

Quantitative Analysis of Lead in Food Coloring Agents

By

Nishat Nowreen
15146019

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

Department of Pharmacy
Brac University
September 2019

© 2019. Brac University
All rights reserved.

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.

Student's Full Name & Signature:

Nishat Nowreen
15146019

Approval

The project titled “Quantitative Analysis of Lead in Food Coloring Agents” submitted by Nishat Nowreen (15146019) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 10 October, 2019.

Examining Committee:

Supervisor:
(Member)

Marzia Alam
Lecturer, Department of Pharmacy
Brac University

Program Coordinator:
(Member)

Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

Dr. Eva Rahman Kabir
Professor and Chairperson, Department of Pharmacy
Brac University

Ethics Statement

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

Abstract

Lead poisoning occurs when lead builds up in the body, often over months or years. Even small amounts of lead can cause serious health problems. Children younger than 6 years are especially vulnerable to lead poisoning, which can severely affect their mental and physical development. At very high levels, lead poisoning can be fatal. The aim of this study is to find out levels of lead in food colors, which are commonly used in street food. According to WHO the maximum permissible limit of lead is 0.05 mg/L. Seven samples of food color were taken namely; S01 (green), S02 (yellow), S03 (chocolate), S04 (red), SS01 (green), and SS02 (yellow) SS03 (red). S01, S02, S03 and S04 were in solid form. Rest of the samples was in liquid form. To find the Pb levels in the samples, Direct Air-Acetylene Flame method was followed. The analysis showed that, out of seven samples, the Pb levels of four samples (S01, S02, S03 and S04) were out of limits which were respectively 0.15 mg/kg, 0.15 mg/kg, 0.15 mg/kg, and 0.10 mg/kg. Therefore, we can say that the usage of these colors can be harmful for us and cause severe toxicity.

Keywords: Lead; Heavy metals; Vulnerable; ICP-MS; Food colors.

Dedication (Optional)

This work is dedicated to my parents for their constant love and support

Acknowledgement

All praise is for the Almighty who is the source of our life and strength of our knowledge and wisdom, has helped me to continue my study in full diligence, which I hope, will reflect in my project.

This research could not also have been completed without the support of many people who are gratefully acknowledged here.

I am thankful to my supervisor, Marzia Alam, Lecturer, Department of Pharmacy, Brac University, for her amiability to provide me with untiring guidance, whole cooperation and for her extensive knowledge in research that helped me in all the spheres to perform the project work.

I would like to put forward my most sincere regards and profound gratitude to Dr. Eva Rahman Kabir, Chairperson and Professor, Department of Pharmacy, Brac University, for giving me the opportunity to conduct such an interesting project and for facilitating a smooth conduction of my study.

I also want to show my gratitude to Dr. Sharmind Neelotpol, Associate Professor, Department of Pharmacy, Brac University, for her guidance and thoughtful suggestions that helped us a lot to conduct the research work.

I would also like to extend my thanks to all the research students in the lab, lab officers and other staffs of the Department of Pharmacy for their help and assistance, friendly behavior and earnest co-operation which enabled me to work in a very friendly and comfortable ambiance. I owe special thanks to my family for their immense support, contribution and continuous motivation in my project work.

Thank you.

Table of Contents

Declaration.....	ii
Approval	iii
Ethics Statement.....	iv
Abstract.....	v
Dedication (Optional)	vi
Acknowledgement	vii
Table of Contents	viii
List of Tables	x
List of Figures.....	xi
List of Acronyms	xii
Glossary	xiii
Chapter 1 Introduction.....	1
1.1 Background	1
1.2 Heavy Metals	1
1.3 Sources of Heavy Metals	2
1.4 Effect of heavy metals contamination	3
1.5 Pharmacokinetics of Lead.....	3
1.6 Toxicity of Lead.....	6
1.6.2 Hematologic toxicity	7
1.6.3 Cardiovascular toxicity	7

1.6.4 Other toxicity	8
1.7 Carcinogenicity	8
1.8 Method for Heavy Metal Detection	8
1.8.1 Direct Air-Acetylene Flame Method	8
1.9 Rationale of the study	9
1.10 Literature Review.....	10
Chapter 2	14
Methodology	14
2.1 Collection of Samples	14
2.2 Labeling of Samples	14
2.3 Sample Analysis.....	15
Chapter 3	16
Result.....	16
Chapter 4	18
Discussion.....	18
Chapter 5	21
Conclusion	21
References:.....	22

List of Tables

Table 1: Labeling of Food Color Samples	15
Table 2: Lead concentrations obtained in the given samples	16

List of Figures

Figure 1: Sources of Heavy Metals	2
Figure 2: Different organs exposed due to heavy metals.....	3
Figure 3: Pharmacokinetics of Lead	5
Figure 4: Target organs toxicity of lead.....	7
Figure 5: Direct Acetylene Flame Method	9
Figure 6: Sample conc vs Permissible limit.....	17
Figure 7: Possible effects of lead toxicity in different organs	19
Figure 8: Blood lead concentrations in different ages (geometric mean).....	20

List of Acronyms

MPL Maximum Permissible Limit

Pb Lead

WHO World Health Organization

Se Selenium

Zn Zinc

Cr Chromium

As Arsenic

Ti Titanium

APHA American Public Health Association

ICP-MS Inductively coupled Plasma Mass Spectroscopy

BSTI Bangladesh Standard and Testing Institute

INARS Institute of National Analytical Research and Service

BCSIR Bangladesh Council of Scientific and Industrial Research

EPA Environmental Protection Agency

NTP National Toxicology Program

Glossary

Bioaccumulation:	Bioaccumulation is the gradual accumulation of substances, such as heavy metals, or other chemicals in an organism. Bioaccumulation occurs when an organism absorbs a substance at a rate faster than that at which the substance is lost by catabolism and excretion.
Ferrochelatase:	Ferrochelatase is the terminal enzyme of the heme biosynthetic pathway in all cells. It catalyzes the insertion of ferrous iron into protoporphyrin IX, yielding heme. In eukaryotic cells, ferrochelatase is a mitochondrial inner membrane-associated protein with the active site facing the matrix.
Aminolaevulinic acid Synthetase (ALAS):	δ -Aminolevulinic acid (also dALA, δ -ALA, 5ALA or 5-aminolevulinic acid), an endogenous non-proteinogenic amino acid, is the first compound in the porphyrin synthesis pathway, the pathway that leads to heme, in mammals, as well as chlorophyll in plants. 5ALA is used in photodynamic detection and surgery of cancer.

Chapter 1 Introduction

1.1 Background

Metallic chemical elements that are comparatively of high density and are toxic at low concentrations are termed as heavy metals. Some examples of heavy metals are mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and thallium (Tl). Heavy metals are naturally found on the crust of the earth. These elements cannot be destroyed. They enter into our bodies via air, food and drinking water to a small extent. Some heavy metals (e.g. zinc, copper, selenium) are trace elements are essential for the metabolism of the human body. At elevated concentrations, lead is dangerous. At elevated air concentrations or intake of food through food chain can result in heavy metal poisoning, for example, contamination from drinking water (e.g. lead pipes). As heavy metals have a tendency to bioaccumulate, they are dangerous for the body. Compounds that assemble in the body can be stored or taken up faster than they are broken down for excretion and metabolism. Heavy metals enter the water supply by industrial or product waste, or even when acid rain combines with groundwater, streams, rivers and lakes (Mohod & Dhote, 2013).

1.2 Heavy Metals

Although there is no particular explanation of a heavy metal contamination, several researches have explained that it has got a high mass and thickness that is 5 times than that of water. Among all the contaminants, heavy metals have gotten an immense consideration regarding ecological scientific experts because of their dangerous nature. In natural water, however, heavy metals are sometimes discovered in trace quantities; in regular water, however, some are deadly, even at very small amount. Metals like Pb, Co, Hg, Cd, Ni, Cr, As, and Se are extremely deadly even in

small amount. Increasing amount of heavy metals in our resources is presently an issue of greater concern, especially since a larger than usual number of industries are releasing their metal discharge into water with non-satisfactory treatment (Masindi & Muedi, 2018)

Heavy metals become toxic once they are not processed by the body and gather inside the soft tissue. Lead will enter the body through food, water, air or through the skin once they come in accessible contact with people working in water and fuel, primary and secondary smelting, plumbing and exposure to lead-bearing paint or contaminated food, car battery industry, manufacturing of ceramic. Industrial exposure accounts for a typical route of exposure for grown-ups as they are most vulnerable to this exposure. Normal human activities are contaminating the environment and resources by discharging waste materials into the surroundings (Masindi & Muedi, 2018)

1.3 Sources of Heavy Metals

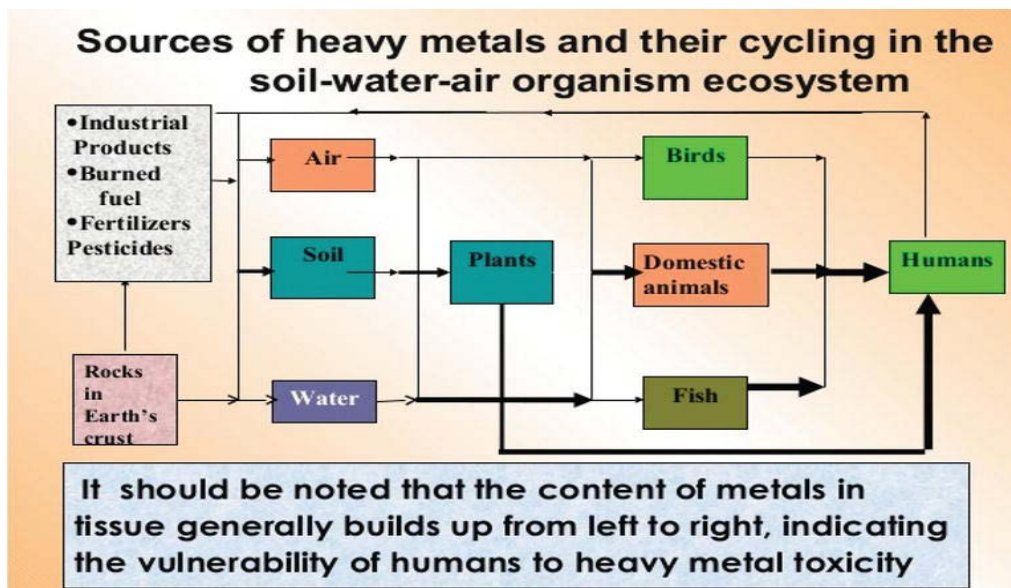


Figure 1: Sources of Heavy Metals

(Torabi et al., 2016)

1.4 Effect of heavy metals contamination

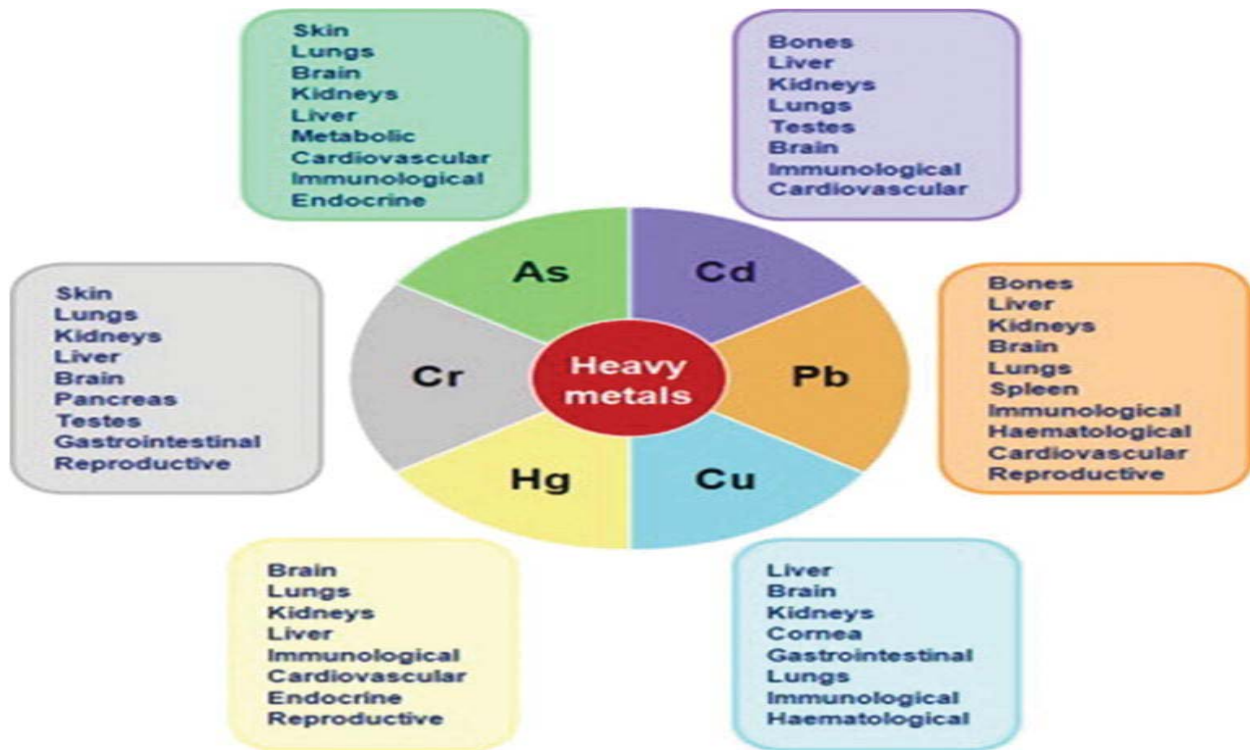


Figure 2: Different organs exposed due to heavy metals.

(Torabi et al., 2016)

1.5 Pharmacokinetics of Lead

Lead enters the human body from nature by inhalation, by eating and drinking and also through skin. After absorption of lead into lungs or gastrointestinal tract, it goes to the blood stream, at first, it is attached to the blood free proteins and then the proteins carry it to different tissues and bones. RBC is linked to most of the lead present in blood streams. Physicians can tell the lead level after diagnosis by measuring the amount of lead in the blood. These quantities are stated as a quantity per volume unit (microgram per deciliter). (Kim et al., 2015)

Lead is spread throughout the body to many tissues and organs. It is important that lead is not lost or converted into something else in the body. The amount of lead contained in the body has been identified as the "body burden" of lead. Adults consider more than 95% of total body stores of lead. Approximately 70% of lead is stored in children's bone. This lead is not necessarily permanently deposited in the bone, but as the body normally works, it travels in and out. When children restructure their bones, for example, to allow normal forms to develop (Kim et al., 2015)

As shown in Figure, there are three main compartments in which lead is distributed: bone, soft tissue, and blood. Once absorbed from the lungs and gastrointestinal tract, lead reaches the red blood cells (RBCs) first, where zinc is replaced by specific hematopoietic enzymes at the active site. Blood lead accounts for only 1–5% of the total body load of lead. 95–99% of blood lead is linked to RBCs at lower lead levels and only 1% is ionized in plasma. Plasma lead can easily be exchanged in soft tissues, particularly in the kidney and brain, where it has the most toxic effects. This binds to the cell membranes, changes the protein structure, and can interfere with gene transcription. Plasma lead is also important in hydroxyapatite crystals for conversion to bone mineral where calcium substitutes for lead are used. Bone lead accounts for 70–80% of the total body burden of lead for children and 90–95% of the total body burden for adults. Because of the bone's relatively slow metabolic turnover, accumulated lead remains for years and bone lead stores slowly accumulate during life; thus, bone lead can be a more accurate aggregate biomarker of lead body burden than either bone blood or soft tissue lead. Bone lead is an endogenous source of lead and may pose a health risk long after exposure to other tissues has ended. In situations of high bone turnover such as osteoporosis, pregnancy, lactation, and some hyperendocrine conditions, bone release will increase (Blumberg, Eisinger, Lamola, & Zuckerman,

1977). Nevertheless, observations of acute lead poisoning (without recent exposure) associated with hyperthyroidism have been reported in the literature, and skeletal lead release has been quantitatively demonstrated in pregnant and lactating women. Even in normal metabolic conditions, slow release of lead from the bone can maintain chronically increased blood lead values in a highly exposed person long after external exposure has stopped (Blumberg et al., 1977).

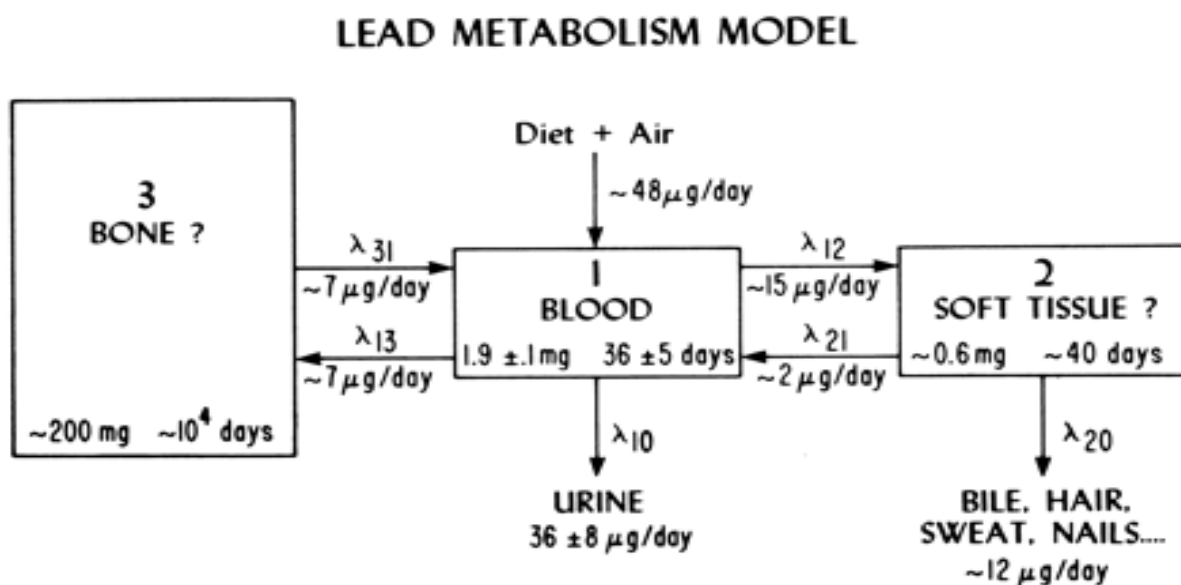


Figure 3: Pharmacokinetics of Lead

(Rabinowitz, Wetherill, & Kopple, 1976)

The steady state kinetics of lead metabolism was observed by five healthy men with stable isotope tracers. In a metabolic process, people ate constant low-lead diets. Their diet was supplemented every day with 79--204 mug of enriched lead-204 as nitrate, eaten with meals for 1--124 days. The concentration and isotopic composition of lead was measured serially in blood, urine, feces, and diet based on isotopic dilution, mass spectrometric examination, and less commonly in hair, teeth, sweat, bone, and food tract secretions. The data suggests a three-part

model for lead metabolism. The first compartment is 1, 5--2, 2 times the blood mass. It has about 1.7--2.0 mg of lead and an average life of 35 days. This reservoir deals with ingested water, urinary lead, and two and three streams in particular. The second compartment consists mainly of soft tissue, contains approximately 0.3--0.9 mg lead and has an average lifespan of approximately 40 days. In hair, teeth, blood, and salivary, gastric, pancreatic, and biliary secretions, this mixture produces lead Pool three exists mostly in the heart, comprises the body's large amount of lead, and has a very long mean life. Through their lead turnover rates, bones seem to vary. The coefficients of transfer between the pools remained constant in the relatively small changes in blood lead observed in this study (Rabinowitz et al., 1976).

1.6 Toxicity of Lead

When lead enters into the body, it is accumulated into specific organs, for instance in the liver or the urinary organ, and is discharged at a slow rate in comparison to its accumulation (Doany et al., 2018). The toxicity of those metals has 2 principal aspects: (a) they do not need any modified or identified route to get inside the body and once they are inside, they cause toxicities to body and different organs. This procedure of bioaccumulation of metals happens to all animals, consisting of animals like fish and oxen as appropriately as to general public (Doany et al., 2018). (b) The potential to accumulate mercury and lead in natural tissues, particularly heavy metals is high than other heavy metals in terms of bioaccumulation (Doany et al., 2018).

In distinguishable minor cases, side effects like solid tremors, migraines, crabbiness and loss of memory occurs while extraordinary cases may result in distraction, writhing, or coma (Mason, Harp, & Han, 2014). Though issues will show up in each the central and periphery nervous system, the periphery nervous system is ordinarily influenced in adults, while in aldoscents, the central nervous system is influenced. Ponders have advised that, in addition the scopes of

continuous development, neurobehavioral improvement has a terrible outcome as low I.Q. and also side effects like ADHD (Mason et al., 2014).

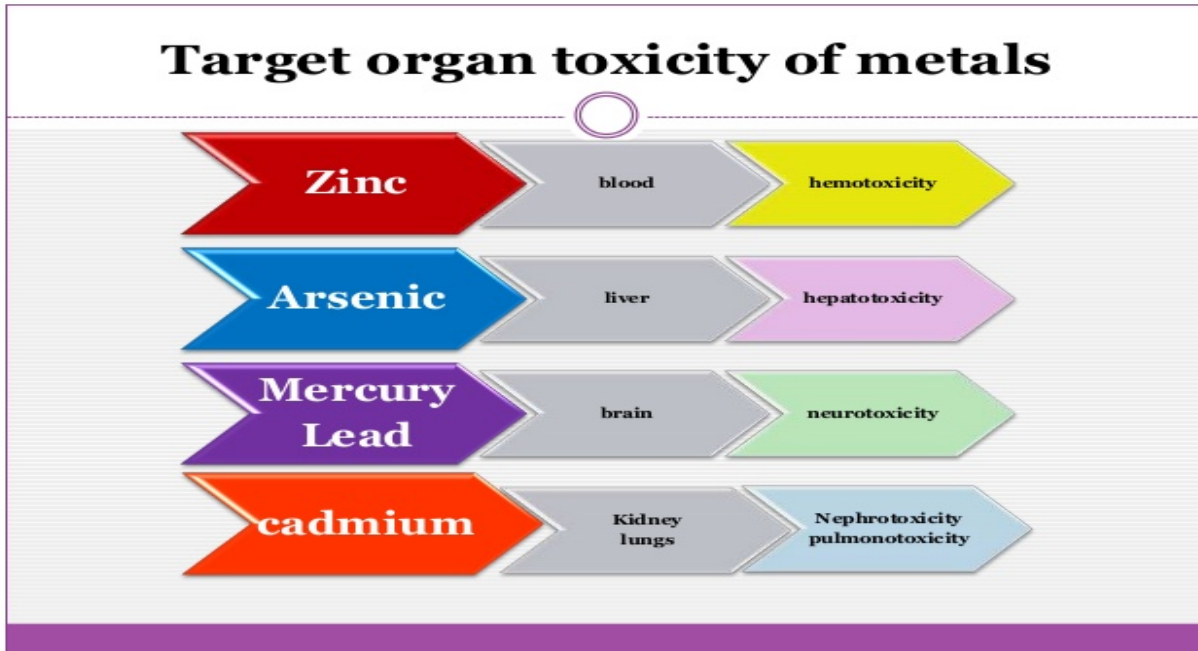


Figure 4: Target organs toxicity of lead

1.6.2 Hematologic toxicity

Lead causes anemia by exploitation dose-dependently inhibits up the action of ferrochelatase, aminolaevulinic acid synthetase (ALAS), and ALAD, 3 necessary enzymes associated to the synthesis of protoheme. Once ALAD is inhibited, aminolaevulinic acid accumulates, and blood lead level rises to ten $\mu\text{g}/\text{dL}$ and even decrease onset that is to be detected. (Kim et al., 2015).

1.6.3 Cardiovascular toxicity

There has been an amazing inquiry agreement that has led to the association between low stages of lead substance and essential sign, and while there are tons to be exchanged, continuing examination has shown that low levels of lead introduction would result in the increase of the

necessary toxicities. A few discoveries have shown to demonstrate a tremendous level of connection between blood lead stages, heartbeat and heartbeat essential signs. (Kim et al., 2015)

1.6.4 Other toxicity

A few examinations have found reason be a thought process of procreative toxicity in every adults. In men, it causes a rebate in sexual desire, in addition as a markdown in sperm and strength, while in female there is associate degree of association within the frequency abortion and miscarriage. For sure, lead would aim on vitamin D deficiency by manner of disturbing the transformation of fat-soluble vitamin into its living human kind, 1,25-dyhydroxyvitamin D (Kim et al., 2015).

1.7 Carcinogenicity

The Worldwide Organization for examination on threat (IARC) inorganic reason is clearly undermining neoplastic contamination to people (IARC gather 2A), supported adequate confirmation from animal testing and limited confirmation with human subjects. Natural lead has a position in IARC cluster three, which means that there is not enough confirmation to think about whether or not individuals believe toxicities that are harmful. (Kim et al., 2015).

1.8 Method for Heavy Metal Detection

1.8.1 Direct Air-Acetylene Flame Method

This method is relevant to the testing of calcium, cadmium, bismuth, cesium, cobalt, copper, antimony, chromium, iridium, gold, lead, manganese, nickel, palladium, lithium, magnesium, iron, manganese, zinc, sodium, nickel, platinum, silver, tin, strontium, ruthenium, thallium, and potassium, rhodium, (APHA, 2005; Federation, 1999; Willis, 1962). Turning the instrument and

let the instrument warm up until the supply of strength stabilizes, usually about 10-20 minutes. The manufacturer has recommended the hollow-cathode light today. Calibration curves for Ca and Mg shall be formed earlier than lanthanum solution dilution based on original needs. The HNO₃/L inhalation water is rinsed with 1.5 ml nebulizer. For trace elements in microgram per liter and for other specific metals in milligrams per liter, each ion metal concentration must be measured. The concentration is also directly read from the read-out system if the instrument is equipped.

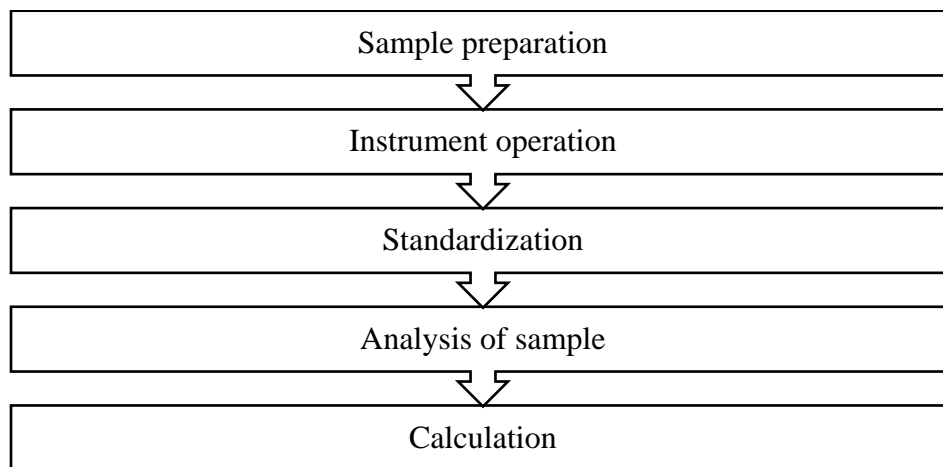


Figure 5: Direct Acetylene Flame Method

1.9 Rationale of the study

This study aims at achieving the following objectives:

1. To make an approach towards the studies of quantifying heavy metals in other different colors we consume in our regular life
2. To make people aware so that they remain cautious about using food colorings as their children are prone to risk of developing diseases by consuming these food additives.

1.10 Literature Review

In this research of, “Lead toxicity: a review” published in the journal of Interdisciplinary toxicology lead harmfulness is a significant surroundings illness and its consequences for the human body are obliterating. There is no capacity in the human body, which is not influenced by lead poisonous quality. In spite of the fact that in nations like US and Canada the utilization of lead has been controlled up to a limited degree, it is yet utilized eagerly in the creating nations. Lead is profoundly diligent in the environment and on account of its consistent utilize its levels ascend in pretty much every nation, presenting genuine dangers (Wani, Ara, & Usmani, 2015).

In this research of, “Health-Related Quality of Life in Early-Onset Scoliosis Patients Treated Surgically: EOSQ Scores in Traditional Growing Rod Versus Magnetically Controlled Growing Rods” published in the journal of Spine, MCGR decreases surgical sessions related to early onset scoliosis treatment, trusting to minimize the burdens seen with dreary intrusive surgeries in TGR treatment. Although there has been broad agreement on the clinical signs for these medications, there is a need to consider their effect on patients and families ' quality of life. The 24-part early scoliosis survey, which has already been approved, has been used to assess quality of life (Doany et al., 2018).

In this research of “Surroundings Contamination by Heavy Metals” published in the journal of Heavy Metals, Worldwide sightseers to Penang conducted this thing about to distinguish the long-haul travel inspiration. Information was analyzed utilizing SPSS computer program 22 adaptation. The discoveries, ‘knowledge and oddity seeking’ were the most thrust components that drove long-haul travel by worldwide tourists to Penang. In the interim, the most drag figure that pulls in long- pull travel by universal sightseers to Penang was its ‘culture and history (Masindi & Muedi, 2018).

In this research of “Lead: the Silent Killer in Our Favorite Street Food” published in the journal of Journal of Evidence Based Medicine and Healthcare, Street foods are a common consumable product for over ages It covers a large extent of an assortment of food things to suit every need, and area unit hugely documented everywhere in the state. They can, in any case, contain harmful overwhelming metals, like lead, which can posture genuine wellbeing risks, counting neuropathy, cardiovascular, renal as well as bone infections. This origin of lead, in any case, is one viewpoint of the wellbeing risks that has not been broadly investigated (Goswami & Mazumdar, 2016).

In this research of “Pb neurotoxicity: neuropsychological effects of lead toxicity” published in the journal BioMed research international the term neurotoxicity could be used to explain neurophysiological changes caused by poisonous substances. The introduction may lead to neurocognitive signs and/or troubling psychiatric influences. Heavy metals, medicines, organophosphates, bacterial and virus neurotoxins are introduced by popular harmful substances. Among the vast exposures of metals, lead is one of the most common exposures that can contribute to significant declines in humans in neuropsychology and usefulness. In this review, the pathophysiology, etiology and disease transmission study of neurotoxic lead exposure was investigated (Mason et al., 2014).

In this research of “Mercury, Lead, Cadmium, Tin and Arsenic in Food” published in the journal Toxicology Factsheet Series, metals and other elements can be shown in food as a result of human activities such as mechanical or agrarian processes. Metals of particular concern are arsenic, cadmium, lead, copper, and mercury in association with harmful effects on health. Mercury and lead are often referred to as "heavy metals." The toxic nature of these metals is partly due to the reality they store in natural tissues known as bioaccumulation. Metal

bioaccumulation occurs in all forms of life as a result of the introduction of metals into food and the atmosphere as well as humans (Ireland, 2009).

In this research of “African Journal of Pharmacy and Pharmacology Review Toxicity of food colors and additives: A review” "Food added substances include dyes, sweeteners, additives and anti-caking operators in this way. There have been a few questions about the security of added nutrients and a few test batteries, and studies are available in writing. In arrange to achieve these focused-on assessments, a few tests have been endorsed by Universal conference on harmonization, organization for financial participation and advancement and European food security specialist. Description of the reading, revealed that once a few confirmed inserted substances or colors ended up being a topic of security concerns, thorough assessments were carried out by analysts and these have periodically contributed to the declassification of a few informative operators as non-genotoxic and non-carcinogenic to this stage. After a thorough evaluation of mutagenicity and genotoxicity studies in vitro and a few in vivo experiments in mammalian tissues, declassification of a few food colors and additional substances as human carcinogens is often performed (Thomas & Adegoke, 2015).

In the Research of “Health-Related Quality of Life in Early-Onset Scoliosis Patients Treated Surgically: EOSQ Scores in Traditional Growing Rod Versus Magnetically Controlled Growing Rods” published in the journal Spine, Lead could be a solid, silver-gray, unavoidable genuine component and is commonly identifiable as adequately as in most organic structures in inactive surroundings. Lead used in products such as batteries for electrical processing, lead solder, radiation filters, tubes and sheaths for hopped-up electrical cable. It should try to connect with completely different metals to make combinations of elements for mechanical assembly of channels. In a range of products such as plastics, rubber compounds, pigments, paints, glassware,

pesticides and pigments, these compounds are used. Chemically, silver components may occur as pure metal, e.g. tin and lead, or combinations formed by combining a metal problem with a non-elemental issue such as mixing Na with chemical element or mercury element, gold, lead, arsenic or cadmium. Because of their high relative atomic mass, mercury and lead are usually brought up as (heavy metals) (Doany et al., 2018)

In this research “A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment” from the journal Environmental Science and Pollution Research even cases with no significant side effects can be found though. In cases of non-stop presentation, signals seem to return to be rationally extra ridiculous in view of the fact that the weeks are passing, although in instances of extreme initiation hard indicators will appear suddenly. Signs in particular show that they do in children in adults. While symptoms usually occur when blood lead levels reach $40 \mu\text{g} / \text{dL}$ in adults and exceed $60 \mu\text{g} / \text{dL}$ in children. In addition, the symptoms occur on the patient's characteristics at distinct range. Wherever an excessive amount of lead has been consumed by the biological processing machine in a very short time, the loss of heaps of water in the digestive system can lead to shock, lysis, and additional symptoms such as anemia and symptoms. (Kim et al., 2015).

Chapter 2

Methodology

The purpose of this research was to quantify lead, a well-known heavy metal in food coloring agents. Different stepwise approaches were carried out to obtain the ultimate outcome of this research. Those steps are described below.

2.1 Collection of Samples

A total of 7 food-coloring samples were collected from two different places. 4 samples of them were in powder form. They were collected from M/S. Haji A. Kalam Store which is situated at Chawk Bazar in Old Dhaka. The colors were Green, Yellow, Chocolate and Red. There were 3 more samples which were in Liquid form. They were collected from Mawola Traders which is situated at D.N.C.C Market in Gulshan-1. Those colors were Green, Yellow and Red. Moreover, these liquid colors were branded products.

2.2 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 understand. They were labeled according to the following table.

Table 1: Labeling of Food Color Samples

Serial No	Food Coloring Sample	Labeled As	Sample Form
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

2.3 Sample Analysis

The food colors were analyzed in the INARS laboratory of BCSIR by following Direct Air-Acetylene Flame method.

Chapter 3

Result

The quantitative analysis of lead was carried out by following Direct Air-Acetylene Flame method. This method is also known as APHA 3111.B method. When the test was completed, the attained concentrations of lead in the samples were recorded in the following table.

Table 2: Lead concentrations obtained in the given samples

Particulars of Sample	Parameter	Conc. Of lead	Permissible limit or equivalent of that value	Exceeding the Limit?
Food Color Sample (S-01)	Lead	0.15 mg/Kg	0.05 mg/Kg	Yes
Food Color Sample (S-02)		0.15 mg/Kg	0.05 mg/Kg	Yes
Food Color Sample (S-03)		0.15 mg/Kg	0.05 mg/Kg	Yes
Food Color Sample (S-04)		0.10 mg/Kg	0.05 mg/Kg	Yes
Food Color Sample (SS-01)		0.03 mg/L	0.05 mg/L	No
Food Color Sample (SS-02)		0.01 mg/L	0.05 mg/L	No
Food Color Sample (SS-03)		0.03 mg/L	0.05 mg/L	No

According to WHO, the maximum permissible limit of lead is 0.05 mg/kg or equivalent of this value. Here, the concentrations of Lead in the non-branded solid samples, S-01, S-02, S-03 and S-04 are respectively 0.15 mg/kg, 0.15 mg/kg, 0.15 mg/kg and 0.10 mg/Kg. All these concentrations of lead are higher than the permissible concentration given by WHO. Again, the concentrations of Lead in the branded liquid samples, SS-01, SS-02 and SS-03 are respectively

0.03 mg/L, 0.01 mg/L and 0.03 mg/L. All these concentrations of Lead are lower than the permissible concentration given by WHO (ATSDR, 2019).

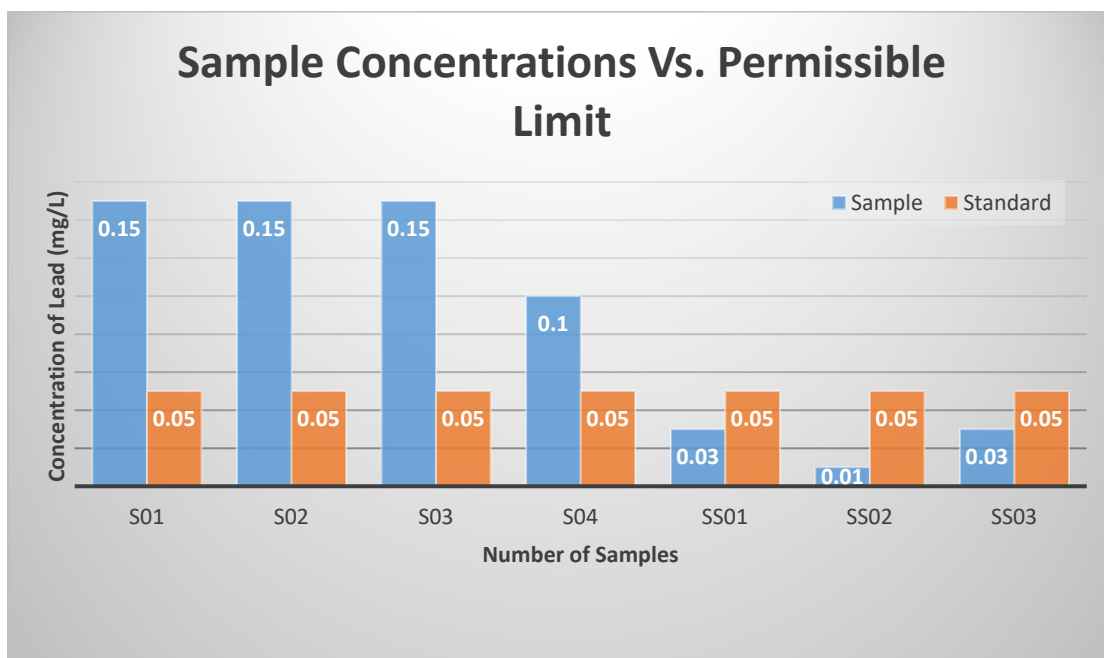


Figure 6: Sample conc vs Permissible limit

Chapter 4

Discussion

During the quantitative analysis of lead in food colors by following Direct Air-Acetylene Flame method, lead levels in four samples were higher than permissible limit of lead proposed by WHO. Several toxic reactions in the human and animal bodies can occur while the body is exposed to lead contaminants at excessive level by consuming foods contains lead contaminants, through inhalation, skins and different other pathways. The most affected areas of the body because of lead contamination are brain, heart, blood cells, kidneys, stomach and bones as well. Moreover, the reproductive system can also be affected. When the brain is affected by lead contaminants, severe consequences can be occurred such as damage of nerves, hearing loss, loss of body movements, coma and even death. When a person is exposed to lead in childhood, that person is at high risk of severe cardiovascular diseases within the age of 50 (Jung & Peddinti, 2018). The blood system is also affected due to lead toxicity. Production of hemoglobin is inhibited so that anemia occurs. Renal systems are also impaired leading to kidney failure, bloody urine, urinary changes etc. Lead toxicity is also responsible for bone disorder which inhibits the regular growth of the body. Along with these effects, hormonal disorder such as impaired cell growth, reproductive system disorders such as lower sperm counts, motility of sperms and stomach disorders such as abdominal pain, cramping and many other rapid and prolonged diseases can be occurred because of the toxicity of lead (Jung & Peddinti, 2018). In the following diagram (Figure 5), illustration of different affected areas of body due to lead toxicity is given as appropriately as possible.

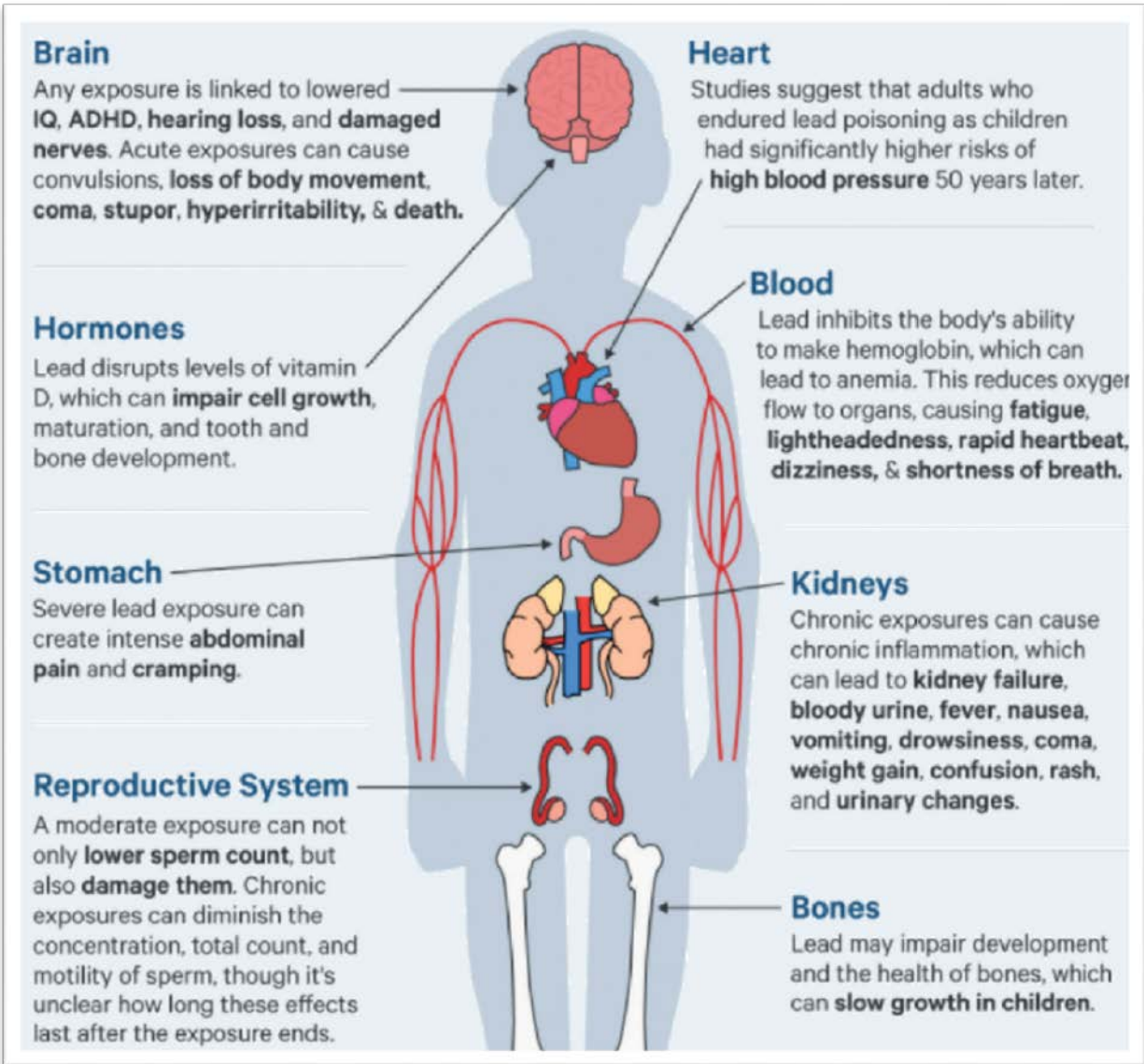


Figure 7: Possible effects of lead toxicity in different organs

(Jung & Peddinti, 2018)

In another bar graph shown in (Figure 6), the blood lead concentration is shown to be increased with the age. The deposition rate of lead is much higher than the excretion rate (ATSDR, 2019). When a child is consuming food products containing lead, lead is deposited in the tissues. As the child is growing up, he is being exposed to lead through different others sources of leads and other heavy metals. Thus, the level of lead in the blood is increased day by day.

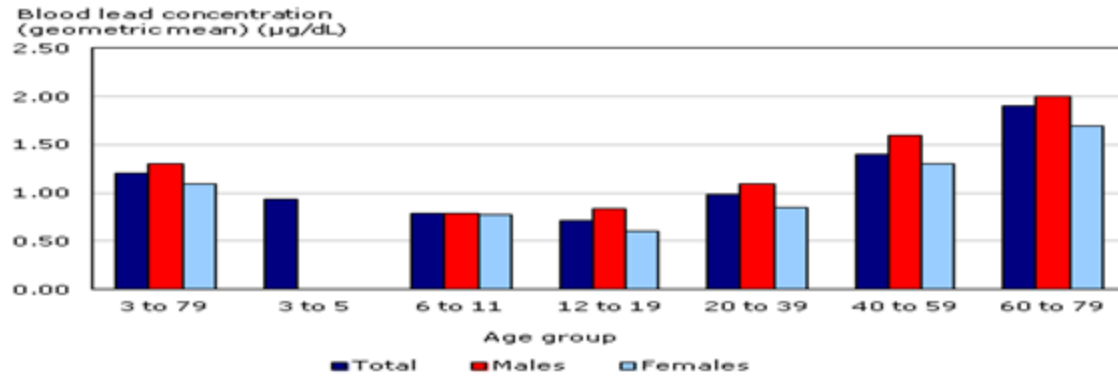


Figure 8: Blood lead concentrations in different ages (geometric mean)

(“Blood lead concentrations in Canadians, 2009 to 2011,” n.d.)

Chapter 5

Conclusion

In this study, the presence of lead in food colors is determined and quantified. Here, quantity of lead in some of the samples was out of the permissible limit while some were within the limit. Although, those food colors are within the permissible limit and harmless to consume compared to others, there are a small amount of lead there. This fact must be considered with high concern. These food colors are less costly and easily accessible so that most of the people can afford these. In addition, foods like bakeries, ice creams, chocolates are found to contain huge quantity of heavy metals such as cadmium, mercury, lead etc. Children are attracted to these foods a lot. It is proven that the food industry is not being regulated properly. Many laws and regulations are set by BSTI but those are not being implemented properly. Necessary steps must be taken by authority and the individuals for the sake of lives. In this study, in vitro testing is conducted. In future, in vivo test for lead can be performed to observe the concentration of lead in the body.

References:

- APHA. (2005). 3111 B. Metals by Flame Atomic Absorption Spectrometry. *Standard Methods for the Examination of Water and Wastewater*, 552.
- ATSDR, A. for T. S. and D. R. (2019). Toxicological Profile for Lead (Draft for Public Comment). *ATSDR's Toxicological Profiles*, (May).
https://doi.org/10.1201/9781420061888_ch106
- Blood lead concentrations in Canadians, 2009 to 2011. (n.d.). Retrieved December 18, 2019, from <https://www150.statcan.gc.ca/n1/pub/82-625-x/2013001/article/11779-eng.htm>
- Blumberg, W. E., Eisinger, J., Lamola, A. A., & Zuckerman, D. M. (1977). The hematofluorometer. *Clinical Chemistry*, 23(2 PT. 1), 270–274. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/832391>
- Doany, M. E., Deniz Olgun, Z., Kinikli, G. I., Bekmez, S., Kocyigit, A., Demirkiran, G., ... Yazici, M. (2018). Health-Related Quality of Life in Early-Onset Scoliosis Patients Treated Surgically: EOSQ Scores in Traditional Growing Rod Versus Magnetically Controlled Growing Rods. *Spine*, 43(2), 148–153. <https://doi.org/10.1097/BRS.0000000000002274>
- Federation, W. E. (1999). Standard Methods for the Examination of Water and Wastewater Standard Methods for the Examination of Water and Wastewater. *Public Health*, 51(1), 940–940. <https://doi.org/10.2105/AJPH.51.6.940-a>
- Goswami, K., & Mazumdar, I. (2016). Lead: the Silent Killer in Our Favourite Street Food. *Journal of Evidence Based Medicine and Healthcare*, 3(22), 969–971.

<https://doi.org/10.18410/jebmh/2016/221>

Ireland, F. safety A. of I. C. (2009). Mercury, Lead, Cadmium, Tin and Arsenic in Food.

Toxicology Factsheet Series, (1), 1–13. <https://doi.org/10.1097/BRS.0000000000002274>

Jung, J. M., & Peddinti, R. (2018). Lead toxicity in the pediatric patient with sickle cell disease:

Unique risks and management. *Pediatric Annals*, 47(1), e36–e40.

<https://doi.org/10.3928/19382359-20171218-01>

Kim, H.-C., Jang, T.-W., Chae, H.-J., Choi, W.-J., Ha, M.-N., Ye, B.-J., ... Hong, Y.-S. (2015).

Evaluation and management of lead exposure. *Annals of Occupational and Environmental Medicine*, 27, 30. <https://doi.org/10.1186/s40557-015-0085-9>

Masindi, V., & Muedi, K. L. (2018). Environmental Contamination by Heavy Metals. *Heavy*

Metals. <https://doi.org/10.5772/intechopen.76082>

Mason, L. H., Harp, J. P., & Han, D. Y. (2014). Pb neurotoxicity: neuropsychological effects of

lead toxicity. *BioMed Research International*, 2014, 840547.

<https://doi.org/10.1155/2014/840547>

Mohod, C. V., & Dhote, J. (2013). Review Of Heavy Metals In Drinking Water And Their Effect

On Human Health. *International Journal of Innovative Research in Science, Engineering and Technology*, 2(7), 2992–2996.

Rabinowitz, M. B., Wetherill, G. W., & Kopple, J. D. (1976). Kinetic analysis of lead

metabolism in healthy humans. *Journal of Clinical Investigation*, 58(2), 260–270.

<https://doi.org/10.1172/JCI108467>

- Thomas, O. E., & Adegoke, O. A. (2015). *African Journal of Pharmacy and Pharmacology Review Toxicity of food colours and additives: A review*. 9(36), 900–914. <https://doi.org/10.5897/AJPP2015.4385>
- Torabi, M., Drahansky, M., Paridah, M. ., Moradbak, A., Mohamed, A. ., Owolabi, F. abdulwahab taiwo, ... Abdul Khalid, S. H. . (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 % . *Intech, i(tourism)*, 13. <https://doi.org/http://dx.doi.org/10.5772/57353>
- Wani, A. L., Ara, A., & Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary Toxicology*, 8(2), 55–64. <https://doi.org/10.1515/intox-2015-0009>
- Willis, J. B. (1962). Determination of Lead and Other Heavy Metals in Urine by Atomic Absorption Spectroscopy. *Analytical Chemistry*, 34(6), 614–617. <https://doi.org/10.1021/ac60186a008>