^{d}	47.64 ± 0.63^{f}	8.64 ± 0.50^{cd}	60.15 ± 1.32^{f}	0.92 ± 0.14^{e}	ND
Poultry droppings	13.60 ± 0.29^{b}	36.86 ± 1.43^{b}	20.65 ± 1.57^{c}	71.07 ± 0.93^{e}	34.78 ± 0.76^{a}	148.12 ± 2.79^{d}	7.06 ± 0.06^{a}	ND
Sewage sludge	13.87 ± 0.20^{b}	312.74 ± 11.52^{a}	29.08 ± 3.85^{ab}	112.63 ± 2.15^{c}	18.58 ± 0.82^{d}	238.55 ± 3.53^{b}	6.12 ± 0.11^{b}	ND
NPK	22.02 ± 0.11^{a}	ND	31.73 ± 1.11^{a}	186.22 ± 0.43^{b}	22.62 ± 1.30^{bc}	441.12 ± 2.86^{a}	4.09 ± 0.08^{c}	0.13 ± 0.01^{a}

Note: Different letters in the same column per plant species column indicate significant difference at $p < 0.05$; NS refers to No definite stem; ND: Not detected (Ni < 15 mg/kg; U < 0.10 mg/kg).

The concentration of Cu recorded in this study ranged from 11.16 ± 1.22 mg/kg to 433.91 ± 5.01 mg/kg. The lowest Cu concentration was recorded in the stem of *A. afra* harvested from biosolid-treated soil while the highest concentration of Cu was recorded in the stem of *C. roseus* harvested from the untreated soil (Table 2c).

Furthermore, the concentration of Co ranged from 0.82 ± 0.14mg/kg to 123.76 ± 0.81 mg/kg. The lowest concentration of Co was recorded in the stem of *W. somnifera* harvested from cow dung-treated soil while the highest was found in the root of *C. asiatica* harvested from the same cow dung-treated soil (Table 2a & 2c).

The concentration of Pb in all the plant parts ranged from 2.01 ± 0.01 mg/kg to 66.00 ± 0.30 mg/kg. The highest concentration of Pb was recorded from the root of *T. officinale* harvested from NPK-treated soil (Table 2a), while the lowest concentration was recorded in the stem of *C. roseus* harvested from sewage sludge-treated soil (Table 2c). The concentration of Pb varied significantly ($p < 0.05$) in the leaves of all the plants except in the biosolids and the control across the five anticancer medicinal plants (Table 2b). The highest concentration of Pb in the leaves across the five anticancer medicinal plants was recorded in *A. afra* harvested from cow dung-treated soil (Table 2b).

Lastly, the concentration of U in this study ranged from 0.06 ± 0.03 mg/kg to 6.49 ± 0.07 mg/kg. The least mean concentration of toxic trace metals was recorded for U in the leaves of *A. afra* harvested from sewage sludge-treated soil (Table 2b).

Generally, the order of trace metal accumulation for all the plants across the treatments is roots > leaves > stem. The order of concentration of trace metals in the roots of *C. roseus* harvested from NPK and *A. afra* harvested from the control was similar i.e. Mn > Zn > Cu > Cr > Ni > Co > Pb > U (Table 2a). The order of trace metal concentrations in the root of *W. somnifera* for the plants treated with NPK and the control was Mn > Zn > Cr > Cu > Ni > Co > Pb > U (Table 2a). U concentration was below the detectable limit (U < 0.10 mg/kg) in the stem of *C. roseus*, *A. afra* and *W. somnifera* harvested from biosolids, poultry droppings and sewage sludge-treated soils (Table 2c). Generally, the concentrations of all the trace metals in the roots, stems and leaves of the five anticancer medicinal plants used in this study were all above the recommended limit set by the WHO.

Bio-accumulation Factor (BAF) of the anticancer plants against the different treatment

Bioaccumulation factor (BAF) ranged from 0.05 to 7.12 (Table 3). The Bioaccumulation factor (BAF) of Co, Cu, Mn, U and Ni was below 1 in all the plants across all the treatments except in *A. afra* harvested from poultry dropping-treated soil (Table 3). The BAF of Zn, Pb and Cr was above 1 in all the plants across the treatments (Table 3). The BAF of Pb in the leaves of *A. afra* harvested from poultry droppings treatment was highest (7.12) among all the plants, while the least BAF was recorded for Co in *C. roseus* harvested from soil treated with biosolid, poultry droppings and sewage sludge (Table 3). The BAF of Ni across the five anticancer medicinal plants and all the treatments ranged from 0.11 – 0.85 (Table 3).

Translocation Factor (TF) of the trace metals from the roots to the leaves

The translocation factor (TF) ranged from 0.08 to 10.23 (Table 4). Chromium from *W. somnifera* harvested from sewage sludge-treated soil recorded the lowest TF of 0.05, while the highest TF (10.23) was recorded in Pb of *A. afra* harvested from cow dung-treated soil (Table 4).

Correlation analysis of the trace metal concentrations in the plants

The correlation matrix of the 2-Factor Principal Component Analysis for the trace metals in the leaves are presented in (Table 5). All the trace metals showed a positive correlation except Co against Pb, Cu against Mn, Mn against Pb and U, and lastly Pb against Ni (Table 5).

DISCUSSION

The soil pH in water and $CaCl_2$ were slightly acidic while the soil organic matter (OM) was high relative to other studies. For example, the study of Lo *et al*. [30] on the effect of organic matter on the specific adsorption of heavy metals reported values of 1.47% and 1.13% OM as high; hence, our inference in the present study that our OM values are higher relatively. Metal uptake from the

Table 3. Bio-accumulation Factor (BAF Leaves) of the five anticancer medicinal plants in the different organic and inorganic fertilizers.

	Pb	Ni	Cu	Mn	Cr	Zn	Co	U
Catharanthus roseus								
C. roseus CTR	**2.60**	0.19	0.74	0.14	0.91	**2.50**	0.06	0.23
C. roseus BIS	0.54	0.16	0.24	0.19	0.57	**1.09**	0.05	0.38
C. roseus CWD	**1.31**	0.09	0.13	0.09	0.42	**1.50**	0.04	ND
C. roseus PLD	0.66	0.30	0.30	0.23	0.52	**1.38**	0.05	ND
C. roseus SWS	**1.54**	0.24	0.48	0.18	0.21	**1.52**	0.05	ND
C. roseus NPK	**1.41**	0.26	0.26	0.22	0.72	**2.20**	0.06	ND
Artemisia afra								
A. afra CTR	**2.30**	0.40	0.56	0.36	**4.61**	**4.14**	0.22	0.84
A. afra BIS	0.84	0.26	0.44	0.32	**1.39**	**3.04**	0.06	ND
A. afra CWD	**4.63**	0.32	0.41	0.37	0.74	**4.51**	0.15	0.14
A. afra PLD	**7.12**	0.45	0.95	0.65	**6.71**	**4.36**	0.18	**1.29**
A. afra SWS	0.65	0.36	0.75	0.37	0.91	**3.19**	0.14	0.04
A. afra NPK	0.95	0.20	0.28	0.35	0.42	**1.95**	0.11	0.04
Taraxacum officinale								
T. officinale CTR	**1.78**	0.34	0.49	0.27	**1.57**	**2.94**	0.19	0.39
T. officinale BIS	**1.47**	0.60	0.54	0.57	**6.15**	**1.86**	0.38	1.07
T. officinale CWD	**1.13**	0.13	0.16	0.13	**1.31**	**1.57**	0.09	0.20
T. officinale PLD	**1.99**	0.85	0.37	0.37	**2.89**	**2.71**	0.23	0.17
T. officinale SWS	**2.44**	0.77	0.75	0.54	**1.08**	**2.86**	0.49	0.98
T. officinale NPK	**1.87**	0.17	0.30	0.34	**4.70**	**1.83**	0.13	0.25
Withania somnifera								
W. somnifera CTR	**1.17**	0.11	0.26	0.09	0.57	**1.12**	0.04	0.21
W. somnifera BIS	0.92	0.19	0.66	0.19	**1.81**	**1.78**	0.19	0.67
W. somnifera CWD	**1.65**	0.28	0.24	0.18	0.89	**1.60**	0.06	0.95
W. somnifera PLD	**1.22**	0.18	0.35	0.15	**1.54**	**1.77**	0.07	0.93
W. somnifera SWS	0.52	0.17	0.31	0.10	0.27	**1.14**	0.04	ND
W. somnifera NPK	0.51	0.14	0.18	0.12	0.55	0.92	0.04	ND
Centella asiatica								
C. asiatica CTR	**1.44**	0.61	0.63	0.48	**4.46**	**3.37**	0.32	0.93
C. asiatica BIS	**1.78**	0.41	0.51	0.51	**3.74**	**3.10**	0.35	0.78
C. asiatica CWD	**1.84**	0.34	0.50	0.31	**3.97**	**3.23**	0.19	0.79
C. asiatica PLD	**1.28**	0.26	0.34	0.32	**1.25**	**2.17**	0.19	0.51
C. asiatica SWS	**1.43**	0.31	0.35	0.31	**2.54**	**1.89**	0.11	0.45
C. asiatica NPK	0.88	0.32	0.33	0.78	**1.15**	**2.22**	0.15	0.14

CTR: Control; BIS: Biosolids; CWD: Cow dung; PLD: Poultry droppings; SWS: Sewage sludge; ND: Not determined.

Table 4. Translocation Factor (TF) of the trace metals from the roots to the leaves of five anticancer medicinal plants.

Catharanthus roseus								
	Pb	**Ni**	**Cu**	**Mn**	**Cr**	**Zn**	**Co**	**U**
C. roseus CTR	0.81	0.16	0.94	**1.06**	0.44	0.93	0.83	0.44
C. roseus BIS	0.38	0.26	0.42	0.40	0.24	0.81	0.15	0.41
C. roseus CWD	**3.76**	0.42	0.57	0.76	0.47	**2.27**	0.51	ND
C. roseus PLD	0.67	0.45	0.47	0.41	0.20	**1.13**	0.11	ND
C. roseus SWS	**1.25**	0.10	0.05	0.32	0.04	0.75	0.12	ND
C. roseus NPK	**1.53**	0.64	0.42	0.47	0.32	**1.22**	0.19	ND
Artemisia afra								
A. afra CTR	**1.99**	**1.25**	**1.19**	**1.35**	**1.89**	**1.75**	**1.16**	0.60
A. afra BIS	**1.68**	0.30	**1.16**	0.51	0.17	**1.16**	0.15	ND
A. afra CWD	**10.23**	0.86	0.9	**1.04**	0.77	**4.59**	0.45	0.26
A. afra PLD	**1.45**	0.22	0.42	0.40	0.42	**2.02**	0.14	**1.00**
A. afra SWS	**1.44**	0.10	**1.23**	**1.08**	0.98	**2.91**	0.47	0.13
A. afra NPK	**1.61**	**1.98**	**1.27**	**2.56**	0.74	**1.67**	**1.52**	0.19
Taraxacum officinale								
T. officinale CTR	0.75	0.33	**1.01**	0.62	0.15	**1.35**	0.62	0.34
T. officinale BIS	**1.33**	0.42	0.90	0.96	0.34	**1.30**	0.93	**1.63**
T. officinale CWD	**2.00**	0.30	0.34	0.36	0.44	**1.59**	0.28	0.24
T. officinale PLD	**1.70**	0.34	0.72	0.72	0.27	**2.29**	0.61	0.92
T. officinale SWS	0.70	0.37	0.35	0.48	0.17	0.74	0.60	0.41
T. officinale NPK	0.44	0.17	0.30	0.36	0.21	0.43	0.22	0.12
Withania somnifera								
W. somnifera CTR	**1.26**	0.61	0.54	0.32	0.11	0.76	0.38	0.24
W. somnifera BIS	0.48	0.19	0.53	0.14	0.15	0.77	0.20	0.33
W. somnifera CWD	0.92	0.39	0.29	0.16	0.08	0.75	0.14	0.35
W. somnifera PLD	**2.11**	0.29	0.70	0.25	0.20	**1.53**	0.20	0.85
W. somnifera SWS	0.29	0.15	0.34	0.13	0.08	0.47	0.10	ND
W. somnifera NPK	0.45	0.32	0.31	0.23	0.14	0.49	018	ND
Centella asiatica								
C. asiatica CTR	0.74	**1.57**	**1.03**	**1.20**	0.31	0.92	**1.69**	0.35
C. asitica BIS	**1.19**	0.40	0.60	0.40	0.16	**1.28**	0.46	0.41
C. asiatica CWD	0.67	0.21	0.29	0.19	0.32	0.99	0.15	0.21
C. asiatica PLD	**1.78**	0.52	0.94	0.70	0.65	**2.63**	0.67	0.81
C. asiatica SWS	0.78	0.51	0.53	0.34	0.36	**1.16**	0.28	0.42
C. asiatica NPK	0.43	2.72	**1.49**	**5.42**	**1.00**	0.96	**3.81**	**1.00**

CTR: Control; BIS: Biosolids; CWD: Cow dung; PLD: Poultry droppings; SWS: Sewage sludge; ND: Not determined.

Table 5. Correlation matrix of trace metals.

	Cobalt	**Copper**	**Zinc**	**Manganese**	**Lead**	**Uranium**	**Nickel**	**Chromium**
Cobalt	1.00	0.17	0.21	**0.45**	-0.00	0.05	**0.62**	0.36
Copper	0.17	1.00	**0.53**	-0.02	0.29	**0.35**	0.14	0.00
Zinc	0.21	**0.53**	1.00	0.10	**0.50**	0.32	0.25	0.25
Manganese	**0.45**	-0.02	0.10	1.00	-0.27	-0.02	**0.36**	0.20
Lead	-0.00	0.29	**0.50**	-0.27	1.00	0.33	-0.06	0.05
Uranium	0.050	**0.35**	0.32	-0.02	0.33	1.00	0.03	0.27
Nickel	**0.62**	0.14	0.25	0.36	-0.06	0.03	1.00	**0.67**
Chromium	**0.36**	0.00	0.25	0.20	0.05	0.27	**0.67**	1.00

The figures in bold represent a strong positive correlation between trace metals.

soil is a function of factors such as soil pH, organic matter contents, clay content, soil redox potentials, and the concentrations of trace metals in the soil. The uptake and accumulation of trace metals are also guided by other conditions such as metal species in solution and adsorption balance between the solid-liquid phase beneath the topmost layer of the soil [31-34].

In the present study, the pH of the soil and the level of organic matter content might not favour the mobility of these trace metals in the soil; however, the concentrations of trace metals reported from the study were all very high and this might have accounted for their mobility *via* the root uptake as noted in the study. The study of Cataldo and Wildung, [35] and Navarro-Pedreño *et al*. [36] reported that the concentrations of trace metals in the soil, organic matter is a factor to be considered for uptake aside from the soil pH. High levels of metals in soils raise their (metals) absorption and uptake by plants. The uptake of metals from soils are influenced by factors such as soil pH, clay contents, and organic matters and these factors (soil pH, clay contents and organic matters) are often modified due to fertilization [34].

Generally, the concentrations of trace metals in the soil were high as noticed from the soil used as the control. Previous reports by Olowoyo *et al.* [23] showed that soils from the area in Pretoria have elevated concentrations of toxic trace metals with specific reference to Cr from the study area. The area where the soil samples were collected is close to a mining and quarry site, which might have affected the level of the trace metals in this study due to the anthropogenic input. High concentrations noted for Mn, Zn, Cr, Ni, and U among others trace metal concentrations as recorded in this study may be associated with their abundance in nature and anthropogenic sources [37-39].

From the organic materials used in the study, cow dung seems to have introduced the highest amount of toxic trace metals in the soil. Studies have shown that it is possible to have an elevated amount of toxic metals in the faeces of animals and this might have accounted for the increase as observed in this present study. Cow dung is an undigested residue of consumed food material excreted by herbivorous bovine animal species and it contains different minerals, and a substantial amount of trace metals like Mg, Cu, Co, Mn and Pb [40]. It has also been noted that organic fertilizers may be a rich source of heavy metals in soils due to ingested plants from the herbivores [33, 41].

The concentrations of all the trace metals reported in this study were all above the permissible limit set by the WHO. For instance, the permissible limit for Mn is set at 200 mg/kg and most of the plants in this study showed values higher than this WHO limit [42]. Even though manganese is a significant contributor to various biological systems including photosynthesis, respiration, and nitrogen assimilation in plants and humans, lack of Mn may lead to skeletal and reproductive abnormalities and its excess could result in lung and brain damage [43].

Zn on the other hand is a micronutrient in plants and it is essential for proper plant growth while its toxicity may result in leaves turning purplish-red [44]. In humans, Zn is the foundational component of a host of different enzymes in the human body and it functions in several regulatory and catalytic roles [45]. Zinc plays a definitive role in DNA synthesis, bone formation, wound healing and human brain development [46]. Although Zn toxicity is rare in humans, its excess ingestion may interfere with the uptake of Cu; hence, its toxic effects include stomach pain and diarrhoea [47]. The concentration of Zn recorded in this study was above the safe human consumption limit of medicinal plants as approved by WHO. The WHO approved limit for Zn in medicinal plants for human consumption is 50 mg/kg [42].

South Africa has the largest Cr reserve in the world [48]. High concentrations of Cr in South African soils may be responsible for the high concentration recorded in this present study. Olowoyo *et al*. (2011) have also reported a higher concentration of Cr in their study of uptake and translocation of heavy metals by medicinal plants (*Datura stramonium* and *Amaranths spinosus*) growing around a waste dumpsite in Pretoria. Cr is a plant stimulator helping to promote growth and increased biomass at moderate concentration; however, excess Cr in soil reduces the seed germination rate [49]. Cr is a mutagenic element and can cause respiratory problems, compromised immune system, liver and genetic material damage in humans at high concentration [38, 48].

Ni toxicity in plants may result in alterations of physiological functions and can lead to chlorosis and necrosis in plants [50]. Ni concentration recorded in this study was above the WHO permissible limit in medicinal plants which is 1.5 mg/kg [42]. The finding of the present study corroborates the report of Nogueira *et al*. [51], who reported Ni as one of the most abundant elements in the earth crust and in organic fertilizers. Diseases associated with excessive ingestion of Ni in humans include allergic dermatitis, disorders of the nasal cavities and lung-related issues [38].

Copper performs a crucial role in CO_2 assimilation and ATP in plants; and plants excessive exposure to Cu may cause disturbance in metabolic pathways and macromolecules' destruction [39, 52]. The amount recorded for Cu in this study exceeds the WHO permissible limit in medicinal plants. The WHO approved limit for Cu in medicinal plants is 3 mg/kg [42]. Erdogan *et al*. [53] reported that soils in South Africa are highly contaminated with toxic elements such as Cu, Pb, Mn, Ni and Cr. In humans, excessive ingestion of Cu may result in hepatic necrosis, salivary gland swelling, haemolysis and nephrotoxic effects, although, it is an essential element in humans at a moderate level [23, 54]. Copper in association with Fe enables the body to form red blood cells as well as helps to maintain healthy bones, blood vessels, nerves, and immune function [54].

Cobalt is an important element for stem growth, leaf disc expansion and plays a critical role in plant attainment of maturity and healthy bud development [55]. The concentration of Co recorded in this study is above the 0.48 mg/kg permissible limit set by WHO [42]. Ingestion of Co at an excessive amount has been implicated for human toxicity which may cause nausea and vomiting, and excessive intake over a long period may result in cardiomyopathy and deafness [56]. Furthermore, Co with other elements in humans causes hypertrophy of interstitial Leydig cells, degeneration of spermatogonial cells and necrosis of both the seminiferous tubules and interstitial tubules [57].

The Pb concentration recorded in this study was above the permissible limit (10 mg/kg) set by the WHO [42]. Lead has no known positive contribution to plants and humans. Pb toxicity causes inhibition of ATP production, lipid peroxidation, and DNA damage in plants [58]. Also, Pb inhibits seed germination, root elongation, plant development, chlorophyll production, and protein content [58]. Ingestion of Pb may result in symptoms such as decreased fertility or increased chances of miscarriage or birth defects, kidney damage and death [38, 59]. Lastly, uranium is among the naturally occurring elements on earth and it is among the most abundant trace metal in nature [60]. Excessive intake of U in humans results in renal abnormalities, although, it is excretable from the human system through urine and faeces but it is a carcinogen especially when it enters into the blood [61].

Although the medicinal plants used in this study have been established in the literature as anticancer plants, the result of our current study showed that they are also hyper-accumulators for Zn, Pb and Cr. This is evident from the report obtained for BAF and TF values that were all > 1. Higher BAFs and

TFs (i.e. BAF > 1; TF > 1) implies a higher degree of accumulation of these trace metals, owing to their large concentration in the soil. According to Maharia *et al*. [62], higher BAFs and TFs such as reported in this study will facilitate the uptake of trace metals by plants.

It must also be reported that other studies have pointed out that the introduction of organic fertilizers such as those used in this study may inhibit the mobility of these trace metals in the soil, improve the soil pH and the soil organic matter; however, this was not the case in our study since the levels of the toxic trace metals were also high in the organic material used [63-65].

The correlation value of 0.67, 0.62, 0.53, 0.50 and 0.45 between Cr and Ni, Co and Ni, Cu and Zn, Zn and Pb, Mn and Cu, respectively suggests that the trace metals were from a common source. This high correlation matrix was observed between Cr and Ni, Co and Ni, Cu and Zn, Zn and Pb and Mn and Cu, which only indicate that the trace metals could be linked to a common source. This observation is similar to those reported by Olowoyo *et al*. [39] where a positive and strong correlation was recorded among the trace elements. The poor correlation values recorded for trace metals like Co and Pb, Mn and Pb, Mn and U, and Ni and Pb may be an indication that the trace metals are from other sources.

CONCLUSION

It could be deduced from the results obtained in this study that the medicinal plants cultivated on both organic and inorganic fertilizers are not safe for human consumption because they all recorded values above the permissible limit approved by WHO. The study showed that all the medicinal plants used in the treatment of cancer in this study can bio-accumulate trace metals in their tissue. It is therefore important to check the concentration of trace metals in soil or any material that will be used as soil amendment/fertilizer since the medicinal plants used in this study can bio-accumulate toxic trace metals from the soil. This study further revealed that though organic material may be added to soil to improve soil fertility thereby increasing plant growth and yield, it may be necessary to consider the source of the organic material because their presence may introduce toxic trace metals in the soil and may be available for plant uptake thereby rendering the plant unsafe for human consumption as noticed in our study. The uptake and translocation of these trace metals by the plants make public enlightenment crucial to provide proper information on the dangers associated with the use of organic materials when collected from a polluted environment. This study provides critical information regarding the potential/possibility of the anticancer medicinal plants as phytoremediation plants for elements such as Zn, Pb and Cr because of their abilities to bio-accumulate and store trace metals in their tissues (leaves and stems).

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

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