Determination of heavy metals (Pb, Cd, Mn, Zn and Cu) in Green tea leaves and their infusions.

Dissertation submitted in partial fulfillment for the degree of

Master of Science in Biotechnology

Submitted By

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MAY - 2018

CERTIFICATE

This is to certify the dissertation entitled "***DETERMINATION OF HEAVY METALS (Pb, Cd, Cu, Mn and Zn) IN GREEN TEA AND THEIR INFUSIONS***" Submitted by ***Ms. SUCHARITA MISHRA*** in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology, KIIT School of Biotechnology, KIIT University, Bhubaneswar bearing Roll No. ***1661027*** & ***Registration No. 16529550260*** is a *bona fide* research work carried out by her under my guidance and supervision from ***18-12-2017*** **to** 04***-05-2018***.

(**Devendra J Haware**)

Sr. Scientist

Endorsed By

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Chief Scientist and Head
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CERTIFICATE

This is to certify that the dissertation entitled "***DETERMINATION OF HEAVY METALS (Pb, Cd, Mn, Cu AND Zn) IN GREEN TEA AND THEIR INFUSIONS***" submitted by **Sucharita Mishra, Roll - 1661027, Regd.no. - 16529550260** to the School of Biotechnology, KIIT University, Bhubaneswar-751024, for the degree of Master of Science in Biotechnology is her original work, based on the results of the experiments an investigations carried out independently by her during the period from ***18 - 12- 2017*** to ***04 - 05 - 2018*** under my guidance.

Further, it is also to certify that the above said work has not been previously submitted for the award of any degree, diploma, or fellowship in any Indian or foreign University.

Date:

Place: (**Devendra J Haware**)

Sr Scientist

<u>DECLARATION</u>

I hereby declare that the dissertation entitled "***DETERMINATION OF HEAVY METALS (Pb, Cd, Cu, Mn and Zn) IN GREEN TEA AND THEIR INFUSIONS***" submitted by me, for the degree of Master of Science to KIIT University is a record of *bona fide* work carried by me under the supervision and guidance of **DEVENDRA J HAWARE**, **Senior Scientist, Food safety and Analytical Quality Control Laboratory, Department in CSIR - Central Food Technological Research Institute, Mysuru, Karnataka**. I further declare that, the results of the work have not formed the basis for the award of any other degree to any candidate of any university.

Date:

Place: ***(Sucharita Mishra)***

Abstract

Tea has been one of the most popular non - alcoholic beverages in the world. Of the all variety of tea, Green tea has become popular among people because of its role in weight loss, prevention of cancer and neuro - degenerative diseases. As tea plays a major role in uptake of essential trace elements, it is important to analyse the trace elements that are present in tea leaves and how much leaching occurs through their infusions. With this study, we have tried to determine the amount of amount of heavy metals, mainly Pb, Cd, Cu, Mn and Zn, present in various brands of Green tea and their infusions.

The presences of Pb, Cd, Mn, Zn and Cu in 38 green tea samples purchased from markets in Mysuru, Karnataka and Bhubaneswar, Odisha were evaluated by Atomic Absorption Spectrometry (AAS).This study was performed both for tea leaves and their infusions. The results provide data that permits an evaluation of exposure or dietary intake of these trace elements. The samples were prepared by adding 4 ml HNO_3 and digesting it in microwave digester. Infusions were prepared by soaking the leaf or tea bags in 100 ml of boiled water and the solution was filtered using Whattman no.42 filter paper prior to the analysis by AAS.

Results of the study showed that the concentration of Manganese was higher in the green tea samples and ranged from 12.7 - 961.3 mg/kg. Lead and Cadmium were below the limit of detection in all the samples. Contents of Cu and Zinc ranged from 4.2- 47.1 mg/kg and 4.0 - 74.6 mg/kg, respectively. Concentration of Mn was seen to be in the range of 5.0 mg/kg - 23.3 mg/kg in tea infusions. The concentrations of Zinc and Copper were from 0.11 mg/kg - 1.34 mg/kg and 0.07 mg/kg - 0.22 mg/kg, respectively.

Acknowledgement

It is my proud privilege to express my profound sense of gratitude and sincere indebtedness to **Shri Devendra J Haware**, Senior scientist, Food Safety and Analytical Quality Control Laboratory Department in CSIR - CFTRI, Mysuru, Karnataka. I am highly obliged to him for his guidance, constructive criticism and valuable advices which he provided to me throughout the tenure of my project.

I am also thankful to **Dr. Siva Shankar Reddy Singham**, Technical officer, Food Safety and Analytical Quality Control Laboratory Department in CSIR - CFTRI, Mysuru, Karnataka for his support, guidance and valuable advice.

I am very grateful to **Shri Jitendra J Jodhav**, Director, CSIR - CFTRI for providing me an opportunity to work in this esteemed institution. I also extent my thanks to **Dr R.P.Singh**, Co-ordinator, HRD, CSIR - CFTRI and **Dr. Alok Kumar Srivastav**, Head of the Department, Food Safety and Quality control laboratory, for providing me the opportunity to carry out my work in this department.

I sincerely acknowledge **Dr. Mrutyunjaya Suar**, Director, KIIT School of Biotechnology, Bhubaneswar, Odisha for his support throughout my project. I thank all my faculties at KIIT School of Biotechnology for their guidance and support.

I acknowledge and will always remember my friend and support in the lab, **Mrs. Pavitra Prakash** and my colleagues **Ms. Sherja K Rapheal** and **Ms. Anu John** who made my stay in this institution a memorable one and helped me a lot throughout my tenure. I would like to thank my friend **Ms Shreedipti Sahoo** for her support and encouragement throughout my dissertation work.

Last but not the least I would like to thank my parents for their support and belief in me.

Date:

Place: ***(Sucharita Mishra)***

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Abbreviations

EGCG - epigallocatechin -3-gallate

EGC - epigallocatechin

ECG - epicatechin - 3- gallate

EC - epicatechin

AAS - Atomic Absorption Spectrometry

ICP MS - Inductively coupled plasma mass spectrometry

ICP AES - Inductively Coupled Plasma Emission Spectrometry with axial plasma configuration

THQ - Target Hazard Quotient

HI - Hazard Index

WHO - World Health Organisation

GFAAS - Graphite Furnace Atomic Absorption Spectrometry

LOD - Limit of detection

MPC - Maximum permissible concentration

US FDA - US Food and Drug Administration

FSSAI - Food Safety and Standards Authority of India

BDL - Below Detection Limit

1 Introduction

1.1 Background and Context

Tea is mainly available in four varieties- Black tea, Oolong tea, Green tea and white tea. *Camellia sinensis* is the plant from which all kinds of tea are produced. The economic and social interest of tea is understood easily from the fact that almost 18 - 20 billion cup of teas are consumed daily worldwide. Among all the varieties of tea, green tea is the most popular one because of its association with weight loss and prevention of various kinds of diseases. It accounts for 20% of the manufactured tea leaves in the world . India is the second largest producer of green tea with approximately 8 million kg of green tea being produced in India, annually. [*Tanmoy Karak and R.M Bhagat 2010*]

Figure 1 - *Camellia sinensis* Leaves

Figure 2 - Green Tea

Green tea is a 'non-fermented tea' and contains more catechins than black and oolong tea. With no fermentation, green tea retains its green color and most of its original polyphenols. Green tea has been produced and consumed in India, China, Japan and Thailand for ages. Due to its various medicinal properties, there has been a growing interest in green tea these days. They are known for having anti - oxidant, anti - carcinogen, anti - inflammatory and anti - radiation biochemical effects in - vitro. [*Carmen Carbera et.al;2006,W.C.Chan et.al; 2011, Parmar Namita et.a;l 2012*]

Kingdom	Plantae
Order	Ericales
Family	Theacae
Genus	Camellia
Species	C.sinensis
Binomial Name	*Camellia sinensis*

Table 1 - Scientific Classification of tea. [*Parmar namita et al 2012*]

Green tea is considered to be a rich source of anti - oxidant. Studies have shown that green tea contains more anti - oxidant than any other form of tea. Other compounds that are present in green tea are alkaloids (caffeine, theophylline, theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds (chemicals that readily produce vapours and contributes to the odour of tea), fluoride, aluminium, minerals and trace elements. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits that have been attributed to green tea. They are also known for their anti - bacterial activities. In general, antibacterial activity decreases when the extent of tea fermentation is increased which implies a stronger anti-bacterial activity in green tea than black tea. Aside from polyphenols, the essential trace elements in humans can be supplemented through green tea as they contain potassium, manganese, selenium, boron, zinc and copper. [*Parmar namita et.al; 2012*]

Green tea mainly consists of flavanols or catechins of epigallocatechin -3-gallate (EGCG), epigallocatechin (EGC), epicatechin - 3- gallate (ECG), and epicatechin (EC). The most active and abundant catechin in the green tea is EGCG. [*Parmar namita et al, 2012*]

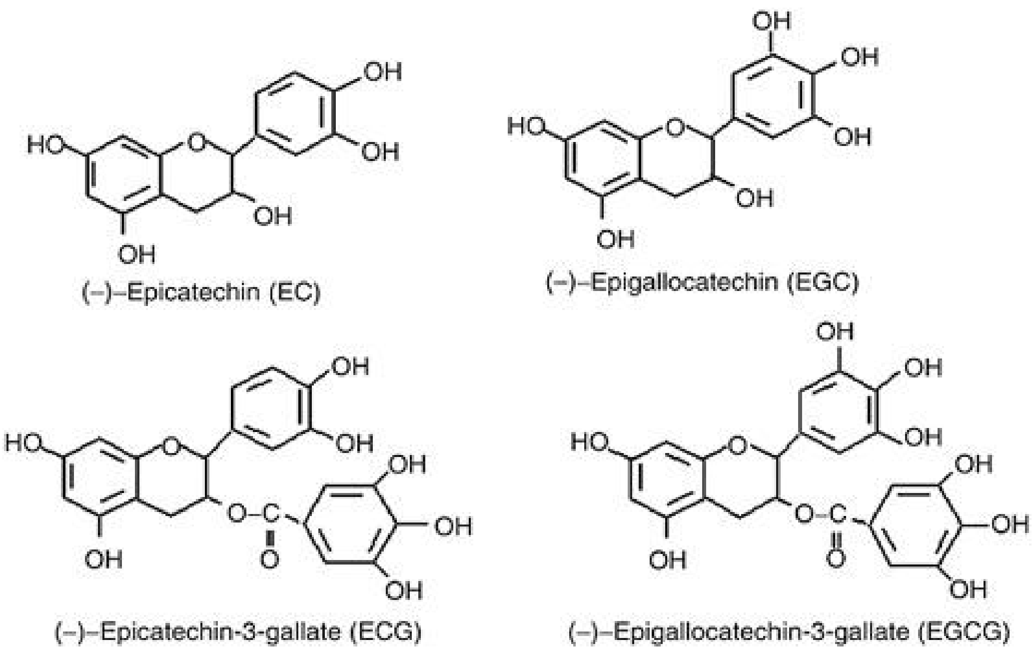


Figure 3 - Structure of Catechins present in green tea [*source - researchGate*]

The most active and abundant compound in green tea is Epigallocatechin - 3 - gallate which is a ester of epigallocatechin and gallic acid. It is found in very high content in green tea (approximately 7380mg per 100g) and plays a very crucial role in various health effects of green tea. [*source - wikipedia*]

Health Benefits of Green Tea

Green tea has been long associated with weight loss and is the reason why it has remained popular worldwide. Other than weight loss, green tea also plays an important role in reduction of risk of cardiovascular disease and some forms of cancer. Green tea is also helpful in preventing tooth decay and other oral diseases by inhibiting oral bacteria. They have also been associated with prevention of neuro - degenerative diseases like Alzheimer's and Parkinson's disease. Other traditional uses of green tea include regulating body temperature and blood sugar, promoting digestion and improving mental health. The antibacterial action of tea is useful for treating infections and wounds. [*Parmar namita et al 2012, Eric W.C. Chan et al 2011*]

Anti - Cancer Activity of Green Tea

Green tea has a reputed role in prevention of cancer as tea catechins have shown to inhibit tumor cell proliferation and promote the destruction of leukemia. Many studies on cultures of tumor cells and mice given carcinogenic chemicals showed green teas potential to inhibit cancer cell growth. A study using 8522 residents, representative of Japan's population tested to see the anti-carcinogenic effect of green tea showed a decreased relative risk of cancer incidence for those consuming over ten cups, compared with those consuming below 3 cups of green tea per day. [*Namita Parmar et al 2012, Nakachi et al 2000*]

Neurodegenerative Diseases

Recent studies have proven the role of green tea in the prevention of Alzheimer's and Parkinson's disease. Studies on animal and cell culture models have suggest that EGCG from green tea may affect several potential targets associated with Alzheimer's disease progression. Various studies on the role of green tea on Parkinson's disease has shown that green tea and EGCG has significantly prevented these pathologies in animal models. [*Parmar namita et al 2012*]

Weight - Loss

Green tea has been long marketed as a weight loss aid. It has remain a very popular drink among people because of its role as a calorie burner but there has been no solid evidence proving this aspect of green tea. An open study found that an 80% ethanol extract of green tea standardized to 25% catechins reduced weight in moderately obese by 4.6% and waist circumference by 4.5% after 3 months of use. However another double blind placebo controlled parallel trial of 46 women showed no difference between the placebo and green tea groups over 87 days in either weight loss or metabolic parameters. [*Parmar namita et al 2012*]

Skin Disorders

Using different animal models, many laboratories have shown that green tea extract, taken orally or applied to the skin inhibits skin tumor formation induced by chemical carcinogen or ultra - violet radiation. The extract also possess anti - inflammatory activity that similarly to the anti - cancer forming activity, is owed to the polyphenolic constituents present therein. The polyphenol mainly responsible for the prevention of cancer formation is EGCG. When applied to the mouse skin, EGCG prevents UVB - induced oxidative stress and suppression of the immune system. Mouse skin models have illustrated extensive beneficial effects of green tea extracts. [*Namita Parmar et al 2012, Katiyar et al 2001*]

1.1 Heavy Metals

Heavy metals are the natural constituents of the earth crust and originate from natural and anthropogenic sources. They are common environmental pollutants. The term heavy metal refers to high density metallic chemical elements that are toxic or poisonous at low concentration and cannot be degraded or destroyed. They are defined as those metals whose density is more than 5gm/cm^3, atomic weight 63.546 - 200.590 and a specific gravity greater than 4.0 or a metal of relatively high density (Specific gravity is greater than 5) or of high atomic weight. [*Metal contamination of food, Conor Riley 1980*]

Figure 4 - Heavy Metal - Mercury [*source - Google images*]

In terrestrial ecosystem, soils are the major recipient of metal contaminants. Metals in soil can come from the rocks in which the soil was formed, fertilizers, sewage sludge and other materials added in the course of agricultural activities. In aquatic system, sediments are the major sink for metals. In fresh water system, water is contaminated due to runoff and drainage via sediment disposal. Ground water is impacted through leaching or transport via mobile colloids.

They can enter the human body by ingestion, inhalation and absorption through skin and mucous membrane. And when these metals are not metabolized by the body, they get accumulated in soft tissues and become toxic. Toxic heavy metals are defined as relatively dense metals which are toxic even in smaller amount. The most common toxic heavy metals are cadmium, mercury, lead and arsenic, all of which appear in the WHO's list of 10 chemical of major public concern. Other examples include chromium, zinc, copper, nickel, cobalt, manganese, selenium, thallium, etc. [*Brathwaite & Rabone, 1985*]

Living organisms require varying amount of heavy metals such as Iron, Cobalt, Manganese, Copper, Molybdenum and Zinc. Small quantities of Iron, Copper, Zinc and Manganese are nutritionally essential for healthy life. These elements are commonly found in fruits and vegetables and in various commercially available multivitamin products.

Physical Properties	**Heavy Metal**
Density	Usually high density
Hardness	Most are quite hard
Thermal expansivity	Low thermal expansivity
Melting point	Low to very high
Tensile strength	Mostly Higher

Table 2- Physical Properties of Heavy Metals *(source - Wikipedia)*

Chemical Properties	**Heavy Metal**
Periodic table location	All found in groups 3 through 16
Reactivity	Less Reactive
Sulfides	Extremely insoluble
Hydroxides	Generally insoluble
Salts	Mostly form colored solution in water
Complexes	Mostly colored

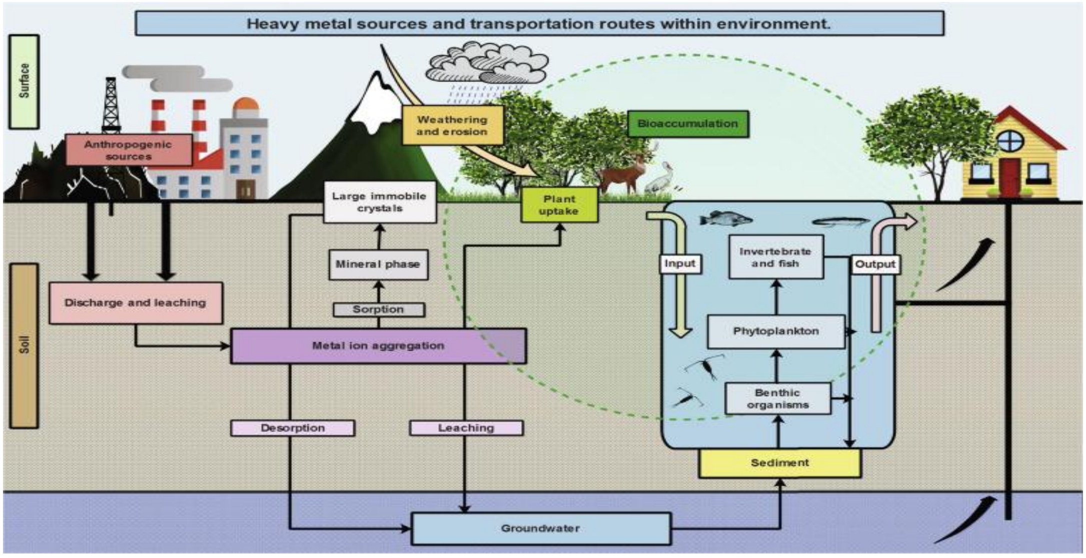
Table 3- Chemical Properties of Heavy Metals *(source - Wikipedia)*

1.1.2 Sources of Heavy Metals

Heavy metals are present in all parts of the earth crust. They are emitted in a continuous manner by various natural and anthropogenic sources. In recent decades, there contamination has increased dramatically because of continuous discharge in sewage and untreated industrial effluents. As they cannot be degraded or destroyed, they are continuously kept “in play” thus entering the physical and biological cycles. Common natural sources of heavy metals are volcanic activities, forest fires, erosion of rock and leaching processes.

The anthropogenic sources of heavy metals are mining and industrial wastes, vehicle emission, municipal waste, fertilizers, paints, chemical conversion and their modes of deposition to pollute the environment. These metals can easily penetrate to cell membranes and internal organs and can cause health effects.

Some heavy metals like iron, cobalt, copper, manganese, zinc, etc. are required by human but can be damaging to the organism at excess levels. Other heavy metals like lead and mercury are highly toxic and their accumulation over time in the body of organisms can cause serious illness. Heavy metals are widely dispersed in the environment at excessive levels and are very toxic to humans.



<u>**Figure 5 - Heavy Metal sources and Transportation through environment**</u>[*source - google images*]

Metal	**Common Sources**
Chromium	Chrome plating, petroleum refining, electroplating industry, leather, tanning, textile manufacturing and pulp processing units. It exists in both hexavalent and trivalent forms.
Nickel	Galvanization, paint and powder, batteries processing units, metal refining and super phosphate fertilizers.
Lead	Lead petrol based materials, pesticides, leaded gasoline and mobile batteries.
Copper	Electroplating industry, plastic industry, metal refining and industrial emissions.
Zinc	Rubber industries, paints, dyes, wood preservatives and ointments.
Cadmium	Batteries, electroplating industry, phosphate fertilizers, detergents, refined petroleum products, paint pigments, pesticides, galvanized pipes, plastics, polyvinyl and copper refineries.
Iron	From metal refining, engine parts
Aluminium	Industries preparing insulated wiring, ceramics, automotive parts, aluminium phosphate and pesticides.
Arsenic	Automobile exhaust/ industrial dust, wood preservatives and dyes
Mercury	Electric/light bulb, wood preservatives, leather tanning, ointments, thermometers, adhesives and paints.

Table 4 - Common Heavy Metals and its sources

1.1.3 Heavy Metals In Green Tea

Green tea is said to play a major role in terms of the intake of a number of nutritional trace elements in human. In addition to anti - oxidants, green tea are also good source of essential trace elements like potassium, manganese, selenium, boron, zinc and copper. But besides the presence of essential micro- and macro-elements, various studies have demonstrated that the accumulation of significant amount of excess nonessential trace elements in tea leaves may eventually increase the metal body burden in human body. The presence of trace elements, especially Pb and Cd is due to tea plants being grown in highly acidic soils, where trace elements are potentially more bio - available for root uptake. In addition to acidic soil, use of fertilizers and pesticides can result in presence of excess amount of trace elements in tea plants. [*Tanmoy Karak and R.M.Bhagat 2010, Raquel F Milani et al 2015*]

Figure 6 - *Camellia sinensis* plant

Processes during machinery tea preparation are another reason for metal contamination in tea. Food - processing equipment and containers have long been recognized as a source of chemical contamination of food. [*Metal Contamination of food, Conor Riley 1980*]

Excess Presence of trace elements in tea may have adverse effect on human health. For example, accumulation of Aluminium in tea infusions is associated with Alzheimer's disease. Accumulation of Pb and Cd can cause permanent damage to key enzymes and many systems of the body including circulatory, renal and central nervous system. [*Tanmoy Karak and R.M Bhagat 2010, Justyna Brzezicha Cirocka et al 2016*]

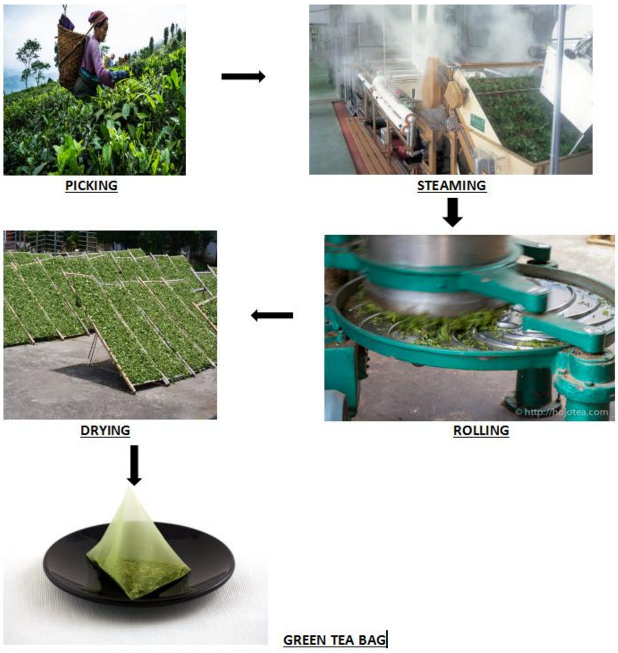


Figure 7- Green Tea Production Process

The physiological roles of essential metals are well known. For example, Iron (Haemoieties of haemoglobin and cytochromes), copper (amine oxidases, caeruloplasmin, dopamine hydrolase and collagen synthesis), Manganese (superoxide dismutase), Zinc (Protein synthesis, stabilization of DNA and RNA), with requirement of Cr (Glucose homeostasis). The physiologic roles of essential metals are due to the fact that these metals are components of enzymes and proteins. The deficiency of these elements could induce disease conditions e.g. Cu deficiency is known to induce hypertension, increase in blood cholesterol (hypercholesterolemia) and low density lipoproteins fraction increment in blood which add to the conditions favoring heart attack. Deficiency of manganese has been associated with chronic disease like osteoporosis, epilepsy and diabetes mellitus.

1.1.4 How heavy metals get into food system

Heavy metals are a group of elements that are present in both solid and liquid state. The multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment. The toxicity of heavy metals depends on various factors like the dose, route of exposure and chemical species, as well as the age, gender, genetics and nutritional status of exposed individuals.

People can be exposed to these metals from the environment or by ingestion of contaminated food and water. The metal content of food, be of animal or of plant origin depends on many factors ranging from environmental conditions to methods of production and processing.

Heavy metals can enter into our food system via soil. These heavy metals are present in soil and were absorbed by the plants and the heavy metals get accumulated in the plant products, thus entering into our food. Some of the fertilizers, pesticides and the fungicides contain such heavy metals which enter into the food web. Mercury and Cadmium are the only metals which is capable of polluting our food supply after passage through water.

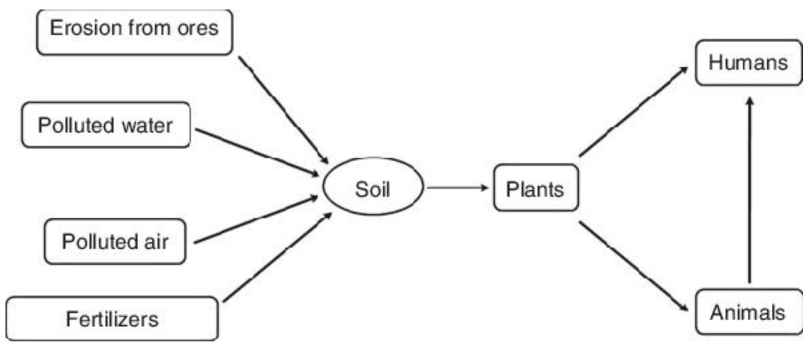


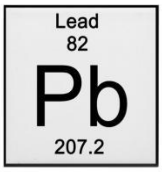
Figure 8 - Pathway of Heavy Metals into Food

1.1.5 Heavy Metals and Human Body

Heavy metals have proven to be a major threat and there are several health risk associated with it. They sometimes act as the pseudo elements which attaches to the receptors and interfere with the proper functioning of the body. Heavy metal toxicity can result in various ill health effects in humans. Inorganic arsenic is carcinogenic and can cause cancer of skin, lungs, liver and bladder. Cadmium and its compounds are also known as human carcinogens. Breathing high levels of Cadmium may cause severe damage to the lungs. Long term exposure to Chromium can cause damage to liver, kidney, and nerve tissues as well as skin irritation. Exposure of high levels of mercury can permanently damage brain, kidney and developing fetus and Lead causes damage to nervous system, hematopoietic system and to renal system. [*Chromium toxicity* https://www.atsdr.cdc.gov, https://niehs.nih.gov]

Five common heavy metals are discussed here in brief - Lead, Cadmium, Copper, Manganese and Zinc. These are all naturally occurring substances which are often present in the environment at low levels. In larger amounts, they can be dangerous.

1.1.5.1 Lead



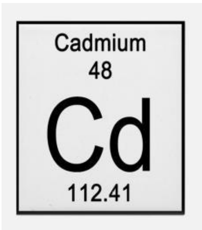
Lead is element number 82, lying in group IVB of the periodic table, with an atomic weight of 207.19. It is one of the heavier of the elements, with a density of 11.4. it is soft and bendable and can be beaten flat with a hammer and cut with a knife. Lead melts at a relatively low temperature of 327.5℃. Its boiling point is 1725℃. The metal is a poor conductor of heat and electricity. Its oxidation states are 0, +2 and +4. In inorganic compounds lead is usually in state +2. Lead has the ability to form alloys with other metals. Some of these, such as solder which is made with tin, are of considerable economic importance.

Leads are used in two main groups, first in metallic form and in chemical compounds. The largest use of lead throughout the world today is lead batteries, metal products, chemical pigments and miscellaneous uses. Metal products such as ammunition and solder follow by casting materials, alloys, sheet lead and others. Chemicals are tetra ethyl and tetra methyl lead. It is important because of their extensive use as antiknock agent in petrol. The use of lead as pigments in decorative paints such as white lead and colors has declined significantly. However consumption for anti - corrosive and high ways traffic safety paint (as red lead and lead chromate) is increasing because of their excellent versatility and relatively low cost. Other uses are automotive wheel weight, ship ballast, rust inhibitors, ammunition, glaze and plastic stabilizer.

Health effects of Lead - The effect of lead exposure are the same whether it is breathed or swallowed. Low levels of Lead have been identified with anaemia as it causes injury to the blood forming systems while high levels cause severe dysfunction of the kidneys, liver, the central and peripheral nervous system and high blood pressure.

Children are most sensitive to the effects of lead than adults. A child who swallows large amount of lead may develop blood anaemia, kidney damage, severe stomach - ache, muscle weakness and brain damage. The lower IQ levels and other neuro - psychological deficiencies among the children exposed to higher levels have been well documented. Lead is toxic at all levels, hence lead based petrol, toys and paints have been banned.

1.1.5.2 Cadmium



Cadmium is number 48 in the periodic table, with an atomic weight of 112.4. It is a fairly dense silvery white, malleable metal which melts at 320.9℃ and boils at 765℃. It has an oxidation state of 2. Cadmium forms a number of inorganic compounds, several of which are quite soluble in water, such as the chloride, sulphate and acetate.

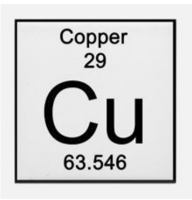
There are no ores of commercial significance which contain cadmium alone. Cadmium is most commonly associated with Zinc in carbonate and sulphide ore. Cadmium is also obtained as a by - product in the refining of other metals especially Zinc but also Copper and Lead. Cadmium has a number of important industrial applications such as electroplating, pigments, plastic stabilizers, batteries and other uses. Cadmium phosphorous as tube in television sets, fluorescent lamps, X - ray screens, cathode ray tube and phosphorescent tape, etc.

Cadmium is a toxic trace element which may accumulate in soils from various human activities. Natural source of cadmium is weathering of rocks while some cadmium enters air, through forest fires and volcanoes. Cadmium and its compounds are extremely toxic at all levels and tend to bio accumulate in organisms and ecosystems.

In human, long - term exposure is associated with renal dysfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer.

Health effects of Cadmium - Cadmium and its compounds are known human carcinogens. Long term exposure to lower levels to a build - up in the kidney and possible kidney disease and lung damage. Cadmium poisoning causes softening of bones and kidney failures and was responsible for the ***itai - itai*** disease (a name derived from the painful screams in Japanese language) due to the severe pain in the joints and spine. Cadmium was released into rivers by mining companies in the mountains of Japan in the late 1940s. The disease arose from increased uptake of cadmium in locally consumed rice grown in paddy fields irrigated with cadmium - contaminated river water. [*CSEM May 2008*]

1.1.5.3 <u>Copper</u>



Copper has an atomic weight of 63.54 and is number 29 in the periodic table of the elements. Its density is 8.96 and it melts at 1083^{0}C. It is a tough though soft and ductile metal, second only to silver in its thermal and electrical conductivities. Oxidation states of copper are normally 1 and 2 and it forms two series of compounds: Cu (I), cuprous and Cu (II), cupric.

The primary use of copper is in the manufacture of electrical goods, electrical cables and similar equipment where excellence of electrical conductivity is required. It has many commercial uses because of its versatility. Copper compounds are used as or in fungicide, algaecides, insecticides and wood preservatives. Copper compound can be added to fertilizers and animal feed as nutrition to support plant and animal growth.

Health effects of Copper - Copper can be released into the environment by both natural sources (e.g. wind - blown dust, decaying vegetation, forest fires and sea spray) and human activities (mining, metal production, wood production and phosphate fertilizer production). Because copper is related both naturally and through human activities; it is very widespread in the environment. Coppers often found near mines, industrial settings, landfills and waste disposals.

Long term exposure to copper can cause irritation of the nose, mouth and eyes and headaches, stomach - aches, dizziness, vomiting and diarrhea. Intentional high uptakes of copper may cause liver and kidney damage and even death. The WHO (1996) recommends a minimal acceptable intake of approximately 1.3 mg per day. According to EFSA the recommended tolerable upper intake limit is 5 mg/kg.

1.1.5.4 Manganese

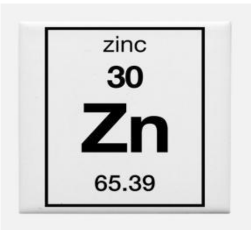
Manganese is element number 25 in the periodic table and has an atomic weight of 54.94. The chemical and physical properties of manganese are very similar to those of Iron, which it immediately precedes in the first transition series. However, it is harder and more brittle than iron and is also less refractory, with a melting point of 1247^{0}C. It is a reactive metal which dissolves readily in dilute, non - oxidizing acids. It burns in chlorine to give $MnCl_2$ and reacts with oxygen at high temperatures, producing Mn_3O_4. It also combines directly with boron, carbon, sulphur, silicon and phosphorus.

Manganese has three principle industrial uses, in steel making, manufacturing of electrical accumulators and as oxidizing agents in the chemical industry. Manganese is used in the original type of dry cell battery as an electron acceptor from zinc, and is the blackish material in carbon - zinc type flashlight cells. Manganese compounds have been used as pigments and for the coloring of ceramics and glass. The brown color of ceramic is sometimes the result of manganese compounds.

Health effect of Manganese - Organic matter decomposition aids manganese solubility. Toxic concentration of manganese is more likely than that of Zn, Fe or Cu. Toxic levels occur only in strongly acidic soils. Exposure to manganese dusts and fumes should not exceed the ceiling value of 5 mg/m^3 even for short periods because of its toxicity level. Manganese poisoning has been linked to impaired motor skills and cognitive disorders.

Prolonged inhalation of high levels of manganese negatively affects the central nervous system, visual reaction time, hand steadiness and eye - hand co-ordination. The proposed mechanism for manganese toxicity is that dysregulations leads to oxidative stress, mitochondrial dysfunction, glutamate - mediated excitoxicity and aggregation of proteins. WHO sets the maximum tolerable ingestion limit of Manganese between 2 - 11 mg/day depending on age. An intake of more than 11 mg/day is considered to be unsafe for adults.

1.1.5.5 <u>Zinc</u>



Zinc is the first element in group 12 of the periodic table. In some aspects Zinc is chemically similar to magnesium. Some 80 enzymes have been shown to contain Zinc. It is an essential mineral in human. Zinc is a bluish - white color, lustrous, diamagnetic metal through most common commercials grade of the metals have a dull finish. It is somewhat less dense than iron and has a hexagonal crystal structure. It does not exhibit multiple valences and is softer and lower melting than neighbouring transition metals triad, copper (Cu), Silver (Ag) and Gold (Au) group Ib. Zinc is intermediate between hard and soft acceptors in its chemicals interaction with ligands. Zinc form complexes of both hard (oxygen donor) bases and soft (sulphur) bases. This is reflected in the occurrence of zinc in nature, both as sulphide and carbonate ores.

Its density is 7.14, with a boiling point of 907.0^0C and a melting point of 420.0^0C like mercury and to a lesser extent cadmium, its two neighbours in the group II b elements, zinc is a remarkably volatile for a heavy metals. It combines readily with non - oxidizing acids, releasing hydrogen and forming zinc salts. Zinc is an essential element for mammals.

The principle ore, zinc sulphides are widely distributed in the world. Zinc metal frequently occurs in association with other metals as lead, cadmium and copper. Zinc imparts an undesirable astringent taste to water. The largest use of zinc is in galvanizing iron and steel products. This provides corrosion - resistant coating which can be finished with electroplated metal coating or organic coating. New alloy such as zinc - aluminium has been developed as protective coatings. Zinc oxide used in rubber as a white pigment. Zinc is important in the pharmaceutical industry, where it is used in nutrient supplements, ointment, shampoos and other preparations. Zinc compounds are used in a number of industrial operations such as manufacture of fiber board.

***Health effects of Zinc* -** Zinc is an essential trace element for humans. Nutritional zinc deficiency in humans has been reported in a number of countries. Acute toxicity arises from the ingestion of excessive amounts of zinc salts, either accidentally or deliberately as an emetic or dietary supplement. The adult human body contains about 2.3 g of zinc which occurs mostly in over 100 enzymes. The normal daily requirement for zinc is 15mg for adult and 5 mg for children.

Acute toxic effects of inhaled zinc are pulmonary distress, fever, chills and gastroenteritis. A zinc deficiency after birth may result in dwarfism, poor appetite and mental lethargy, etc. Excess amount of zinc on the other hand can cause stomach cramps, nausea, vomiting, central nervous system disorder. Prolonged exposure to high level of zinc can cause copper deficiency and subsequent anaemia. It can also interfere with iron metabolism. Similar effects may be produced by ingestion of high doses of zinc supplements, an intake of more than 15 mg/kg in this way is not recommended. [*Arif tasleem et al, 2015*]

1.2. Scope and Objectives

In recent years, there has been a growing interest in green tea. Various study claims that green tea plays an important role in weight loss and cancer prevention. Apart from anti - oxidants, tea also contains many bio - elements as well as toxic metals like Pb and Cd. Heavy metals are very toxic and are present in various levels in the environment, e.g. soil, water and atmosphere. Hence it is important to monitor the presence of heavy metals that are present in green tea.

Determination of heavy metals will help us understand two very important things. It will be helpful to judge their nutritional value and guard us against any probable ill - effects.

1.3 Achievements

Results of the study showed that the concentration of Manganese was higher in the green tea samples and ranged from 12.685 - 961.285 mg/kg. Lead and Cadmium were below the limit of detection in all the samples. Contents of Cu and Zinc ranged from 4.22 - 47.13 mg/kg and 4.05 - 74.645 mg/kg, respectively.

For the green tea infusions Concentration of Mn was seen to be in the range of 5.05 mg/kg - 23.37 mg/kg and was higher than any other heavy metals. The concentrations of Zinc and Copper were from 0.112mg/kg - 1.345mg/kg and 0.066mg/kg - 0.218mg/kg, respectively. Lead and Cadmium were below the limit of detection in infusions as well.

The information obtained from this study could further help for health risk assessment of green tea and their infusions. The study will also help to determine the best brand of green tea available in the market.

1.4 Overview of Dissertation

The main goal of this dissertation was to determine the amount of heavy metals (Pb, Cd, Mn, Zn and Cu) present in various brands of green tea and their infusions.

The presence of Pb, Cd, Mn, Zn and Cu in 38 green tea samples purchased from markets in Mysuru, Karnataka and Bhubaneswar, Odisha were evaluated by Atomic Absorption Spectrometry (AAS).This study was performed both for tea leaves and their infusions. The results provide data that permits an evaluation of exposure or dietary intake of these trace elements. The samples were prepared by adding 4 ml HNO_3 and digesting it in microwave digester. Infusions were prepared by soaking the leaf or tea bags in 100 ml of boiled water and the solution was filtered using Whattman no.42 filter paper prior to the analysis by AAS.

The result showed a high amount of Mn was present in all the samples as well as in their infusions. Pb and Cd were below the detection level.

2 Review of Literature

This review is concerned with the summary and evaluation of the results from the heavy metals investigation conducted on green tea by various researchers. 13 research paper were included in this review of literature. Most of the studies evaluated are compositions, heavy metals and minerals present in green tea. Metals are present in almost all samples either naturally or as a result of human activities such as mining process, agricultural practice, industrial emission and it may also occur in rivers, lakes and oceans due to the contamination of pollutants.

In several countries, similar studies on green tea and their infusions were previously reported concerning heavy metals as in the case in the current study, Brzezicha-Cirocka et al 2016, Lanhai Li et al 2015, Milani et al 2015, Wen Si Zhong et al 2015.

H Colak and others (2005) analysed the trace metal content of various herbal and fruit teas produced and marketed in turkey. Manganese, iron, zinc, copper and nickel contents were determined by Flame Atomic Absorption Spectrometry. The concentration range of Mn, Fe, Zn, Cu and Ni was found to be 67.5 - 1,610.0 µg/g, 23.0 - 1028.5 µg/g, 3.3 - 32.5 µg/g, 5.5 - 31.5 µg/g and 11.3 - 37.0 µg/g, respectively. The levels of Cr, Cd, Pb and Co in the samples were below the detection limit of FAAS.

Wen - Yan Han et al; (2005) investigated the scale and causes of Pb contamination in Chinese tea. 1225 tea samples were collected from 17 tea producing province in China and the Lead concentration was determined by ICP - AES. The concentration of Pb in the 1225 tea samples varied from Below the Detection level to 97.9 mg/kg, with a mean and median of 2.7 and 1.4 mg/kg.

Renee Street et al; (2006) analysed the status of micronutrients (Cu, Fe, Mn, Zn) in tea and tea infusions in selected samples imported to the Czech Republic. A total of 30 tea samples of different origins, thirteen green tea samples, thirteen black tea samples, two semi - fermented tea samples and one white tea, imported to the Czech Republic, were collected and analyzed for the total content of copper, iron, manganese and zinc in tea leaves and tea infusions. The contents of Cu, Fe, Zn and Mn in the plant digests and tea infusions were determined using Inductively Coupled Plasma Emission Spectrometry with axial plasma configuration (ICP - AES). The total contents of metals in tea leaves differ according to the type of tea (green or black) and are probably influenced by many other factors, e.g. soil properties. The total contents of Mn were much higher compared to the total contents of Cu, Fe and Zn, and varied between 511 - 2220 mg/kg. The proportion of the element contents in the infusions from the respective total contents in leave were 30 ± 60% Cu, 26 ± 10% Zn, 18 ± 10% Mn and 1.5 ± 0.8% Fe.

F Qin and W.Chen (2007) analyzed lead and copper levels in 57 tea samples marketed in Beijing, China. The lead concentrations in tea samples varied from 0.198 - 6.345 mg/kg. The highest level of lead was found in Qimen Black tea (Anhui Province) and the lowest level of lead was found in Longzing green tea (Zhejiang Province). The average amount of lead in all the samples were below the maximum permissible concentration (MPC) of 5mg/kg dry weight (Chinese Ministry of Health). The copper content in the investigated teas ranged widely from 1.790 - 48.19 mg/kg. The copper content of all the tea samples in the current study were below the upper limits imposed on tea by China.

Chong Wei Jin et al; (2008) studied the concentration of Cu in tea leaves produced at Yuyao County, China. Copper concentrations in all tea leaves sampled from tea gardens were below 60 mg/kg, the permissible level given by the Chinese Ministry of Agriculture. The study indicates that Cu concentrations in tea leaves from the investigated producing areas are acceptable but are still a concern. The factors that affected the Cu accumulation in tea leaves were further analyzed. The Cu availability in soil was found to be closely correlated with the soil's H^+ activity, followed by organic matter content. The soils in the tea gardens were found to be severely acidic with the lowest pH of 3.58. The tea garden soils, if fertilized with animal manure, could also contribute to the risk of Cu contamination. Cu concentrations in the final products of tea leaves were greatly increased by the machinery processing in factories that used copper boards at the twisting stage.

Study done by **Karimi and others (2008)** on concentrations of heavy metals on tea samples marketed in Iran showed that the highest (908.30 - 377.70 µg/kg) and lowest (0.09 - 0.02 µg/kg) amount were related to Aluminium and Arsenic respectively. Metal content of black tea was found to be higher than there infusions. Concentration of As in tea samples varied from 0.08 - 0.12 µg/kg and Cu content ranged from 17.59 - 32.80 µg/kg. All samples contained As and Cu at levels below those set as the maximum values by Iranian Ministry of Health. The percent release of Hg to infusion was 70% whereas only 2.6% was found for Pb.

Albert Cosmas Achudume and Dayo Owoeye (2010) studied the bio - accumulation of heavy metals in tea marketed in Nigeria. Four major and most consumed brand of tea were selected for the study. The metals present in lowest order were Zn, Cd, Cu and Pb. The metals present in greatest concentrations were Fe (442 - 1344 mg/kg), followed closely by Mg (123 - 239 mg/kg) and highly toxic element Cu (2-7 mg/kg). It was concluded that the variations in heavy metals content of tea brands may be due to geographical, seasonal changes and the chemical characteristics of the growing regions.

Filiz Korkmaz Gorur et al; (2011) analysed the radionuclides and heavy metals concentration in tea marketed in Turkish Market. Since this region was contaminated by the Chernobyl accident in 1986, a comprehensive study was planned and carried out to determine the radioactivity level in the tea growing region. The activity concentration of ^{232}Th, ^{238}U, ^{40}K and ^{137}Cs were measured in 29 black tea and one green tea from local Turkish markets using gamma spectrometry with HpGe detector. The average activity concentration of 232Th, 226Ra, 40K and 137Cs were found 3.2 ± 0.6 Bq/kg, 445.6 ± 17.8 Bq/kg and 42.0 ± 1.4 Bq/kg in tea samples, respectively. In addition, the concentration of five heavy metals including Fe, Mn, Zn, Cu and Pb were determined by inductively coupled plasma spectroscopy (ICP/OES) on tea samples. Among the investigated metals, Mn was the highest levels. The levels of manganese were in the range of 1850.75 - 292.65 µg/g. Levels of Pb in the tea samples analyzed were below the detection limits. The concentrations of all elements for daily intake are below safety levels for human consumption.

Khakhathi L. Mandiwana et al; (2011) did the speciation analysis of black, green and herbal teas by treating the samples with 0.1M Na_2CO_3. The total Chromium content was determined with the help of Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The results of the investigation showed that the concentration of total Cr(VI) in black teas varied between 0.03 and 3.15 µg/g, in green tea ranged between 0.03 and 0.14 µg/g and that in herbal tea was below the limit of detection(LOD), thereby indicating that Cr(VI) levels increase in the order of herbal tea, green tea and black tea. It was also found that up to 17.5 µg/g of Cr(VI) could be consumed per unit cup of black tea (200ml) when standard tea bag (2.0g) or 2.0g leaf was used for the preparation of tea.

Wen - Si Zhong et al; (2015) studied the content of lead, cadmium, chromium, copper and nickel in Chinese teas. The contents of Pb, Cd, Cr, Cu and Ni were determined in

25 tea samples from China, including green, yellow, white, oolong, black, Pu'er and jasmine tea products, using high - resolution continuum source graphite furnace atomic absorption spectroscopy. The lead contents in tea leaves were 0.48 - 10.57 mg/kg and 80% of these values were below the maximum values stated by the guidelines in China. The contents of Cadmium and Chromium ranged from 0.01 mg/kg - 0.39 mg/kg and from 0.27 mg/kg - 2.45 mg/kg, respectively, remaining in compliance with the limits stipulated by China's Ministry of Agriculture. The copper contents were 7.73 - 63.71 mg/kg; only 64% of these values compiled with the standards stipulated by Ministry of Agriculture. The nickel contents ranged from 2.70 mg/kg - 13.41 mg/kg.

Lanhai Li et al; (2015) studied the potential health risk of Aluminium and other heavy metals in tea leaves and tea infusions of commercially available green tea in Jiangxi, China. In the study, contents of Al, Cd, Co, Cr, Cu, Ni and Pb were measured by ICP - MS and ICP - AES. Target Hazard Quotient (THQ) and Hazard Index (HI) were employed to assess the Potential health risk of studied metals in tea leaves and tea infusions to drinkers. The results showed the content of Al, Cd, Co, Cr, Cu, Ni and Pb in tea leaves were 487.57, 0.055, 0.29, 1.63, 17.04, 7.71 and 0.92 mg/kg, respectively. Metal contents were within their limit maximum limits except for Cu. Concentrations of metals in tea infusions were all below their maximum limits. The concentration decreased with increase with infusion times. The THQ from 2.33×10^{-5} to 1.47×10^{-1} and HI from 1.41×10^{-2} to 3.45×10^{-1} values in tea infusions were all less than 1, suggesting that consumption of tea infusions would not cause significant health risk for consumers.

Raquel F. Milani et al; (2015) analysed the presence of heavy metals (Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn) in 30 tea samples (Black tea - 9, Green tea - 9, White tea - 9 and Red oolong tea - 3) and their infusions found in the markets of Brazil. They were evaluated by ICP - MS. The concentration of Aluminum in tea leaves ranged from 750 - 3937 mg/kg, Iron and Manganese concentration was observed to be 61 - 987 mg/kg and 108 - 1960 mg/kg, respectively. A high concentration of Cu and Zn was observed in black and green tea samples and high level of Lead was detected in oolong tea samples. In tea infusions, high level of manganese and Aluminium were observed in all types of teas.

Justyna Brzezicha - Cirocka et al; (2016) studied the concentrations of toxic metals (Cd, Pb) and other elements (Ca, K, Mg, Na, P, Mn, Fe, Zn, Cu, Co, Cr, Ni) in green tea leaves and their infusions from various geographical locations. The metal contents were determined by Atomic Absorption Spectroscopy. Phosphorous content was measured by using an ultraviolet - visible - spectrophotometer. Green tea from India had the lowest concentration of Cd and Pb among the analyzed samples. Among the macro - elements, K was present at the highest concentration, with values of 2215 mg/100g in Indian tea and 2006 mg/100g in Chinese tea. Manganese was the most abundant micro - element in the teas analyzed (39.0 - 126 mg/100g). The average Pb and Cd levels in a 200ml beverage were 0.002 and 0.003 mg, respectively. Indian teas had the highest percentage of Cd leaching (43.8%) and Chinese tea had the lowest (94.1%).

3 Aims and Objectives

The main aim of this study was to determine the amount of heavy metals that are present in various brands of green tea and their infusions. Heavy metals are a group of elements that are present in both solid and liquid state. The multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment. The toxicity of heavy metals depends on various factors like the dose, route of exposure and chemical species, as well as the age, gender, genetics and nutritional status of exposed individuals. In recent years there has been a rapid increase in the consumption of green tea. This is due to various studies claiming its role in weight loss, prevention of cancer, prevention of neuro - degenerative diseases like Parkinson's and Alzheimer's and prevention of tooth decay.

Green tea is rich in anti - oxidant. But other than those, there are various bio elements present in tea leaves. Most toxic of which are lead and cadmium. Presence of Lead and Cadmium can be explained because of the acidic soil in which tea leaves are grown. Cadmium and Lead are abundant in acidic soil and are available for root uptake. Contamination of other trace elements in green tea can occur at processing levels. Hence it is important to determine the concentration of the heavy metals in green tea.

Determination of the concentration of heavy metals in green tea can also help us to determine the nutritional value of green tea of various brands. It will also help us to avoid any probable ill effect that can happen from the intake of green tea

Studying the concentration of heavy metals in green tea infusions will help us to analyse the amount of leaching that occurs from tea leaves to the infusions.

4 Materials and Methods

The method used for the examination of heavy metals in green tea was adopted from AOAC. AOAC international is an organization that begun in 1884 to serve the analytical methods needed by government regulatory and research studies. The goal of AOAC international is to provide methods that will be fit for their intended purpose (i.e. it will be performed with the necessary accuracy and precision under usual laboratory conditions).

A total of 38 random green tea samples were purchased from Mysore supermarket, Karnataka and Bhubaneswar supermarket, Odisha. They were analysed by Flame Atomic Spectrophotometer (AAS) for the determination of levels of toxic elements such as Lead(Pb), Cadmium (Cd), Manganese (Mn), Zinc (Zn) and Copper (Cu).

4.1 Samples

38 Green tea samples were collected from markets of Mysuru, Karnataka and Bhubaneswar, Odisha. All the available brands of green tea were collected for analysis of heavy metals.

The samples were taken directly from the supermarket to the food safety and analytical quality control laboratory in CSIR-CFTRI, Mysuru. They were noted down on a log book with its name, brand name, batch number, manufacturing date and expiry date. Each sample was given a unique code for easy handling of the material during the investigation.

<u>Figure 9- Green tea samples collected for the study</u>

4.2 <u>Sample Preparation</u>

The samples were carefully opened and around 20 - 30gm of the samples were transferred to polythene pouches to avoid any spoilage to the original sample. These samples were homogenized with the help of mortar and pestle to obtain an uniform mixture. 0.25 gm of the homogenized sample (for microwave digestion) were weighed and taken in a clean quartz tube. The rest of the samples were neatly wrapped in air-tight pouches and stored.

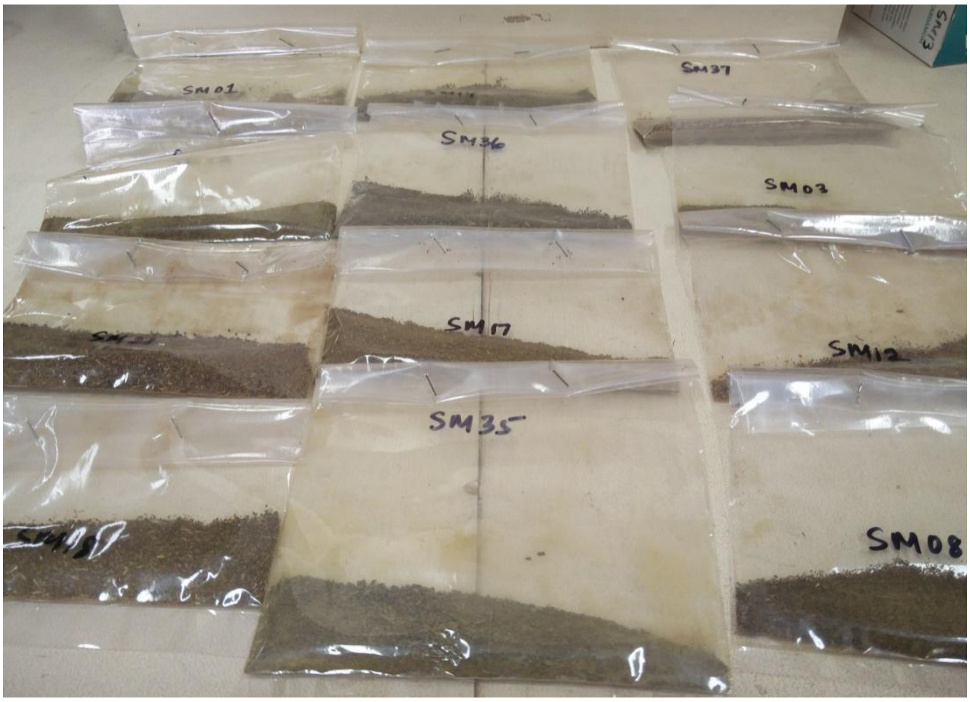


Figure 10 - Samples preserved in air - tight pouches

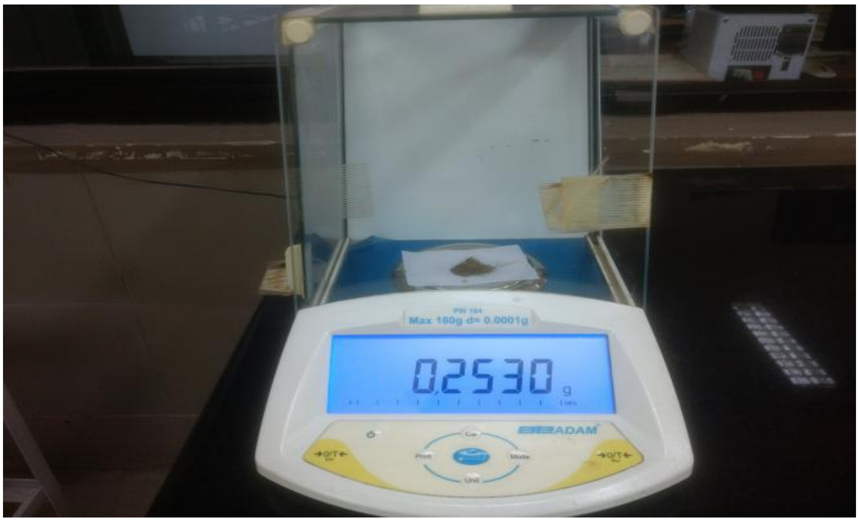


Figure 11- Weighing of the samples

4.2.1 Infusion Samples

For infusion study 15 green tea samples were selected randomly. Tea infusions were prepared using 2.0g of the material and 100 ml of boiled deionised water. Tea was infused for 5 min and was filtered using Whattman no.42 filter paper. The solution was then concentrated up to 1 ml using Water bath. It was filtered again and made up to 30 ml. Infusion samples were ready for analysis by AAS.

Figure 12- Green Tea Infusion Samples for Analysis

4.3 Sample Digestion

The digestion of the green tea leave samples were done by Microwave digestion, prior to their analysis.

4.3.1. Microwave Digestion

For the microwave digestion, clean deionized quartz tubes were taken. 0.25 gm of the homogenized powdered samples were weighed and taken in the quartz tubes. Equal ratio of conc. HNO_3 (65% ultra-pure, Merck) and deionized water were taken in the tubes along with the sample. After the required reagents were added, the tubes were kept in the fume-hood for 25 minutes which allows the removal of acid fumes which releases the pressure (<10 bar) from the tubes. Afterwards stoppers were placed on the tubes which were fixed to the rotor unit tightly and were covered with its shield. It is then kept in the microwave digestion system with high temperature (450℃) and high pressure (80 bar) for 45 minutes leading to the digestion of the sample followed by cooling. The digested solution obtained is filtered through Whattman no.42 filter paper with 3 - 4 continuous rinsing of the quartz tube and transferring it to 30 ml test tube. The sample was ready for determination of heavy metals by using Atomic Absorption Spectrophotometer (AAS).

<u>Figure 13 - Microwave Digester</u>

Figure 14- Green tea leave samples for Analysis

4.4 **Calibration**

For Calibration, different standards of known concentrations were prepared from 1000ppm standard by serial dilution. All the reagents used were of Ultra-Pure Metal free and deionized Milli Q water was used during all the experimental procedure. All heavy metals such as Lead (Pb), Cadmium (Cd), Manganese (Mn), Zinc (Zn) and Copper (Cu) standards were from E - Merck which is traceable to National Institution of Standards and Technology (NIST), USA.

4.5 **Flame Atomic Absorption Spectrophotometer (FAAS)**

For the determination of Lead, Cadmium, Manganese, Zinc and Copper, Absorption Spectrophotometer, iCE 3000 Thermo scientific series was used.

Atomic Absorption Spectrophotometer or Atomic Absorption is a very common technique for detecting metals or metalloids in environmental and food samples. The technique is based on the fact that a ground state metal absorbs light at specific wave lengths. Metal ions in a solution are converted to atomic state by means of a flame.

Light of the appropriate wavelength is supplied and the amount of light absorbed can be measured against a standard curve. Atomic Absorption spectroscopy was first used as an analytical technique and the underlying principles were established in the second half of the 19th century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg, Germany. The modern form of AAS was largely developed during the 1950s by a team of Australian chemists led by Sir Alan Walsh at the commonwealth Scientific and Industrial Research organization (CSIRO), Division of Chemical Physics, Melbourne, Australia.

Figure 15- Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy have many uses in different areas of Chemistry. Such as -

- Clinical Analysis - Analyzing metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue and semen.
- Pharmaceuticals - In some pharmaceutical manufacturing processes, minute quantities of a catalyst that remain in the final drug products.
- Water and Food samples - Analyzing water and food samples for its metal contents.

4.5.1 Basic Principle

The technique of Atomic Absorption Spectrophotometer (AAS) requires a liquid sample to be aspirated, aerosolized and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. Sample solution is usually aspirated with the gas flow into nebulizing/ mixing chamber to form small droplets before entering the flame. The mixture is ignited in a flame whose temperature ranges from 2100^0C - 2800^0C. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelength.

The characteristics wavelength are element specific and accurate to 001 - 0.1 nm. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A device such as photon multiplier can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the sample. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer - Lambert Law.

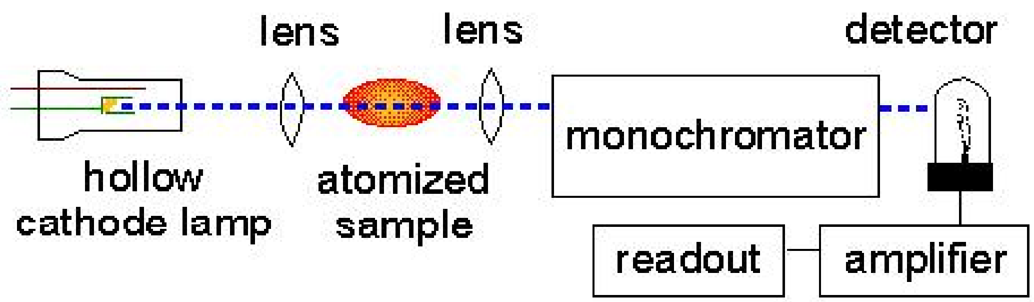


Figure 16- Working Principle Of AAS

4.5.2 Instrumentation

During 1950s the research work of Walsh and Alkenade, led to the introduction of Atomic Absorption Spectroscopy for the analysis of trace metal at ppm levels and it is used to estimate the concentration of elements in a given sample.

Flame absorption spectrometer with attached graphite furnace in particular is designed to operate either with a flame or with graphite. The graphite furnace is additionally equipped with an auto sampler. In order to analyse a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electro - thermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element - specific line radiation source or a continuum radiation source. The radiation then passes through a monochromator in order to separate the element - specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

The basic instrumental parts of atomic absorption spectrometer are -

- Radiation source, usually a Hollow cathode lamp.
- Atomiser, usually a nebulizer - burner system or electro thermal furnace
- Flame, usually 1700 - 3150C for AAS.
- Chopper
- Monochromator, usually an ultraviolet - visible (UV-Vis) grating monochromator.
- Detector, usually a photomultiplier tube (PMT)
- Read - out unit, usually Computer

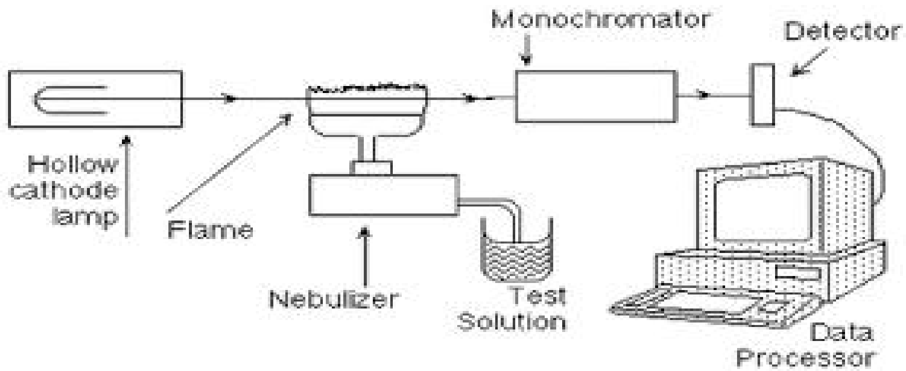
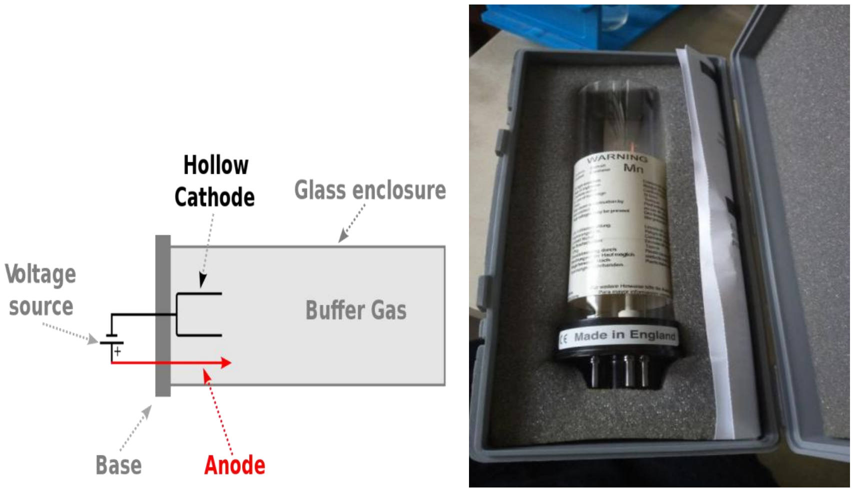


Figure 17- AAS Instrument Parts

4.5.2.1 <u>Hollow Cathode Lamp (HCL)</u>

It is a stable light source, which is necessary to emit the sharp characteristic spectrum of the element to be determined. A different cathode lamp is needed for each element, although there are some lamps that can be used to determine three or four different elements if the cathode contains all of them. Each time a lamp is changed, proper alignment is needed in order to get as much light as possible through the flame, where the analyte is being atomized, and into the monochromator. To HCL, high voltage is applied across anode and cathode. As and He usually will be filters, this gas will be ionized at anode and attracted towards cathode. Cathode is made up of element that is to be analyzed. The gas ions reaching the cathode displacing atoms of that metal to atmosphere, where this atom gets excited and emits the characteristics radiations. Cathodes will be cylindrical shape to concentrate radiations in a limited region in the tube. The radiations are formed by application of electric current through the vapours of metal atoms.



<u>Figure 18- Hollow Cathode Lamp</u>

4.5.2.2 <u>Atomization Source</u>

The flame source consists of burner and flame. Most widely used flame for AAS is air acetylene flame or nitrous oxide acetylene flame. Argon and Hydrogen are used as fuel in case, where wavelength is below 200 nm as in case for As (193.50nm) and Se (197.00 nm) because air acetylene absorbs radiations below 200 nm of wavelength. These metals from hydride (AsH_3 and H_2Se) to enter the flame.

4.5.2.3 Chopper

Chopper is a motor drive device that has open and solid regions alternating regions. It is like a rotating wheel between Hollow Cathode Lamp and flame. Modulation is the main function of the chopper. Modulation of the lamp beam from a DC to AC signal, while the flame signal remains DC and the detector is tuned to pick up only the lamp signal. It is used to avoid the problem that most of the elements emit some radiation of the same wavelength when they absorb it. But by modulating the beam from the lamp and tuning the detector to this frequency, the emitted light is no longer detected and full level of absorption can be detected. This is achieved by using the mechanical chopper rotating in a controlled frequency to which the detector is tuned. The chopper blades allow through blocks of light alternating with darkness and this produces an alternating current in the detector.

4.5.2.4 Monochromator

Prism and grating are most commonly used monochromators. Its function is to select a given absorbing line from spectral lines emitted from the Hollow Cathode Lamp. The monochromator isolates the specific spectrum line emitted by the light source through spectral dispersion, and focuses it upon a photomultiplier detector, whose function is to convert the light signal into an electrical signal. The processing of electrical signal is fulfilled by a signal amplifier. The signal could be displayed for readout, or further fed into a data station for printout by the requested format.

4.5.2.5 Detector

Film and photo-multiplier tube are more commonly used detectors. Photo-multiplier tube converts radiant energy to electrical signals.

4.5.2.6 Amplifier

Lock in amplifier is preferred which provides narrow frequently band and helps to achieve excellent signals to noise ratio.

Sl.no	Parameters	Lead	Cadmium	Copper	Manganese	Zinc
1	Hollow Cathode Lamp	NC30481	NC30821	NC30291	NC30251	NC30301
2	Lamp Current	10mA	10mA	10mA	10mA	10mA
3	Wavelength	217.0nm	228.8nm	324.8nm	279.5nm	213.9nm
4	Slit Width	0.7nm	0.7nm	0.7nm	0.7nm	0.7nm
5	Range	Linear	Linear	Linear	Linear	Linear
6	Fuel Mixture	Air - Acetylene	Air - Acetylene	Air - Acetylene	Air - Acetylene	Air - Acetylene
7	Fuel Pressure Control	Acetylene - 12-14psi, Air - 60psi	Acetylene - 12-14psi, Air - 60psi	Acetylene - 12-14psi, Air - 60psi	Acetylene - 12-14psi, Air - 60psi	Acetylene - 12-14psi, Air - 60psi
8	Acetylene Tank Pressure	Not less than 70-75	Not less than 70-75	Not less than 70-75	Not less than 70-75	Not less than 70-75
9	Standards in ppm	0.5, 1.0, 2.0, 4.0	0.1, 0.2, 0.4, 0.8	0.25, 0.50, 1.0, 2.0	0.5, 1.0, 2.0, 4.0	0.5, 1.0, 2.0, 4.0
10	Reading Replicate	03	03	03	03	03
11	AOAC 20TH Edition	999.11	999.11	985.35	985.35	985.35

Table 5 - Instrumental Parameters used by FAAS (iCE 3000 Thermo scientific)

5 Results

The monitoring work was carried out to study the level of toxic metals (Lead, cadmium, Copper, Manganese and Zinc) in commercially available Green teas and their infusions. 38 green tea samples from different brands were procured from the markets of Mysuru, Karnataka and Bhubaneswar, Odisha. From the analysis it was found that some samples were exceeding the US FDA limits, WHO limits and the FSSAI limits.

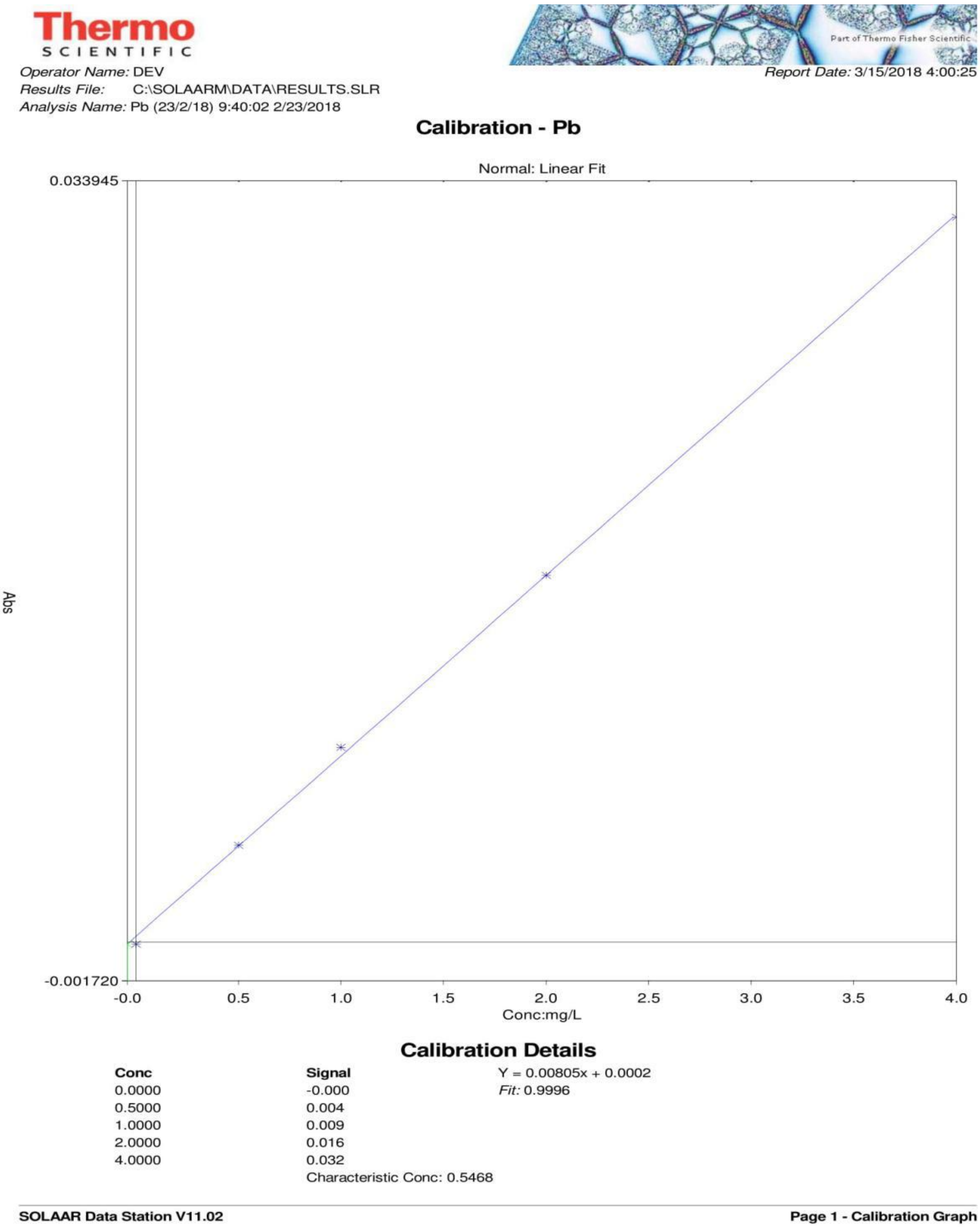


Figure 19 - Standard Calibration Curve for Pb

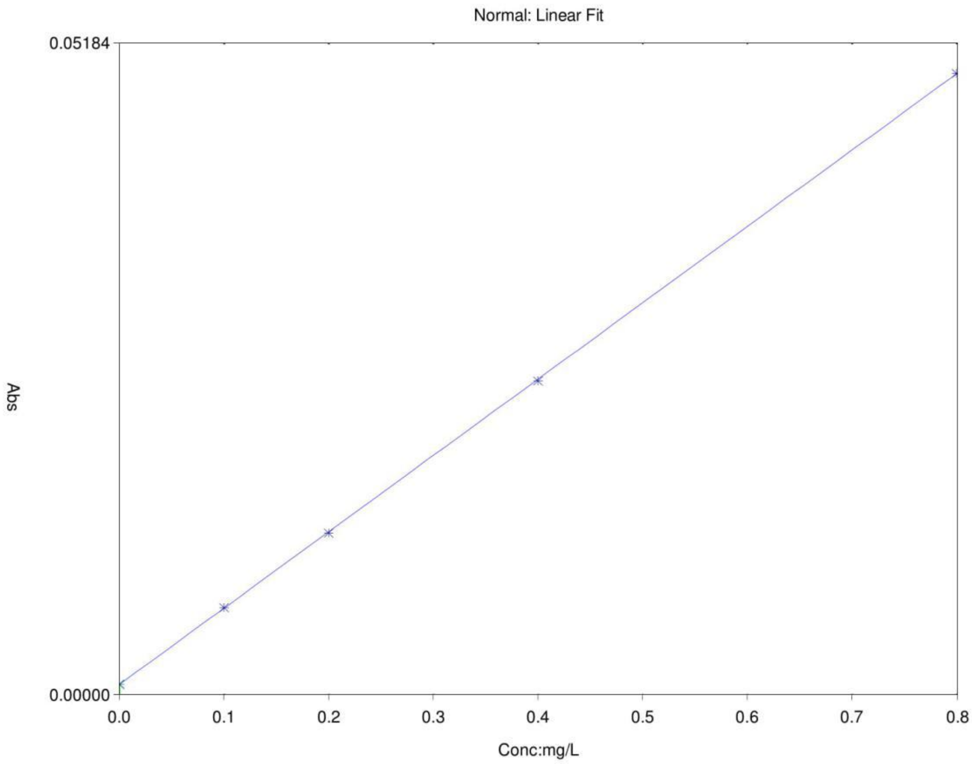
Thermo
SCIENTIFIC

Operator Name: DEV *Report Date:* 3/15/2018 3:53:08

Results File: C:\SOLAARM\DATA\RESULTS.SLR

Analysis Name: Cd 20.02.18 10:11:53 2/20/2018

Calibration - Cd



Calibration Details

Conc	Signal	Y = 0.06072x + 0.0008
0.0000	0.001	*Fit:* 1.0000
0.1000	0.007	
0.2000	0.013	
0.4000	0.025	
0.8000	0.049	
	Characteristic Conc: 0.0725	

SOLAAR Data Station V11.02 **Page 1 - Calibration Graph**

Figure 20 - Standard Calibration Curve for Cadmium

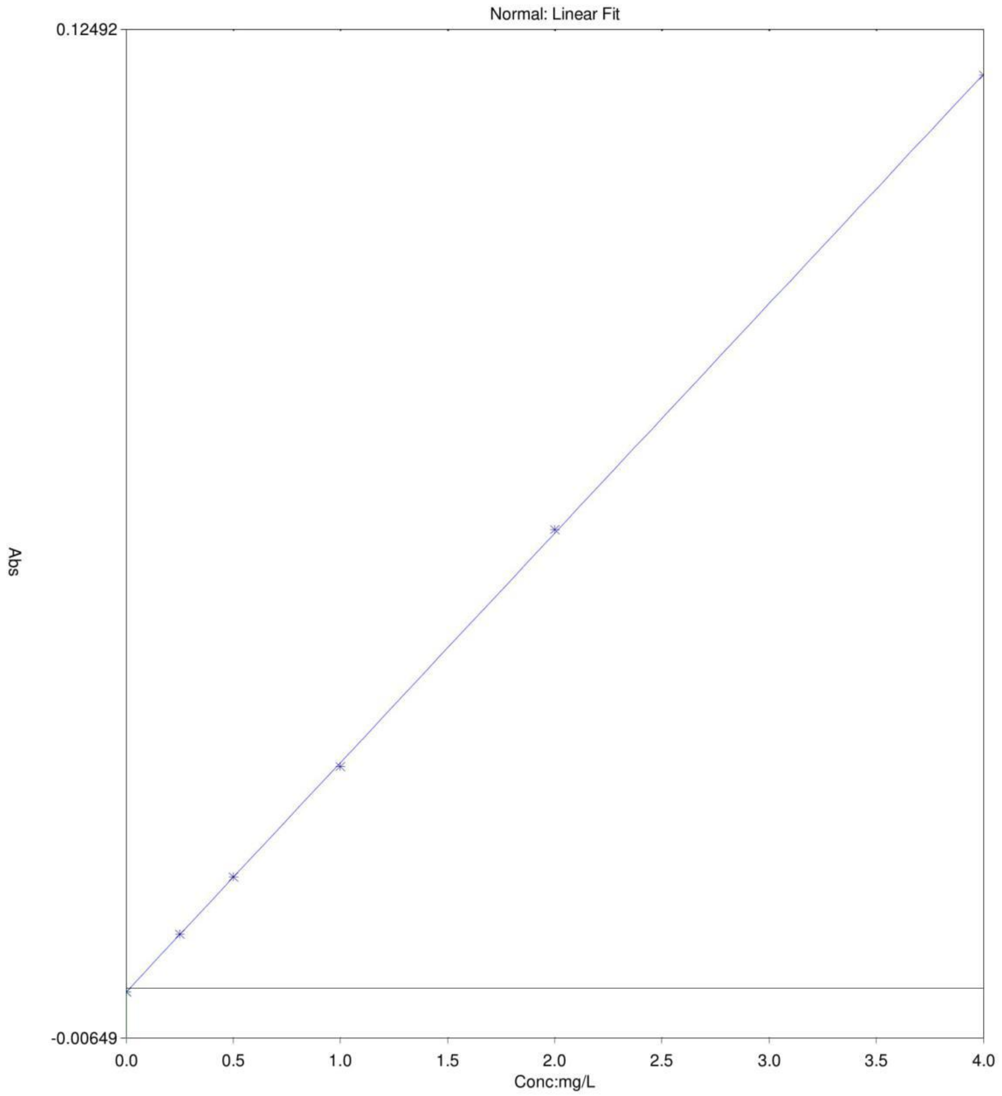
Thermo
SCIENTIFIC

Part of Thermo Fisher Scientific

Operator Name: DEV
Results File: C:\SOLAARM\DATA\RESULTS.SLR
Analysis Name: Cu (22/2/18) 3:07:58 2/22/2018

Report Date: 3/15/2018 4:07:10

Calibration - Cu



Calibration Details

Conc	Signal	
0.0000	-0.001	Y = 0.02990x - 0.0005
0.2500	0.007	*Fit:* 0.9999
0.5000	0.015	
1.0000	0.029	
2.0000	0.060	
4.0000	0.119	
	Characteristic Conc: 0.1471	

SOLAAR Data Station V11.02 **Page 1 - Calibration Graph**

Figure 21 - Standard Calibration Curve for Copper

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific

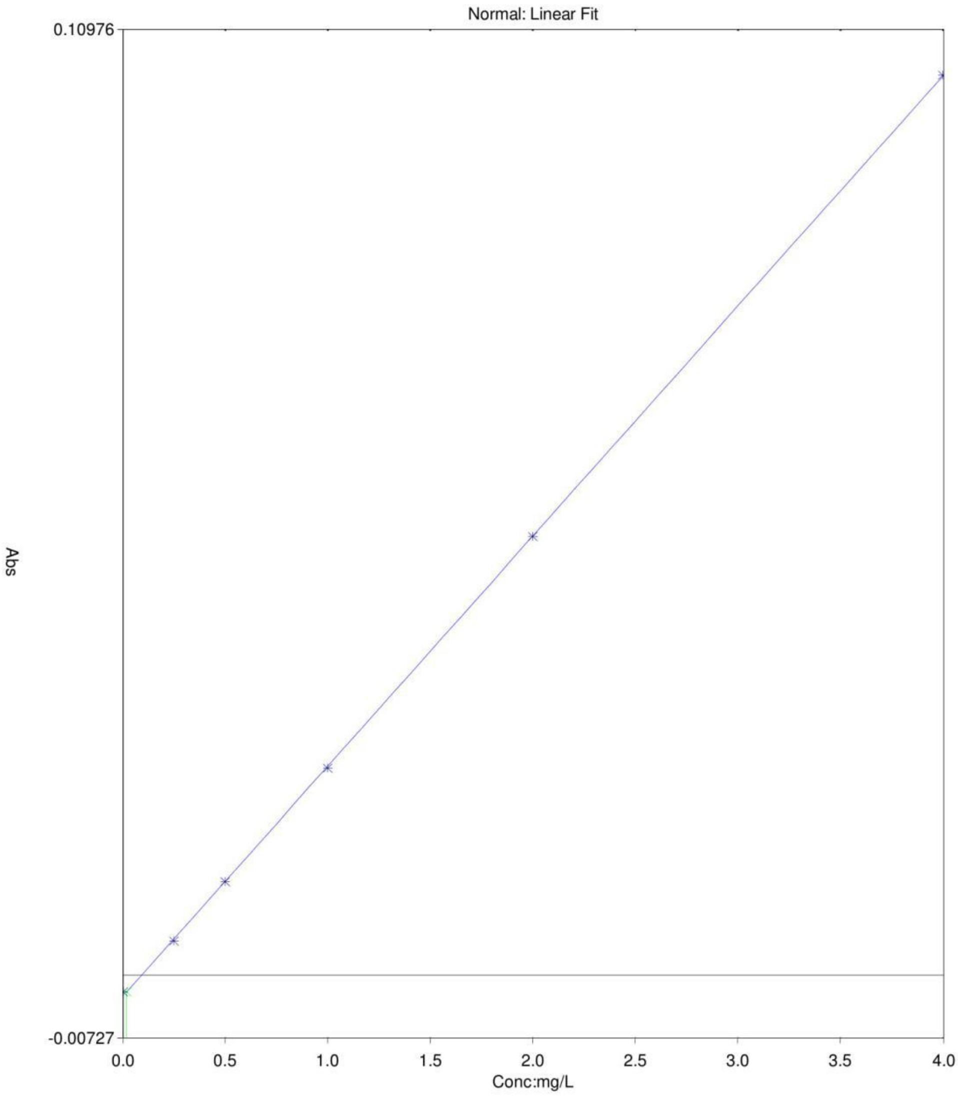
Operator Name: DEV

Results File: C:\SOLAARM\DATA\RESULTS.SLR

Analysis Name: Mn (28/2/18) 9:52:02 2/28/2018

Report Date: 3/15/2018 3:58:06

Calibration - Mn



Calibration Details

Conc	Signal	
0.0000	-0.002	Y = 0.02670x - 0.0024
0.2500	0.004	*Fit:* 1.0000
0.5000	0.011	
1.0000	0.024	
2.0000	0.051	
4.0000	0.104	
	Characteristic Conc: 0.1648	

SOLAAR Data Station V11.02 **Page 1 - Calibration Graph**

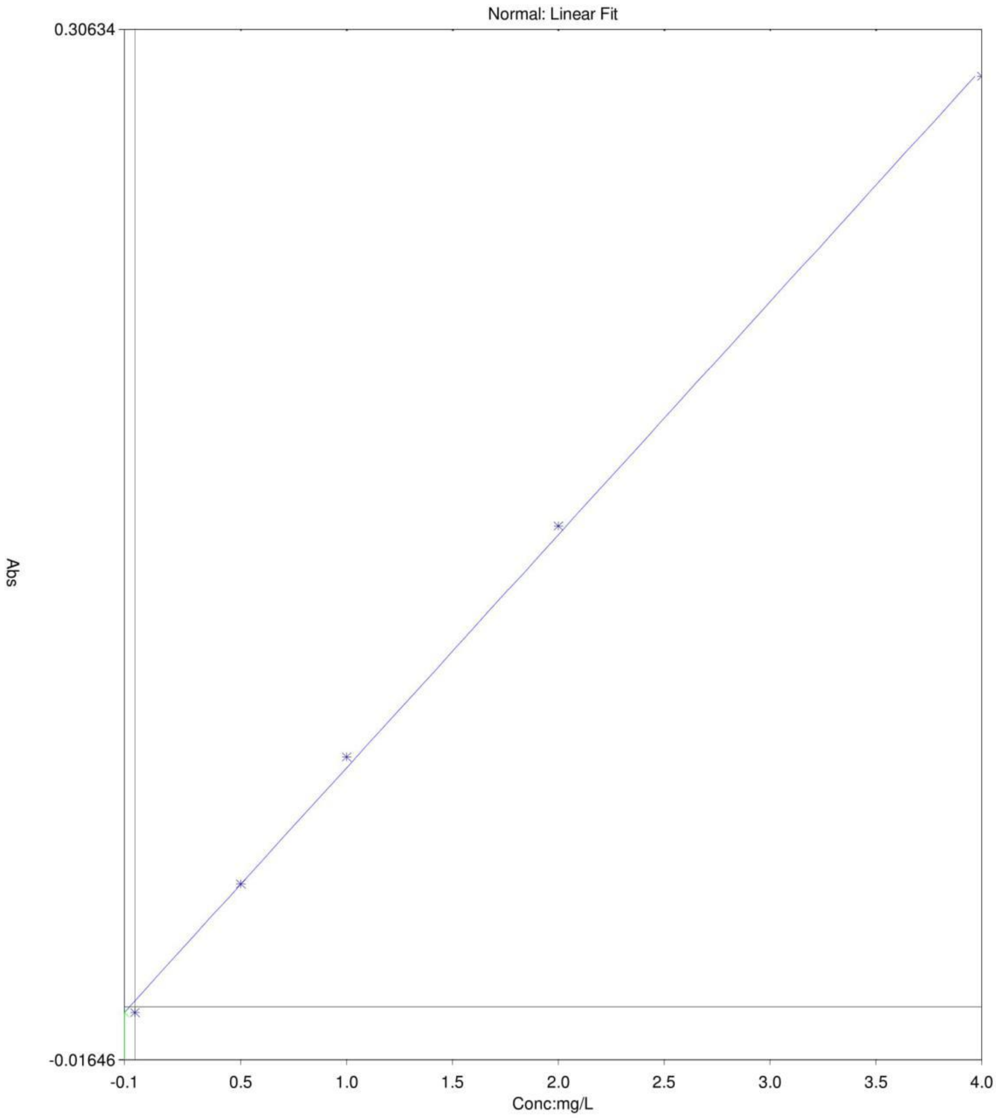
Figure 22 - Standard Calibration Curve for Manganese

Thermo SCIENTIFIC

Operator Name: DEV
Results File: C:\SOLAARM\DATA\RESULTS.SLR
Analysis Name: Zn 08.03.18 2:45:07 3/8/2018

Report Date: 3/15/2018 4:04:33

Calibration - Zn



Calibration Details

Conc	Signal	
0.0000	-0.002	Y = 0.07295x + 0.0020
0.5000	0.038	*Fit:* 0.9993
1.0000	0.078	
2.0000	0.151	
4.0000	0.292	

Characteristic Conc: 0.0603

SOLAAR Data Station V11.02 **Page 1 - Calibration Graph**

Figure 23 - Standard Calibration Curve for Zinc

The atomic absorption spectroscopy was calibrated to Lead, Cadmium, Copper, Manganese and Zinc prior to the analysis by using the standard solutions of the corresponding metals and the absorbance was noted for the five concentrations. The calibration curve was obtained with good linearity and perfection for all metals in all ranges of concentration.

Results for Green Tea Leave Sample

The data obtained from the analysis of green tea leaves on different parameters such as Lead (Pb), Cadmium (Cd), Copper (Cu), Manganese (Mn) and Zinc (Zn) are represented in the table.

Sample ID	Concentration (mg/kg)				
	Pb	*Cd*	*Cu*	*Mn*	*Zn*
SM01	BDL of 0.5	BDL OF 0.1	19.3	345.4	33.6
SM02	BDL of 0.5	BDL OF 0.1	15.2	923.4	21.8
SM03	BDL of 0.5	BDL OF 0.1	21.7	661.6	74.6
SM04	BDL of 0.5	BDL OF 0.1	25.0	556.9	33.2
SM05	BDL of 0.5	BDL OF 0.1	19.9	309.9	29.2
SM06	BDL of 0.5	BDL OF 0.1	15.9	961.3	23.9
SM07	BDL of 0.5	BDL OF 0.1	5.7	155.7	11.3
SM08	BDL of 0.5	BDL OF 0.1	20.8	743.5	42.8
SM09	BDL of 0.5	BDL OF 0.1	21.6	173.8	41.2
SM10	BDL of 0.5	BDL OF 0.1	16.1	543.7	24.2
SM11	BDL of 0.5	BDL OF 0.1	19.5	612.1	42.6
SM12	BDL of 0.5	BDL OF 0.1	25.7	255.8	22.9
SM13	BDL of 0.5	BDL OF 0.1	17.3	341.9	23.5
SM14	BDL of 0.5	BDL OF 0.1	35.9	339.3	36.9
SM15	BDL of 0.5	BDL OF 0.1	24.8	97.2	35.4
SM16	BDL of 0.5	BDL OF 0.1	20.7	650.9	18.8
SM17	BDL of 0.5	BDL OF 0.1	22.2	189.5	30.6
SM18	BDL of 0.5	BDL OF 0.1	16.6	613.2	17.5
SM19	BDL of 0.5	BDL OF 0.1	18.7	896.2	19.9
SM20	BDL of 0.5	BDL OF 0.1	12.12	845.1	18.9
SM21	BDL of 0.5	BDL OF 0.1	16.815	191.9	34.1
SM22	BDL of 0.5	BDL OF 0.1	13.785	883.9	10.7
SM23	BDL of 0.5	BDL OF 0.1	19.52	823.2	22.7
SM24	BDL of 0.5	BDL OF 0.1	20.82	855.9	23.7
SM25	BDL of 0.5	BDL OF 0.1	18.205	675.3	22.0
SM26	BDL of 0.5	BDL OF 0.1	21.525	456.9	34.9

SM27	BDL of 0.5	BDL OF 0.1	***21.7***	***370.6***	***29.1***
SM28	BDL of 0.5	BDL OF 0.1	4.2	12.7	4.05
SM29	BDL of 0.5	BDL OF 0.1	21.0	528.9	27.4
SM30	BDL of 0.5	BDL OF 0.1	47.1	411.9	48.3
SM31	BDL of 0.5	BDL OF 0.1	19.2	532.5	33.7
SM32	BDL of 0.5	BDL OF 0.1	20.8	289.6	22.8
SM33	BDL of 0.5	BDL OF 0.1	15.5	684.5	17.4
SM34	BDL of 0.5	BDL OF 0.1	20.0	686.9	24.1
SM35	BDL of 0.5	BDL OF 0.1	19.1	139.8	46.0
SM36	BDL of 0.5	BDL OF 0.1	13.6	467.1	15.9
SM37	BDL of 0.5	BDL OF 0.1	6.0	170.8	BDL of 0.5

Table 6- Results obtained for green tea leaves sample

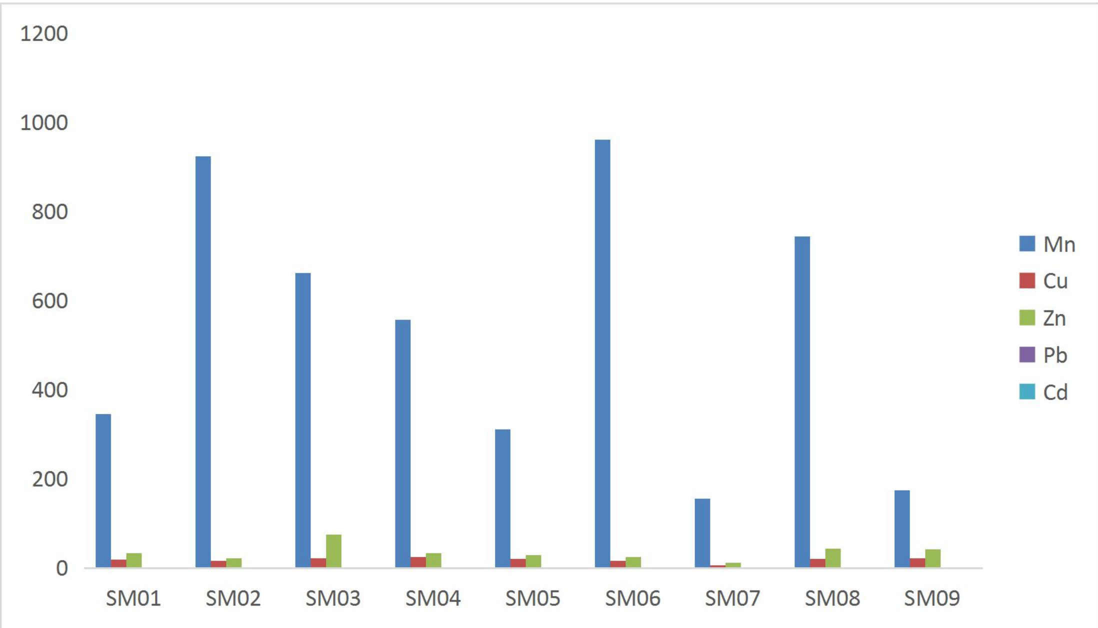


Figure 24- Graphical Representation of Heavy metal concentration in sample 1 - 9

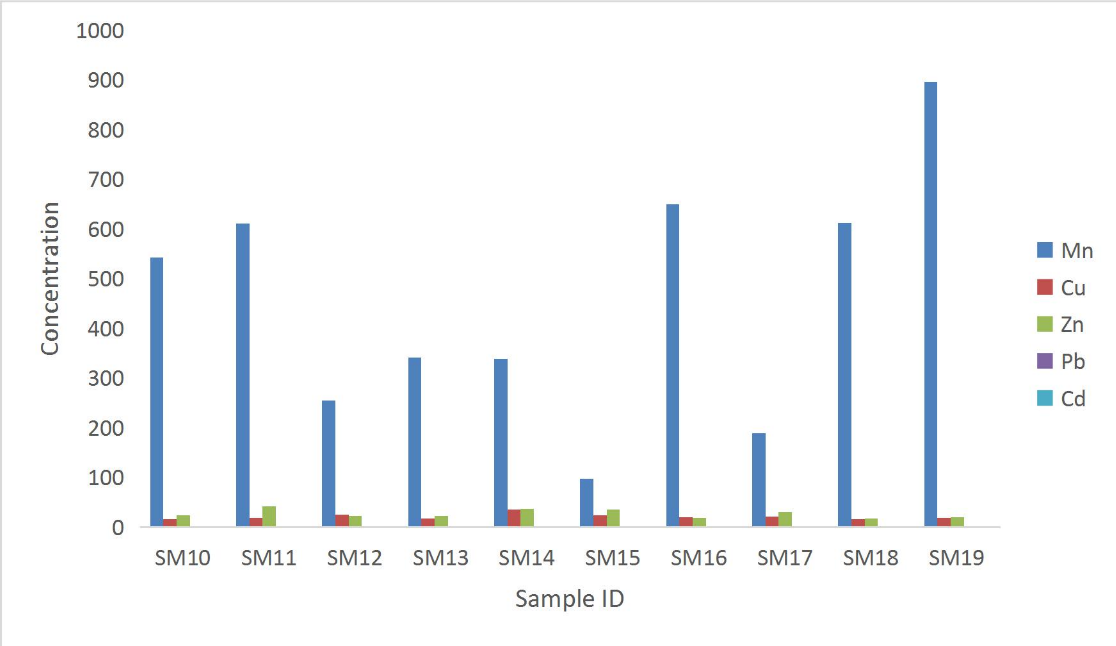


Figure 25- Graphical Representation of Heavy metal concentration in sample 10 - 19

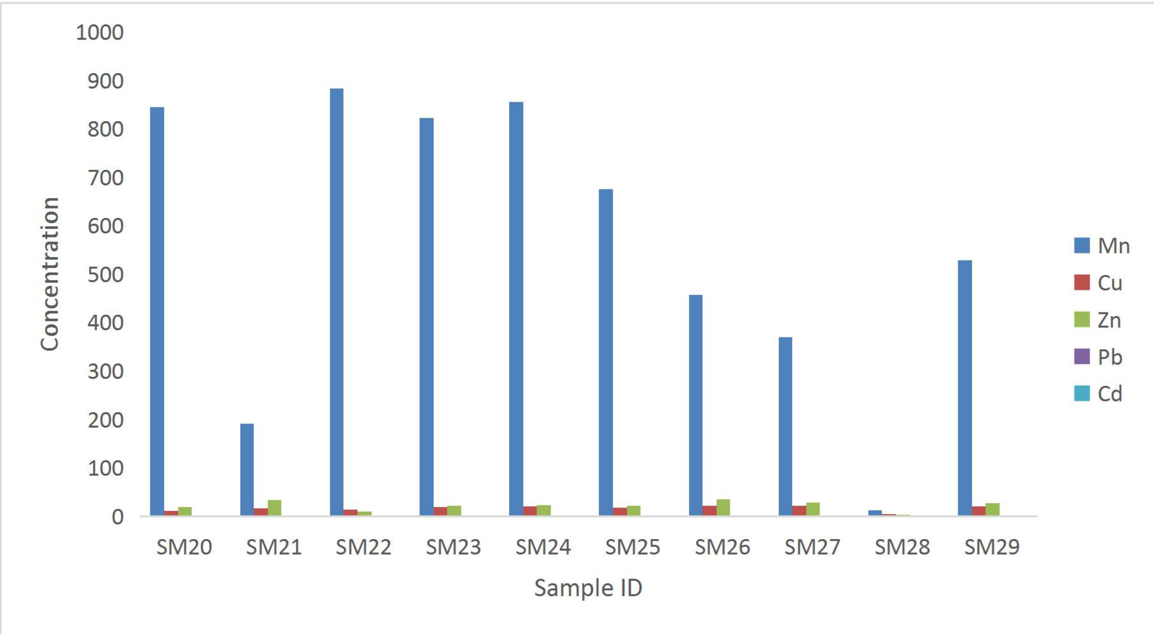


Figure 26 - Graphical Representation of Heavy metal concentration in sample 20 - 29

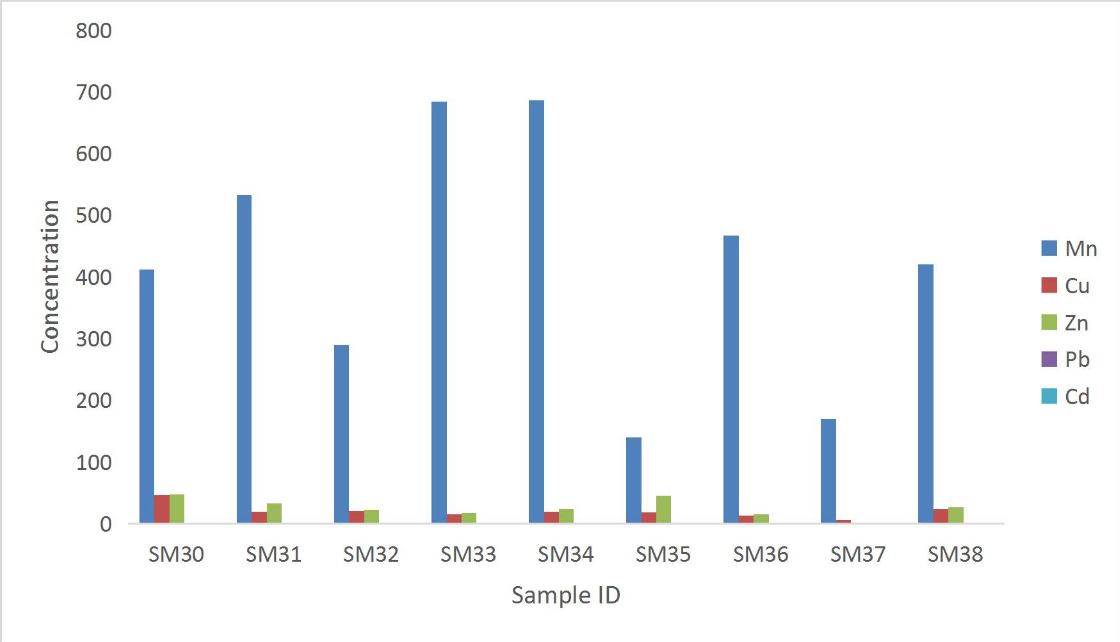


Figure 27- Graphical Representation of Heavy metal concentration in sample 30 - 38

The graph (fig24; fig25; fig26; fig27) represents the combined results of all the 38 green tea leave samples. In the five parameters analyzed, the manganese concentration was comparatively higher than the other metal content. Out of the 38 samples analyzed, Sample SM06 (961.3 mg/kg) showed the elevated level of Manganese concentration.

The level of Copper was highest in the sample SM30 (47.1 mg/kg) and was lowest in the sample SM28 (4.2 mg/kg). Zinc level was highest in sample SM03 (74.6 mg/kg). The Cadmium level was recorded as Below Detectable Limit (BDL of 0.1 mg/kg) and the Lead concentration was also Below Detectable Limit (BDL of 0.5 mg/kg).

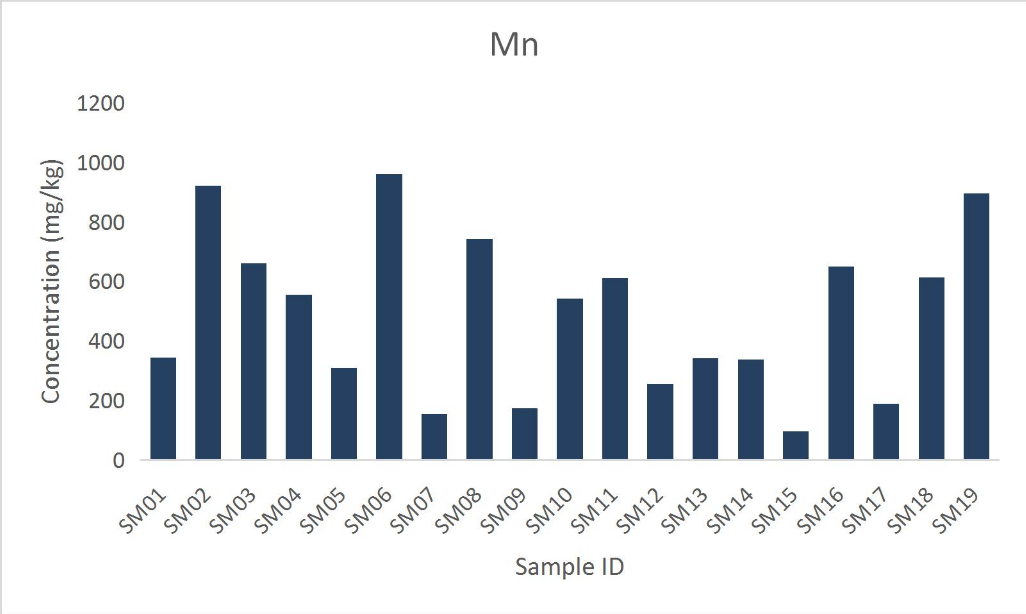


Figure 28- Graphical representation of Mn concentration in samples 1 - 19

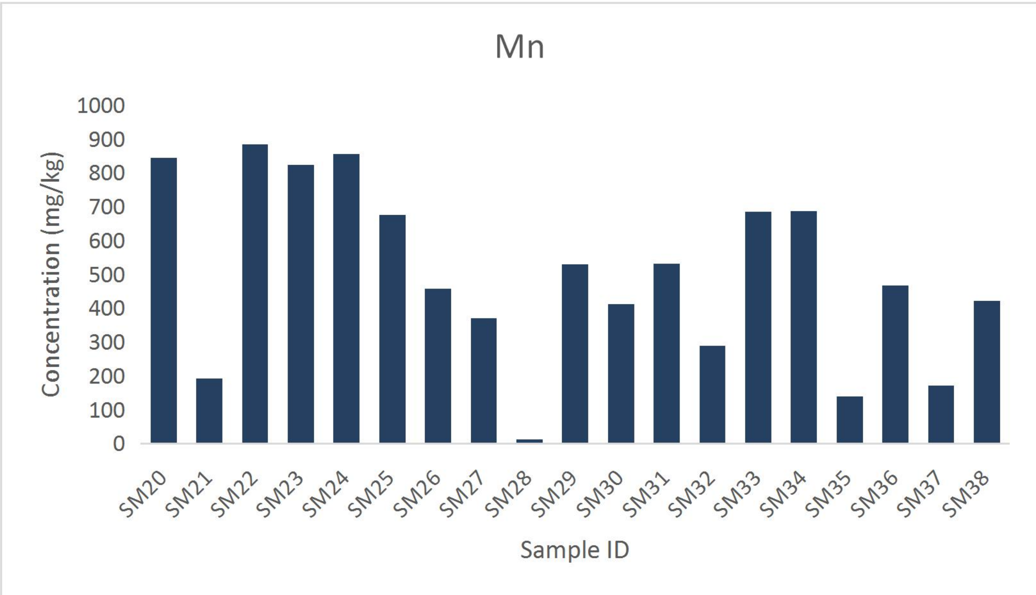


Figure 29- Graphical Representation of Mn concentration in sample 20 - 38

The two graphs (fig28; fig29) demonstrates the high level of Manganese found in various green tea leave samples. The concentration of Manganese in the green tea sample ranged from 12.7 - 961.3 mg/kg. Sample 06 had the highest level of manganese from all 38 samples while Sample 28 had the lowest amount present.

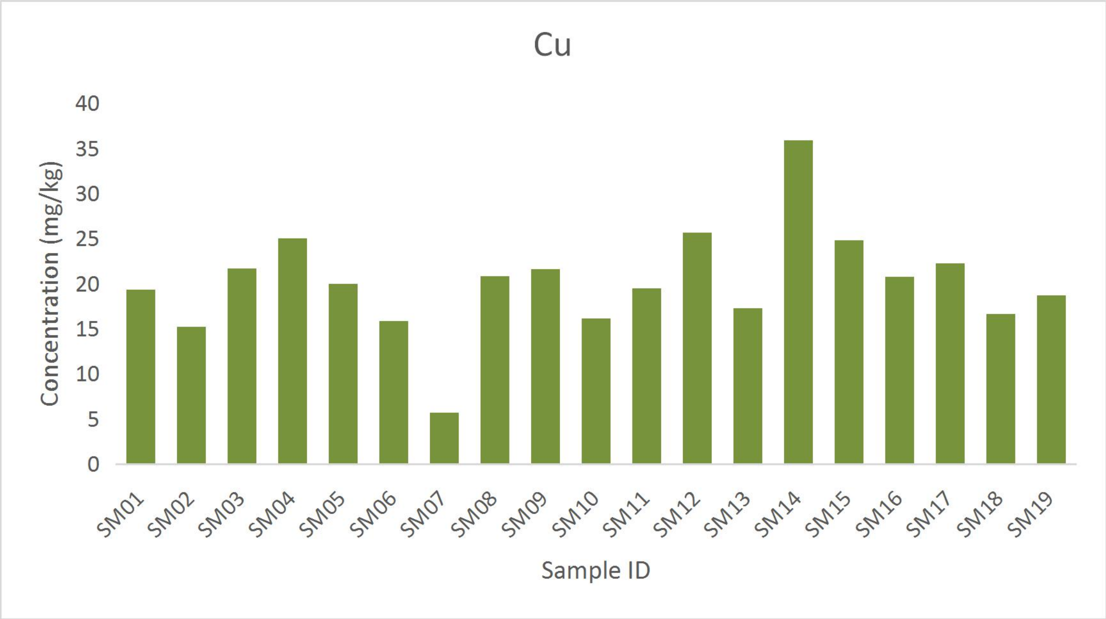


Figure 30- Graphical Representation of Copper concentration in sample 1 - 19

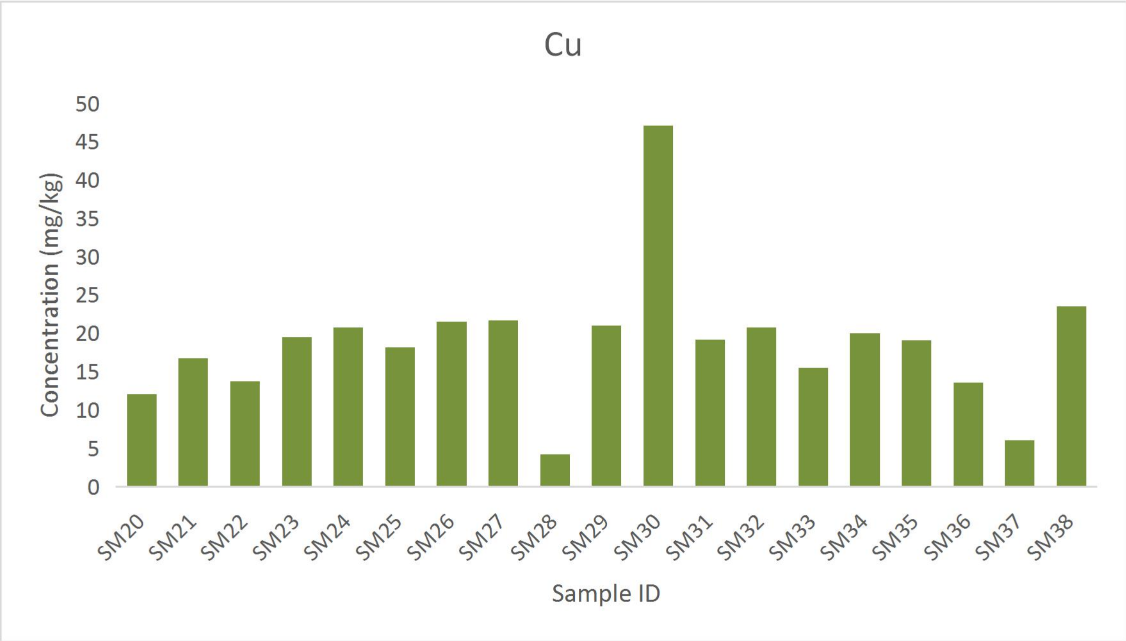


Figure 31- Graphical Representation of Copper concentration in samples 20 - 38

The above two graphs (fig30; fig31) demonstrate the level of copper found in green tea samples. The concentration of copper ranged from 4.2 - 47.1 mg/kg. Sample 30 had the highest level of Copper present whereas Sample 28 had the lowest level.

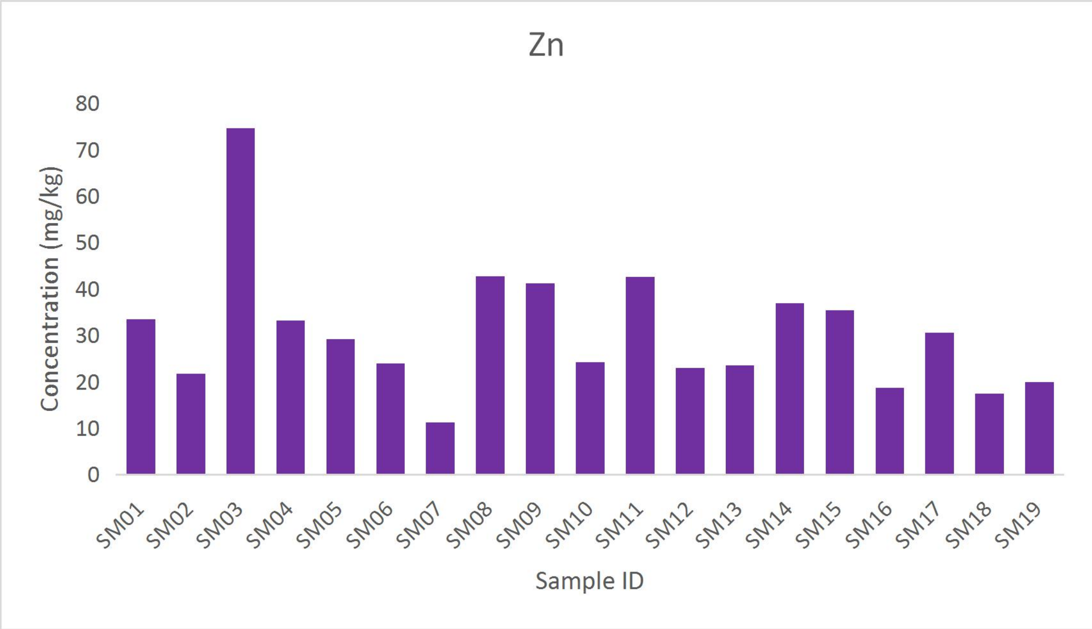


Figure 32- Graphical Representation of Zinc Concentration in Sample 1 - 19

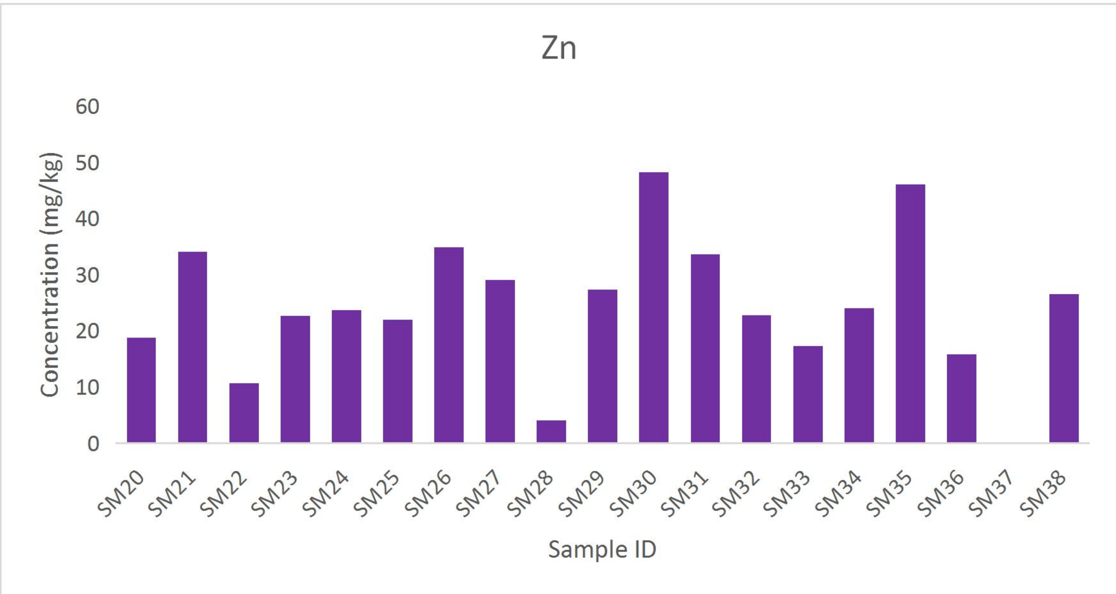


Figure 33- Graphical representation of Zinc concentration in sample 20 - 38

The above two graphs (fig32; fig33) demonstrates the level of zinc found in the green tea samples. The concentration of Zinc ranged from 0 - 74.6 mg/kg. The highest level of Zinc was observed in sample 03 while Sample 37 had 0 concentration of Zinc.

Results for Green tea Infusions

The data obtained from the analysis of Green tea infusions on 15 randomly selected samples on different parameters are represented in the table.

Sample ID	**Concentration (mg/kg)**				
	Pb	**Cd**	**Cu**	**Mn**	**Zn**
SM01	BDL of 0.5	BDL of 0.1	0.13	5.0	0.4
SM02	BDL of 0.5	BDL of 0.1	0.16	6.8	0.3
SM03	BDL of 0.5	BDL of 0.1	0.18	8.9	1.3
SM04	BDL of 0.5	BDL of 0.1	0.18	10.3	1.0
SM05	BDL of 0.5	BDL of 0.1	0.13	6.2	0.7
SM06	BDL of 0.5	BDL of 0.1	0.09	8.7	0.1
SM10	BDL of 0.5	BDL of 0.1	0.1	11.2	0.4
SM14	BDL of 0.5	BDL of 0.1	0.2	4.4	0.5
SM21	BDL of 0.5	BDL of 0.1	0.1	4.6	0.7
SM24	BDL of 0.5	BDL of 0.1	0.1	15.4	0.8
SM25	BDL of 0.5	BDL of 0.1	0.2	23.4	0.9
SM27	BDL of 0.5	BDL of 0.1	0.2	11.5	0.5
SM28	BDL of 0.5	BDL of 0.1	0.06	BDL of 0.5	0.009
SM31	BDL of 0.5	BDL of 0.1	0.2	5.7	0.4
SM37	BDL of 0.5	BDL of 0.1	0.1	4.5	0.4

Table 7- Results obtained for Green Tea infusions

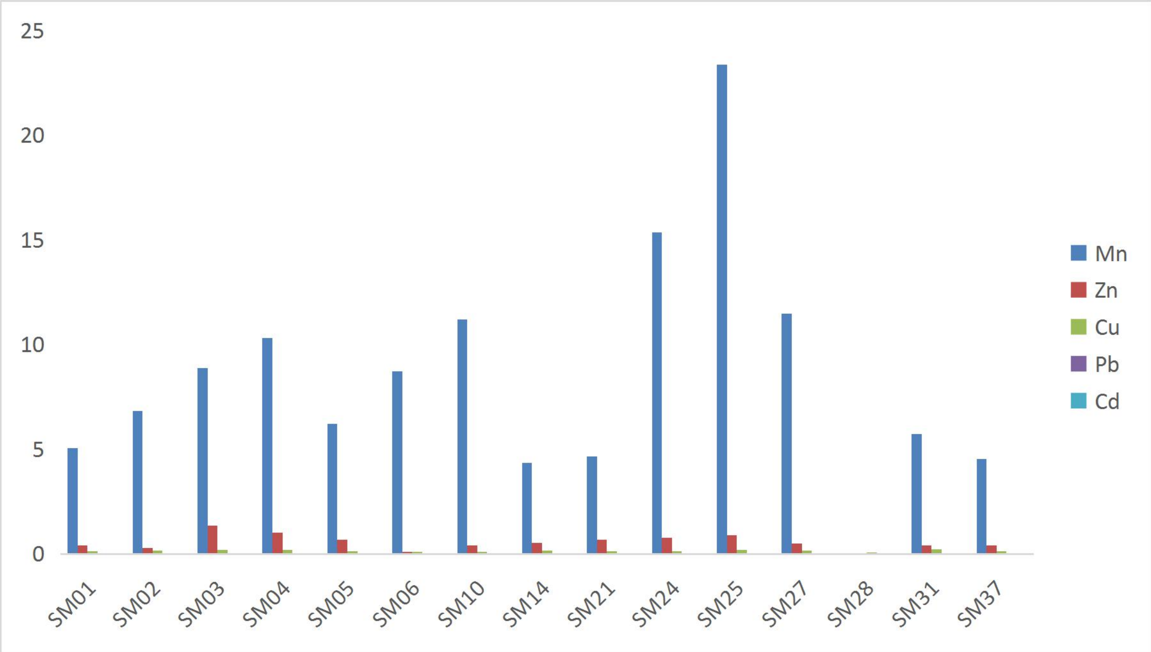


Figure 34- Graphical Representation of Heavy metal concentration in Green tea infusion

The above graph represents the combined results of 15 green tea leave infusions. In the five parameters analyzed, the manganese concentration was comparatively higher than the other metal content. Out of the 15 samples analyzed, Sample SM25 (23.4 mg/kg) showed the elevated level of Manganese concentration.

The level of Copper was highest in the sample SM31 (0.2 mg/kg). Zinc level was highest in sample SM03 (1.3 mg/kg). The Cadmium level was recorded as Below Detectable Limit (BDL of 0.1 mg/kg) and the Lead concentration was also Below Detectable Limit (BDL of 0.5 mg/kg).

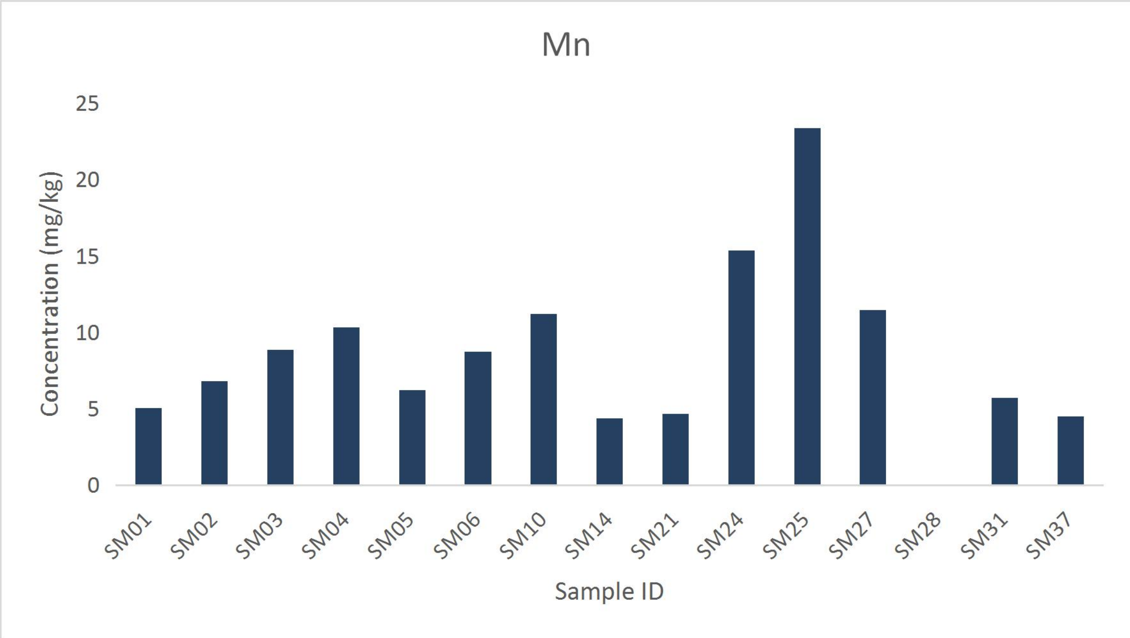


Figure 35- Graphical Representation of Manganese concentration in tea infusions

Figure 35 demonstrates the large range of concentration of manganese in the tea infusions. Most of the samples have the concentration of manganese in the range of (4.3 - 10.3) except the samples SM10, SM24 and SM25 which had a value of 11.2 mg/kg, 15.4 mg/kg and 23.4 mg/kg, respectively. The level of manganese was below the limit of detection in sample SM28. The maximum tolerated ingestion limit for manganese varies from 2 - 11 mg/day depending on age.

Manganese is essential to the formation of bones and the metabolism of amino acids, cholesterol and carbohydrates. But a higher oral intake of manganese than the recommended dose can result in impaired motor skills and cognitive diseases.

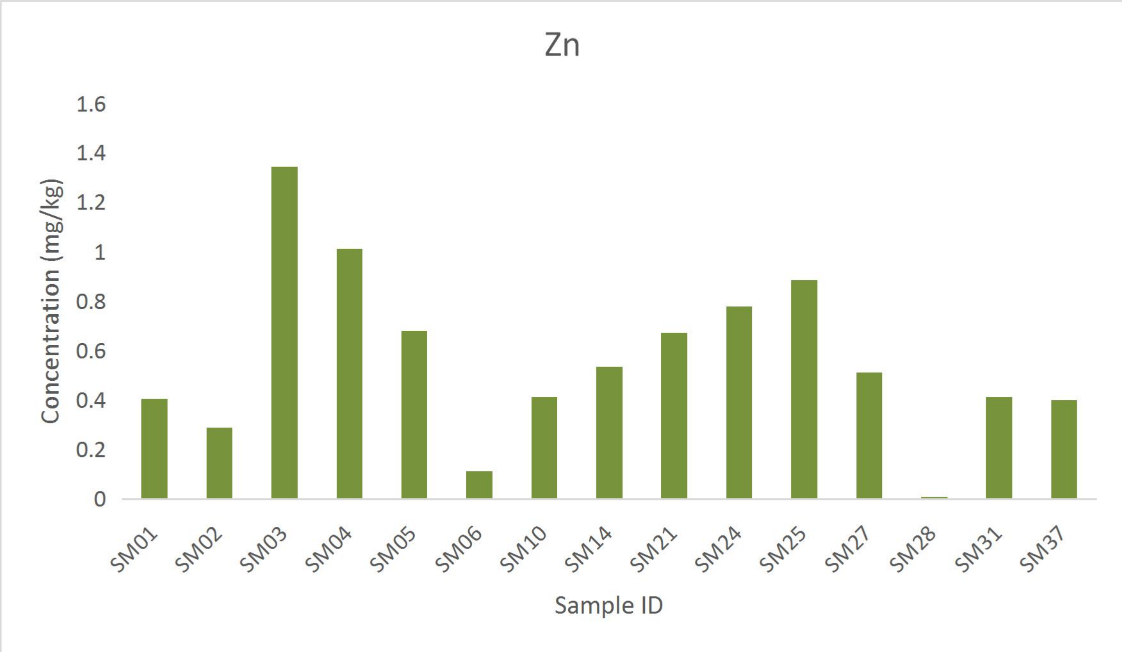


Figure 36- Graphical Representation of Zinc concentration in tea infusions

The zinc concentration in the tea infusions ranged from 0.09 - 0.8 mg/kg. Zinc concentration was maximum in sample SM 25 (0.9 mg/kg) and minimum in sample SM28 (0.009mg/kg). According to FSSAI, a maximum limit of 50 mg/kg is prescribed for zinc, under the 'Food not specified' section.

Zinc plays a key role during physiological growth and also fulfills an immune function. It also acts as an anti - oxidant and plays a role in cancer prevention. Zinc toxicity in human is minimal even though it interferes with copper metabolism.

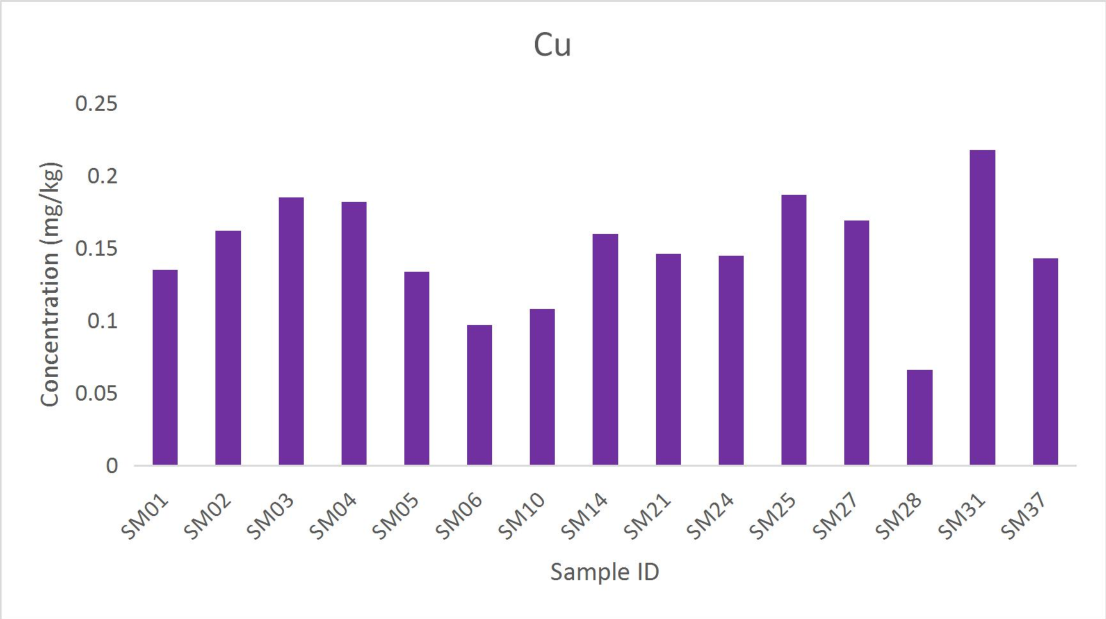


Figure 37- Graphical representation of copper concentration in tea infusions

In all the samples analyzed, there were no elevated concentrations of Copper. The concentration of copper ranged from 0.06 - 0.2 mg/kg. These were all below the maximum limit of 150 mg/kg recommended by FSSAI under "Tea" section.

Copper is an essential trace element in human body since it is necessary for proper growth, development and maintenance of bone, connective tissue, brain, heart and many other body organs. It can also help to neutralize free radicles and prevent cell damage.

An approximate daily intake of more than 5 mg/kg can be toxic to human. The WHO recommends a minimal acceptable intake of approximately 1.3 mg per day. According to EFSA the recommended tolerable upper intake limit is 5 mg/kg.

6.DISCUSSION

The recent study on determination of heavy metals in green tea indicated an elevated level of manganese in all the green tea samples and there infusions. In green tea leaves the concentration ranged from 12.7 - 961.3 mg/kg. Highest concentration of manganese was present in sample SM06. In case of green tea infusions, the concentration of Mn ranged from 4.3 - 23.4 mg/kg. Manganese present in the tea infusions exceeded the permissible daily intake of 2 - 11mg per day. This high level of Mn in tea confirms the suggestion made by **Dambiec et all** (2013) that tea is the accumulator of Mn. The result of our study was similar to that done by **Justyna Brzezicha - Cirocka and others**(2016) where the Mn level ranged from 39 - 126 mg/kg. High level of manganese taken orally can result in impaired motor skills and cognitive diseases.

The concentration of Pb and Cd were below the limit of detection in both tea leaves and their infusions which is similar to the report given by **Chen, Yu, Xu, Chen and Shi**(2009). As green tea are grown in acidic soils where Pb and Cd are easily available for root uptake, it is necessary to monitor the levels of Pb and Cd in tea. On the basis of existing literature, it has been observed that Cd in tea infusion are generally low. **Shen and Chen**(2008) reported that only 52.8% of the total cadmium was released from eighteen samples of Oolong tea, 40.3% from fifteen samples of black tea, while no Cd was extracted from green tea samples from Taiwan.

Although the concentration of Copper and Zinc were high in tea leaves, the amount decreased in the infusions. The concentration of Copper varied from 4.2 - 47.1 mg/kg in tea leaves while the concentration was observed to be between 0.06 - 0.2 mg/kg in tea infusions. According to **Gallaher, Gallaher, Marshall and Marshall** (2006) Cu content of tea infusion varies from 0.05 ± 0.003 mg/l. In the case of Zinc, the concentration in tea leaves ranged from 0 - 74.6 mg/kg and in the infusions it varied from 0.009 - 1.3 mg/kg. The amount of Zn and Cu present in tea leaves and infusions were below the maximum limit set by the FSSAI.

7. Conclusion

Nowadays there has been an increase of interest among the consumers regarding the food safety and quality of the food they are consuming. Recently there has been an increase in the green tea due to its many beneficial characteristics. Many studies done on green tea link green tea and anti - oxidants present in them to the prevention of various diseases. It has been suggested that green tea plays an important role prevention of various types of cancer, prevention of neuro - degenerative diseases like Alzheimer’s and Parkinson’s. Green tea also plays some role in total body weight loss. But recent studies on green tea and their infusions have indicated the presence of various heavy metals in tea. Through this study we analysed the presence of certain heavy metals in green tea of various brands that are available in markets of Mysuru, Karnataka and Bhubaneswar, Odisha with the help of AAS. As green tea is taken as a beverage, it is also important to determine the percentage of leaching into the infusions.

7.1 Summary

The presences of Pb, Cd, Mn, Zn and Cu in 38 green tea samples purchased from markets in Mysuru, Karnataka and Bhubaneswar, Odisha were evaluated by Atomic Absorption Spectrometry (AAS).This study was performed both for tea leaves and their infusions. The results provide data that permits an evaluation of exposure or dietary intake of these trace elements. The samples were prepared by adding 4 ml HNO_3 and digesting it in microwave digester. Infusions were prepared by soaking the leaf or tea bags in 100 ml of boiled water and the solution was filtered using Whattman no.42 filter paper prior to the analysis by AAS.

7.2 Evaluation

The recent study indicated that almost all samples of green tea contained heavy metal. Manganese was the most abundant metal found in almost all tea leave samples and their infusions. In worldwide, there is no specific maximum residue limit (MRL) level for these heavy metals. So it can be laid under the FSSAI “Tea” category. In the infusion study, it was observed that the manganese content was still very high in the tea infusions. The concentration varied from 4.3 - 23.3 mg/kg. The highest amount of manganese was found in sample SM25.The maximum ingestion limit set by WHO for Mn is 2 - 11 mg/kg depending on age. Hence regular intake of green tea can cause bio -

accumulation of Mn in the body. An intake of more than 11 mg per day is considered unsafe for adults. Exposure to high level of Manganese over a long period of time can develop diminished memory, attention deficit, aggression, hyperactivity. Prolonged exposure can also cause Parkinson's disease, lung embolism and bronchitis.

Other than Manganese, trace elements like Zn and Cu were also detected in the tea leaves and their infusions. Although the concentrations of both elements were high in tea leaves, but decreased in the infusions. The infusion values came under the maximum limit set by FSSAI. Pb and Cd were below the detection level. Even though the level of Cu and Zn were below the limit prescribed by FSSAI, more consumption can cause bio - accumulation of these heavy metals. Bio - accumulation of zinc and copper can cause various physiological disorders like hypertension, sporadic fever, nausea, renal damage, cramps, etc.

7.3 Future Work

From this investigation, further study on the Human Health Risk Assessment of heavy metals like Manganese, Zinc and Copper intake can be done. The study done on green tea showed us the presence of Manganese in alarming level which can be harmful. Other than Manganese, Zinc and Copper were also found in the samples. This study has helped us to determine the nutritional value of the green tea available in the market.

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