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ANALYSIS OF METAL CONTENT IN BLACK PEPPER POWDER AVAILABLE IN THE SRI LANKAN MARKET

P.T. Sandanayake[*], I. Wickramasinghe, R.M.G.B. Rajanayake, A. Bamunuarachchi
[*]Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

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ABSTRACT

Black pepper (*Piper nigrum* L.), is a highly valued spice. It is one of the major items exported by Sri Lanka. In recent years, monitoring metal contamination in pepper has been a growing interest and it is reported to contain significant quantities of some metals which cause detrimental effects on human health. In this study concentrations of some macro, micro and toxic metals in five common brands and unbranded Black pepper samples available in the Sri Lankan market were determined. Three batches from each brand were subjected to analyze. Whole Black pepper samples were analyzed as a control to study the possible metal contaminations during the process of powdering. AOAC Official method of 975.03 (1996) was used to determine the metal content by Atomic absorption spectroscopy and X-ray Fluorescence Spectroscopy. The results were statistically analyzed using analysis of variance (ANOVA).The Study shows differences in metal concentrations of their mean values as Potassium> Calcium> Magnesium>Sodium>Iron> Manganese>Copper> Zinc> Cadmium> Lead. Cadmium was significantly above the WHO maximum permissible limit (MPL), inferring that the agricultural practices might be affecting cadmium contamination in pepper. The study highlighted possibility of wear and tear of machineries causing high Iron content in powdered pepper.

KEYWORDS: **pepper powder, macro metals, micro metals, and toxic metals.**

I. INTRODUCTION

Pepper is described as the "king of spices," as it shares on most dinner tables with salt. Pepper is grown in Indonesia, Malaysia, Sri Lanka, Vietnam, and Kampuchea as well as the West coast of India. The United States is the largest importer while India is the largest exporter of this spice.

Black and White pepper come from the shrub classified as *Piper nigrum*. *Piper nigrum* is one of about 1,000 species in the Piper genus and is a part of the larger family of peppers called Piperaceae. A berry-like fruits produces peppercorns contains a cluster seeds. It is indigenous to southern India and Sri Lanka. It has been cultivated in other countries with uniformly warm temperatures and moist soil conditions.

Black pepper is a favourite spice and flavouring agent of cooks because of its dark colour and pungent aroma and flavour. Peppercorns contain an alkaloid called piperine. The Sri Lankan Pepper has higher piperine content which gives it a superior quality and pungency. The hot taste sensation in pepper comes from chavicine in the peppercorns.

As a natural medicinal plant, Black pepper has been used for relieving arthritis, nausea, fever, migraine headaches, poor digestion, strep throat, and even coma (Peter, 2004). It has also been used for non-medical applications such as an insecticide, pharmaceutical and perfumery industries.

Pepper processing includes many steps. First step is screening and cleaning to remove dust, dirt and stones. Next is drying. This is the most important part in the process. If it is not dried adequately, it may lead to mould growth. Then pepper is graded by colour, size, and relative density. Grinding may also add value, but must be done carefully. There is a market resistance to ground pepper due to fear of adulteration. Last step is packaging and ground pepper requires polypropylene.

In Sri Lanka pepper is grown in the wet and intermediate zones as mixed crops. It has been reported to contain significant quantities of some metals. Metals such as iron, zinc and copper have biological importance. Iron transports oxygen in red blood cells and in muscles. Zinc helps in optimum functioning of many enzymes that

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involved in catalytic reactions and also maintenance of structural stability and regulatory functions. Copper acts as an antioxidant by protecting the brain and the nervous system. Chronic exposure to mercury can damage the kidneys and central nervous system. The food that contaminated with trace and heavy metals may result in accumulation of these metals in human organs (Darko B, 2010).Exposure to lead (Pb) may lead to higher risks of heart attacks and strokes in adults. The food that contaminated with trace and heavy metals may result in accumulation of these metals in human organs. Trace and heavy metals above the permissible level affect the human health and may result in illness to human fetus, abortion and preterm labour, and mental retardation in children.
Pepper plants can be contaminated with heavy metals through environmental pollution. Contamination could result from the type of soil for cultivation, source of water used for irrigation, place of the spices processed, stored or sold. Also contaminates from the soil or aerial depositions can be happened as these are dried on the ground or on roof tops, especially with small scale producers. Commercial mills may also introduce some amount of metals due to wear and tear of the machinery.
Metal content was determined using Atomic Absorption Spectroscopy (AAS) which is a combination of flame; Graphite furnace Spectroscopy method and X- ray Fluoresce Spectroscopy. The data were compared with WHO international standard limits. The major objective of this study is to analyzed macro metals{Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca)}trace metals {Manganese (Mn), Iron (Fe), Copper (Cu) and Zinc (Zn)}and toxic metals {Lead (Pb), Cadmium Cd)}in Black pepper, commonly available in the Sri Lankan market. The specific objectives are to check whether these metal contents in Black pepper samples are within the standard limits recommended by International (WHO standards) standards, to find out whether there is a significant difference in metal content among the brands, unbranded sample and whole pepper.

Table 1: Maximum limits for micro and toxic heavy metals in spices

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

Source: WHO (2005)

II. MATERIALS AND METHODS

Sample Collection for the Analysis
Five brands of Black pepper samples named A, B, C, D and E and an unbranded sample from the local market in Sri Lanka were selected for analysis. Samples were purchased from different locations during a period of six months. Three batches from each brand and three samples from each batch were selected leaving the time period of 2 months within batches. An unbranded sample was randomly purchased and an ungrounded Black pepper sample was collected as the control.

Sample preparation for Atomic absorption spectrometric analysis
Materials
Glassware, Containers and Apparatus

- Volumetric flasks (50 mL), Pyrex beakers (150 mL), Pipettes, porcelain dishes, polypropylene bottles
- Drying Oven (CascinaTorchio, B 3535S) maintained at 105 ^{0}C
- Analytical Balance (RADWAG, AS 220.R2)
- Hot Plate (VELP Scientifica, VSS2FCR2)
- Fume hood
- No 41 ash less filter papers
- Muffle Furnace
- Desiccators

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Reagents

Analytical grade chemicals were used throughout the experiment to prepare the reagents. De-ionized water was used to prepare the standards.

- Nitric acid (HNO_3) solution (1+1)
- Perchloric acid ($HClO_4$) solution (60%)
- Hydrochloric acid (HCl) solution (1+1)
- Lanthanum stock solution (50 g La/ L)

Cleaning Glassware and containers

All glassware including pipettes, beakers, Petri dishes, watch glasses, crucibles, glass rods and poly propylene bottles were soaked overnight in a plastic basin containing 10%v/v Nitric acid. After soaking they were triple-rinsed with de-ionized water.

Method

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

Black pepper sample in a porcelain dish was oven dried at 105^{0}C for 2 hours, until constant weight obtained. Then accurately weighed about 1 g of oven dried sample into a 150 mL Pyrex beaker and 10 mL of HNO_3 (1+1) was added. It was allowed to soak thoroughly and 3 mL of 60% $HClO_4$ was added. Then it was heated on a hot plate in a fume hood, slowly at first, until frothing ceased. The heating continued at 100 ^{0}C, until almost all HNO_3 evaporated. The addition of 10 mL of HNO_3 and heating were repeated another five times, until clear solution was obtained. Then it was cooled and 10 mL of HCl (1+1) was added, this was quantitatively transferred into a 50 mL volumetric flask. La solution (5%, 10 mL) was added to settle silica. Then it was filtered through an acid washed No. 41 filter paper. Prepared solutions were then transferred into pre-cleaned labeled polypropylene bottles with no metal linings until taken for analysis. As a measure of quality control, blanks were prepared by following the same procedure as described above without adding the sample.

Atomic Absorption Spectrometer (AAS) analysis

Flame Atomic Absorption Spectrometer (FAAS)

Materials

Glassware, Apparatus and Instruments

- Atomic absorption spectrometer (Thermo Scientific,iCE 3000 series)
- Hollow cathode lamps of Na, K, Mg, Ca, Fe, Mn, Cu, Zn.
- Volumetric Flasks (50mL, 100mL)

Reagents

- Standard stock solutions (1000 ppm) of Na, K, Mg, Ca, Fe, Mn, Cu, Zn,
- Trace metal grade hydrochloric acid (HCl) solution (10%)
- De-ionized water

Method

The standard series of each metal was prepared, using the stock solutions.

Ca standard series were prepared 10mL of 5% La solution was added to the standard in 50 mL volumetric flask, so that final dilutions contained 1 % La.

Thermo Scientific iCE 3000 series AAS equipped with robust flame sample introduction system was used for analysis of Na, K, Mg, Ca, Fe, Mn, Cu, and Zn in Black pepper samples. Thermo scientific SOLAAR software allowed quick and easy optimization of the method. Hollow cathode lamp with each metal was used at the specific wavelength.

Spectrometer parameters used are shown in the Table 2.

Table 2- Spectrometer parameters used for AAS

Element	Wave length (nm)	Fuel flow rate (L/ min)	Flame type	Slit width (nm)	Lamp current (mA)
Na	589.6	0.9 - 1.2	Air / acetylene	1.0	5
K	766.5	1.1 - 1.3	Air / acetylene	1.0	5
Mg	285.2	0.9 - 1.2	Air / acetylene	0.5	4
Ca	422.7	4.0 -4.4	Nitrous oxide / acetylene	0.5	10
Fe	248.3	0.8 - 1.0	Air / acetylene	0.2	5
Mn	279.5	0.9 - 1.2	Air / acetylene	0.2	5
Cu	324.8	0.8 - 1.1	Air / acetylene	0.5	4
Zn	213.9	0.9 - 1.2	Air / acetylene	1.0	5

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The digested solutions of pepper samples were aspirated and the concentrations were read. The blank was used to establish 0absorbance. For K and Ca determination the samples were diluted 50 times with 10% HCl to obtain reading within the range of the instrument.

Graphite furnace Atomic Absorption Analysis
Materials
Reagents

- De-ionized water
- Standard stock solutions (1000ppm) of Pb and Cd

Thermo scientific iCE 3000 series Atomic Absorption Spectrometer equipped with graphite furnace atomizer was used to analyze Pb and Cd levels in pepper samples. All the spectrometer parameters were controlled by SOLAAR software.
Furnace parameters used in the analysis is given in the Table 3.

Table 3 - Graphite Furnace parameters used in analysis of Pb and Cd

Element	Wave-length (nm)	Lamp current (mA)	Slit width (nm)	Atomize temperature (^{0}C)
Pb	217.0	5	1.0	1200
Cd	228.8	5	0.5	900

Calculations
The concentration values in the original sample calculated as follows.

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

Where,
C = Concentration of the metal given by AAS reading (µg/mL)
V = Final volume of the digested sample (mL)
W = Weight of the dried sample (g)
Dilution factor was considered in K and Ca analysis

Sample preparation for X-Ray fluorescence Spectrometric analysis
Black pepper samples from each brand were oven dried at 105 ^{0}C for 2 hours, in silica dishes, until obtaining a constant weight. About 20 g of oven dried sample was ashed in the muffle furnace at 300 ^{0}C for one day. Again weighed to obtain the ash weight. Metal contents were analyzed according to the validated method used by the Atomic Energy Board of Sri Lanka.

Apparatus and Materials

- Analytical balance (RADWAG, AS 220 R2)
- Muffle Furnace (Thermolyne, F 627 30) maintained at 300^{0}C
- Oven (CascinaTorchio, B35358) maintained at 105^{0}C
- Pelletizing press (Spectro Press 4312 E)
- Cylindrical pellet die
- Hard plastic motor and pestle
- Oil papers
- Silica dishes

X-ray fluorescence Spectrometric analysis
Materials

- **Prepared pellets**
- **X-Ray Fluorescence (XRF) Spectrometric analysis (Seifert, 00221)**

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Method

Ashed sample (0.50 g) was weighed into a clean oil paper. Then using a mortar, it was ground into a homogeneous powder. Then powder was transferred into a cylindrical pellet die and evenly distributed. This was pressed manually operated bench top pelletizing press (Spectro Press, 4312 E) to form a pellet which had a smooth, homogeneous sample surface. The prepared pellets were loaded into the sample holder. Then the Energy dispersive X – ray fluorescence spectrometer setup (Seifert, 00221) instrument was set up using the operating parameters given in the Table 4. The AXIL software was used to run the programme. The sample was run 500 seconds using the molybdenum target.
Using this method K, Ca, Fe, Mn, Cu, Zn was analyzed.

Table 4: 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

The analysis was carried out for the above mentioned metals and they were identified by their characteristic fluorescent energy.

Calculation

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

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 Data Analysis

Minitab analysis of variance (ANOVA) at 95% confidence interval was carried out to determine the statistical significance of differences in mean metal concentrations among brands. Using the Tukey's pair wise comparisons, the significant differences of metal contents among brands were compared. According to the Dunnet's comparison statistical analysis, a significant difference was evaluated with whole pepper (Control).Bartlett's statistical analysis was used to analyze the significant difference among the batches. Finally paired t test was carried out to compare the two methods of metal analysis (AAS method and XRF method).

III. RESULTS AND DISCUSSION

In this study, macro, micro and toxic metal contents of Black pepper powder samples were analyzed using selected five brands of Black pepper power samples and an unbranded sample which are commonly available in the Sri-Lankan markets. An ungrounded whole Black pepper sample was selected as the control.

Analysis of macro metals

The macro metals (Na, Mg, K and Ca) contents in Black pepper batch wise and brand wise which were determined by AAS are listed in Table 5and 6 respectively. All the results of metal concentrations were expressed as µg/g on dry weight basis.

Table 5- Macro metal content in Black pepper (batch wise)by flame AAS{µg/g, Dry Weight (DW)}

Brand	Batch	Na(µg/g) DW	K(µg/g) DW	Mg(µg/g) DW	Ca(µg/g) DW
A	1	133.7±1.1[a]	20394.7±0.8[a]	2192.4±0.5[a]	2938.6±0.2[a]
	2	123.5±0.6[a]	20381.7±1.0[a]	2188.3±0.4[a]	2940.5±0.3[a]
	3	131.0±0.8[a]	20377.4±0.9[a]	2190.4±0.5[a]	2937.8±0.3[a]
B	1	142.7±0.4[b]	20320.0±0.6[b]	2161.7±0.5[b]	2590.3±0.2[b]
	2	148.7±0.8[b]	20325.2±0.6[b]	2160.1±0.4[b]	2588.5±0.2[b]
	3	140.7±0.7[b]	20347.8±0.4[b]	2158.1±0.3[b]	2592.5±0.4[b]
C	1	152.9±0.5[c]	20411.7±0.7[c]	2015.2±0.3[c]	2280.6±0.3[c]
	2	160.1±0.5[c]	20423.2±0.8[c]	2013.5±0.4[c]	2275.2±0.3[c]
	3	161.3±1.0[c]	20437.8±0.7[c]	2018.2±0.3[c]	2278.6±0.5[c]
D	1	173.0±0.7[d]	20378.7±0.7[a]	1887.7±0.5[d]	1825.2±0.3[d]
	2	182.2±0.6[d]	20379.9±0.7[a]	1881.8±0.4[d]	1822.4±0.5[d]
	3	172.4±0.8[d]	20403.8±0.7[a]	1884.9±0.3[d]	1827.2±0.3[d]
E	1	167.5±1.0[c]	20421.6±0.4[c]	2263.2±0.3[e]	2319.4±0.3[e]
	2	157.1±0.8[c]	20420.9±0.6[c]	2261.8±0.4[e]	2320.7±0.5[e]
	3	163.7±0.6[c]	20443.7±0.6[c]	2265.3±0.4[e]	2317.8±0.3[e]

Results are mean ± 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).
Contents of macro metals present as brand wise in Black pepper is given below.

Table 6 - The macro metals of Black pepper brands {µg/g,dry weight (DW)} –AAS

Brand	Na(µg/g) DW	K(µg/g) DW	Mg(µg/g) DW	Ca(µg/g) DW
A	129.4±4.6[a]	20384.6±7.8[a]	2190.4±1.8[a]	2938.9±1.2[a]
B	144.1±3.7[b]	20331.0±12.8[b]	2160.0±1.6[b]	2590.4±1.8[b]
C	158.1±4.0[c]	20424.2±11.4[c]	2015.6±2.1[c]	2278.1±2.4[c]
D	175.9±4.8[d]	20387.5±12.3[a]	1884.8±2.6[d]	1824.9±2.1[d]
E	162.8±4.6[c]	20428.7±11.3[c]	2263.4±1.6[e]	2319.3±1.3[e]
Unbranded	107.9±0.2[e]	16604.3±0.2[d]	1806.3±0.5[f]	1783.4±0.4[f]
Raw (control)	243.3±0.2[f]	17190.0±0.2[e]	1746.8±1.4[g]	2696.1±0.3[g]

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

When analysis is carried out using atomic absorption spectrometry the mean Na content of Black pepper brands were in the range of129.4 to 175.9 µg/g on dry weight basis (Table 6). These values were higher than unbranded samples whereas it is lower than raw Black pepper (control). When considering the results obtained by AAS for Na, whole pepper (control) has the highest value among all the tested pepper samples. This means processing may affect the Na content present in the ground pepper samples.
The mean K content according to the Table 6 in pepper brands ranged from 20331 to 20428.7 µg/g (on dry weight basis). All these values for K are higher than unbranded and raw Black pepper samples. Looking at the data for K, it is the highest concentrated metal in the plant as it is required large quantities for growth and fruiting. It has been reported that most of the fertilizer added to pepper plants include K ion. According to research data, mean of the unbranded sample is the lowest value meaning small scale growers may not much add fertilizer to their vines.
When considering the results obtained by ASS method (Table 6), mean Mg contents obtained is ranged from 1884.8µg/g to 2263.4µg/g (on dry weight basis).As depicted by the results, in all brands, Mg contents are higher than the unbranded and whole black pepper samples. Adulterations may cause for higher Mg content in ground pepper .Also highlights that fertilizer mixtures which are added to the plant may contain Mg ion.
According to Table 6 the mean Calcium content of pepper types ranged from1824.9µg/g to 2938.9µg/g on dry weight basis among the brands. Wide variation was found within the unbranded and branded Calcium contents.

When compared with the previously published data, it shows that the nutrients consumed by Black pepper, N uptake is the highest followed by K and Ca and the magnitude of the nutrients removed is in order: N>K>Ca>Mg>P>S>Fe>Mn>Zn (Hamza *et al.*, 2007). Similarly it is reported that the nutrient removal by pepper plants was N-255, P-22.8, K-208.2, Ca-54.5 and Mg-36.4 kg ha^{-1}. The application of 400-600 g $plant^{-1}$ N,P,K and Mg (12:12:17:2) three times a year, with 500 g $plant^{-1}$ dolomite applied two times a year is reported to be the best fertilizer dose for productive pepper plantations in Bangka.

This complies with the research data revealing high levels of macro nutrients in Black pepper as well as all the branded ground pepper show higher levels than the unbranded samples, meaning the small scale producers may not apply fertilizers much to their vines.

Contents of Na, Mg, K and Ca in each Black pepper powder brands, unbranded powder and raw pepper by flame AAS are graphically represented in the Figures 1, 2and 3.

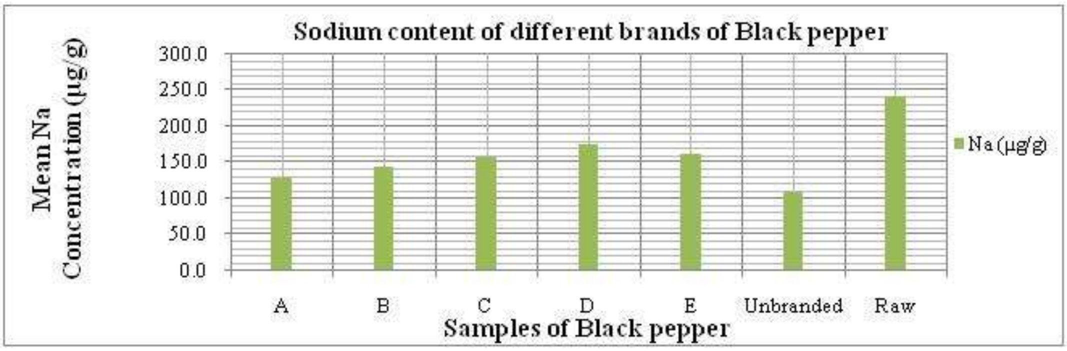

Figure 1- Na content in Black pepper by flame AAS

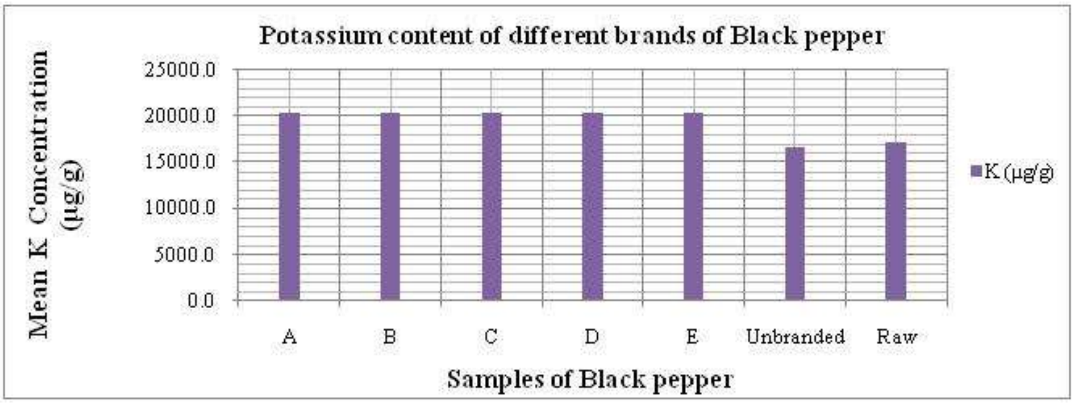

Figure 2- K content in Black pepper by flame AAS

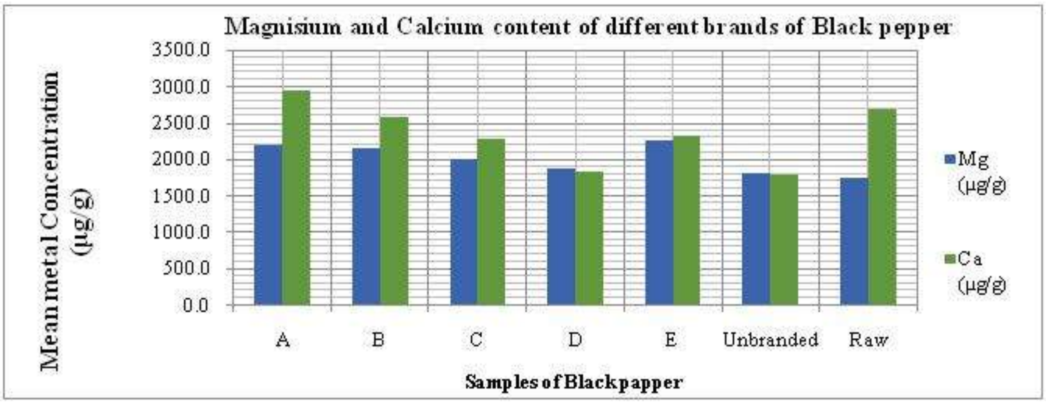

Figure 3-Mg and Ca content in Black pepper by flame AAS

Table7 shows the macro metal contents in Black pepper powder samples, according to the XRF spectrometric results. The results are expressed as µg metal /g of dried pepper powder (ppm).

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Table 7 - The macro metals of Black pepper brands (brand wise) {μg/g,dry weight (DW)}-XRF

Brand	K(μg/g) DW	Ca(μg/g) DW
A	20294.0±225.7	2938.6±74.3
B	20319.5±147.2	2590.3±43.5
C	18470.1±134.9	2280.3±34.8
D	16556.2±125.2	1815.5±38.4
E	20465.7±129.6	2319.6±39.1
Unbranded	16604.1±108.1	1783.3±33.2
Raw (control)	17190.1±168.1	2696.0±66.6

(Results are metal content ± measurement uncertainty of each sample, calculated on dry weight basis.)

The results show that these two methods were giving practically very close values. Statistically, according to the paired t test (P> 0.05) the results obtained by XRF method for K and Ca were similar to the results obtained by AAS for these 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 Black pepper in different brands (Table 6). Using Tukey's pair wise comparison, the significant difference of metal contents between different brand pairs was compared. There was no significant difference in Na and K contents between brand C and E, while between brand A and D there was no significant difference in K content.
According to the Dunnet's comparison statistical analysis for all macro metals, brands and unbranded pepper powder exhibited a significant difference from whole pepper (Control). Also, statistical analysis show that there is no significant difference in macro metal content among batches when considering all the branded Black pepper samples.

Analysis of Micro metals.
The micro metals in Black pepper samples were analyzed using flame AAS and XRF spectrometric techniques. Table 8and 9 show the Zn, Cu, Fe and Mn contents in each batch wise and brands wise obtained using the flame AAS technique.

Table 8 - Micro metal content in Black Pepper powder by flame AAS -Batch wise{dry weight (DW)}

Name of the sample: Brand	Batch	Zn(μg/g) DW	Cu(μg/g) DW	Fe(μg/g) DW	Mn(μg/g) DW
A	1	11.7±0.1^a	15.2±0.2^a	71.1±0.4^a	33.2±0.1^a
	2	11.6±0.2^a	13.6±0.2^a	71.3±0.1^a	31.7±0.2^a
	3	11.8±0.2^a	16.5±0.3^a	69.6±0.2^a	32.4±0.5^a
B	1	12.5±0.1^b	12.5±0.4^b	75.8±0.2^b	27.2±0.3^b
	2	12.1±0.3^b	13.6±0.2^b	74.5±0.3^b	26.7±0.2^b
	3	13.0±0.1^b	11.7±0.2^b	76.3±0.5^b	24.5±0.3^b
C	1	10.5±0.1^c	11.5±0.4^b	80.4±0.6^c	34.8±0.2^a
	2	10.2±0.1^c	13.6±0.3^b	80.3±0.6^c	32.5±0.4^a
	3	10.7±0.2^c	12.5±0.3^b	78.4±0.2^c	33.5±0.4^a
D	1	13.6±0.2^d	9.4±0.3^c	143.8±0.9^d	51.1±0.8^c
	2	13.3±0.2^d	8.6±0.2^c	142.3±0.4^d	50.5±0.3^c
	3	14.0±0.1^d	10.5±0.4^c	141.1±0.7^d	52.3±0.3^c
E	1	13.1±0.1^e	13.4±0.3^a	224.1±0.5^e	60.3±0.3^d
	2	12.9±0.2^e	14.6±0.4^a	223.5±0.3^e	61.5±0.4^d
	3	13.5±0.2^e	15.6±0.3^a	226.4±0.3^e	59.4±0.3^d

Results are mean ± 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).

Table 9- Mean micro metal content in Black pepper samples by flame AAS –brand wise{dry weight (DW)}

Brand	Zn(µg/g) DW	Cu(µg/g) DW	Fe(µg/g) DW	Mn(µg/g) DW
A	11.7±0.1[a]	15.1±1.3[a]	70.6±0.8[a]	32.5±0.7[a]
B	12.5±0.4[b]	12.6±0.8[b]	75.5±0.8[b]	26.2±1.3[b]
C	10.5±0.3[c]	12.5±0.9[b]	79.7±1.1[c]	33.6±1.0[a]
D	13.6±0.3[d]	9.5±0.9[c]	142.4±1.3[d]	51.3±0.9[c]
E	13.2±0.3[e]	14.5±1.0[a]	224.7±1.4[e]	60.4±0.9[d]
Unbranded	9.5±0.2[f]	11.5±0.4[b]	154.7±0.3[f]	49.2±0.1[e]
Raw	8.2±0.2[g]	11.3±0.4[b]	44.1±0.2[g]	42.5±0.4[f]

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 ($p < 0.05$).

According to Table 9 the mean Fe content obtained from AAS method was ranged between70.6-224.7µg/g (on dry weight basis) and similar range was reported with XRF analysis too (Table 10). The levels of Fe in ground pepper were much higher when compared with the whole pepper (Figure4).The high level of Fe in pepper powder could be due to contamination during milling. Research indicates that commercial grinding of spices contaminates them to about between 3 and 5 folds, due to wear and tear of the machine parts (Janitha *et al.*, 1988). The high levels of Fe could be due to presence of Fe which occurs naturally in plants in the form of metalloproteins, plant ferritins and the fact that iron forms part of the structural component of plants. Fe helps in the transport of oxygen from the lungs to cells and also is required for a number of vital functions, including growth, reproduction, healing and immune function in the body (Wardlaw, 2003).Even though Iron is an essential element needed by the body, consumption of an excessive amount can lead to health effects. The WHO recommended tolerance limit for Fe is 300mg/kg. Wide variation in Fe content found between the powder and whole Black pepper may be due to wear and tear of machineries and it confirms the reported findings. Although the Fe content is considerably high, it is below the maximum permissible limit. For an average adult (60 kg body weight) the provisional tolerable daily intake (PTDI) for Fe is 48mg (Joint FAO/WHO 1998).

According to the results obtained from AAS method the mean Zn content of the powder Black pepper brands ranged between 10.5 and13.6µg/g (on dry weight basis).Similar pattern was observed in XRF data. Whole pepper Zinc content was lower than all the ground pepper samples. The differences in Zn content from various brands could be due to type of soil for cultivation and also due to adulterations or contaminations from drying environment.

Research results on heavy metal contamination was studied in Nigeria, Ghana and Saudi-Arabia and found that Zn ion content is 68µg/g in black pepper available at local markets in these countries (Etonihu *et al*., 2013). Umar *et al*., (2014) reported in his study carried out for samples obtained from Abuja, Nigeria Zn content is 40.233mg/kg. It was reported that high rainfall status of Black pepper growing areas, especially on the slopes of Western Ghats made the soil less productive, more acidic and low P, K, Ca and Zn status due to leaching and erosion losses of these nutrients.

According to WHO recommendations the maximum Zn level permitted for Black pepper is 50 mg/kg and all the samples were well below the permissible limit which means there is no harm on health from the Zn content in Black pepper. High amount of Zn could be more deleterious than nutritious; but the WHO limit is not to be exceeded. The proposed value for Zn MRL is 0.3mg/kg/day.

The mean Cu content from AAS method ranged between 9.5 and 15.1µg/g (on dry weight basis) and similar range was reported with XRF analysis too. Compliance within the two methods suggests the practical errors are minimize and the difference among the brands may be due to differences in soil type.

In the literature sources, the recommended adequate foliar content of Cu for Black pepper in Brazil is 8-11 mg/kg (Partelli, 2009).A similar value was reported from studies carried out in Nigeria, Ghana and Saudi-Arabia which is 16 mg/kg (Etonihu *et al*, 2013).

Although Cu is an essential element in trace amount it can be toxic at excess level. Cu build can result in a tendency for hyperactivity in autistic children.

In this study the Cu values are in agree with literature values. The maximum Cu level permitted for spices is 20mg/kg according to WHO recommendations. The FAO/WHO has set limits for intake of metals based on body weight. For an average adult (60 kg body weight) the provisional tolerable daily intake (PTDI) for Cu is 3mg (Joint FAO/WHO, 1998). As revealed by analytical results Cu content of these samples are below the permissible limit. Thus, there is no harm on health due to intake of Cu ion from Black pepper. The waste water from electronic industry is a significant source of Cu ion in soil and water (Batool *et al.*, 2014).

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Manganese acts as catalyst and co factor in many enzymatic processes involved in the synthesis of fatty acids and cholesterol, mucopolysaccharide synthesis, and in the synthesis of glycoproteins, which coat body cells and protect against invading viruses (Umar *et al*., 2014). The mean Mn content was ranged between 26.2 and 60.4µg/g (on dry weight basis). XRF readings show similar pattern to AAS readings. It was reported that the recommended adequate foliar content of Mn ion for Black pepper in Brazil is 60-200µg/g (Partelli, F.L., 2009).When comparing with the WHO limit which is 100mg/kg, all the branded, unbranded and raw samples status is below the WHO limit. Hence it can be recommended that there would not be any harm to body from Mn ion by utilizing pepper. Deficiency of Mn cause skeletal abnormalities; defective muscular coordination; impaired glucose tolerance and management of blood sugar levels (Umar *et al*., 2014).
Mean Fe contents in Black pepper samples are graphically represented in the Figure 4, while mean Zn and Cu contents are represented in the Figure 5.Figure 6 represents the Mn content in Black pepper samples.

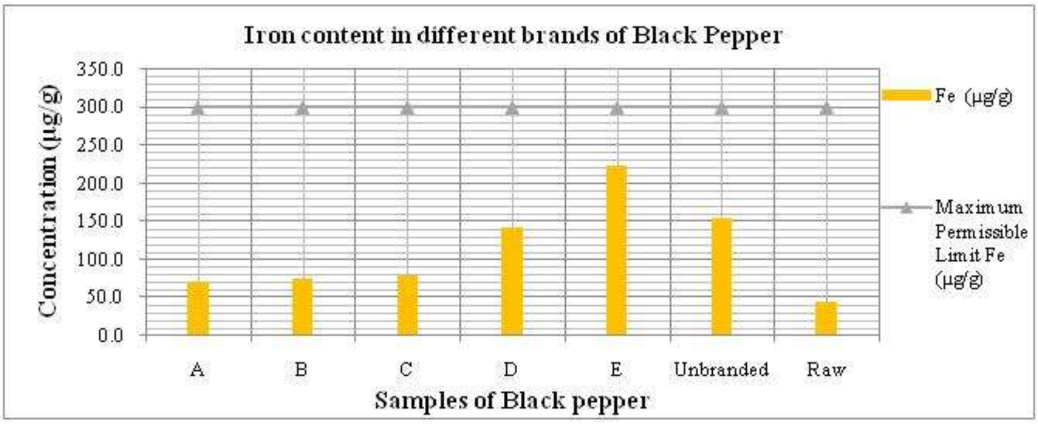

Figure 4-Fe content in Black pepper by flame AAS

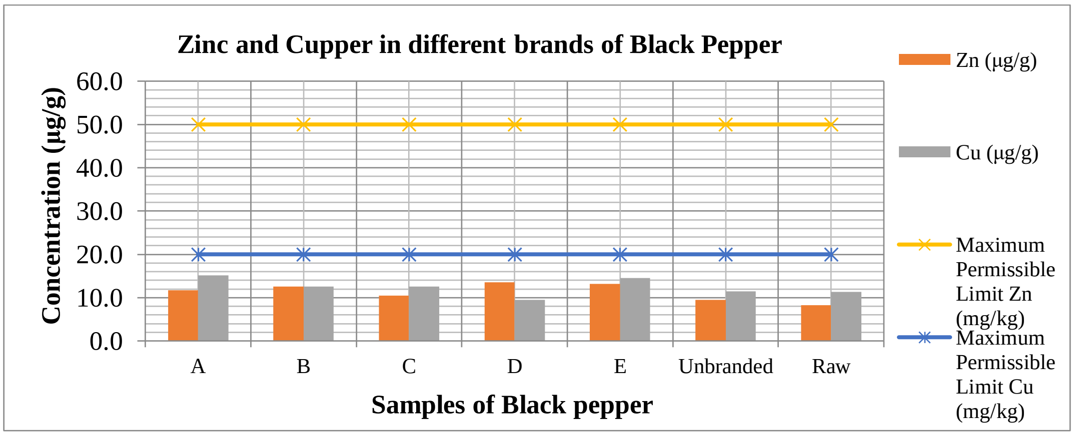

Figure 5- Zn and Cu contents in Black pepper by flame AAS

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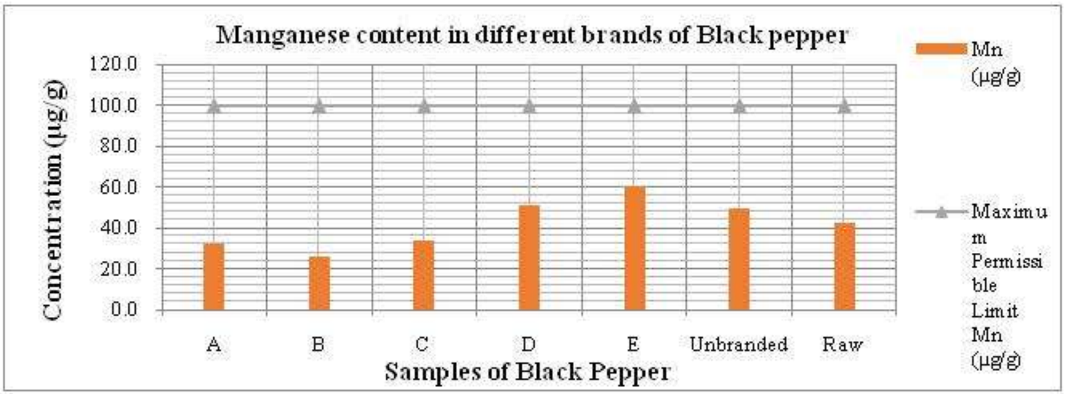

Figure6-:Mn content in Black pepper by flame AAS

Table 10 shows the Fe, Zn, Cu and Mn contents in Black pepper samples, according to the XRF spectrometric results.

Table 10: Micro metal content in different Black pepper brands by XRF {dry weight (DW)}

Brand	Zn(µg/g) DW	Cu(µg/g) DW	Fe(µg/g) DW	Mn (µg/g) DW
A	11.8±0.5	15.2±0.5	71.5±2.2	33.2±1.6
B	12.6±0.3	12.6±0.3	75.6±1.7	27.5±1.2
C	10.7±0.2	11.7±0.3	80.6±1.3	35.0±1.0
D	13.8±0.3	9.7±0.3	144.7±2.1	50.3±1.1
E	13.0±0.3	13.1±0.3	224.6±3.6	60.6±1.5
Unbranded	9.6±0.3	11.1±0.3	155.1±2.2	49.2±1.3
Raw	8.2±0.2	11.1±0.4	44.3±1.2	42.8±1.2

Results are metal content ± measurement uncertainty of each sample, calculated on dry weight basis.

As shown by the results, practically these two methods have given very close values. Statistically according to the paired t test ($P>0.05$) to the results obtained by XRF method for iron, manganese, copper and zinc were similar for the results obtained by AAS. According to the analysis of variance (ANOVA) at 95% confidence interval there were significant differences ($p<0.05$) among the means of Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn) contents in different brands for AAS method.
Using the Tukey's pair wise comparisons, the significant differences of metal contents among brands were compared and they are presented in the Table 9.As revealed by the results brand B, C and unbranded samples did not exhibit a significant difference in Cu. Tukey's pair wise comparisons showed a significant different for Fe and Mn among the brands. According to Dunnet's comparison statistical analysis for Fe, Zn and Mn were significantly different in all branded and unbranded pepper powder from whole pepper. However for Cu, some brands of pepper powder were significantly different from raw pepper whereas some were not.

Analysis of Toxic metals
The toxic heavy metals content (Pb and Cd) of Black pepper determined by graphite furnace AAS is given in the Table 11.

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Table 11 -Toxic heavy metal content in Black pepper powder by graphite furnace AAS ({µg/kg (DW)}

Name of the sample		**Pb (µg/kg)**	**Cd (µg/kg)**
Brand	Batch		
A	1	261.7±0.2^{a}	363.4±0.4^{a}
	2	262.4±0.6^{a}	365.1±0.3^{a}
	3	262.9±0.5^{a}	364.9±0.4^{a}
B	1	220.6±0.3^{b}	405.4±0.4^{b}
	2	222.8±0.7^{b}	404.7±0.6^{b}
	3	223.7±0.5^{b}	405.5±0.8^{b}
C	1	253.4±0.2^{c}	319.3±0.2^{c}
	2	251.3±0.5^{c}	319.4±0.9^{c}
	3	254.5±0.6^{c}	320.2±0.7^{c}
D	1	196.4±0.5^{d}	356.0±0.9^{d}
	2	195.3±0.5^{d}	355.1±0.4^{d}
	3	197.3±0.7^{d}	355.2±0.6^{d}
E	1	250.5±0.3^{e}	221.5±0.6^{e}
	2	251.4±0.6^{e}	221.5±0.7^{e}
	3	248.2±0.7^{e}	222.0±0.2^{e}

Results are means ± **s**tandard 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).

Toxic Metal content from GFAAS method in branded, unbranded pepper powder and whole pepper is given below.

Table 12- Average toxic heavy metal content in different Black pepper brands by graphite furnace AAS **{µg/kg (DW)}**

Brand	**Pb (µg/kg)**	**Cd (µg/kg)**
A	262.3±0.6^{a}	364.5±0.9^{a}
B	222.4±1.4^{b}	405.2±0.6^{b}
C	253.1±1.5^{c}	319.7±0.7^{c}
D	196.3±1.0^{d}	355.4±0.7^{d}
E	250.0±1.5^{e}	221.7±0.5^{e}
Unbranded	344.5±0.4^{f}	312.2±0.5^{f}
Raw	155.5±0.1^{g}	212.6±0.2^{g}

Results are means ± **s**tandard 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 data given in Table 12 the mean Pb content of pepper was between155.5 µg/kg and 344.5µg/kg (on dry weight basis). The highest Pb level was found in Unbranded sample and the lowest was in the whole pepper (Raw).The Maximum Permitted level for spices is 10mg/kg according to WHO recommendations. The level of Pb in all sample means were below the permissible limit .The FAO/WHO has set limits for intake of metals based on body weight. For an average adult (60 kg body weight) the provisional tolerable daily intakes (PTDI) for Pb are 214µg (Joint FAO/WHO, 1998).Spices are reputed to acquire Pb during growth in lead contaminated soils or in the course of milling or other processing procedures. The use of pesticides contaminated with heavy metals during growing of herbs and spices may also be a source of Pb contamination in the final product (Galal- Gorchev, 1991). The Pb content in seasonings could also be attributed to the addition of Pb during processing to impart colour, sweetness to taste or to increase the weight of these products. Also it may indicate that traffic density increases the Pb burden in the environment thereby increasing the Pb content in vegetation (Rodriguez- Flores *et al.*, 1982)
According to the research data Pb content in all powder samples was higher than the raw (whole) pepper. Pb is a non –essential toxic heavy metal (Schroeder et al., 1973). Increased levels of Pb in man result in poor mental development in children, cardiovascular diseases, and renal dysfunction and encephalopathy seizures.
As shown in Table 12, the mean Cd content was ranged from 212.6 and 405.2µg/kg, dry weight basis in pepper. The maximum permissible level according to WHO recommendations is 0.2mg/kg. This data reveals that all pepper samples were above the permissible level (200µg/kg) set by WHO for Cd in spices. Cd is said to be

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easily transported from the soil to the edible parts of plants (Mengel *et al.*, and Kirkby, 1982), hence high Cd levels in the pepper could be due to high amount of Cd in the soil.

The Cd present in food is mostly derived from various sources of environmental contaminations and has no biological importance. Though Cd content in market available pepper could be due to contamination of raw materials, technological processes or colouring agents used also contain Cd salts (Cabrera *et al.*, 1995). The use of phosphate fertilizers may increase the naturally occurring Cd in the soil which may in turn increase the Cd content in plants. Soil amended with sewage sludge shows high Cd content (Wajahat *et al.*, 2006) which may have a direct affect on plants.

In human Cd toxicity is characterized by chest pain, cough with foamy and bloody sputum and death of the lining of the lung tissues due to excessive accumulation of watery fluids.

XRF method is carried out in the range of ppm and Pb and Cd present in the study is ppb range. Therefore these two metals in pepper could not be determined by XRF method.

Contents of Pb and Cd in each Black pepper powder brands, unbranded powder and raw pepper analyzed by graphite furnace AAS are graphically presented in the Figure 7.

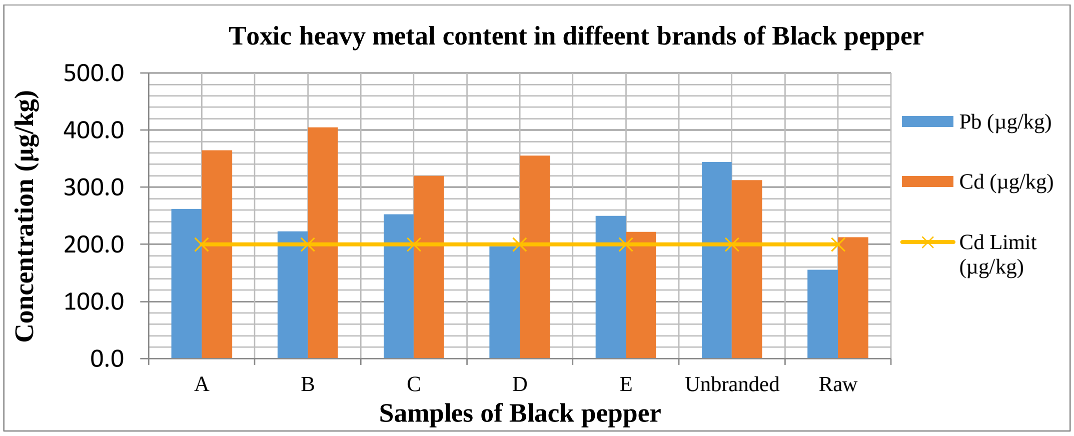

Figure 7-Pb and Cd content in Black pepperby GF AAS

Using the Tukey's pair wise comparisons, the significant differences of metal contents among brands were compared and they are presented in the Table 12.As revealed by the results there is a significant difference among all brands with respect to Pb and Cd contents. According to the Dunnet's comparison statistical analysis for all toxic metals, brands and unbranded pepper powder exhibited a significant difference with whole pepper (Control).

According to one sample t test the Cadmium content in all powdered samples were significantly higher ($P< 0.05$) than the maximum permissible limit of 200 µg/kg recommended by WHO.

IV. CONCLUSION

The present study provided important information of metal content in terms of macro, micro and toxic metal content (Na, K, Ca, Mg, Fe, Cu Zn, Mn, Pb, Cd) in ground pepper and whole pepper. Even though the levels of most metals in the test were below the safe limit, the means of Cd was above the safe limit. This clearly highlights the Cd contamination in pepper. Furthermore significant statistical difference was observed with WHO permissible limits proving that agricultural practice may affect Cd contamination in pepper. The Fe content in ground pepper did not exceed the safe limit but it was significantly higher than in whole pepper. These high levels could be due to contamination during milling and wear and tear of the machine parts. Fe, Pb, Cd and Zn in ground pepper revealed a significant difference with whole pepper concluding possible contamination during grinding of the product. Among the major nutrients studied, K is the highest metal in Black pepper followed by Calcium and Magnesium as most of the fertilizers include these metals. Statistical analysis shows that K and Mg contents in branded powder samples were significantly higher than the unbranded pepper. This could be due to added nutrients. When considering all the analyzed metals in the Black pepper powder samples there were no significant differences among the 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 K, Ca, Fe, Zn, Cu and Mn contents. The mean distribution of these metals in black pepper were K>Ca>Mg>Na>Fe>Mn>Cu>Zn>Cd>Pb. The results of

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this study provide initial evidence that Black pepper powder in the market has high levels in some metal contents than the whole pepper. It is emphasized to take preventive measures through regular monitoring to control the buildup of metals at toxic levels in the human body.

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VI. REFERENCES

[1] AOAC, Association of Analytical Communities, Official Methods of Analysis of AOAC,1996

[2] Batool, S., Khan, N., "Estimation of heavy metal contamination and antioxidant potential of Pakistani condiments and spices" in *journal of Biodiversity and Environmental Sciences (JBES)* ISSN: 2220-6663(Print) 2222-3045 (online) Vol.5, No.3,p.340-346, 2014

[3] Cabrera, C., Lorenzo, M. L. and Lopez, C. M., " Lead and Cadmium Contamination in Diary Products and its repercussions on Dietary Intake", *J. Agri. Food Chem*., 43: pp.1605-1609, 1995.

[4] Darko, B., Heavy metal content and phytochemicals in some seasonings in Kumasi metropolis (Ghana), 2010

[5] Etonihu, A.C., Obelle, F.N. and Nweze,C.C., "Chemical perspectives on Some Readily Consumed Spices and Food Condiments" : *A review in Food Science and Qulity Management*, Issn 2224-6088 (Paper) Issn 2225- 0557 (online), Vol. 15, 2013

[6] Galal-Gorchev, H., Dietary intake of pesticide residues: cadmium, mercury and lead. Food AdditContam., : pp.793 –806, 1991

[7] Hamza, S., Srinivasan, V. and Dinesh, R., "Nutrient diagnosis of of black pepper (*Piper nigrum L.*) Gardens in Kerala and Karnataka" in *Journal of Spices and Aromatic Crops* Vol.16 (2): 77-81, 2007 .

[8] Janitha, P. P., Chaman, D. K .I. and Samarajeewa, U. , Iron contamination during grinding of spices. *J. Natn. Sci. Coun*., 16(1): 105-114, 1998.

[9] Mengel, K. and Kirkby, E. A., Principles of plant nutrition. International Potash Institute. Berne, Switzerland, 1982.

[10] Partelli,F.L., Nutrition of black pepper (*Piper nigrum* L.)-a Brazillian experience, *Journal of Spices and Aromatic Crops*, Vol. 18 (2), pp.73-83, 2009.

[11] Peter, K. V. Handbook of spices. Vol. 2. Abington: WoodheadPublising Limited. Pp.15-20, 2004.

[12] Rodriguez-Flores, M. and Rodriguez-Castellon, E., Lead and Cadmium levels in soil and plants near highways and their correlation with traffic density. Environmental Pollution (series B), 4: 281-290. 1982.

[13] Schroeder, H. A., Balassa, J. J. and Tipton, I. H., Essential trace metals in man: Manganese. A study in homeostasis. *J Chron Dis*., 19: 545-571, 1973.

[14] Umar, M.A. and Salihu, Z.O.O., "Heavy metals content of some spices available within FCT-Abuja, Nigeria." in *International Journal of Agricultural and Food Science*, ISSN 2249-8516, 2014.

[15] Wajahat , N., Sajida .P. and Syed, A . S., Evaluation of Irrigation water for Heavy metals of Akbarpura Area. *Journal of Agricultural and Biological Science*, 1(1): 1-7, 2006.

[16] Wardlaw, G. M., Contemporary Nutrition, Issues and Insight. 5thedition. New York: The McGraw Hill Companies. pp 317-325, 2003.

[17] WHO., Sixteenth Report of the Joint FAO/WHO Expert Committee on Food Additives, Evaluation of mercury, lead, cadmium and the food additives, amaranth, diethylpyrocarbonate and octylgallate. WHO. techn. Rep. Ser., No. 4 . WHO, Geneva,1972

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

[18] WHO., Cadmium. In: Toxicological evaluation of certain foods and contaminants. Geneva. WHO, Food Additives Series No. 24, 163-219, 1989.

[19] World Health Organization, Quality Control methods for medicinal plant materials, Genava, 2005.

[20] Vitamin and mineral requirements in human nutrition, 2nd Edition. Joint FAO/WHO consultation on human vitamin and mineral requirement. Bangkok, Thailand. pp. 59-272, 1998

	**Ms. Prashani T. Sandanayake** Assistant Director, Sri Lanka Standards Institution; M.Sc. Degree in Food Science and Technology in the Faculty of Applied Sciences, University of Sri Jayewardenepura; Holding the B.Sc. Special Degree in Chemistry, University of Colombo, Sri Lanka; Member of the Royal Society of Chemistry, UK; Member of the Institute of Chemistry, Ceylon.
	**Dr. (Ms.) Indira Wickramasinghe** 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 Bio Technology, 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

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	**Mr. R. M. G. B. Rajanayake** 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 & Technology, University of Sri Jayewardenepura, Sri Lanka, Agriculture & 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 & Nutrition; Council member of the Institute of Chemistry Ceylon.
	**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.

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