

**ORIGINAL RESEARCH****Residual levels and potential health risk assessment of heavy metals in varieties of teff (*Eragrostis tef* (Zucc.) Trotter) using ICP-OES from Becho District, Ethiopia**Fantahun Gebeyehu<sup>1</sup>, Molla Tefera, Mengstu Mulu<sup>2</sup>, Atnafu Guadie<sup>2\*</sup><sup>1</sup>Department of Chemistry, College of Natural and Computational Sciences, Wolkite University, Wolkite, Ethiopia.<sup>2</sup>Department of Chemistry, College of Natural and Computational Sciences, University of Gondar, Gondar, Ethiopia\*Corresponding author: [atnafu.guadie@uog.edu.et](mailto:atnafu.guadie@uog.edu.et), OR [atnafuguad@gmail.com](mailto:atnafuguad@gmail.com)

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**ABSTRACT**

This study determined the levels of heavy metals and associated health risks from frequently consumed red, mixed, and white teff grown in Ethiopia's Becho area. The sample was wet-digested and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Further data acquisition and analysis, such as spike experiments, were conducted to validate the method's performance. The levels of Fe, Mn, Zn, Cu, Cr, Co, Ni, Pb, and Cd ranged from 83.67 to 330.10, 157.02 to 299.16, 25.88 to 44.60, 2.84 to 8.03, 0.0014 to 2.50, 0.024 to 0.35, 0.0012 to 19.73, 0.0022 to 0.40, and 0.022 to 0.34 mg/kg in all teff varieties, respectively. The percentage recovery of the examined metals in the spiked samples ranged from 88.65 to 118.80%, showing the good validity of the optimized digested procedures. Non-carcinogenic health risks to exposed adults were also assessed. The target hazard quotient (THQ) values of Fe and Mn in red teff and Mn and Ni in mixed teff were greater than unity, indicating that the consumption of red and mixed teff may cause possible non-carcinogenic health risks to exposed adults. The values of hazard index (HI) for white, red, and mixed teff were 3.95, 8.302, and 8.84, respectively, indicating that exposure of the population to heavy metals leads to potential adverse health risks. The target cancer risk (TCR) values of Ni and Cr in mixed teff showed high cancer risk, with Cd indicating a moderate effect, and the values of Ni, Cd, Pb, and Cr in red teff; Pb in mixed teff; and Cd, Cr, and Ni in white teff showed low cancer risks in the exposed adult population in the area.

**Keywords/phrases:** Heavy Metals, ICP-OES, Risk Assessment, Teff.**Introduction**

Teff (*Eragrostis tef*) is the most common and endemic cereal crop in Ethiopia and has been used as one of the main sources of nutrients in Ethiopia for centuries (Adane et al., 2020). It

is mainly grown in the central and northern parts of the country. Teff is reported to have higher amino acids, fibers, minerals, vitamins, and fatty acids compared to grains like wheat, rice, oats, and barley (Abraha et al., 2020; Dame, 2020). Nowadays, interest in

growing teff is increasing in Europe. In Ethiopia, teff is consumed in the form of fermented bread called “injera” (Callejo et al., 2016). The injera is fine; it is made from teff flour, water, and a starter (liquid collected from a previously fermented mixture) after successive fermentations (Gebregewergis, 2021). It is considered a low-risk crop because it can be grown in a variety of ecological settings and in harsh environmental conditions where most other crops fail to grow (Weldehawaria, 2022). In recent years, teff has become increasingly popular in the markets of developed countries due to its attractive nutritional properties and gluten-free nature. Food for babies has been prepared in the food industry by mixing teff with chickpeas, soybeans, and other grains to enhance their nutritional values as teff (Lianne et al., 2020). People consuming teff have higher hemoglobin levels in their blood and do not suffer from hookworm anemia, even if infested. In addition, consuming teff prevents pregnancy-related anemia (Habte et al., 2020).

There are three main varieties of teff identified in Ethiopia, described as white, red, and mixed teff with almost the same composition, except the red one is rich in iron content and recommended to treat iron deficiency (Callejo et al., 2016; Gebregewergis, 2020; Habte et al., 2022). White teff typically most effectively grows within the highlands of the country and requires relatively appropriate growing situations. Even though white teff has increased consumer preference and is the most expensive variety, red teff, which is considered nutritious, has also been gaining popularity among health-conscious consumers in Ethiopia (Gebregewergis, 2020). Literature reports have shown that red teff is well known for its higher contents of iron and calcium than mixed or white teff (Lianne et al., 2020). Besides coffee, teff is an important source of revenue and income for local farmers in Ethiopia, generating about \$500 million annually (Alemneh et al., 2022; Gebregewergis, 2021).

Trace metal contamination is a global

environmental concern because it widely occurs on the earth's crust, water, air, and food, and the levels of this contamination have increased due to human and natural activities (Akele et al., 2017; Eghbaljoo-Gharehgheshlaghi et al., 2020). In rural areas, trace metal contamination comes from waste disposal, sewage sludge, pesticides, herbicides, and fertilizers (Akele et al., 2017). Contamination of the environment with heavy metals, especially soil, has been the most serious pollution problem due to the severe toxicity, wide distribution, persistence, and transferability of these metals to plants compared to other contaminants, which can lead to various diseases (Mihretu et al., 2021; Fan et al., 2017). The growing demand for food safety inspired researchers to examine the risks associated with consuming food contaminated with heavy metals. Currently, food safety issues and potential health risks are the most serious environmental issues (Rai et al., 2019). Depending on the physiochemical properties like organic matter, clay fraction, pH, mineral composition, and binding capacity of the soil, foods can absorb large amounts of heavy metals from the soil they grow in (Adjei-Mensah et al., 2021).

Prolonged exposure to excessive amounts of potentially toxic metals from food and dietary supplements can have harmful effects on human health (Woldetsadik et al., 2020). In addition to natural input from a source rock, heavy metal contamination of agricultural soils is caused by anthropogenic activities such as industrialization, metal mining, unskilled application of fertilizers and pesticides, and sewage irrigation (Guo et al., 2020). Unlike organic contaminants, these inorganic chemical hazards such as As, Cd, Pb, Sn, Al, and Hg are not biodegradable and can therefore accumulate in the soil at dangerous levels. Generally, these contaminants can pose a risk to human health through the consumption of food (Sibuar et al., 2022). Teff is a precious supply of minerals; especially Ca, Fe, Mn, and Zn, which are found in larger quantities (Koubová et al., 2018).

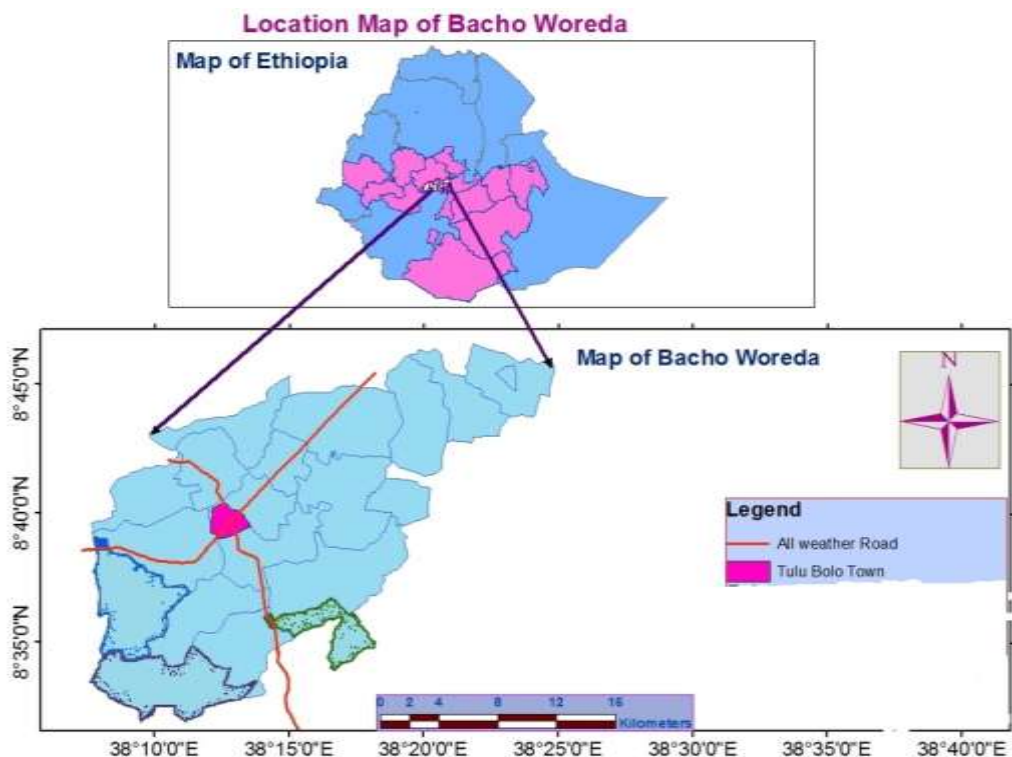
In Ethiopia, limited research was available to analyze the concentration of heavy metals in teff samples (Dame, 2020; Gebregewergis, 2020; Mulugeta & Mohammed, 2015). However, there were no reports on associated health risk assessments in red, mixed, and white teff samples in Becho District (Tullu Bollo). Therefore, this study aimed to determine the concentration of heavy metals (Fe, Co, Ni, Zn, Mn, Cd, Pb, Cr, and Cu) in samples of red, white, and mixed teff collected in the Becho District, Ethiopia, and to estimate the heavy metal-related health risks to the community from teff consumption. The findings of this research would also pave the ways to conduct further investigation on human health risks of the heavy metals associated with the long-

term exposures to the heavy metals up on consumption of teff in the study areas. This study is limited to the study of heavy metals in varieties of teff samples from Becho District, Ethiopia.

## Materials and methods

### *Description of the study area*

The study was conducted in the Becho District of the Oromia region of Ethiopia (Fig. 1). It is located in the southwestern territory of Shewa and is bordered by Saden Sodo to the south, Woliso to the west, Dawo to the northwest, Elu to the north, and Tole to the east. The capital of Becho is Tulu Bolo.



**Fig.1.** Location map of the study area

### **Sample collection and preparation**

The three varieties of teff samples (red, white, and mixed) were collected from the local market of Becho (Tullu Bollo). The samples had been randomly taken from different farmers within this marketplace. For each sample type, 0.25 kg sub-samples were collected from twelve different teff suppliers originating from the Becho District and mixed according to sample type so as to make 3 kg composite samples of each of the three teff varieties. The collected teff samples were stored in polyethylene bags and transported to the Chemistry Laboratory of the University of Gondar. To remove dust and adsorbed particles, both teff samples were washed with water. The dried samples were ground with a mill and sieved to a mesh size of 0.5 mm. Powdered samples were packed in clean, dry plastic (polyethylene) bags under airtight conditions until digestion.

### **Reagents and standards**

All chemicals were analytical reagent grade, and deionized water was used throughout the analysis.  $\text{HNO}_3$  (70%) and  $\text{HClO}_4$  (70%) (Research Lab Fine Chem. Industries, Mumbai 400 002, India) were used to digest the sample matrix; standard stock solutions with 1000 mg/L (Buck Scientific Puno. Graphic™) of each metal have been used for calibration and spike recovery tests. Plastic-based materials were soaked in 20%  $\text{HNO}_3$  for 24 h and rinsed with deionized water before use. The glasswares were also immersed in 10%  $\text{HNO}_3$  for 24 h, rinsed with deionized water, and dried in a dust-free place until analysis.

### **Digestion of teff samples**

Wet acid digestion was one of the most widely used methods to recover free metal ions in dissolved form from a complex organic matrix based on changing various digestion parameters (volume ratio of added reagents, digestion temperature, and time). Digestion is completed when the solution is clear and colorless. Various digestion procedures were performed for teff samples using acid mixtures of  $\text{HNO}_3$  and  $\text{HClO}_4$

with varying volumes, digestion times, and digestion temperatures (Gebregewergis *et al.*, 2020). Applying the optimized procedure, 2 g of dried and homogenized teff samples have been transferred into a 100 mL round bottom flask containing a 6 mL combination of  $\text{HNO}_3$  and  $\text{HClO}_4$  (5:1, v/v), and the mixtures were digested at the temperature first at 120°C for a half-hour, then elevated to 300°C for the remaining 2:30 hr. The digests were cooled for 10 min at room temperature without dismantling the condenser from the flask, and then for an additional 5 min after putting off the condenser. Deionized water was added, and the solution was filtered into a 50 mL volumetric flask with Whatman No. 125-mm filter paper. Subsequently, the solution was rinsed with deionized water in a 50 mL volumetric flask. Then, 1% of the lanthanum nitrate solution was introduced and filled to the mark with deionized water. Blank solutions were digested with the same procedure as the sample in triplicate. The digested samples had been stored in the refrigerator until the levels of metals in the sample solutions were determined through ICP-OES.

All instrumental conditions have been optimized for maximum sensitivity, as described by the manufacturer. Calibration curves were constructed using standard solutions for each respective metal, and triplicate measurements were performed for every sample. The same analytical procedure was used for elemental determination in the digested blank solutions.

### **Method performance and validation**

The limit of detection (LOD) was determined by summing the mean of the blank signal plus three times the standard deviation of the nine blanks. The instrument detection limits were obtained directly from the instrument manual for each item examined, and the limit of quantification (LOQ) was obtained from the triplicate analysis of nine reagent blanks digested using the same procedure as for the teff samples. Method validation was established by spiking the samples with a known standard concentration of each metal. Spiked and

non-spiked samples were digested under similar conditions and analyzed to determine the percent analyte recovery.

### **Health risk assessment of metals due to teff consumption**

The evaluation of health risk assessment to estimate cancer and non-cancer risks from exposure to heavy metals (Fe, Mn, Zn, Cu, Ni, Co, Cr, Pb, and Cd) in teff was based on the US EPA Human Health Assessment method (USEPA, 2011). Although there are many routes by which humans are exposed to heavy metals, including drinking, soil uptake, skin contact, and inhalation routes, ingestion has been identified as the main route contributing to more than 90% of health risks (Guo et al., 2020). The Target Hazard Quotient (THQ) and Hazard Index (HI) have been used to describe potential human health risks (Guadie et al., 2022).

### **Non-carcinogenic analysis**

**Estimated daily intake of metals (EDI):** EDI is measured in (mg/kg body weight/day) (Javed & Usmani, 2016) and calculated according to Eqn. 1:

$$EDI = \frac{C \times IR}{Bw} \times 10^{-3} \quad (1)$$

Where, C is the metal concentration in the teff samples (mg/kg dry weight), IR is the intake rate assumed to be 180 g/day based on previous studies (Gebregewergis et al., 2020) and BW is the average body weight of Ethiopian adults, assumed to be 65 kg as described by Sinaga et al. (2019).

**Target Hazard Quotient (THQ):** THQ was used to assess the health risk resulting from consuming teff contaminated with heavy metals and it was calculated based on Eqn. 2:

$$THQ = \frac{C \times IR \times 10^{-3} \times EF \times ED}{Rfd \times Bw \times ATn} \quad (2)$$

where THQ is a non-carcinogenic risk and is dimensionless. EF is the exposure frequency (365

days/year), ED is the exposure duration (68 years), and Rfd (Oral reference doses) were 0.001, 0.004, 0.3, 0.04, 0.14, 0.02, 0.7, 0.043 and 1.5 mg/kg/d for Cd, Pb, Zn, Cu, Mn, Ni, Fe, Co and Cr, respectively (USEPA IRIS, 2011) (Temiotan et al., 2018). ATn is the averaging time for non-carcinogens (365 days/year  $\times$  ED) (United States Environmental Protection Agency, (USEPA), 2011).

A THQ < 1 signifies that no adverse health risk will occur, while THQ  $\geq$ 1 signifies that non-cancer effects will occur (Morakinyo et al., 2021).

**Hazard Index (HI):** HI value was obtained by adding the THQ values of each metal, and the health risk posed by more than one metal is added together and referred to as the hazard index (HI) (USEPA 2011), and calculated through Eqn.3:

$$HI = \sum THQ \quad (3)$$

HI < 1 stands for no significant risk of non-carcinogenic effects, while HI  $\geq$ 1 shows the occurrence of non-carcinogenic effects (Morakinyo et al., 2021).

### **Carcinogenic analysis**

Target cancer risk (TCR) is used to indicate the carcinogenic risk. The method that is used to estimate TR is provided in the USEPA Regional Screening Levels (RSLs) Risk-Based Concentration Table (USEPA, 2021). TR was calculated based on Eqn.4:

$$TCR = \frac{C \times IR \times 10^{-3} \times CPSo \times EF \times ED}{Bw \times ATn} \quad (4)$$

where CPSo is the carcinogenic potency slope, oral (mg/kg BW-day<sup>-1</sup>). Ant is the averaging time carcinogen (365 days/year  $\times$  68 years). Since Mn, Fe, Co, Cu, and Zn do not cause any carcinogenic effects as their CPSo have yet to be established (USEPA, 2011) (Javed & Usmani, 2016), Thus, only the TR values of Ni, Cd, Cr, and Pb were calculated to show the carcinogenic risk. The CPSc oral cancer slope factor for Cd, Cr, Ni, and

Pb was 0.38, 0.5, 1.7, and 0.0085 (mg/kg/day)<sup>-1</sup>, respectively (Gebeyehu & Bayissa, 2020).

### Statistical analysis

All data were analyzed using Microsoft Excel 2016 and SPSS 20.0. Pearson's correlation analysis, one-way ANOVA, and LSD were performed to determine the relationships between the heavy metals in the teff samples examined. The level of significance was set to  $p < 0.05$ .

## Results and discussion

### Method performance and validation

**Table 1:** Recovery test (%R) LOD (mg/kg), and LOQ (mg/kg) of analyzed heavy metals in three varieties of Teff samples

Elements	Fe	Mn	Zn	Cu	Cr	Co	Ni	Pb	Cd
Red	115.43	88.65	116.74	118.80	116.78	112.27	110.08	108.61	96.42
Mixed	109.7	107.95	112.23	107.75	106.27	115.88	103.08	106.8	95.45
White	105.11	95.83	108.43	103.03	118.8	116.79	118.33	118.80	98.33
LOD	0.039	0.009	0.015	0.021	0.0004	0.003	0.0003	0.0022	0.00014
LOQ	0.13	0.03	0.05	0.07	0.0013	0.01	0.001	0.0072	0.0047

### The concentration of heavy metals in Teff samples

The average concentrations of heavy metals (Fe, Mn, Zn, Cu, Cr, Co, Ni, Pb, and Cd) in the examined teff samples are shown in Table 2. Fe (330.10 mg/kg) accumulated the most, followed by Mn (299.16 mg/kg), Zn (40.52 mg/kg), Cu (5.51 mg/kg), Pb (0.370), Co (0.350 mg/kg), Ni (0.0022 mg/kg), Cd (0.021 mg/kg) and Cr (0.0020 mg/kg) in red teff. The concentration of Mn (222.10 mg/kg) was the highest in mixed teff, followed by Fe (207.07 mg/kg), Zn (44.60 mg/kg), Ni (19.73 mg/kg), Cu (8.03 mg/kg), Cr (2.50 mg/kg), Pb (0.403 mg/kg), Cd (0.338 mg/kg), and Co (0.0024 mg/kg).

The concentration of Mn (157.67 mg/kg) was the highest in the white teff sample, followed by Fe

The results of the spiked experiment and the sensitivity of the method are presented in Table 1. The LOD values were 0.039, 0.009, 0.015, 0.021, 0.0004, 0.003, 0.0003, 0.0022, and 0.00014 mg/kg, while the LOQ values were 0.13, 0.03, 0.05, 0.07, 0.013, 0.01, 0.001, 0.0072, and 0.0047 mg/kg for Fe, Mn, Zn, Cu, Cr, Co, Ni, Pb and Cd, respectively. The percentage recovery of analyzed metals in the spiked samples ranged from 88.65% to 118.80% (Table 1), which is within the acceptable range of recoveries of 80-120% for metal analysis (Ebere et al., 2020). For this reason, the spiking experiments showed good precision and accuracy of the method for heavy metal analysis in the three teff samples.

(83.67 mg/kg), Zn (25.88 mg/kg), Cu (2.84 mg/kg), Co (0.05 mg/kg), Cd (0.0023 mg/kg), Ni (0.0015 mg/kg), and Cr (0.0014 mg/kg). However, Pb in white teff was found to be below LOD. Among the three varieties of teff samples, the concentrations of heavy metals were relatively lowest in the white teff sample. Except for Pb in red and mixed teff and Cd in mixed teff, the levels of all heavy metals were found below the maximum permissible limit set by WHO/FAO (Tegegne, 2015). Based on one-way (ANOVA), Fe, Mn, Zn, Cu, and Co showed significant differences ( $p < 0.05$ ) in all teff samples (Table 2). The paired samples t-test did not show significant differences ( $p > 0.05$ ) for concentrations of Pb in red and mixed teff samples.



**Table 2:** Mean concentration of heavy metals (Mean  $\pm$ SD, n = 3, mg/kg dry weight) in Teff samples analyzed by ICP-OES

Metals	Red	Mixed	White	Min.	Max.	WHO/FAO
Fe	330.10 $\pm$ 3.78 <sup>a</sup>	207.07 $\pm$ 2.23 <sup>b</sup>	83.67 $\pm$ 2.74 <sup>c</sup>	83.67	330.10	425.5
Mn	299.16 $\pm$ 5.97 <sup>a</sup>	222.10 $\pm$ 2.79 <sup>b</sup>	157.02 $\pm$ 4.46 <sup>c</sup>	157.02	299.16	500
Zn	40.52 $\pm$ 0.11 <sup>a</sup>	44.60 $\pm$ 1.87 <sup>b</sup>	25.88 $\pm$ 1.08 <sup>c</sup>	25.88	44.60	99.4
Cu	5.51 $\pm$ 1.01 <sup>a</sup>	8.03 $\pm$ 0.47 <sup>b</sup>	2.84 $\pm$ 0.42 <sup>c</sup>	2.84	8.03	73.3
Cr	0.0020 $\pm$ 0.001 <sup>a</sup>	2.50 $\pm$ 0.4 <sup>b</sup>	0.0014 $\pm$ 0.0004 <sup>a</sup>	0.0014	2.50	2.3
Co	0.350 $\pm$ 0.08 <sup>a</sup>	0.024 $\pm$ 0.004 <sup>b</sup>	0.05 $\pm$ 0.01 <sup>c</sup>	0.024	0.35	50
Ni	0.0022 $\pm$ 0.001 <sup>a</sup>	1.973 $\pm$ 0.186 <sup>b</sup>	0.0015 $\pm$ 0.001 <sup>a</sup>	0.0012	19.73	67
Pb	0.370 $\pm$ 0.03 <sup>a*</sup>	0.403 $\pm$ 0.01 <sup>a*</sup>	BDL	0.0022	0.403	0.3
Cd	0.021 $\pm$ 0.002 <sup>a</sup>	0.338 $\pm$ 0.04 <sup>b</sup>	0.023 $\pm$ 0.01 <sup>a</sup>	0.022	0.338	0.2

\*The values in the same row followed by different letters were significantly different ( $p < 0.05$ ) confidence levels computed by one-way ANOVA, BDL = below the detection limit, and <sup>a\*</sup> = paired samples t-test for Pb concentration in red and mixed teff.

The mean concentration of Fe obtained from this study was higher than previously reported values in white, red, and mixed teff samples from Ethiopia (Mulugeta & Mohammed, 2015). However, except for Mn (20–45 mg/kg), the concentrations of Fe (252–1195 mg/kg), Zn (73–90 mg/kg), Cu (13–15 mg/kg), Cd (0.8–1.8 mg/kg), and Pb (1.8–2.8 mg/kg) in white, red, and mixed teff samples were higher than in the present study (Gebregewergis et al., 2020). A similar report indicated that the concentration of Fe (330.10 mg/kg) in red teff was higher than the concentrations (217–239 mg/kg) reported by Habte et al. (2020), but comparable in mixed and white teff. The concentrations of Mn (56–99 mg/kg) in all types of teff grains were lower than in the present study (Habte et al., 2020). The concentrations of Zn (35–85 mg/kg) were slightly higher than in the present study. A study conducted in the southern part of Ethiopia showed that the concentrations of Fe and Zn in the three varieties of teff were comparable with the findings of the present study. The levels of Mn and Cu in both teff samples were higher and lower than reported values, respectively. Lead concentrations were also higher than reported by Habte et al. (2020). Similarly, the levels of metals were analyzed in teff (red and white) purchased from Addis Ababa market, Ethiopia (Dame, 2020). The findings of the levels of Fe, Mn, Zn,

Cu, Co and Pb in this study were lower than the reported values by Dame (2020). However, the levels of Cr, Cd and Pb were higher than reported by Dame (2020).

Reports in Bolivia, the USA, and Europe showed the concentrations of Fe, Zn, Pb, Cd, and Mn in white and mixed teff were lower than the results of this study. While, the concentrations of Cr, Cu, Co and Ni reported by Koubová et al. (2018) were higher than the values of our study (Koubová et al., 2018). In addition, reported data by Neela & Fanta (2020) showed that concentrations of Fe (763 mg/kg), Mn (924 mg/kg), Zn (363 mg/kg), and Cu (81 mg/kg) in red teff were greater than the present study. Pearson's correlation was applied to determine the relationships between different metal contents in the teff samples and the results are presented in Table 3. Strong positive correlations have been observed between the concentrations of Fe with Cd, Co, and Ni; Mn with Zn; Cu with Cr, Ni, and Pb; Cr with Ni and Pb; Co with Cd; Ni with Pb in red teff; Fe with Cu and Ni; Mn with Zn, Co, Pb and Cd; Cu with Cr and Ni; Cr with Cd and Ni; Co with Cd, Zn and Pb, Cd with Pb, and Zn with Cd and Pb in mixed teff, and Fe with Mn and Ni; Zn with Cr; Cu with Cr, Co, Ni, and Cd; Cr with Co and Cd, and Co with Cd in white teff. This correlation could suggest the origin of metals was from

similar sources (Guadie et al., 2022). While, strong negative correlations were detected between Mn with Cu, Cr, Ni, and Pb and Zn with Cu, Cr, Ni, and Pb in red teff; Fe with Mn, Zn, and Cd; Mn with Cu and Ni; and Zn with Cu and

Ni in mixed teff, and Fe with Z, Cr, and Co; Mn with Zn, Cr, Co, and Cd; and Zn with Ni in teff, which indicates the lack of common origin between metals (Adefa & Tefera, 2020).

**Table 3.** Pearson's correlation matrices for heavy metals in three teff samples

	Fe	Mn	Zn	Cu	Cr	Co	Ni	Pb	Cd	
<b>Red Teff</b>	Fe	1								
	Mn	-0.122	1							
	Zn	-0.514	0.914	1						
	Cu	0.133	-0.987	-0.919	1					
	Cr	-0.088	-0.978	-0.809	0.975	1				
	Co	0.982	0.070	-0.340	-0.059	-0.277	1			
	Ni	0.585	-0.876	-0.996	0.882	0.756	0.419	1		
	Pb	0.024	-0.995	-0.870	0.994	0.994	-0.168	0.825	1	
	Cd	0.967	0.135	-0.278	-0.124	-0.339	0.998	0.359	-0.232	1
<b>Mixed Teff</b>	Fe	1								
	Mn	-0.988	1							
	Zn	-0.958	0.953	1						
	Cu	0.656	-0.642	-0.845	1					
	Cr	0.101	-0.082	-0.381	0.817	1				
	Co	-0.941	0.948	0.806	-0.363	0.240	1			
	Ni	0.585	-0.569	-0.792	0.996	0.866	-0.277	1		
	Pb	-0.930	0.937	0.787	-0.334	0.271	0.999	-0.247	1	
	Cd	-0.738	0.751	0.515	0.024	0.596	0.923	0.115	0.934	1
<b>White Teff</b>	Fe	1								
	Mn	0.817	1							
	Zn	-0.998	-0.775	1						
	Cu	0.061	-0.526	-0.130	1					
	Cr	-0.643	-0.967	0.587	0.726	1				
	Co	-0.542	-0.927	0.482	0.806	0.992	1			
	Ni	0.739	0.216	-0.784	0.717	0.041	0.165	1		
	Cd	-0.447	-0.881	0.383	0.866	0.973	0.994	0.273	-0.825	1

#### Health risk assessment of heavy metals in teff grains

Table 4 shows the values of EDI, THQ, HI, and CR of non-carcinogenic and carcinogenic risks to adults caused by consumption of red, mixed, and

white teff. The highest EDI values in red teff were found for Fe, Mn, and Co, the highest EDI values in mixed teff were recorded for Zn, Cu, Cr, Ni, Pb, and Cd. However, among the three varieties, the lowest EDI for all metals was found in white teff.



The THQ values of Fe, Mn, Zn, Cu, Cr, Co, Ni, Pb, and Cd were recorded as 1.306, 5.914, 0.373, 0.375,  $3.9 \times 10^{-6}$ , 0.023,  $3.0 \times 10^{-4}$ , 0.250, and 0.061; 0.819, 4.393, 0.413, 0.550, 0.175,  $1.5 \times 10^{-3}$ , 1.279, 0.275, and 0.940; and 0.331, 0.240, 0.200,  $2.6 \times 10^{-6}$ ,  $3.0 \times 10^{-3}$ ,  $2.1 \times 10^{-4}$ , ND, and 0.064 mg kg<sup>-1</sup> day<sup>-1</sup> in red, mixed, and white teff, respectively. The THQ values of Fe (1.306 mg kg<sup>-1</sup> day<sup>-1</sup>) in red teff, Mn in red teff (5.914 mg kg<sup>-1</sup> day<sup>-1</sup>), mixed (4.393 mg kg<sup>-1</sup> day<sup>-1</sup>) and white (3.107 mg kg<sup>-1</sup> day<sup>-1</sup>), and Ni (1.279 mg kg<sup>-1</sup> day<sup>-1</sup>) in mixed teff were higher than 1, suggesting that ingestion of these heavy metals in these teff samples might pose serious risks to the local population. The variations between the THQ values could be attributed to the variations in different geographic locations (Guo et al., 2020).

The HI (total THQ) of red, mixed, and white teff is mainly attributable to the THQ values of Mn and Fe. The HI values for red (8.302), mixed (8.840) and white teff (3.950) samples were > 1, indicating that adults are exposed to these metals, which leads to potential adverse health risks in the study area.

The TCR carcinogenic risk to adults caused by red, mixed, and white teff consumption is indicated in Table 4. The TCR value of Cd was the highest followed by Ni, Pb, and Cr for red teff; while for mixed teff, Ni displayed the highest TCR value, followed by Cr, Cd, and Pb. However, Cd has the highest TCR in white teff followed by Ni and Cr. As can be seen in Table 4, Ni demonstrated the highest role in the total TCR value in mixed teff. According to the New York State Department of Health Center for Environmental Health (Javed & Usmani, 2016), the TCR category is described as low if TCR ≤ 10<sup>-6</sup>; 10<sup>-4</sup> to 10<sup>-3</sup> is moderate; 10<sup>-3</sup> to 10<sup>-1</sup> is high, and ≥ 10<sup>-1</sup> is very high. In this study, Ni (0.0935) and Cr ( $3.5 \times 10^{-3}$ ) in mixed teff show high cancer risk for the exposed population, and Cd ( $3.6 \times 10^{-4}$ ) shows a moderate effect (Song et al., 2021). However, the TCR values of red teff ranged from 1.0 × 10<sup>-5</sup> to 8.5 × 10<sup>-6</sup>; in mixed teff, TCR ranged from 9.4 × 10<sup>-6</sup> to 0.0935, and white teff ranged from 1.3 × 10<sup>-6</sup> to 2.4 × 10<sup>-5</sup>. Except for Ni, Cr, and Cd in mixed teff, the carcinogenic risk for Ni, Cd, Pb, and Cr was between the 10<sup>-6</sup> and 10<sup>-4</sup> ranges. These results revealed that the intervals signify the predicted tolerable lifetime risks for carcinogens (Peters et al., 2018).

**Table 4:** Estimated daily intake (EDI) values for adult consumers, hazard quotient (THQ), and target carcinogenic human health risk (TCR) of trace metals due to consumption of teff

Metal	Red Teff			Mixed Teff			White Teff		
	EDI	THQ	TCR	EDI	THQ	TCR	EDI	THQ	TCR
Fe	0.914	1.306		0.573	0.819		0.232	0.331	
Mn	0.828	5.914		0.615	4.393		0.435	3.107	
Zn	0.112	0.373		0.124	0.413		0.072	0.240	
Cu	0.015	0.375		0.022	0.55		$8.0 \times 10^{-3}$	0.200	
Cr	$5.8 \times 10^{-6}$	$3.9 \times 10^{-6}$	$2.9 \times 10^{-6}$	$7 \times 10^{-3}$	0.175	$3.5 \times 10^{-3}$	$3.9 \times 10^{-6}$	$2.6 \times 10^{-6}$	$1.3 \times 10^{-6}$
Co	$9.7 \times 10^{-4}$	0.023	-	$6.6 \times 10^{-5}$	$1.5 \times 10^{-4}$	-	$1.3 \times 10^{-4}$	$3.0 \times 10^{-3}$	
Ni	$6.1 \times 10^{-6}$	$3.0 \times 10^{-4}$	$1.0 \times 10^{-5}$	0.055	1.279	0.0935	$4.2 \times 10^{-6}$	$2.1 \times 10^{-4}$	$7.1 \times 10^{-6}$
Pb	$1.0 \times 10^{-3}$	0.250	$8.5 \times 10^{-6}$	$1.1 \times 10^{-3}$	0.275	$9.4 \times 10^{-6}$	-	-	-
Cd	$6.1 \times 10^{-5}$	0.061	$2.3 \times 10^{-5}$	$9.4 \times 10^{-4}$	0.94	$3.6 \times 10^{-4}$	$6.4 \times 10^{-5}$	0.064	$2.4 \times 10^{-5}$
HI		8.302			8.84			3.95	
$\sum TCR$			$4.44 \times 10^{-5}$			$9.74 \times 10^{-2}$			$3.24 \times 10^{-5}$

## Conclusion

In this study, the levels of nine heavy metals in red, mixed, and white teff samples in the Becho area were determined by using ICP-OES. The concentrations of all metals except Pb in red and mixed teff and Cd in mixed teff were within the safe limit set by the WHO/FAO allowable limits. The individual THQ values except for Fe and Mn in red teff, Mn and Ni in mixed teff, and Mn in white teff were all below 1 for adults consuming teff, suggesting a tolerable level of non-carcinogenic adversative risk. The HI values of heavy metals in all teff samples exceeded 1, which may cause potential health risks, including cancer for the exposed adult population in the area. Iron and Mn in red, mixed, and white teff and Ni in mixed teff were the most significant contributors to total THQ or HI values for the exposed population. The values of TCR in this study, Ni and Cr in mixed teff, suggest high cancer risk in the exposed population, and Cd was in the range of moderate effect. The data and findings gave a clear picture of trace metal concentrations and their associated health risks in teff samples consumed by the inhabitants of the study areas. There are significant health effects to the population from consuming teff at the study areas. Thus, the health risks of the studied metals should not be ignored. The findings in this report can also be used as an imperative reference for guiding policymakers in focusing on agricultural management and food quality control agencies in the region. Future studies of trace metal concentrations in the study areas from soil samples are of paramount importance to provide the sources and complete human health risk assessment.

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## Conflict of interest

The authors declared that they have no conflict of interest in the work reported in this paper.

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