DETERMINATION OFSELECTED HEAVY METALS CONTENT IN TOBACCO LEAVES AND LOCAL CIGARETTE FROM KOTA MARUDU, SABAH

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ABSTRACT

Tobacco (*Nicotiana tabaccum*) is a commercial plant that is naturally accumulate and concentrate relatively high levels of heavy metals. It is one of the basic agricultural products with social and economic importance. However, there is no much report in the literature on the determination of heavy metals levels in tobacco leaves and local cigarettes. Hence this study is an initial attempt to investigate the accumulation level of selected heavy metals (Cd, Co, Cr, Cu, Fe, Mg, Ni, Pb & Zn) concentration in tobacco leaves and local cigarettes of Kota Marudu, Sabah. Digestion of all samples was done using an acid digestion method which is using nitric acid (HNO_3). However, the methods that used to digest the samples of tobacco leaves and local cigarettes are different. Digestion of tobacco leaves was undergo 85°C-150°C for 2 hours whereas for local cigarettes was 100°C for 2 hours. *Inductively Coupled Plasma-Optical Emission Spectrometry* (ICP-OES) is used to determine the concentration of heavy metals. Analysis from ICP-OES shows the accumulation of heavy metals in tobacco leaves are Co<Pb<Cr<Cd<Cu<Ni<Zn<Fe<Mg whereas the sequence for local cigarettes is Co<Pb<Cd<Cr<Ni<Cu<Zn<Fe<Mg. Highest heavy metals found in both tobacco leaves samples and local cigarettes samples are Mg with the value of 217.395mg/L ±13.072 and 217.743mg/L ± 9.374 respectively. On the other hand, the lowest heavy metals that found in both samples is Co with the concentration of 0.022mg/L ± 0.017 in tobacco leaves and 0.033mg/L ± 0.001 in local cigarettes. Using Pearson's Two Tailed Correlation, Co, Cu, Fe and Ni in tobacco leaves is no significant correlation with the metals in local cigarettes. The only significant correlation was observed between them for some metals.

TABURAN LOGAM BERAT TERPILIH DI DALAM DAUN TEMBAKAU DAN ROKOK TEMPATAN DI KOTA MARUDU, SABAH

ABSTRAK

Daun tembakau (*Nicotina tabaccum*) adalah tumbuhan komersial yang boleh mengumpul logam berat yang agak tinggi secara semula jadi. Tumbuhan ini adalah salah satu produk asas pertanian yang mempunyai kepentingan dalam sosial dan ekonomi. Walau bagaimanapun, tidak banyak laporan dalam kajian penentuan tahap logam berat dalam daun tembakau dan rokok tempatan. Oleh itu kajian ini dijalankan untuk mengenal pasti taburan logam berat di daun tembakau dan rokok tempatan di Kota Marudu, Sabah. Logam-logam yang menjadi kajian adalah kadmium, kobalt, kromium, kuprum, magnesium, nikel, besi, plumbum dan zink. Penghadaman kesemua sampel ini telah dilakukan menggunakan asid nitrik (HNO_3). Walaubagaimanapun, penghadaman bagi daun tembakau dan rokok tempatan adalah berbeza. Penghadaman bagi daun tembakau adalah menggunakan 85°C-150°C selama 2 jam manakala 100°C selama 2 jam bagi rokok tempatan. *Inductively Coupled Plasma-Optical Emission Spectrometry* (ICP-OES) telah digunakan untuk menjalankan analisis elemen logam berat. Keputusan yang terdapat melalui ICP-OES menunjukkan elemen logam berat di dalam daun tempatan meningkat mengikut susunan Co<Pb<Cr<Cd<Cu<Ni<Zn<Fe<Mg manakala susunan bagi rokok tempatan adalah Co<Pb<Cd<Cr<Ni<Cu<Zn<Fe<Mg. Kepekatan logam berat tertinggi dalam kedua-dua sampel daun tembakau dan rokok tempatan adalah Mg, masing-masing dengan nilai 252.550mg/L ±3.105 dan 217.743mg/L ± 5.520. Kepekatan logam berat yang paling rendah adalah Co dengan nilai 0.020mg/L ± 0.000 dalam daun tembakau dan 0.033mg/L ± 0.000 dalam rokok tempatan. Berdasarkan nilai faktor translokasi, keputusan telah menunjukkan Co, Cu, Fe dan Ni dalam daun tembakau menunjukkan hubungan yang signifikan.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF FORMULA

LIST OF SYMBOL AND ABBREVIATIONS

%	percent
°C	degree Celsius
cm	centimeter
g	gram
g/mol	gram per molecular mass
kg	kilogram
min	minute
mL	milliliter
mm	millimeter
s	second
M	Molarity
>	More than
<	Less than
r	Correlation Coefficient
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
Mg	Magnesium
Ni	Nickel
Pb	Lead
Zn	Zinc
AAS	Atomic Absorption Spectrometry
ATSDR	Agency for Toxic Substance and Disease Registry
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization

GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GSA	General Services Administration
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ITIS	Integrated Taxonomic Information System
IZA	International Zinc Association
MHA	Ministry of Health Malaysia
UNEPA	United States Environmental Protection Agency
WHO	World Health Organization

LIST OF APPENDIX

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Metal is a group of globally-distributed pollutants that are naturally excreted from the Earth's crust. It is widely dispersed in the environment and can be accumulated in some selected tissues of human body. Some metals have no function in human physiology but will turn toxicity at high levels of concentration (Duffus, 2002).

Although adverse health effects of heavy metals have been know for a long time, uses of heavy metals in daily life still continue increasing. Applications of heavy metals can be seen through building materials, pigment for glazing ceramics and pipes for transporting water (Jarup, 2003).

People may be exposed to the harmful chemical, physical and biological agents in environment surrounding. The effects will only show out if the agent and outer boundary of human body have the direct contact. This includes airways, skin or the mouth. Tobacco smoking can contribute to the negative impacts of human health via the heavy metals that in the cigarette (Jarup, 2003).

Nowadays, tobacco smoking not only happened on adults, but also common on the women and children. Tobacco becomes the main agricultural crop for many countries, including Bulgaria. There are several factors that could be affect the content of metals in the tobacco such as soil type and pH genotype, use of a metal-containing pesticides, and fertilizers. Metals are absorbed from the soil and the leaves are the primarily concentrated of the metals (Zaprjanova *et al.*, 2006)

Tobacco plants seem to occur all around geographical of the world due to the habit of tobacco use. It has become one of the most frequently used plants in the study of heavy metal analysis. This indicates that habit of smoking has increase the probability of people to exposure the heavy metals (Addo *et al.*, 2011).

Metals from soil, fertilizers and even industrial pollution will be absorbed as nutrients for growing tobacco plants. Smoking can liberates all these metals from tobacco leaves into the smoke. When smoking, people have the high probability to inhale all these harmful elements. Heavy metals in tobacco will be inhaled by the bystanders and second hand smokers at the all same time. Chemical form of metals are varies during the burning process. The metals can be completely transformed from low toxicity to high toxicity after burning process. Thus the health risk that faced by people is relatively depends on the chemical form of heavy metal (Campbell, 2014).

Out of 4,000 chemical components in cigarette, there are at least 250 of them are harmful to human health. Some main chemical components are include 1,2-butadine, acrolein, arsenic, benzene, cadmium, chromium (VI), formaldehyde, polonium-210, tar, carbon dioxide and nicotine. Nicotine in tobacco can be absorbed quickly into bloodstream and this product can me people get into addiction to continue smoking (Hamrah, 2015).

Many scientific paper is proved that tobacco bring serious effect on human health (Musharaf *et al.*, 2012; Caruso *et al.*, 2013; Muhammad, 2012). Different information of heavy metal contents in tobacco should elicit a concern. Due to increasing consumption of tobacco products, a study of the heavy metals in tobacco is necessary.

1.2 Objective of Study

The objective in this study is to determine and compare the concentration of nine selected heavy metals (Cd, Co, Cr, Cu, Fe, Mg, Ni, Pb & Zn) in the tobacco leaves from Kota Marudu farm, Sabah and local cigarette using Inductively Coupled Plasma-Optical Emission Spectrometry.

1.3 Scope of Study

Heavy metals are chosen to be determined in this research because it can be naturally occurring in the environment especially in a geological formation that contains a certain amount of heavy metal. Besides heavy metals in at higher concentrations and in larger doses heavy metals can lead to poisoning and may be toxic to organisms. Increasing in tobacco smoking has been associated with health issues, thus the research of heavy metals in tobacco is chosen. Health problems such as overwhelmingly cardiovascular disease, cancers and respiratory diseases will happen. The effects of taking tobacco are essential to be known to support smoking cessation. All tobacco-related diseases will be reduced when people reduce smoking (Sloan & Gelband, 2007).

The samples will be analysed by using instrument which is ICP-OES. ICP-OES is an excellent technique for identifying and quantifying trace elemental constituents in various sample matrices. ICP-OES is an atomic emission process and plasma is used in ICP-OES (Tyler, 1991). This comparative study is used to determine the heavy metals content in tobacco for human health. Besides, the accuracy, precision and sensitivity of this technique are important to ensure the correct data.

1.4 Significance of Study

There are many research had been studied metal concentration in tobacco either in cigarettes or tobacco leaves. In this study, analysis of heavy metals concentration in tobacco leaves and local cigarette will be identified. The study focuses on the Cd, Co, Cr, Cu, Fe, Mg, Ni, Pb and Zn contents in the tobacco leaves and local cigarettes.

Excess heavy metal accumulation in tobacco is toxic to human especially heavy smokers. These heavy metal contaminations could be transferred into human body via smoking and indirectly via taking the water contamination and metal uptake by plants. Cadmium will primarily take the routes of tobacco smoking to exposure to the human bodies. From the study paper of Jarup (2003) stated that blood cadmium level in cigarette smokers is 4-5 times higher than non-smokers.

Long term exposure to heavy metals would cause serious health problems such as asthma, inflammation, heart disease, stroke, chronic bronchitis and other airway infections. Thus the study of the heavy metals in tobacco is crucial as it would directly bring a huge impact to human health.

The study is also important as the number of smokers in the world is increasing throughout the developed country. Level of awareness of tobacco smoking should be increased among smokers if they can understand the effects of heavy metals that contained in the tobacco.

Study of heavy metals concentration in tobacco will benefit majority of the individuals who are willing to quit smoking. Through this study the contents and impacts of tobacco can be indicated. Ten heavy metals are chose in this study to investigate which are Cd, Co, Cr, Cu, Fe, Mg, Ni, Pb and Zn. These heavy metals selected due to there are commonly presence in the tobacco leaves based on the literature study. Kota Marudu, Sabah is chosen because it is one of the main tobacco plantation in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

According to Duffus (2002), heavy metal is often used as a group name for metals and semimetals that have been related with contamination and potential toxicity or ecotoxicity. There are many heavy metals in the world and cannot be degraded or destroyed. It is the group of elements with atomic number greater than 20 excluding alkali metal, alkaline earths, lanthanoids and actionoides (Agarwal, 2009). Heavy metal can be defined as group of metals and metalloids with its molecular weight are above 40. Normally any metal which its weight is less than 40 are called as light metal (Chen, 2013). Heavy metal has high density, which is above 5g/cm^3 and high thermal conductivity.

Heavy metals can be divided into three groups which are essential light metals, essential heavy metals and toxic heavy metals (non-essential heavy metals). They are grouped according to their importance to human beings. These are the elements that can be found in essential heavy metals such as manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), molybdenum (Mo) and selenium (Se). Essential heavy metals are important for living organisms to maintain their optimum condition to survive. If the concentration of heavy metals is oversupplied, essential heavy metals would become toxic. Examples of non-essential heavy metals are including arsenic (As), lead (Pb), mercury (Hg) and nickel (Ni). It will only become toxic when its concentration is above a toxic level. The toxic level is often regulated by the national environmental protection agencies (EPAs) or World Health Organization (WHO) (Chen, 2013). Table 2.1 shows the essential heavy metal macronutrients and micronutrients that needed for human body.

Table 2.1 Macronutrients and micronutrients of essential heavy metal.

Essential heavy metal macronutrients (~100mg or more per day)	Essential heavy metal micronutrients (a few mg or µg per day)
Ca, Cu, Mg, P, K, Na, S	As, Co, Cr, Cu, Fe, Mn, Mo, Se, V, Zn

2.2 Heavy Metals Selected

Metals have characteristics that are different from non-metals. They are physical properties which include the ability to conduct heat, and an electrical resistance that is directly proportional to temperature, malleability, ductility and even luster. Heavy metals generally exhibit good electrical and thermal conductivity especially in pure form (Housecroft & Sharpe, 2008).

Generally, the chemical elements become more metallic when moving towards the lower left corner of the periodic table and non-metallic towards the upper right corner (Klaus, 2010). In other words, metallic character decreases from left to right and from the bottom to the top of the table. A metal is grouped according to the last electronic subshell in its atom. In the fundamental review paper written by Duffus (2002), 13 different works were cited that used lower limits on the density of a "heavy" metal ranging from $3.5gcm^{-3}$ to $7gcm^{-3}$.

2.2.1 Cadmium (Cd)

Cadmium (Cd) is a chemical element with atomic number of 48. It appears as lustrous, bluish-white in color. It has low melting point with 321°C and low boiling point with 767°C. Cd is quite similar with zinc but forms complex compounds. The special of the Cd is that it is resistant to corrosion so it usually used as a protective layer of metals. Cd is insoluble in water and alkalis but soluble in acids. It is not flammable (ATSDR, 2012).

Natural sources of Cd metal are zinc carbonate and sulfide ores or copper carbonate and sulfide ores (Lee & Kunitake, 2012). Through the waste combustion and burning of fossil fuels, Cd may enter the air. Production of artificial phosphate fertilizers is one of the important sources of cadmium emission (Godt *et al.*, 2006).

Cd has been used widely in electroplating industries, television sets, photography, pigments and textiles. Since it is resistant to the erosion, it is always used to electroplate steel and protect it from corrosion (Sharma *et al.*, 2015).

Cd is chemically similar to zinc. Once absorbed, Cd is efficiently retained and accumulates in the human body. It can cause irreversible damage to the biological system throughout life (Chen, 2013). Bernard (2008) stated that high concentration of Cd bring toxic to the kidney and accumulate in there. This is because Cd is primarily toxic to the kidney, especially to the proximal tubular cell which is the main site of accumulation.

According to Godt *et al.* (2006), low dosage of Cd will stimulate ovarian progesterone biosynthesis, while high dosages inhibit it. This will definitely affect the productions of progesterone and testosterone thus the reproduction is affected. Besides, the relationship of bone damage and Cd is shown by the Itai-itai disease. This is a disease with low grade of bone mineralization, high rate of fractures, increased rate of osteoporosis, and intense bone associated pain. This was observed that in the 1940s the patients were found to eat the rice that is irrigated highly with the Cd. The research paper of Inaba *et al.* (2005) has stated that the most serious stage of chronic Cd poisoning is that Itai-itai disease that occurs at Japan.

Cd binds with organic matter in soil and enters the food chain. Insoluble forms of Cd will deposit and thus absorb to sediments. Besides, it contaminates water, urban soil and agricultural runoff (ATSDR, 2012; Dokmeci *et al.*, 2009).

2.2.2 Cobalt (Co)

According to ATSDR (2004), properties of Co are similar to those naturally-occurring elements such as Fe and Ni. It appears as silver grey hard metal. Co is an element which has 27 atomic number and Co-59 is the only one stable isotope of Co.

This element can naturally found in rocks, soil, water, plants and animals. However, most of Co only can typically found in small amounts. Besides, Co appears in meteorites. Dissolved form or ionic form of Co can found in water (ATSDR, 2004).

Alloys formed by mixing Co metal with other metals. Military and industrial applications such as aircraft engines, magnets and grinding or cutting tools need a huge supply of alloys. In addition, they are widely used in artificial hip, knee joints, colorants in glass, ceramics, paints, catalyst and additives in agriculture and medicine. Other than that, Co can exist in radioactive form which can change it into different isotopes (ATSDR, 2004).

EPA (2016) stated that Co enters environment and then react with other particles. However, it cannot be destroyed and will be absorbed on soil particles and water sediments.

2.2.3 Chromium (Cr)

In the Earth's mantle, Cr is the 17th most abundant element in the earth. The common forms of Cr are Cr(0), the trivalent Cr(III) and the hexavalent Cr(VI) species although it can exists in several oxidation states (Oliveira, 2012).

Oliveria (2012) stated that in naturally form, it will occurs as chromite ($FeCr_2O_4$) in ultramafic and serpentine rocks or complexed with other metals like crocoites ($PbCrO_4$), bentorite $Ca_6(Cr,Al)_2(So_4)_3$ and tarapacaite (K_2CrO_4), vauquelinite ($CuPb_2CrO_4PO_4OH$), among others.

This abundant element is widely used in plating, alloying, tanning of animal hides, inhibition of water corrosion, textile dyes and mordants, pigments, ceramic glazes, refractory bricks and pressure-treated lumber (Oliveria, 2012). However, WHO (2000) stated that excess Cr will cause air pollution and water pollution.

2.2.4 Copper (Cu)

Copper is one of the earliest known metals with symbol Cu. Cu is one of the transition metals with atomic mass of 63.54. It has atomic number of 29. Its melting point is around 1084.62°C and boiling point is 2560°C (Prasad, 2008). Due to its very high thermal and electrical conductivity, it is usually used as a conductor of heat and electricity. It is most commonly seen in the coin and electrical making. Besides, it is malleable and ductile. Cu is bright reddish-brown in color but it turns into green when exposed to the atmosphere (GSA, 2014). The green pigments is copper sulphate (Cu_2SO_4) and used as mordant in dyeing (Saxena, 2011).

There are two sources of Cu which are natural sources and human activities. Examples of natural sources such as wind-blown dust, decaying vegetation, forest fires and sea spray. Most of them are come from sulfides and oxide minerals. Chalcocite (Cu_2S), covellite (CuS), malachite ($CuCo_3$–$Cu(OH)_2$) and chalcopyrite (Cu_2S-Fe_2S_3) are the main sources of copper. For the human activities, the examples include mining, metal production, wood production and phosphate fertilizer production (Prasad, 2008).

According to Angelova *et al.* (2011), Cu is an essential micronutrient for human health. Normally, the daily intake of Cu is 2mg-5mg. The Cu content is varies and different based on the gender and age. It is necessary for the normal iron metabolism and the formation of red blood cells. Besides, it is also necessary for the formation of haemoglobin and to assist in the mobilization in the iron (Saxena, 2011). In addition, Kouremenou-Dona *et al.* (2006) stated that Cu is an essential trace element for the enzymes superoxide dismutase, lysyl oxidase and ceruploplasmin, which protect cells from oxidative damage.

Fraga (2005) has stated that human can get the sources of Cu from liver, oysters, nuts, seeds, dark chocolate and whole grains. Cu is important for the development of connective tissue, nerve coverings and bone. In the enzymes, Cu acts as a reductant to reduce molecular oxygen. According to the Osredkar & Sustar (2011), Cu can act as both an antioxidant and a pro-oxidant. When Cu acts as an antioxidant, Cu may neutralize free radicals and help prevent some of the damage they cause. On the other hand, as a pro-oxidant, Cu promotes free-radical damage and thus contributes to the development of Alzheimer's disease.

High dose intake of Cu can be extremely harmful. When the body cannot excrete the excess Cu, the high levels of Cu concentration can interference with the action of many other nutrients leading to overall tiredness. Drinking water that contaminated with Cu can cause acute gastrointestinal symptoms, liver and kidney damage. Free copper ions (Cu^{2+}) enter the water cycle and become toxic forms in aquatic life. The chronic toxicity of Cu produces neurotoxicity which leads to Wilson's disease. Patients with Wilson's disease may suffer nervous disorders, tremors, difficulty in swallowing and still joints (Saxena, 2011). Fraga (2005) stated that excessive dietary zinc can cause copper deficiency. The study paper stated by the Angelova *et al.* (2011), deficiency of Cu can cause anemia, Menkens syndrome, Parkinson's disease, impaired intestinal restoration, parenteral, nutrition, protein loss and others.

Excess Cu brings impacts to environment. According to Solomon (2009) and Garcia *et al.* (2013), excess Cu will kill aquatic organisms especially blue-green algae. Besides, it enters the water and sediments then accumulated by benthic animals.

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