Quantitative Analysis of Mercury in Some Food Colors

By

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A project submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The project submitted is my own original work while completing degree at Brac University.
2. The project does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The project does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled “Quantitative Analysis of Mercury in Some Food Colors” submitted by Kifayat Faizah (13346042) of Summer, 2013 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 27 February, 2020.

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Ethics Statement

This study does not involve any kind of human or animal trial.

Abstract

Mercury is one of the very well-known heavy metals. Mercury toxicity occurs due to the long-term exposure of mercury compounds. The exposure may lead to accumulation of precipitated mercury in the body, which may result in fatal condition over the time. The research finding shows that mercury contamination in food colors can occur due to the environmental pollution. Last but not the least mercury also can be added purposely because of their antimicrobial properties. Therefore, the aim of this study is to find out the presence of mercury both in the local and branded food colors used in Dhaka city, Bangladesh. According to EU specification the maximum permissible limit of mercury is 1 µg/g. Seven food color samples were collected from the retail market of Dhaka. S01 (green), S02 (yellow), S03 (chocolate), S04 (red) were local solid powders and SS01 (green), SS02 (yellow), SS03 (red) were branded liquids. The *in-vitro* test, called Direct Cold vapor atomic absorption spectrometric method is used to quantify the presence of mercury. The final result shows that all these seven color samples contain mercury under the permissible limit. Consequently, it shows that the consumption of these colors might harmful in term of mercury toxicity due to the long-term use of them.

Keywords: Mercury; toxicity; exposure; permissible; food colors; fatal

Dedication

Dedicated to my beloved family and respected teachers who supported and guided me through thick and thin to achieve my goals

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Table of Contents

List of Tables

List of Figures

List of Acronyms

Hg	Mercury
Co	Cobalt
Fe	Iron
Mn	Manganese
Mo	Molybdenum
Zn	Zinc
Cd	Cadmium
Pb	Lead
THMs	Toxic Heavy Metals
NFSL	National Food Safety Laboratory
WHO	World Health Organization
EU	European Union
BSTI	Bangladesh Standards and Testing Institution
APHA	American Public Health Association
BCSIR	Bangladesh Council of Scientific and Industrial Research
INARS	Institute of National Analytical Research and Service
FDA	Food and Drug Administration
FIHRI	Food Ingredient and Health Research Institute

Chapter 1

Introduction

The contamination caused by heavy metals is a concerning global issue now a days, which is producing deleterious health hazard both in human and animal species through the polluted environment. The exalted quantity of heavy metal contamination produces hazardous effects when they have been taken up through plants and eventually in foods and that is too ingested by human (Onakpa, Njan, & Kalu, 2018).

Hundreds of sources for dangerous heavy metals include soil, water, air and the surrounding atmosphere which are natural ones. And also by the implication of the pesticides, herbicides and fertilizer in agricultural purposes are the indication of the anthropogenic activities (Onakpa et al., 2018).

The environment is the most important pathway for Hg emission. Volcanic eruption, cinnabar, fossil fuels as likely petroleum, coal etc. and forest fires are the sources from where mercury can be obtained naturally. Also ocean and land play a key role to reallocate Hg in marine ecosystem and fresh water (Driscoll, Mason, Chan, Jacob, & Pirrone, 2013).

The human health also become susceptible to mercury, mainly methyl mercury when they ingest marine fish (Driscoll et al., 2013).

On the other hand, Cobalt (Co), manganese (Mn), iron (Fe), molybdenum (mo) and zinc (Zn) are few of those heavy metals which are vital for the metabolic activity at lower concentration for the body and also contemplated as trace minerals for metabolic processes. Besides whenever the concentration limit of these metals cross a incontrovertible threshold ,the negative impacts are more likely to be occurred (Rahman & Singh, 2019).

At higher concentration generally toxic effects are exerted by all the heavy metals. Some known non-threshold heavy metals are promoted as the most toxic and dangerous ones, which include lead (pb), cadmium (Cd), chromium (Cr), mercury (Hg), and Arsenic (As). These heavy metals are marked among the priority of the metals due to their high toxicity characteristics and also these are frequently known as toxic heavy metals (THMs) (Rahman & Singh, 2019).

There are some aspects where adversely are produced carcinogenic and toxic effects by these heavy metals which are often includes several mechanical attributes that are not properly elucidated. Furthermore every heavy metals tends to have distinctive physiochemical properties which ensure particular mechanism of action that promote toxicity (Tchounwou, Yedjou, Patlolla, & Sutton, 2012).

Mainly the heavy metals induce toxicity via air, water and food when they enter into our body. Then they start to form complex cellular compounds and mostly they contain nitrogen, oxygen and sulfur. These types of cellular complexes disable the systemic enzymes and also promote protein structures which are complex. Cellular death occurs due to the dysfunction. Heavy metals significantly target the central and peripheral nervous system, cardiovascular system, gastro-intestinal system, renal and hematopoietic systems in our bodies (Ibrahim, Froberg, Wolf, & Rusyniak, 2006).

Different heavy metals produce different types of health hazards. Such as; Lead promotes complications in nervous system and RBC; Cadmium promotes renal tubular dysfunction, breast cancer, osteoporosis etc.; Mercury causes cardiovascular disease, neurotoxicity, immunotoxicity, carcinogenicity etc. (Thompson, 2019).

Usually mercury is a very common environmental and anthropogenic pollutant and toxicant that provokes serious types of tissue alteration in our body and deploy harmful diseases. Both the human and animals are widely susceptible to the several types of mercury in our surroundings. These include elemental mercury vapor, inorganic mercury, and the organic mercury compounds. The

omnipotent presence of mercury in the surroundings causes the plants, humans and animals to become extremely vulnerable to the different types of mercury (Tchounwou et al., 2012)**.**

Mercury toxicity causes mainly neurological damage , cardiovascular disease, autism in children last but not the least it produces reproductive effect (Eisler, 2006)

Heavy metals are found in different products in Bangladesh, such as; foods, cosmetics and in other important consuming and usable products. Cr, Ni, Cu, Zn, As, Cd, and Pb (mg/kg), which are determined in the composite samples in several concentration in most commonly consumed cereals (rice, wheat, and maize) and pulses (lentil and black gram) by the Bangladeshi population. The National Food Safety Laboratory (NFSL) has found high level of lead in dairy products recently in Bangladesh (Islam, Ahmed, & Habibullah-Al-Mamun, 2014).

These types of findings indicate the higher carcinogenic risk among the Bangladeshi people. Also indicate nutritional deficiency of essential elements, which should be present in foods (Islam et al., 2014).

Some of very popular techniques are being used for the quantification of these heavy metals. Such as atomic absorption spectroscopy (AAS), coupled plasma / atomic emission spectroscopy, mass spectroscopy etc. (Koller, Saleh, Saleh, & Koller, 2018)**.**

1.2 Aim of the study

Therefore, the aim of this study is to evaluate the concentration of mercury used in some food colors in Dhaka city, Bangladesh.

1.3 Objectives of the study

The objectives of the study are:

1. to find out whether persistent exposure of mercury in food color can produce any toxicity in human body.
2. to determine the presence of mercury in food additives.

Chapter 2

Background of the study

2.1 Heavy Metals

Heavy metals are the natural components of earth's crust and also the human's oldest toxins. Heavy metals include natural sources (eg; metal ores, ground water), commercial products, contaminated foods, herbal products, folk remedies and industrial processes as their potential exposures. Nearly all available heavy metals are toxic in adequate amounts (Ibrahim et al., 2006).

Heavy metals have multiple implications in various purposes, such as: agricultural, domestic, technological and medical etc. These wide ranges application of heavy metals, result in huge distribution of them in the environment, which elevate the concerns over the potential consequences both on the environment and human health. The harmful toxicity depends on various factors. Which include the doses, route of exposures and the chemical species, ages, genders, genetics and nutritional statuses of the exposed folk (Tchounwou et al., 2012).

Heavy metals have received a paramount attention to environmental chemists due to their toxic nature. Heavy metals are usually present in trace amounts in natural waters but many of them are toxic even at very low concentrations. Metals such as arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, zinc and selenium are highly toxic even in minor quantity. Raising amount of toxic heavy metals in our environment is recently a sector of major concern and threat too. Mainly a huge amount of industries are discharging the metal containing effluvium into the environmental and water system without treating them adequately (Tchounwou et al., 2012).

The chemists, environmentalists and health professionals have given their prime attention for the heavy metals regarding of having extreme toxic nature. Generally heavy metals are found in trace quantities in natural waters. But sometimes they can be noxious even at very low concentration. Heavy metals tend to be severely toxic even in minor quantities, such as; lead, cadmium, chromium, nickel, mercury, cobalt, zinc, arsenic and selenium (Muedi, 2018).

The habitual weathering processes promote the release of heavy metals from their regional spheres to various environmental compartments. These metals also can be found in different forms of oxides, hydroxides, sulfates, sulfides, silicates, phosphates and other organic compounds. The mostly found and used heavy metals are lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), mercury (Hg), zinc (Zn) and copper (Cu) (Muedi, 2018).

Some heavy metals have magnificent technological performances out there in the in daily life. Those metals are zinc, iron, tin, lead, tungsten, copper etc. In recent times the artificially constructed “bioinorganic” catalysts for specific chemical transformations and that too include various heavy metals which act as fundamental atom. Furthermore gold, silver, iridium, rhodium, or platinum are few of prestigious noble elements to be found. On different note mercury, cadmium, arsenic, chromium, thallium, lead, and other heavy metals classically portray the “dark side of chemistry”, because at low concentration they exert toxicity (Koller et al., 2018).

2.2 Sources of Heavy Metals

Heavy metals are derived from both natural and anthropogenic processes and later on ended up in different environmental compartments (soil, water, air and their interface) (Muedi, 2018). Natural sources, which include radiations from volcanic eruptions, forest fires, sea salt sprays, biogenic sources, wind borne soil particles and rock weathering (Muedi, 2018).

Industrial, agricultural, waste water, metallurgical and mining processes. And also runoffs of exerted heavy metals in the environment are the anthropogenic processes. Automobile exhaustion which releases lead, insecticides which releases arsenic, smelting which releases arsenic, copper, zinc and fuels of burnt fossils which release nickel, vanadium, mercury, selenium and tin, these type of contamination in the environment are significant anthropogenic source of heavy metals (Muedi, 2018).

And also, last but not the least some researchers have been found that the huge amount of daily manufactured goods producing environmental pollution (Muedi, 2018).

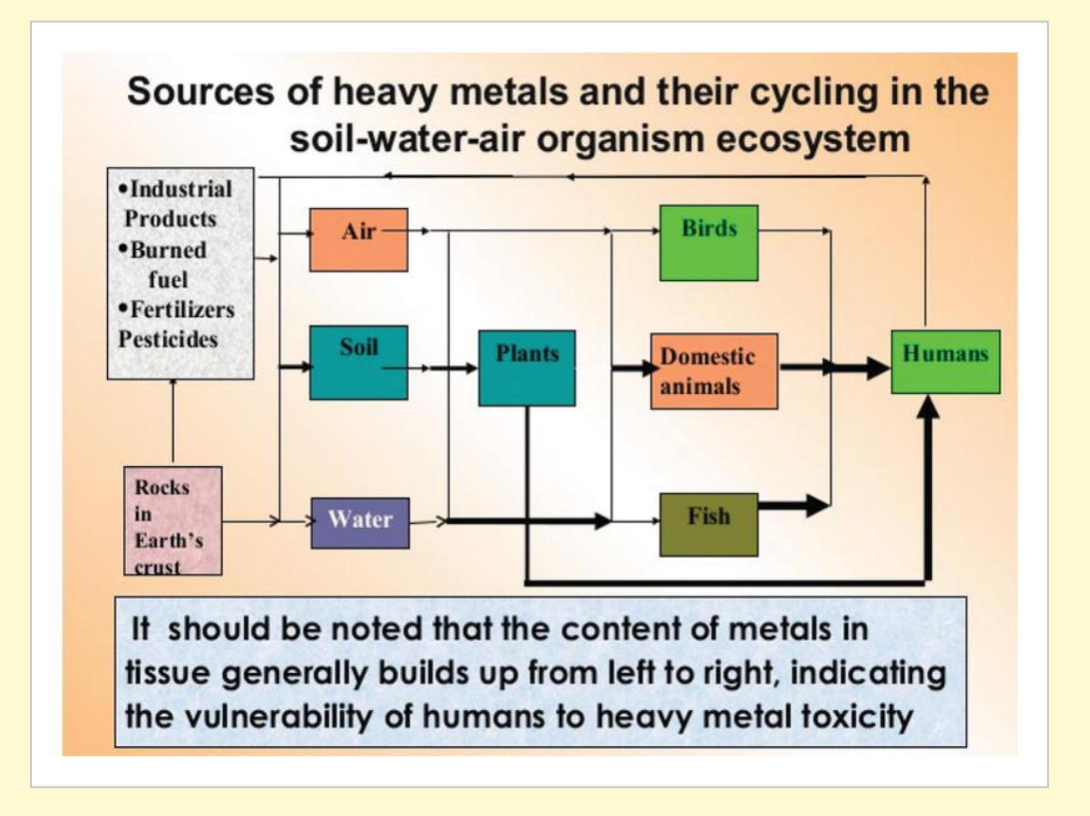

Figure 1: Heavy metal sources and cycle (Muedi, 2018)

Therefore, the heavy metals tend to have relatively higher density and toxicity even when they have been present in ppb levels. They are particularly in nature of being non-biodegradable. And tend to accumulate in the soft tissue of living organism. Also as matter of fact they have been identified as potential carcinogens (Tchounwou et al., 2012).

These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Also as matter of fact they have been identified as potential carcinogens .They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer (Tchounwou et al., 2012).

2.3 Mercury

Mercury (Hg) is liquid at room temperature and pressure. The atomic number of mercury is 80. Mercury freezes at -38.9°C and it starts to boil at 357°C. Mercury is also known as quick silver, which is too alloyed with other metals easily, such as gold, silver and tin (Rafati-Rahimzadeh, Rafati-Rahimzadeh, Kazemi, & Moghadamnia, 2014).

Mercury is a poisonous and non-essential metal for the human body. It is pervasively found in the environment, determined in natural products and also distributed substantially in our daily life. Mercury is considered as one of the major environmental pollutants. It has also great agricultural, industrial, medicinal use. Besides it also regulates in our ecosystem but never gets destroyed (Park & Zheng, 2012)

2.3.1 Sources of Mercury

Mercury can be obtained naturally from various sources, such as, fossil fuels as likely petroleum, coal etc., cinnabar, forest fires and volcanic eruption. Also airborne mercury found on the ground as raindrops, dust etc. (Rafati-Rahimzadeh et al., 2014).

Mercury also undergoes anthropogenic release to the environment. Discharge from hydroelectric, pulp, mining, paper industries tend to increasing level of mercury in the environment. The emission from coal-using power plants and also the burning result of municipal and medical waste promote into high levels of mercury (Rafati-Rahimzadeh et al., 2014)

Mercury also can be released to the environment anthropogenically. Levels of mercury in the environment are increasing due to discharge from hydroelectric, mining, pulp, and paper industries. Incineration of municipal and medical waste and emissions from coal-using power plants also contribute to high levels of mercury. Mercury released from ongoing human activity in the U.S. can be separated into four broad categories. The first category is “area sources”. Landfills, dental preparations, and laboratory use are defined as area sources. The second category is combustion processes. These include coal-fired power generation, medical waste incinerators, and municipal waste combustors. The third category is the manufacture of metals, alkali, and cement. Other industrial processes fall into the fourth category (Rafati-Rahimzadeh et al., 2014)

2.3.2 Types of Mercury

Mercury exists in the environment in three forms:

1) Elemental mercury (poisonous as vapor)

2) Organic mercury (methyl mercury and ethyl mercury) and

3) Inorganic mercury (mercuric mercury)

Mercury could be found in different commercial forms. Mercury and its related compounds are being circulated and concentrated in soil and distributed into the air via coal fuels, industrial furnaces or active volcanoes. It then returns to the soil, water, or living organisms. Re-cycling from atmospheric emission, deposition in water reservoirs and exposure and bioaccumulation in animals and humans is a known example of mercury cycle in the environment (Rafati-Rahimzadeh et al., 2014)

In recent years, due to abundant availability of various chemicals, the rate of intoxication of mercury has been surprisingly increased. People can overuse or misuse drugs, chemicals, and

may get poisoned intentionally or accidentally. Similarly, heavy metals, either released from natural sources or from industries wastes pose a consistent health threat to human being (Rafati-Rahimzadeh et al., 2014).

2.4 Mercury Exposure to Human

Humans are generally exposed to mercury chronically and also in a low dose series. Exposure to high doses of mercury can occur for extremely short periods in industrial accidents (Park & Zheng, 2012).

Mercury chemically exists in several forms, such as elemental or metallic Hg, inorganic mercury compounds and organic mercury compounds (Park & Zheng, 2012).

Elemental mercury is liquid in room temperature and also can be easily released into the environment as vapor due to its high vapor pressure. Inorganic mercuric compounds are found in two oxidative states, which are mercurous Hg+ and mercuric Hg++. Generally these are mercurous or mercuric salts in solid states and also mercury compounds with chlorine, sulfur or oxygen. Both methyl and ethyl mercury are common organic forms of mercury and carbon. By methylation of inorganic mercury methyl mercury is formed with the help of the microorganism in the environment (Park & Zheng, 2012)..

The exposure of methyl mercury (Me-Hg) from seafood, mercury vapor (Hg) from dental amalgam and inorganic mercury (I-Hg) from food are the main forms in the population. The way of exposure, absorption, delivery and target organ toxicity is mostly determined by the different types of mercury (Park & Zheng, 2012).

The human exposure to mercury can be occurred by the mercury-based thermometers and sphygmomanometers. Also the usage of batteries and fluorescent lights can cause mercury exposure (Park & Zheng, 2012).

The intoxication of metallic mercury had been in knowledge by the time of Aristotle. And also in the 8th century in Japan wide range of mercury poisoning took place by the time of the great statue of Buddha of Narawas construction (Rafati-Rahimzadeh et al., 2014).

In the 20th century two major catastrophes throughout the mercury poisoning were recorded. The 1st one is was Mianamata illness, where death of 2200 people happened due to the consumption of mercury contaminated fish and shellfish. The another one happened in Niigata, where nearly the victims were 700 in the year of 1950s and 1960s (Clifton, 2007)(Watanabe & Satoh, 1996).

The third outbreak of mercury poisoning in Iraq happened between 1955-1957 and 1959-1960. And the rural population in 1971-1972 has suffered from the huge outbreak due to the contaminated homemade products. The official announcements were 459 deaths and 6530 were hospitalized (Watanabe&Satoh,1996).

Few countries such as Brazil's amazon, the Faroen islands, Peru, New Zealand, Slovenia, Seychelles, pitwork at goldmines in Tanzania, The Philippines and in Indonesia are dangerously affected by the dumping of inorganic mercuric compound (Rafati-Rahimzadeh et al., 2014).

Chronic use of sea foods such as fishes can lead to mercury poisoning by their long term concentration in human organs. Similar cases have been seen in coastal provinces of Iran. There mercury has been detected both in tap and agricultural water. Generally the global standard of mercury is little in the area of Caspian Sea. But the huge and continuous consumption can scale up the health risk (De Mora, Sheikholeslami, Wyse, Azemard, & Cassi, 2004).

2.5 Mechanism of Toxicity

The impact of mercury compounds on human health has been very dangerous. They demonstrate troublesome dysfunction to human organs, such as; the intracellular equilibrium

alteration of calcium and its membrane capacity, breakdown the formation of microtubules, prohibition of enzymes, alteration of the cell membrane, triggering of oxidative stress, inconvenience of the immune functions (Doull, 2017).

Biologically, mercury is connected to phosphoryl, carboxylic and amide groups. In the presence of methyl mercury due to free radicals and oxidative stress the neurotoxicity is triggered. And also due to the methyl mercury induction, the accumulation of aspartate, glutamate and serotonin promote neurotoxicity. In CNS methyl mercury connects sulphuryl containing molecules into organic form. The bond thiol comprising protein such as glutamine, albumin, cysteine, inorganic and methyl mercury induces mercury distribution in the body. By adding to the thiol groups, mercury distribution is promoted in the body. This protective mechanism restrict compounds to from binding to other proteins (Rafati-Rahimzadeh et al., 2014).

2.5.1 Clinical manifestations

Various kind of mercury have different clinical manifestations and adverse effect (Rafati-Rahimzadeh et al., 2014). Some are described below;

2.6 Elemental mercury

Inhalation of elemental vapor can cause cough, fever, chilling and respiration disturbance. It also triggers the GIT condition and produce nausea, vomiting. As well affect the metallic taste followed by dysphagia, salivation, weakness, headaches and vision problems. When mercury is imbibed, it is rarely found in ordinary GIT mucosa. A serious irritation occurs when an abnormal GIT mucosa absorbs sufficient elemental mercury due to exposure. Then it began to produce corrosive wound rapidly. Moreover, mouth inflammation, loose teeth, ulcerative gingivitis, metallic taste, gingival bleeding etc. different issues with oral cavity can be seen. Other than that also grayish mucous membrane, discoloration, oropharygeal pain, bloody

diarrhea might be observed. Last but not the least, the mercury released from dental amalgam can also result in stomatitis (Dantzig, 2003; Rafati-Rahimzadeh et al., 2014).

Elemental mercury easily passes through the alveolar membrane and absorbs easily through the blood. Then distributed and transferred to the tissue during respiration (Glezos, Albrecht, & Gair, 2006).

Dyspnea, chest pain, dry cough, hypoxemia ,ventilation and modified carbon monoxide diffusive capacity are the implication of intoxication (Rafati-Rahimzadeh et al., 2014).

Bronchitis, bronchiolitis and pneumonitis are caused by mercury vapor in higher concentrations. In addition to this pulmonary edema, respiratory failure and death occurs. Serious pulmonary difficulties progressed in the survivors. Those are interstitial fibrosis, pulmonary restrictive residual diseases. By using metallic mercury solutions in subcutaneous injections produce local abscesses and the granuloma. And also acute pulmonary embolism and systemic respiratory failure occur from IV injections (Rafati-Rahimzadeh et al., 2014).

2.7 Inorganic Mercury

Inorganic mercury salts can cause gastroenteritis. Cause also metallic taste, nausea, local oropharyngeal, vomiting, abdominal colic, bloody diarrhea and renal failure etc. Sometimes post existing stomatitis and hematemesis may cause inorganic chronic mercury salt poisoning, which may later develop liptremor, severe salivation, anorexia, loss of teeth, and weight loss. Also oral and throat damages such as gingivitis, gingival bleeding, corrosive wounds occur. Thoracic pain, dyspnea, strain, dry toxins, acute chemical pneumonitis and bronchiolitis are symptoms of acute inorganic mercury inhalation. It may also lead to massive fluid loss and acute tubular necrosis. Chronic inorganic mercury intoxication also promotes GI symptoms, neurological abnormalities and renal dysfunction (Rafati-Rahimzadeh et al., 2014).

As well the inorganic mercury toxicity leads to myocardial infarction (MI), coronary heart disease (CHD), increased in carotid intimal medial thickness (IMT), carotid obstruction and hypertension (Rafati-Rahimzadeh et al., 2014) .

Nearly 85% to 90% of inorganic mercury is found in the kidney, when they are absorbed into the blood stream. Chronic inorganic absorption can lead to polyurea, proteinuria, nephritis, glomerular immunological illness etc. (Rafati-Rahimzadeh et al., 2014).

2.8 Organic mercury

A little exposure may not generate any serious symptoms. But continuous chronic exposure produce acute GIT symptoms, neurotoxicity, neuron destruction (Clarkson, Magos, & Myers, 2003). Autism syndrome is common due to the ethyl mercury exposure (Bernard, Enayati, Redwood, Roger, & Binstock, 2001).

Seafood consumption may lead to organic mercury poisoning. However, organic mercury is classified into three forms, such as; aryl, brief alkyl chains and long alkyl chains. The aryl and alkyl long chains are comparable toxic characters but mildly corrosive whereas organic mercury is a little less corrosive than inorganic mercury. It’s also reported that in contaminated areas both the fresh and salt water fish contain methyl mercury (Rafati-Rahimzadeh et al., 2014).

2.9 Toxic effects on CNS

Mercury's toxic impacts on the human central nervous system are well-knotted and experimental animals have demonstrated that mercury crosses the placenta and reaches the fetal brain and accumulates in CNS (Rafati-Rahimzadeh et al., 2014).

2.10 Toxic effects on skin

Skin is differently affected by the toxicity of the mercury, such as; light swelling, scaling, irritation, erythema and urticaria are the most common symptoms of mercury exposure. The both topical and systemic exposure to dermatitis might be painful, which is most common type of mercurial skin response (Dantzig, 2003). Due to injection hyperpigmentation and purpura may be seen in early stages (Rafati-Rahimzadeh et al., 2014).

2.11 Biomarkers to evaluate mercury

Mercury may undergo biotransformation in typical conditions, both chemically and physically. The discontinuation of mercury concentration from complete mercury in the clinical labs, without taking any physical or chemical forms onto consideration (Rafati-Rahimzadeh et al., 2014).

2.11.1 Urine

Urine is an important sample for testing inorganic and elemental mercury. Methyl mercury is excreted on feces. Just because the urine sample test does not imply the organic mercury levels in the body (Rafati-Rahimzadeh et al., 2014).

2.11.2 Blood

In red blood cell methyl mercury is found in acute intoxication but there is a difference in chronic toxicity. The total level of blood mercury is below 10μg / L, but that can rises upto 20μg / L. So after long exposure to mercury vapor blood mercury level might be risen up to 35μg / L (Rafati-Rahimzadeh et al., 2014).

2.11.3 Hair and nail

Hair contains the elevated group of sulfur dioxide, whereas mercury has the tendency to bond with the sulfur. The total mercury concentration is assessed in skin and blood by the level of

intoxication of the methyl mercury exposure. In hair, mercury levels simply do not goes beyond 10mg/kg. During moderate intoxication mercury levels in hair are 200-800mg/kg. But due to serious toxicity they rise to 2400mg/kg. NRC has recommended cord blood mercury concentration to be the best accessible biomarker for fatal methyl mercury exposure. For methyl mercury intoxication identification in human, the nail can be used as a biomarker (Rafati-Rahimzadeh et al., 2014).

Chapter 3

Methodology

To complete this research few structured literature reviews and a Cold vapor atomic absorption spectrometric method have been followed.

3.1 Sources of information

A huge range of data mining was gathered from numerous texts. Such as research articles, review papers and some specific websites related to the topic.

The following data bases were searched.

• PubMed

• Science direct

• Medline

• Scopus

• Science.Gov

• Google scholar etc.

3.2 Place from where samples are collected

Total of 7 food coloring samples were collected from two different places. 4 of the samples were in solid powder form. They were collected from Chawk Bazar in Old Dhaka on 27 July, 2019. The colors were Green, Yellow, Chocolate and Red. These powder colors were non-branded products purchased from a normal grocery shop.

There were 3 more samples too, which were in liquid form. They were collected from D.N.C.C Market in Gulshan-1 on 26 August, 2019. Those colors were Green, Yellow and Red. Moreover, these liquid colors were branded products which were Foster Clark food colors.

3.3 Labeling of samples

After collecting these seven samples from two different places, the samples were labeled so that the results after being tested would be easier to evaluate.

They were labeled according to the following table.

Table 1: Labeling of Food Color Samples

Samples	**Colors**	**Labeled as**	**Sample forms**
01	Green	Food Color Sample (S-01)	Solid
02	Yellow	Food Color Sample (S-02)	Solid
03	Chocolate	Food Color Sample (S-03)	Solid
04	Red	Food Color Sample (S-04)	Solid
05	Green	Food Color Sample (SS-01)	Liquid
06	Yellow	Food Color Sample (SS-02)	Liquid
07	Red	Food Color Sample (SS-03)	Liquid

3.4 Analysis of samples

After labeling, the samples were brought to Bangladesh Council of Scientific and Industrial Research (BCSIR) for further analysis which was on 27th August 2019.

The samples were received by the authorized officers. All the paper works and formalities were done properly. After all these, the samples were shifted to the Institute of National Analytical Research & Services (INARS) laboratory.

The scientists of Institute of Analytical Research & Services (INARS) laboratory, BCSIR, have analyzed the samples.

The exclusive method was applied to get the accurate result. At the end, APHA 3112.B which is a standard Atomic Absorption Spectroscopic method was followed to complete the analysis.

3.5 Name of the method

Cold vapor atomic absorption spectrometric method (APHA, 1999)

3.5.1 General discussion

For determination of mercury in the sample, this specific method is applied (APHA, 1999).

3.5.2 List of the name of the apparatus

1. Atomic absorption spectrometer:

It is a special instrument which consists, a hollow cathode lamp or electrodeless discharge lamp. This is a light source which emits the line of the spectrum of an element.

Also a monochromator and adjustable slit is used to produce flame to vaporize the sample to isolate the absorption line.

Lastly a photoelectric detector which confirms the electronic amplify and also measure the equipment (APHA, 1999)**.**

2. Burner:

The commonly used burner is premix. It promotes the spray into the condensing chamber to eliminate the larger droplets.

It contains a single slot or three slot blotting head. Which is required directly aspire with an air-acetylene flame.

Maybe also another head to use with nitrous oxide and acetylene (APHA, 1999).

3. Digital Meter Readout machine:

Frequently it is assigned with either a null or a digital meter readout machine. Generally new technological equipments are associated with a microprocessor. Or sometimes with a stand-alone control computers which is used to integrate absorption signals over time and also linear calibration curve at higher concentration can be made (APHA, 1999).

4. Lamps:

An electrodeless discharge lamp (EDL) or a hollow cathode lamp can be used.

Lower sensitivity can be produced by multi element hollow cathode lamp than single the ones.

EDL is a time consuming one to heat up and stabilize (APHA, 1999).

5. Pressure-reducing valves:

It supplies oxidant and fuel under pressure. Sometimes higher pressure is used to control operating pressure of the equipment by the valves.

For each gas an individual reducing valve is used (APHA, 1999).

6. Vent:

To eliminate the fumes and vapors a vent is introduced about 15 to 30 cm top of the burner.

It is used as a precautionary step. It helps the personnel to provide safety from the toxic vapors. Also provide protection to the equipment from corrosive vapors. Also protect the stability of the flame (APHA, 1999).

Some specifically designed apparatus are used in cold vapor AAS. Such as;

a) Absorption cell:

A tube is used, which can be glass or plastic and in diameter of 2.5cm approximately. The preferred diameter is 15 cm long tube.

A grind tube is perpendicular to the longitudinal axis and cement quartz windows on place.

Also contain a gas inlet and outlet ports a diameter of 6.4 mm (APHA, 1999).

b) Cell support:

A cell is strapped to the flat nitrous –oxide burner head. Then to provide maximum transmittance it is aligned in the light beam (APHA, 1999).

c) Air pumps:

A peristaltic pump is used with the capacity of providing 2L air/min with electronic speed.

Otherwise regulated air compressed air cylinder or air system also preferred (APHA, 1999).

d) Flow meter:

It measures an air flow of 2L/min (APHA, 1999).

e) Aeration tubing:

It is a straight glass frit, which has a coarse porosity to use in a reaction flask (APHA, 1999).

f) Reaction flask:

A BOD bottle or Erlenmeyer flask of 250mL is adjusted by a rubber stopper to hold the aeration tube (APHA, 1999).

g) Drying tube:

It consists 20g $Mg(ClO_4)_2$ and its diameter is 150mm X 18mm. To reduce the condensation of moisture inside the absorption cell a 60-W light bulb is used. And a ambient temperature of 10°C is to be maintained (APHA, 1999).

h) Connecting tubing:

This particular tube is used to pass the mercury vapor from the reaction flask to the absorption cell. And also interconnect all the other components. Clear vinyl plastic tube also can be used as a substitution (APHA, 1999).

Reagents

1. Metal-free water:

Metal free water is used to prepare all the reagents, calibration standards and as dilution of water.

Metal free water is prepared by de-ionizing tap water, single distillation, re-distillation or sub boiling.

To determine the under analyzed element is present in a trace amount, the de ionized water needed to be checked always (APHA, 1999).

(NOTE: If the source water contains Hg or other volatile metals, single- or redistilled water may not be suitable for trace analysis because these metals distill over with the distilled water. In such cases, use sub-boiling to prepare metal-free water) (APHA, 1999).

b. Stock mercury solution:

0.1354 g mercuric chloride, $HgCl_2$ in 70mL water and by adding 1mL conc. HNO_3 is dissolved. And diluted to 10mL with water.1.00mL = 1.00 mg Hg (APHA, 1999).

c. Standard mercury solutions:

A series of standard mercury solutions containing 0 to 5 µg/L are prepared by appropriate dilution of stock mercury solution with water containing 10mL conc. HNO_3/L. Standards are prepared daily (APHA, 1999).

d. Nitric acid:

Concentrated HNO_3 is used (APHA, 1999).

e. Potassium permanganate solution:

50 g $KMnO_4$ is dissolved in water and diluted to 1 L (APHA, 1999).

f. Potassium persulfate solution:

50 g $K_2S_2O_8$ is dissolved in water and diluted to 1 L(APHA, 1999).

g. Sodium chloride-hydroxylamine sulfate solution:

120g NaCl and 120 g $(NH_2OH)2{\cdot}H_2SO_4$ is dissolved in water and diluted to 1 L.

A 10% hydroxylamine hydrochloride solution might be substituted for the hydroxylamine sulfate (APHA, 1999).

h. Stannous ion (Sn2+) solution:

Either 1) stannous chloride or 2) stannous sulfate, is used to prepare this solution containing about 7.0 g Sn2+/100mL.

1)10 g SnCl2 is dissolved in water containing 20mL conc. HCl and diluted to 100mL.

2)11 g SnSO4 is dissolved in water containing 7mL conc. H_2SO_4 and diluted to 100mL.

Both of these solutions are decomposed with aging. If a suspension forms then continuous stirring might help.

The reagent volume is enough to process about 20 samples. And adjustment volumes are prepared to accommodate number of samples processed (APHA, 1999).

i. Sulfuric acid:

Concentrated H_2SO_4 is used (APHA, 1999).

Procedure

a. Instrument operation:

A wavelength to 253.7nm is set. Absorption cell is installed and aligned in light path to provide maximum transmission. Associated equipments are connected to the absorption cell with a glass or vinyl plastic tube.

Then air is turned on. And the flow rate is adjusted to 2L/min. The air is allowed to flow continuously.

NOTE: Fluorescent lighting might increase baseline noise (APHA, 1999).

b. Standardization:

100mL of each of the 1.0, 2.0, and 5.0 µg/L Hg standard solutions and a blank of 100mL water are transferred to 250-mL Erlenmeyer reaction flasks. 5mL conc. H_2SO_4 and 2.5mL conc. HNO_3 are added to each flask. 15mL $KMnO_4$ solution is added to each flask. Then stand at least for 15 minutes.

8mL $K_2S_2O_8$ solution is added to each flask and heated for 2h in a water bath at 95°C.Then cooled in room temperature.

Enough NaCl-hydroxylamine solution is added to reduce excess KMnO4. Then 5mL $SnCl_2$ or $SnSO_4$ solution is added and the flask is then attached to the aeration instrument. Each flask is treated individually.

As Hg is volatilized and carried into the absorption cell, absorbance will increase to a maximum within a few seconds.

The recorder as soon as possible returned to the base line. Then the stopper is removed from the flask. It is replaced with a flask-water.

The system is flushed for a few seconds and also the next standard is run in the same process. A standard curve is processed by plotting peak height versus micrograms Hg (APHA, 1999).

c. Analysis of samples:

100mL sample or diluted portion is transferred to 100mL containing but not more than 5.0 µg Hg/L to a reaction flask.

Seawaters, brines, and effluents high in chlorides require as much as an additional 25mL $KMnO_4$ solution.

During oxidation step, chlorides are converted to free chlorine, which absorbs at 253 nm.

All free chlorine is removed before the Hg is reduced and swept into the cell by using an excess (25mL) of hydroxylamine reagent.

Free chlorine is removed by sparging sample gently with air or nitrogen after adding hydroxylamine reducing solution. A separate tube and frit is used to avoid carryover of residual stannous chloride, which could cause reduction and loss of mercury (APHA, 1999).

5. Drawing inference:

The peak height of sample is determined from recorder chart and mercury value from the standard curve is read (APHA, 1999).

Chapter 4

Results

Afterwards the analysis of all seven samples by using the cold vapor atomic absorption spectrometric method, the presence of mercury is detected. There is no sample left with the existence of a little amount of mercury. All of the samples, respectively S-01, S-02, S-03 S-04, SS-01, SS-02 and SS-03 were quantified with less or permissible amount of mercury.

According to EU specification the permissible limit of mercury is 1 µg/g. And 1 µg/g = 1 mg/kg (Ogimoto, Uematsu, Suzuki, Kabashima, & Nakazato, 2009).

Therefore, in the samples the concentration of mercury we have achieved are in S-01, S-02, S-03, S-04, SS-01, SS-02, SS-03 are 0.31mg/kg, 0.14mg/kg, 0.38mg/kg, 0.73mg/kg, 0.11mg/kg, 0.18mg/kg and 0.02mg/kg. All of these concentrations of mercury are lower than the permissible concentration given by EU (Ogimoto et al., 2009).

Table 2: Concentrations of mercury in the samples

Particulars of sample	**Parameter**	**Concentrations**	**Test method (APHA)**
Food color sampleS-01	Mercury	0.31 mg/kg	3111.B
Food color sampleS-02		0.14 mg/kg	
Food color sample S-03		0.38 mg/kg	
Food color sampleS-04		0.73 mg/kg	

Food color sampleSS-01		0.11 mg/l	
Food color sampleSS-02		0.18 mg/l	
Food color sampleSS-03		0.02 mg/l	

According to EU specification the permissible limit of mercury is 1 µg/g. And 1 µg/g = 1 mg/kg (Ogimoto et al., 2009). So here in samples the found quantity of mercury are in S-01, S-02, S-03, S-04, SS-01, SS-02, SS-03 are 0.31mg/kg, 0.14mg/kg, 0.38mg/kg, 0.73mg/kg, 0.11mg/kg, 0.18mg/kg and 0.02mg/kg. All these concentrations of mercury are lower than the permissible concentration given by EU.

All the seven samples with the concentration of mercury present in them have been shown in a graphical representation below.

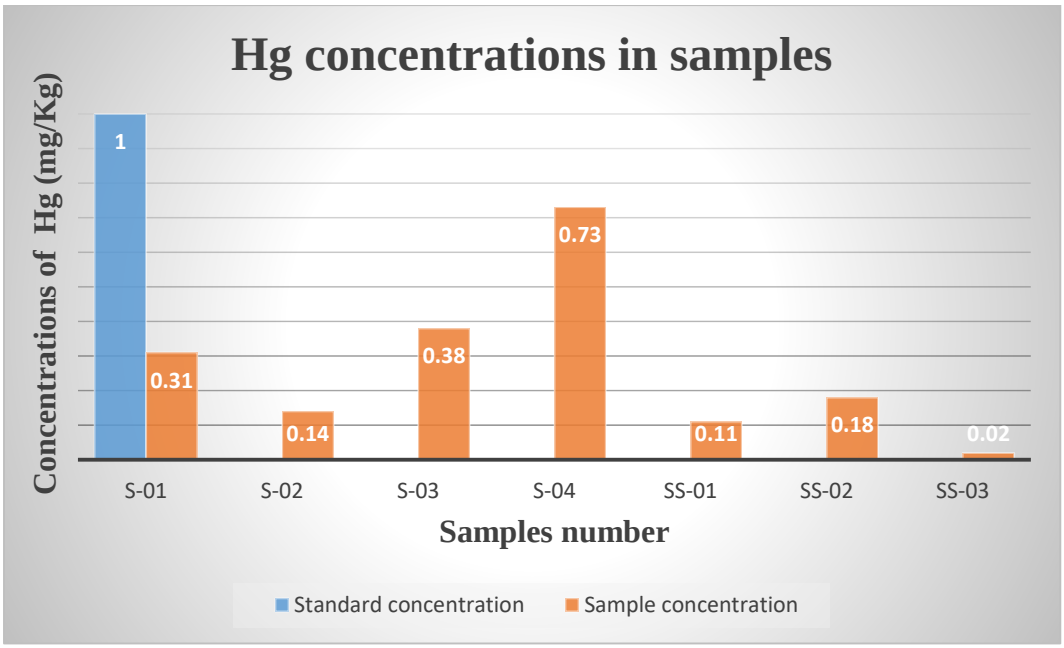

Figure 2: Sample concentrations Vs permissible limit

Chapter 5

Discussion

During the quantitative analysis of mercury in food colors by following Direct Cold vapor atomic absorption spectrometric method, the level of mercury present in all samples were lower than the permissible limit of mercury proposed by EU.

Heavy metals, such as; mercury (Hg) is not an essential element in food because of producing toxicity in low concentration. The significant causes of Hg contamination in food colors include: different types of environmental pollution, such as: urbanization, industrialization, agriculture etc. The unpredictability of Hg concentration presents in food color, shows the level of pollution present in the environment (WHO 1990) (Nordin & Selamat, 2013).

The research findings showed that mercury is used in amalgam fillings due to its antibacterial properties. However, possessing antimicrobial and antibacterial properties mercury has also been added to food colors intentionally in order to increase the shelf life of the product and also to diminish the probable spoilage (Leistevuo et al., 2000)

Furthermore, a food color safety petition is submitted by FIHRI (Food Ingredient and Health Research Institution) on 22nd March, 2011 to FDA (Food and Drug Administration) which proposes that the US CFR (Code of Federal Regulation) has been authorized mercury to be present as impurity in few food colors. Nevertheless, the limit does not stand out 1ppm (part per million). Moreover mercury occasionally has been added as an active ingredient in food colors to reduce or eliminate microbial growth (Leistevuo et al., 2000).

Therefore, according to the research findings, if permissible limit of mercury is present in food color that can be ingested safely. However, their continuous and long term intake may accumulate the mercury into our organ and demonstrate disorders and difficulties to the human species.

Primarily elemental mercury absorption occurs by inhalation of the mercury vapor. After that it transports easily across the pulmonary alveolar membranes and then enters to the circulation and then distributed to the most of the tissues. These tissues include red blood cells, brain and liver. The fast pervasion from air to blood befalls, because elemental mercury is an uncharged, lipid soluble, intermediate sized atom. The intracellular catalyzes oxidize the mercuric vapor to the divalent from Hg++, when present in RBC. Furthermore it is complexed with hemoglobin by the reaction of sulfa hydryl groups. After all, the mercury vapor remains in the elemental form for few minutes. The reaction of catalyze is saturable. However the rate of diffusion into RBC is not so fast to pull out the solubilized mercury vapor immediately and also a proportion of the vapor still dispersed into the blood, which may cross the blood brain barrier into the CNS. In CNS the Hg0 becomes Hg++, which is the toxic proximate agent. And finally the charged mercuric form retains in the brain tissue and sometime for longer years. This may lead to disorder in DNA, RNA, protein synthesis, microtubular polymerization, cell division and cell migration (Clifton, 2007).

97% of mercury vapor absorption takes place via lungs; on the other hand only 3% diffuses across the skin. Studies also found by using radioactive mercury vapor, 75% to 80% inhaled mercury retains within the body. After inhalation of mercury vapor roughly 9 hours is needed to reach a peak plasma concentration. When there is 4% of the estimated dose is present in the plasma by that time. And then round about the 7% is distributed across the blood brain barrier into the CNS. And the 80% is deposited in the kidney. The overall half-life in adult is nearly 60 days (Clifton, 2007).

Metallic mercury is mainly absorbed (80%) in human body through inhalation, then 7% to 10% absorption occurs due to ingestion and almost 1% occurs through the skin contact. Mercury vapor binds to the sulfahydral group due to greater attraction right after entering the body. Then bind to all the sulfur containing amino acids present in the body (Bernhoft, 2012).

However mercury vapor then transported to brain by dissolving either in serum or adhere to red cell membranes. Metallic mercury crosses readily the blood brain barrier and the placenta. However it is embedded in the fetal brain. It is rapidly oxidized to mercuric mercury and enters to the blood stream. Staying in metallic form it prevents considerable uptake by the central nervous system so cannot quickly oxidized. It also can be accumulated in thyroid, muscles, myocardium, breasts, skin, kidney, liver, adrenals, lungs, adrenals, sweat glands, salivary glands, Prostates, testes, enterocytes and pancreas. Moreover causes dysfunctions of mentioned organs. The deposition can also be seen in breast milk too (Bernhoft, 2012).

Widely metallic mercury is excreted as mercuric form. The rate of excretion and half-life depend on the redox state and the deposited organ. The half-life can be exceeded from few days to several months and also to several years as well. Its excretion is mainly seen through urine and stool. However they also excreted through saliva, breast milk, tears and sweat (Bernhoft, 2012).

Sometimes half-lives are seemed to be multiphasic. Considering an oral tracer dose, an effective half live can be measured of 42 days long for 80% dose and the rest of 20% do not have a measureable rate of excretion (Bernhoft, 2012).

Organic mercury such as; methyl mercury is very common in human exposure, which is appeared to humans due to the food mainly sea fish and other food substances. Otherwise ethyl mercury is almost similar to methyl mercury in the cellular level. Moreover ethyl mercury has one third of long excretory half-life than methyl mercury (Bernhoft, 2012).

It accumulates in brain, kidney, placenta, liver and fetus. Specially, it remains in the fetal brain and also in the peripheral nerves and bone marrow. Due to demethylation the methyl mercury slowly converted to inorganic mercury (Bernhoft, 2012).

Furthermore, the half-life of excretion of methyl mercury in human is about 70 days. The 90% of it is being excreted through the stool. Moreover apparently the enterohepatic circulation occurs. Then 20% of it is excreted through breast milk due to the severity of exposure (Bernhoft, 2012).

Inorganic mercury demonstrates toxicity by altering the structures of tertiary and quaternary proteins. Later on binding with sulfhydryl and selenohydral groups, it can possibly diminish the functions of any organ or sub cellular structure. The prime target of mercury vapor is the brain. Other than that peripheral nerve function, immune function, renal function, muscle function, endocrine function and different kind of dermatitis have also been seen. Respiration dysfunction, neurological disorder can occur due to chronic exposure to inorganic mercury (Bernhoft, 2012).

In mercuric mercury brain disorders are less common to be occurred than the other forms. Due to its deposition in testicles, mercuric mercury inhibits the spermatogenesis and also produces atrophy and capillary damage in the thigh muscle (Bernhoft, 2012).

Organic mercury binds with sulfhydryl group throughout the body and then intercedes into the cellular or sub cellular structure. It also interferes with protein synthesis and DNA transcription. Also many cellular dysfunctions occur in different types of organ systems. Specially in peripheral nervous system (Bernhoft, 2012).

Persistent exposure to huge amount of metallic mercury (mercury vapor) produces pneumonitis. Furthermore, it also includes disorders like micromercurialism, immune dysfunction, polyarthritis, different form of dermatitis, syndrome imitate pheochromocytoma,

myocardial infarction, carotid atherosclerosis, hypertension, stroke and ischemic heart disease (Bernhoft, 2012).

Organic mercury (methyl and ethyl mercury) demonstrates wide range of dangerous diseases. The symptoms depend on the immensity of methyl mercury retention than the rate of deposition. The severity of exposure is likely to have a latency period of one or more weeks. After that toxic doses are cleared slowly. Massive prenatal toxicity forms cerebral palsy. Postnatal poisoning establishes symptoms like paresthesia. When moderately exposed it causes visual, auditory and extra pyramidal impairment. High exposure may cause clonic seizures and sometimes the little exposure may cause ataxia (Bernhoft, 2012).

Chapter 6

Conclusion

This particular study is involved in the determination and quantification of the level of mercury (Hg) presents both in local and branded food colors found in Bangladesh. These examined different food colors samples contain mercury less than the permissible limit. Which implicates these colors is completely harmless from mercury toxicity in term of consumption. May be occasional intake of these colors might seem quite normal. But the simultaneous and prolong consumption of these colors with the presence of other sources of mercury may accumulate mercury precipitation in the body. Which may lead to many fatal conditions of human body, which includes various diseases, organ disorders, autism, and many more.

The law enforcement agencies and BSTI should work hand in hand to take necessary steps to strictly control the heavy metal contamination to the food colors.

6.1 Future research Plan

Here in this study, only *in vitro* test was performed. However, in future the *in-vivo* tests can also be carried out to demonstrate the concentration of mercury present in the body.

6.2 Limitations of the study

While carrying out the whole analytical procedure, we were only able to perform the method once. However, it should have been carried out three times to get more accurate observations and specific results.

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