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ANALYSIS OF METAL CONTENT IN TURMERIC POWDER AVAILABLE IN THE SRI LANKAN MARKET

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ABSTRACT

Turmeric (*Curcuma longa* L*)* powder is a yellow coloured powder made by grinding of dried mature turmeric rhizomes. Turmeric is used in culinary preparations, cosmetic industries and medicinal preparations. It is available in Sri Lankan market both as branded and unbranded powders. This study was carried out to assess the levels of macro, micro and toxic heavy metals in some of those widely used branded and unbranded turmeric powders available in Sri Lankan market. Three batches from five different brands and an unbranded turmeric powder and a pure turmeric rhizome were subjected to the analysis. AOAC Official method of 975.03 (1996) was carried out to determine the metal content using Atomic Absorption Spectrometry (AAS). X-Ray Fluorescence Spectrometry (XRF) method was also carried out. The results were statistically analyzed using analysis of variance (ANOVA). According to the results, Potassium was the most abundant macro metal in turmeric. Except Iron the other micro metals were present in very low level in turmeric. Iron and Cadmium contents in some brands were significantly exceeded the maximum limit recommended by World Health Organization (WHO). There were significant differences among all the brands and unbranded turmeric powder in metal contents. The variation among batches in same brand for all the metals was not significant.

KEY WORDS: Turmeric powder, macro metals, micro metals, toxic heavy metals

INTRODUCTION

Spices are generally used to enhance the colour, flavor and the other organoleptic properties which increase the acceptability of food. Turmeric is a well known spice which is added to foods in cooking mainly as a coloring agent. It is produced from the dried rhizome of *Curcuma longa* L which belongs to the ginger family. Turmeric is widely distributed in tropical and subtropical regions especially in South and Southeast Asian countries, Africa and America. In Sri Lanka turmeric is cultivated in nearly every part of the island except in areas of very high elevations. The yellow colour of turmeric is due to a compound known as curcumin. Sometimes turmeric is contaminated with trace and heavy metals excessively. Regular usage of these contaminated turmeric result in accumulation of these metals in human organs and may results in serious health problems. Trace metals composition of foods are very important because of their essentiality or the toxic nature. Heavy metal contamination in the food chain is mainly caused by environmental pollution. Trace metal contents dependent upon their introduction in the growing, transport, processing and fortification of food and the levels vary according to the food item. In addition to these, the other processes which are used to convert the raw food to the final product can significantly increase the total trace metal contents of the food. There is very limited information about the heavy metal contamination of these spices. Since rice and curry is the main meal of countries like Sri Lanka, usage of spices like turmeric is relatively high in daily meals. Due to this high consumption it is important to estimate the toxic metal contents in spices. Sri Lanka is one of the major turmeric importing countries in the world and therefore ensuring the quality of imported turmeric is very important.

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Table 1: Metals in food

Macro metals	Micro metals	Toxic heavy metals
Na	Fe	Ni
Mg	Mn	Pb
K	Cu	Cd
Ca	Zn	
	Cr	

Table 2: Maximum limits for trace and toxic heavy In spices

Metal	Maximum Permissible Limit (µg/g)
Fe	300
Mn	100
Cu	20
Zn	50
Cr	30
Ni	50
Pb	10
Cd	0.2

Source: WHO (2005)

MATERIALS AND METHOD

Sample Selection

Five brands of turmeric powder samples namely A, B, C, D, E, and an unbranded sample were subjected to this study. Three batches from five different brands and an unbranded turmeric powder were purchased randomly from the Sri Lankan market within January to April in 2015. A genuine sample of turmeric obtained from turmeric rhizome was selected as the control. A total of fifty one samples (51) were collected (three from each type) and analyzed for metals using Atomic Absorption Spectroscopy (AAS). Totally seven samples, one sample from each brand, unbranded and pure rhizome were selected to determine the metal content using X-Ray Fluorescence (XRF) method.

Sample Preparation

Fifty one samples were subjected to wet digestion for metal analysis using atomic absorption spectroscopy. Dry ashing at 300 ^{0}C was carried out for seven samples which were analyzed using X-Ray Fluorescence method.

Atomic Absorption Spectrometric (AAS) analysis

AOAC – Official method 975.03 (AOAC, 1996)

This method is applicable to determine sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), copper (Cu), iron (Fe), zinc (Zn), chromium (Cr), nickel (Ni), lead (Pb) and cadmium (Cd) in food stuffs.

Heavy metals which are present in trace amounts were analysed using graphite furnace AAS at ppb level.

Table 3: Operating parameters of elements for flame

Element	Wave Length (nm)	Fuel Type	Slit Width (nm)	Fuel Flow Rate (L/min)
Na	589.6	Air-C_2H_2	1.0	0.9 - 1.2
Mg	285.2	Air-C_2H_2	0.5	0.9 - 1.2
K	766.5	Air-C_2H_2	1.0	1.1 -1.3
Ca	422.7	Air-C_2H_2	0.5	4.0 - 4.4
Fe	248.3	Air-C_2H_2	0.2	0.8 – 1.0
Mn	279.5	Air-C_2H_2	0.2	0.9 – 1.2
Cu	324.8	Air-C_2H_2	0.5	0.8 – 1.1
Zn	213.9	Air-C_2H_2	1.0	0.9 – 1.2

Table 4: Operating parameters of elements for graphite furnace AAS

Element	Wave Length (nm)	Slit Width (nm)	Furnace Temperature (^{0}C)
Cr	357.9	0.2	2500
Ni	232.0	0.2	2500
Pb	217.0	1.0	1200
Cd	228.8	0.5	900

Procedure

Accurately around 1 g of oven dried (105 ^{0}C for three hours) turmeric sample was weighed into a 150 mL Pyrex beaker. Then 10.0 mL of HNO_3 (1+1) was added and let soak thoroughly. Then 3.0 mL of 60% $HClO_4$ was added to it and heated on a hot plate placed in a fume hood, slowly at first, until frothing ceases. Heating was done until HNO_3 is evaporated at 150 ^{0}C. When it was charred, cooled and 10.0 mL of HNO_3 was added and continued heating. The sample was heated to white fumes of $HClO_4$. Then it was cooled and 10.0 mL of HCl (1+1) was added. The digested sample was quantitatively transferred to a 50.0 mL volumetric flask.

The above procedure was repeated without adding turmeric powder or rhizome to prepare the blank solution.

Determination

To the solution in 50.0 mL volumetric flask, 10 mL of 5% Lanthanum solution was added and diluted to volume using deionized water. It was set to

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settle silica and filtered through an acid washed No. 41 filter paper. Necessary dilutions were done with 10% HCl to obtain solutions within range of instruments. In the determination of potassium fifty time dilution was done. These prepared samples were stored in polypropylene bottles until the analysis by AAS.

Calculation

Element, ppm (μg/g) on dry basis=

$$\frac{\text{Reading (μg/mL)} \times 50.0\ \text{mL} \times \text{DF}}{\text{Dry Weight of sample (g)}}$$

DF=Dilution Factor

X-Ray Fluorescence Spectrometric (XRF) analysis

This method is applicable to potassium (K), calcium (Ca), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni) and lead (Pb).

Table 5: Operating parameters of XRF

Parameter	Value
Run time/s	500
X-Ray tube voltage/kV	40
X-Ray photon emitting current/mA	20
Tube current/mA	10
Detector voltage/V	-500
The target	Molybdenum

Procedure

Around 20 g of dried sample was accurately weighed into a crucible and it was incinerated at 300 ^{0}C in the muffle furnace for 16 hours. After ashing the total ash content was weighed.

Pellet preparation

Pressed pellet method was used to prepare pellets. Therefore accurately 5.0000 g of ash was weighed into a cleaned oil paper and transferred to a hard plastic mortar to ground to a uniform particle size. These ground samples were converted to pellets using a special apparatus called cylindrical pellet die. Samples were pressed using manually operated bench top pelletizing press under same conditions each time to obtain a hard pellet.

Determination

After switching on the instrument wait one hour to warm up before proceeding to stabilize the machine. The prepared pellets were kept on the sample holder of X-Ray Fluorescence spectrophotometer. Then the instrument was set up for the operating parameters as given in the Table 5 and the sample was run 500 seconds and spectrum of count per channel against channel number was obtained. The analysis was carried out for the above mentioned metals and they were identified by their characteristic fluorescent energy.

Calculation

Element, ppm (μg/g) on dry basis=

$$\frac{\text{Reading (μg/g)} \times \text{Total ash content (g)}}{\text{Dry weight of sample (g)}}$$

Statistical Analysis

The results were statistically analyzed using analysis of variance (ANOVA). Tuckey's pair wise comparison was used to compare the difference among brands in metal content, Dunnet's comparison was used to determine the differences of branded and unbranded with the pure sample and the equal variance test was used to determine the variation among the batches in same brand. Then one sample t test was used to determine whether the levels of these metals are exceeding the maximum permissible limit recommended by World Health Organization (WHO) or not. Finally to compare the results obtained by AAS and XRF whether they have a significant difference or not, the paired t test was used.

RESULTS AND DISCUSSION

The results that are given below were obtained from the analysis, which was carried out to quantify macro, micro and toxic heavy metals in branded and unbranded turmeric powder available in the Sri Lankan market. Therefore five different brands of turmeric powder, an unbranded turmeric powder and dried pure turmeric rhizome were analyzed. Three batches from each brand were subjected to the analysis. The metals Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Nickel (Ni), Chromium (Cr), Lead (Pb) and Cadmium (Cd) were determined by AAS and XRF methods.

Estimation of macro metals

The macro metals (Na, Mg, K and Ca) contents in batch wise and brand wise which were determined by AAS are listed in Table 6 and 7 respectively. The macro metals (K and Ca) which were determined by XRF are listed in table 8.

All the results of metal concentrations were

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expressed as µg/g on dry weight basis.

Table 6 : Macro metal content in turmeric powder by flame AAS{µg/g, Dry Weight (DW)}

Name of the sample		Na (µg/g)	Mg (µg/g)	K (µg/g)	Ca (µg/g)
Brand	Batch				
A	1	367.2±0.2 [a]	1600.5±0.2 [a]	36155.9±0.5 [a]	785.7±0.2 [a]
	2	371.7±0.2 [a]	1602.0±0.2 [a]	36155.8±0.3 [a]	785.7±0.1 [a]
	3	373.3±0.3 [a]	1597.9±0.2 [a]	36155.8±0.4 [a]	785.8±0.1 [a]
B	1	350.8±0.3 [b]	1810.7±0.3 [b]	41126.9±0.2 [b]	584.5±0.2 [b]
	2	354.3±0.3 [b]	1805.5±0.2 [b]	41126.9±0.3 [b]	584.4±0.2 [b]
	3	360.1±0.2 [b]	1796.7±0.3 [b]	41126.8±0.2 [b]	584.5±0.2 [b]
C	1	478.0±0.3 [c]	2200.4±0.2 [c]	42788.7±0.2 [c]	687.8±0.2 [c]
	2	465.6±0.2 [c]	2204.7±0.4 [c]	42788.7±0.2 [c]	687.7±0.2 [c]
	3	471.8±0.3 [c]	2195.9±0.2 [c]	42788.8±0.2 [c]	687.8±0.2 [c]
D	1	314.5±0.2 [d]	2070.7±0.2 [d]	42739.9±0.3 [d]	684.7±0.2 [d]
	2	317.5±0.2 [d]	2065.6±0.2 [d]	42740.0±0.2 [d]	684.7±0.2 [d]
	3	312.9±0.2 [d]	2073.9±0.2 [d]	42739.9±0.2 [d]	684.6±0.2 [d]
E	1	346.7±0.3 [e]	1980.1±0.3 [e]	41353.1±0.2 [e]	701.9±0.2 [e]
	2	343.9±0.2 [e]	1973.1±0.2 [e]	41353.5±0.3 [e]	701.9±0.2 [e]
	3	337.2±0.2 [e]	1981.8±0.4 [e]	41353.7±0.3 [e]	701.9±0.2 [e]

Results are means ± standard deviation of three determinations and calculated on a dry weight basis. Means within the same column that have no common letters are significantly different ($p < 0.05$).

Table 7: Average Macro metal content in different turmeric brands by flame AAS {µg/g, Dry Weight (DW)}

Brand	Na (µg/g)	Mg (µg/g)	K (µg/g)	Ca (µg/g)
A	370.7±2.8[a]	1600.2±1.8[a]	36155.8±0.4[a]	785.8±0.1[a]
B	355.1±4.1[b]	1804.3±6.1[b]	41126.9±0.2[b]	584.4±0.2[b]
C	471.8±5.4[c]	2200.3±3.8[c]	42788.8±0.2[c]	687.7±0.2[c]
D	315.0±2.1[d]	2070.1±3.7[d]	42739.9±0.2[d]	684.6±0.2[d]
E	342.6±4.2[e]	1978.3±4.0[e]	41353.4±0.3[e]	701.9±0.2[e]
Unbranded	363.0±0.2[f]	1746.1±0.2[f]	40616.6±0.3[f]	885.8±0.2[f]
Pure Rhizome	203.2±0.2[g]	1915.1±0.2[g]	25651.9±0.3[g]	869.9±0.2[g]

Results are means ± standard deviation of nine determinations and calculated on a dry weight basis. Means within the same column that have no common letters are significantly different ($p < 0.05$).

As revealed by observed results, mean value of Na content in different brands of turmeric powder and pure turmeric rhizome ranged between 203.3 - 471.8 µg/g on dry weight basis. The highest mean level of Na was found in brand C and lowest mean value was found in pure turmeric rhizome. In all the other samples the concentration ranged between 315 - 370 µg/g. According to the results grinding may increase the levels of Na in turmeric.

This may be happening due to the addition of salt in the grinding process. According to the Literature, Na levels in spices have been reported in the range of 30-4500 µg/g (Millican, 2012). In these studied samples of turmeric, it is an evident that these Na levels are in accordance with the values mentioned in the literature (Millican, 2012).

In case of Mg the highest mean concentration value was also found in the brand C and the lowest mean concentration in brand A. The Mg levels ranged between 1600.2 - 2200.3 µg/g in all the analyzed brands. Magnesium content of the pure rhizome was also within that range. According to these results grinding has no effect on the Mg content in turmeric. Obtained levels of Mg in the relevant turmeric samples were found to be within

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the range of 90-5520 µg/g which is also supported by the literature (Millican, 2012). Enzymes which are involved in biochemical reactions in the body such as protein synthesis, muscle and nerve function, blood glucose control contain magnesium as the co-factor (FAO/WHO, 1998).

Potassium is required for the normal functioning of the nerves and muscle, the sugar metabolism, acid-base balance and oxygen metabolism in the brain (Tolonen, 1990). Results indicated that K was the highest element detected in all brands and turmeric rhizome compared to the other metals. The K content of all the five brands and unbranded turmeric powder ranged from 36155.8 to 42788.8 µg/g. The highest mean level was found in brand C and the brand A was the lowest for K. However K content in pure turmeric powder was 25651.9 µg/g and it was lower compared to the other samples. Generally plant materials contain high levels of K. In addition, turmeric is subjected to high doses of nitrogen and K in a balanced manner to get a higher yield. When turmeric is cultivated industrially powder form or granular form of Muriate Of Potash (MOP) which contain more than 50% soluble K_2O was applied. That may be the reason for the higher K content in branded and unbranded turmeric powder compared to the pure turmeric rhizome as it was not industrially cultivated turmeric (Meerabai, 2000). According to the relevant literature K content in spices ranged from 270 -90260 µg/g which is justifying this study (Millican, 2012).

In case of Ca the situation is quite different as pure turmeric rhizome contained the second highest level (869.9±0.2 µg/g) of Ca. The highest mean concentration was found in unbranded turmeric powder and lowest concentration in brand B. The level was in the range between 584.4 - 885.8 µg/g. As revealed by the results there were not an effect on Ca content in the grinding process. Body use bone tissues as a Ca source. Bones and teeth store remaining 99% of body's Ca to maintain their structure and function.

Contents of Na, Mg, K and Ca in each turmeric powder brands, unbranded powder and pure rhizome by flame AAS are graphically presented in the Figures 1 and 2.

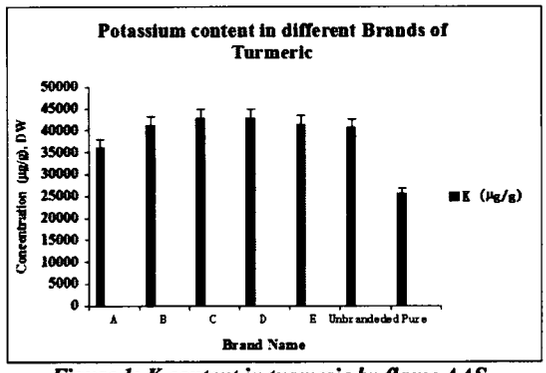

Figure 1: K content in turmeric by flame AAS

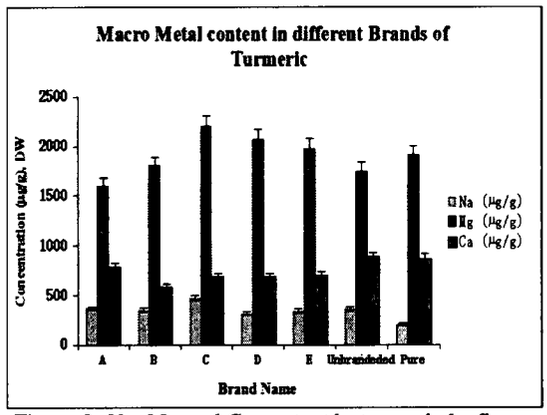

Figure 2: Na, Mg and Ca content in turmeric by flame AAS

Table8: Macro metal content in different turmeric brands by XRF {µg/g, dry weight (DW)}

Brand	K (µg/g)	Ca (µg/g)
A	36156.8±274.5	790.2±106.7
B	41130.0±221.6	585.2±47.4
C	42790.2±240.0	690.6±48.1
D	42740.0±266.7	692.0±59.4
E	41353.3±289.7	702.0±44.9
Unbranded	40616.6±284.2	886.6±54.3
Pure Rhizome	25652.3±207.0	870.7±66.5

As revealed by the results, practically these two methods gave very close values with minor differences. Statistically, according to the paired t test the results obtained by XRF method for K and Ca were similar (P> 0.05) to the results obtained by flame AAS for those metals.

According to the analysis of variance (ANOVA) at 95% confidence interval, there was a significant difference ($p<0.05$) among the mean K, Ca, Na and Mg contents of turmeric in different brands (Table 7). Using Tukey's pair wise comparison, the significant difference of metal contents among different brands was compared and there were

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significant differences for all macro metals among the all brands. According to the Dunnet's comparison method the all macro metals in branded and unbranded turmeric powder were significantly different from the macro metal content in pure turmeric rhizome. According to statistical analysis, macro metal content of all the branded turmeric powders exhibits an equal variance among batches (P>0.05).

Estimation of micro metals

The micro metal (Fe, Mn, Cu, Zn and Cr) content in batch wise and brand wise were determined by AAS were listed in Table 9 and 10 respectively. The micro metals which were determined by XRF were listed in Table 11.

Table 9: Micro metal content in turmeric powder by AAS {dry weight (DW)}

Name of the sample		Fe (µg/g)	Mn (µg/g)	Cu (µg/g)	Zn (µg/g)	Cr (µg/kg)
Brand	Batch					
A	1	297.5±0.1 [a]	22.0±0.2 [a]	2.0±0.1 [a]	9.2±0.1 [a]	59.0±0.1 [a]
	2	297.5±0.2 [a]	21.9±0.1 [a]	2.2±0.1 [a]	9.4±0.1 [a]	58.9±0.3 [a]
	3	297.5±0.1 [a]	21.9±0.1 [a]	1.8±0.1 [a]	9.4±0.1 [a]	58.9±0.2 [a]
B	1	230.7±0.2 [b]	20.2±0.1 [b]	3.4±0.1 [b]	10.2±0.1 [b]	138.5±0.1 [b]
	2	230.7±0.1 [b]	20.1±0.1 [b]	3.5±0.1 [b]	10.5±0.1 [b]	138.6±0.1 [b]
	3	230.8±0.1 [b]	20.3±0.1 [b]	3.5±0.1 [b]	10.4±0.1 [b]	138.6±0.2 [b]
C	1	276.2±0.1 [c]	19.7±0.1 [c]	4.1±0.1 [c]	10.9±0.1 [c]	53.9±0.2 [c]
	2	276.2±0.2 [c]	19.8±0.1 [c]	4.0±0.1 [c]	10.9±0.1 [c]	54.0±0.2 [c]
	3	276.1±0.1 [c]	19.8±0.1 [c]	4.1±0.1 [c]	11.0±0.1 [c]	53.9±0.2 [c]
D	1	277.1±0.2 [d]	27.6±0.2 [d]	3.6±0.1 [b]	12.4±0.1 [d]	49.8±0.1 [d]
	2	276.9±0.1 [d]	27.6±0.2 [d]	3.5±0.1 [b]	12.4±0.1 [d]	50.0±0.1 [d]
	3	277.1±0.2 [d]	27.5±0.2 [d]	3.5±0.1 [b]	12.5±0.1 [d]	49.9±0.2 [d]
E	1	219.9±0.1 [e]	14.8±0.1 [e]	3.2±0.1 [d]	10.3±0.1 [b]	52.8±0.2 [c]
	2	220.0±0.1 [e]	14.8±0.2 [e]	3.2±0.1 [d]	10.3±0.1 [b]	52.7±0.2 [c]
	3	220.0±0.2 [e]	14.7±0.1 [e]	3.2±0.1 [d]	10.3±0.1 [b]	52.8±0.2 [c]

Results are means ± standard deviation of three determinations and calculated on a dry weight basis. Means within the same column that have no common letters are significantly different (p <0.05).

Table 10:Average Micro metal content in different turmeric brands by AAS {dry weight (DW)}

Brand	Fe (µg/g)	Mn µg/g)	Cu µg/g)	Zn (µg/g)	Cr (µg/kg)
A	297.5±0.1[a]	21.9±0.1[a]	2.0±0.2[a]	9.3±0.1[a]	58.9±0.2[a]
B	230.8±0.1[b]	20.2±0.1[b]	3.5±0.1[b]	10.3±0.2[b]	138.6±0.1[b]
C	276.2±0.1[c]	19.8±0.1[c]	4.0±0.1[c]	10.9±0.1[c]	53.9±0.2[c]
D	277.0±0.1[d]	27.6±0.2[d]	3.5±0.1[b]	12.4±0.1[d]	49.9±0.2[d]
E	220.0±0.1[e]	14.8±0.1[e]	3.2±0.1[d]	10.3±0.1[b]	52.8±0.1[e]
Unbranded	461.7±0.2[f]	24.7±0.2[f]	4.5±0.1[e]	12.2±0.1[e]	56.3±0.1[f]
Pure	203.6±0.2[g]	21.6±0.2[g]	4.4±0.1[e]	17.0±0.1[f]	48.4±0.1[g]

Results are means ± standard deviation of nine determinations and calculated on a dry weight basis. Means within the same column that have no common letters are significantly different (p <0.05).

According to the results Fe content of turmeric samples ranged between 203.6 - 461.7µg/g. The highest mean concentration was found in the unbranded sample and the lowest mean concentration in pure turmeric rhizome. It indicates that all the turmeric powders contain higher Fe content than pure rhizome and there may be an addition of Fe up to a certain extent during the grinding process. This addition of Fe can be caused by the wearing of grinding equipment during the processing. Iron has a relatively high maximum permissible limit for spices (300 µg/g)

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as recommended by World Health Organization (WHO). Iron contents mentioned in the literature have been reported as 210.0 ± 2.3 µg/g dry weight in turmeric (Ibrahim, 2012). Iron is also added in the form of $FeSO_4$ at the planting of turmeric as a micro nutrient. According to the results in the Table 10 the unbranded turmeric powder contained more than permissible level of Fe and the brand A was in the marginal area but not exceeded the limit.

In case of Mn a high variation was not observed in the mean concentration for all turmeric samples ranging from 14.8 - 27.6 µg/g. The highest mean concentration of Mn was found in the brand D and the lowest content was in brand E. As revealed by the results the Mn content in turmeric were below the WHO limit of Mn 100 µg/g. Manganese contents in the literature have been also reported as 26.8±1.4 µg/g, dry weight in turmeric from Pakistan (Ibrahim, 2012).

When consider about Cu, the mean copper concentration was ranged between 2.0 - 4.5 µg/g in studied turmeric samples. Unbranded turmeric powder was containing the highest mean Cu content while the brand A was the lowest. The pure rhizome was the second highest for the Cu. According to the WHO regulations the maximum Cu level permitted for spices is 20 µg/g. The Cu levels of studied turmeric were within the specified limits. Copper contents mentioned in the literature have been reported as 23.8 ± 2.1µg/g, dry weight in turmeric, which is higher than the limit (Ibrahim, 2012). Recommended daily allowance of Cu is 2 mg. About 24-60% of Cu is orally absorbed. The amount of Cu in the diet and the competition with other metals such as Fe and Zn affect to the Cu absorption (Geiger, 2012).

In case of Zn, there was a small variation in concentration, ranging between 9.3 - 12.2 µg/g of studied turmeric samples except pure turmeric rhizome; pure turmeric rhizome contained a relatively high amount of Zn 17.0 µg/g. The lowest Zn content was in brand A. According to the literature the Zn content in spices are ranged from 10 -1010 µg/g (Millican, 2012). The experimental results were at the lower margin of that range.

At the time of planting turmeric, Zn may also be applied as a fertilizer in the form of $ZnSO_4$. It can be affected to the level of Zn in turmeric. The WHO limit of Zn is 50 µg/g. Therefore according to the study the concentration of Zn was below the limit and may be considered tolerable.

In case of Cr the concentrations were below the detection limit of flame AAS. Therefore Cr was analyzed by graphite furnace AAS. The highest mean concentration of Cr was 138.6 µg/kg found in the brand B. Mean Cr concentrations of other samples were ranging between 48.4 - 58.9 µg/kg. The lowest mean concentration was found in pure turmeric rhizome. Chromium particularly Cr (III) plays an important role in the body. It function in trace amount, however, it is toxic in excess amount (Mubeen, 2009). However, according to the literature Cr level is 16.0 ± 0.3 µg/g in turmeric (Ibrahim, 2012), which is higher than the levels resulted by this study. The WHO limit of Cr is 30 µg/g for spices. According to the results the levels of Cr in studied turmeric samples were in ppb range and it was very well below the limit. The possible explanation for the reduction of Cr in the turmeric rhizomes is due to the take up of Cr into their cells by the microbial bio fertilizers. Additionally, the reduced concentrations of Cr in the rhizome were possible because microbial bio fertilizers applied can able to resist or restrict the uptake of heavy metals by plant roots (Sumathi, 2012).

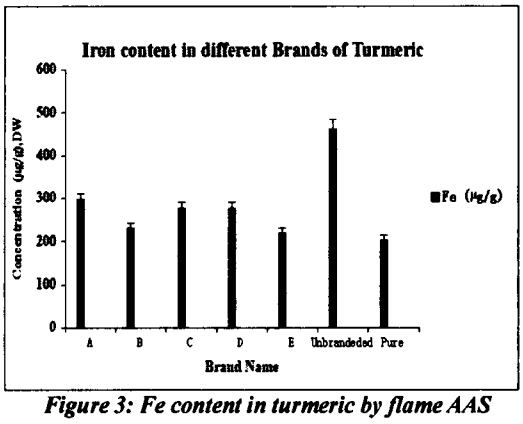

Figure 3: Fe content in turmeric by flame AAS

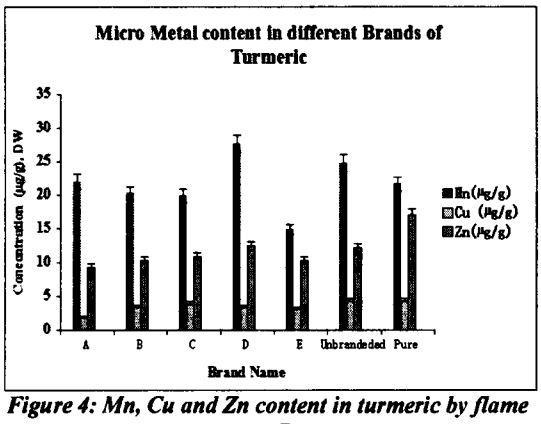

Figure 4: Mn, Cu and Zn content in turmeric by flame AAS

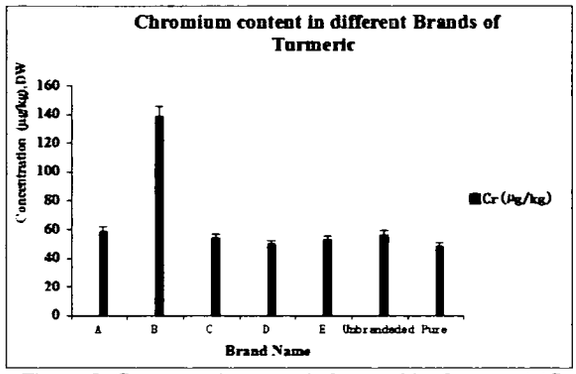

Figure 5: Cr content in turmeric by graphite furnace AAS

Table 11: Micro metal content in different turmeric brands by XRF {dry weight (DW)}

Brand	Fe (µg/g)	Mn µg/g)	Cu µg/g)	Zn (µg/g)	Cr (µg/g)
A	299.5±4.4	22.2±1.8	2.4±0.2	9.6±0.5	ND
B	235.2±3.9	21.0±1.4	3.6±0.3	10.6±0.4	ND
C	279.0±3.6	20.3±1.4	4.2±0.3	11.1±0.3	ND
D	279.7±3.4	30.0±1.6	3.5±0.3	12.5±0.4	ND
E	222.1±3.1	15.3±1.2	3.2±0.2	10.4±0.3	ND
Unbranded	464.0±5.2	24.8±1.5	4.7±0.3	12.2±0.5	ND
Pure	205.6±3.0	22.0±1.4	4.3±0.3	17.0±0.4	ND

ND-Not Detected

As depicted by the results, practically these two methods were giving very close values with small differences except Cr. XRF method is carried out in the range of ppm and the detection limits of Cr is 40 µg/g. However Cr was present in the studied turmeric in ppb range. Therefore Cr in turmeric cannot be determined by XRF.

Statistically according to the paired t test the results obtained by XRF method for Fe, Mn, Cu and Zn were similar (P> 0.05) to the results obtained by flame AAS for those metals.

According to the analysis of variance (ANOVA) at 95% confidence interval there were significant differences ($p<0.05$) among the mean Fe, Mn, Cu, Zn and Cr contents of different brands of turmeric. Using the Tukey's pair wise comparisons, the significant differences of metal contents among brands were compared and they are presented in Table 10. As revealed by the results brand B and C were not exhibited a significant difference in Cu content and in the same way brand B and E were not significantly different in Zn content. There were significant differences among all the brands and unbranded for the other metals. According to the Dunnet's comparison statistical analysis the brand E was not exhibited a significant difference with pure rhizome in Cu content. However, all the other micro metals are in branded and unbranded turmeric powder were significantly different from pure turmeric rhizome.

According to Bartlett's statistical analysis, micro metal content of all the branded turmeric powder has an equal variance among batches ($P>0.05$).

According to the one sample t test the Fe content in unbranded sample was significantly higher ($P<0.05$) than the maximum permissible limit of 300 µg/g recommended by WHO.

Estimation of toxic heavy metals

The toxic heavy metal content (Ni, Pb and Cd) of turmeric determined by graphite furnace AAS is given in the following table.

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Table 12: Toxic heavy metal content in turmeric powder by graphite furnace AAS (DW)

Name of the sample		Ni (µg/kg)	Pb (µg/kg)	Cd (µg/kg)
Brand	Batch			
A	1	44.4±0.1 [a]	ND	33.7±0.1 [a]
	2	44.4±0.2 [a]	ND	33.8±0.2 [a]
	3	44.3±0.2 [a]	ND	33.8±0.2 [a]
B	1	15.2±0.2 [b]	ND	423.3±0.2 [b]
	2	15.2±0.3 [b]	ND	423.2±0.2 [b]
	3	15.2±0.2 [b]	ND	423.4±0.1 [b]
C	1	58.9±0.2 [c]	247.2±0.2 [a]	788.0±0.2 [c]
	2	59.0±0.2 [c]	246.4±0.1 [a]	788.1±0.2 [c]
	3	58.8±0.1 [c]	248.0±0.2 [a]	788.0±0.2 [c]
D	1	64.3±0.2 [d]	ND	23.2±0.1 [d]
	2	64.2±0.2 [d]	ND	23.0±0.2 [d]
	3	64.3±0.2 [d]	ND	23.1±0.3 [d]
E	1	79.0±0.2 [e]	ND	126.7±0.2 [e]
	2	78.9±0.1 [e]	ND	126.8±0.1 [e]
	3	79.0±0.2 [e]	ND	126.8±0.2 [e]

Results are means ± standard deviation of three determinations and calculated on a dry weight basis. Means within the same column that have no common letters are significantly different ($p < 0.05$).

Table 13: Average toxic heavy metal content in different turmeric brands by graphite furnace AAS

Brand	Ni (µg/g)	Pb (µg/kg)	Cd (µg/kg)
A	44.4±0.1[a]	ND	33.7±0.1[a]
B	15.2±0.2[b]	ND	423.3±0.2[b]
C	58.9±0.2[c]	247.2±0.7 [a]	788.0±0.2[c]
D	64.3±0.2[d]	ND	23.1±0.2[d]
E	78.9±0.1[e]	ND	126.7±0.1[e]
Unbranded	56.2±0.2[f]	ND	34.4±0.2[f]
Pure	14.4±0.1[g]	380.2±0.3 [b]	195.3±0.2[g]

Results are means ± standard deviation of nine determinations and calculated on a dry weight basis. Means within the same column that have no common letters are significantly different ($p < 0.05$).

As revealed by the results mean Ni concentration of turmeric ranged between 14.4 – 78.9 µg/kg. The highest mean level of Ni was found in the brand E and the lowest mean value was found in pure turmeric rhizome. Nickel accumulates in soil primarily through the disposal of industrial effluents, sewage sludge and fertilizers. Even moderate concentrations of this metal can severely limit the growth of plants. *Azopirillum sp* treatment was remarkably effective to reduce Ni concentration in turmeric rhizomes (Sumathi, 2012). The maximum level of Ni permitted for spices is 50 µg/g and compared to the levels in studied turmeric this limit is very high.

As revealed by the received data the highest mean Pb concentration was found in pure turmeric rhizome and the value was 380.2 µg/kg. Except the pure sample only brand C contained Pb to a level of 247.2 µg/kg. Lead was not detected in other turmeric brands even by graphite furnace under detection limit of 0.03 µg/kg. According to WHO specifications the maximum permissible level of Pb for spices is 10 µg/g and the analyzed turmeric contained Pb very well below the limit. In the literature the reported value for Pb in turmeric from Sri Lankan market was 26 µg/kg (Senanayake, 2013). Certainly, there is a reduction in Pb concentrations when treated with microbial bio fertilizers. That is because the micro organisms have the potential to reduce the Pb content in the rhizosphere soil (Sumathi, 2012). To the greater extent, the Pb concentrations were less when

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treated with *Azospirillum sp* in turmeric. The bio fertilizer treatments also have the potential to reduce the Pb accumulation inside the turmeric rhizome. Lead is heavy metal poison which forms complexes with oxo- groups in enzymes to affect virtually all steps in the process (Sumathi, 2012).

In the case of Cd there was a large variation in concentration among the turmeric brands. The concentration ranged between 33.7 - 788.0 µg/kg. The highest mean concentration of Cd was found in brand C and the lowest mean concentration was found in brand A. The concentration of Cd in brand B was 423.3 µg/kg, which was the secondly highest brand. Both these brands C and B were exceeding the 200 µg/kg which is the maximum permissible level of Cd recommended by WHO.

In addition, the pure turmeric rhizome was also in the marginal area and the value was 195.3 µg/kg. In this study, levels of Cd in brands A, D, E and unbranded were lower than WHO limits. The high level of Cd might be due to the use of Cd containing fertilizers. According to the literature the Cd level of turmeric from Pakistan market was 12.00 ±0.01 µg/kg, which is lower than this study. The presence of heavy metals in rhizosphere soils and turmeric rhizomes proves that due to rapid industrialization, heavy metals have been a regular and deliberate constituent in agricultural products and environment. Rhizome containing species were found to be most susceptible food groups contaminated with heavy metals. Usually the problem arises when the concentration of these heavy metals increased than the permissible limits (Sumathi, 2012).

Contents of Ni, Pb and Cd in each turmeric powder brands, unbranded powder and pure rhizome by graphite furnace AAS are graphically presented in Figure 6.

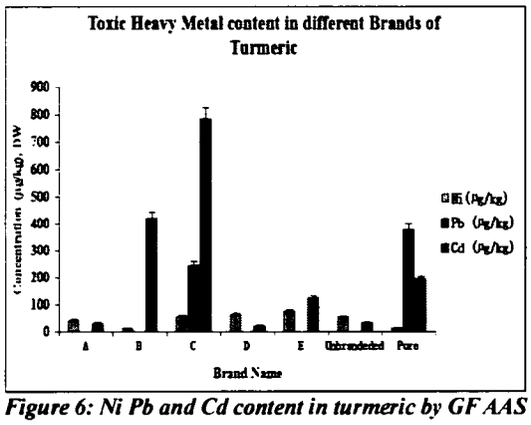

Figure 6: Ni Pb and Cd content in turmeric by GF AAS

Table 14: Average toxic heavy metal content in different turmeric brands by XRF

Brand	Ni (µg/g)	Pb (µg/g)
A	ND	ND
B	ND	ND
C	ND	ND
D	ND	ND
E	ND	ND
Unbranded	ND	ND
Pure	ND	ND

As revealed by results both Ni and Pb were not detected in studied turmeric by XRF. Both Ni and Pb were not in the range of ppm in turmeric and they were in samples ppb level. The detection limit for both Ni and Pb is 3.0 µg/g in XRF. Therefore both Ni and Pb cannot be detected by XRF.

According to the analysis of variance (ANOVA) at 95% confidence interval there were significant differences ($p<0.05$) among the mean Ni and Cd content of different brands of turmeric. According to the Tukey's pair wise comparisons, the significant difference in Ni and Cd contents was existed among turmeric brands and the difference is presented in the Table 13. According to the Dunnet's comparison statistical analysis the brand C exhibited a significant difference with pure rhizome in Pb content and the Pb was not detected in the other brands. Nickel and Cadmium in branded and unbranded turmeric powder was significantly different from pure turmeric rhizome. According to statistical analysis, toxic heavy metal content of all the branded turmeric powder has an equal variance among batches ($P>0.05$. According to the one sample t test the Cd content in turmeric powder of brand B and C were significantly higher ($P<0.05$) than the maximum permissible limit of 200 µg/kg recommended by WHO.

CONCLUSION AND FURTHER WORK

On the basis of analyzed results it can be concluded that the majority of widely used turmeric brands in Sri Lanka are not contaminated with heavy metals except a few cases. The contents of toxic metals in turmeric were generally found to be low. According to the statistical analysis only Cd content in few brands of turmeric exceeded the maximum permissible limit significantly recommended by WHO and the exceeding values were 423.3 µg/g and 788.0 µg/g. Lead was not present in most of the turmeric brands except only one brand.

The content of micro metals in turmeric was generally found to be low except Fe. It was evident that the increment of Fe content in turmeric powder was happened during the grinding process. Even though there was high level of Fe content in all the studied samples, statistical analysis proves that only the unbranded sample exceeded the maximum permissible limit significantly as the value 461.7 µg/g. The other micro metals were present in turmeric below the maximum limit.

The analyzed turmeric samples were shown to be elevated in K content and the values ranged from 36155.8 to 42788.8 µg/g. In addition as a plant material, K is introduced to the turmeric rhizome through fertilizers. Among the macro metals Na was the lowest in turmeric and powdered turmeric contains a higher content of Na ranged from 315.0 to 471.8 µg/g than pure rhizome (203.2 µg/g). As reported in Fe, addition of Na is also occurred in the grinding process may be as salts.

As depicted by the statistical analysis it can be concluded that there was no variation in the contents of macro, micro and toxic heavy metal levels among the batches of each brand where as a significant variation was existed among individual brands. According to the statistical analysis the results given by AAS and XRF were not significantly different.

Finally it is worth to mention that the samples reported with high levels of Cd and Fe has to be subjected to more research studies. In order to eradicate the possible metal contamination it is vital to identify the possible causes by extensively studying each unit operation starting from the soil where these turmeric is cultivated and up to the level of final packaging. There after the future research should be directed towards elimination of identified root causes.

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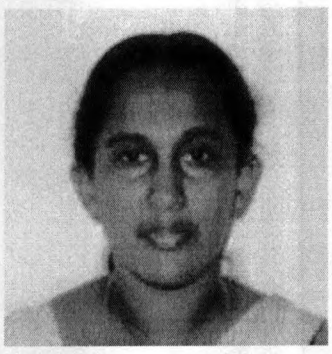

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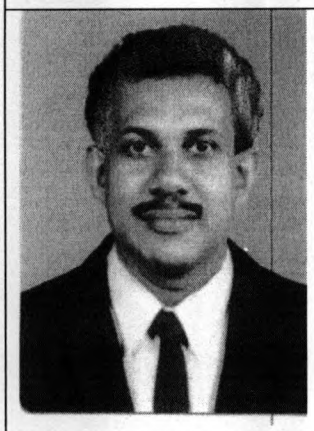

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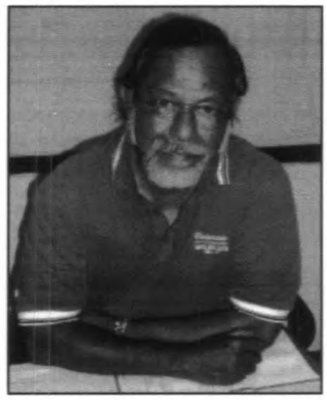

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