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Levels of Heavy Metals in Common Medicinal Plants Collected from Environmentally Different Sites

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Abstract: The use of herbal medicine is increasing dramatically worldwide. However, environmental pollution especially with heavy metals poses serious problem on quality of medicinal plants and their products. In Ethiopia, where more than 85% of the population relies on traditional medicine, data on the level of Ni, Co, Cu, Cr and As in plants used for production of herbal medicines is unavailable. Therefore, the purpose of this study was to assess the Ni, Co, Cu, Cr and As contents of eight medicinal plants (*Allium sativum, Dodonea angustifolia, Hagenia abyssinica, Ocimum lamifolium, Ocimum utricifolium, Ruta chalapensis, Thymus serrulatus* and *Zingiber officinale*) grown in different parts of Ethiopia. Out of 26 samples analyzed, three (11.5%) for Ni (10.42 ± 0.21 to 11.25 ± 0.01 mg/kg) and nine (~34.6%) for Cr (2.77±0.06 to 13.24±0.21 mg/kg) contained concentrations above the maximum limits, 10 and 2 mg/kg respectively. None of the studied samples were found to contain cobalt, copper and Arsenic concentrations in above WHO limits for safe human consumption (25, 40 and 5 mg/kg respectively).

Key words: Medicinal plants · Plants' metal contamination · Herbal medicine · Shirka · Heavy metals contamination · Traditional medicine

INTRODUCTION

Herbal medicines have been used for treatment of diseases for many thousands of years and are recognized as valuable, readily available resources for health care. Many reports show that the use of these products is increasing in both developing and developed countries in the last few decades due to their reasonable price and above all due to the assumption that natural products are safe [1, 2]. In some parts of the world, traditional medicine is the main option for primary health care as in Ethiopia where more than 85% of the population relies on such products [3]. To have the desired therapeutic outcomes, the quality of finished products and plant raw materials must be ensured. Reports from many countries have shown that one of the major quality problems frequently encountered is high heavy metals content of herbal medicines that can be associated to extensive pollution of the environment where medicinal plants used as raw materials grow [4-7]. Evaluating and monitoring heavy metal contamination is an essential step in improving the overall safety and quality of widely used medicinal plants which will in turn result in safeguarding the consumer [8].

Pollution of the atmosphere and soil, followed by plants and animals with Ni originate from sources such as stainless steel, glass and ceramic production industries, catalytic converters, cigarette smoke and medical prostheses and utensils. Polluted irrigation water, automobile and industrial exhausts, pesticides and fertilizers play important roles in contamination of medicinal plants with Co and Cu [9]. Metals under study are included in the thirteen elements of highest concern by the European Community. Although, some of them are micronutrients and required for the normal function of the body, continued ingestion together with medicines and food can cause accumulation in organisms producing serious health hazards especially on vital organs [10]. The common health problems caused by high level of Ni in the body are cancer, asthma, cardiovascular disorders

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and allergic reactions; whereas high level of Co causes asthma, anexiety, cardiovascular problems, abnormal thyroid function, polycythemia and over production of red blood cells. On the other hand, common toxicities of Cu include anorexia, fatigue, premenstrual syndrome, depression, anxiety, migraine headaches, allergies, childhood hyperactivity and learning disorders [9, 11].

Cr contamination is caused by metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production and pulp and paper production. The health hazards associated with exposure to Cr are dependent on its oxidation state. Common health problems are skin rashes, stomach upset and ulcer, respiratory problems, weakened immune systems, kidney and liver damage, alteration of genetic material, cancer and death [1, 12, 13].

Arsenic contamination is caused from natural geological sources leaching into aquifers, contaminating water and may also occur from mining, pesticide manufacturing and other industrial processes. It is present as a contaminant in many traditional remedies. Arsenic toxicity is a global health problem affecting many millions of people. Arsenic exerts its toxicity by inactivating up to 200 enzymes, especially those involved in cellular energy pathways and DNA synthesis and repair. Acute poisoning is associated initially with nausea, vomiting, abdominal pain, severe diarrhea, encephalopathy and peripheral neuropathy. Chronic toxicity results in multisystem disease including carcinogen affecting numerous organs [14].

Due to these health problems, WHO is emphasizing emphatically that herbal drugs should not be used without qualitative and quantitative analysis of their heavy metals content [15].

In addition, these and other heavy metals are claimed to affect the secondary metabolites production in plants which may be important for the medication [16].

Therefore, it is mandatory to assess Ni, Co, Cu, Cr and As concentrations in medicinal plants before using them for herbal drugs preparation. Though similar studies have been conducted in different parts of the world [8, 17, 18], there is no report on Ethiopian flora except our previous report on Pb and Cd level [3]. With this notion the work was done to measure the three metals in plants largely used for preparation of herbal medicines and food items in different parts of Ethiopia with the objective to compare the metals' accumulation in different plant species and its variation in the same species collected from urban and rural places.

MATERIALS AND METHODS

All chemicals used were of analytical grade. Heavy metal standard stock solutions (Atomic absorption spectrophotometeric grade) of cobalt nitrate, copper nitrate, nickel nitrate, chromium oxide and tri-arsenic oxide corresponding to 1000 mg/L of each metal and distilled-deionized water were from BDH, England. Stock solutions of the metals were serially diluted and used to construct calibration curves. Distilled-deionized water was used for dilution and other analytical works. Glass-wares used were washed with 2% Extran solution, soaked in 3N HCl for 24 hrs and rinsed with distilled-deionized water before use. Perkin-Elmer A-Analyst 600 Flame Atomic Absorption Spectrophotometer (FAAS) with appropriate hollow cathode lamps was used for analysis of samples.

A total of 26 samples belonging to 8 different plant species were collected in their fresh form from four different places in Ethiopia (Addis Ababa, Bonga, Kombolcha and Shirka). The places were chosen on the basis of the possible presence and absence of extensive environmental pollution. Shirka and Bonga were selected to represent exhaust free (rural and green) areas while the other two were suspected to contain high level of environmental pollution due to high traffic and presence of industries. The studied plants were selected based on their frequent use by the society in the preparation of herbal drugs and as food additives. The most commonly used part of each plant was considered as experimental target.

Freshly collected plant samples were stored separately in polythene bags and transported to the laboratory for processing. The plants investigated, sites of collection and plant parts analyzed are given in Table 1.

The plant samples were washed using distilled water and dried under shade.

The protocol used to determine levels of Ni, Co, Cu and Cr in the plant samples was the one described by Rai *et al*., (2001, 2005) with slight modification. In summary: washed, dried and finely powdered samples (2.00 g each) were digested in Nessler tube containing 15 ml of a mixture of nitric acid and perchloric acid (3:1) for 2 hours on a water bath at 80°C until formation of brownish gas ceased and volume finally adjusted to 25 mL using distilled-deionized water. Solutions prepared were then diluted with distilled-deionized water until concentrations fall with in the calibration range and stored in labeled acid-washed glass vials. Ni, Co, Cu and

Table 1: Plants investigated, places of collection and parts analyzed

Plant Name	Area of collection: Addis Ababa	Bonga	Kombolcha	Shirka	Part Analyzed
Allium sativum	✓	✓	✓	✓	Bulb
Dodonea angustifolia	✓	✓	✓	✓	Leaves
Hagenia abyssinica	✓	†	†	✓	Flowers
Ocimum lamifolium	✓	✓	†	✓	Leaves
Ocimum utricifolium	†	✓	✓	†	Leaves
Ruta chalepensis	✓	✓	✓	✓	Leaves
Thymus serrulatus	✓	✓	✓	✓	Leaves
Zingiber officinale	✓	✓	✓	†	Rhizome

† place where the plant is unavailable

Cr analysis was carried out immediately on the resultant digests using FAAS, with the use of prepared standards (run before each batch) to determine sample concentrations.

Arsenic determination was performed by hydride generation technique reported by Delgado-Andrade *et al*. [19] which is summarized as follows. Dried and finely powdered samples (300 mg each) was placed in a 100 ml volumetric flask and digested by addition of 5.0 ml concentrated HNO_3 and heating at 90°C for 45 min in a beaker. Another 5.0 ml of a 4: 1 mixture of HNO_3 and $HClO_4$ were added and heating continued at 130°C for an additional 2 h until the sample was completely digested. The digest was then cooled and the resulting solution diluted to 25 ml with double-distilled water. A second dilution was made by taking different aliquots of the first solution and diluting with 1.5% HCl depending on the original concentration of arsenic in the sample. Total arsenic content was determined in triplicates using the HG-AAS technique (Perkin-Elmer A-Analyst 600 atomic absorption spectrometer equipped with a MHS-10 hydride generation system). Hydride generation was performed using a 3% (w/v) $NaBH_4$ in 1% NaOH solution.

Analysis was carried out in triplicates to ensure precision of results. Metal concentrations were calculated from each replicate absorbance value, which was then used to calculate the average sample metal concentration. All metal concentrations were expressed in mg/kg on a dry-weight basis of the plant sample. All analyses were run in batches, which included known standards, method blanks and plant samples. The absorbance of a blank sample was determined for background correction. Accepted recoveries ranged from 85% to 105% and batches with recoveries less than 85% were reanalyzed.

Precision and accuracy of analysis were ensured through repeated analysis of samples.

RESULTS AND DISCUSSION

Concentrations of Ni, Co, Cu, Cr and As in the plant samples investigated are presented in Table 2. All the 26 analyzed samples were found to contain detectable levels of Ni, Co, Cu and Cr but As was not detected in *A. sativum* and *R. chalapensis* both from Shirka and *T. serrulatus* collected from Bonga.

Evaluation of the results obtained was done according to three measuring parameters, namely type of studied metals, plant species and site of collection.

Concentrations of Ni in 3 of the samples (11.5%) were observed to exceed the limit of 10 mg/kg specified by WHO (2005) showing obvious signs of environmental contamination.

According to the result, plant samples highly contaminated with nickel were *O. lamifolium* collected from Addis Ababa (11.25 ± 0.01 mg/kg), Bonga (10.83 ± 0.12 mg/kg) and Shirka (10.42 ± 0.21 mg/kg). Moreover, analysis done for the levels of Ni revealed that all *O. lamifolium* samples contained the metal above the maximum safe level set. The maximum being 11.25±0.01 mg/kg for the *O. lamifolium* sample and the minimum (1.25±0.03 mg/kg) was observed in *A. sativum* collected from Addis Ababa. The correlation analysis on these and other results in Table 2 clearly illustrated selective accumulation of Ni in samples of the same species collected from different localities. This shows that the genetic factor seem to exhibit more influence on the specific accumulation of Ni than environmental factors. Both the lowest (1.25±0.03 mg/kg in *A. sativum*) and highest (11.25±0.01 mg/kg in *O. lamifolium*) Ni concentrations were found from samples collected from Addis Ababa.

The result also showed that nine samples (34.6%) contained Cr above the maximum safe limit specified

Table 2: Summary of results of Co, Ni, Cu, Cr and As concentrations in mg/kg ± SD (n=3)

Plant	Collection site	Co	Ni	Cu	Cr	As
Allium sativum	Addis Ababa	0.17±0.05	1.25±0.03	5.29±0.04	3.74±0.07	0.41±0.03
	Bonga	0.63±0.00	2.50±0.01	5.58±0.07	0.22±0.03	0.07±0.01
	Kombolcha	0.75±0.03	2.92±0.23	4.54±0.11	1.97±0.04	0.13±0.03
	Shirka	0.71±0.01	3.75±0.10	5.08±0.14	0.31±0.03	Nd[a]
Dodonea angustifolia	Addis Ababa	2.17±0.14	5.00±0.01	8.50±0.21	8.14±0.14	2.63±0.03
	Bonga	1.54±0.02	8.75±0.01	6.29±0.14	1.73±0.07	1.07±0.03
	Kombolcha	1.71±0.04	2.50±0.00	6.67±0.07	7.08±0.11	1.33±0.06
	Shirka	2.13±0.01	3.33±0.11	5.58±0.14	0.77±0.05	1.24±0.10
Hagenia abyssinica	Addis Ababa	0.95±0.07	3.75±0.01	9.63±0.10	13.24±0.21	3.32±0.12
	Shirka	1.08±0.03	3.33±0.11	12.36±0.13	2.77±0.06	2.74±0.04
Ocimum lamifolium	Addis Ababa	3.04±0.07	11.25±0.01	11.50±0.15	1.43±0.01	0.11±0.01
	Bonga	2.75±0.00	10.83±0.12	13.04±0.10	0.22±0.06	0.09±0.01
	Shirka	2.71±0.02	10.42±0.21	11.25±0.11	0.11±0.03	0.08±0.04
Ocimum utricifolium	Bonga	2.67±0.07	6.25±0.02	20.46±0.21	0.14±0.01	0.09±0.02
	Kombolcha	3.04±0.14	7.50±0.01	15.33±0.11	0.93±0.06	0.07±0.00
Ruta chalapensis	Addis Ababa	2.13±0.09	5.00±0.00	9.54±0.07	11.37±0.13	0.22±0.04
	Bonga	1.75±0.12	6.67±0.17	9.63±0.13	0.63±0.08	0.07±0.02
	Kombolcha	2.17±0.07	5.00±0.06	8.29±0.07	6.01±0.16	0.13±0.05
	Shirka	2.33±0.06	8.75±0.03	10.08±0.14	1.82±0.11	ND[a]
Thymus serrulatus	Addis Ababa	2.50±0.11	5.83±0.12	12.22±0.03	0.67±0.02	0.09±0.02
	Bonga	3.00±0.13	7.50±0.04	13.67±0.07	0.08±0.01	ND[a]
	Kombolcha	3.58±0.14	6.25±0.11	11.50±0.13	0.43±0.02	0.07±0.01
	Shirka	1.88±0.11	4.58±0.17	13.63±0.13	0.23±0.04	0.03±0.01
Zingiber officinale	Addis Ababa	1.00±0.10	2.50±0.01	9.88±0.05	4.06±0.21	0.98±0.04
	Bonga	2.38±0.13	5.00±0.01	6.50±0.00	1.44±0.06	0.75±0.03
	Kombolcha	1.08±0.07	3.75±0.05	5.50±0.12	3.68±0.08	0.92±0.07

[a]ND-not detected

Table 3: Average metal content of the studied plant specie

Plants	Co	Ni	Cu	Cr	As
Allium sativum	0.57	3.47	5.12	1.56	-
Dodonea angustifolia	1.89	4.90	6.76	4.43	1.57
Hagenia abyssinica	1.02	3.54	10.10	8.01	3.03
Ocimum lamifolium	2.83	10.83	11.93	0.59	0.09
Ocimum utricifolium	2.86	6.88	17.90	0.56	0.08
Ruta chalapensis	2.10	6.36	9.39	4.96	-
Thymus serrulatus	2.74	6.04	12.76	0.35	-
Zingiber officinale	1.49	3.75	7.29	3.06	0.88

i.e. 2 mg/kg. Namely, *A. sativum* (3.74+0.07mg/kg), *D. angustifolia* (8.14+0.14mg/kg), *H. abyssinica* (13.24+0.21mg/kg), *R. chalapensis* (11.37+0.13mg/kg) and *Z. officinale* (4.06+0.21mg/kg) all collected from Addis Ababa as well as *R. chalapensis* (6.01+0.16 mg/kg) and *Z. officinale* (3.68+0.08 mg/kg) collected from Kombolcha. The lowest Cr level was observed in *T. serrulatus* (0.08+0.01 mg/kg) taken from Bonga. Overall, results obtained for Cr indicate that environmental factors are more dominant than genetic factors for its accumulation. This is evidenced by Addis Ababa and Kombolcha samples are found to contain the highest Cr level in all species investigated. However, samples from sites included to represent pollution free areas (Shirka and Bonga) found to contain relatively lower Cr concentration.

On the other hand, none of the samples analyzed were observed to contain Cu, Co and As beyond the maximum safe limits set. The highest amount of Co was found in *T. serrulatus* (3.58+0.14 mg/kg) collected from Kombolcha whereas the lowest amount was found in *A. sativum* (0.17+0.05 mg/kg) collected from Addis Ababa. On the contrary, the highest level of Cu was found in

O. utricifolium (20.46+0.21 mg/kg) collected from Bonga (though the reason was not known) but the lowest was in *A. sativum* (4.54+0.11 mg/kg) from Kombolcha. Maximum As level was found in H. abyssinica (3.32+0.12 mg/kg) sampled from Addis Ababa.

Close examination of average metal contents of the data obtained showed that some plants like *O. lamifolium* tend to accumulate relatively large amount of Ni, Co and Cu whereas plants like *A. sativum* were seen to have minimum ability of accumulating the three metals irrespective of their site of collection.

On the other hand, some plants tend to accumulate some metals in large amounts like *H. abyssinica* for Cu, Cr and As but hate to accumulate Co.

Close examination of the averages of each metal in each plant species (Table 3) showed maximum Co, Ni, Cu, Cr and As contents in *O. utricifolium* (2.86 mg/kg), *O. lamifolium* (10.83mg/kg), *O. utricifolium* (17.90 mg/kg), *H. abyssinica* (8.01 mg/kg) and *H. abyssinica* (3.03 mg/kg) respectively. Lowest averages of Co, Ni and Cu were observed in *A. sativum* and Cr in *T. serrulatus*.

According to the result obtained, it seems that leaf samples tend to accumulate relatively larger amount of Co and Ni whereas lower amount of these metals was observed in bulb, rhizome and flower. This is some how inline with the finding of Korkmaz *et al.* [20].

By and large, the concentrations of Ni, Co, Cu, Cr and As found in this work are inline with those reported from different parts of the glob [21-30].

Data were statistically treated by analysis of Variance (ANOVA). The results of the treatments are summarized as follows.

One way and two-way analysis of Variance (ANOVA) were made at 95% confidence interval for different variables. The result showed that: significant difference was seen between metal contents when all the areas of collection and plant species were considered in the calculations (calculated F-values are greater than the tabulated F-values). Similarly, significant difference was observed on the metal contents of different plant samples collected from the same area and also significant difference was observed when samples of different species were compared for the same metal content. However, no significant difference was observed for samples of the same species collected from different areas.

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