DF-528
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RESEARCH ARTICLE
Agricultural Science Digest

Determination of the Levels of Selected Heavy Metals in Finger Millet (*Eleusine coracana*) Flour in West Gojjam Zone, Amhara Region

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10.18805/ag.DF-528

ABSTRACT

Background: Finger millet is a native and indigenous food crop in Ethiopia and has been cultivated in different parts of the country for centuries. However, its heavy metal contents in other parts of Ethiopia were not well studied, especially in the study area. The study was conducted in the Mankussa and Finoteselam district of the western Gojjam zone, Amhara region Ethiopia, to determine the heavy metal (Mn, Fe, Zn, Cu and Pb) levels of finger millet by using inductively coupled plasma optical emission spectroscopy.

Methods: A wet digestion procedure was developed using a mixture of HCl, HNO_3 and H_2O_2 to decompose a mixed powdered finger millet sample.

Result: Mn, Fe, Cu, Zn and Pb mean levels were 3.751, 1.699, 0.072, 0.4485 and 0.024 mg/L, respectively, in Mankussa Black finger millet samples. In Mankussa mixed finger millet, the mean metal levels were 3.573 mg/L, 2.7585 mg/L, 0.0585 mg/L, 0.799 mg/L and 0.024 mg/L for Mn, Fe, Cu, Zn and Pb, respectively and the mean metal levels of Mankussa White Finger millet were found to be 2.326, 2.289, 0.045, 0.5985 and 0.014 mg/L of Mn, Fe, Cu, Zn and Pb respectively. While the mean metal level of Finoteselam black Finger millet samples was found to be 5.651 mg/L, 2.1955 mg/L, 0.0435 mg/L, 0.744 mg/L and 0.011 mg/L for Mn, Fe, Cu, Zn and Pb respectively, in the mixed finger millet it was 3.023 mg/L, 2.155 mg/L, 0.0525 mg/L,1.259 mg/L of Mn, Fe, Cu and Zn. In white finger millet, it was found to be 2.8695 mg/L, 1.412 mg/L, 0.0525 mg/L and 1.0735 mg/L of Mn, Fe, Cu and Zn. Pb was not detected by the instrument in Finoteselam mixed and white finger millet samples. The metal levels investigated in this study were comparable with international standards.

Key words: Finger millet, Heavy metal, ICP-OES, Wet digestion.

INTRODUCTION

Cereals are sources of energy and minerals for humans and are also the most commonly consumed foods (Salihu *et al*., 2014) and contain a sufficient amount of carbohydrates, trace elements, vitamins, oil and protein (Doe *et al*., 2013). Consumers can require food quality and safety, a complex characteristic of food that determines its value or acceptability (Chang *et al*., 1984; Zahir *et al*., 2009; Ismail *et al*., 2011). The concentration of the metals in cereal crops is based on plant species, genetics, types of soil and metal, soil conditions, weather, environment, stage of maturity and supply route to the market (Judy *et al*., 2012). Among metals, heavy metals can cause human health problems if the excess amount is ingested through food (Haware *et al*., 2011).

Crops and vegetables grown in soils contaminated with heavy metals have a moresignificant accumulation than those grown in uncontaminated soils (Barbeau and Hilu, 1993) because farmlands situated in industrialized areas are prone to pollution by releasing chemicals into the farmlands leading to contamination of plant crop (Abdulrazak and Oniwapele, 2014). Elements such as cadmium (Cd) and chromium (Cr), are considered carcinogenic, while Iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and nickel (Ni) are considered essential trace elements. The intake of heavy metal-contaminated cereal crops may pose a risk to human health (Ismail *et al*., 2011). Trace elements do not contribute to calories, but they play an essential role in the metabolic regulations of the human body if they exist in required amounts. For example, they are co-enzymes and co-factors in the human system, playing different roles in growth, metabolism and immune system development (Elbagermi *et al*., 2012). Metal analysis of foods is an essential aspect of food quality (Edem *et al*., 2009).

While several metals like Cu, Zn and Fe are crucial micronutrients, metals such as Cd and Pb constitute non-essential and toxic elements (Luis *et al*., 2015). Trace elements play a vital role in the proper functioning of enzymes by modulating reactions, constituting their active

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How to cite this article: Fetene, D., Lelago, A., Bibiso, M. and Bosha, A. (2023). Determination of the Levels of Selected Heavy Metals in Finger Millet (*Eleusine coracana*) Flour in West Gojjam Zone, Amhara Region. Agricultural Science Digest. doi:10.18805/ag.DF-528

Submitted: 20-12-2022 **Accepted:** 28-04-2023 **Online:** 29-05-2023

sites, stabilizing their structure and mediating the oxidation-reduction process (Fraga *et al*., 2005). They also play an essential role in the proper functioning of the immune system (Rolla *et al*., 1983). Therefore, the present researchers are highly inspired to research for the following reasons: there is a scarcity of the study conducted on the heavy metals concentration of finger millet.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Jabithehinan woreda, specifically at Mankusa and Finoteselam, in the West Gojjam Administrative Zone of Amhara Regional State of Ethiopia (Fig 1). in 2021. The area is situated at 13°37′48.5″N and 34°23′39.3″E at an altitude of 1500-2300 meters above sea level.

Sample collection and preparation

In each study area, Finoteselam and Mankusa, the farmers randomly collected three composite finger millet samples, each 2 kg. The samples were separately packed into different Polyethylene plastic container bags, labeled and transported to B.D.U. Laboratory for further investigation.

The samples were handpicked and sifted by 2 mm mesh to remove the hull, stones and unwanted materials. Each finger millet sample was thoroughly washed with tap water in plastic containers and rinsed in distilled water to remove surface contaminants like soil, dust and spray residues. The samples were placed in acid-washed clean porcelain crucibles labeled according to the sample and oven dried at a temperature of 45°C for 12 hrs. At this stage, care is taken to avoid any source of contamination, especially micronutrients. The dried finger millet samples were later ground, powdered, sieved and filtered with a sieve of 2 mm mesh to get fine powder and homogenized into a fine powder with a grinding device and stored in polyethylene bags for digestion.

Digestion procedure

This experimental activity (determination of heavy metals from finger millet) was carried out at the Bahir Dar University of, the Postgraduate Chemistry Laboratory. A series of procedures were applied by changing the reagent volume, digestion temperature and time until a clear and colorless solution was formed. 0.5 g of powdered black, mixed and white finger millet samples were measured and each dissolved in 25 ml of a mixture of HCl (36-38%), HNO_3 (69-72%) and H_2O_2 in a 3:1:1 ratio by volume. The mixtures were first heated at 180°C for 2 hr. and the temperature increased to 300°C for about 3:00 hr unit the color of the solution became colorless to indicate the removal of organic

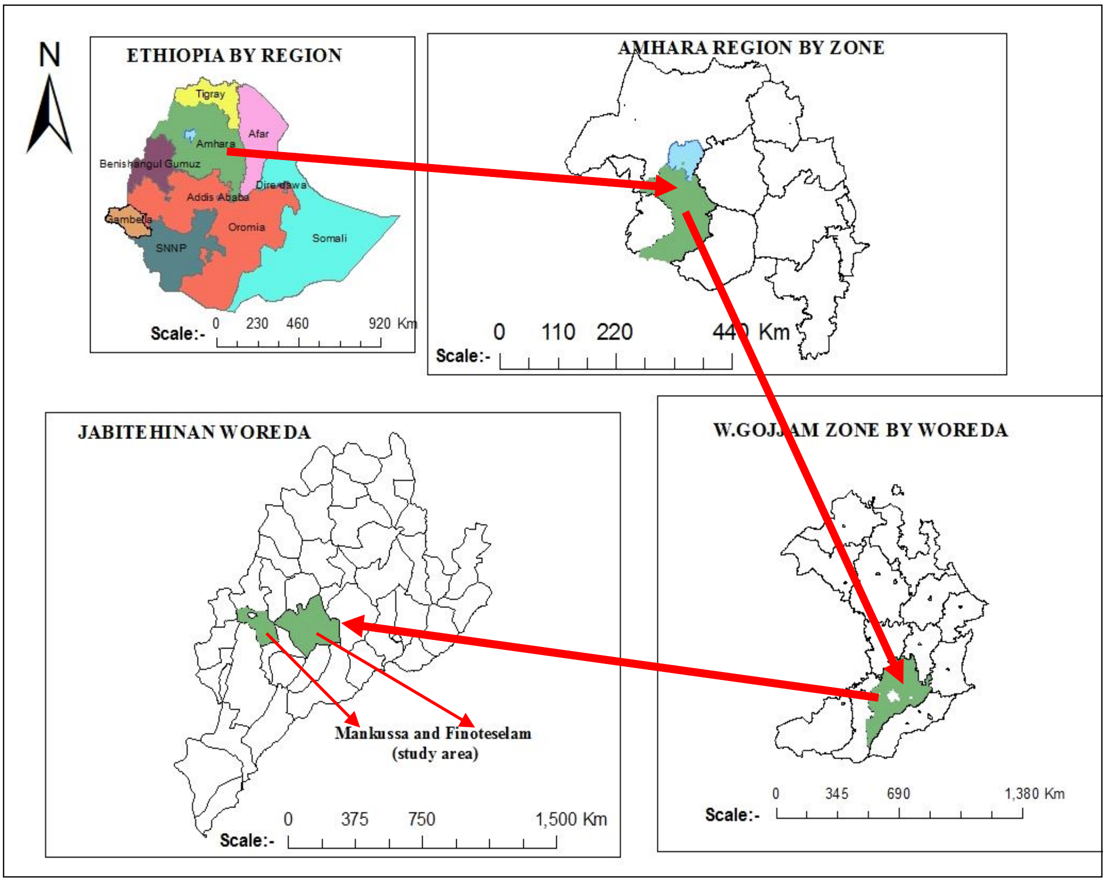

Fig 1: Map of the study area.

matter from the sample; hence it is the optimum condition for the digestion process of finger millet. Finally, the digested solution was allowed to cool for 15 minutes and filtered through Whatman filter paper (pore size 11µm) and deionized water was added to the filtrate until the solution volume reached 50.

Instrumental calibration

Optical- 8000 (Perkin Elmer, U.S.A.) (ICP-OES) was used to determine the metal concentrations in the finger millet sample. The quality of results obtained for heavy metals analysis using ICP-OES is seriously affected by calibration and standard solution preparation procedures. Instrument calibration was done using the standards before the determination of the sample. So, the instrument was calibrated using seven working standards and a blank solution. The stock solution which is 100 ppm of each metal was taken and 10 ppm was prepared as an intermediate for preparing the working standards. The standard working solutions of each metal were prepared freshly by diluting the intermediate standard solutions. That is, instrument calibration was done using the standards before the determination of the sample. By using the working standards, the instrument was calibrated with a good correlation coefficient. After making sure the instrument was calibrated correctly, the concentration of the metals in each sample was measured.

Statistical analysis

Nutritional quality data were analyzed with MS Excel 2007. An independent sample t-test using SPSS software version 22 followed by Tukey's post hoc multiple comparison tests was used to -check the statistical differences among quality indicator parameters of the samples from different types of finger millets. The t-value, P-value, mean difference and the standard error of the mean (S.E.M.) of the metal contents of black, mixed and white finger millet were taken from Finoteselam and Mankussa studying areas were evaluated by independent sample t-test at 95% confidence level. The results of all experiments were expressed as mean±S.D. of triplicate measurements. For comparison of the means of treatments, the Fisher's least significant difference (LSD) test was used at α = 0.05 significance level.

RESULTS AND DISCUSSION

Levels of metal contents in the analyzed finger millet samples

The results indicated that the samples had a variable concentration of analyte metals in three types of finger millet from the two study areas.

Table 1 indicates variation in heavy metal concentration in three types of finger millet. The manganese concentration was the highest of all the analyzed metals in all samples. Its mean concentration ranges from 2.3260 to 5.6510 mg/L, while Lead (ND to 0.024 mg/L) was the least mean concentration of all samples among the two study areas from the analyzed metals. Therefore, the trend of the analyzed metal concentration determined in all types of finger millet samples from the two study areas was almost Mn>Fe>Zn>Cu>Pb. So, The probable reasons for the variation of these metal concentrations in all three types of finger millet samples between the two study areas may be due to their genetic potential to absorb and accumulate these metals from the soil, the availability of the minerals in the soil that exist in usable forms, the degree of contamination of the soil with heavy metals, the use of different types of fertilizers, pesticides, herbicides and other chemicals, *etc*

Table 1: Average levels of selected heavy metals in powdered finger millet samples.

Analyte	Concentration (mg/l), SD and RSD (%)	Mankussa			Finoteselam		
		Black finger millet	Mixed finger millet	White finger millet	Black finger millet	Mixed finger millet	White finger millet
Mn	Mean (Mg/L)	3.7510	3.5730	2.3260	5.6510	3.0230	2.8695
	SD	0.0296	0.01815	0.01045	0.0157	0.0039	0.0108
	RSD	0.7900	0.5100	0.0450	0.2800	0.1300	0.3750
Fe	Mean (Mg/L)	1.6945	2.5785	2.2890	2.1955	2.1550	1.4120
	SD	0.0120	0.0152	0.0172	0.0246	0.0119	0.0106
	RSD	0.7250	0.5000	0.4425	0.6150	0.5750	0.0730
Cu	Mean (Mg/L)	0.0720	0.0585	0.0450	0.0435	0.0525	0.0525
	SD	0.0003	0.0002	0.0003	0.0004	0.0068	0.0004
	RSD	0.4200	0.2150	0.6250	0.8600	11.5950	0.7400
Zn	Mean (Mg/L)	0.4485	0.7990	0.5985	0.7740	1.2590	1.0735
	SD	0.0015	0.0025	0.0009	0.0011	0.0072	0.0032
	RSD	0.3450	0.3750	0.1550	0.1600	0.5650	0.5050
Pb	Mean (Mg/L)	0.0240	0.0240	0.0140	0.0110	-0.0140	-0.0245
	SD	0.0007	0.0007	0.0036	0.0005	0.0044	0.0005
	RSD	2.8800	1.4250	46.0650	5.6900	42.2950	1.9350

*Mean (mg/L)= Mean metal level of the analyte; SD= Standard deviation; RSD= Relative standard deviation.

(Chandravanshi and Feleke, 2015 and Yohannes and Chandravanshi, 2015).

Therefore, as shown in Table 2, an independent t-test at a 95% confidence level indicated that there were significant differences between the mean values of black finger millet samples in Mn, Fe, Cu, Zn and Pb, mixed finger millet samples in Mn, Zn and Pb, white finger millet samples in Mn, Fe, Cu and Pb. And there was no significance between the mean values of mixed finger millet in Fe, Cu and white finger millet in Zn in both studying areas.

Manganese status

The concentration of manganese in black finger millet from Mankusa varied from 3.693 to 3.820 mg/L with a mean value of 3.751 mg/L. Mixed finger millet ranges from 3.507 to 3.643mg/L with a mean value of 3.573 mg/L; in the white finger millet, it was 2.302 to 2.337mg/L with a mean value of 2.326 mg/L. In the case of Finoteselam, the concentration of black finger millet varied from 5.591 to 5.702 mg/L with a mean value of 5.651 mg/L. while the mixed finger millet was found to be from 2.953 to 3.097 mg/L with a mean value of 3.023 mg/L and in the white finger millet, it ranges from 2.856 to 2.886 mg/L with a mean value of 2.8695 mg/L. So, the concentration of Mn was found to be the highest of all heavy metals measured in finger millet samples. The pattern of the mean concentration of Mn in finger millet varieties was in the order of white finger millet<mixed finger millet< and black finger millet among the two study areas. From the analysis, as shown in Table 2, it was observed that the concentration of Mn with mean values of black, mixed and white finger millets from both study areas have significant differences ($P<0.05$). The highest and lowest mean values were recorded in Finoteselam black finger millet (5.651 mg/L) and Mankussa white finger millet (2.326 mg/L), respectively.

The result (2.326 to 5.651 mg/L) of Mn in this study was not agreed with the value (52±3mg/L to75.3± 0.3 mg/L) reported by (Desta and Bhagwan 2014). According to the safe limit adopted by FAO (2010), almost all of the finger millet samples in the two study areas were above the safe limit (2 mg/L). The finding of this study is in contrast to what is recommended by FAO. The daily intake amount of these metals differs from person to person based on the developmental levels, sex and the standards of the different countries they set. Therefore, According to U.S.A. standards, the R.D.I. of Mn is (2.3 mg/day) for matured adults and (2.6 mg/day) for lactating females, respectively. Because of the mean concentration of Mn in this study as compared to the FAO standard, most of the finger millet samples are not safe for consumption. But in the case of Mankussa and Finoteselam, white finger millets are safe for consumption.

Iron status

The concentration of Iron in Makussa, black finger millet, varied from 1.493 to 1.892 mg/L with a mean value of 1.695 mg/L and mixed finger millet ranged from 1.676 to 3.508 mg/L with a mean value of 2.579 mg/L and white finger millet samples vary from 1.488 to 3.082 mg/L with a mean value of 2.289 mg/L. But in the case of Finoteselam, it was 1.696 to 2.698 mg/L with a mean value of 2.196 mg/L, 1.910 to 2.389 mg/L with a mean value of 2.155 mg/L and 1.187 to 1.642 mg/L with a mean value of 1.412 mg/L for black, mixed and white finger millet samples respectively. So, the concentration of Fe was found to be the second highest of all heavy metals measured in finger millet samples. The pattern of the mean concentration of Fe in finger millet varieties was in the order of white finger millet < mixed finger millet < black finger millet in the Mankussa study area while in the case of Finoteselam, it was Black finger millet < white finger millet < mixed finger millet. From the analysis, it was observed that the concentration of Fe with mean values in mixed finger millets from both areas had no significant difference because the P value of the samples was $P=0.188$ which is greater than 0.05 at 95% confidence level while the concentration of Fe in black and white finger millets from both areas have significant difference because the P value of the sample was $P=0.011$ and $p=0.036$ respectively these P values are less than 0.05 at 95% confidence level. Fe's highest and lowest mean values were recorded in Mankussa mixed finger millet (2.5785 mg/L) and Finoteselam white finger millet (1.412 mg/L), respectively. The result (1.412 to 2.5785 mg/L) of Fe in this study was not agreed with the

Table 2: Pair-wise expression for various finger millet samples by independent samples t-test at 95% confidence level.

Study areas	Samples	Parameters* For expression	Metals to be analyzed				
			Mn	Fe	Cu	Zn	Pb
Mankussa and Finoteselam	Black finger millet	D_m	-1.900	-0.501	0.0287	-0.325	0.128
		t_p	0.000	0.011	0.000	0.021	0.000
		Conclusion	S	S	S	S	S
	Mixed finger millet	D_m	-0.5502	0.4272	0.0062	-0.459	0.378
		t_p	0.000	0.188	0.103	0.000	0.000
		Conclusion	S	NS	NS	S	S
	White finger millet	D_m	-0.5432	0.877	-0.0072	-0.476	0.375
		t_p	0.000	0.005	0.000	0.119	0.000
		Conclusion	S	S	S	NS	S

* D (m): differences between means; tp: P value at 95% confidence level; S: significant; NS: not significant.

value (156±18 mg/L to 775.3±42 mg/L) reported by (Birhanu *et al*., 2015). According to the permissible limit adopted by FAO (2010), almost all of the finger millet samples in the two study areas were below the safe limit (3.6 to 6.4 mg/L). The finding of this study is in contrast to recommend the value of FAO (2010). Because of the lower mean concentration of Mn, the finger millet is not safe for consumption. So, According to Vijayakumari (2003) finger millet is the richest source of iron. Iron deficiency leads to anemia, which can be overcome by introducing finger millet and other food items that can be rich in iron to our daily diet.

Copper status

When we looked at copper status in the two study areas, the concentration of Copper varied from 0.058 to 0.086 mg/L with a mean value of 0.072 mg/L, 0.053 to 0.064 mg/L with a mean value of 0.0585 mg/L and 0.043 to 0.047 mg/L with a mean value of 0.045 mg/L in black finger millet, mixed finger millet and white finger millet, respectively from Mankussa. while its concentration is also varied from 0.042 to 0.045 mg/L with a mean value of 0.044 mg/L,0.046 to 0.074 mg/L with a mean value of 0.0525 mg/L and 0.052 to 0.053 mg/L with a mean value of 0.0525 mg/L in Finoteselam black, mixed and white finger millet respectively. The trend of the mean concentration of Cu in finger millet varieties among the Mankussa study area was in the order of black finger millet > mixed finger millet >white finger millet. In contrast, in the case of Finoteselam, it was mixed finger millet = white finger millet >Black finger millet. There is a significant difference in the concentrations of Cu in black and white finger millets between both areas (p=0.000) each P values are less than 0.05 at 95% confidence level. While there is no significant difference in the concentration of Cu in mixed finger millet in both areas because the P value of the sample is p=0.103. This P value is greater than 0.05 at a 95% confidence level. The highest and lowest mean values of Cu are recorded in Mankussa black finger millet (0.072 mg/L) and Finoteselam black finger millet (0.0435 mg/L) respectively. The result (0.0435 to 0.072 mg/L) of Cu in this study is not agreed with the safe limit value of (30 mg/L) reported by FAO (2010). The daily intake amount of Cu differs from person to person based on the developmental levels, sex as well as the standards of the different countries they set. So, According to U.S.A. standards, R.D.I. of Cu is (0.9 mg/day) for matured adults and (1.3 mg/day) for lactating females respectively.

Zinc status

The Zinc concentration varies from 0.336 to 0.561 mg/L with a mean value of 0.449 mg/L,0.521 to 1.077 mg/L with a mean value of 0.799 mg/L and 0.514 to 0.682 mg/L with a mean value of 0.599 mg/L in Mankussa black, mixed and white finger millet samples respectively. while its concentration is varied from 0.410 to 1.139 mg/L with a mean value of 0.774 mg/L,1.152 to 1.365 mg/L with a mean value of 1.259 mg/L and 0.210 to 1.941 mg/L with a mean value of 1.0735 mg/L in Finoteselam black, mixed and white finger millet samples respectively. From the analysis, it was observed that the concentration of Zn with mean values in black and mixed finger millets from both areas have significant differences because the P value of the samples was p=0.021 and P=0.00 respectively each P values are less than 0.05 at 95% confidence level while the concentration of Zn in mixed finger millets from both areas have no significant difference because the P value of the sample was p=0.119)this P value is greater than 0.05 at 95% confidence level. In the present study, the concentration of zinc was found to be high in mixed finger millet from Finoteselam (1.259 mg/L), while a low concentration of zinc was observed in black finger millet from Mankussa (0.4485 mg/L). The result (0.4485 to 1.259 mg/L) of Zn in this study was not agreed with the value (15±3 to 24±3 mg/L) reported by (Birhanu *et al*.,2015). The permissible limit of Zn is 27.4 mg/L and the average daily intake of zinc is 7-16.3 mg per day, the recommended dietary allowance is 15 mg per day for men and 12 mg per day for women, almost all of the finger millet samples among the two study areas were found to be below the safe limit. As it falls below the safety limit, finger millet which contains Zn can be consumed in addition to other types of food which include Zn to obtain all of the following importance of Zn. which is essential in wound healing, nervous system, reproductive and immune systems, metabolic function, malaria treatment and as well as the treatment of diabetes Mellitus's (Djama *et al*., 2011).

Lead status

The concentration of Lead is varied from 0.023 to 0.024 mg/L with a mean value of 0.024 mg/L,0.022 to 0.026 mg/L with a mean value of 0.024 mg/L and 0.003 to 0.02 mg/L with a mean value of 0.014 mg/L in Mankussa black, mixed and white finger millet samples respectively. while its concentration is varied from 0.007 to 0.014 mg/L with a mean value of 0.011 mg/L,-0.018 to (-0.001) mg/L with a mean value of -0.014 mg/L and -0.025 to (-0.023) mg/L with a mean value of -0.0245 mg/L in Finoteselam black, mixed and white finger millet samples respectively. The pattern of the mean concentration of Pb in finger millet varieties was in the order of black finger millet = mixed finger millet >White finger millet in the Mankussa study area while in the case of Finoteselam, it was Black finger millet >white finger millet (N.D.)= mixed finger millet (N.D.). From the analysis, it was observed that the concentration of Zn with mean values in black, mixed and white finger millets from both study areas have significant differences because the P value of each sample was p=0.000 which is less than 0.05 at 95% confidence level. In the present study, the concentration of Mean values of Pb was found to be high in Mankussa black and mixed finger millet which is 0.024 mg/L for each, while a low concentration of mean values of Lead was observed in Finoteselam mixed and white finger millet (-0.014 and -0.0245 mg/L) respectively.

CONCLUSION

The elemental composition of Finger millet samples taken from two locations in Mankussa and Finoteselam like Fe, Cu, Zn and Pb are below the minimum tolerable limit given by WHO/FAO. It contains Mn that fits with the standards. The Finoteselam mixed and white finger millet sample does not contain Pb, since this metal is toxic and harmful to our biological system, its absence in the finger millet samples makes it good for health the soil is free from heavy metal contamination. Therefore, farmers should keep their land from contamination to produce crops that are not harmful to society.

Conflict of interest: None.

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