

**INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH  
TECHNOLOGY****ANALYSIS OF TRACE METAL CONTENT IN CHILLIE POWDER AVAILABLE IN  
THE SRI LANKAN MARKET****W.A.A.V.S. Amaradivakara\*, L. Wickramasinghe, R.M.G.B. Rajanayake, A. Bamunuarachchi**  
Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri  
Jayewardenepura, Sri Lanka**ABSTRACT**

Chillie (*Capsicum annum* L.) powder is the dried, pulverized fruit of chillies and used as a spice to add pungency flavour to the dish. This study was conducted to investigate the trace metal content in chillie powder samples commonly available in the Sri Lankan market. Five brands of chillie powder samples named A, B, C, D and E, and an unbranded sample were selected for this study. Three batches from each brand and three samples from each batch were purchased. An ungrounded whole dry chillie sample was selected as the control. The trace metal content was determined using AAS and XRF spectrometric techniques. Results were statistically analyzed using one-way ANOVA, at 0.05 probability level with MINITAB-14 software package. According to this study, there was no significant difference between the batches in each brand in mean metal contents. But there were significant differences among the brands in mean metal contents. The mean Fe content of brand A, brand B and unbranded samples were significantly higher than the WHO maximum permissible limit. This may be due to the metal (Fe) contamination during the grinding process. Thus brand A, B and unbranded samples are not suitable for daily consumption.

**KEYWORDS:** Chillie powder, Trace metals.**INTRODUCTION**

Chillie crop is one of the most important commercial spice crops. Botanical name of chillie is *Capsicum annum* L. There are more than 400 different varieties of chillies found all over the world. Chillie belongs to the genus capsicum, under the solanaceae family. Chillies occur referred to as chillies, hot peppers, bell peppers, red peppers, pod peppers, cayenne peppers, paprika, pimento, and capsicum in different parts of the world (MAGI, 2009).

Currently, chillies are used throughout the world as a spice and also in the making of beverages and medicines. Some varieties of chillies are famous for red colour because of the pigment 'capsanthin,' others are known for biting pungency attributed to 'capsaicin'. Chillie is very important as an anti-oxidant and anti-inflammatory agent. Chillie has become an essential ingredient in Sri Lankan meals. Per capita consumption of chillie in the form of dry chillie is estimated 2.32 kg per annum and the national annual requirement of dry chillie is around 42,634 Mt. The annual production of dry chillie in Sri Lanka is about 18,616 Mt, therefore, an amount of 31,242 Mt is being imported (Year 2007 figures). Chillie is mainly imported from India, as dry chillie (DASL, 2006).

In the chillie powder manufacturing process in Sri Lanka dried chillies are cleaned manually to remove impurities and then washed. After drying them, they are pulverized in two or more grinders to convert them into the powder form. Then chillies in powder form are passed through sieves to obtain uniform mesh size (45-65 mesh). Some manufactures use metal detectors to detect and remove metal particles from the ground chillie. Finally, packing is done in polythene bags using automated powder fillers, and bags are sealed. Due to major part of chillie being imported from India, many branded chillie powders available in the Sri Lankan market are products of imported dry chillies from India. The main environmental pollutants in whole chillie are thought to be toxic trace metals from agronomic sources particularly agricultural, chemicals and processing operations. Some of the important trace metal pollutants are lead, copper, arsenic, tin, zinc, cadmium, mercury and methyl mercury. Thus imported chillie may contain these toxic metals. When processing these chillie within Sri Lanka to produce powdered chillie, trace amounts of metals may be added during the manufacturing process.

The term "trace metals" refers to the metals which may be present in foods in amounts well below 50 mg/kg and which have some toxicological or nutritional significance (FSSAI, 2012). Iron (Fe) is one of the most investigated and best understood of nutrients. The metal Fe is essential for oxygen transport, electron transfer reactions, gene regulation and regulation of cell growth and differentiation (Ranaweera, 2007). Excess Fe intake can cause the

proliferation of foreign bacteria in the gut and producing discomfort from gas and bloating. Symptoms of Fe toxicity are vomiting, diarrhea and intestinal damage (Deshpande and Dekker, 2002). Copper (Cu) is a micronutrient required by our body. It is involved in the absorption, storage and metabolism of Fe. Manganese (Mn) is a vital trace element necessary in the metabolism and involved in antioxidant activities, maintaining fertility, forming strong healthy bones, nerves and muscles. Zinc (Zn) helps to maintain normal cell-mediated immune function and is present in all cells, particularly in the nucleus. Chromium (Cr) is an essential nutrient and a trace element required for normal sugar and fat metabolism.

Nickel (Ni) exposure introduces free radicals which lead to oxidative damage and may also affect the kidneys and liver. The trace metal Ni has been identified as a toxin that severely damages reproductive health and can lead to infertility, miscarriage, birth defects, and nervous system defects. Although lead (Pb) may have beneficial effects in small amounts, Pb toxicity is of more concern than Pb deficiency. Plant foodstuffs grown under high Pb conditions can include Pb and Pb toxicity results in anaemia, kidney damage and central nervous abnormalities (Ranaweera, 2007). Cadmium (Cd) is a relatively toxic metal, which soon after ingestion is liable to cause acute gastritis with vomiting and diarrhea. The toxic metal Cd can be included in plant foods, those grown on high Cd soils, and Cd has a long half life in the body and thus high intakes can lead to accumulation resulting in damage in some organs, especially the kidney (Ranaweera, 2007).

In Sri Lankan standards, maximum permissible limits for trace metals in chillie powder or even in spices have not been established yet. Hence, WHO standards in 2005 have to be considered. According to the WHO standards in 2005, the maximum permissible limits of metals in spices are summarized in the Table 1.

*Table 1: Maximum permissible limits of metals in spices*

Metal	Maximum permissible limit (in ppm)
Iron	300
Copper	20
Manganese	100
Zinc	50
Chromium	30
Nickel	50
Lead	10
Cadmium	0.2
Mercury	0.5

Source: WHO (2005)

Many research have been carried out globally to investigate the trace metal content in common spices, including chillie powder, available in foreign markets. Hisfa *et al* (2009) investigated the concentrations of some heavy metals such as iron, copper, chromium, lead, cadmium and cobalt present in common spices of two brands widely used in Pakistan. Baley (2014) has determined the lead, cadmium and chromium content in Ethiopian spices. But in Sri Lanka, there is lack of research about this area. Hence it is very important to carry out such a study to investigate trace metal content in chillie powder, commercially available in the Sri Lankan market. Thus the major objective of this study was to determine the content of some selected trace metals in chillie powder. The specific objectives of this study were to determine the Fe, Mn, Cu, Zn, Cr, Ni, Pb and Cd content in chillie powder available in the Sri Lankan market, using selected brands; to check whether these metal contents are within the standard limits (according to WHO standards); to find out whether there is a significant difference in metal content among the brands, with the unbranded sample and with the control; and to find out whether there is a significant difference among batches in a same brand.

## MATERIALS AND METHODS

### Sample selection

Five brands of chillie powder samples named A, B, C, D and E, and an unbranded sample were selected for this study. Three batches from each brand and three samples from each batch were purchased from the local markets in

Sri Lanka, within the time period of January to April in 2015. Three samples of the unbranded sample were also collected. An ungrounded whole dry chillie sample was selected as the control.

#### Sample preparation for atomic absorption spectrometric analysis

Wet digestion method was carried out according to the AOAC official method 975.03 (AOAC, 1996).

##### Method

Chillie powder sample was placed in a porcelain dish and oven dried at 105 °C for 2 hours, until obtaining a constant weight. Accurately about 1 g of oven dried chillie powder sample was weighed into a 150 mL pyrex beaker. Then 10 mL of HNO<sub>3</sub> (1+1) was added. It was allowed to soak thoroughly and 3 mL of 60% HClO<sub>4</sub> was added. Then it was heated on a hot plate, placed in a fume hood, slowly at first, until frothing ceased. The heating was carried out at 150 °C, until HNO<sub>3</sub> was almost evaporated. Then it was cooled and 10 mL of HNO<sub>3</sub> was added again and continued heating due to the charring of the sample. The addition of 10 mL of HNO<sub>3</sub> and heating were repeated another four times, until obtaining a clear solution, without charring. It was heated to white fumes of HClO<sub>4</sub>; cooled; 10 mL of HCl (1+1) was added and quantitatively transferred into 50 mL volumetric flask and diluted to volume using de-ionized water. Then it was filtered through an acid washed No. 41 filter paper after discarding the first few drops of filtrate. This digested solution was stored in a polypropylene bottle for the AAS analysis. A blank was prepared by following all the above steps, at the same conditions, without adding the sample

#### Atomic absorption spectrometric (AAS) analysis

##### Flame AAS analysis - Method

The standard series of each metal was prepared, using the stock solutions. The metals in the digested solutions of chillie powder samples, namely Fe, Mn, Cu, Zn and Cr were analyzed using the flame atomic absorption spectrometric technique. This metal analysis was performed using Thermo Scientific, iCE 3000 series model double beam atomic absorption spectrometer, with deuterium background correction. The instrument was set up using the operating parameters given in the Table 2. The Solaar software was used to run the programme. Hollow cathode lamp with each metal was used at the specific wavelength.

Table 2: Operating parameters of element for flame AAS

Element	Wave length (nm)	Flame type	Fuel flow rate (L/ min)	Lamp current (mA)	Slit width (nm)
Fe	248.3	Air / acetylene	0.8 – 1.0	5	0.2
Mn	279.5	Air / acetylene	0.9 – 1.2	5	0.2
Cu	324.8	Air / acetylene	0.8 – 1.1	4	0.5
Zn	213.9	Air / acetylene	0.9 – 1.2	5	1.0
Cr	357.9	Nitrous oxide / acetylene	4 - 4.4	7	0.2

The calibration curve was prepared by aspirating the standard series. Then digested solutions of chillie powder samples were aspirated and the concentration was read from the plot of absorbance against concentration (µg/mL). The prepared blank was used to establish the 0 absorption point. The burner was flushed off with de-ionized water between samples.

##### Graphite furnace AAS analysis - Method

The master standard of each metal was prepared, using the stock solutions. The metals Ni, Pb and Cd in the digested solutions of chillie powder samples, were analyzed using the graphite furnace atomic absorption spectrometric technique in the Thermo Scientific, iCE 3000 series model double beam atomic absorption spectrometer, with deuterium background correction. The instrument was set up using the operating parameters given in the Table 3

Table 3: Operating parameters of element for graphite furnace AAS

Element	Wave length (nm)	Lamp current (mA)	Slit width (nm)	Atomize temperature (°C)
Ni	232.0	4	0.2	2500
Pb	217.0	5	1.0	1200
Cd	766.5	5	1.0	900

The samples were loaded into the autosampler. The master standard, blank and de-ionized water were loaded into the R1, R2 and R3 positions of the autosampler respectively. The standard series was prepared by the instrument using the master standard and established a calibration curve of absorbance against concentration (µg/L), to read the concentrations of the samples.

*Determination of the original metal content in the sample on dry basis*

$$\text{Metal content in the original sample (}\mu\text{g/g, DW)} = \frac{C \times V}{W}$$

Where,

C = Concentration of the metal given by AAS reading (µg/mL or µg/L)

V = Final volume of the digested sample (mL)

W = Weight of the dried sample (g)

**Sample preparation for X-ray fluorescence spectrometric analysis**

For the sample preparation, palletizing technique was used, after dry ashing the sample at 300 °C, according to the validated method of Atomic Energy Board of Sri-Lanka.

*.Method*

Chillie powder sample was placed in a silica dish and oven dried at 105 °C for 2 hours, until obtaining a constant weight. Accurately about 20 g of oven dried chillie powder sample was weighed into another silica dish and ashed in the muffle furnace (Thermolyne, F62730) at 300 °C for 16 hours. Then the dish was weighed again to obtain the ash weight. From the ashed sample, 0.50 g of ash was weighed into a clean oil paper. Then it was transferred into a hard plastic mortar and ground into a homogeneous powder. Then this powder was transferred into a cylindrical pellet die, as evenly distributed and pressed using a manually operated bench top pelletizing press (Spectro Press, 4312 E) to form a supported pellet which ideally had a smooth, homogeneous sample surface and good physical stability.

**X-ray fluorescence (XRF) spectrometric analysis**

This method was used to analyze Fe, Mn, Cu, Zn, Cr, Ni and Pb contents in the chillie powder samples. One sample from the each batch, with the unbranded sample and the control was analyzed.

*Method*

The instrument was switched on and wait one hour to warm up before proceeding, to stabilize the machine. The prepared pellets were loaded into the sample holder. The instrument was set up using the operating parameters given in the Table 4. The AXIL software was used to run the programme.

*Table 4: Operating parameters for XRF*

Operating parameter	
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
Target	Mo

The sample was run 500 seconds using the molybdenum target and the spectrum of count per channel against channel number was obtained. The metals were identified using their characteristic fluorescent energy value. The quantification of identified metals was carried out by the calibration curve method, obtained with many standard samples.

*Determination of the original metal content in the sample on dry basi*

$$\text{Metal content in the original sample (}\mu\text{g/g, DW)} = \frac{C \times W_a}{W_t}$$

Where,

C = Concentration of the metal given by XRF (µg/g)

Wt = Weight of the dried sample (g)

Wa = Weight of the ash obtained (g)

#### Statistical analysis

Results were statistically analyzed using one-way analysis of variance (ANOVA), at 0.05 probability level with MINITAB-14 software package. The Bartlett's test (normal distribution) was performed to check whether there is any significant difference of mean metal content, between the batches in each brand. One-way ANOVA was used to check whether there is a significant difference among the brands in mean metal contents. Tukey's pairwise comparison was performed to select the brand pairs which are significantly different in mean metal contents. Dunnett's comparison test was performed to check whether selected chillie powder samples are significantly different from the control sample in the mean metal content. To statistically analyze whether the metal contents are significantly higher than the WHO maximum permissible limit, one sample t test was performed. The results of metals obtained by the AAS and XRF techniques were compared using the paired t test, to check whether there is any significant difference between these two methods.

## RESULTS AND DISCUSSION

In this study, trace metal content of chillie powder samples was analyzed using selected five brands of chillie power samples and an unbranded sample, commonly available in the Sri-Lankan market. An ungrounded whole dry chillie sample was selected as the control.

#### Micro metals in chillie powder

The micro metals in chillie powder samples were analyzed using flame AAS and XRF spectrometric techniques. Table 5 shows the mean Fe, Mn, Cu, Zn and Cr contents in each batch of five chillie powder brands, obtained using the flame AAS technique. The results are expressed as  $\mu\text{g}$  metal /g of dried chillie powder (ppm).

Table 5: Mean micro metal content in chillie powder samples (flame AAS) – batchwise

Brand	Batch	Mean metal content ( $\mu\text{g/g}$ , DW)				
		Fe	Mn	Cu	Zn	Cr
A	1	729.5 $\pm$ 0.9 <sup>a</sup>	19.9 $\pm$ 0.06 <sup>a</sup>	5.9 $\pm$ 0.1 <sup>a</sup>	12.8 $\pm$ 0.3 <sup>a</sup>	11.1 $\pm$ 0.2 <sup>a</sup>
	2	727.0 $\pm$ 0.5 <sup>a</sup>	19.6 $\pm$ 0.1 <sup>a</sup>	5.5 $\pm$ 0.1 <sup>a</sup>	12.7 $\pm$ 0.3 <sup>a</sup>	11.2 $\pm$ 0.2 <sup>a</sup>
	3	728.5 $\pm$ 0.7 <sup>a</sup>	19.8 $\pm$ 0.2 <sup>a</sup>	5.5 $\pm$ 0.2 <sup>a</sup>	12.5 $\pm$ 0.1 <sup>a</sup>	11.6 $\pm$ 0.1 <sup>a</sup>
B	1	618.7 $\pm$ 0.8 <sup>b</sup>	18.0 $\pm$ 0.2 <sup>b</sup>	10.3 $\pm$ 0.2 <sup>b</sup>	18.8 $\pm$ 0.2 <sup>b</sup>	17.1 $\pm$ 0.1 <sup>b</sup>
	2	614.5 $\pm$ 0.7 <sup>b</sup>	17.7 $\pm$ 0.2 <sup>b</sup>	10.2 $\pm$ 0.2 <sup>b</sup>	18.7 $\pm$ 0.2 <sup>b</sup>	16.7 $\pm$ 0.2 <sup>b</sup>
	3	616.5 $\pm$ 0.7 <sup>b</sup>	17.9 $\pm$ 0.2 <sup>b</sup>	10.3 $\pm$ 0.3 <sup>b</sup>	18.7 $\pm$ 0.2 <sup>b</sup>	17.0 $\pm$ 0.06 <sup>b</sup>
C	1	167.5 $\pm$ 0.7 <sup>c</sup>	12.4 $\pm$ 0.06 <sup>c</sup>	8.7 $\pm$ 0.2 <sup>c</sup>	16.7 $\pm$ 0.2 <sup>c</sup>	18.1 $\pm$ 0.1 <sup>c</sup>
	2	164.8 $\pm$ 0.7 <sup>c</sup>	12.2 $\pm$ 0.2 <sup>c</sup>	8.6 $\pm$ 0.2 <sup>c</sup>	16.7 $\pm$ 0.2 <sup>c</sup>	17.8 $\pm$ 0.06 <sup>c</sup>
	3	166.6 $\pm$ 0.7 <sup>c</sup>	12.4 $\pm$ 0.2 <sup>c</sup>	8.3 $\pm$ 0.2 <sup>c</sup>	16.7 $\pm$ 0.2 <sup>c</sup>	17.6 $\pm$ 0.1 <sup>c</sup>
D	1	199.4 $\pm$ 0.9 <sup>d</sup>	10.5 $\pm$ 0.2 <sup>d</sup>	5.1 $\pm$ 0.1 <sup>d</sup>	10.8 $\pm$ 0.2 <sup>d</sup>	18.3 $\pm$ 0.06 <sup>d</sup>
	2	196.9 $\pm$ 0.3 <sup>d</sup>	10.4 $\pm$ 0.2 <sup>d</sup>	5.1 $\pm$ 0.2 <sup>d</sup>	10.7 $\pm$ 0.2 <sup>d</sup>	18.1 $\pm$ 0.1 <sup>d</sup>
	3	197.6 $\pm$ 0.6 <sup>d</sup>	10.5 $\pm$ 0.2 <sup>d</sup>	5.2 $\pm$ 0.06 <sup>d</sup>	10.6 $\pm$ 0.2 <sup>d</sup>	18.5 $\pm$ 0.06 <sup>d</sup>
E	1	84.2 $\pm$ 0.4 <sup>e</sup>	9.2 $\pm$ 0.2 <sup>e</sup>	6.0 $\pm$ 0.2 <sup>e</sup>	11.8 $\pm$ 0.2 <sup>e</sup>	5.9 $\pm$ 0.1 <sup>e</sup>
	2	81.4 $\pm$ 0.8 <sup>e</sup>	9.6 $\pm$ 0.1 <sup>e</sup>	6.0 $\pm$ 0.2 <sup>e</sup>	11.7 $\pm$ 0.2 <sup>e</sup>	5.3 $\pm$ 0.06 <sup>e</sup>
	3	83.7 $\pm$ 0.8 <sup>e</sup>	9.3 $\pm$ 0.2 <sup>e</sup>	6.1 $\pm$ 0.2 <sup>e</sup>	11.6 $\pm$ 0.1 <sup>e</sup>	5.7 $\pm$ 0.06 <sup>e</sup>

Results are mean  $\pm$  standard deviation of three replicates and calculated on dry weight basis. Means within the same column that have same common letters are not significantly different ( $p > 0.05$ ).

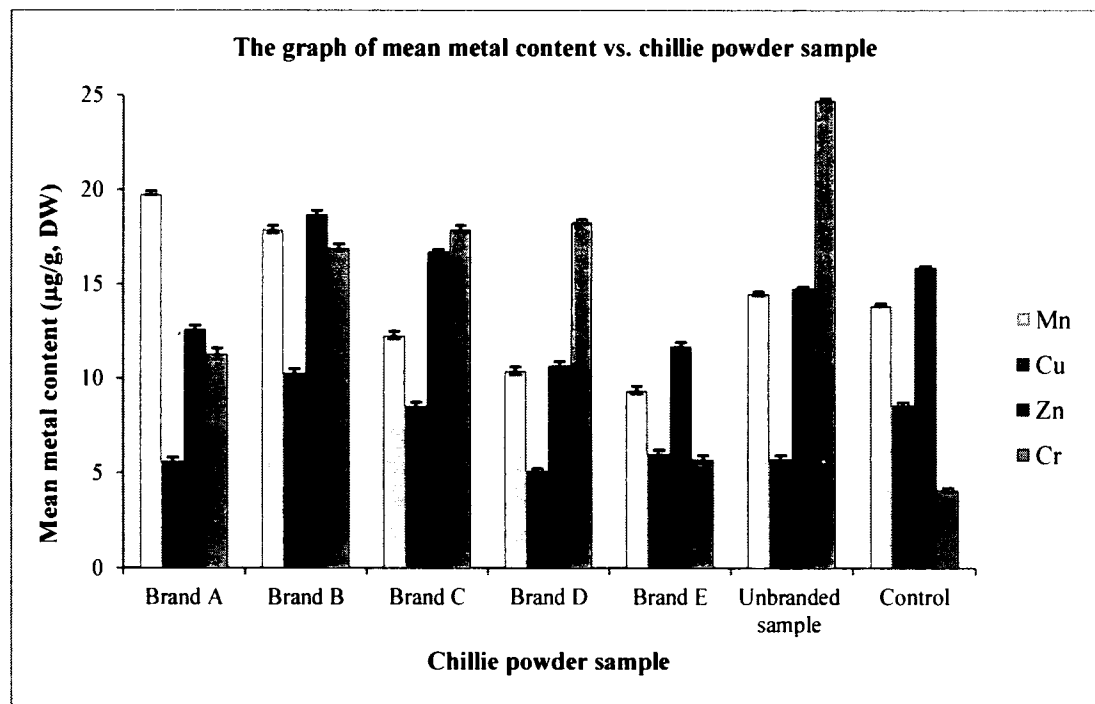
In the statistical analysis of variance (ANOVA), according to the Bartlett's test (normal distribution) results, there was no significant difference ( $p > 0.05$ ) between the batches in each brand, A, B, C, D and E, in mean Fe, Mn, Cu, Zn and Cr contents. Table 6 shows the mean Fe, Mn, Cu, Zn and Cr contents in each chillie powder brand and unbranded sample with the control, obtained using the flame AAS technique.

**Table 6: Mean micro metal content in chillie powder samples (flame AAS) - brandwise**

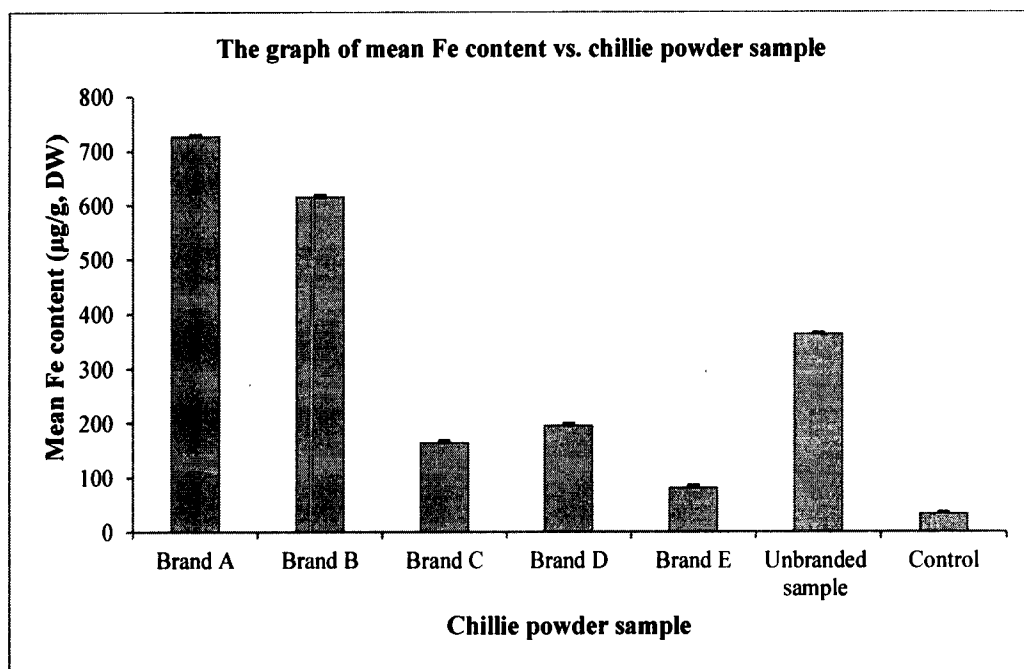
Chillie powder sample	Mean metal content (µg/g, DW)				
	Fe	Mn	Cu	Zn	Cr
Brand A	728.3 ± 1.2 <sup>a</sup>	19.8 ± 0.1 <sup>a</sup>	5.6 ± 0.2 <sup>a</sup>	12.6 ± 0.2 <sup>a</sup>	11.3 ± 0.3 <sup>a</sup>
Brand B	616.6 ± 1.9 <sup>b</sup>	17.9 ± 0.2 <sup>b</sup>	10.3 ± 0.2 <sup>b</sup>	18.7 ± 0.2 <sup>b</sup>	16.9 ± 0.2 <sup>b</sup>
Brand C	166.3 ± 1.3 <sup>c</sup>	12.3 ± 0.2 <sup>c</sup>	8.5 ± 0.2 <sup>c</sup>	16.7 ± 0.1 <sup>c</sup>	17.9 ± 0.2 <sup>c</sup>
Brand D	197.9 ± 1.2 <sup>d</sup>	10.4 ± 0.2 <sup>d</sup>	5.1 ± 0.1 <sup>d</sup>	10.7 ± 0.2 <sup>d</sup>	18.3 ± 0.1 <sup>d</sup>
Brand E	83.1 ± 1.4 <sup>e</sup>	9.4 ± 0.2 <sup>e</sup>	6.0 ± 0.2 <sup>e</sup>	11.7 ± 0.2 <sup>e</sup>	5.7 ± 0.2 <sup>e</sup>
Unbranded sample	365.3 ± 0.5 <sup>f</sup>	14.5 ± 0.1 <sup>f</sup>	5.7 ± 0.2 <sup>e</sup>	14.8 ± 0.06 <sup>f</sup>	24.7 ± 0.1 <sup>f</sup>
Control	35.2 ± 0.3 <sup>g</sup>	13.9 ± 0.06 <sup>g</sup>	8.6 ± 0.1 <sup>c</sup>	15.9 ± 0.06 <sup>g</sup>	4.1 ± 0.1 <sup>g</sup>

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

One way ANOVA was used to check whether there is a significant difference between brands in mean metal contents. Due to p-value is less than 0.05, there are significant differences among brands in the mean Fe, Mn, Cu, Zn and Cr contents. According to the Tukey's pairwise comparison test results, all brands are significantly different to each other and with the unbranded sample in mean Fe, Mn, Zn and Cr contents. When considering the Cu content there is no significant difference between brand E and the unbranded sample, while all the other brands are significantly different to each other in Cu content. According to the Dunnett's comparison test results, all brands are significantly different from the control in mean Fe, Mn, Zn and Cr contents. When considering the Cu content, there is no significant difference of brand C with the control, while all the other brands are significantly different. Mean Mn, Cu, Zn and Cr content in chillie powder samples are graphically represented in the Figure 1, while mean Fe content is represented in the Figure 2.



**Figure 1: Variation of Mn, Cu, Zn and Cr contents in chillie powder samples (flame AAS)**



*Figure 2: Variation of Fe content in chillie powder samples (flame AAS)*

Table 7 shows the Fe, Mn, Cu, Zn and Cr, contents in chillie powder samples, according to the XRF spectrometric results. The results are expressed as µg metal /g of dried chillie powder (ppm).

*Table 7: Micro metal content in chillie powder samples (XRF) - brandwise*

Chillie powder sample	Metal content (µg/g, DW)				
	Fe	Mn	Cu	Zn	Cr
Brand A	730.9 ± 9.0	22.0 ± 2.3	6.0 ± 0.5	13.1 ± 0.6	ND
Brand B	621.3 ± 5.1	18.4 ± 1.3	10.7 ± 0.4	19.0 ± 0.5	ND
Brand C	170.0 ± 2.6	12.6 ± 1.3	12.6 ± 1.3	17.0 ± 0.4	ND
Brand D	205.8 ± 2.5	11.0 ± 0.8	5.4 ± 0.2	11.3 ± 0.3	ND
Brand E	85.0 ± 1.7	9.8 ± 0.9	9.8 ± 0.9	12.2 ± 0.3	ND
Unbranded sample	376.4 ± 3.9	14.8 ± 1.2	14.8 ± 1.2	14.8 ± 0.3	ND
Control	36.7 ± 1.6	14.1 ± 2.8	9.0 ± 0.4	15.8 ± 0.5	ND

Results are metal content ± measurement uncertainty of each sample, calculated on dry weight basis. ND stands for "not detected" (Instrumental detection limit for Cr is 0.0054 ppm).

When comparing the results of micro metals obtained by the AAS and XRF techniques, according to the paired t test results, there was no significant difference ( $p > 0.05$ ) between these two methods, AAS and XRF in Fe, Mn, Cu and Zn contents. The mean Fe content of selected chillie powder samples was in the range from 83.1 to 728.3 µg/g (on dry weight basis). But the control sample was having mean Fe content of 35.2 µg/g, thus it is clear that mean Fe content of some brands and unbranded sample is significantly high. According to the WHO standards in 2005, the maximum permissible limit of Fe for spices is 300 ppm. In the brands A, B and in the unbranded sample Fe content is higher than the maximum permissible limit. To statistically analyze whether this increment is significant or not, one sample t test was performed. According to the test results, p-values are less than 0.05 and therefore Fe content in A and B brands and unbranded sample are significantly higher than the maximum permissible limit. This significantly high value of Fe content may be due to metal contamination during the grinding process. In the chillie grinding process, grinding machinery and equipment with metal parts that can break loose (e.g., moving wire mesh belts, screens and portion control equipment, and metal ties) are likely sources of metal that may enter chillie powder during processing (LOMA systems, 2013). Once introduced into a product, metal (Fe) fragments may be

removed from the product by passing it through an electronic metal (Fe) detector. Although many chillie powder manufactures use metal detectors during the manufacturing process, modern metal detectors are still unable to detect every particle of metal passing through them. Therefore it is clear that brand A, brand B and unbranded sample contain iron particles, added during the grinding process, which is not suitable for the human health. The mean Mn content of chillie powder samples varies between 9.4 and 19.8  $\mu\text{g/g}$  (on dry weight basis). The maximum permissible limit of Mn is 100  $\mu\text{g/g}$  (WHO, 2005), and Mn content of all the chillie samples are below this limit. The mean Cu content of chillie powder samples was ranged between 5.1 and 10.3  $\mu\text{g/g}$  (on dry weight basis), while the mean Zn content between 10.7 and 18.7  $\mu\text{g/g}$  (on dry weight basis). The maximum permissible limits for Cu and Zn are 20 and 50  $\mu\text{g/g}$  respectively (WHO, 2005). Thus in the chillie powder samples both these metals are below the maximum permissible limit. The mean Cr content varies between 5.7 and 24.7  $\mu\text{g/g}$  (on dry weight basis). The unbranded sample was having significantly higher amount of Cr than the control sample, and it indicates some sort of Cr contamination in the unbranded sample. But it is below the maximum permissible limit of 30  $\mu\text{g/g}$  (WHO, 2005), and therefore it is not highly considerable.

#### Toxic metals in chillie powder

The toxic metals in chillie powder samples were analyzed using graphite furnace AAS and XRF spectrometric techniques. Table 8 shows the mean Ni, Pb and Cd contents in each batch of five chillie powder brands, obtained using graphite furnace AAS technique. The results are expressed as  $\mu\text{g}$  metal /kg of dried chillie powder (ppb).

Table 8: Mean toxic metal content in chillie powder samples (graphite furnace AAS) – batchwise

Brand	Batch	Mean metal content ( $\mu\text{g/kg}$ , DW)		
		Ni	Pb	Cd
A	1	173.3 $\pm$ 0.3 <sup>a</sup>	120.0 $\pm$ 0.3 <sup>a</sup>	519.3 $\pm$ 0.7 <sup>a</sup>
	2	170.8 $\pm$ 0.4 <sup>a</sup>	115.9 $\pm$ 0.4 <sup>a</sup>	516.0 $\pm$ 0.2 <sup>a</sup>
	3	173.1 $\pm$ 0.1 <sup>a</sup>	118.9 $\pm$ 0.3 <sup>a</sup>	517.8 $\pm$ 0.3 <sup>a</sup>
B	1	221.0 $\pm$ 0.1 <sup>b</sup>	55.8 $\pm$ 0.3 <sup>b</sup>	495.9 $\pm$ 0.7 <sup>b</sup>
	2	218.2 $\pm$ 0.3 <sup>b</sup>	52.7 $\pm$ 0.3 <sup>b</sup>	490.9 $\pm$ 0.7 <sup>b</sup>
	3	220.2 $\pm$ 0.1 <sup>b</sup>	54.3 $\pm$ 0.1 <sup>b</sup>	493.6 $\pm$ 0.5 <sup>b</sup>
C	1	99.5 $\pm$ 0.2 <sup>c</sup>	531.5 $\pm$ 0.1 <sup>c</sup>	528.9 $\pm$ 0.4 <sup>c</sup>
	2	96.2 $\pm$ 0.4 <sup>c</sup>	525.9 $\pm$ 0.3 <sup>c</sup>	525.2 $\pm$ 0.5 <sup>c</sup>
	3	97.6 $\pm$ 0.2 <sup>c</sup>	528.4 $\pm$ 0.2 <sup>c</sup>	527.8 $\pm$ 0.4 <sup>c</sup>
D	1	208.2 $\pm$ 0.2 <sup>d</sup>	ND	247.0 $\pm$ 0.2 <sup>d</sup>
	2	207.6 $\pm$ 0.2 <sup>d</sup>	ND	245.7 $\pm$ 0.5 <sup>d</sup>
	3	206.2 $\pm$ 0.2 <sup>d</sup>	ND	242.7 $\pm$ 0.5 <sup>d</sup>
E	1	54.7 $\pm$ 0.1 <sup>e</sup>	146.1 $\pm$ 0.2 <sup>e</sup>	230.8 $\pm$ 0.2 <sup>e</sup>
	2	55.2 $\pm$ 0.2 <sup>e</sup>	142.8 $\pm$ 0.3 <sup>e</sup>	225.8 $\pm$ 0.4 <sup>e</sup>
	3	54.2 $\pm$ 0.06 <sup>e</sup>	144.9 $\pm$ 0.1 <sup>e</sup>	228.0 $\pm$ 0.2 <sup>e</sup>

Results are mean  $\pm$  standard deviation of three replicates and calculated on dry weight basis. Means within the same column that have same common letters are not significantly different ( $p > 0.05$ ). ND stands for "not detected" (Instrumental detection limit for Pb is 0.03 ppb).

Table 9: Mean toxic metal content in chillie powder samples (graphite furnace AAS) – brandwise

Chillie powder sample	Mean metal content ( $\mu\text{g/kg}$ , DW)		
	Ni	Pb	Cd
Brand A	172.4 $\pm$ 1.2 <sup>a</sup>	118.2 $\pm$ 1.9 <sup>a</sup>	517.7 $\pm$ 1.5 <sup>a</sup>
Brand B	219.8 $\pm$ 1.2 <sup>b</sup>	54.3 $\pm$ 1.4 <sup>b</sup>	493.5 $\pm$ 2.2 <sup>b</sup>
Brand C	97.8 $\pm$ 1.4 <sup>c</sup>	528.6 $\pm$ 2.5 <sup>c</sup>	527.3 $\pm$ 1.7 <sup>c</sup>
Brand D	207.3 $\pm$ 0.9 <sup>d</sup>	ND	245.1 $\pm$ 1.9 <sup>d</sup>
Brand E	54.7 $\pm$ 0.4 <sup>e</sup>	144.6 $\pm$ 1.5 <sup>d</sup>	228.2 $\pm$ 2.2 <sup>e</sup>
Unbranded sample	148.8 $\pm$ 0.2 <sup>f</sup>	1147.0 $\pm$ 0.2 <sup>e</sup>	784.7 $\pm$ 0.2 <sup>f</sup>
Control	14.8 $\pm$ 0.1 <sup>g</sup>	ND	196.1 $\pm$ 0.1 <sup>g</sup>

According to the Bartlett's test (normal distribution) results, there was no significant difference ( $p > 0.05$ ) between the batches in each brand, A, B, C, D and E, in mean Ni, Pb and Cd contents. Table 9 shows the mean Ni, Pb and Cd



contents in each chillie powder brand and unbranded sample with the control, obtained using graphite furnace AAS technique.

Results are mean  $\pm$  standard deviation of nine replicates and calculated on dry weight basis. Means within the same column that have no common letters are significantly different ( $p < 0.05$ ).

According to one way ANOVA test results ( $p < 0.05$ ), there are significant differences among brands in the mean Ni, Pb and Cd contents. According to the Tukey's pairwise comparison test results, all brands are significantly different to each other and with the unbranded sample in mean Ni, Pb and Cd contents. According to the Dunnett's comparison test results, all brands are significantly different from the control in mean Ni and Cd contents. When considering the Pb content, Pb was not detected in the control sample and also in the brand D. Mean Ni, Pb and Cd contents in chillie powder samples are graphically represented in the Figure 3.

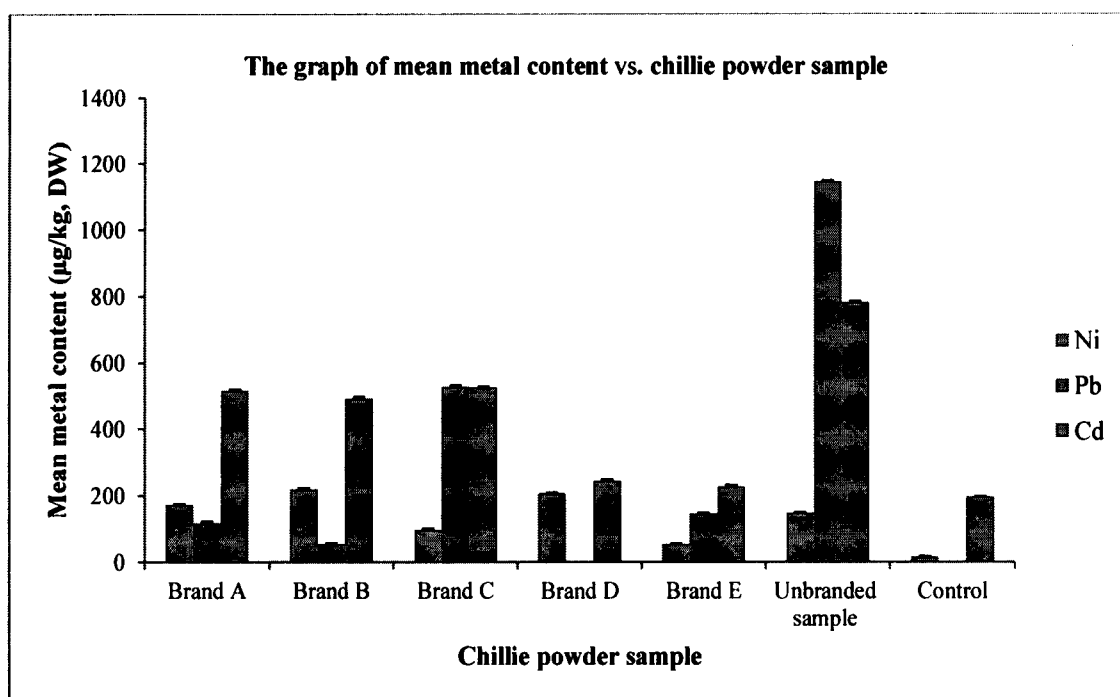


Figure 3: Variation of Ni, Pb and Cd contents in chillie powder samples (graphite furnace AAS)

The mean Ni content of chillie powder samples was ranged between 54.7 and 219.8  $\mu\text{g}/\text{kg}$  (on dry weight basis), while the mean Pb content between 54.3 and 1147.0  $\mu\text{g}/\text{g}$  (on dry weight basis). The maximum permissible limits for Ni and Pb are 50 000 and 10 000  $\mu\text{g}/\text{kg}$  respectively (WHO, 2005). Thus in the chillie powder samples both these metals are far below the maximum permissible limit. The mean Cd content of chillie powder samples was ranged between 228.2 and 527.3  $\mu\text{g}/\text{kg}$  (on dry weight basis). The maximum permissible limit of Cd for spices is 200  $\mu\text{g}/\text{kg}$  (WHO, 2005). In the all branded samples and the unbranded sample Cd content exceeds the maximum permissible limit. In the statistical analysis, according to the one sample t test results, p-values are less than 0.05 and therefore Cd contents in each brand and unbranded sample are significantly higher than the maximum permissible limit. This is not a favourable condition because Cd is a relatively toxic metal, and Cd uptake by plants is enhanced due to the use of superphosphate fertilizers. The toxic metal Cd also can be included in chillies, those grown on high Cd soils (Pearson, 1976). Therefore Cd content in chillie powder brands can be significantly different due to the differences in soil conditions where they were grown, and also the types of fertilizers used. The toxic metals, Ni, Pb and Cd were not detected by the XRF technique. XRF has detection limits in the ppm range. Therefore it is not a suitable technique for the detection of metals in the ppb range.

## CONCLUSION

This study was carried out to investigate the trace metal content present in the selected chillie powder samples, commonly available in the Sri Lankan market. According to the results, the mean Fe content of brand A ( $728.3 \pm 1.2 \mu\text{g/g}$ ), brand B ( $616.6 \pm 1.9 \mu\text{g/g}$ ) and unbranded sample ( $365.3 \pm 0.5 \mu\text{g/g}$ ) were significantly higher than the WHO maximum permissible limit ( $300 \mu\text{g/g}$ ). This may be due to the metal (Fe) contamination during the grinding process. Although many chillie powder manufactures use metal detectors to remove metal parts during the manufacturing process, modern metal detectors are still unable to detect every particle of metal passing through them. Thus brand A, B and unbranded samples are not suitable for daily consumption. In all the chillie powder samples the micro minerals Mn, Cu, Zn and Cr were found to be present in ppm level, and they were below the maximum permissible limits. In these chillie powder samples toxic metals, Ni, Pb and Cd were also present in ppb level. In the tested samples Ni and Pb were below the maximum permissible limits, but in all samples Cd content exceeded the maximum permissible limit. The mean Cd content of chillie powder samples ranged between 228.2 and 527.3  $\mu\text{g/kg}$ . The control sample also contained relatively high amount of Cd content ( $196.1 \pm 0.1 \mu\text{g/kg}$ ), but below the maximum limit ( $200 \mu\text{g/kg}$ ). The content of Cd in chillie powder samples can be significantly higher in those which are grown on high Cd soils and differences occur due to the types of fertilizers used. When considering all the analyzed metals in the chillie powder samples there were no significant differences between batches in each brand. But significant differences present among the brands. When comparing the results obtained by the AAS and XRF techniques, there was no significant difference between these two techniques in Fe, Mn, Cu and Zn contents. XRF has detection limits in the range of ppm. Therefore it is not a suitable technique for the detection of metals in the ppb range, like Ni, Pb, Cd etc.

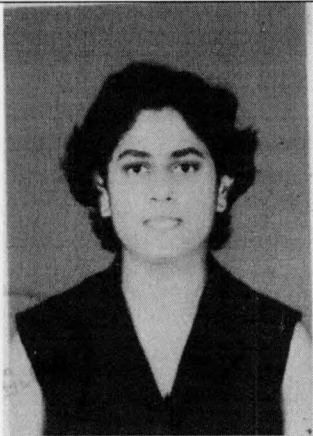

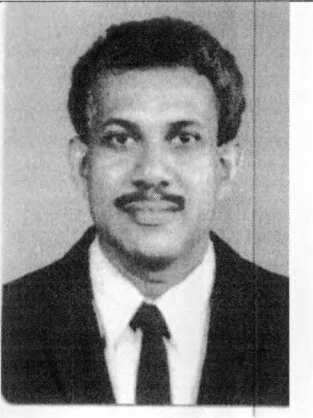
#### ACKNOWLEDGEMENTS

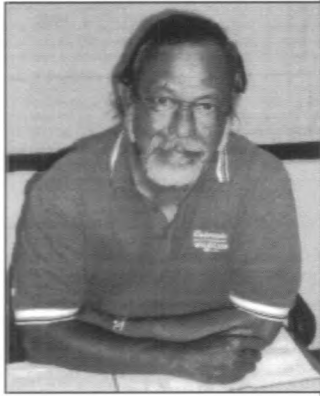
The authors thank the Atomic Energy Board, Sri Lanka for providing the necessary facilities for the XRF spectrometric analysis.

#### REFERENCES

- [1] AOAC (1996), Association of Analytical Communities, Official Methods of Analysis of AOAC International, 16<sup>th</sup> edition, Vol. II
- [2] Balcerzak, M. (2002), Sample Digestion Methods for the Determination of Traces of Precious Metals by Spectrometric Techniques, *Analytical Sciences*, Vol. 18, pp. 737-750
- [3] Belay, K. (2014), Analysis of Lead, Cadmium and Chromium in Ethiopian Spices after Wet (acid) Digestion using Atomic Absorption Spectroscopy, *Global Journal of Science Frontier Research : (B) Chemistry*, Vol. 14, Iss.4 No. 1.0, pp. 1-5
- [4] Belay, K. (2014), Determination of Pb (II), Cr (VI) and Cd (III) contents in Ethiopian Spices after Microwave Digestion using Atomic Absorption Spectroscopy, *International Journal of Technology Enhancements and Emerging Engineering Research*, Vol. 2, Iss. 8, pp.87-91
- [5] Buhrke, V.E., Jenkins, R. and Smith, D.K. (1998), A Practical Guide for the Preparation of Specimens for X-Ray Fluorescence and X-Ray Diffraction Analysis, pp.39
- [6] DASL (2006), Department of Agriculture - Sri Lanka, Crop Recommendations – Chillie
- [7] Deshpande, S.S. and Dekker, M. (2002), Hand book of Food Toxicology, *New York*
- [8] FSAI (2009), Food Safety Authority of Ireland, Mercury, Lead, Cadmium, Tin and Arsenic in Food, *Toxicology Factsheet Series*, No. 1, pp. 1-13
- [9] FSSAI (2012), Food Safety and Standards Authority of India, Manual of Methods of Analysis of Food Metals
- [10] Garcia, R. and Baez, A.P. (2012), Atomic Absorption Spectrometry (AAS), pp. 1-11
- [11] Inam, F., Deo, S. and Narkhede, N. (2013), Analysis of Minerals and Heavy Metals in Some Spices Collected from Local Market, *IOSR Journal of Pharmacy and Biological Sciences*, Vol. 8, Iss. 2, pp. 40-43
- [12] Krejpcio, Z., Krol, E. and Sionkowski, S. (2007), Evaluation of Heavy Metals contents in Spices and Herbs Available on the Polish Market, *Polish Journal of Environmental Studies*, Vol.16, No.1, pp. 97-100
- [13] LOMA Systems (2013), A guide to metal detection in the food manufacturing industry
- [14] MAGI (2009), Ministry of Agriculture – Government of India, Post Harvest Profile of Chillie, pp. 6-16
- [15] Pearson D. (1976), *The Chemical Analysis of Foods*, 7<sup>th</sup> edition, pp. 73-97
- [16] Ranaweera, K.K.D.S. (2007), *All About Foods*, Godage International Publishers (Pvt) Ltd, pp. 153-176

## AUTHOR BIBLIOGRAPHY

	<p><b>Ms. W.A.A.V.S. Amaradivakara</b> Assistant City Analyst, Colombo; Following M.Sc. Degree in Food Science and Technology in the Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka, Holding the B.Sc. Special Degree in Chemistry (First class Hons.); Member of the Royal Society of Chemistry, UK; Life Member of the Institute of Chemistry, Ceylon; Member of Sri Lanka Association for the Advancement of Science, Sri Lanka.</p>
	<p><b>Dr. (Ms.) Indira Wickramasinghe</b> Senior Lecturer, Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewadenapura, Sri Lanka; Former Head of the Department of Food Science and Technology, Lecturer MSc in Food Science and Technology and Department of Plant BioTechnology, University of Colombo; Member of the Editorial Board of National Aquatic Resources Research and Development Agency (NARA) Journal, Colombo; Life member of Sri Lanka Association for the Advancement of Science, Sri Lanka and Nutrition society of Sri Lanka; Member of the Development Group and Advisory committee of the National Diploma in Food Technology NVQ Level 5 for National Competency Standards for food Technology in Sri Lanka.</p>
	<p><b>Mr. R. M. G. B. Rajanayake</b> City Analyst Colombo; Chartered Chemist; Fellow member, Past Chairman, Sri Lanka section of the Royal Society of Chemistry, UK.; Fellow member of the Institute of Chemistry, Ceylon; Visiting lecturer in Food Science &amp; Technology, University of Sri Jayewardenepura, Sri Lanka, Agriculture &amp; Environment Science, University of Kelaniya Sri Lanka, Community Medicine, Post Graduate Institute of Medicine, University of Colombo; Editor and visiting lecturer for Food technology programs, Open University of Sri Lanka, Nawala; Member of the Food Advisory Committee, Ministry of Health &amp; Nutrition; Council member of the Institute of Chemistry Ceylon.</p>



**Prof. Arthur Bamunuarachchi**

Emeritus Professor, University of Sri Jayewardenepura, Sri Lanka; National and International Consultant Food Scientist and Technologist; Specialist Post Harvest, Food Processing and Agribusiness Trainer; D.Sc (Hon.Causa), Sabaragamuwa University of Sri Lanka; D.Sc(Hon.Cauza),University of Sri Jayewardenepura, Sri lanka; Presidential awards for Research Publications – 2009/10/11; Member, Australian Institute of Food Technologist(Ex.); Member, American Institute of Food Technologist(Ex.); Member, American Institute of Cereal Chemists.(Ex.); Member, Indian Association of Food Scientist and Technologists. (Ex.); Member, Pakistan Association of Food Science and Technologists.(Ex.); Member, Institute of Chemistry, Sri Lanka.



## INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

### ANALYSIS OF TRACE METAL CONTENT IN CHILLIE POWDER AVAILABLE IN THE SRI LANKAN MARKET

W.A.A.V.S. Amaradivakara\*, I. Wickramasinghe, R.M.G.B. Rajanayake, A. Bamunuarachchi  
Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri  
Jayewardenepura, Sri Lanka

#### ABSTRACT

Chillie (*Capsicum annum* L.) powder is the dried, pulverized fruit of chillies and used as a spice to add pungency flavour to the dish. This study was conducted to investigate the trace metal content in chillie powder samples commonly available in the Sri Lankan market. Five brands of chillie powder samples named A, B, C, D and E, and an unbranded sample were selected for this study. Three batches from each brand and three samples from each batch were purchased. An ungrounded whole dry chillie sample was selected as the control. The trace metal content was determined using AAS and XRF spectrometric techniques. Results were statistically analyzed using one-way ANOVA, at 0.05 probability level with MINITAB-14 software package. According to this study, there was no significant difference between the batches in each brand in mean metal contents. But there were significant differences among the brands in mean metal contents. The mean Fe content of brand A, brand B and unbranded samples were significantly higher than the WHO maximum permissible limit. This may be due to the metal (Fe) contamination during the grinding process. Thus brand A, B and unbranded samples are not suitable for daily consumption.

**KEYWORDS:** Chillie powder, Trace metals.

#### INTRODUCTION

Chillie crop is one of the most important commercial spice crops. Botanical name of chillie is *Capsicum annum* L. There are more than 400 different varieties of chillies found all over the world. Chillie belongs to the genus capsicum, under the solanaceae family. Chillies occur referred to as chillies, hot peppers, bell peppers, red peppers, pod peppers, cayenne peppers, paprika, pimento, and capsicum in different parts of the world (MAGI, 2009).

Currently, chillies are used throughout the world as a spice and also in the making of beverages and medicines. Some varieties of chillies are famous for red colour because of the pigment 'capsanthin,' others are known for biting pungency attributed to 'capsaicin'. Chillie is very important as an anti-oxidant and anti-inflammatory agent. Chillie has become an essential ingredient in Sri Lankan meals. Per capita consumption of chillie in the form of dry chillie is estimated 2.32 kg per annum and the national annual requirement of dry chillie is around 42,634 Mt. The annual production of dry chillie in Sri Lanka is about 18,616 Mt, therefore, an amount of 31,242 Mt is being imported (Year 2007 figures). Chillie is mainly imported from India, as dry chillie (DASL, 2006).

In the chillie powder manufacturing process in Sri Lanka dried chillies are cleaned manually to remove impurities and then washed. After drying them, they are pulverized in two or more grinders to convert them into the powder form. Then chillies in powder form are passed through sieves to obtain uniform mesh size (45-65 mesh). Some manufactures use metal detectors to detect and remove metal particles from the ground chillie. Finally, packing is done in polythene bags using automated powder fillers, and bags are sealed. Due to major part of chillie being imported from India, many branded chillie powders available in the Sri Lankan market are products of imported dry chillies from India. The main environmental pollutants in whole chillie are thought to be toxic trace metals from agronomic sources particularly agricultural, chemicals and processing operations. Some of the important trace metal pollutants are lead, copper, arsenic, tin, zinc, cadmium, mercury and methyl mercury. Thus imported chillie may contain these toxic metals. When processing these chillie within Sri Lanka to produce powdered chillie, trace amounts of metals may be added during the manufacturing process.

The term "trace metals" refers to the metals which may be present in foods in amounts well below 50 mg/kg and which have some toxicological or nutritional significance (FSSAI, 2012). Iron (Fe) is one of the most investigated and best understood of nutrients. The metal Fe is essential for oxygen transport, electron transfer reactions, gene regulation and regulation of cell growth and differentiation (Ranaweera, 2007). Excess Fe intake can cause the

proliferation of foreign bacteria in the gut and producing discomfort from gas and bloating. Symptoms of Fe toxicity are vomiting, diarrhea and intestinal damage (Deshpande and Dekker, 2002). Copper (Cu) is a micronutrient required by our body. It is involved in the absorption, storage and metabolism of Fe. Manganese (Mn) is a vital trace element necessary in the metabolism and involved in antioxidant activities, maintaining fertility, forming strong healthy bones, nerves and muscles. Zinc (Zn) helps to maintain normal cell-mediated immune function and is present in all cells, particularly in the nucleus. Chromium (Cr) is an essential nutrient and a trace element required for normal sugar and fat metabolism.

Nickel (Ni) exposure introduces free radicals which lead to oxidative damage and may also affect the kidneys and liver. The trace metal Ni has been identified as a toxin that severely damages reproductive health and can lead to infertility, miscarriage, birth defects, and nervous system defects. Although lead (Pb) may have beneficial effects in small amounts, Pb toxicity is of more concern than Pb deficiency. Plant foodstuffs grown under high Pb conditions can include Pb and Pb toxicity results in anaemia, kidney damage and central nervous abnormalities (Ranaweera, 2007). Cadmium (Cd) is a relatively toxic metal, which soon after ingestion is liable to cause acute gastritis with vomiting and diarrhea. The toxic metal Cd can be included in plant foods, those grown on high Cd soils, and Cd has a long half life in the body and thus high intakes can lead to accumulation resulting in damage in some organs, especially the kidney (Ranaweera, 2007).

In Sri Lankan standards, maximum permissible limits for trace metals in chillie powder or even in spices have not been established yet. Hence, WHO standards in 2005 have to be considered. According to the WHO standards in 2005, the maximum permissible limits of metals in spices are summarized in the Table 1.

*Table 1: Maximum permissible limits of metals in spices*

Metal	Maximum permissible limit (in ppm)
Iron	300
Copper	20
Manganese	100
Zinc	50
Chromium	30
Nickel	50
Lead	10
Cadmium	0.2
Mercury	0.5

Source: WHO (2005)

Many research have been carried out globally to investigate the trace metal content in common spices, including chillie powder, available in foreign markets. Hisfa *et al* (2009) investigated the concentrations of some heavy metals such as iron, copper, chromium, lead, cadmium and cobalt present in common spices of two brands widely used in Pakistan. Baley (2014) has determined the lead, cadmium and chromium content in Ethiopian spices. But in Sri Lanka, there is lack of research about this area. Hence it is very important to carry out such a study to investigate trace metal content in chillie powder, commercially available in the Sri Lankan market. Thus the major objective of this study was to determine the content of some selected trace metals in chillie powder. The specific objectives of this study were to determine the Fe, Mn, Cu, Zn, Cr, Ni, Pb and Cd content in chillie powder available in the Sri Lankan market, using selected brands; to check whether these metal contents are within the standard limits (according to WHO standards); to find out whether there is a significant difference in metal content among the brands, with the unbranded sample and with the control; and to find out whether there is a significant difference among batches in a same brand.

## MATERIALS AND METHODS

### Sample selection

Five brands of chillie powder samples named A, B, C, D and E, and an unbranded sample were selected for this study. Three batches from each brand and three samples from each batch were purchased from the local markets in

Sri Lanka, within the time period of January to April in 2015. Three samples of the unbranded sample were also collected. An ungrounded whole dry chillie sample was selected as the control.

#### Sample preparation for atomic absorption spectrometric analysis

Wet digestion method was carried out according to the AOAC official method 975.03 (AOAC, 1996).

#### Method

Chillie powder sample was placed in a porcelain dish and oven dried at 105 °C for 2 hours, until obtaining a constant weight. Accurately about 1 g of oven dried chillie powder sample was weighed into a 150 mL pyrex beaker. Then 10 mL of HNO<sub>3</sub> (1+1) was added. It was allowed to soak thoroughly and 3 mL of 60% HClO<sub>4</sub> was added. Then it was heated on a hot plate, placed in a fume hood, slowly at first, until frothing ceased. The heating was carried out at 150 °C, until HNO<sub>3</sub> was almost evaporated. Then it was cooled and 10 mL of HNO<sub>3</sub> was added again and continued heating due to the charring of the sample. The addition of 10 mL of HNO<sub>3</sub> and heating were repeated another four times, until obtaining a clear solution, without charring. It was heated to white fumes of HClO<sub>4</sub>; cooled; 10 mL of HCl (1+1) was added and quantitatively transferred into 50 mL volumetric flask and diluted to volume using de-ionized water. Then it was filtered through an acid washed No. 41 filter paper after discarding the first few drops of filtrate. This digested solution was stored in a polypropylene bottle for the AAS analysis. A blank was prepared by following all the above steps, at the same conditions, without adding the sample

#### Atomic absorption spectrometric (AAS) analysis

##### Flame AAS analysis - Method

The standard series of each metal was prepared, using the stock solutions. The metals in the digested solutions of chillie powder samples, namely Fe, Mn, Cu, Zn and Cr were analyzed using the flame atomic absorption spectrometric technique. This metal analysis was performed using Thermo Scientific, iCE 3000 series model double beam atomic absorption spectrometer, with deuterium background correction. The instrument was set up using the operating parameters given in the Table 2. The Solaar software was used to run the programme. Hollow cathode lamp with each metal was used at the specific wavelength.

Table 2: Operating parameters of element for flame AAS

Element	Wave length (nm)	Flame type	Fuel flow rate (L/ min)	Lamp current (mA)	Slit width (nm)
Fe	248.3	Air / acetylene	0.8 – 1.0	5	0.2
Mn	279.5	Air / acetylene	0.9 – 1.2	5	0.2
Cu	324.8	Air / acetylene	0.8 – 1.1	4	0.5
Zn	213.9	Air / acetylene	0.9 – 1.2	5	1.0
Cr	357.9	Nitrous oxide / acetylene	4 - 4.4	7	0.2

The calibration curve was prepared by aspirating the standard series. Then digested solutions of chillie powder samples were aspirated and the concentration was read from the plot of absorbance against concentration (µg/mL). The prepared blank was used to establish the 0 absorption point. The burner was flushed off with de-ionized water between samples.

##### Graphite furnace AAS analysis - Method

The master standard of each metal was prepared, using the stock solutions. The metals Ni, Pb and Cd in the digested solutions of chillie powder samples, were analyzed using the graphite furnace atomic absorption spectrometric technique in the Thermo Scientific, iCE 3000 series model double beam atomic absorption spectrometer, with deuterium background correction. The instrument was set up using the operating parameters given in the Table 3

Table 3: Operating parameters of element for graphite furnace AAS

Element	Wave length (nm)	Lamp current (mA)	Slit width (nm)	Atomize temperature (°C)
Ni	232.0	4	0.2	2500
Pb	217.0	5	1.0	1200
Cd	766.5	5	1.0	900

The samples were loaded into the autosampler. The master standard, blank and de-ionized water were loaded into the R1, R2 and R3 positions of the autosampler respectively. The standard series was prepared by the instrument using the master standard and established a calibration curve of absorbance against concentration (µg/L), to read the concentrations of the samples.

*Determination of the original metal content in the sample on dry basis*

$$\text{Metal content in the original sample (}\mu\text{g/g, DW)} = \frac{C \times V}{W}$$

Where,

C = Concentration of the metal given by AAS reading (µg/mL or µg/L)

V = Final volume of the digested sample (mL)

W = Weight of the dried sample (g)

**Sample preparation for X-ray fluorescence spectrometric analysis**

For the sample preparation, palletizing technique was used, after dry ashing the sample at 300 °C, according to the validated method of Atomic Energy Board of Sri-Lanka.

*.Method*

Chillie powder sample was placed in a silica dish and oven dried at 105 °C for 2 hours, until obtaining a constant weight. Accurately about 20 g of oven dried chillie powder sample was weighed into another silica dish and ashed in the muffle furnace (Thermolyne, F62730) at 300 °C for 16 hours. Then the dish was weighed again to obtain the ash weight. From the ashed sample, 0.50 g of ash was weighed into a clean oil paper. Then it was transferred into a hard plastic mortar and ground into a homogeneous powder. Then this powder was transferred into a cylindrical pellet die, as evenly distributed and pressed using a manually operated bench top pelletizing press (Spectro Press, 4312 E) to form a supported pellet which ideally had a smooth, homogeneous sample surface and good physical stability.

**X-ray fluorescence (XRF) spectrometric analysis**

This method was used to analyze Fe, Mn, Cu, Zn, Cr, Ni and Pb contents in the chillie powder samples. One sample from the each batch, with the unbranded sample and the control was analyzed.

*Method*

The instrument was switched on and wait one hour to warm up before proceeding, to stabilize the machine. The prepared pellets were loaded into the sample holder. The instrument was set up using the operating parameters given in the Table 4. The AXIL software was used to run the programme.

*Table 4: Operating parameters for XRF*

Operating parameter	
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
Target	Mo

The sample was run 500 seconds using the molybdenum target and the spectrum of count per channel against channel number was obtained. The metals were identified using their characteristic fluorescent energy value. The quantification of identified metals was carried out by the calibration curve method, obtained with many standard samples.

*Determination of the original metal content in the sample on dry basi*

$$\text{Metal content in the original sample (}\mu\text{g/g, DW)} = \frac{C \times W_a}{W_t}$$

Where,

C = Concentration of the metal given by XRF (µg/g)



Wt = Weight of the dried sample (g)

Wa = Weight of the ash obtained (g)

#### Statistical analysis

Results were statistically analyzed using one-way analysis of variance (ANOVA), at 0.05 probability level with MINITAB-14 software package. The Bartlett's test (normal distribution) was performed to check whether there is any significant difference of mean metal content, between the batches in each brand. One-way ANOVA was used to check whether there is a significant difference among the brands in mean metal contents. Tukey's pairwise comparison was performed to select the brand pairs which are significantly different in mean metal contents. Dunnett's comparison test was performed to check whether selected chillie powder samples are significantly different from the control sample in the mean metal content. To statistically analyze whether the metal contents are significantly higher than the WHO maximum permissible limit, one sample t test was performed. The results of metals obtained by the AAS and XRF techniques were compared using the paired t test, to check whether there is any significant difference between these two methods.

## RESULTS AND DISCUSSION

In this study, trace metal content of chillie powder samples was analyzed using selected five brands of chillie power samples and an unbranded sample, commonly available in the Sri-Lankan market. An ungrounded whole dry chillie sample was selected as the control.

#### Micro metals in chillie powder

The micro metals in chillie powder samples were analyzed using flame AAS and XRF spectrometric techniques. Table 5 shows the mean Fe, Mn, Cu, Zn and Cr contents in each batch of five chillie powder brands, obtained using the flame AAS technique. The results are expressed as  $\mu\text{g}$  metal /g of dried chillie powder (ppm).

Table 5: Mean micro metal content in chillie powder samples (flame AAS) – batchwise

Brand	Batch	Mean metal content ( $\mu\text{g/g}$ , DW)				
		Fe	Mn	Cu	Zn	Cr
A	1	729.5 $\pm$ 0.9 <sup>a</sup>	19.9 $\pm$ 0.06 <sup>a</sup>	5.9 $\pm$ 0.1 <sup>a</sup>	12.8 $\pm$ 0.3 <sup>a</sup>	11.1 $\pm$ 0.2 <sup>a</sup>
	2	727.0 $\pm$ 0.5 <sup>a</sup>	19.6 $\pm$ 0.1 <sup>a</sup>	5.5 $\pm$ 0.1 <sup>a</sup>	12.7 $\pm$ 0.3 <sup>a</sup>	11.2 $\pm$ 0.2 <sup>a</sup>
	3	728.5 $\pm$ 0.7 <sup>a</sup>	19.8 $\pm$ 0.2 <sup>a</sup>	5.5 $\pm$ 0.2 <sup>a</sup>	12.5 $\pm$ 0.1 <sup>a</sup>	11.6 $\pm$ 0.1 <sup>a</sup>
B	1	618.7 $\pm$ 0.8 <sup>b</sup>	18.0 $\pm$ 0.2 <sup>b</sup>	10.3 $\pm$ 0.2 <sup>b</sup>	18.8 $\pm$ 0.2 <sup>b</sup>	17.1 $\pm$ 0.1 <sup>b</sup>
	2	614.5 $\pm$ 0.7 <sup>b</sup>	17.7 $\pm$ 0.2 <sup>b</sup>	10.2 $\pm$ 0.2 <sup>b</sup>	18.7 $\pm$ 0.2 <sup>b</sup>	16.7 $\pm$ 0.2 <sup>b</sup>
	3	616.5 $\pm$ 0.7 <sup>b</sup>	17.9 $\pm$ 0.2 <sup>b</sup>	10.3 $\pm$ 0.3 <sup>b</sup>	18.7 $\pm$ 0.2 <sup>b</sup>	17.0 $\pm$ 0.06 <sup>b</sup>
C	1	167.5 $\pm$ 0.7 <sup>c</sup>	12.4 $\pm$ 0.06 <sup>c</sup>	8.7 $\pm$ 0.2 <sup>c</sup>	16.7 $\pm$ 0.2 <sup>c</sup>	18.1 $\pm$ 0.1 <sup>c</sup>
	2	164.8 $\pm$ 0.7 <sup>c</sup>	12.2 $\pm$ 0.2 <sup>c</sup>	8.6 $\pm$ 0.2 <sup>c</sup>	16.7 $\pm$ 0.2 <sup>c</sup>	17.8 $\pm$ 0.06 <sup>c</sup>
	3	166.6 $\pm$ 0.7 <sup>c</sup>	12.4 $\pm$ 0.2 <sup>c</sup>	8.3 $\pm$ 0.2 <sup>c</sup>	16.7 $\pm$ 0.2 <sup>c</sup>	17.6 $\pm$ 0.1 <sup>c</sup>
D	1	199.4 $\pm$ 0.9 <sup>d</sup>	10.5 $\pm$ 0.2 <sup>d</sup>	5.1 $\pm$ 0.1 <sup>d</sup>	10.8 $\pm$ 0.2 <sup>d</sup>	18.3 $\pm$ 0.06 <sup>d</sup>
	2	196.9 $\pm$ 0.3 <sup>d</sup>	10.4 $\pm$ 0.2 <sup>d</sup>	5.1 $\pm$ 0.2 <sup>d</sup>	10.7 $\pm$ 0.2 <sup>d</sup>	18.1 $\pm$ 0.1 <sup>d</sup>
	3	197.6 $\pm$ 0.6 <sup>d</sup>	10.5 $\pm$ 0.2 <sup>d</sup>	5.2 $\pm$ 0.06 <sup>d</sup>	10.6 $\pm$ 0.2 <sup>d</sup>	18.5 $\pm$ 0.06 <sup>d</sup>
E	1	84.2 $\pm$ 0.4 <sup>e</sup>	9.2 $\pm$ 0.2 <sup>e</sup>	6.0 $\pm$ 0.2 <sup>e</sup>	11.8 $\pm$ 0.2 <sup>e</sup>	5.9 $\pm$ 0.1 <sup>e</sup>
	2	81.4 $\pm$ 0.8 <sup>e</sup>	9.6 $\pm$ 0.1 <sup>e</sup>	6.0 $\pm$ 0.2 <sup>e</sup>	11.7 $\pm$ 0.2 <sup>e</sup>	5.3 $\pm$ 0.06 <sup>e</sup>
	3	83.7 $\pm$ 0.8 <sup>e</sup>	9.3 $\pm$ 0.2 <sup>e</sup>	6.1 $\pm$ 0.2 <sup>e</sup>	11.6 $\pm$ 0.1 <sup>e</sup>	5.7 $\pm$ 0.06 <sup>e</sup>

Results are mean  $\pm$  standard deviation of three replicates and calculated on dry weight basis. Means within the same column that have same common letters are not significantly different ( $p > 0.05$ ).

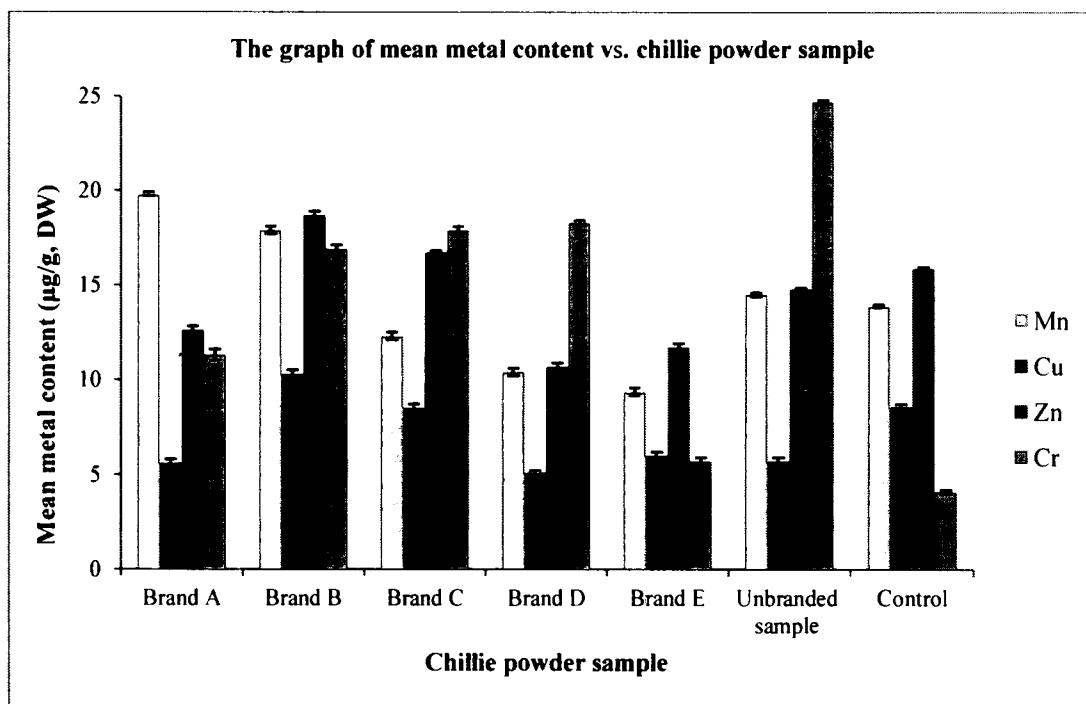
In the statistical analysis of variance (ANOVA), according to the Bartlett's test (normal distribution) results, there was no significant difference ( $p > 0.05$ ) between the batches in each brand, A, B, C, D and E, in mean Fe, Mn, Cu, Zn and Cr contents. Table 6 shows the mean Fe, Mn, Cu, Zn and Cr contents in each chillie powder brand and unbranded sample with the control, obtained using the flame AAS technique.

**Table 6: Mean micro metal content in chillie powder samples (flame AAS) - brandwise**

Chillie powder sample	Mean metal content (µg/g, DW)				
	Fe	Mn	Cu	Zn	Cr
Brand A	728.3 ± 1.2 <sup>a</sup>	19.8 ± 0.1 <sup>a</sup>	5.6 ± 0.2 <sup>a</sup>	12.6 ± 0.2 <sup>a</sup>	11.3 ± 0.3 <sup>a</sup>
Brand B	616.6 ± 1.9 <sup>b</sup>	17.9 ± 0.2 <sup>b</sup>	10.3 ± 0.2 <sup>b</sup>	18.7 ± 0.2 <sup>b</sup>	16.9 ± 0.2 <sup>b</sup>
Brand C	166.3 ± 1.3 <sup>c</sup>	12.3 ± 0.2 <sup>c</sup>	8.5 ± 0.2 <sup>c</sup>	16.7 ± 0.1 <sup>c</sup>	17.9 ± 0.2 <sup>c</sup>
Brand D	197.9 ± 1.2 <sup>d</sup>	10.4 ± 0.2 <sup>d</sup>	5.1 ± 0.1 <sup>d</sup>	10.7 ± 0.2 <sup>d</sup>	18.3 ± 0.1 <sup>d</sup>
Brand E	83.1 ± 1.4 <sup>e</sup>	9.4 ± 0.2 <sup>e</sup>	6.0 ± 0.2 <sup>e</sup>	11.7 ± 0.2 <sup>e</sup>	5.7 ± 0.2 <sup>e</sup>
Unbranded sample	365.3 ± 0.5 <sup>f</sup>	14.5 ± 0.1 <sup>f</sup>	5.7 ± 0.2 <sup>e</sup>	14.8 ± 0.06 <sup>f</sup>	24.7 ± 0.1 <sup>f</sup>
Control	35.2 ± 0.3 <sup>g</sup>	13.9 ± 0.06 <sup>g</sup>	8.6 ± 0.1 <sup>c</sup>	15.9 ± 0.06 <sup>g</sup>	4.1 ± 0.1 <sup>g</sup>

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

One way ANOVA was used to check whether there is a significant difference between brands in mean metal contents. Due to p-value is less than 0.05, there are significant differences among brands in the mean Fe, Mn, Cu, Zn and Cr contents. According to the Tukey’s pairwise comparison test results, all brands are significantly different to each other and with the unbranded sample in mean Fe, Mn, Zn and Cr contents. When considering the Cu content there is no significant difference between brand E and the unbranded sample, while all the other brands are significantly different to each other in Cu content. According to the Dunnett’s comparison test results, all brands are significantly different from the control in mean Fe, Mn, Zn and Cr contents. When considering the Cu content, there is no significant difference of brand C with the control, while all the other brands are significantly different. Mean Mn, Cu, Zn and Cr content in chillie powder samples are graphically represented in the Figure 1, while mean Fe content is represented in the Figure 2.



**Figure 1: Variation of Mn, Cu, Zn and Cr contents in chillie powder samples (flame AAS)**

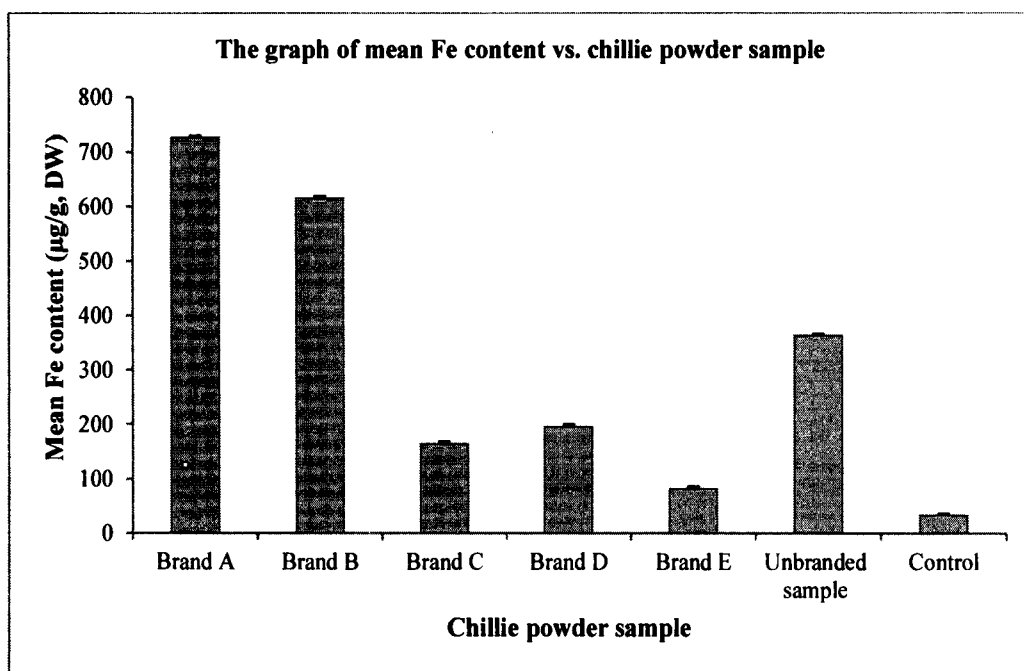


Figure 2: Variation of Fe content in chillie powder samples (flame AAS)

Table 7 shows the Fe, Mn, Cu, Zn and Cr, contents in chillie powder samples, according to the XRF spectrometric results. The results are expressed as µg metal /g of dried chillie powder (ppm).

Table 7: Micro metal content in chillie powder samples (XRF) - brandwise

Chillie powder sample	Metal content (µg/g, DW)				
	Fe	Mn	Cu	Zn	Cr
Brand A	730.9 ± 9.0	22.0 ± 2.3	6.0 ± 0.5	13.1 ± 0.6	ND
Brand B	621.3 ± 5.1	18.4 ± 1.3	10.7 ± 0.4	19.0 ± 0.5	ND
Brand C	170.0 ± 2.6	12.6 ± 1.3	12.6 ± 1.3	17.0 ± 0.4	ND
Brand D	205.8 ± 2.5	11.0 ± 0.8	5.4 ± 0.2	11.3 ± 0.3	ND
Brand E	85.0 ± 1.7	9.8 ± 0.9	9.8 ± 0.9	12.2 ± 0.3	ND
Unbranded sample	376.4 ± 3.9	14.8 ± 1.2	14.8 ± 1.2	14.8 ± 0.3	ND
Control	36.7 ± 1.6	14.1 ± 2.8	9.0 ± 0.4	15.8 ± 0.5	ND

Results are metal content ± measurement uncertainty of each sample, calculated on dry weight basis. ND stands for "not detected" (Instrumental detection limit for Cr is 0.0054 ppm).

When comparing the results of micro metals obtained by the AAS and XRF techniques, according to the paired t test results, there was no significant difference ( $p > 0.05$ ) between these two methods, AAS and XRF in Fe, Mn, Cu and Zn contents. The mean Fe content of selected chillie powder samples was in the range from 83.1 to 728.3 µg/g (on dry weight basis). But the control sample was having mean Fe content of 35.2 µg/g, thus it is clear that mean Fe content of some brands and unbranded sample is significantly high. According to the WHO standards in 2005, the maximum permissible limit of Fe for spices is 300 ppm. In the brands A, B and in the unbranded sample Fe content is higher than the maximum permissible limit. To statistically analyze whether this increment is significant or not, one sample t test was performed. According to the test results, p-values are less than 0.05 and therefore Fe content in A and B brands and unbranded sample are significantly higher than the maximum permissible limit. This significantly high value of Fe content may be due to metal contamination during the grinding process. In the chillie grinding process, grinding machinery and equipment with metal parts that can break loose (e.g., moving wire mesh belts, screens and portion control equipment, and metal ties) are likely sources of metal that may enter chillie powder during processing (LOMA systems, 2013). Once introduced into a product, metal (Fe) fragments may be

removed from the product by passing it through an electronic metal (Fe) detector. Although many chillie powder manufactures use metal detectors during the manufacturing process, modern metal detectors are still unable to detect every particle of metal passing through them. Therefore it is clear that brand A, brand B and unbranded sample contain iron particles, added during the grinding process, which is not suitable for the human health. The mean Mn content of chillie powder samples varies between 9.4 and 19.8  $\mu\text{g/g}$  (on dry weight basis). The maximum permissible limit of Mn is 100  $\mu\text{g/g}$  (WHO, 2005), and Mn content of all the chillie samples are below this limit. The mean Cu content of chillie powder samples was ranged between 5.1 and 10.3  $\mu\text{g/g}$  (on dry weight basis), while the mean Zn content between 10.7 and 18.7  $\mu\text{g/g}$  (on dry weight basis). The maximum permissible limits for Cu and Zn are 20 and 50  $\mu\text{g/g}$  respectively (WHO, 2005). Thus in the chillie powder samples both these metals are below the maximum permissible limit. The mean Cr content varies between 5.7 and 24.7  $\mu\text{g/g}$  (on dry weight basis). The unbranded sample was having significantly higher amount of Cr than the control sample, and it indicates some sort of Cr contamination in the unbranded sample. But it is below the maximum permissible limit of 30  $\mu\text{g/g}$  (WHO, 2005), and therefore it is not highly considerable.

### Toxic metals in chillie powder

The toxic metals in chillie powder samples were analyzed using graphite furnace AAS and XRF spectrometric techniques. Table 8 shows the mean Ni, Pb and Cd contents in each batch of five chillie powder brands, obtained using graphite furnace AAS technique. The results are expressed as  $\mu\text{g}$  metal /kg of dried chillie powder (ppb).

Table 8: Mean toxic metal content in chillie powder samples (graphite furnace AAS) – batchwise

Brand	Batch	Mean metal content ( $\mu\text{g/kg}$ , DW)		
		Ni	Pb	Cd
A	1	173.3 $\pm$ 0.3 <sup>a</sup>	120.0 $\pm$ 0.3 <sup>a</sup>	519.3 $\pm$ 0.7 <sup>a</sup>
	2	170.8 $\pm$ 0.4 <sup>a</sup>	115.9 $\pm$ 0.4 <sup>a</sup>	516.0 $\pm$ 0.2 <sup>a</sup>
	3	173.1 $\pm$ 0.1 <sup>a</sup>	118.9 $\pm$ 0.3 <sup>a</sup>	517.8 $\pm$ 0.3 <sup>a</sup>
B	1	221.0 $\pm$ 0.1 <sup>b</sup>	55.8 $\pm$ 0.3 <sup>b</sup>	495.9 $\pm$ 0.7 <sup>b</sup>
	2	218.2 $\pm$ 0.3 <sup>b</sup>	52.7 $\pm$ 0.3 <sup>b</sup>	490.9 $\pm$ 0.7 <sup>b</sup>
	3	220.2 $\pm$ 0.1 <sup>b</sup>	54.3 $\pm$ 0.1 <sup>b</sup>	493.6 $\pm$ 0.5 <sup>b</sup>
C	1	99.5 $\pm$ 0.2 <sup>c</sup>	531.5 $\pm$ 0.1 <sup>c</sup>	528.9 $\pm$ 0.4 <sup>c</sup>
	2	96.2 $\pm$ 0.4 <sup>c</sup>	525.9 $\pm$ 0.3 <sup>c</sup>	525.2 $\pm$ 0.5 <sup>c</sup>
	3	97.6 $\pm$ 0.2 <sup>c</sup>	528.4 $\pm$ 0.2 <sup>c</sup>	527.8 $\pm$ 0.4 <sup>c</sup>
D	1	208.2 $\pm$ 0.2 <sup>d</sup>	ND	247.0 $\pm$ 0.2 <sup>d</sup>
	2	207.6 $\pm$ 0.2 <sup>d</sup>	ND	245.7 $\pm$ 0.5 <sup>d</sup>
	3	206.2 $\pm$ 0.2 <sup>d</sup>	ND	242.7 $\pm$ 0.5 <sup>d</sup>
E	1	54.7 $\pm$ 0.1 <sup>e</sup>	146.1 $\pm$ 0.2 <sup>e</sup>	230.8 $\pm$ 0.2 <sup>e</sup>
	2	55.2 $\pm$ 0.2 <sup>e</sup>	142.8 $\pm$ 0.3 <sup>e</sup>	225.8 $\pm$ 0.4 <sup>e</sup>
	3	54.2 $\pm$ 0.06 <sup>e</sup>	144.9 $\pm$ 0.1 <sup>e</sup>	228.0 $\pm$ 0.2 <sup>e</sup>

Results are mean  $\pm$  standard deviation of three replicates and calculated on dry weight basis. Means within the same column that have same common letters are not significantly different ( $p > 0.05$ ). ND stands for "not detected" (Instrumental detection limit for Pb is 0.03 ppb).

Table 9: Mean toxic metal content in chillie powder samples (graphite furnace AAS) – brandwise

Chillie powder sample	Mean metal content ( $\mu\text{g/kg}$ , DW)		
	Ni	Pb	Cd
Brand A	172.4 $\pm$ 1.2 <sup>a</sup>	118.2 $\pm$ 1.9 <sup>a</sup>	517.7 $\pm$ 1.5 <sup>a</sup>
Brand B	219.8 $\pm$ 1.2 <sup>b</sup>	54.3 $\pm$ 1.4 <sup>b</sup>	493.5 $\pm$ 2.2 <sup>b</sup>
Brand C	97.8 $\pm$ 1.4 <sup>c</sup>	528.6 $\pm$ 2.5 <sup>c</sup>	527.3 $\pm$ 1.7 <sup>c</sup>
Brand D	207.3 $\pm$ 0.9 <sup>d</sup>	ND	245.1 $\pm$ 1.9 <sup>d</sup>
Brand E	54.7 $\pm$ 0.4 <sup>e</sup>	144.6 $\pm$ 1.5 <sup>d</sup>	228.2 $\pm$ 2.2 <sup>e</sup>
Unbranded sample	148.8 $\pm$ 0.2 <sup>f</sup>	1147.0 $\pm$ 0.2 <sup>e</sup>	784.7 $\pm$ 0.2 <sup>f</sup>
Control	14.8 $\pm$ 0.1 <sup>g</sup>	ND	196.1 $\pm$ 0.1 <sup>g</sup>

According to the Bartlett's test (normal distribution) results, there was no significant difference ( $p > 0.05$ ) between the batches in each brand, A, B, C, D and E, in mean Ni, Pb and Cd contents. Table 9 shows the mean Ni, Pb and Cd

contents in each chillie powder brand and unbranded sample with the control, obtained using graphite furnace AAS technique.

Results are mean  $\pm$  standard deviation of nine replicates and calculated on dry weight basis. Means within the same column that have no common letters are significantly different ( $p < 0.05$ ).

According to one way ANOVA test results ( $p < 0.05$ ), there are significant differences among brands in the mean Ni, Pb and Cd contents. According to the Tukey's pairwise comparison test results, all brands are significantly different to each other and with the unbranded sample in mean Ni, Pb and Cd contents. According to the Dunnett's comparison test results, all brands are significantly different from the control in mean Ni and Cd contents. When considering the Pb content, Pb was not detected in the control sample and also in the brand D. Mean Ni, Pb and Cd contents in chillie powder samples are graphically represented in the Figure 3.

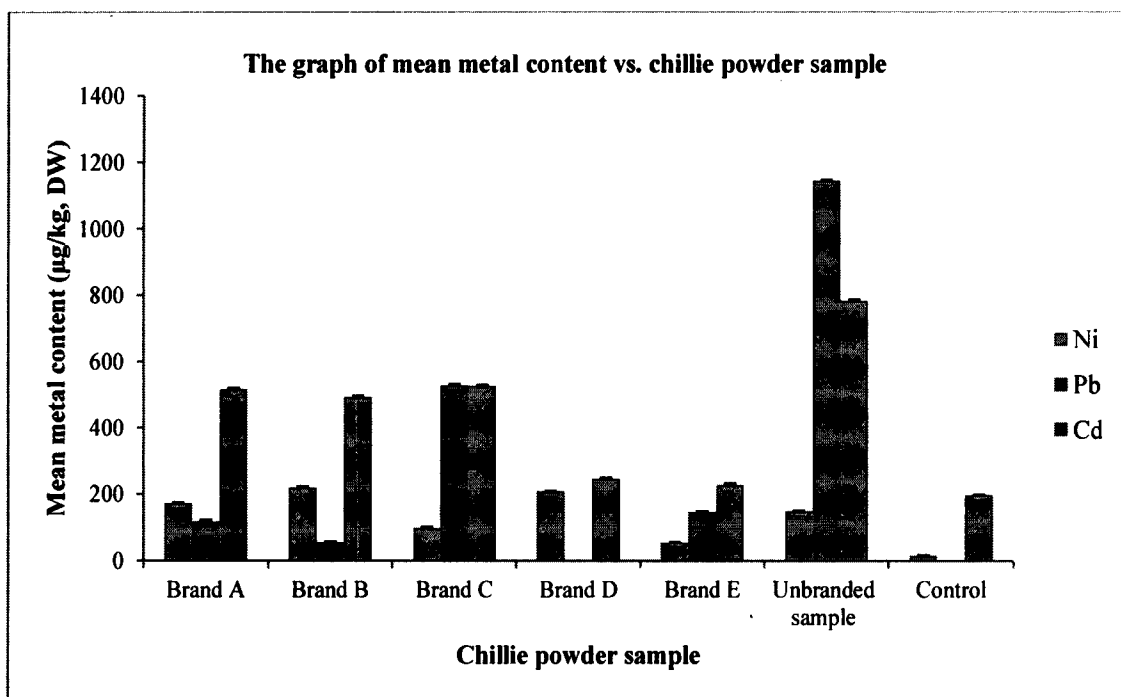


Figure 3: Variation of Ni, Pb and Cd contents in chillie powder samples (graphite furnace AAS)

The mean Ni content of chillie powder samples was ranged between 54.7 and 219.8  $\mu\text{g}/\text{kg}$  (on dry weight basis), while the mean Pb content between 54.3 and 1147.0  $\mu\text{g}/\text{g}$  (on dry weight basis). The maximum permissible limits for Ni and Pb are 50 000 and 10 000  $\mu\text{g}/\text{kg}$  respectively (WHO, 2005). Thus in the chillie powder samples both these metals are far below the maximum permissible limit. The mean Cd content of chillie powder samples was ranged between 228.2 and 527.3  $\mu\text{g}/\text{kg}$  (on dry weight basis). The maximum permissible limit of Cd for spices is 200  $\mu\text{g}/\text{kg}$  (WHO, 2005). In the all branded samples and the unbranded sample Cd content exceeds the maximum permissible limit. In the statistical analysis, according to the one sample t test results, p-values are less than 0.05 and therefore Cd contents in each brand and unbranded sample are significantly higher than the maximum permissible limit. This is not a favourable condition because Cd is a relatively toxic metal, and Cd uptake by plants is enhanced due to the use of superphosphate fertilizers. The toxic metal Cd also can be included in chillies, those grown on high Cd soils (Pearson, 1976). Therefore Cd content in chillie powder brands can be significantly different due to the differences in soil conditions where they were grown, and also the types of fertilizers used. The toxic metals, Ni, Pb and Cd were not detected by the XRF technique. XRF has detection limits in the ppm range. Therefore it is not a suitable technique for the detection of metals in the ppb range.

## CONCLUSION

This study was carried out to investigate the trace metal content present in the selected chillie powder samples, commonly available in the Sri Lankan market. According to the results, the mean Fe content of brand A ( $728.3 \pm 1.2 \mu\text{g/g}$ ), brand B ( $616.6 \pm 1.9 \mu\text{g/g}$ ) and unbranded sample ( $365.3 \pm 0.5 \mu\text{g/g}$ ) were significantly higher than the WHO maximum permissible limit ( $300 \mu\text{g/g}$ ). This may be due to the metal (Fe) contamination during the grinding process. Although many chillie powder manufactures use metal detectors to remove metal parts during the manufacturing process, modern metal detectors are still unable to detect every particle of metal passing through them. Thus brand A, B and unbranded samples are not suitable for daily consumption. In all the chillie powder samples the micro minerals Mn, Cu, Zn and Cr were found to be present in ppm level, and they were below the maximum permissible limits. In these chillie powder samples toxic metals, Ni, Pb and Cd were also present in ppb level. In the tested samples Ni and Pb were below the maximum permissible limits, but in all samples Cd content exceeded the maximum permissible limit. The mean Cd content of chillie powder samples ranged between 228.2 and 527.3  $\mu\text{g/kg}$ . The control sample also contained relatively high amount of Cd content ( $196.1 \pm 0.1 \mu\text{g/kg}$ ), but below the maximum limit ( $200 \mu\text{g/kg}$ ). The content of Cd in chillie powder samples can be significantly higher in those which are grown on high Cd soils and differences occur due to the types of fertilizers used. When considering all the analyzed metals in the chillie powder samples there were no significant differences between batches in each brand. But significant differences present among the brands. When comparing the results obtained by the AAS and XRF techniques, there was no significant difference between these two techniques in Fe, Mn, Cu and Zn contents. XRF has detection limits in the range of ppm. Therefore it is not a suitable technique for the detection of metals in the ppb range, like Ni, Pb, Cd etc.



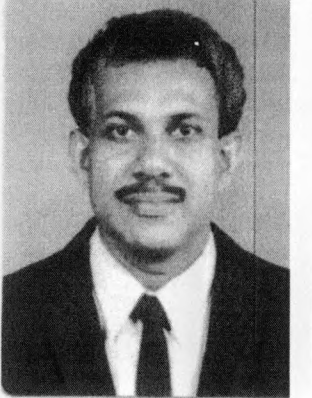
#### ACKNOWLEDGEMENTS


The authors thank the Atomic Energy Board, Sri Lanka for providing the necessary facilities for the XRF spectrometric analysis.

#### REFERENCES

- [1] AOAC (1996), Association of Analytical Communities, Official Methods of Analysis of AOAC International, 16<sup>th</sup> edition, Vol. II
- [2] Balcerzak, M. (2002), Sample Digestion Methods for the Determination of Traces of Precious Metals by Spectrometric Techniques, *Analytical Sciences*, Vol. 18, pp. 737-750
- [3] Belay, K. (2014), Analysis of Lead, Cadmium and Chromium in Ethiopian Spices after Wet (acid) Digestion using Atomic Absorption Spectroscopy, *Global Journal of Science Frontier Research : (B) Chemistry*, Vol. 14, Iss.4 No. 1.0, pp. 1-5
- [4] Belay, K. (2014), Determination of Pb (II), Cr (VI) and Cd (III) contents in Ethiopian Spices after Microwave Digestion using Atomic Absorption Spectroscopy, *International Journal of Technology Enhancements and Emerging Engineering Research*, Vol. 2, Iss. 8, pp.87-91
- [5] Buhre, V.E., Jenkins, R. and Smith, D.K. (1998), A Practical Guide for the Preparation of Specimens for X-Ray Fluorescence and X-Ray Diffraction Analysis, pp.39
- [6] DASL (2006), Department of Agriculture - Sri Lanka, Crop Recommendations – Chillie
- [7] Deshpande, S.S. and Dekker, M. (2002), Hand book of Food Toxicology, *New York*
- [8] FSAI (2009), Food Safety Authority of Ireland, Mercury, Lead, Cadmium, Tin and Arsenic in Food, *Toxicology Factsheet Series*, No. 1, pp. 1-13
- [9] FSSAI (2012), Food Safety and Standards Authority of India, Manual of Methods of Analysis of Food Metals
- [10] Garcia, R. and Baez, A.P. (2012), Atomic Absorption Spectrometry (AAS), pp. 1-11
- [11] Inam, F., Deo, S. and Narkhede, N. (2013), Analysis of Minerals and Heavy Metals in Some Spices Collected from Local Market, *IOSR Journal of Pharmacy and Biological Sciences*, Vol. 8, Iss. 2, pp. 40-43
- [12] Krejpcio, Z., Krol, E. and Sionkowski, S. (2007), Evaluation of Heavy Metals contents in Spices and Herbs Available on the Polish Market, *Polish Journal of Environmental Studies*, Vol.16, No.1, pp. 97-100
- [13] LOMA Systems (2013), A guide to metal detection in the food manufacturing industry
- [14] MAGI (2009), Ministry of Agriculture – Government of India, Post Harvest Profile of Chillie, pp. 6-16
- [15] Pearson D. (1976), The Chemical Analysis of Foods, 7<sup>th</sup> edition, pp. 73-97
- [16] Ranaweera, K.K.D.S. (2007), All About Foods, Godage International Publishers (Pvt) Ltd, pp. 153-176

## AUTHOR BIBLIOGRAPHY

	<p><b>Ms. W.A.A.V.S. Amaradivakara</b> Assistant City Analyst, Colombo; Following M.Sc. Degree in Food Science and Technology in the Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka, Holding the B.Sc. Special Degree in Chemistry (First class Hons.); Member of the Royal Society of Chemistry, UK; Life Member of the Institute of Chemistry, Ceylon; Member of Sri Lanka Association for the Advancement of Science, Sri Lanka.</p>
	<p><b>Dr. (Ms.) Indira Wickramasinghe</b> Senior Lecturer, Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewadenapura, Sri Lanka; Former Head of the Department of Food Science and Technology, Lecturer MSc in Food Science and Technology and Department of Plant BioTechnology, University of Colombo; Member of the Editorial Board of National Aquatic Resources Research and Development Agency (NARA) Journal, Colombo; Life member of Sri Lanka Association for the Advancement of Science, Sri Lanka and Nutrition society of Sri Lanka; Member of the Development Group and Advisory committee of the National Diploma in Food Technology NVQ Level 5 for National Competency Standards for food Technology in Sri Lanka.</p>
	<p><b>Mr. R. M. G. B. Rajanayake</b> City Analyst Colombo; Chartered Chemist; Fellow member, Past Chairman, Sri Lanka section of the Royal Society of Chemistry, UK.; Fellow member of the Institute of Chemistry, Ceylon; Visiting lecturer in Food Science &amp; Technology, University of Sri Jayewardenepura, Sri Lanka, Agriculture &amp; Environment Science, University of Kelaniya Sri Lanka, Community Medicine, Post Graduate Institute of Medicine, University of Colombo; Editor and visiting lecturer for Food technology programs, Open University of Sri Lanka, Nawala; Member of the Food Advisory Committee, Ministry of Health &amp; Nutrition; Council member of the Institute of Chemistry Ceylon.</p>

	<p><b>Prof. Arthur Bamunuarachchi</b> Emeritus Professor, University of Sri Jayewardenepura, Sri Lanka; National and International Consultant Food Scientist and Technologist; Specialist Post Harvest, Food Processing and Agribusiness Trainer; D.Sc (Hon.Causa), Sabaragamuwa University of Sri Lanka; D.Sc(Hon.Cauza),University of Sri Jayewardenepura, Sri lanka; Presidential awards for Research Publications – 2009/10/11; Member, Australian Institute of Food Technologist(Ex.); Member, American Institute of Food Technologist(Ex.); Member, American Institute of Cereal Chemists.(Ex.); Member, Indian Association of Food Scientist and Technologists. (Ex.); Member, Pakistan Association of Food Science and Technologists.(Ex.); Member, Institute of Chemistry, Sri Lanka.</p>
---	--