Heavy Metal Analysis and Health Risk Assessment of Heavy Metal in Selected Soft Drinks in Local Market of Malaysia

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DECLARATION

I declare that this thesis entitled 'Heavy metal Analysis and Health Risk Assessment of Heavy Metal in Selected Sof Drinks in Local Market of Malaysia ' is the results of my own research except as cited in the references.

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ABSRACT

Soft drink has been deemed as one of the most refreshing beverages in the world. They are widely consumed by all ages of people around Malaysia. Heavy metal pollution of soft drink is caused by man made activities and natural occurrence. Thus, this purpose of study is to analyse the heavy metal residue (Pb,Zn,Cd,Cu) in 5 samples of soft drinks that will purchased from the local supermarket by using Atomic Absorption Spectrometer (AAS) and health risk assessments are also done to all people in Malaysia to assess the hazard level of soft drinks towards human. The health risk assessment are also evalauated using Hazard Index (HI) and Provisional Daily Intake (PDI) and Provisional tolerable weekly intake (PTWI).The results indicates the average mean concentration for the soft drinks were arranged in decreasing order Pb>Zn>Cd>Cu, Cu was not included in the health risk assessment as it is not detected. In addition, the estimated weekly intake and was within the range of PTWI, while the average THQ for Pb is 0.4463; Cd is 0.1033 and Zn is 2.32x10^{-3} respectively. The HI calculated was 0.1023, which is less than 1. The results suggests that intake of the soft drinks posed no carcinogenic danger of the four metals.

Keywords: soft drinks, contamination, heavy metal, extraction, health risk assessment, Atomic Absorption Spectrometer, Concentration

Analisis Logam Berat dan Penilaian Risiko Kesihatan Logam Berat dalam Minuman Ringan Terpilih di Pasaran Tempatan Malaysia

ABSTRAK

Minuman ringan telah dianggap sebagai salah satu minuman paling menyegarkan di dunia. Mereka digunakan secara meluas oleh semua orang di seluruh Malaysia. Pencemaran logam berat minuman ringan berpunca daripada aktiviti buatan manusia dan kejadian semula jadi. Justeru, tujuan kajian ini adalah untuk menganalisis sisa logam berat (Pb,Zn,Cd,Cu) dalam 5 sampel minuman ringan yang akan dibeli dari pasar raya tempatan dengan menggunakan Spektrometer Penyerapan Atom dan penilaian risiko kesihatan juga dilakukan. kepada semua rakyat di Malaysia untuk menilai tahap bahaya minuman ringan terhadap manusia. Penilaian risiko kesihatan juga dinilai menggunakan Indeks Hazard dan Pengambilan Harian Sementara dan Pengambilan Mingguan Boleh Ditolak Sementara. Keputusan menunjukkan kepekatan purata untuk minuman ringan disusun dalam susunan menurun Pb>Zn>Cd>Cu, Cu tidak termasuk dalam pengiraan penilaian risiko kesihatan kerana ia tidak dikesan. Selain itu, anggaran pengambilan mingguan dan berada dalam julat PTWI, manakala purata THQ bagi Pb ialah 0.4463; Cd ialah 0.1033 dan Zn ialah $2.32x10^{-3}$ masing-masing. Indeks Bahaya yang dikira ialah 0.1023, iaitu kurang daripada 1. Keputusan menunjukkan bahawa pengambilan minuman ringan tidak menimbulkan bahaya karsinogenik bagi empat logam tersebut.

Kata kunci: minuman ringan, pencemaran, logam berat, pengekstrakan, penilaian risiko kesihatan, Spektrometer Penyerapan Atom, Kepekatan

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LIST OF SYMBOLS

>	More than
%	Percentage
°C	Degree Celsius
µ	Micro
+	Addition
-	Subtraction
✖	Multiplication
÷	Division
±	Standard error
RM	Ringgit Malaysia

LIST OF ABBREVATIONS

EC	European Comission
WHO	World Health Organization
MFA	Malaysian Food Act
MOH	Ministry of Health
AAS	Atomic Absorption Spectrometer
Pb	Lead
Cu	Copper
Zn	Zinc
Cd	Cadmium
µg	Microgram
Mg	Milligram
MRL	Maximum Residue Limits
µm	Micrometre
L	Litre
mL	Millilitre
µg/L	Microgram per Litre
mg/L	Milligram per Litre
HNO_3	Concentrated nitric acid
H_2SO_4	Sulphuric acid
H_2O_2	Hydrogen peroxide
$Pb(NO_3)_2$	Lead nitrate
$Cu(NO_3)_2$	Copper nitrate

$Zn(NO_3)_2$	Zinc nitrate
$Cd(NO_3)_2$	Cadmium nitrate
µg/L/day	Microgram per Litre per day
µg/L/week	Microgram per Litre per week
USEPA	United States Environmental Protection Agency
TDI	Tolerable Daily Intake
EDI	Estimated Daily Intake
EWI	Estimated Weekly Intake
PTWI	Provisional Tolerable Weekly Intake
THQ	Target Hazard Quotient
HI	Hazard Index

CHAPTER 1

INTRODUCTION

1.1 Research Background

Soft drink has been known as a trendy drink throughout the whole world. It is known for its sweet and refreshing taste. Soft drinks are known as non-alcoholic drinks that mix sweetness and a hint of acidity (Ashurst, Hargitt, & Palmer, 2017). Water, sugars, citric acid, fruit extract, preservatives, flavours, syrups, caffeine, CO_2, and caramel as a colouring agent are everything contained in soft drinks. (Lebda, et al., 2017). Even though the soft drink is being recognised as unhealthy drinks, many people, especially children, like soft drinks, have led to many health problems. Many research has shown that one can of carbonated soft drink with added sugar produces large amount of calories if consumed daily. (Tahmassebi & BaniHani, 2020). According to statisticsMexico has been named the country with the highest soft drink intake in 2019. It has achieved a record of 6308 ounces or the same as 186.55 litres of soft drinks consumed per year. These statistics show that the phenomenon of consuming soft drinks is getting even worse each year. According to the World Health Organisation, the soft drink must be reduced as it will not giving any fullness and thirst-quenching for us (Lobstein, 2014).

The first step in soft drink processing is syrup preparation. The syrup is prepared by mixing glucose or sugars with water, while diet drinks syrup is prepared using artificial sweeteners. These raw materials can be found in liquid or powder form. Next, few additional steps such as dissolving, mixing, heating, and filtration steps to make the syrup base used in the processing later. Once the syrup is done, it is then bottled. During the bottling process, a specific amount of water and syrup are mixed to form the soft drink. Then, the soft drink is then infused with carbon dioxide. Then the drink is packaged in cans or bottles. After packaging, they are delivered into multiple stores and finally sold the products to the final customers (Demartini, Pinna, Aliakbarian, Tonelli, & Terzi, 2018).

Rapid urbanisation and industry malpractice causes a series of heavy metal pollution in food production. The origins of heavy metal in canned beverages can be traced back to waste water, chemical residue in fermented drinks, bioaccumulation in marine species, and agricultural emissions into the processed drink prior to canning. (S.G., O.O.Adekoyeni, & T.B.Hammed, 2016). Some studies alsos tated that the sources of heavy metal contamination in soft drinks include water, sweeteners, flavour agents, colouring dyes, or manufacturing procedures (Ataie, Alkhatib, & Mounir, 2020). Thus, the quality of food and beverages is at stake. The contamination of heavy metal in the soft drink will cause considerable problems to humans if no remedy or control measure is applied. In cans, the cans used for packaging usually are made of aluminium and steel, and the drinks are not directly in contact with the can. However, the transferring of heavy metal to the drink might happen (Geuke, 2018).

Finally, heavy metal pollution in soft drinks is a grave matter throughout the food and beverages industry. Every people must recognise the awareness of heavy metal presence in the soft drink. Health risk assessment of humans must be determined to prevent this matter from happening again as the accumulation of heavy metals will significantly affect the body physiology, mental state, reproductive system, and other body functions (Godwill, et al., 2015).

This study will be performed to determine the level of heavy metals in soft drinks within the permissible level of WHO, MFA and EC to perform risk assessment heavy metal pollution among soft drinks among Malaysian.

1.2 Problem Statement

Soft drinks are a type of non-alcoholic drink that is consumed in our daily life. It is known to be refreshing and thirst-quenching to us. Thus, soft drink is liked by all ages of people. In soft drink production, heavy metal transmission in soft drinks will happen for many reasons. Heavy metal pollution can come from water sources to raw materials (Godwill, et al., Determination of some soft drink constituents and contamination by some heavy metals in Nigeria, 2015). Thus, more heavy metal analysis must be done on soft drinks to ensure the soft drink's safest quality is delivered to the people.

Furthermore, the pollution of water sources and raw materials also causing heavy metals in soft drinks. The most common problem of heavy metal contamination in a soft drink is water source pollution. The pollution of the water supply with heavy metals has attracted worldwide interest due to the severity of the case and the toxicity of the heavy metal (Siddiqui & Pandey, 2019). Various human-made activities mainly cause pollution of water. This waste contains much heavy metal that is harmful to any life form. Therefore, these heavy metals will affect the health if the heavy metal flows into the food products (Tang, et al., 2016).

Lastly, people must be aware of the heavy metal contents in soft drinks. In this urbanisation era, people must know about food safety in every food product. Many previous studies have found that heavy metals contaminate soft drink products (Abdel-Rahman, Ahmed, Sabry, & Ali, 2019). Thus, quality control and assessment in the soft drink industry must be done to minimise heavy metals contamination in the soft drink.

1.3 Significance of Research

In this modern era, soft drink has been one of the most popular drinks in the world. Due to its popularity, the soft drinks industry is booming since the beginning of the soft drink. According to statistics, the world carbonated soft drink market size is estimated to achieve USD 320.1 billion by 2028 (Inc, 2021). Therefore, many consumers like to drink soft drinks due to their processing technology and marketing innovations towards new flavours (J.F.Thamassebi & Hani, 2020). Even though soft drink causes health problems on general and dental health, soft drink consumptions still going on (J.F.Thamassebi & Hani, 2020).

Heavy metal is a dangerous component that is present around the environment. Due to massive urbanisation and human activities, heavy metal pollution cases have been going on for decades. As the consumption of soft drinks is going on, a few heavy metal pollution in the soft drink product has been discovered among the community. The contamination of heavy metals can come from water, packaging materials, raw ingredients, and other possible factors (Godwill, et al., 2015) . Natural occurrences and human activities contribute to heavy metal pollution within the processing line (Fakhri, et al., 2017).

To spread awareness about the heavy metal composition in soft drinks, monitoring soft drink products must be done frequently. This is because even a low level of heavy metal enters our body, it will cause severe toxicity in our body (Fakhri, et al., 2017). Frequent quality checks on soft drink products must be done often within the processing line to ensure that the heavy metal level in soft drink products is not more than the safety levels by WHO and MOH of Malaysia.

To address this problem, proper research and technique must be done to curb the problem. To detect heavy metal levels present in the soft drink, Atomic Absorption Spectrometer (AAS) must be used in this research. The Atomic Absorption Spectrometer (AAS) results are compared to the heavy metal level guidelines regulated by the WHO and Malaysia's MOH. Finally, this research also determined to identify the heavy metal levels in the soft drink, so the soft drink contamination awareness is spread around the people.

1.4 Objective of Study

The objective of the study is

1. Determination of heavy metals that is present in the soft drink by using an Atomic Absorption Spectrometer (AAS) and compare with WHO, EC and MFA
2. To assess human health risk in the intake of soft drinks in Malaysia

1.5 Scope of Study

The most crucial objective in this research is the extraction of heavy metals in the soft drink samples such as cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), arsenic (As), tin (Sn), chromium (Cr) and mercury (Hg). Several selected soft drink brands will be purchased from the local market for heavy metal extraction and heavy metal residue analysis. Furthermore, the heavy metal analysis of soft drinks will be conducted using an Atomic Absorption Spectrometer (AAS).

Also, the readings obtained from the Atomic Absorption Spectrometer (AAS) are compared with the guidelines that are set by the World Health Organization (WHO) and the Ministry of Health (MOH) Malaysia. Consumers frequently consume these selected soft drinks. Moreover, a health risk assessment will also be conducted on the people in Malaysia about soft drinks.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy metals

Heavy metals are a type of element that is present in the earth's crust. They are formed naturally and have many uses for humans. Heavy metals that are found commonly are lethal even if a small amount is consumed in the body. Heavy metals are defined as transition and post-transition metals (J.Hawkes, 1997). They usually appear as metalloids and have the same properties as metal such as Iron (Fe) and Copper (Cu). They have the physical property of heat conductivity and electrical resistance (Appenroth, 2010). What makes them different from the metal we know is their destructive properties and multiple biological roles in living things. Heavy metals now play an important part in our lives.

The example of heavy metals that we familiar with are Lead (Pb), Mercury (Hg), Arsenic (Ar), Cadmium (Cd), and lots more. Even though heavy metal is considered toxic to all living things, some heavy metals such as iron (Fe) and copper (Cu) provide benefits. For instance, iron that is present in our body is essential for forming red blood cells. Overdose or lacking these nutrients will cause sickness in our bodies. Humans must consume fruits or leafy vegetables to obtain these nutrients (Can, Ozyigit, Can, Hocaoglu-Ozyigit, & Yalcin, 2020). Apart from that, heavy metals also are beneficial elements in our everyday life. For example, copper is very useful in making conductors in our electric wires. Some heavy metals are used for making electrical components, building construction, automobile production, and many others.

However, heavy metals are often lethal if they are found in high concentrations in living organisms and the atmosphere.. High amounts of heavy metals bring toxic effects on soil and plant growth (Yadav, et al., 2021). This causes the plants to lose their nutrients and causes nutrient deficiencies in plants (Weissmanová & Pavlovskỳ, 2017)**.** As a result, some crops such as paddy and vegetables will be significantly affected. This will make the crops lose their crop yield due to a lack of nutrients in them. As for humans, a high amount of heavy metal will interact with our enzymes and cell in our body, deactivate the body system, thus shutting down the organs (Jiao, et al., 2021). Humans were getting in contact with heavy metal through inhalation, touching, and digestion from food. Heavy metal in contact with our skin also causes skin diseases in humans (Sanaei, et al., 2020). Thus, cross-contamination of heavy metals into human bodies must be avoided at all costs.

2.1.1 Classification of heavy metals

In the depths of the earth, there are many metal elements present in this world. However, heavy metals are the ones that are dangerous and problematic to humans. Heavy metal elements in the environment will contact various living organisms due to humankind and environmental activities. Heavy metals can be divided into three groups, which is Toxic metals, precious metals, and radionuclide (Tasharrofi, Hassani, Taghdisian, & Sobat, 2018). As we know, toxic metals and radionuclide are very harmful to us, toxic metals such as mercury (Hg), lead (Pb), and Nickel (Ni). In contrast, radionuclides such as Uranium (U) and Radium (Ra) are very potent for humans and the environment. However, some heavy metals that benefit us, like Iron (Fe) and copper (Cu), are exceptionally beneficial if small amounts are present. For example, there are some metals such as Copper (Cu), Zinc (Zn), and Cobalt (Co) that are very important in the process of oxygen utilisation in our body (Kim, Kim, & Kumar, 2019)**.**

2.2 Heavy metal in soft drinks

In this world of technology, heavy metal pollution is a prevalent problem in this world. The contamination of heavy metals in soft drinks can come from any raw material source to the processing line. Numerous soft drink processing steps have contributed to the heavy metal ions diffusing into the soft drink products. Heavy metal products can also come from the water source, soil, or packaging (Zucchi, Moreira, J.Salvador, & L.Santos, 2005). Many research institutions have engaged in research about heavy metal content in soft drinks to this day. This research in this field alerted people to concern about the safety of the food and beverages product.

One of the most crucial elements present in soft drinks is water. According to a report published by the Institute of Medicine of the National Academy of Sciences (IOM), 90 % of soft drinks are mainly water. In comparison, diet soft drinks are composed of 99 % water (Hydration). Thus, water sources are also very crucial in the soft drink industry. Various drinking waters obtained from rivers, wells, or lakes can be the raw material for the soft drink production site. The sources of heavy metal may come from natural occurring such as weathering of rocks that cause heavy metal elements to flow into the water bodies but mostly human-made pollution that affects the quality of the drinking water sources (Cobbina, Myilla, & Michael, 2013). Thus, the potential of heavy metal enters into the soft drink is highly possible.

Moreover, the soft drinks' packaging method is also one of the main problems that soft drinks contain heavy metal. The primary material for any canned food or beverages used is solder. Solder is used in the production of cans and it is composed of different types of metals such as tinplate, chromium coated steel, or aluminium (Massadeh & Al-Massaedh, 2017). Therefore, the possibilities of heavy metal that are transported into the soft drink are very high throughout the soft drink packaging.

Many studies have researched the heavy metal elements that are present in soft drinks. In a research by Aleti Jaya Sree at 2019, there are many types of metal that is present in the soft drink. However, the quantity of the metal is not high enough to become a threat (Sree, B.Aruna, Narayana, & Rao, 2019). In a research by Angèle N. Tchana (2018) , the random samples of soft drinks in Cameroon were have an exceptionally high amount of heavy metal in it which is way too high than the permissible level set by WHO (Tchana, et al., 2018). Moreover, in a research by H.L.Al-Mudhaf at 2016, the research found that the heavy metal levels in soft drink that is sold in Kuwait was lower than the documented ones in the previous research (H.L.Al-Mudhaf, H.M.Alzaid, & A.I.Abu-Shady, 2016).

Soft Drinks	The contamination of heavy metal residues (mean± standard deviation) (mg/L)							Location	References
	Mercury (Hg)	**Lead (Pb)**	**Cadmium (Cd)**	**Zinc (Zn)**	**Arsenic (As)**	**Tin (Sn)**	**Chromium (Cr)**		
Bottled Soft Drink	0.35 ± 0.28	0.85 ± 0.4	1.71 ± 1.42	7.36 ± 6.11	-	-	-	Yaoundé,Cameroon	Angèle N. Tchana, 2018
Canned and Bottled Soft Drink	ND	0.07±0.04	0.01±0.01	3.72±3.6	-	-	0.25±0.11	Kuwait	H. F. Al-Mudhaf, 2016
Canned and Bottled Soft Drinks	-	0.82-41.86	0.86-7.74	-	-	-	0.4-4.31	Andhra Pradesh, India	Aleti Jaya Sree, 2019

Canned and Bottled Soft drink	-	ND	ND	0.01	-	-	ND	Abeokuta,Nigeria	Adewale Matthew Taiwo, 2020
Canned and Bottled Soft Drink	-	0.006	0.004	0.50	-	-	0.01-0.08	Kazaure,Nigeria	Sunusi.S. , 2020
Canned Soft Drink	-	0.01-0.07	-	0.09-0.1	-	-	0.025-0.44	Okada, Nigeria	Hassan,A , 2018

Table 2.1 : Previous research on the accumulation of heavy metal residues in soft drinks

Table 2.2: Summary of WHO (2008), Malaysian Food Act 1983 & Regulations 1985, EU (2006)-reported overall residual limits (MRL) for heavy metals concentration (mg/L) in drinking water products.

Types of heavy metals	**WHO**	**EC**	**MFA**
Mercury (Hg)	0.006	0.05	0.05
Lead (Pb)	0.01	0.01	0.01
Cadmium (Cd)	0.003	-	0.003
Copper (Cu)	2.00	-	1
Zinc (Zn)	3.00	-	3.00
Arsenic (As)	0.01	-	0.1
Tin (Sn)	-	100	-
Chromium (Cr)	0.05	-	-

Source: ((EC), 2006), ((WHO), 2008), ((MFA), 2020)

The World Health Organization (WHO), the European Comission (EC), and the Malaysia Food Act have all detailed the Maximum Residue Limits (MRL) in Table 2.2. (MFA). To guarantee that the shrimp we eat are safe, the approved requirements must be observed.

2.3 Toxicity of heavy metals

2.3.1 Lead (Pb)

Lead is one of the most lethal heavy metals that we know today. It is present in the earth's crust or the products of human-made activities. Lead is present as the element of Group 14 in the Periodic Table. Lead has a density of 11.34 g/cm^3, a melting point of 327.4℃, and a bluish-grey solid. Lead has a high density, low melting point, and soft and malleable property. This makes lead is useful in our industry process where lead can be made into different kinds of products. In life, lead can be made into pipes, solder, weights, and batteries (Abadin, et al., 2007). In life, the authorities have limited the concentration of lead present in any material is zero (Registry A. f., 2019). However, the environment contains a small amount of lead. As lead is formed by nature, the lead present in the soil generally ranges from 15-40 ppm (Amherst). Surprisingly, we also consumed lead unexpectedly through water and food. Any toxic substances entering the body are being stored in our body, and it needs a long time to break down and excreted.

For an average human, the amount of lead present in the blood usually is 10 µg/dL for adults, as for children, it is 5µg/dL. Any amount that is higher than these readings is considered exposure to lead (Khatri, 2021). Ingestion of lead is associated with inflammatory reactions in the human immune system. Lead can reconfigure the cells in the immune system and caused cancer. Thus, lead is deemed carcinogenic for this reason (Ebrahimi, et al., 2020). Apart from that, lead also causes osteoarthritis, which leads can also cause delayed fracture healing and bone recovery (Park & Choi, 2019).

In contrast, lead can also be damaging pregnant women. As lead is present in the mother's body, it passes the placental barrier and harms the foetus. The concentration of Pb contained in umbilical cord blood is estimated to be even 80–100% of the maternal average (L.Gulson, J.Mizon, J.Korsch, M.Palmer, & B.Donelly, 2003). Apart from that, lead can also be causing neurotoxicity. A human that is affected by lead can also damaging their brains. This causes the slowing of reaction time, lowering of IQ, Alzheimer's, and depression. As a result, exposure to lead is hazardous for us. The lead element must be controlled and prohibited to flow into the environment at all costs.

2.3.2 Copper (Cu)

Copper perhaps is one of the most valuable metals ever discovered. It is mainly located underground. It can be used in various industry types, from electric, accessories, buildings, and others. Known for its high value and accessibility, copper is quite a popular metal around us. Copper has also appeared as one of the metals that are located in group 14. Copper also contains a boiling point of 1083.4℃, which makes copper a heat-resistant material. Copper appears as a solid with a reddish-orange solid, and it has a shiny metallic surface. Copper is also very malleable and ductile. This makes copper are easy to shape into different kinds of products (Helmenstine, 2019).

Copper is highly resistant to corrosion as copper takes the lowest place in the reactivity series. Its high corrosion resistance is used to make water pipes, jewellery, electrical cables, and radiators. Copper is also widely used in marine and aquaculture industries due to its corrosion resistance from water and air. Apart from that, copper also easily alloys with other metals. It can combine with other metals to serve different purposes. For example, copper and zinc can be integrated into brass. Brass is also one of the metal alloys widely used in industries (Association, 2018). Moreover, copper is also known for its excellent electrical conductivity. It posses a low electrical resistance than other metals. Thus, copper is widely used in electrical wires and cables (Association, 2018).

In the human body, we humans also needed a small amount of copper in our body. This is because that copper also catalyses our body's biological enzyme reaction. Copper is related to the production of the enzyme in the metabolism of several body components (Xia, et al., 2020). However, there will be side effects if a large amount of copper is ingested into a human body. Due to advanced mining, water pipes leaching, chemical pollution, some coppers had entered into water and food sources. This caused humans to ingest copper elements into their bodies unintentionally (Battogtokh, Jae.M.Lee, & Woo, 2014). A large amount of copper will cause Wilson disease if its present in our body (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Wilson disease will cause abnormalities in the human liver and disturbance in the nerve (Huster, 2010)

In a nutshell, copper is beneficial for us in small amounts but lethal in large quantities. Thus, control towards the amount of copper leaked into people’s livelihood must be enforced to prevent the deterioration of human health.

2.3.3 Mercury (Hg)

Mercury is one of the infamous toxic heavy metals that are present in our life. It is one of the typical metals that is known to humans due to its appearance. Mercury is known to exist as a liquid form even at room temperature. It belongs to the Periodic Table's 12th group. It has a low melting point of -38.829°C and behaves as a liquid silvery metal. (Mercury). Like every metal, mercury is also naturally present in the environment. Mercury is produced through weathering of rocks and from the eruption of the volcano. Mercury can be found in cinnabar ores. To obtain the mercury, the cinnabar is heated, and the vapour during the heating is then condensed to form mercury (Mercury). However, mercury is abundant in today’s society through coal-burning factory activities (What is Mercury ? Where does it originate ?, 2009).

Mercury also provides some uses in everyday human life. Due to its high density and high coefficient expansion, mercury is used in thermometers. It can react quickly towards any temperature changes. This makes us read the thermometer easily due to the expansion and contraction of the thermometer (Peshin, 2019). Many kinds of devices, such as barometers and diffusion pumps, include mercury. Switches, electrical apparatus, and batteries all also contain mercury in it (Sheffield). Furthermore, gaseous mercury is also used in mercury-vapour lamps and advertising signs. In some industries, mercury is also used in chlorine production and dental amalgams (Sheffield).

Though mercury is helpful in everyday life, it is known that mercury is poisonous to humans. Mercury can be present in food or water sources through environmental contamination. An average human must contain 10µg/L or below 20µg/L of mercury in blood. Humans will be deemed as overexposed if the mercury in the blood is present by 35µg/L (Ye, et al., 2016). The most common mercury exposure is through water or fish sources. Some water sources, such as lakes and rivers, contain mercury from pollution or biological sources. Our daily seafood has the best example of mercury contamination since mercury bonded onto these sea organisms. Furthermore, mercury can come through exhalation or touching (A.Bernhoft, 2012)

Intoxication of mercury can be caused many detrimental effects. Ingested mercury can destroy cellular function and configuring the enzymes in the body. This will be causing organ failure and destruction of cellular structure (A.Bernhoft, 2012). Moreover, different states in mercury are toxic to humans. The vapour state of mercury can result in human poisoning. Exposure to it can be causing coughing, fever, hallucination, and death if severe poisoning happens. In terms of inorganic mercury (Hg^{++}), inorganic mercury in the body can be reacting in the kidneys and causes an inflammatory reaction that stresses the immune system in our body (Carocci, Rovito, Sinicropi, & Genchi, 2014).

2.3.4 Chromium (Cr)

Chromium is also known as one of the heavy metal elements that are present in our environment. It is known as the symbol Cr, and it is situated in group 6, period 4 of the element periodic table. Chromium appears as a steely-grey and shiny metal. It contains a glossy surface and highly resistant to corrosive properties. Chromium is also very unstable under oxygen. It forms a protective layer that protects the metal below to prevent corrosion. Thus, chromium is a good material for alloys due to its corrosive resistance ability (Lennetech).

Chromium is also widely used in automobile industries. Chromium is used as a plating on cars and other vehicles. This is to prevent the metal on the car body to corrode by environmental factors. Moreover, chromium is also widely used in some industrial processes as a catalyst. Chromium is also used in some paints and dyes to make them look shiny (Stewart, 2012). Chromium also presents in our body in small amounts. Humans obtain chromium through everyday food sources such as seafood, vegetable, or mushrooms. As chromium is ingested in small amounts, the chromium will be reduced by our gastrointestinal tract from Cr (VI) into Cr (III). The Cr (III) ions will then bind with the plasmas in the blood and circulate in the body. In our body, Cr(III) ions are then bound onto nucleic acid, which then prevents the RNA from denaturation. Then, some Cr(VI) and Cr(III) ions are then excreted through milk, sweat, hair, and nails (Achmad, Budiawan, & Auerkari, 2017).

However, chromium is deemed carcinogenic and toxic by the authorities. A large amount of ingestion of chromium into our body can cause many side effects. Chromium in large amounts can irritate and obstructing the airway. Inhalation of chromium gases will also cause asthma and chronic bronchitis. This will affect the respiratory system significantly. If the chromium is in contact with the skin, chromium will also cause skin allergies in some people. If a person is allergic to chromium, swelling and irritation of the skin can be seen. In terms of carcinogenic properties, chromium can cause nose and lung cancer. Cr(VI) reaction with reductant and oxidiser in our body will cause the DNA to be damaged and mutated (Achmad, Budiawan, & Auerkari, 2017).

There are some cases of chromium contamination that are happening in this world. The primary source of chromium contamination is the contamination in the drinking water. There is some industrial that produces tanning salt, which is known as chromium sulphate. These industries will release chromium-based waste into nearby water sources or ground. This is due to uncontrollable causes of chromium-based waste disposal happens (Bhattacharya, et al., 2019). The authorities should enforce more to prevent chromium contamination from happening around us.

2.3.5 Zinc (Zn)

Zinc is also one of the metals that are widely used in our life. Zinc is a metal that appears as a bluish-white coloured metal. It is located among the group 11 elements in the Periodic Table. Zinc is also malleable and elastic when it is heated to 100℃. Zinc is a very reactive material that can react with both acid and alkali materials; thus, it is deemed amphoteric metal. If zinc is exposed to air, it reacts with air to form a soft grey-coloured zinc oxide coating layer. Due to its reactivity, zinc also can combine with other materials to form zinc compounds (Chemistry).

Due to its high resistance towards corrosive, it is widely used in the construction and metal industry. Zinc is generally used as coatings in pipes, building parts, or parts on a vehicle. This enables to protect them from severe corrosion. Zinc can also be found in castings by combining other materials to form a zinc compound (AZoM, 2001). Zinc is also one of the beneficial metals that are present in our body. Zinc is deemed as an essential element for cell building and organ function. Its role as a catalyst in many enzyme reactions in our body. Besides, zinc also essential in the gene transcription process. It provides a functional domain for the DNA transcription process. Furthermore, zinc is also vital in early childhood brain development (Dıaz-Gomez, et al., 2003).

Zinc is present in every part of the environment. For example, there is approximately 1.2 % of zinc element present in the river sediment, but the amount of zinc will be increased after a long time (Shikazono, Zakir, & Sudo, 2008). Zinc contamination is also one of the primary concerns in today's society. Many of the zinc contamination is related to automobile emissions. For instance, zinc is one of the main components of car tyres. As the tyres kept wearing out, zinc will be released into the environment.

Zinc can be fatal to us when it is ingested too much. This phenomenon usually is happening in the industrial field. As humans inhale many zinc particles, they will experience fever, nausea, muscle pain, and respiratory problems. However, these symptoms can be reduced if proper treatment is administered. However, too little zinc in our body will cause health problems too. Zinc deficiency will cause decreased wound healing, decreased nerve conduction and even infertility (M.Plum, Rink, & Haase, 2010).

2.3.6 Arsenic (As)

Arsenic is also one of the infamous lethal heavy metals that is present in the environment. Arsenic is a type of metalloid that appears in a bright silver-grey colour. It is very brittle and has no mechanical properties. Under the high temperature of 270℃, arsenic will form several isotopes such as black, yellow and brown arsenic. Arsenic is commonly found under the soil and present in water or underground (Habashi, 2013).

Arsenic is always used as alloys to develop a more rigid and durable metal. For transistors, arsenic is used as a doping agent normally. Arsenic also combines with other elements to form arsenic compounds. For example, Gallium arsenide is used in laser to convert electricity into coherent light. In agriculture, arsenic compounds such as Paris green and calcium arsenate are used to produce pesticides. In pyrotechnics, arsenic is widely used to change the colour of the flame (Pedersen, 2016). Arsenic is found in the natural environment as a compound such as arsenopyrite, realgar and orpiment. These compounds have to undergo an extraction process to obtain pure arsenic (Pedersen, 2016).

Arsenic is very famous due to its toxicity towards the human. It is very lethal even ingested in a small amount. Arsenic contamination is spread throughout the environment through human-made activities such as mining, factory processing and overuse of pesticides. This has caused the contamination of arsenic on water, soil, and even food sources. Authorities have limited the arsenic content in various food and water sources (Singh, Singh, Parihar, Singh, & Prasad, 2015). Arsenic is mainly contaminated through inhalation or ingestion. Some of our organ parts can be found having arsenic in them such as skin, lungs, liver, and kidneys. Prolonged exposure to arsenic will cause skin lesions and disturbance in the neurons. As in the respiratory system, arsenic will cause chronic cough, bronchitis, and shortness of breath. In short, arsenic can affect various body systems and causes severe illness (Sudheera, Ediriweera, & Jayasumana, 2015).

2.3.7 Tin (Sn)

Tin is deemed as one of the most popular metals that we know today. Tin or known as Sn is located in group 14 of the periodic table. It has existed as a metal with a melting point of 232℃. It appears as a metallic solid which is silver-white colour looking. Tin exists in 2 forms, α-tin, and ß-tin. ß-tin is known to be very malleable while α-tin is cooled to a temperature below 13℃ to change into a crumbly grey powder (Tin). Tin is not exceedingly abundantly available in the natural environment. It only exists at some places in some regions in southeast Asia (Tin).

Tin is handy since the beginning of the industrial era. The most famous uses of tin are the production of food cans. Tin that is made into tinplate are being used in food can beverage cans. In the electrical and piping industry, tin is used as solders to produce various electrical appliances. Meanwhile, in the piping industry, tin is used as a stabiliser in PVC and polymer production. As in the automobile industry, tin is used in lead-acid batteries and some parts in vehicles (association, 2020). One of the main reasons for tin contamination is canned food and beverages. Some of the tin content will dissolve in this food or drinks if the plain uncoated surface in the cans is used. Thus, people that consume canned foods or beverages have a high risk of ingesting tin into their bodies (Blunden & Wallace, 2003).

As far as it goes, inorganic tin is not dangerous to our health. If a human ingested or inhaled a large amount of inorganic tin into their body, their respiratory system and caused skin and eye irritation. Moreover, tin would also be causing problems in the liver, kidney, and stomach if ingested in a large amount (Registry, 2005). Thus, the control towards tin contamination is crucial to prevent contamination cases.

2.3.8 Aluminum (Al)

Aluminium is one of the most useful metals that is present in society nowadays. Aluminium is situated in the 13th group of the element periodic table. It appears as a metallic silvery-white solid. Aluminium is widespread around the earth's crust; thus, aluminium is very easy to obtain. Aluminium has ductile, malleable and very durable properties. The aluminium is also corrosive-resistant and light-weighted, making it a convenient material to transport and process. Apart from that, aluminium also able to combine with other metals to form alloys. Aluminium is obtained through the bauxite ores, and it is produced through electrolysis smelting of the ores and obtaining the solid aluminium (RUSAL).

Aluminium can be seen in everyday life. In electronic industries, aluminium has recently become the primary conductor in some electrical appliances and music amplifiers. This makes them a good substitute for coppers. The most famous uses for aluminium are in food packaging. Cans, tins, and tetra bricks are produced using aluminium brick, making them resistant to heat and prevent oxygen from entering the drinks. Thus, aluminium-based packaging is being famous for preserving beverages product. Additionally, aluminium is also used in automobile industries to build frameworks for cars and bicycles (STAC).

As aluminium is ubiquitous in our lives, the contamination of aluminium can be happening any time. Aluminium is deemed to be lethal of a large amount of it is congested. Aluminium ingestion could have come from occupational hazards and leaching of aluminium-based food packaging and other sources. Thus, WHO authorities have limited that the amount of aluminium in our body is limited to 2mg/kg of body weight. If someone is contaminated with aluminium, aluminium is a potent neurotoxin for humans. In clinical reports, aluminium can affect the human brain and nervous system. People affected are suffering from memory impairments and dementia if severe contamination happens (Klotz, et al., 2017). Thus, it is advisable to look after the aluminium contamination cases that are happening in everyday life.

2.4 Impact of heavy metal on environment

As we know, heavy metal has many side effects on the human body. However, research has found that heavy metal element has also brought a massive impact towards the environment. Normally, the heavy metal that is under the earth comes from volcano eruption and weathering of rocks. Through the modernisation of society, heavy metals may leak into the environment, thus changing the quality of the environment (Tchounwou, Yedjou, Patlolla, & Sutton, 2012).. In today, sea ecology toxicity is the most widespread source of heavy metal pollution in nature.. Most of the leading reasons that cause heavy metal contamination in the marine ecology are oil spilling. As a result, these heavy metals will be absorbed by the marine organisms through ingesting and breathing. Eventually, many kinds of seafood like molluscs, fish, prawns contain many heavy metal sediments in their organ and body. As humans eat them, these heavy metals will go into the body (Naser, 2013). Thus, frequent monitoring and law enforcement must be done to protect the marine ecosystem.

Moreover, heavy metal also having a detrimental effect on the agriculture system. As human activities are occurring, the heavy metal components are entering the soil through these activities. Heavy metal elements present under the earth can change the soil's biological properties. This is because the microorganism in the ground is affected in terms of their activity and the numbers. As this phenomenon occurs, the health of the plants is also at stake. The abundant heavy metal in soils can cause cell structure damage in plants causing many enzymes to be inhibited. This can cause death to the plants after a long time exposed to the heavy metal. Thus, bioremediation on heavily polluted soil is being researched to minimise heavy metal pollution (S.C.Obiora & Chibuike, 2014).

2.5 Method of study on heavy metal residue

There are many studies about heavy metals that have been done nowadays. Since heavy metal is a global concern, higher study institutes keep developing new methods to study heavy metal residues. The most famous method of studying heavy metal residue is through an Atomic absorption spectrometer (AAS). AAS works by absorbing the radiation that is emitted by the chemical element (Farrukh, 2011). Many types of AAS, such as Flame Atomic Absorption Spectrometer (FAAS), Graphite Funnel Atomic Absorption Spectrometer (GFAAS), Electrothermal atomisation atomic absorption spectrometer (ETAAS), and Chemical vapour generation atomic absorption spectrometer (CVAAS), are being used nowadays in any research institute (Ferreira, et al., 2017). In the heavy metal determination of soft drink, using FAAS and GFAAS are the most popular methods.

FAAS is a type of AAS that using the flame as an atomiser. It can absorb radiation that is below 230nm. FAAS can usually detect some elements like sodium, potassium, calcium, and magnesium in other research. However, FAAS can only detect sample that has the amount of 1–100 mg/L and only needs the volume of 1-2µL (Sperling, 2006).

FAAS is operated through series of operations. First is through nebulisation, the sample is reduced and mixed well with the fuel and oxidant gases. Then, the solution is in aerosol form. As the liquid passes the flame, it undergoes evaporation, leaving solid particles. As heat is more substantial, condensation will take place. The higher heat will then evaporate the sample. At this point, the target metal is still bound onto an anion. As stronger heat is applied, the molecule dissociates into individual atoms, making the FAAS easier to detect (Kerber, Beaty, & D., 1993).

The advantage of using FAAS is that it requires low cost as this equipment is cheaper than the others. Moreover, FAAS also easily to be controlled and handled. It is easier to manage than the rest of the AAS model. Furthermore, the FAAS also had the advantage of the speed of operation. Since FAAS requires less time to analyse the samples, it can significantly shorten the time to obtain the results (Spiver, 2015). However, FAAS also has its disadvantage. FAAS is less sensitive and requires a larger sample to detect as it can only detect a small amount of sample only (Kerber, Beaty, & D., 1993).

GFAAS is also a machine that is widely used in the research field. GFAAS is popular in using detect minerals in ecological samples. The sample volume in GFAAS is 20-30µL, which is higher than the volume in FAAS. GFAAS is prepared by integrating the peak areas for absorbance measurements (Béni, Karosi, & Posta, 2007; Doker, Uzun, & Denizli, 2013).

GFAAS is mainly measuring and dispensing a specific volume of sample into the furnace. The sample is firstly undergoing a multi-step temperature program. When it reaches a particular temperature, the sample will start to atomise, and the absorbance reading is done (Kerber, Beaty, & D., 1993). The advantage of using a GFAAS is that the sensitivity is far greater than the FAAS. This is because that the atomised sample is being focused on a heated cell. Furthermore, the GFAAS operation procedure is much more automated than the rest. This enables the process to run automatically, thus reducing the work burden. Finally, GFAAS also analyses a wide variety of samples, making it a very applicable technique (Kerber, Beaty, & D., 1993).

2.6 Atomic Absorption Spectrometer

Atomic Absorption Spectrometer (AAS) is a very popular instrument that is present in today's world. It is a very applicable technique for determining some substances in a very small amount. Today, AAS is used in various industries to assess certain compounds. AAS have many models and containing many different components to determine the amount of samples present. In general, the AAS is working by which free gaseous samples absorb electromagnetic waves to produce a measurable signal (E.H.Ivanova, 2005).

AAS works in a very specific way. AAS consists of a light source that is known as a hollow cathode lamp (HCL) which giving out particular frequency of light which only can be absorbed by the analyte, which analyte will convert the sample into gases form that absorbs light of HCL, which then also serves to isolate and determine the specific wavelength. AAS also attached to a digital system to control the instrument operation and collect and process the analysis data. To prepare the samples for AAS, the samples are converted into an aqueous solution through digestion procedures to minimise the error and provides the accuracy of the data (D.J.Butcher, 2005).

Although AAS are widely used nowadays, there is a more improved and modern type of AAS that are being introduced nowadays, such as Graphite Furnace Atomic Absorption Spectrometer (GFAAS). However, AAS also contains some advantage and disadvantage in it. For the advantage of AAS, AAS is cheap and easy to handle. Moreover, the AAS are also sensitive towards the elements. This is because that the specific element atoms are only able to absorbed by a specific wavelength of light (J.O, 2016). This will ensure there are no isotopes or any foreign atoms that are accidentally determined in the AAS. As for the disadvantage, AAS can only detect a limited amount of elements, including earth metals. Moreover, AAS are not able to detect non-metal (J.O, 2016).

2.7 Health risk assessment

The key goal of health risk assessment is to determine the extent and likelihood of negative health consequences in humans that could be exposed to toxins in the environment. (EPA, 2016). It is a type of program which undergoes multiple steps to calculate scientific data to estimate the risk on human health. It contains a range of data from different studies that can inform an assessment in health risk assessment. Exposure data are then calculated with different pharmacokinetics and pharmacodynamic models to understand the dose, mechanism, target, and other aspects of the toxicity. In health risk assessment, it contains many steps. First of all, key issues and some questions about toxicity are addressed. Next, hazard identification is made to identify the environmental agent that causes hazards towards the human. Furthermore, dose-response is addressed to identify the uncertainty and variability of the data (Cote, Vandenberg, Druwe, & Angrish, 2019)

Target Hazard Quotient (THQ)	Mercury (Hg)	Lead (Pb)	Cadmium (Cd)	Zinc (Zn)	Arsenic (As)	Tin (Sn)	Chromium (Cr)	References
	NA	0.42	0.35	NA	NA	NA	0.38	(Nabi Shariatifar,2020)
	0.0057-0.0343	0.0014-4.15	0.0097-10.6	NA	0.019-6.0	NA	NA	(Rose Ngozi Asomugha , 2021)
	NA	0.0266-0.077	0.23-1.088	0.003-0.008	0.267-0.751	NA	NA	(John Adekunle Oyedele Oyekunle, 2019)
	NA	$8.79x10^{-5}$	$1.07x10^{-4}$	$3.60x10^{-5}$	$1.35x10^{-3}$	NA	$2.4x10^{-4}$	(Mahmoud Ghuniem, 2020)
Estimated Daily Intake	NA	NA	NA	$5.28E^{-4}$	NA	NA	NA	(Nabi Shariatifar,2020)

(EDI) (µg/L/day)	NA	$8.9x10^{-5}$ – $27x10^{-5}$	$13x10^{-5}$ – $44x10^{-5}$	$65.2x10^{-5}$ - $250.3x10^{-5}$	$1.4x10^{-5}$ - $22.4x10^{-5}$	NA	NA	(John Adekunle Oyedele Oyekunle, 2019)
	$7x10^{-6}$ – $3.43x10^{-5}$	$5.7x10^{-6}$ – $1.6x10^{-2}$	$9.7x10^{-6}$ – $1.06x10^{-2}$	NA	$5.7x10^{-6}$ – $1.5x10^{-3}$	NA	NA	(Rose Ngozi Asomugha , 2021)
	NA	$5.63x10^{-7}$	$2.8x10^{-7}$	$1.96x10^{-5}$	$1.05x10^{-6}$	NA	$7.95x10^{-7}$	(Mahmoud Ghuniem, 2020)

*NA: Not available

Table 2.3 shows the previous studies in health risk assessment of Soft drink

CHAPTER 3

METHODOLOGY

3.1 Analytical Instrumentation

Using the Atomic Absorption Spectrometer (AAS) (Perkin-Elmer (PE) 3300), the concentration level of heavy metals is calculated and then monitored by a computer auto-sampler (PE AS 60).

3.2 Chemical Reagents

Table 3.1 below lists all of the chemicals and reagents that were used to perform in this study. All of the chemicals and reagents used were in analytical grade

Table 3.1 List of chemical reagents used

No.	Chemicals and Reagents
1	Concentrated nitric acid (HNO_3)
2	Lead nitrate (Pb(NO3)2)
3	Cadmium nitrate, Cd(NO3)2
4	Zinc nitrate, Zn(NO3)2
5	Copper Nitrate $Cu(NO_3)^2$
6	Deionised water

3.3 Apparatus

All the apparatus, scientific instruments and equipment used in the laboratory tabulated in Table 3.2.

Table 3.2 List of laboratory apparatus

No	Apparatus
1.	Volumetric flask 50 mL
2.	Micropipette P 1000 and P 10 (5 µL to 1000 µL)
4.	Laboratory beaker
5.	Conical flask 100 mL
6.	Atomic Absorption Spectrometry
7.	Winlab 32^{TM} AAS computer software
8.	Hot Plate
9.	0.45µm syringe filter
10.	Syringe
11.	Stirring rod
12.	Laboratory fume hood
13.	50mL Volumetric Flask
14.	50 mL Falcon Tube
15.	Glass Dropper

3.4 Collection of Samples

The samples of soft drink are brought from a grocery store. 5 different brand of canned soft drinks is brought from the grocery store in Jeli, Kelantan.

3.5 Preparation of blank reagent solution

The blank samples are prepared by mixing 5mL distilled water with 5 mL of HNO_3 and was made up to 50mL by using distilled water in a conical flask.

3.6 Preparation of Standard Calibration Solution

Stock solution for each metal (Pb, Cd, Zn, Cu) at the concentration of 1000 mg/L was prepared. Standard calibration solution for each metal at a concentration ranging from 5 µg/L to 100 µg/L was prepared by diluting the stock solution of heavy metals with deionised water

3.7 Standard Calibration Solution for Pb, Cd, Zn, Cu

3.7.1 Standard Calibration solution for Pb

For the standard calibration preparation of Pb with a concentration range from 0.50 mg/L to 10.00 mg/L, a constant volume of the Pb standard stock solution was prepared and diluted with deionised water. The diluted Pd standard calibration solution with a concentration range from 0.50 mg/L to 10.00 mg/L was installed into the AAS

3.7.2 Standard Calibration solution for Zn

For the standard calibration preparation of Zn with a concentration range from 0.50 mg/L to 4.00 mg/L, a fixed volume of the Zn standard stock solution was prepared and diluted with deionised water. The diluted Zn standard calibration solution with a concentration range from 0.50 mg/L to 4.00 mg/L was installed into the AAS.

3.7.3 Standard Calibration solution for Cd

For the standard calibration preparation of Cd with a concentration range from 0.00 mg/L to 0.376 mg/L, a fixed volume of the Cd standard stock solution was prepared and diluted with deionised water. The diluted Cd standard calibration solution with a concentration range from 0.00 mg/L to 0.376 mg/L was installed into the AAS.

3.7.4 Standard Calibration Solution for Cu

A fixed volume of Cd standard stock solution was diluted with 40 L of 0.5 % v/v nitric acid in a 50 mL volumetric flask and then diluted to the required volume with deionised water for standard calibration at concentrations ranging from 2.00 mg/L to 20.00 mg/L. After that, AAS was set with normal calibration solution concentrations ranging from 2.00 mg/L to 20.00 mg/L.

3.8 Preparation of Sample

The soft drink that is purchased from the grocery store were stored at room temperature until it is ready to be analysed by using an Atomic Absorption Spectrometer (AAS).

3.9 Sample Analysis for Determination of Heavy Metal Residues by Atomic Absorption Spectrometer (AAS)

First of all, 5 mL of soft drinks was added with the addition of 5mL of concentrated nitric acid (HNO_3) and 5mL of distilled water. The solution was poured into a conical flask. The conical flask was heated with a hot plate till the volume is about 5 mL to break the complex bond of the components in the soft drink. Red fumes will appear due to the release of nitric acid. Then, the solution is filtered into a 100mL beaker, and distilled water was added to made-up into 50mL by using a 50mL volumetric flask. The solution was mixed well and poured into a 50 mLfalcon tube. The solution is also done with serial dilution before analysis. The solution is then left to analysis by AAS (O.O., O.E., A.E., & O.O., 2015).

3.10 Calculation and evaluation of the result

The concentration (C) of each heavy metals in the sample will be calculated according to the equation (3.1) below:

$$\boldsymbol{C = \frac{(a - b) \times V}{m \times 1000}}$$

Where,

C= Concentration in the test samples (µg/L)

a= Concentration in the test solution (µg/L)

b= Average concentration in the blank solution (µg/L)

V= Volume of the test solution (mL)

m= Weight of the test portion

3.11 Survey conducted

There was a survey conducted made by using Google Forms targeting people all around Malaysia as respondents. The survey comprises two sections, A and B. Both sections are on the socio-demographic and to know the perceptions of Kelantan residents on the seafood intake as food resources and frequency of intake of seafood species in a week and a month. There are a total of 300 respondents taking part in this survey all around Malaysia.

3.12 Estimated daily intake of soft drink in Malaysia

The estimated daily intake is to estimate the amount of soft drink that Malaysian people take for one day (Dee, Abdullah, Nasir, & Appalasamy, 2019).

3.12.1 Estimated Daily Intake (EDI)

Estimated daily intake (EDI) (μg/kg body weight) of heavy metals from soft drinks consumption was obtained using the following equation:

$$EDI = \frac{(C_{metal} \times IR)}{BW}$$

Where,

C_{metal} = Heavy metal content weighted average (μg/g)

IR = Ingestion rate daily soft drink consumption (gram/day person)

BW= Average body weight

3.12.2 Estimated Weekly Intake (EWI)

The average weekly consumption was calculated by multiplying the EDI by a factor of seven, which equals seven days (EWI). Locals, on the other hand, eat soft drink on two days a week, according to 43 questionnaires. (Dee, Abdullah, Nasir, & Appalasamy, 2019). Therefore, using the following equation, EWI was obtained:

$$EWI = EDI \times 7 \times \frac{2}{7}$$

3.12.3 Target Hazard Quotient (THQ)

The target hazard quotient has long been used to determine the danger of eating metal-contaminated food.The target hazard quotient (THQ) was calculated using the following equation to determine if heavy metal toxicity in soft drinks poses a non-carcinogenic risk. (Dee, Abdullah, Nasir, & Appalasamy, 2019)

$$THQ = \frac{EF \times ED \times IR \times MC}{RfD \times BW \times AT} \times 10^{-3}.$$

Where,

EF = Exposure frequency for average consume

ED = Exposure duration (70 years) is comparable to a human lifetime.

IR = Ingestion rate (g/person/day)

MC = Metal concentration in soft drink (µg/kg,ww)

RfD = Oral Reference Dose

(Pb: 3.5µg/L/day , Cd: 1µg/L/day , Zn: 300µg/L/day, Cu:400 µg/L/day)

BW= Average Body Weight (Kg)

AT= Averaging time for non-carcinogens

3.12.4 Hazard Index (HI)

The sum of the hazard quotients is the hazard index determined from THQ. By using this formula, the hazard index can be determined. If the THQ is less than 1, there is no obvious risk (Dee, Abdullah, Nasir, & Appalasamy, 2019).

$$HI = THQ_{Pb} + THQ_{Cd} + THQ_{Zn} + THQ_{Cu}$$

Where,

THQPb = Targat Hazard Quotient of Lead, Pb

THQCu = Target Hazard Quotient of Cooper, Cu

THQZn = Target Hazard Quotient of Zinc, Zn

THQCd = Target Hazard Quotient of Cadmium. Cd

RESEARCH FLOW CHART

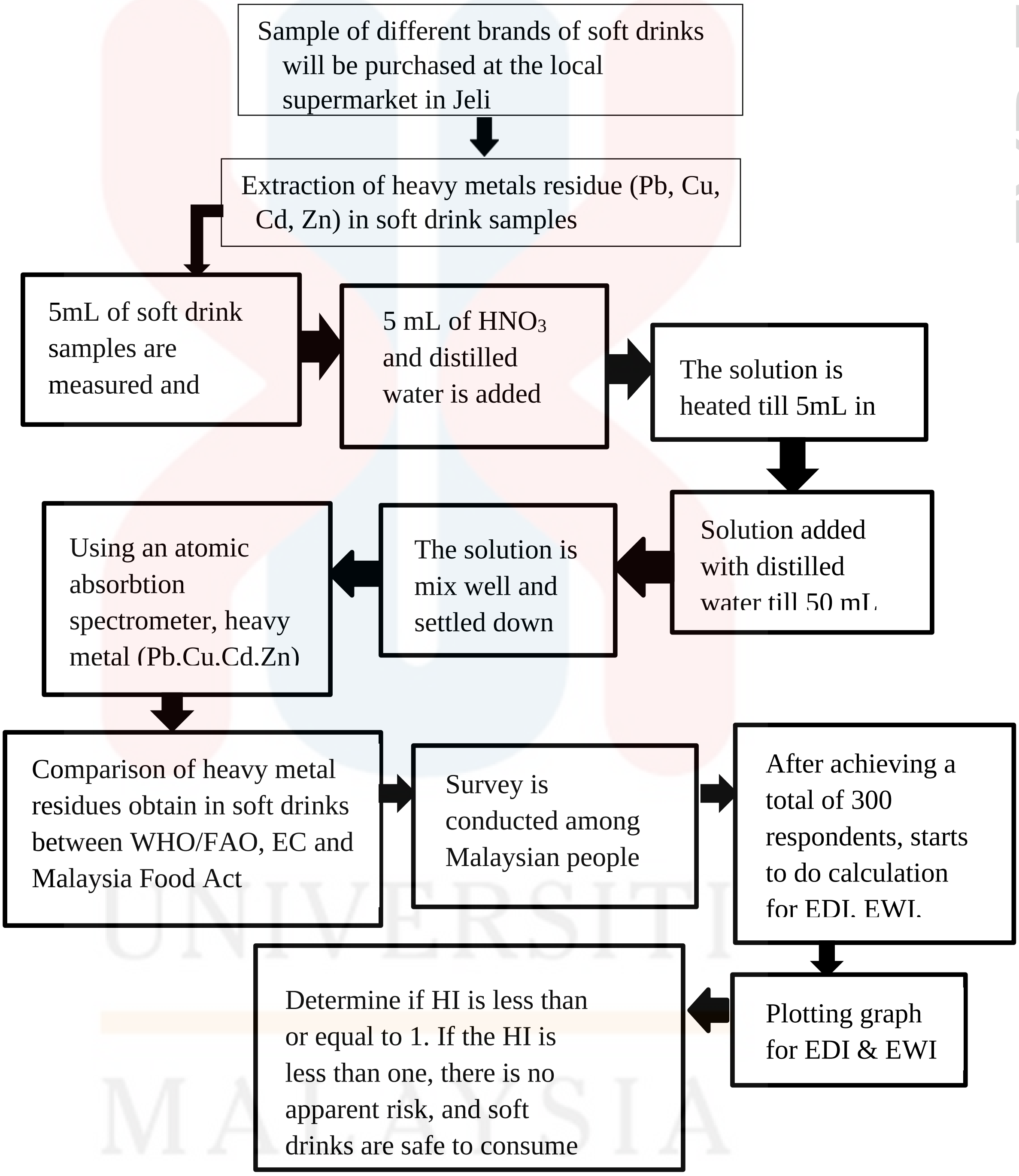


Figure 3.1: Flow chart of research activities.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Sample Collection

The samples were bought from local supermarket and grocery store in Jeli, Kelantan.

4.2 Samples Extraction

First of all, 5 mL of soft drinks was added with the addition of 5mL of concentrated nitric acid (HNO_3) and 5mL of distilled water. The solution was poured into a conical flask. The conical flask was heated with a hot plate till the volume is about 5 mL to break the complex bond of the components in the soft drink. Red fumes will appear due to the release of nitric acid. Then, the solution is filtered into a 100mL beaker, and distilled water was added to made-up into 50mL by using a 50mL volumetric flask. The solution was mixed well and poured into a 50 mLfalcon tube. The solution is also done with serial dilution before analysis. The solution is then left to analysis by AAS (O.O., O.E., A.E., & O.O., 2015).

4.3 Metal concentration in different soft drink samples

In many soft drink brands, 5 random soft drink were selected as the samples in this experiment. The samples were analysed to determine the Lead, Copper, Cadmium and Zinc concentration in it. The average concentration of the heavy metal in the samples were presented by mean and standard deviation are tabulated and compared in Table 4.1. The measurements are recorded on a wet weight basis of microgram per liter (µg/L).

Among the heavy metal concentration, each of every metal in the soft drink samples were varied. For instance, Zinc (Zn) has the highest concentration which give an amount of 0.509µg/L while the Copper (Cu) has the least amount in the samples which have to be declared as not detected (ND). This is related to the AAS machine's limited detection (Table 4.1) in determining the presence of heavy metal residues in soft drink samples.

Determined on the basis metal concentrations in various soft drink brands (Table 4.1), the highest concentration of lead (Pb) is in the Sample A brand which is 0.355µg/L; level for zinc is in sprite which is 0.509µg/L.; level for Cadmium (Cd) is in Sample B which is 0.036µg/L. While for Copper (Cu), every sample have a very little amount for copper which results in negative value in the AAS machine. Thus, the copper metal concentration in every soft drink samples were declared as not detected (ND).

The calculation of average concentration of heavy metal (Pb,Cu,Zn,Cd) evaluated in the samples were recorded in µg/L unit provided the following outcomes; Pb: 0.2194, Cu: ND, Zn: 0.1708, Cd: 0.0254. This gives the ranking Pb>Zn>Cd>Cu. The presence of heavy metals in each of the soft drink samples was compared to the authorised limits by Malaysian Food Regulations (MFA) (1985),European Comission (EC) (2006) and WHO (2008). The levels for all the heavy metal were within the permissible limits of MFA,WHO and EC except for lead in Sample A and Sample B and all of Cadmium metal.

Table 4.1: Level of heavy metal concentration (µg/L) in different soft drink samples

Soft Drink Brands	Heavy metal concentration (µg/L) Mean±SD			
	Vital Element		Toxic Element	
	Zn	Cu	Pb	Cd
Sample A	0.071±0.0018	ND	0.309±0.00114	0.035±0.0009
Sample B	0.111±0.0023	ND	0.355±0.0129	0.036±0.0006
Sample C	0.051±0.0011	ND	0.159±0.0051	0.018±0.0007
Sample D	0.509±0.001	ND	0.162±0.0083	0.021±0.0015
Sample E	0.112±0.0001	ND	0.112±0.0036	0.017±0.0012
Permissible Limit (MFA)	3.0	1.0	0.2	1
Permissible Limit (EC)	-	-	0.01	-
Permissible Limit (WHO)	3.0	2.0	0.01	0.003

*ND: Not Detected

4.4 Result Analysis of vital elements

4.4.1 Copper (Cu)

Copper is a vital element that is needed in our bodies. In the soft drink samples, all the samples were contain a very little amount of copper in it which is beyond the AAS machine's detection limit. Thus, the amount of copper that is detected in the samples were known as not detected. Based on the table 4.2, the amount of the copper were very low till it is undetecetable. The amount of the copper that is detected in the samples were way below the permissible limits of MFA and WHO.

Table 4.2: Comparison of level of Copper (Cu) detected in soft drinks analysed with the maximum permitted level set by regulations

Soft Drink Brands	**Level of Cu (µg/L) Mean±SD**	**Maximum permitted level of Cu in soft drink**		
		MFA (1985)	EC (2006)	WHO (2008)
Sample A	ND			
Sample B	ND			
Sample C	ND	1.0	-	2.0
Sample D	ND			
Sample E	ND			

*ND: Not Detected

Copper is a essential mineral to our body. It activates the fundamental metabolic protein synthesis which helps our body metabolism. Anemia, weight loss, and abnormal physiological development are among symptoms of copper deficiency. Abnormal eating habits result in neurological, hepatic, and renal problems (Castro & Baccan, 2004). Copper has been found as a contaminant in a variety of environmental samples, including water, soil, and food, and is one of the Environmental Protection Agency's priority pollutants (EPA) (Xu, Zhu, Shao, Huang, & Luo, 2018). In a recent research done by Angèle N. Tchana, the concentration of copper in their research is 18±12.41μg/L, which is way higher than the result in this research. By comparison, the concentration of copper in this research is much lower (Tchana, et al., 2018).

4.4.2 Zinc (Zn)

Zinc is also one of the essential mineral in our body. In the samples, the level of zinc is way less than the permitted level of zinc by MFA and WHO. As shown in Figure 4.1, all of the samples were did not exceed the permissible level. In the samples, Sample D has the highest concentration of zinc which gives 0.509±0.001μg/L while Sample C has the lowest concentration of zinc which gives a number of 0.051±0.0011μg/L. All of the concentration were not exceeded the permissible liit of MFA and WHO which is 3.0μg/L.

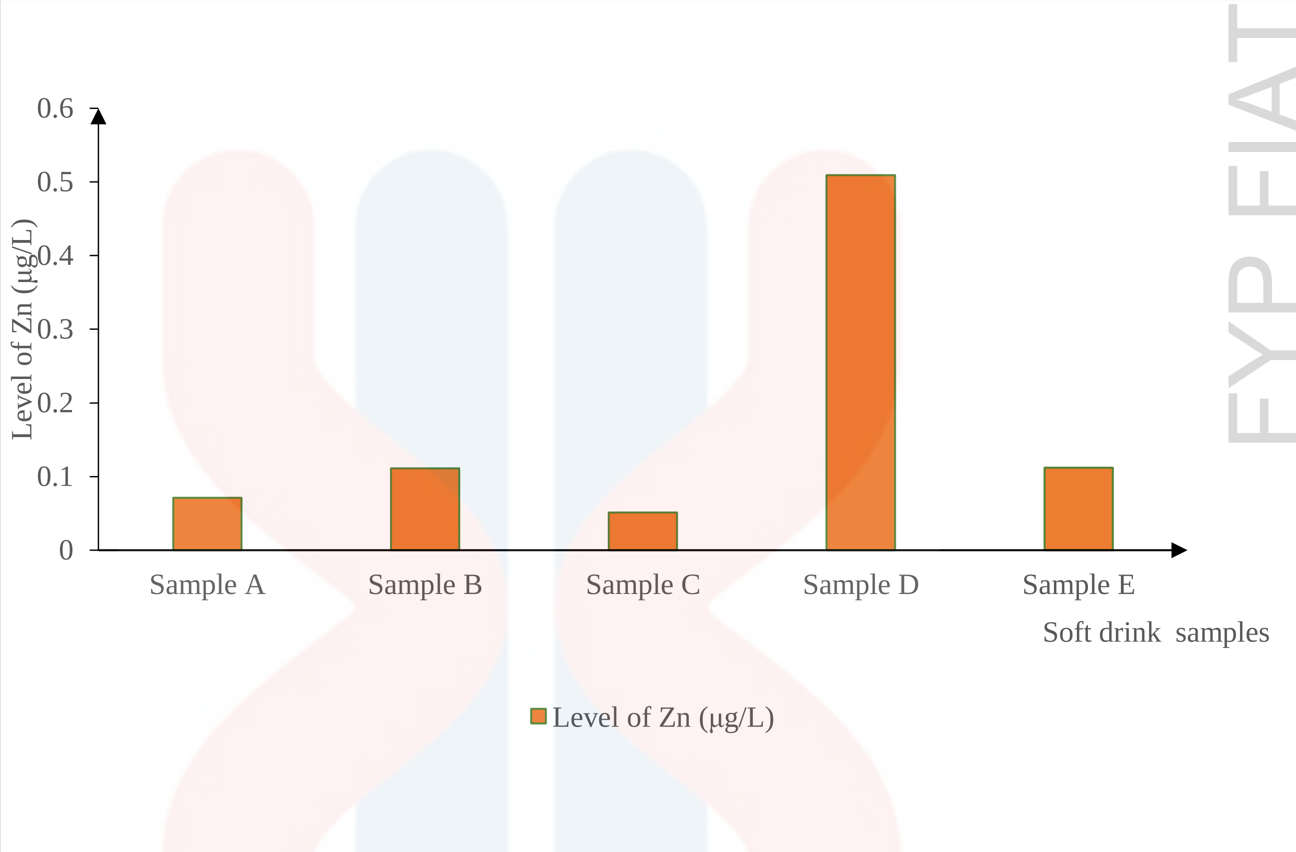


Figure 4.1: Zinc,Zn concentration in soft drink samples

From the result obtained in Table 4.3, the zinc metal concentration in every soft drink samples were do not exceed the limit of MFA (1985) and WHO (2008). The highest mean concentration that has been observed is Sample D which is 0.509±0.001µg/L, followed by Sample E which is 0.112±0.0001µg/L, then by Sample B which is 0.111±0.0023µg/L, then followed by Sample A which is 0.071±0.0018µg/L. Lastly, Sarsi has the least number which is 0.051±0.0011µg/L.

Table 4.4: Comparison of level of Zinc (Zn) detected in soft drinks analysed with the maximum permitted level set by regulations

Soft Drink Brands	**Level of Zn (µg/L) Mean±SD**	**Maximum permitted level of Zn in soft drink**		
		MFA (1985)	EC (2006)	WHO (2008)
Sample A	0.071±0.0018	3.0	-	3.0
Sample B	0.111±0.0023			
Sample C	0.051±0.001			
Sample D	0.509±0.001			
Sample E	0.112±0.0001			

In natural environment, Zinc (Zn) metal is commonly occurred below the earth's crust. For zinc, Zinc is essential to our body. Zinc is normally required for wound healing (tissue repair, such as collagen) and reproduction. Some minerals such as iron, zinc, and calcium,are used in diverse formulations as supplemental meals. Zinc oxide is the most often used chemical in product fortification because it is easily absorbed, generates organoleptic changes, and is much less costly than other zinc compounds (K.Lutter & Dewey, 2003). However, Sideroblastic anaemia, hypochromic microcytic anaemia, leukopenia, lymphadenopathy, neutropenia, hypocupraemia, and hypoferraemia were all symptoms of ingesting too much zinc (Ngirau, 2007). In the previous research, the mean concentration of zinc in that research is 3.72 µg/L which is way lower than our mean zinc concentration which is 0.1708µg/L (H.L.Al-Mudhaf, H.M.Alzaid, & A.I.Abu-Shady, 2016).

4.5 Result Analysis of toxic elements

4.5.1 Lead (Pb)

Lead was detected in all of the soft drink samples. Only two samples , Coke and Pepsi shows a high amount of lead concentration compared to the other samples. The highest sample is Sample B which is 0.355±0.0129µg/L , followed by Sample A which is 0.309±0.00114µg/L. Both of the samples were exceeded the permissible limits of MFA(1985), EC (2006) and WHO(2008) . As for the other samples, the lead concentration were exceeded the permissible limits of WHO (2008) and EC (2006). All of the concentration can be seen in figure 4.2.

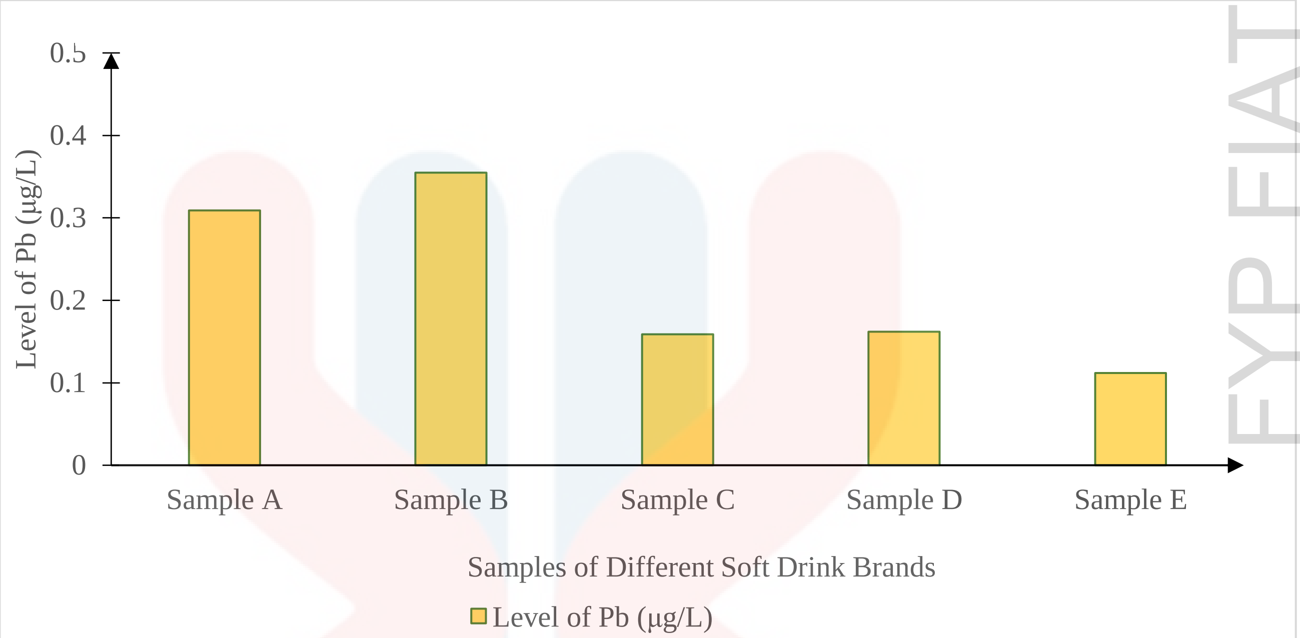


Figure 4.2: Lead,Pb Concentration in different soft drink samples.

As the results were compared in Table 4.4, two of the soft drink samples were exceeded all the permissible limit stated. While the other samples exceed the permissible limits of WHO (2008) and EC (2006). Those two samples that exceed all the permissible limits were Sample A and Sample B which gives the concentration of a total of 0.309±0.00114µg/L and 0.355±0.0129µg/L respectively. Both of these samples were exceed the permissible limits that is regulated by MFA (1985), EC (2006) and WHO (2008). Which is 0.2µg for MFA (1985) and 0.01 for both EC (2006) and WHO (2008) respectively.

Table 4.4: Comparison of level of Lead (Pb) detected in soft drinks analysed with the maximum permitted level set by regulations

Soft Drink Brands	Level of Pb (µg/L) Mean±SD	Maximum permitted level of Pb in soft drink		
		MFA (1985)	EC (2006)	WHO (2008)
Sample A	0.309±0.00114	0.2	0.01	0.01
Sample B	0.355±0.0129			
Sample C	0.159±0.0051			
Sample D	0.162±0.0083			
Sample E	0.112±0.0036			

Lead is a very toxic metal that is present in the natural environment. Due to massive economic growth, the lead production has increased significantly. This phenomeneon which cause several sources of lead exposure in the environment (L.Jones, et al., 2009). In drinking water, lead has became a significant problem in this matter. Lead is used in plumbing materials and solder which that contacted into drinking water. Lead is leached into raw water as a result of the corrosion of lead-based plumbing material, which in turn increase the risk of lead pollution (Brown & Margolis, 2012). Lead builds up in the bones, teeth, brain, spleen, liver, and lungs, among other places. Lead removal from soft tissues is predicted to take around a month, but lead removal from bones might take up to ten years (Wani, Ara, & Usmani, 2015). Once you've been exposed to lead for a long time, it'll take even longer for you to get rid of it. In a recent case, several people have chronic lead exposure in shooting ranges, these people are suffer from fatigue, dizziness and other illness (Laubner & Stražnickaitė, 2022). In recent research done by Sunusi.S., the average concentration is 0.006 while our average lead concentration is 0.2194 (S., Abdulmumin.Y, & A.U., 2020). This shows our lead concentration in soft drinks is much higher compared to other research.

4.5.2 Cadmium (Cd)

Cadmium were also detected in all of the soft drink samples. All of the samples were have very low concentration of cadmium in it. However, all of the samples were exceeded the permissible limit of cadmium that is regulated by WHO which is 0.003µg/L. The samples were also didn't exceed the limit by MFA (1985) which is 1µg/L. The samples were presented in a graph at Figure 4.3.

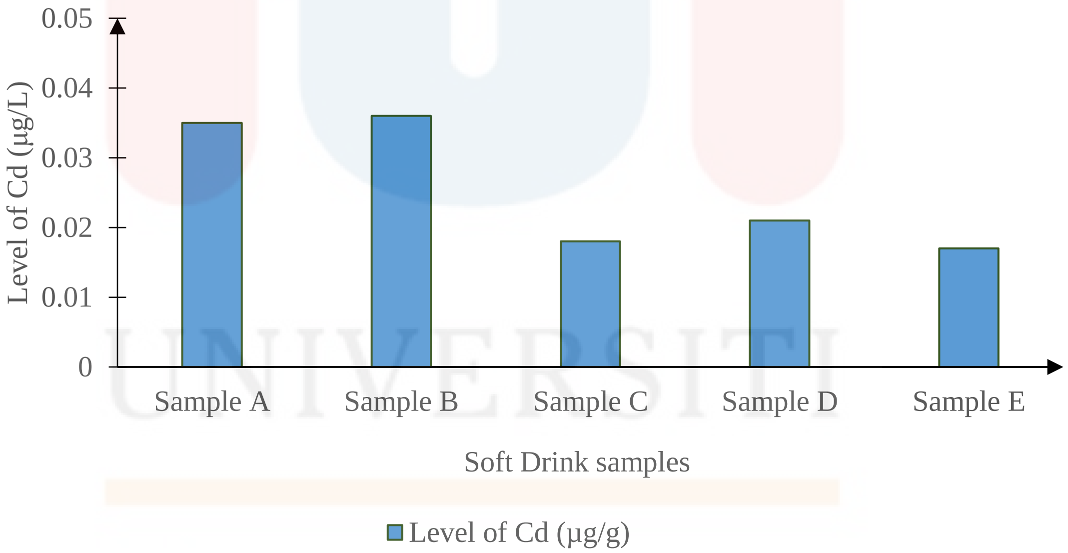


Figure 4.3: Cadmium,Cd concentration in different brand of soft drink samples

Throughout the result that is presented in figure 4.3, the highest concentration of Cadmium is belong to Sample B which is 0.036±0.0006µg/L. Next is Sample A and

Sample D which is 0.035±0.0009µg/L and 0.021±0.0015µg/L respectively. Then Sample C has the second last concentration which is 0.018±0.0007µg/L. Last but not least, Sample E has the least amount of concentration which is 0.017±0.0012µg/L. All of the samples were exceed the permissible limit of WHO (2008) which is 0.003µg/L but does not exceed the permissible limit of MFA (1985) which is 1µg/L. The sample concentration will be compared with the permissible limits at Table 4.5.

Soft Drink Brands	**Level of Cd (µg/L) Mean±SD**	**Maximum permitted level of Cd in soft drink**		
		MFA (1985)	EC (2006)	WHO (2008)
Sample A	0.035±0.0009			
Sample B	0.036±0.0006	1.0	-	0.003
Sample C	0.018±0.0007			
Sample D	0.021±0.0015			
Sample E	0.017±0.0012			

Table 4.5 Comparison of level of Cadmium (Cd) detected in soft drinks analysed with the maximum permitted level set by regulations

Cadmium is also a type of lethal heavy metal. It is extremely toxic to all living things. However, Cadmium is also can be found in the environment. In nature, this element is found in trace levels, however anthropogenic activities have helped to raise its concentration in the environment. Mining, zinc refining, plastic manufacture, production, and distribution are all examples of industries that produces high amount of cadmium (Garcı´a-Reyes, Ortega-Barrales, & Molina-Dı´az, 2006). Cadmium has been shown to harm the kidney, liver, bones, and cardio-pulmonary system, however the kidney is the most vulnerable organ, as cadmium can causes kidney failure if cadmium is ingestedin high concentration (B.Arain, et al., 2015). In a previous research done by Aleti Jaya Sree at 2019, their research has the average concentration of cadmium of 2.063µg for soft drinks while in this research, the average concentration for cadmium is 0.0254µg which means our concentration of cadmium in soft drink is lower than other research (Sree, B.Aruna, Narayana, & Rao, 2019).

4.6 Accumulation of heavy metal in soft drinks

The aim of this research is to quantify the heavy metal concentration in various soft drink brands in Malaysia. This study also aims to determine if heavy metals pose a harmful effects on human health if they are consumed in large quantities in human diets. 5 random brands of soft drink are chosen as these soft drink are favoured by Malaysian. These soft drink are sold and consumed in a large amount by fellow Malaysian.

By conducting this research, it helps to determine the amount of heavy metal in soft drinks as average Malaysian consumes soft drink at 56g/person per day. The number of average intake were also increasing among the children and the people that lives in rural areas (MOH, 2010). In Malaysia, Soft drink vending machines can be found in any public area such as shopping mall, government agencies, schools and so on. The fast food restaurant in Malaysia also mainly serves soft drinks to the customers. It is undeniably that the soft drink are accessible in all around Malaysia.

Heavy metal in soft drinks can be happen due to various reasons. The main reasons of this phenomeneoen is the heavy metal contamination of ingredients, water sources or packaging materials (Godwill, et al., 2015). Since it is possible for the soft drink to accumulate heavy metal, thus it is important to ensure the heavy metal concentration in soft drink dows not exceed the permissible limit in MFA (1985), WHO (2008) and EC (2006).

4.7 Survey Conducted

This survey is conducted to understand the people's perception in all of Malaysia about soft drink consumption. There are 372 respondents, which consist of states from around Malaysia, who responded to this survey altogether. This survey has circulated within social media such as Instagram, Facebook, WhatsApp, and so on. Throughout the survey, we can know about the health risk assessment of the people in Malaysia about the consumption of soft drinks. The survey's findings are broken down into three categories: respondents' socioeconomic status, understanding of heavy metal, and the frequency of soft drink consumption. All the questions are provided with multiple-choice answers to let the respondents select their answers and shorten their time to answer this survey.

4.7.1 Socio-demographic of the respondents

4.7.1.1 Gender of respondents

The first socio-demographic is shown in figure 4.4 below. This Figure shows the gender of all the respondents. Based on the survey conducted in Malaysia, 95 Male respondents and 229 female respondents answered this survey, which makes up 372 respondents altogether. Around 260 female (69.89%) and 112 male respondents (30.1%) answer this survey. This shows that the female is more interested in answering the survey online.

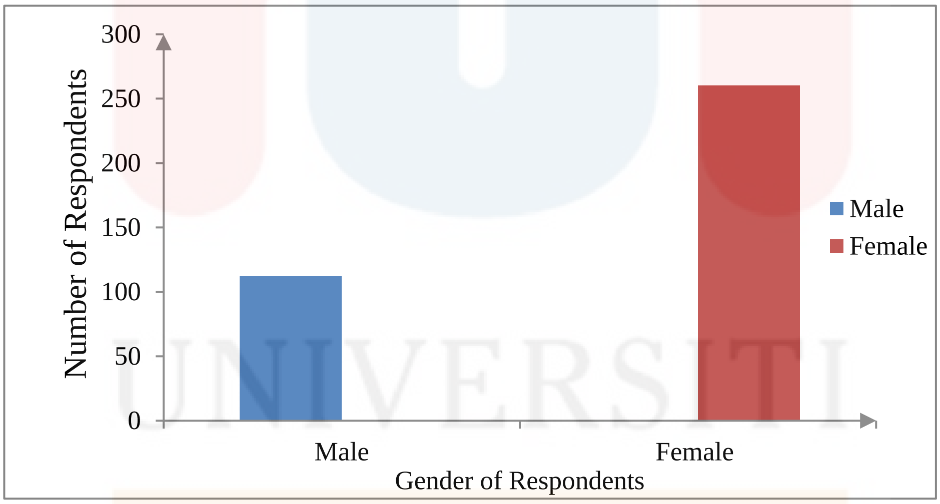


Figure 4.4 shows the gender of the respondents

4.7.1.2 Age of respondents

The next socio-demographic question is shown in figure 4.2, which focuses on the respondents' age. There is a variety of answers received from each different age group. The most answered age group is age group 18-25, next is age group above 55, then is age group 45-50, next is age group 25-30, followed by age group 50-55, followed then by age group 35-40, next is age group 40-45, followed by age group 30-35, and finally is the age group of below 18. The majority of respondents, 97 people (26.07%), originated from 18-25, followed by 58 people (15.59%) from above 55. Next is the age group of age 45-50 which is 12.63%. Furthermore, the next age group is 50-55 which gives 36 (9.67%). Meanwhile, the age group of 25-30, 35-40 and 40-45 have the same number of respondents, 33 (8.87%). Finally, the age group below 18 has the least respondent, 8 people (2.15%).

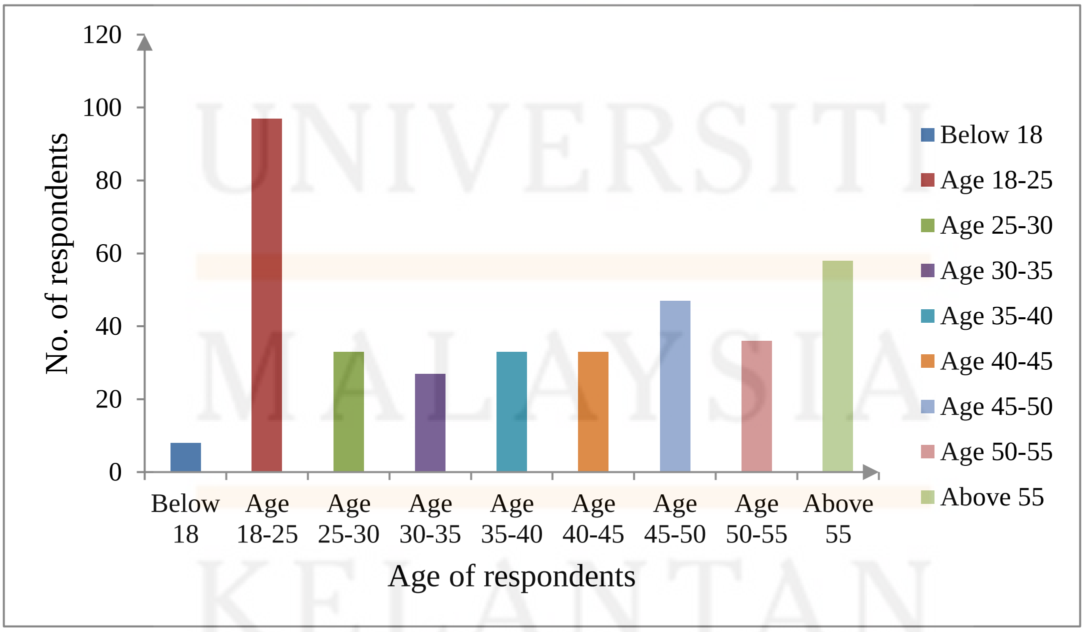


Figure 4.5 shows the age of the respondents

4.7.1.3 Race of respondents

Then, the third socio-demographic is the race of the respondents that is presented in figure 4.3 below. The race of the respondents are consists of Malay, Chinese, Indian, and Others. The amount of races that responded to this survey is shown in figure 5.0 below. In this question, most of the respondents are Chinese, 241 respondents, which gives 64.78%. Next is followed by Malay, which is 78 respondents, which gives 20.97%. Moreover, the next race is Indian, which is 37 respondents with a total of 9.95%. Last but not least, the other races have 16 respondents, which give 4.3% among the total respondents.

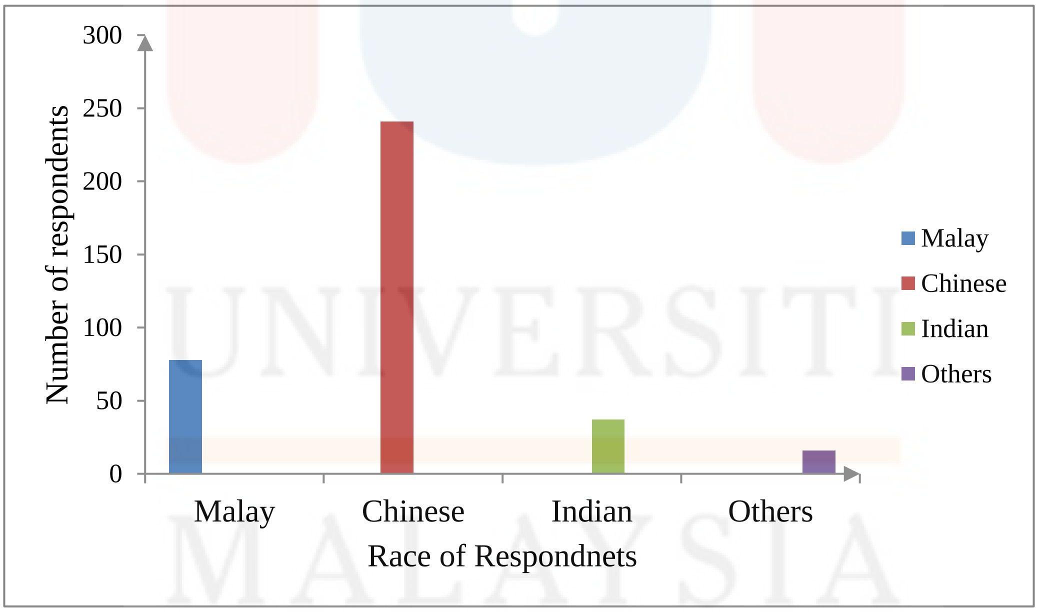


Figure 4.6 shows the race of the respondents

4.7.1.4 The educational level of respondents

The next socio-demographic is the educational level of the respondents which presented in figure 4.4 below. This section is to determine which educational group is most likely to consume soft drinks. Among all the educational groups, the number of people with a bachelor's degree has the highest number, which gives 163 people, equivalent to 43.82%. The second highest is the people with the diploma certificate which gives a number of totals 88 people which is equal to 23.66%. Next, the people with secondary school educational levels have 62 people, similar to 16.67%. The following is the people with a pre-university educational level with 28 people, equal to 7.53%. The next group is those people who have a master educational level which is 15 people altogether and it is also equivalent to 4.03%. Next, those who have elementary school level are recorded 11 people, equal to 2.96%. Finally, those people who have a P.h.D level are the least one, which gives 5 people, equivalent to 1.34%.

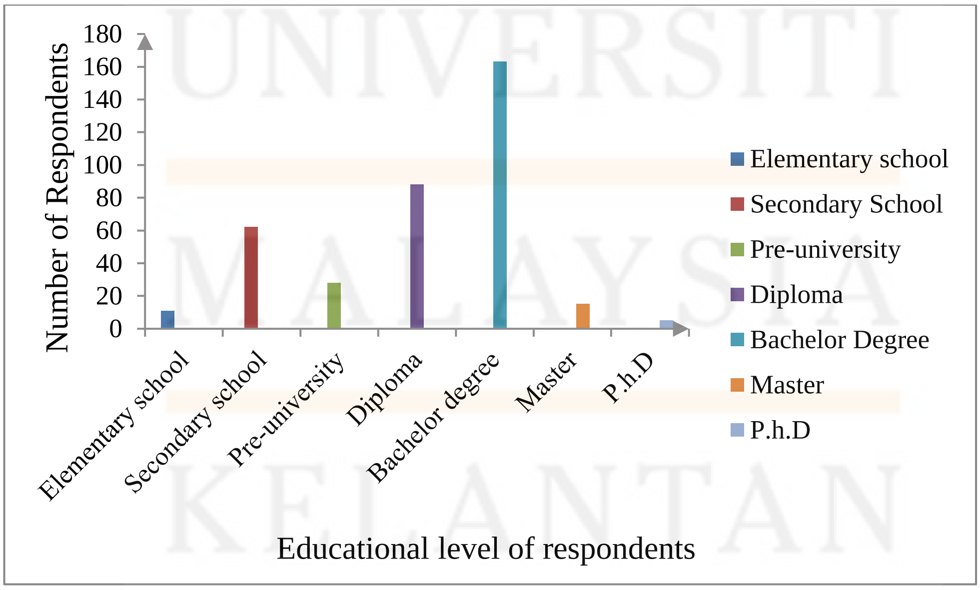


Figure 4.7 shows the educational level of the respondents

4.7.1.5 Residential state of the respondent

In this section, the total respondent from each state in Malaysia is recorded. This is to find out which most of the respondents are originated from which state. The result is recorded in the table below. Throughout the survey, it is known that most of the respondents came from Negeri Sembilan, which has a total of 166 people, which is equal to 44.62%. The second highest is the respondent from Selangor, which has 50 people, similar to 13.44%. Moreover, the third-highest among all is those respondents from Kuala Lumpur, which has 42 people, equal to 11.29%. Perak and Kelantan, the following one, which gives 14 people (3.76%) and 11 people (4.57%) respectively. Next is Kedah, which has a total of 10 people, which is equal to 2.69%.

Both Terengganu, Melaka and Perlis share the same amount of people, which is 8 people in total, equivalent to 2.15%. For Pahang, it is 6 people in total, which is equivalent to 1.61%. The following one is Sabah which gives an amount of 3 people, which is equal to 0.81%. Finally, Labuan and Sarawak have the same amount, which is 2 people, equivalent to 0.54% respectively.

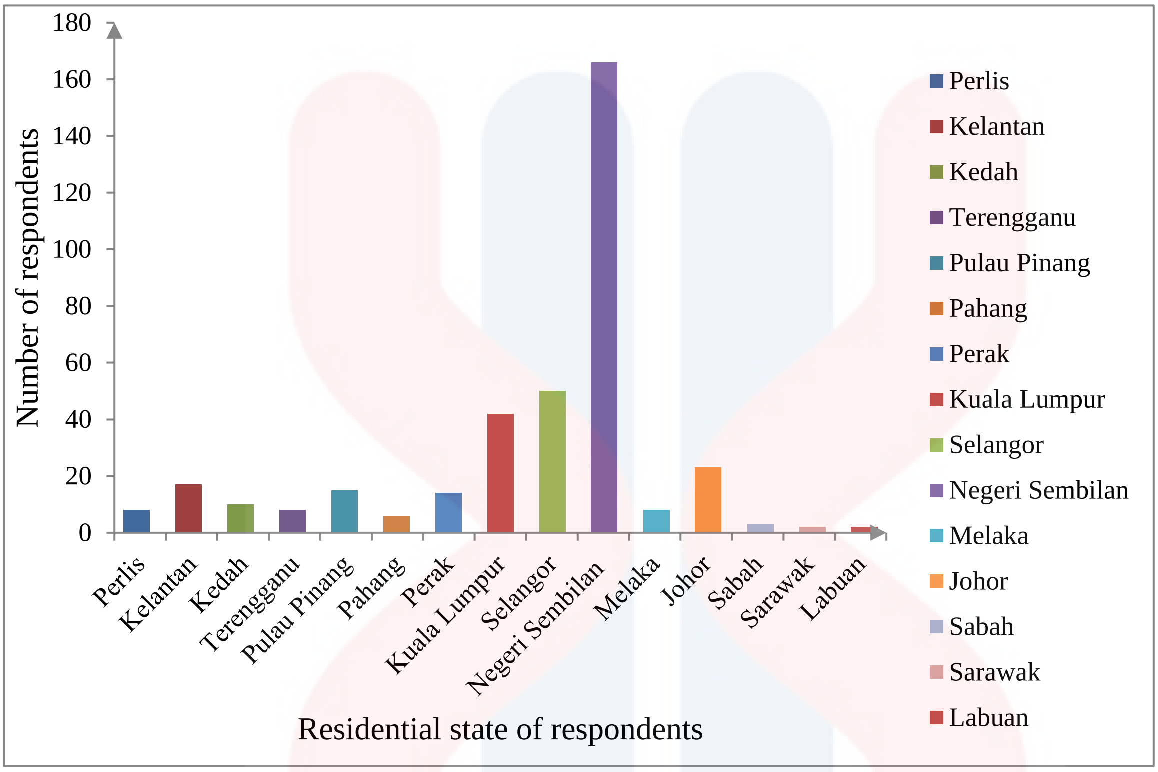


Figure 4.8 shows the state where respondents are from

4.7.1.6 Income Level of respondents

The next socio-demographic is the income level of the respondents which is presented in figure 4.6 below. Each of the respondent's income levels is recorded in the Figure below. This section of the survey is to determine which of the income level answer the survey the most. Throughout the survey, most of the respondents come from income levels below RM500, which is in a total of 81 people and is equivalent to 21.77%. Next does the income level between RM 500 and RM 1000, with 22 people equal 5.91%. Next is the respondent, who is situated in the range of RM 1500- RM 2000. It has an amount of 43 people, which is equal to 11.56%. Next, the respondents who are situated in the income range of RM 2500- RM 3000 have a total of 51 people and are equal to 13.71%. The following are the respondents located within the range of RM 3500- RM 4000, 46 people in total and 12.36%. The following ones are RM 4500- RM 5000 income level, with 37 respondents equalling 9.95%. The next one is the income level of RM 5500-RM 6000, which has a total of 22 people and it is equivalent to 5.91%. The following ones are the income range of RM 6500- RM 7000, which has 18 people as it is also equal to 4.84%. The income level between RM 7500-RM 8000 and RM 8500- RM 9000 has 12 people (3.22%) and 9 people (2.42%), respectively. Next, those people who have an income range between RM 9500- RM 10000 have 12 people, equal to 4.03%. Finally, the people who have an income above RM10000 are 19 people in total, equivalent to 5.11%.

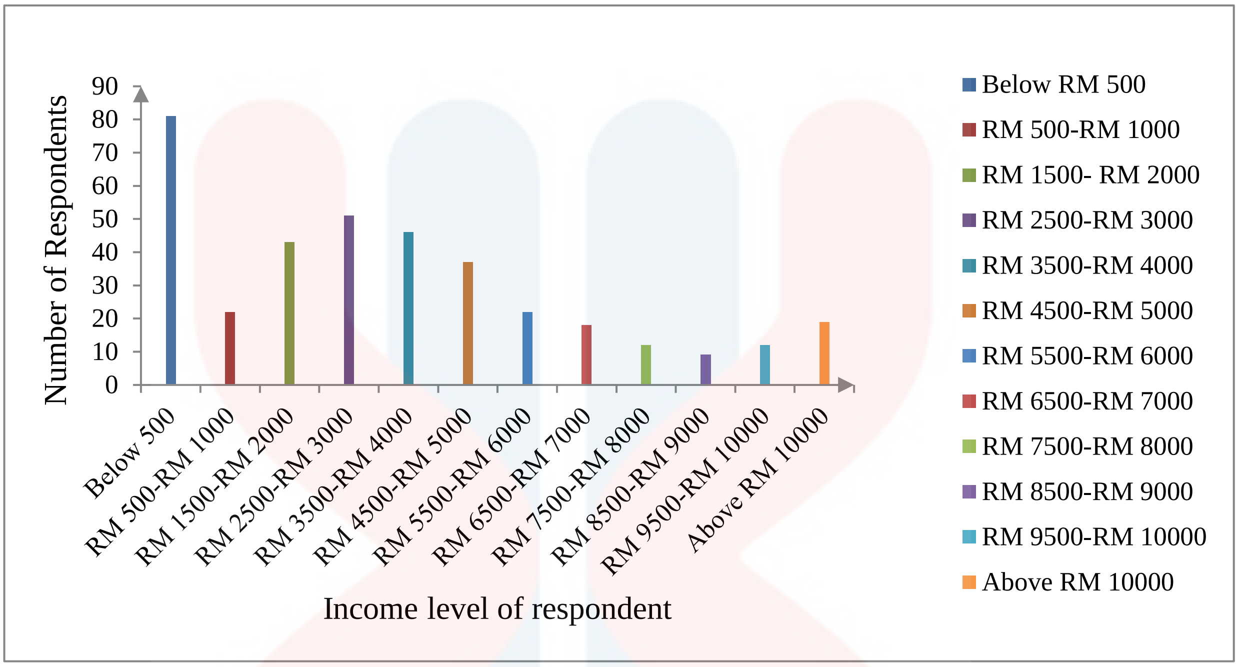


Figure 4.9 shows the income level of the respondents

4.7.2 Knowledge of respondents towards heavy metal

4.7.2.1 Familiar with the term heavy metal

This is the first question shown in the second part of the survey shown in the Figure 4.7 below. It was unexpected that most respondents were familiar with the term "heavy metal" throughout the survey. This is because most respondents answer "Yes" on this question as they know about the word "heavy metal." Among all the respondents, 222 respondents answered “Yes” to this question, equivalent to 59.7%. On the other hand, 150 respondents responded “No” which is equal to 40.32%.

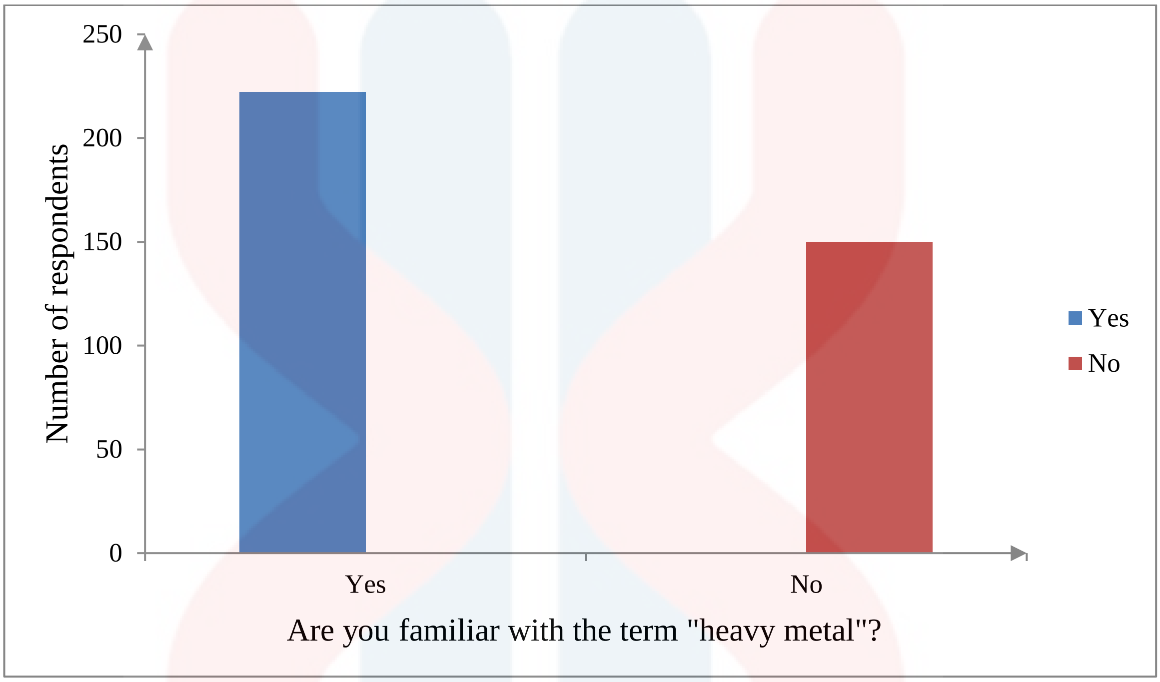


Figure 4.10 shows the respondent’s knowledge towards heavy metal

4.7.2.2 Opinion on the meaning of heavy metal

Next, the Figure 4.8 below shows the opinion of respondents towards the meaning of heavy metal. The answer to this question is selected based on the respondents thinking and understanding of heavy metal. Of all the responses, most of the respondents answered the incorrect definition of heavy metal, which is a substance that is high density and toxic at high concentrations. The total number of respondents who answered this is around 187 people, equivalent to 50.27%. However, only 109 out of 320 respondents responded to the correct definition for heavy metal, a high-density substance and toxic at low concentrations. This is also equal to 34.1% of respondents who answered this question correctly. Next, 37 respondents responded to this selected answer, stating that heavy metal is a low-density substance and toxic in high concentrations, equal to 9.95%. Finally, the people who have answered the substance is low density and toxic at low concentration has a total of 30 people, which is equal to 8.06%.

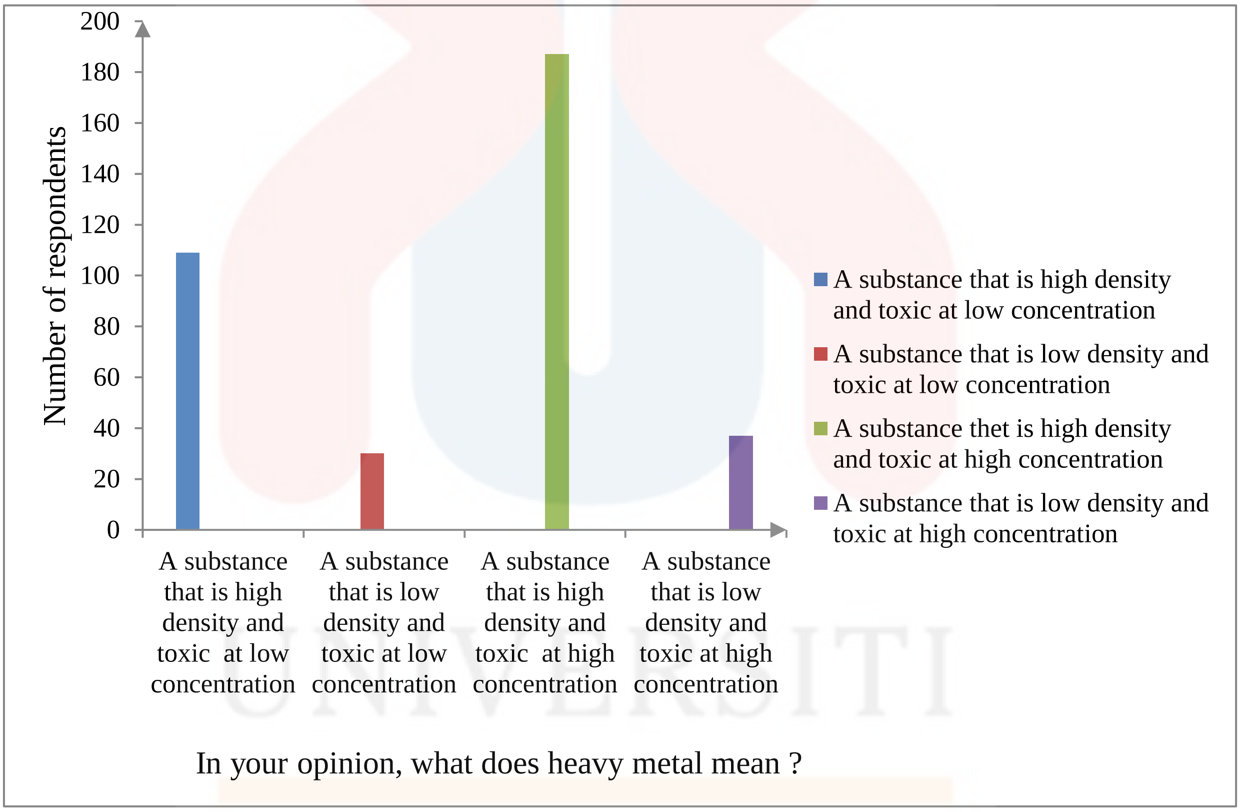


Figure 4.11 shows the opinion of respondents towards the meaning of heavy metal

4.7.2.3 Opinion on the example of heavy metal

The question in part B that is presented in the figure 4.9 below is dedicated to ask the respondents about their opinion on the examples of heavy metals. The question stated that are Lead (Pb), Iron (Fe), and Arsenic (Ar) is an example of heavy metal or not. The respondents have to tick yes or no to answer this question. In this question, about 316 people answered "Yes," which is also equivalent to 84.95%. This shows that most of the respondents do know the names of particular heavy metals are. However, 56 people answer "No" in this question which is equivalent to 15.05%.

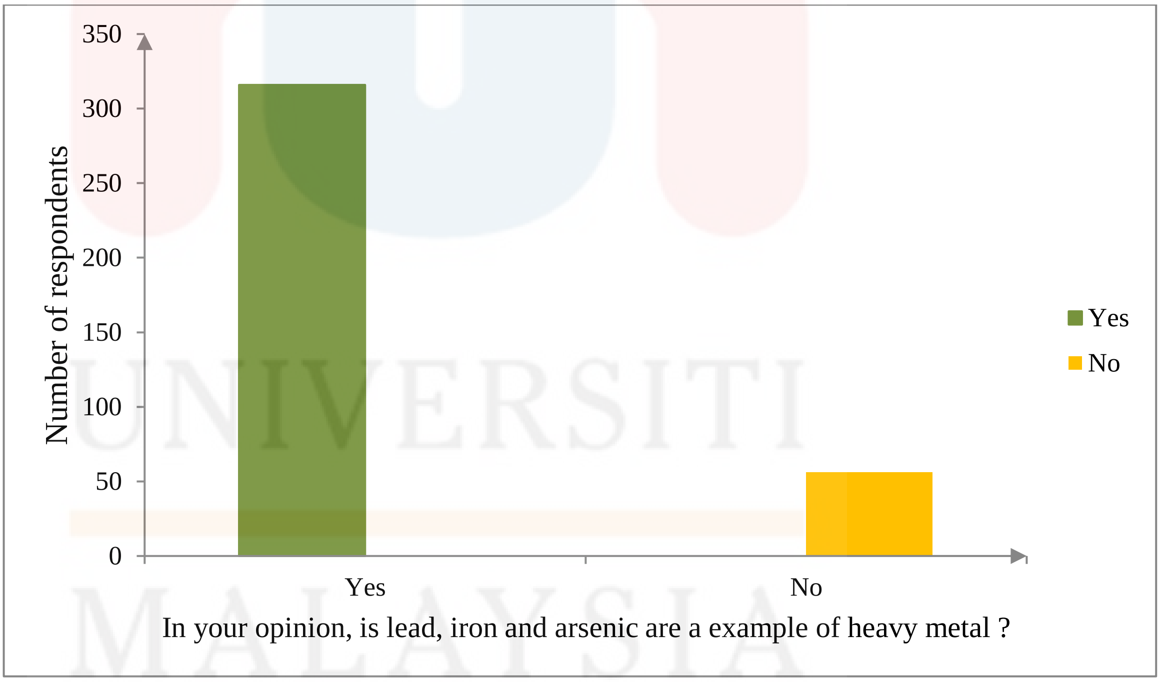


Figure 4.12 shows the knowledge of respondents towards heavy metal example

4.7.2.4 Opinion on Heavy Metals Harming Towards Respondents' Body

The next question is presented in the figure 4.10 below. In this question, the respondents must answer this question based on their own opinion whether heavy metals do bring harmful effect to the human body or not. In this question, 320 people answer "Yes" which is equal to 86.02%. This shows that most of the respondents have a basic knowledge of the heavy metal effect on health. On the other hand, there are only 52 people who answered "No" in this question which is equivalent to 7.2%.

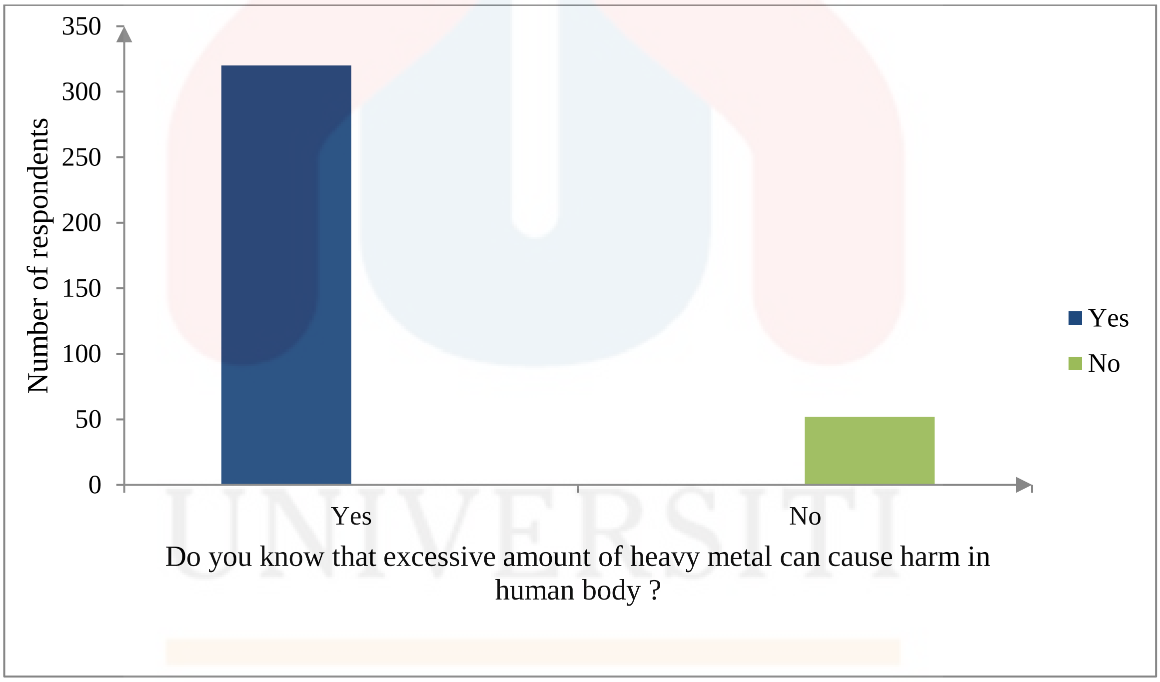


Figure 4.13 shows the opinion on heavy metals harming towards respondents' body

4.7.3 Frequency of soft drink consumption by respondents

4.7.3.1 Frequency of soft drink consumption by respondents per week

This section is to determine that how often the respondents consume the soft drink in a week. The responses were presented in the figure 4.11 below. What is shocking is that most of the respondents do not consume any soft drinks in one week. This shows that most of the respondents practice a healthy lifestyle. About 215 respondents do not consume any soft drinks in a week, equivalent to 57.79%. Next, there are about 118 people who consume soft drinks 1-2 times a week which is equal to 31.72%. Next, about 22 people consume 2-3 times soft drinks per week, equivalent to 5.91%. Next are those respondents who consume soft drinks 3-4 times per week, they are 9 respondents who have this habit, equal to 2.42%. Finally, some respondents consume soft drinks more than 4 times. This habit is considered extreme and very unhealthy. There are only 8 respondents who have this habit, equal to 2.15%

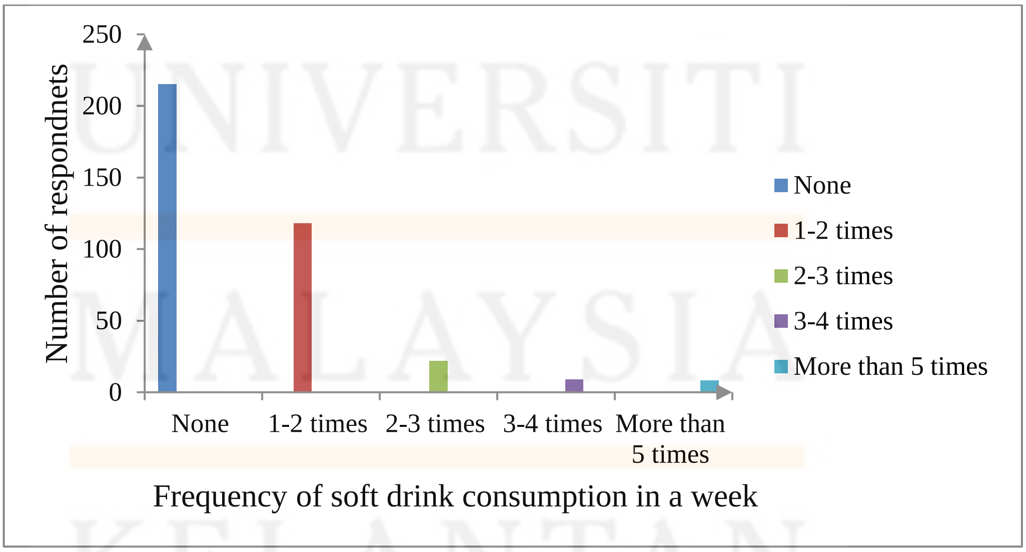


Figure 4.14 shows the frequency of soft drink consumption in a week by respondents

4.7.3.2 Frequency of consuming soft drinks in a month by Respondents

In this last question, the respondents have to choose how much they consume soft drinks in a month. All the responses were presented in the figure 4.12 below. Based on the responses, most of the respondents consume soft drinks 1-2 times per month, which has a total of 159 respondents, equivalent to 42.74%. This shows that most of the respondents have minimum consumption on soft drinks in a month. Next, 92 respondents do not consume any soft drink in a month, equal to 24.73%. Next, 56 respondents consume soft drinks 2-3 times per month, equivalent to 15.05%. The following were those respondents who consume 3 -4 times soft drinks per month, which gives 31 respondents, equal to 8.33%. Finally, some respondents consume soft drinks more than 4 times per month. This habit is also defined as a highly unhealthy habit. The total number of respondents who practiced this habit is 34 respondents, equal to 9.14%.

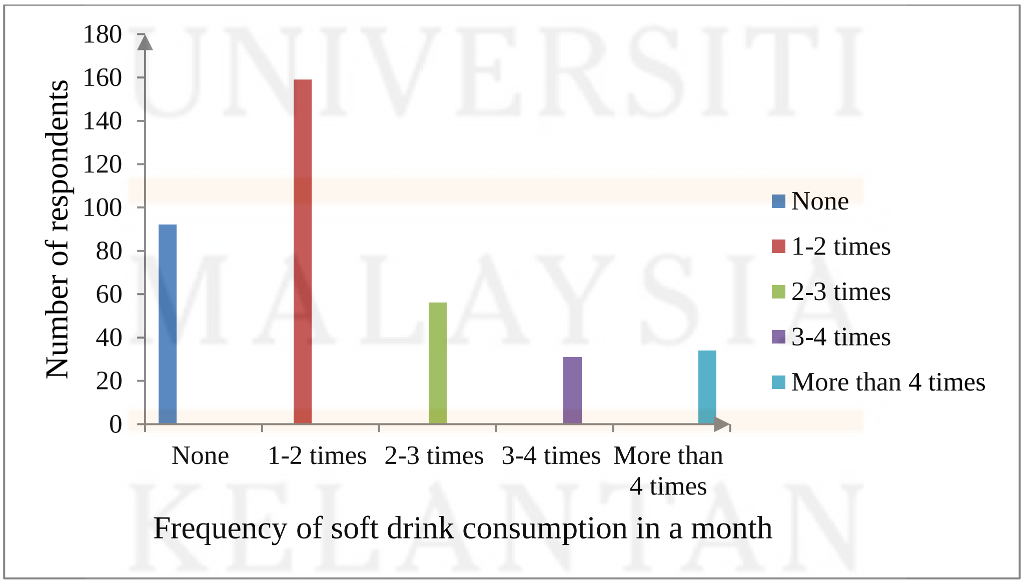


Figure 4.15 shows the frequency of soft drink consumption in a month by respondents

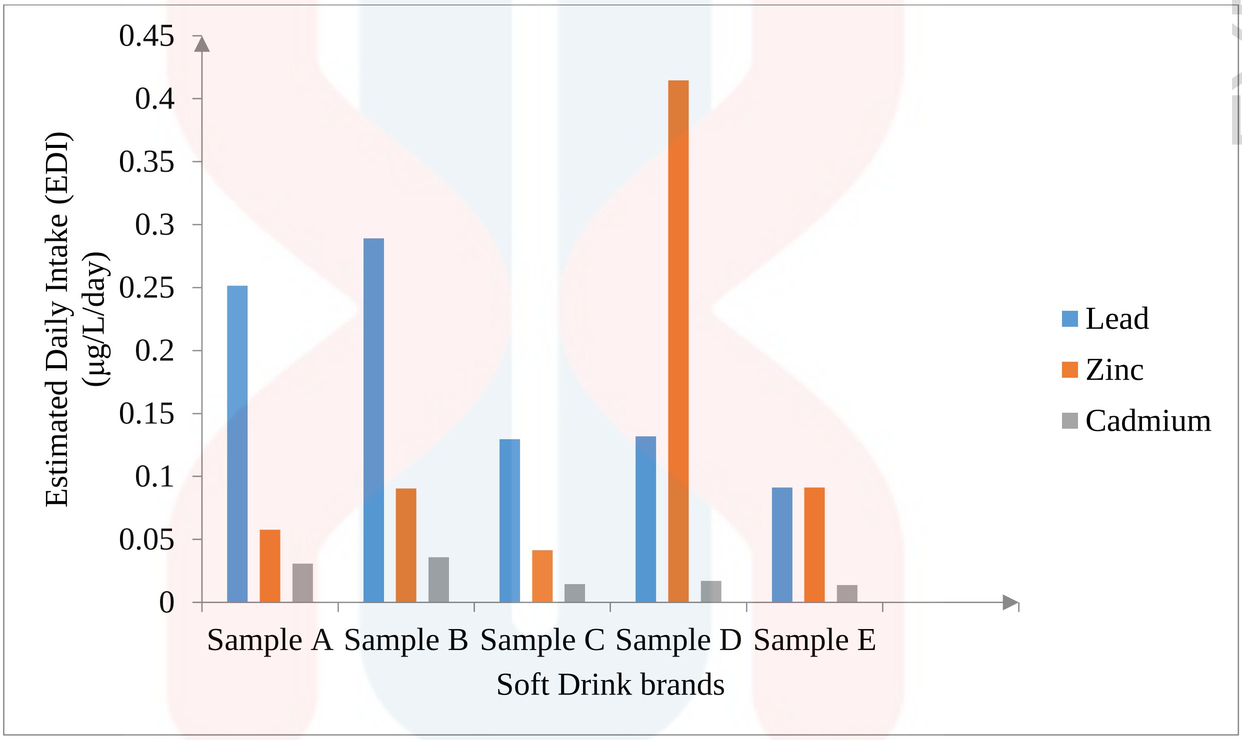
4.8 Health risk assessment

4.8.1 Estimated Daily Intake (EDI) of Soft Drinks

Figure 4.16 below shows the estimated daily intake of various soft drink brands in adults. In human body, Zinc and copper is needed by human body in daily life. However, the estimated daily intake for Copper (Cu) is unavailable as the concentration of copper in the soft drinks were not detected, thus it is unnecessary to calculate the Estimated Daily Intake for copper in the soft drinks. As for zinc, the highest EDI of zinc is in Sample D which is 0.4145µg/L/Day, next is Sample E which is 0.0912µg/L/Day. Then, the EDI for Sample B is 0.0904µg/L/Day. Lastly the lowest EDI is in Sample A which is 0.0578µg/L/Day.Lastly, the least value belongs to Sample C which is 0.0415µg/L/Day. Followed by lead, the highest EDI for lead belongs to the sample in Sample B which is 0.2891µg/L/Day. Next is Sample A which is 0.2516µg/L/Day. Then, the value for lead EDI is the EDI in Sample D which is 0.1319µg/L/Day,. Next, value is in Sample C which is 0.1295µg/L/Day. Last but not least, Sample E has the least value which is 0.0912µg/L/Day.

Next is for cadmium which is a type of toxic metal. For the EDI of cadmium, all of the samples has a relatively low value of EDI. For Sample B, it has the highest EDI of cadmium which is 0.0358µg/L/Day. Next is Sample A which is 0.0309µg/L/Day. Then, the next value is Sample D which is 0.0171µg/L/Day. Furthermore, the next value is Sample C which is 0.0146µg/L/Day. Lastly, Sample E has the least value which is 0.0138µg/L/Day.

Figure 4.16 : Estimated Daily Intake (EDI) (µg/L/Day)



4.8.2 Estimated Weekly Intake (EWI) of soft drinks

Figure 4.18 shows the Estimated Weekly Intake (EWI) for soft drinks and observed heavy metal in this research. In this research, the highest value of EWI belongs to Zinc (Zn), followed by Pb and Cd. Copper (Cu) is not included in the EWI calculation as the concentration of Cu is undetected. For Zinc, the highest EWI intake belongs to Sample D which is 0.829µg/L/Week. Next is Sample E which is 0.1824µg/L/Week. Furthermore, the next EWI is in Sample B which is 0.1808µg/L/Week. Next, the next EWI value is in Sample A which is 0.1156µg/L/Week. Lastly, the least amount is in Sample C which is 0.083µg/L/Week.

Next, the EWI for lead is also calculated. For lead, the highest EWI for lead belongs to Pepsi which is 0.5782µg/L/Week. Next is Sample A which gives a number of 0.5032µg/L/Week. Then,Sample D has the value of 0.2638µg/L/Week. Next, Sample C has the value of 0.259µg/L/Week. Last but not least, the least value belongs to Sample E which is 0.1824µg/L/Week.

Next metal is cadmium. For Cadmium, the highest EWI belongs to Sample B which is 0.0716µg/L/Week. Then the next value is Sample A which is 0.0618µg/L/Week. Furthermore, the next EWI is Sample D which is 0.0342µg/L/Week. Next, the value of EWI for Sample C takes the place which is, 0.0292µg/L/Week. Lastly, the least value of EWI belongs to Sample E which is 0.0276µg/L/Week.

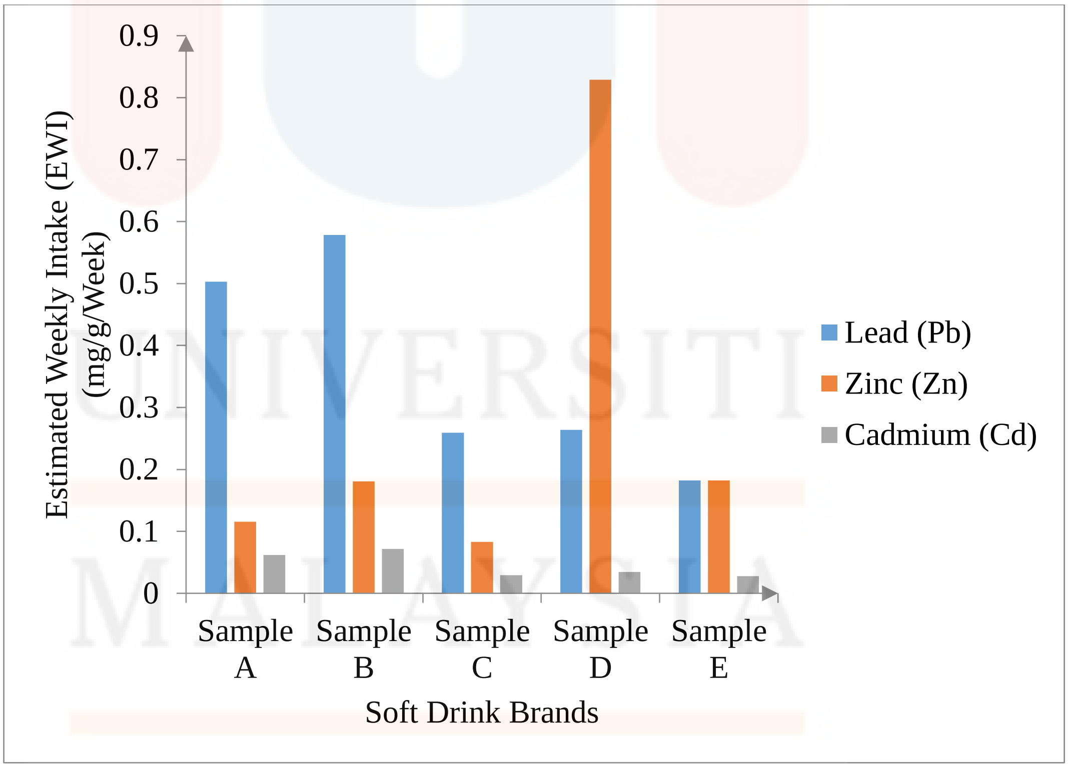


Figure 4.17: Estimated Weekly intake (µg/L/Week)

Table 4.6 shows the comparison of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) for Malaysian in all five soft drink samples were calculated in this research. The quantity of EDI is smaller than the amount of EWI because their timing is different: EDI is used to determine the metal needs in soft drinks for Malaysian in days, whereas EWI is used to determine the metal requirements in soft drinks for Malaysian in weeks. Then, the TDI of soft drinks are taken from WHO (2008). WHO (2008) stated that Lead should be ingested 3.5µg/Kg/day, Zinc should be 1µg/kg/day, Copper should be 2µg/kg/day and Cadmium should be 0.143µg/kg/day.

As for PTWI (µg/kg/week), the PTWI in soft drink for lead is 25µg/kg/week, as for cadmium is around 1µg/kg/week, for Zinc is 7 µg/kg/week and for copper is 14µg/kg/week. By observing table 4.6, the EDI and EWI does not exceed the TDI and PTWI that is regulated by WHO (2008).

Soft Drink Samples	**EDI (µg/L/Day)**			**EWI (µg/L/Week)**		
	Pb	**Cd**	**Zn**	**Pb**	**Cd**	**Zn**
Sample A	0.2516	0.0309	0.0578	0.5032	0.0618	0.1156
Sample B	0.2891	0.0358	0.0904	0.5782	0.0716	0.1808
Sample C	0.1295	0.0145	0.0415	0.259	0.0292	0.083
Sample D	0.1319	0.0171	0.4145	0.2638	0.0342	0.829
Sample E	0.0912	0.0138	0.0912	0.1824	0.0276	0.1824
TDI (µg/L/Day)	3.5	<1	<1	-	-	-
PTWI (µg/L/Week)	-	-	-	25	7	<1

Table 4.6: Comparison of Estimated Daily Intake (EDI) and Estimated Weekly Intake(EWI) for adults

4.8.3 Target Hazard Quotient and Hazad Index in Soft Drinks

Table 4.7 shows the result of Target Hazard Quotient (THQ) and Hazard Index (HI) for adults in 5 soft drink samples. First of all, the Target Hazard Quotient for the heavy metals is calculated. The THQ for copper is yet again unavailable as the concentration of copper is undetected .The average THQ for Pb is 0.05303. Next for Cd is 0.1033. Lastly for Zn is $2.32x10^{-3}$. Then the Hazard Index (HI) is then calculated.. To determine HI, the THQ of all the heavy metal must be added together. Thus, the HI for this research is 0.1503. Since the HI is less than 1, there are no obvious risk for the consumption of soft drink among Malaysian.

Soft Drink Brands	**Target Hazard Quotient (THQ)**			**Hazard Index (HI)**
	Pb	**Cd**	**Zn**	
Sample A	0.0126	0.0285	$1.93x10^{-4}$	0.0413
Sample B	0.0144	0.0293	$3.01x10^{-4}$	0.044
Sample C	$6.47x10^{-3}$	0.0146	$1.38x10^{-4}$	0.0212
Sample D	$6.6x10^{-3}$	0.0171	$1.38x10^{-3}$	0.0251
Sample E	$4.56x10^{-3}$	0.0138	$3.04x10^{-4}$	0.0187
Average value	0.04463	0.1033	$2.32x10^{-3}$	0.1503

Table 4.7: Target Hazard Quotient (THQ)and Hazard Index (HI) of soft drink samples for adults

CHAPTER 5

CONCLUSION AND FUTURE RECOMMENDATION

5.1 Conclusion

Throughout the study, this research helps to let people know the accumulation of heavy metal in soft drinks. The brands of soft drink which taken for sample are Coca-cola, Pepsi, Sarsi, Sprite and Kickapoo. The geavy metals that are found in the soft drinks are Pb, Cd and Zn by using Atomic Absorption Spectrometer (AAS) instrument. There are many reasons which causes the accumulation of heavy metal in the soft drinks such as the quality of the water, quality of the ingredients used, lack of quality control and many more. Since Malaysia are one of the main consumers of soft drink, there is a potential risk if that the heavy metal poisoning will occur.

From the complete analysis, this current research reviews that the soft drink that is sold in all around Malaysia contains various level of heavy metal in it. In all of the heavy metal, lead has the highest concentration in the soft drink samples. Next is followed by zinc and then followed by cadmium. Lastly, copper has the least concentration as it is unable to detect in all of the samples. The average concentration for the heavy metal are Pb (0.2194), Zn (0.1708), Cd (0.0254). Thus, the order for the concentration of the heavy metals ranked decreasingly are Pb > Zn > Cd > Cu. Only 2 samples, Sample A and Sample B has the lead concentration which exceeding the permissible limit of MFA(1985), WHO (2008) and EC (2006). While for Cd, all of the samples exceed the permissible limit of MFA (1985) and WHO (2008).

Moreover the Estimated Daily Intake (EDI) for the consumption of soft drinks are found to be less than the Tolerable Daily Intake (TDI) indicated by WHO (2008). Thus, soft drinks can still be consumed . As for Estimated Weekly Intake (EWI), the value for the Estimated Weekly Intake (EWI) does not exceed the Provisional Tolerable Weekly Intake (PTWI) and Tolerable Daily Intake (TDI) that is regulated by WHO (2008). Therefore the soft drink that consumed by Malaysian does not pose a threat to Malaysian. This research also shows that the consumption of soft drink with a consumption rate of 56g/Person with a weekly intake of 2 days per week does not give any health risk to fellow Malaysian. The Hazard Index (HI) was 0.1503 where it is less than 1 which proposes there is no obvious health risk for the soft drink that is sold in Malaysia.

5.2 Future Recommendation

Firstly,further research on the buildup of heavy metals and other pollutants in soft drinks is strongly suggested. As the consumption of soft drink in Malaysia is still high, the concentration of heavy metals in various soft drinks must be examined to ensure the quality of the soft drink must be assured. This is because thereare also research which proves there are also accumulation of heavy metals who happens in various brands of soft drinks too. However, quality control and strict regulations must be done frequently to ensure the quality of the soft drink must be assured. However, the best solution is to educate the public to reduce the consumption of soft drink.

Next, for the survey part, it is recommended the questionnaire data must be obtained via direct communication. By personally questioning the respondents, it is possible to improve the findings and learn why the respondents opted to consume soft drink once, twice or none in a week. Through the questionnaire, we will not know the true reason why the respondent chose this answer. Throughout direct communication, we can improve the accuracy of the questionnaire data. A good interaction with the respondents will obtain a even more accurate data for the respondents.

Furthermore, prior to executing this research, it is necessary to have information and create awareness of heavy metal buildup in soft drinks. Our own initiative is crucial, therefore study beforehand in this topic before beginning this research. Reading papers or previous research on this topic might be beneficial. Various lab skills must be mastered during the preparation of samples. Safe and accurate lab procedures allowing to complete the lab work on time. Good writing abilities, grammar and research skills should not be taken for granted in order to produce a good thesis.

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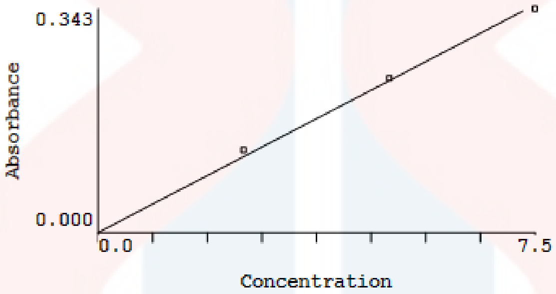
APPENDIX A

Standard calibration curve for each heavy metal using AAS.

i) Lead (Pb)

Method: Pb Std Mtd Page 2 Date: 27-Dec-21 12:17:07 PM

Correlation Coef.: 0.997233 Slope: 0.04663 Intercept: 0.00000



Calibration data for Pb 217.00 Equation: Linear Through Zero

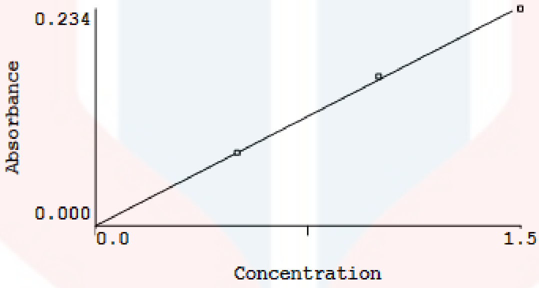
ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1264	2.5	2.711	0.00	0.12
Calib Std 2	0.2370	5.0	5.081	0.00	0.34
Calib Std 3	0.3435	7.5	7.366	0.00	0.37

Correlation Coef.: 0.997233 Slope: 0.04663 Intercept: 0.00000

ii) Copper (Cu)

Method: Cu Std Mtd Manual   Page 2   Date: 27-Dec-21 3:26:39 PM

Correlation Coef.: 0.999338   Slope: 0.15767   Intercept: 0.00000



Calibration data for Cu 324.75   Equation: Linear Through Zero

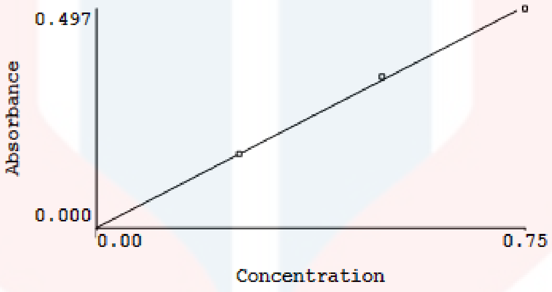
ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	9.31
Calib Std 1	0.0786	0.5	0.498	0.00	0.51
Calib Std 2	0.1610	1.0	1.021	0.00	0.22
Calib Std 3	0.2343	1.5	1.486	0.00	0.70

Correlation Coef.: 0.999338   Slope: 0.15767   Intercept: 0.00000

iii) Zinc (Zn)

Method: Zn Std Mtd Manual Page 2 Date: 27-Dec-21 2:38:11 PM

Correlation Coef.: 0.999243 Slope: 0.66954 Intercept: 0.00000



Calibration data for Zn 213.86 Equation: Linear Through Zero

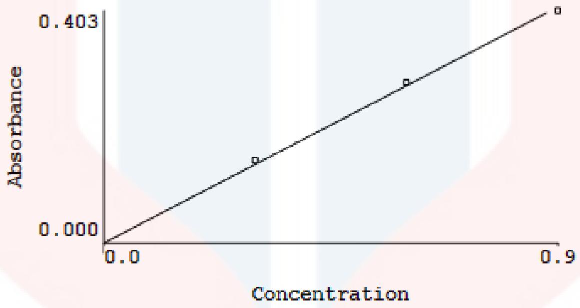
ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1671	0.25	0.250	0.00	0.90
Calib Std 2	0.3424	0.50	0.511	0.00	0.34
Calib Std 3	0.4970	0.75	0.742	0.00	0.61

Correlation Coef.: 0.999243 Slope: 0.66954 Intercept: 0.00000

iv) Cadmium (Cd)

Method: Cd Std Mtd Manual   Page 2   Date: 27-Dec-21 11:49:23 AM

Correlation Coef.: 0.998379   Slope: 0.45523   Intercept: 0.00000



Calibration data for Cd 228.80   Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1436	0.3	0.315	0.00	0.56
Calib Std 2	0.2787	0.6	0.612	0.00	0.22
Calib Std 3	0.4034	0.9	0.886	0.00	0.44

Correlation Coef.: 0.998379   Slope: 0.45523   Intercept: 0.00000

APPENDIX B

TURNITIN SIMILARITY REPORT

Heavy Metal Analysis and Health Risk Assessment of Heavy Metal in Selected Soft Drinks in Local Market of Malaysia

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

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6	Mahmoud M. Ghuniem, Mona A. Khorshed, Eglal R. Souaya. "Method validation for direct determination of some trace and toxic elements in soft drinks by inductively coupled plasma mass spectrometry", International Journal of Environmental Analytical Chemistry, 2019 Publication	<1%

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