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LEVELS OF SELECTED TOXIC HEAVY METALS IN THE ROOT OF *RUMEX ABYSSINICUS*, A TRADITIONAL MEDICINAL PLANT, COLLECTED FROM TWO LOCATIONS IN THE CITY OF ADDIS ABABA

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ABSTRACT. In this study, the contents of selected heavy metals; Fe, Cu, Zn, Cd, Cr, Pb and Ni in the root of *Rumex abyssinicus* (RA) were determined by flame atomic absorption spectroscopy (FAAS) after acid digestion. The digestion required 3:1 HNO_3/HCl (v/v) for 3 h, with temperature of 300°C for complete digestion of 0.5 g RA roots using Kjeldahl apparatus. The levels of metals; Fe, Zn, Cu and Ni were 31.9, 7.76 and 4.73 mg kg^{-1}, respectively, for the samples collected from Addis Ababa University, AAU, Science campus (RA-A samples), and 5.59 Zn, 103 Fe and 3.01 Ni, all in mg kg^{-1}, were found in RA-W sample from Weregenu/Gerji area in AA. Validity of the optimized procedure was evaluated using spiked sample whose recovery varied from 89.8-95.1%. The findings confirmed that only Fe was higher than the tolerance limits. From the health risk perspective, the hazard quotient (HQ) value of Fe for both RA-A and RA-W samples exceeded 1, indicating potential health risks. The hazard index (HI) value suggested that consumption of the root of RA-A and RA-W samples could pose potential health risks over long-term consumption. This may signify the non-carcinogenic health risk associated with consuming RA plant, though continuous regulatory control may necessitate to ensure safety to the consumers.

KEYWORDS: Medicinal plant, Wet digestion, *Rumex abyssinicus*, Trace metals, Health risk assessment, Pearson correlation.

INTRODUCTION

Medicinal plants refer to plants that are either grown and found as wild or planted intentionally, and utilized for their healing properties. Traditional medicines encompass herbal remedies made from various plant components, such as herbs, botanical materials, and prepared herbal products. These remedies often contain active ingredients derived from parts of the plants or other plant materials and sometimes in combination [1]. One of the medicinal plants is *Rumex abyssicus* (RA), which is a flowering plant belonging to the genus Rumex and the Polygonaceae family, originates from tropical Africa [2]. It is medicinal and meals additive plant mainly growing in sufficiently heavy soil that retains moisture. The shoots and leaves are safe to eat and the tuber can be used as a tea to help with meal insecurity while the rhizomes have been used to refine butter, giving it a deep yellow color [3, 4].

The most commonly used RA plant parts as medicinal plant in East African countries, including Ethiopia, is the root and mainly utilized for treatment of different diseases [5]. In Ethiopia, quite a number of Ethno-medicinal uses of the root of RA have also been documented in the literature. A few of these include the treatment for hepatitis by consuming the chewed roots [6]; treatment of gonorrhea by consuming the boiled pieces with water [7]; for tuberculosis after softening with cow butter [8]; treatment of ascariasis infestation by pulverizing the root and mixing with small amount of water, etc. [9]. These demands may be driven by cultural traditions, community trust in the medicinal efficacy of traditional remedies, their affordability, and the challenges in accessing modern healthcare facilities [10].

Plants could become tainted with environmental toxins, particularly heavy metals, causing

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significant health hazards with prolonged exposure for all organisms. One way the herbal products become contaminated is through cultivation of soils containing elevated levels of heavy metals. Farmlands traditionally used for generations to grow medicinal herbs may face direct encroachment from factories, roads, and other high-pollution zones, or they may become contaminated by airborne particles of waste materials [10].

There is a growing concern regarding the safety and potential toxicity of natural herbal products found in the market. Typically, herbal preparations can become contaminated during their growth, harvest, and processing stages. Various sources of heavy metal contamination in the herbal formulations could be attributed to factors such as the water used in irrigation, polluted soils, fertilizers and pesticides, industrial emissions, transportation, as well as harvesting and storage procedures [11]. Certain metals like Zn, Fe, Mn, and Cu are essential elements, serving vital roles in biological systems and only posing harm at elevated levels, while others such as Hg, Pb, As, Cr, and Cd are considered non-essential and toxic even at low concentration levels. Generally, the toxicity associated with these metals stems from their chemical reactivity with cellular structural proteins, enzymes, and membrane systems [12]. Furthermore, metals are non-biodegradable and tend to accumulate over time, making them persistent pollutants [13].

Ensuring the safety and efficacy that medicinal herbal products may have, there are significant concerns for health authorities, pharmaceutical industries, and the public at large. The potential toxicity of herbal plants can be associated with various contaminants, including pesticides, microbes, heavy metals, chemical toxins, and adulterants [14]. Despite being perceived as safe and innocuous, due to their natural origins, herbal products often contain hazardous heavy metals such as Pb, Cd, and Cr, which may pose significant health risks to humans even at low concentration levels [15-17]. Global studies have consistently revealed that herbal preparations frequently exceed the maximum permissible limits set by the World Health Organization (WHO) for heavy metals [18]. For instance, research conducted in Nigeria found that medicinal plant samples were found to contain Cd levels above the WHO's permissible limit of 0.3 mg kg^{-1}, with 33% of the samples exceeding the permissible limit for Pb (10 mg kg^{-1}). Similarly, a study carried out in the United Arab Emirates indicated that most of the analyzed herbs contained levels of heavy metals that surpassed the WHO's permissible limits [19].

Traditional medicine has deep roots in Ethiopia, contributing to the development of diverse disease-fighting techniques over the years. These methods reflect the rich tapestry of cultures, languages, and belief systems present in various regions of the country. The different parts of plants such as leaves, roots, rhizomes, and stems are recognized for their traditional medicinal properties, offering treatment for a wide array of human and animal ailments across the species distributional range [20]. Additionally, the roots, tender shoots and leaves of *Rumex abyssinicus* (RA) are widely consumed as vegetables, appreciated for their delicious taste. In rural settings, herdsmen, farmers, and children commonly snack on the leaves for their tangy flavor, while the stems are chewed akin to sugarcane, valued for their sweetness [21].

Therefore, this study aims to analyze the concentration of heavy metals such as Pb, Cd, Cr, Ni, Fe, Cu and Zn that could be found in the roots of *Rumex abyssinicus* (RA) cultivated/grown in Ethiopia and to evaluate the associated health risks from their daily consumption, aiming to safeguard society from potential adverse health effects resulting from exceeded permissible limits. Collectively, the objective seeks to provide insights into the RA medicinal plants usage and provide scientific information for future extended studies aiming to explore the levels of several heavy metals in the medicinal plant.

EXPERIMENTAL

Chemicals, reagents and standard solutions

All chemicals used in this study were of analytical grade reagents. For the preparation of standard solutions and reagents, distilled-deionized water was used. Acid digestion of the RA root samples

were performed using 69.5% nitric acid (HNO_3) (Scharlau AC 1600, Spain) and 37% hydrochloric acid (HCl) (Riedel-de Haan, Germany) in Kjeldahl apparatus (Gallenkamp, England). Stock standard solution for each metal including Fe, Cd, Pb, Ni, Zn, Cr and Cu, with a concentration of 1000 mg L^{-1}, was used to prepare intermediate or working standard solutions of 10 mg L^{-1} for the calibration standards of each metal. All glass wares were soaked in 5% (v/v) HNO_3 overnight and then rinsed with distilled/deionized water, and dried using laboratory dryer prior use.

Instrumentation and apparatus

Ceramic mortar and pestle (COORS USA, 522-3 E-46) were used for grinding and homogenizing the dried RA root samples. Digital analytical balance (Aczel, CY 224, England), with ± 0.0001 g precision, was used to measure the mass of the samples and polyethylene plastic bags were used for collecting the samples. Quick-fit 24/29 and 29/32 round bottom flasks (250 mL), fitted with reflux condenser, were used in Kjeldahl apparatus to digest the samples. The digests of the sample solution, following digestion, was filtered using Whatman® 110 mm filter paper (Whatman International Ltd., Maidstone, England) both for optimization as well as the solutions prepared for metal analysis. Preparation of the standard solutions and their subsequent dilutions were carried out in 50 mL and 100 mL volumetric flasks (ZEEnit 700p scientific model Analytikjena (Germany)). Flame Atomic Absorption Spectrometer (FAAS), (Zeenit 700P, Germany), equipped with deuterium arc background corrector using air-acetylene flame was utilized for analysis of the target metals including Cr, Cu, Zn, Ni, Cd, Fe and Pb.

Sampling sites

Depending on the availability of the *Rumex abyssinicus* plant, the RA root samples were collected from two sampling sites, within the city of Addis Ababa. The first sites is within the campus of the College of Natural and Computational Sciences of the Addis Ababa University (AAU), Ethiopia: located at 9.0335° N latitude and 38.7637° E longitude, and elevation of 2420 m above sea level, while the second site is located around Gerji/Weregenu area, in the vicinity of Bole International Airport, Addis Ababa, with the geographical location of 9.0062° N latitude and 38.8232° E longitude and lies at elevation of 2320 m above sea level.

The collection sites were chosen, primarily, for two major reasons. First, the sample areas in Addis Ababa were selected because the sites receive significantly more rainfall few months before the sample collection period and the plant grows right after the rainy seasons. Secondly, for comparison of the level of the metal found in this medicinal plant around the area of Woregenu and the campus of the College of Natural and Computational Sciences of the AAU; as the two sites were found to grow fairly abundant quantities of the plant than other areas in the city.

Collection of RA samples

The RA samples were randomly picked from selected study areas separately during November to December, 2022. Collection of the RA root samples, from both sampling sites, was carried out by digging the soil using a hoe around the plant roots with caution and taking out the roots from the soil, Figure 1A, wearing protective hand gloves. About 1.5 kg of the root samples were collected from both sites and stored in cleaned polyethylene plastic bags. One of the plastic bags containing the RA plant root collected from Woregenu sampling site, was labeled as "RA-W; for the Woregenu sample" while the second obtained from the campus of Natural and Computation Science College was labeled as "RA-A; for the AAU sample". The collected root samples were transported to the Analytical Laboratory of the Chemistry Department, AAU, and stored in cool, dark and shaded shelves until used for the subsequent processes and experiments.

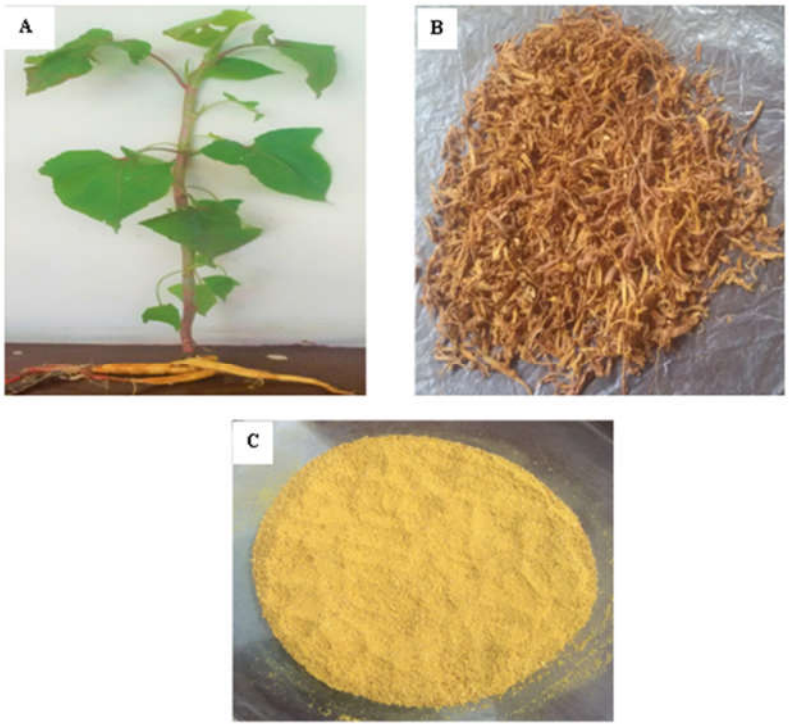

Figure 1. (A) The RA medicinal plant; (B) the RA plant root after drying; and (C) the root sample after ground in mortar and pestle.

Pretreatment of the plant and sample preparation for analysis

To eliminate potential contaminants, particulate matters or natural impurities, the plant roots were cleaned using flowing tap water followed by rinsing with distilled and de-ionized water. The RA root was then cut into small segments using a cleaned stainless steel knife and left to air dry for seven days at room temperature, followed by additional five days in the oven at 50 °C to ensure complete drying (Figure 1B). Subsequently, the dried samples were crushed into powder using a mortar and pestle (Figure 1C). Afterward, the samples were pulverized, sieved with a specific sieve size of 0.5 mm, and stored in a dry, sanitized sample bottle.

Optimization of the digestion procedure

In this study, the Kjeldahl digester heating block was used to prepare the RA root samples for metal analysis following wet digestion. Then, by altering the reagent volume, digestion time, temperature, and reagent composition, various digestion processes were tested. At the end, the final appearance of the digests was resulted in a clear, colorless solution, and then the best possible procedure was chosen and employed. The choices took into account the clear solution of digests, quick digestion, low reagent volume, and low temperature. The optimal process for these samples required 3 h due to the deep pigmentation of the root samples, necessitating high temperatures for complete digestion of the samples from both sites, containing 0.5 g, with 3 mL of 69.5% HNO_3

and 1 mL of 37% HCl. Detailed experimental conditions for the parameters optimized are provided in Table 1 (A-C).

Table 1. Optimization of digestion procedure for 0.5 g of RA root samples.

A. Optimization for reagent volume

No	Reagents used	Reagent volume (mL)	Temp. ºC	Digestion time (h)	Results after filtration and dilution
1	HNO_3:HCl	3:3	300	3	Yellow clear solution
2	HNO_3:HCl	3:2	300	3	Pale yellow clear solution
3	**HNO_3:HCl**	**3:1***	**300**	**3**	**Clear and colorless solution**
4	HNO_3:HCl	6:4	300	3	Pale yellow clear solution
5	HNO_3:HCl	5:3	300	3	Deep yellow clear solution
6	HNO_3:HCl	4:2	300	3	Clear and colorless solution

B. Optimization for temperature

No	Reagents used	Reagent volume (mL)	Temp. ºC	Digestion time (h)	Results after filtration and dilution
1	HNO_3:HCl	3:1	150	3	Yellow clear solution
2	HNO_3:HCl	3:1	180	3	Slightly yellow clear solution
3	HNO_3:HCl	3:1	210	3	Pale yellow clear solution
4	HNO_3:HCl	3:1	240	3	Pale yellow clear solution
5	HNO_3:HCl	3:1	270	3	Almost colorless solution
6	**HNO_3:HCl**	**3:1**	**300***	**3**	**Clear and colorless solution**

C. Optimization for digestion time

No	Reagents used	Reagent volume (mL)	Temp. ºC	Digestion time (h)	Results after filtration and dilution
1	HNO_3:HCl	3:1	300	2:00	Deep yellow clear solution
2	HNO_3:HCl	3:1	300	2:15	Yellow clear solution
3	HNO_3:HCl	3:1	300	2:30	Yellow clear solution
4	HNO_3:HCl	3:1	300	2:45	Pale yellow clear solution
5	**HNO_3:HCl**	**3:1**	**300**	**3:00***	**Clear and colorless solution**
6	HNO_3:HCl	3:1	300	3:15	Clear and colorless solution

Note: * Indicates the optimized volume, temperature and time of plant sample digestion. Digestion of the plant samples.

A 0.5 g of the powdered sample of the plant material was added to a 250 mL round-bottom flask following the aforementioned optimized procedure. Subsequently, 3 mL of 69.5% HNO_3 and 1 mL of 37% HCl were added to the flask containing the powdered plant material. The round-bottom flask was then connected to a reflux condenser and heated for 3 h at a temperature of 300 °C on the Kjeldahl apparatus. Upon completion of digestion, the mixture was diluted with de-ionized water and filtered through Whatman filter paper into a 50 mL volumetric flask. The digestion was conducted in triplicate for each sample. The same analytical technique was applied to extract any possible constituents in the digested blank solutions.

Calibration of the instrument and determination of the metals by FAAS

Intermediate standard solutions of the metals at the concentration level of 10 mg L^{-1} were prepared from the stock solution, initially 1000 mg L^{-1}, for quantitative determination of the metal content using the FAAS. These secondary standards were then diluted with de-ionized water to produce

four working standards for each metal; namely, Cr, Cu, Zn, Fe, Cd, Ni, and Pb, ranging from 0.25-2.0 mg L^{-1}, (Table 2). The wavelengths, limits of detection, working standard solutions, and correlation coefficients of the calibration curves for each metal were provided in Table 2.

The method detection limit (MDL) is the minimum concentration that can be detected using the analytical method with sufficient sensitivity, and the limit of detection is the lowest concentration level that can be determined which is statistically different from the blank (with 95% confidence) [22]. The MDL/LOD is typically determined to be in the region where the signal-to-noise ratio is greater than 3, but not necessarily quantified as exact value. It can be calculated by multiplying the standard deviation of the reagent blank (S blank) by three (MDL = 3 × S blank) [23].

Table 2. Working standard concentrations and correlation coefficient, detection limits for determination of the metals using FAAS.

No	Metal	Working standard (mg L^{-1})	Correlation coefficient; R^2	MDL (mg L^{-1})
1	Cr	0.25, 0.5, 0.75,1	0.9946	*BDL
2	Cd	0.25, 0.5, 0.75, 1	0.9921	BDL
3	Fe	0.25, 0.5, 1, 2	0.9948	0.21
4	Pb	0.25, 0.5, 0.75, 1	0.9988	BDL
5	Zn	0.25, 0.5, 0.75, 1	0.9939	0.012
6	Cu	0.25, 0.5, 0.75, 1	0.9999	0.011
7	Ni	0.25, 0.5, 0.75, 1	0.9949	0.021

*BDL: Below detection limit.

Validation of optimized procedure

Spiking tests were run to confirm the effectiveness of the analytical procedure. To this end, the standard solutions containing 1,000 mg L^{-1} of each metal were utilized, and from these solutions, intermediate standards containing 10 mg L^{-1} of each metal (Fe, Zn, and Ni,) were prepared. The sample from Woregenu site was utilized for the recovery measurement, and the spiking was carried out in three groups in triplicate. In the first group, 515 µL Fe was spiked and introduced to a 250 mL round-bottomed flask that has already contained 0.5 g sample and 4 mL of the acid mixture (containing 3 mL HNO_3 and 1 mL HCl). Similarly, in the second group, 30 µL Zn was spiked, while in the third group, 15 µL Ni was spiked. Then, the same digestion procedure was conducted following the optimal digestion process employed in the sample analysis. The corresponding results are given in Table 2.

Health risk assessment of spices consumption

The potential risk posed by consumption of herbal products contaminated with heavy metals on human health was assessed using the estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI) of the metals. The EDI was calculated to estimate the average daily uptake of metals into the body of consumers with a specified body weight. The EDI value was determined using the following formula [24]:

$$EDI = C^*_{metal}\, IR/BW \quad (1)$$

where, C^*_{metal} (mg kg^{-1}) represents the average weight of heavy metal contents in traditional herbal preparations, IR (ingestion rate) denotes the average daily consumption of herbal products (in g/day/person), and BW represents the average body weight in kg. The typical daily consumption of herbal preparations for an adult is 20 g/person/day of dry weight. The average body weight

considered for adults was 65 kg [24-26].

The hazard quotient (HQ) serves as a tool for evaluating the non-carcinogenic risks posed to humans by prolonged exposure to heavy metals found in vegetables, medicinal plants, and fruits. The HQ value below 1 indicates that exposure is not expected to result in significant health effects, whereas the HQ value exceeding 1 may indicate potential health risks from exposure. HQ is computed as the ratio of the estimated daily intake (EDI) to the reference dose (RfD), as illustrated in the following formula [25]:

$$HQ = EDI/RfD \quad (2)$$

Here, EDI represents the average estimated daily intake of herbal preparations per day in mg kg^{-1} of body weight per day, and RfD denotes the oral reference dose of the metal in mg kg^{-1} of body weight per day. The RfD serves as an estimate of the daily exposure level deemed tolerable without significant risk of adverse effects over lifetime. The RfD values in medicinal plants for Pb, Cu, Cd, and Cr are 0.004 mg/kg/day, 0.04 mg/kg/day, 0.001 mg/kg/day, and 0.003 mg/kg/day, respectively [26].

The hazard index (HI) serves as a tool for estimating the collective non-carcinogenic risk posed to human health by multiple heavy metals. When individuals are exposed to more than one pollutant, their effects combine additively. It is determined by adding together the hazard quotients (HQs) for each heavy metal, as shown in the relation below [27]:

$$HI = HQ(Cr) + HQ(Cd) + HQ(Fe) + HQ(Pb) + HQ(Zn) + HQ(Cu) + HQ(Ni) \quad (3)$$

If the HI values is less than 1, it is unlikely to cause adverse risk effects on human health [25, 27].

RESULTS AND DISCUSSION

In this study, spiking tests were performed to ascertain the response enhancement process effectiveness by adding known quantities of the standard solutions of Fe, Zn, and Ni to the processed RA-W root samples. The results obtained for the recovery analysis are summarized in Table 3 demonstrating that the percentage recoveries ranged between 89.8−95.1%. Consequently, the percentage recoveries for the selected metals in the RA sample were found to be within the range, reported in the scientific literatures [23].

Table 3. Recovery study of the selected metals using the optimized procedure of the root of RA from RA-W samples.

Metal	Level of metal in un spiked sample (mg kg^{-1})	Amount spiked (mg kg^{-1})	Percent spiked (mg kg^{-1})	Level of metal in spiked sample (mg kg^{-1})	Percent (%) recovery
Fe	103	10.3	10	113	95.1
Zn	5.59	0.56	10	6.12	94.2
Ni	3.01	0.30	10	3.28	89.8

Levels of metals in the roots of RA samples

The FAAS technique was employed to analyze the concentrations of seven metals in the samples of RA roots collected from two specified study areas (RA-A and RA-W). Table 4 shows the findings for each sample, presented in milligrams per kilogram (mg kg^{-1}) on a dry weight basis, along with the corresponding standard deviation values.

Table 4. Mean concentration (mg kg^{-1}) and %RSD of the determined metals in RA samples (RA-A and RA-W), analyzed by FAAS; n = 3.

No	Metal	Sample sites, level of metals in mg kg^{-1}, dry weight			
		RA-A	%RSD	RA-W	%RSD
1	Cr	ND	ND	ND	ND
2	Cu	7.76	9.61	ND	ND
3	Zn	31.9	6.98	5.59	3.67
4	Fe	145	2.47	103	9.15
5	Ni	4.73	2.79	3.01	2.64
6	Cd	ND	ND	ND	ND
7	Pb	ND	ND	ND	ND

ND = Not detected.

Level of metals in RA root collected from RA-A and RA-W

As indicated in Table 4 and Figure 2, Fe has the highest concentration in the RA-A plant, amounting to 145 mg kg^{-1}. Zn comes in second place with a concentration of 31.9 mg kg^{-1} followed by Cu with 7.76 mg kg^{-1} and Ni 4.73 mg kg^{-1}. Cr, Cd, and Pb are not detected in the root sample of RA-A. The amount obtained for each of these metals was below the detection limit. Thus, for the RA samples collected from the Campus of Natural and Computational Sciences of the AAU, the metal levels of the plants follow the order; Fe > Zn > Cu > Ni. It has also been noted that in the RA-W samples, Fe was found as the most abundant metallic element, with concentration of 103 mg kg^{-1}, followed by Zn at 5.59 mg kg^{-1}, and Ni at 3.01 mg kg^{-1}. The sequence of metal levels determined was found to be Fe > Zn > Ni. These findings are presented in Figure 2. In the RA-W sample, the other four metals; i.e., Cu, Cd, Cr, and Pb, were found to be below the detection limit of the instrument.

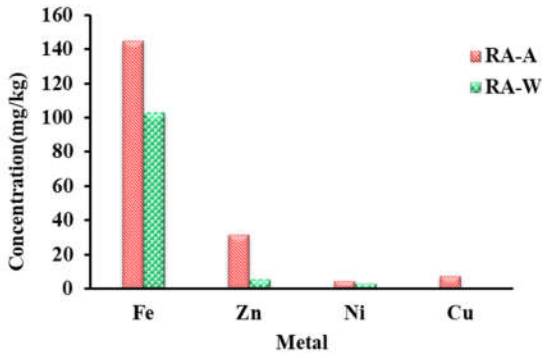

Figure 2. Concentration level of metals determined in the RA-A and RA-W root samples.

Trend of metals in RA root samples

Zinc (Zn). The Zn metal is a dietary trace mineral known for its use in treating the common cold and its vital roles in growth, development, and immune system functions are widely recognized [28]. The Zn levels in RA roots found in the current study varied from 5.59 to 31.9 mg kg^{-1}. In addition, the Zn contents of the RA medicinal plant was falling below the WHO's recommended limit of 50 mg kg^{-1} [29-31]. Further closer observation of the findings in this study, signifies that the results are in good agreement with those reported for other medicinal plants [32, 33].

Copper (Cu). Cu is essential both for plants and mammalian lives, playing crucial roles in carbohydrate and lipid metabolism. Despite its significant importance, Cu can have various biological impacts, both beneficial and harmful [34]. The Cu level determined in the RA-A sample was found to be 4 7.76 mg kg^{-1}, which is lower than the WHO's recommended limit of 20 mg kg^{-1} for Cu in medicinal plants [23]. The quantity of Cu found in RA-A medicinal plant is comparable with the reported values for other similar plants [32, 33].

Iron (Fe). The element Fe plays pivotal roles in energy production, oxygen delivery, and immune function in the human body. Anemia can result from Fe deficiency [26]. Fe concentrations in RA medicinal plants ranged from 103 to 145 mg kg^{-1}, which exceeds the FAO/WHO permitted limits of 20 mg kg^{-1} for medicinal plants [30]. However, for medicinal plants similar to RA, a WHO limit has not been established for the Fe contents [31]. Besides, the iron contents determined in this study were found to be in agreement with the findings reported in the literature [33, 35, 36].

Nickel (Ni). Ni is necessary for enzyme function in both plants and animals and also plays a great role in iron metabolism. Ni deficiency is rare in individuals with a diverse diet [37]. The maximum Ni content permitted by the WHO for medicinal herbs is 1.5 mg kg^{-1} [29]. Ni levels in RA samples of the current study was found to vary from 3.01 to 4.73 mg kg^{-1}, which is above the WHO acceptable limit.

Cadmium (Cd). The element Cd is considered hazardous and non-essential heavy metal due to its presence in the environment from various human activities, posing threats to the ecosystems [38]. Plants exposed to high Cd levels undergo physiological and biochemical alterations. The permissible limit set for Cd in medicinal plants by WHO, China and Thailand was 0.3 mg kg^{-1} [31]. It was observed that Cd was not detected in the RA plant considered in this study.

Chromium (Cr). Although a little is known about Cr, its deficiency in some individuals may be associated with diabetes. High Cr intake has been linked to lung cancer and liver damage [39]. The maximum permissible limit of Cr set by Canada for medicinal plants is 2 mg kg^{-1} [40]. Chromium levels were below the detection limit in this study.

Lead (Pb). Pb poisoning has numerous adverse effects, including cognitive impairment, abdominal discomfort, and damage to vital organs such as kidneys, brain, and reproductive organs [41]. The maximum permissible limits of Pb in the medicinal plants was set by China, Malaysia, Thailand and WHO is 10 mg kg^{-1} [31]. However, Pb was not detected in the RA roots of this study. The levels of several metals in the two RA root samples collected from the two different locations in Addis Ababa, generally fell within the maximum tolerable limits set by the WHO [31, 39], indicating that they are fairly safe for human consumption.

Comparison of levels of selected metals in RA roots with permissible limits by WHO

The findings of this study can be evaluated in light of the established standards set by the WHO. To date, there have been no comprehensive investigations into the metal composition levels of RA roots in Ethiopia or globally. Hence, comparison for this plant are limited to the WHO's permissible values, Table 5.

Pearson correlation analysis

The Pearson correlation analysis conducted on metal pairs within RA samples demonstrated strong relationships. The values of the product moment-correlation coefficient (r) fall between -1

and +1. Additionally, a perfect negative correlation has r-value of -1, meaning that all experimental data points lie on a straight line with a negative slope. Similarly, the r-value of +1 denotes a perfect positive correlation, with each point precisely falling along a line with a positive slope. However, a zero r-value just indicates that the two components (in the formula, y and x) are not linearly connected and not imply that they are completely unrelated [43].

Table 5. Comparison of selected metal levels (mg kg^{-1}) in the root of RA plant of this study with permissible values of WHO [20, 32, 42].

No	Metal	Range of concentration of metal (mg kg^{-1})	
		Ethiopia	*WHO*
1	Zn	5.59 - 31.9	50
2	Fe	103-145	20
3	Cu	7.76	20
4	Ni	3.01-4.73	1.5
5	Cd	ND	0.3
6	Cr	ND	2
7	Pb	ND	10

Table 6 lists the correlation coefficient values between the metal concentrations in RA root, carried out using Micro Soft Excel. Fe-Zn and Fe-Ni revealed perfect relationships in the RA root metal vs RA root metal system, with matching r-values of 0.91 and 0.90, respectively. Additionally, Ni-Zn, Cu-Zn and Cu-Fe have strong relationships, with r-values of 0.63, 0.83, and 0.53, respectively. Furthermore, there was a weak association between Cu-Ni, which means that the presence of one metal within the plant has no effect on the presence of the other.

Table 6. Pearson correlation matrices for metals in the roots of RA sample.

Correlation matrices	Fe	Zn	Ni	Cu
Fe	1			
Zn	0.91	1		
Ni	0.90	0.63	1	
Cu	0.53	0.84	0.11	1

Health risk assessment of metals in Rumex abyssinicus plant

Estimating health risks based on the estimated daily intake (EDI) of heavy metal contaminants is a crucial factor for understanding health risk assessment. It considers factors such as the frequency and duration of exposure, as well as the body weight of the exposed individual. Generally, health risks stemming from metal contamination rely on the average daily dietary intake [24]. The EDI values for Cu, Zn, Pb, Cd, Cr, and Ni fell within the tolerable daily intake reference limits for the samples of RA-A and RA-W. However, for both samples, the EDI for Fe exceeded the tolerable daily intake reference limit, Table 7.

Assessment of the non-carcinogenic risk of heavy metals in herbal preparations was conducted using the hazard quotient (HQ), as shown in Table 7. HQ evaluates the long-term exposure to heavy metal contaminants in herbal preparations. When the HQ is less than 1, consumers are thought to be safe; if it equals or exceeds 1, it is considered to pose as a health risk [44-46]. The findings of the current study indicates that the HQ values for Cu, Zn, Pb, Cd, Cr, and Ni were all below 1, confirming that there is no health risk from these metals in herbal preparations of both RA-A and RA-W samples. However, the HQ for Fe in both samples RA-A (6.37) and RA-W (4.53) have exceeded 1, indicating potential health risks caused by Fe exposure (Table 7).

Similarly, if the hazard index (HI) value of any heavy metals in herbal preparations is less

than 1, it suggests that the exposed population is unlikely to experience adverse health effects over their lifetime. Conversely, if the HQ equals or exceeds 1, there's a potential health risk [47-49]. The HI values for the analyzed herbal samples were below 1, indicating no long-term health risks from the combined effects of heavy metal contaminants in these herbal preparations for adults. However, due to the occurrence level of Fe, higher than the WHO limit, in both the RA-A and RA-W samples, the HI values exceeded 1. Similar findings was also reported for Fe, in other studies, where vegetables and leafy plants were analyzed, that are commonly consumed both around the rural villages and cities of the Eastern industry zone of the central Ethiopia [50].

Table 7. EDI, HQ and HI values of the metals in *Rumex abyssinicus*.

Metal	RA-A		RA-W		
	EDI	HQ	EDI	HQ	HI
Cu	0.002387	0.059675	-	-	0.059675
Zn	0.00981	0.0327	0.00172	0.005733	0.038433
Pb	-	-	-	-	-
Fe	0.044615	6.37357	0.031692	4.527428	10.900998
Cd	-	-	-	-	-
Cr	-	-	-	-	-
Ni	0.001455	0.7275	0.00092615	0.463075	0.535825

CONCLUSIONS

In this study, *Rumex abyssinicus* (RA) root samples from the selected sites around the city of Addis Ababa, were analyzed for Cr, Cu, Zn, Cd, Fe, Ni, and Pb using FAAS. The recovery test, employing optimized sample processing procedure in wet acid digestion method for RA examination, proved effective for the selected metals, with a recovery percentage ranging from 89.8% to 95.1%. Metal concentrations in the RA root samples were expressed in the following order: Fe (103-145 mg kg^{-1}), followed by Zn (5.59-31.9 mg kg^{-1}), Cu (7.76 mg kg^{-1}), and Ni (3.01-4.73 mg kg^{-1}) in both samples, while levels of Cd, Cr, and Pb were below the instrumental detection limits. From a health risk perspective, the HQ value of Fe, both in RA-A and RA-W samples exceeded 1, indicating potential health risks. The HI value suggested that the consumption of RA-A and RA-W samples may pose potential health risks over long-term consumption. This suggests a non-carcinogenic health risk associated with consuming certain herbal preparations marketed in Addis Ababa, Ethiopia, necessitating continuous and stringent regulatory control to ensure their safety. Based on the results, the need for initiating further studies may be thought about and could be undertaken in the future, primarily to know the distributions of these and other metals in several parts of the RA.

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REFERENCES

1. Hailemariam, A.; Bibiso, M. Determination of levels of some metals in selected traditional medicinal plants in Wolaita Zone, Southern Ethiopia. *Int. J. Curr. Res.* **2019***,* 11, 1839–1844.
2. Shifa, M.; Abdissa, D.; Asere, T.G. Chemical constituents of *Rumex abyssinicus* roots and evaluation of its antimicrobial activities. *J. Turkish Chem. Soc.* **2021**, 8, 21–46.
3. Duguma, H.T. Wild edible plant nutritional contribution and consumer perception in Ethiopia. *Int. J. Food Sci.* **2020,** 2020, 2958623.
4. Prakah, M.A.; Sharifi-Rad, M.; Shariati, M.A.; Mabkot, Y.N.; Al-Showiman, S.S.; Rauf, A.; Salehi, B.; Zupunski, M.; Sharifi-Rad, M.; Gusain, P.; Sharifi-Rad, J.; Suleria, H.A.R.; Iriti, M. Bioactive compounds and health benefits of edible Rumex species-A review. *Cell. Mol. Biol.* **2018**, 64, 27–34.
5. Mohammed, S.A.; Panda, R.C; Madhan, B.; Demessie, B.A. Extraction of bio-active compounds from Ethiopian plant material *Rumex abyssinicus* (mekmeko) root, A study on kinetics, optimization, antioxidant and antibacterial activity. *J. Taiwan Inst. Chem. Eng.* **2017**, 75, 228–239.
6. Nigussie, G.; Tola, M.; Fanta, T. Medicinal uses, chemical constituents and biological activities of *Rumex abyssinicus:* A comprehensive review. *Int. J. Second. Metab.* **2022**, 9, 440–456.
7. Limenih, Y.; Umer, S.; Wolde-Mariam, M. Ethno-botanical study on traditional medicinal plants in Dega Damot woreda, Amhara Region, North Ethiopia. *Int. J. Res. Pharm. Chem.,* **2015,** *5*, 258–273.
8. Gidey, M.; Beyene, T.; Signorini, M.A.; Bruschi, P.; Yirga, G. Traditional medicinal plants used by Kunama ethnic group in Northern Ethiopia. *J. Med. Plants Res.* **2015**, 9, 494–509.
9. Gijan, M.; Dalle, G. Ethno-botanical study of medicinal plants in Nagelle Arsi District, West Arsi Zone of Oromia, Ethiopia. *J. Nat. Sci. Res.* **2019**, 9, 1–19.
10. Atinafu, T.; Mekonnen, T.; Somasundaram, J. Determination of some toxic heavy metal accumulation in medicinal plants commonly used in Gondar area district, Northwestern Ethiopia. *Int. J. Pharm. Anal. Res.* **2015**, 4, 399–405.
11. Ababneh, F.A. The hazard content of cadmium, lead and other trace elements in some medicinal herbs and their water infusions. *Int. J. Anal. Chem.* **2017**, 2017, 1–8.
12. Mahurpawar, M. Effects of heavy metals on human health. *Int. J. Res. Granthaalayah* **2015**, 3, 1–7.
13. Okatch, H.; Ngwenya, B.; Raletamo, K.M.; Andrae-Marobela, K. Determination of potentially toxic heavy metals in traditionally used medicinal plants for HIV/AIDS opportunistic infections in Ngamiland District in Northern Botswana. *Anal. Chim. Acta* **2012**, 730, 42–48.
14. Nkansah, A.M.; Hayford, S.T.; Borquaye, L.S.; Ephraim, J.H. Heavy metal contents of some medicinal herbs from Kumasi, Ghana. *Cogent Environ. Sci.* **2016**, 2, 1234660.
15. Ekeanyanwu, R.C.; Njoku, J.O.; Nwodu, P.O.; Njokuobi, A.E. Analysis of some selected toxic heavy metals in some branded Nigerian herbal products. *J. Appl. Pharm. Sci.* **2013**, 3, 6918–6922.
16. Dargan, I.P.; Gawarammana, I.B.; Archer, J.R.; House, I.M.; Shaw, D.; Wood, D.M. Heavy metal poisoning from Ayurvedic traditional medicines: an emerging problem? *Int. J. Environ. Health* **2008**, 2, 463–474.
17. Goroya, K.G.; Mitiku, Z.; Asresahegn, Y.A. Determination of concentration of heavy metals in ginger using flame atomic absorption spectroscopy. *Afr. J. Plant Sci.* **2019**, 13, 163–167.
18. Amde, M.; Megersa, N.; Taddesse, A.M.; Bedassa, T. Determination of the levels of selected metals in seeds, flowers and fruits of medicinal plants used for tapeworm treatment in Ethiopia. *Toxicol. Environ. Chem.* **2013**, 95, 82–100.

19. Samali, A.; Mohammed, M.I.; Ibrahim, M.B Analysis of heavy metals concentration in Kano herbal preparations for major disease conditions. *Chem. Search J.* **2017**, 8, 22–28.
20. Girmay, M.; Lulekal, E.; Bekele, T.; Demissew, S. Use and management practices of medicinal plants in and around mixed woodland vegetation, Tigray Regional State, Northern Ethiopia. *Ethnobot. Res. Appl.* **2021**, 21, 1–26.
21. Eshete, M.A.; Kelbessa, E.; Dalle, G. Ethno-botanical study of medicinal plants in Guji agro-pastoralists, Bule Hora District of Borana Zone, Oromia Region Ethiopia. *J. Med. Plants Stud.* **2016**, 4, 170–184.
22. Tschinkel, P.F.S.; Melo, E.S.P.; Pereira, H.S.; Silva, K.R.N.;Arakaki, D.G.; Lima, N.V.; Fernandes, M.R.; Leite, L.C.S.; Melo, E.S.P.; Melnikov, P.; Espindola, P.R.; de Souza, I.D; Nascimento, V.A.; Junior, J.L.R.; Geronimo, A.C.R.; dos Reis, F.J.M.; Valter A. Nascimento, V.A. The hazardous level of heavy metals in different medicinal plants and their decoctions in water: a public health problem in Brazil. *BioMed. Res. Int.* **2020**, 2020, 1–11.
23. Kumar, M.; Ray, J.; Gupta, P.; Sundar, S.; Bhardwaj, A.; Gautam, S.; Jain, A.; Gaur, A.; Sigh, Y.; Singh, S.; Aggarwal, B.K. A validated method for analysis of toxic heavy metal in food spices by ICP-MS. *Plant Arch.* **2020**, 20, 123–128.
24. Gebreyohannes, F.; Gebrekidan, A. Health risk assessment of heavy metals via consumption of spinach vegetable grown in Elalla River. *Bull. Chem. Soc. Ethiop.* **2018**, 32, 65–75.
25. Sarvestani, R.A.; Aghasi, M. Health risk assessment of heavy metals exposure (lead, cadmium, and copper) through drinking water consumption in Kerman city, Iran. *Environ. Earth Sci.* **2019**, 78, 714.
26. Gebeyehu, H.R.; Danno, B.L. Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia. *PLoS One* **2020**, 15, e0227883.
27. Tefera, M.; Teklewold, A. Health risk assessment of heavy metals in selected Ethiopian spices. *Heliyon* **2021**, 7, e07048.
28. Khudair, Z.J. Assessment of some heavy metals in fruit and vegetables grown in Samawah City, Iraq. *Eur. J. Mol. Clin. Med.* 2**021**, 8, 1600–1612.
29. Nkuba, L.L.; Mohammed, N.K. Heavy metal and essential element in selected medicinal plant commonly used for medicine in Tanzania. *Chem. Sci. Int. J.* **2017**, 19, 1–11.
30. FAO/WHO, ''Contaminants.'' Codex Alimentarius, Vol. XVII, 1st ed. FAO/WHO, Codex Alimentarius Commission: Rome; **1984**.
31. WHO, National Policy on Traditional Medicine and Regulation of Herbal Medicines, Report of WHO Global Survey: Geneva, Switzerland; **2005**.
32. Khan, S.A.; Khan, L.; Hussain, I.; Marwat, K.B.; Akhtar, N. Profile of heavy metals in selected medicinal plants. *Pak. J. Weed Sci. Res.* **2008**, 14, 101–110.
33. Dubale, A.A.; Chandravabshi, B.S.; Gebremariam, K.F. Levels of major and trace metals in the leaves and infusions of *Croton macrostachyus*. *Bull. Chem. Soc. Ethiop*. **2015**, 29, 11-26.
34. Dzomba, P.; Chayamiti, T.; Togarepi, E. Heavy metal content of selected raw medicinal plant materials: implication for patient health. *Bull. Environ. Pharmacol. Life Sci.* **2012**, 1, 28–33.
35. Petel, P.; Petel, N.M.; Patel, P.M. WHO guidelines on quality control of herbal medicines. *Int. J. Res. Ayurveda Pharm.* **2011**, 2, 1148–1154.
36. Ashraf, M.; Hayat, M.Q.; Mumtaz, A.S. Study on elemental contents of medicinally important species of *Artemisia* L. (Asteraceae) found in Pakistan. *J. Med. Plant Res.* **2010**, 4, 2256–2263.
37. Zerihun, A.; Chandravanshi, B.S.; Debebe, A.; Mehari, B. Level of selected metals inleaves of *Cannabis sativa* L. cultivated in Ethiopia. *Springer Plus* **2015**, 4, 359.
38. Mukhopadhyay, G. Atomic spectroscopy analysis of heavy metals in plants. *Int. J. Pharm. Biol. Arch.* **2018**, 9, 1–5.

39. Hagos, M.; Chandravanshi, B.S. Levels of essential and toxic metals in *fenugreek seeds* (*Trigonella Foenum-Graecum* L.) cultivated in different parts of Ethiopia. *Braz. J. Food Technol.* **2016**, 19, e2015059.
40. World Health Organization (WHO). Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. Geneva, Switzerland, **2007**.
41. Wuana, R.A.; Okeieimen, F.E. Heavy metals in contaminated soil, A review of source, chemistry, risks and best available strategies for remediation. *ISRN Ecol*. **2011**, 3, 402647.
42. Randelovic, S.S.; Kostic, D.A.; Zarubica, A.R.; Snezana, S.; Milan, M.N.; The correlation of metal content in medicinal plants and their water extracts, *Hem. Ind.* **2013**, 67, 4585–4591.
43. Adefa, T.; Tefera, M. Heavy metal accumulation and health risk assessment in *Moringa Oleifera* from Awi Zone, Ethiopia. *Chem. Afr.* **2020**, 3, 1073−1079.
44. Aschale, M.; Sileshi, Y.; Kelly-Quinn, M. Health risk assessment of potentially toxic elements via consumption of vegetables irrigated with polluted river water in Addis Ababa, Ethiopia. *Environ. Syst. Res.* **2019**, 8, 29.
45. Kacholi, D.S.; Sahu, M. Levels and health risk assessment of heavy metals in soil, water, and vegetables of Dar es Salaam, Tanzania. *J. Chem.* **2018**, 2018, 1402674.
46. Dee, K.H.; Abdullah, F.; Md Nasir, S.N.A; Appalasamy, S.; Mohd Ghazi, R.; Eh. Rak, A. Health risk assessment of heavy metals from smoked *Corbicula fluminea* collected on roadside vendors at Kelantan, Malaysia. *BioMed. Res. Int.* **2019**, 2019, 1–9.
47. Li, J.; Wang, Y.; Yang, H.; Yu, P.; Tang, Y. Five heavy metals accumulation and health risk in a traditional Chinese medicine *Cortex Moutan* collected from different sitesin China. *Hum. Ecol. Risk Assess.* **2018**, 24, 2288−2298.
48. Khan, S.; Cao, Q.; Zheng, Y.M.; Huang, Y.Z.; Zhu, Y.G. Health risk of heavy metals in contaminated soils and food crops irrigated with waste water in Beijing, China. *Environ. Pollut. Series* **2008**, 152, 686−692.
49. Kohzadi, S.; Shahmoradi, B.; Ghaderi, E.; Loqmani, H.; Maleki, A. Concentration, source, and potential human health risk of heavy metals in the commonly consumed medicinal plants. *Biol. Trace Elem. Res.* **2019**, 187, 41–50.
50. Bahiru, D.G.; Teju, E. Levels of some selected metals (Fe, Cu and Zn) in selected vegetables and soil around eastern industry zone, central Ethiopia. *Afr. J. Agric. Res.* **2019**, 14, 78−91.