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LEAD CONCENTRATION IN LONG-TAILED MACAQUE (*Macaca fascicularis*) HAIR IN KUALA SELANGOR

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by

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DECLARATION

I declare that this thesis entitled "Lead concentration in long-tailed macaque (*Macaca fascicularis*) hair in Kuala Selangor"is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ________________________________

Name : ________________________________

Date : ________________________________

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Lead Concentration in Long-tailed Macaque (*Macaca fascicularis*) Hair in Kuala Selangor

ABSTRACT

Macaque is an animal species that potentially as a good indicator to toxic exposure because of they shared similar to humans both physiologically and behaviorally. Synanthrophy with humans increases the possibility macaques come into contact with anthropogenic toxicants, such as lead. The objectives of this study is to determine the heavy metal (lead) concentration level in the long-tailed macaque (*Macaca fascicularis*) and also to distinguish their health status between those inside and outside Kuala Selangor Nature Park. Sample collection activity was conducted at Kuala Selangor Nature Park (KSNP) and Bukit Melawati. Analysis was carried out by inductively coupled plasma-mass spectrometry (ICP-MS). The results of replicate analysis showed the average mean of Pb concentrations (µg/g): Bukit Melawati (6.3138); KSNP (3.1628). Despite, the concentration of lead (Pb) between the two places insignificant statistically nevertheless Bukit Melawati recorded high lead concentrations than in KSNP. The probability of lead exposure was demographic and the macaque behavioral had a great influence on lead hair concentration among the macaque in Kuala Selangor. Although, lead exposure in this area seems to be low, additional monitoring and possible remediation may be required.

Kepekatan Plumbum dalam rambut Kera (*Macaca fascicularis*) di Kuala Selangor

ABSTRAK

Kera merupakan salah satu spesies haiwan yang berpotensi sebagai sebuah sentinel yang baik untuk pendedahan toksik kerana mereka berkongsi persamaan bersama manusia seperti fisiologi dan tingkah laku. Aktiviti pengumpulan sampel telah dijalankan di Taman Alam Kuala Selangor (TAKS) dan Bukit Melawati. Objektif Kajian ini adalah untuk menentukan tahap kepekatan logam berat (plumbum) pada kera (*Macaca fascicularis*) dan juga untuk membezakan status kesihatan mereka di dalam dan di luar TAKS. Analisis telah dijalankan oleh inductively coupled plasma-mass spectrometry (ICP-MS). Keputusan analisis replika menunjukkan purata min bagi kepekatan Pb (µg/g): Bukit Melawati (6.3138); KSNP (3.1628). Walaupun, kepekatan plumbum antara kedua-dua tempat tidak begitu ketara secara statistik namun begitu Bukit Melawati mencatatkan kepekatan plumbum yang tinggi berbanding di KSNP. Kemungkinan pendedahan plumbum adalah demografi dan tingkah laku Kera mempunyai pengaruh yang besar pada kepekatan plumbum rambut antara Kera di Kuala Selangor. Walaupun pendedahan plumbum di kawasan ini seolah-olah rendah, pemantauan tambahan dan kemungkinan pemulihan mungkin diperlukan.

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LIST OF ABBREVIATION

AAS	Atomic absorption stereoscopy
Ag	Silver
As	Arsenic
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
Hg	Mercury
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IUPAC	International Union of Pure and Applied Chemistry
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
Pb	Lead
Se	Selenium
Sr	Strontium
Tl	Thallium
V	Vanadium
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Heavy metals are naturally occurring elements that are found throughout the Earth's crust (Tchounwou *et al*., 2012). They are metallic chemical elements that has a relatively high density, specific gravity or atomic weight and are toxic or poisonous at low concentrations. But, according to the International Union of Pure and Applied Chemistry or IUPAC, the term "heavy metal" maybe a "meaningless term" because of there is no standardized definition for a heavy metal (Helmenstine, 2014). Typically, the term mostly refers to elements of atomic number 21 or higher and considers all of the transition elements. Their concentrations depend also on local geology and globally distributed pollution (Khan *et al*., 2008; Hang *et al*., 2009).

Examples of heavy metals include; Mercury (Hg), Cadmium (Cd), Arsenic (As), Chromium (Cr), Copper (Cu), Nickel (Ni), Zinc (Zn), Silver (Ag), Thallium (Tl), and Lead (Pb) (Tchounwou *et al*., 2012). As trace elements, the small amount of heavy metal is common in our environment and diet which are needed in very minute quantities for the proper growth, development, and physiology of the organism. These elements include Vanadium (V), Manganese (Mn), Iron (Fe), Cobalt (Co), Selenium (Se), Strontium (Sr) and Molybdenum (Mo). In addition, they cannot be degraded or destroyed.

Furthermore, they play an important role in various oxidation-reduction reactions and also important constituents of several key enzymes (WHO, 1996). However, a large amount of heavy metals can lead to poisoning and cause chronic toxicity. Unlike many organic pollutants, which eventually degrade to carbon dioxide and water, heavy metal will tend to accumulate in the environment (Wang, 2009). Many of the heavy metals are essential, in small quantities, for human health. Eventually, they become concentrated as a result of human caused activities. Common sources are mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilizers; paints; treated woods; and aging water supply infrastructure (Harvey *et al*., 2015). The most common heavy metal pollutants are Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Cadmium (Cd), and Mercury (Hg). Commonly, human and animals may be exposed to the toxicity through any route such as from a contamination of drinking water, food, soil, medicines, improperly coated food containers, the ingestion of lead-based paints, air or water pollution and also dust as well as industrial activities.

It is believed that many toxic metals exert their bad effects by distressing the enzyme systems of animals. Many of them will bind to specific enzymes and proteins necessary for cellular function and thus compete with other substances essential for maintenance and the continued function of cells. Thus, the poisons can also have the effect of inducing mineral deficiencies (Korff, 2013). Additionally, many toxins appear to assist in the formation of the paramagnetic anion, superoxide (O_2-), which itself is toxic and seems widely responsible for the spontaneous cell death (Davis, 2010).

In this study, the heavy metal concentration level should be identified in the body of long-tailed macaque in order to determine their health status. Long-tailed macaques (*Macaca fascicularis*) are an edge species (Gumert *et al.*, 2009), tend to live at the forest borders in a wide range of habitats. Besides that, the long-tailed macaque is a species that can easily and quickly adapt with other species. When they inhabit disturbed areas near human settlement, long-tailed macaques quickly learn to raid gardens or crops and beg for food from humans (Lucas & Corlett, 1991). The long-tailed macaque is an omnivores species where their primary food choice is consistent more on fruits compared with other types of foods (Berenstain, 1986). But they are an opportunistic feeding omnivore type, meaning that they can and will eat a wide variety of animals, plants and other materials.

Nowadays, due to continuity of human activities, their diet may easily be exposed to any heavy metal elements and their diet might be alternate to another food sources. Based on the previous study, it is stated that the long-tailed macaque changes their diet to alternative food sources when fruits are unavailable and therefore some of the researchers classify them as frugivorous (Berenstain, 1986). Furthermore, they are keen to feed on the food wastes in garbage bins; instead of consuming plants as their food sources, their health will be affected.

In addition, Macaque monkeys shared important immunological and physiological similarities with humans, particularly in the way they respond to toxic exposure (Lee *et al.*, 2012). This makes macaques potentially valuable as sentinels for toxic exposures and predictors of physiologic responses to chemicals in humans (Engel *et al*., 2010).

A number of criteria are cited as being important to an organism being a good sentinel. These include a relatively large body size, sensitivity to the particular agents, physiology similar to that of humans with a similar route of exposure, a relatively long life, being non-migratory with a wide distribution in the environment, and having a short latent period between exposure and symptom onset (O'Brien *et al*., 1993). Macaques satisfy all of these criteria for being a good sentinel.

1.2 Problem Statement

Based on the previous studies, there do not seem to be much of research done regarding the heavy metal concentration in long-tailed macaques (*Macaca fascicularis*). So, this study was the first attempt to investigate heavy metal (lead) concentration in long-tailed macaque hair at Kuala Selangor Nature Park and Bukit Melawati. In this field of study, we will investigate and also determine the heavy metal (lead) concentration in the long-tailed macaque hair.

The purpose study of this research is to have a better understanding with more depth investigation and also to look out on observation about the importance of the effects of the heavy metal (lead) in the long-tailed macaques Kuala Selangor Nature Park and Bukit Melawati.

1.3 Objective

The purpose of this research study is: -

1. To determine the heavy metal (lead) concentration level in the long-tailed macaque (*Macaca fascicularis*) hair.
2. To distinguish their health status between those inside and outside Kuala Selangor Nature Park.

CHAPTER 2

LITERATURE REVIEW

2.1 Macaque

The macaque can become a model for physiological studies of human visual perception, including color (Conway *et al.*, 2010) because of the similarities of immunological and physiological with human. Because of that, macaques are good sentinel to humans for environmental pollutions because their similarities in genetic and physiological characteristics (Lee *et al.*, 2012).

Based on the past investigation studies that have been conducted over 35 years ago, it concluded that macaque have excellent color vision that is probably similar with humans (De Valois *et al.*, 1974; Sidley *et al.*, 1965). Consistent with the conclusion, macaques have similar higher order psychophysical chromatic mechanism to those documented in human (Stoughton *et al.,* 2012).

2.2 Long-tailed Macaque (*Macaca fascicularis*)

2.2.1 Background of Long-tailed Macaque

Macaca fascicularis (Raffles, 1821) is also known as long-tailed, crab-eating or cynomolgus macaque. In Indonesia and Malaysia, *M. fascicularis* and other macaque species are known generically as kera, possibly because of their high-pitched cries. The crab-eating macaque differs from other macaque species in the fact that the crab-eating macaque has a long tail which is about the same length as its body. Their long tail helps to balance and their sharp nails and fingers to toes that help with their grip

Macaca fascicularis is the most widespread and ecologically diverse of nonhuman primate species in the world (Fooden, 1995). It is a cercopithecinae primate native to Southeast Asia (Perveen & Anuar, 2014), weighing between 3 to 5 kilograms for female and weighing between 5 to 9 kilogram for male and their body length of adult is 38-55 cm with comparably short arms and legs with tail typically 40-65 cm. Long-tailed macaques is widely distributed in Southeast Asian region includes Thailand, Indonesia, Singapore, Malaysia, Philippines, Vietnam and Laos (Brandon-Jones *et al.*, 2004) (Figure 1.0). The long-tailed macaques can be found everywhere in Peninsular Malaysia, Sabah and Sarawak, especially in lowland areas and along the coasts, plus nowadays they can be easily found in urban areas (Perhilitan, 2006). The population has grown to about 35,000 animals and the long-tailed monkeys is culled and exported for use in biomedical research (Sussman & Tattersall, 1986; Bonnotte, 2001).

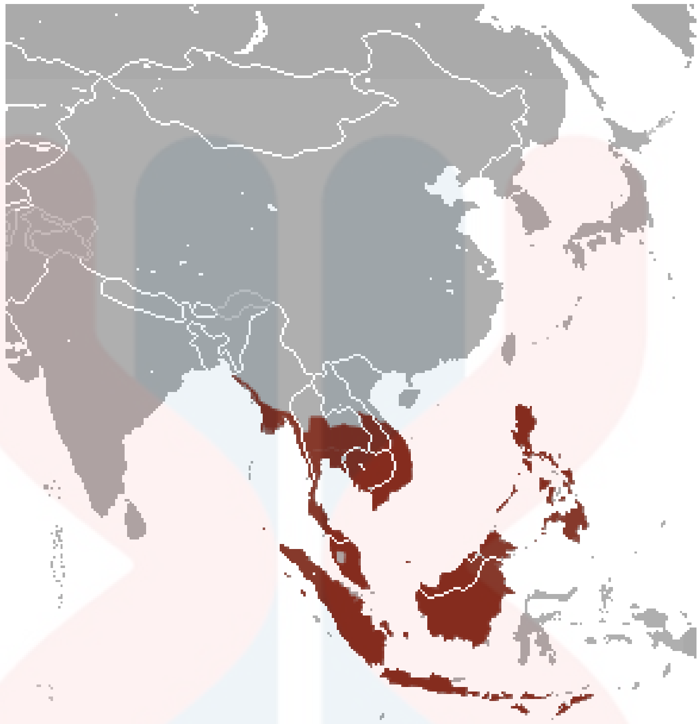

Figure 1.0: The distribution of long-tailed macaque

(Source: Wikipedia)

Long-tailed macaques are the species that live in a large group of about 30 individuals (Lucas, 1995) and also known as a social species. They live in groups and their social structures as well as their behavior are almost as complex as the human when in the wild. Long–tailed macaques can be considered as a good proxy to human for environment pollution because of their similarities in genetic and also physiological characteristics.

2.2.2 Long-tailed Macaques behavior

Long-tailed macaques have unique behavior among other non-human primates because of their ability to show learned or cultural behavior. This cultural behavior can be observed in the preparation of food by long-tailed macaques. For instance, in Sumatera and Kalimantan, there three groups of long-tailed macaque monkey have been observed to catch and eat fish (Stewart *et al.,* 2008). Furthermore, they use fibers, human hair and wooden picks to clean and floss their teeth in Lopburi, Thailand (Watanabe *et al.,* 2007). In Thailand and Myanmar, crab-eating macaques use stone tools to open nuts, oysters, and other bivalves, and various types of sea snails (nerites, muricids, trochids, etc.) along the Andaman sea coast and offshore islands (Gumert *et al.,* 2009).

Besides that, the long-tailed macaques are diurnal animal that is active during the day where they use one full day to travel from one area to another in order to search for food (Hambali *et al.,* 2012). These facts have been supported by past researchers where it was stated that their movement patterns are influenced by the distribution of food resources (O'Brien & Kinnaird, 1997). Long-tailed macaques are opportunistic omnivores (Bonadio & Christopher, 2000), and exploit many different food types, reflecting the diversity of habitats they can utilize. Because of that, they can become a synanthrope, living off human resources. They are known to feed in cultivated fields on young dry rice, cassava leaves, rubber fruit, taro plants, coconuts, mangos, and other crops, often causing significant losses to local farmers (Long, 2003). The main food consists of human food.

The primary food of long-tailed is more on fruit compared with other type of foods but nowadays, (Long, 2003) in villages, towns, and cities, they frequently take food from garbage cans and refuse piles. The species can become unafraid of humans in these conditions, which can lead to macaques directly taking food from people, both passively and aggressively. Furthermore, they are keen to feed from food wastes from garbage containing plants or fruits. This can be attributed to the higher availability of anthropogenic food sources like human garbage and lack of natural food sources in the area of anthropogenic habitats (Sha & Hanya, 2013).

In addition, this may be also caused by habituated factors because people often give food to the long-tailed macaques (Hambali *et al*., 2012 a) such as breads, nuts, snacks and sweets. The long-tailed macaques also were seen to be causing damage to human crops and eating food wastes in urban and housing area (Twigg, 2008).

2.3 Heavy metal concentration

Heavy metals are trace metals which can cause harm to human, animal and other organism health and having a density at least five times that of water (Sardar *et al.*, 2013). Because of indiscriminate human activities widespread and rampant altered heavy metal geochemical and biochemical balance (Singh *et al.*, 2011). Heavy metals cannot be degraded or destroyed (Tchounwou *et al.*, 2012). They can enter a water supply from industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. Also, they can be exposed into our body via inhalation, ingestion and skin absorption (Verma & Pratima, 2013). Besides that, exposures of high heavy metal concentration are not a requirement to produce a state of toxicity in the body, as heavy metal accumulation occurs in body tissues gradually and, over time, can reach toxic concentration levels, much beyond the permissible limits (Khanna, 2011). The bioaccumulation of toxic metals can occur in the body and food chain (Govind & Madhuri, 2014).

In addition, heavy metals can affect the production and the quality of crops and also influences the quality of the atmosphere and water bodies and threatens the health and life of animals and human beings by way of the food chain (Sardar *et al.*, 2013). Heavy metals are also one of the major contaminating agents in our food supply (Zaidi *et al.*, 2005; Khair, 2009). However, some elements otherwise regarded as heavy metals are essential, in small quantities, for human health, if it has arrived from organic or plant resource.

Heavy metals become toxic and harmful if they come from inorganic or metallic sources (Saccoman). Leaf parts accumulate the higher amounts of heavy metals because they absorb these metals in their leaves and then the leaves will be consumed by human and also animals (Santamaria *et al.*, 1999).

The excess quantities of heavy metals are detrimental as these destabilize the ecosystems because of their bioaccumulation in organisms, and elicit toxic effects on biota and even death in most living organisms (Gupta, 2013). Also, due to formation of toxic soluble compounds, certain heavy metals become toxic (Govind & Madhuri, 2014).

2.4 Wildlife as sentinels of environmental contamination

The role of animals as sentinels of environmental contamination is often discussed in the literature by many researchers (Schillaci *et al*., 2011). Sentinels are seen as being unique in that they focus attention on the premise that human and animal health is interrelated (O'Brien *et al*. 1993). However, not all animal species are relevant models for human toxicant exposures (Engel *et al*., 2010) but macaques satisfy all of these criteria.

They share similarities in physiology and life history traits, for example a long juvenile period, are attributable to humans and macaques sharing a recent common ancestor dating to the Oligocene (ca 30–35 mya) (Steiper *et al*., 2004). Because of that similarities, they also known as synanthropic species in South and Southeast Asia region, where they are ecologically associated with humans (Engel *et al*., 2010). Synanthrophy with humans increase the possibility that macaques come into contact with anthropogenic toxicants, such as lead and mercury, and might be appropriate sentinels for human exposures to certain toxic materials (Engel *et al.*, 2010). All of these factors contribute to making macaques potentially valuable as sentinels for toxic exposures and predictors of physiologic responses to chemicals in humans.

2.5 Previous research in Kuala Selangor

Despite the relatively large body of research on macaque biology and behavior cited above, there have been no studies on macaque exposure to environmental contaminants in Kuala Selangor. Animal hair is a good bio-monitoring tool for heavy metal assessment, because it investigates the accumulation and concentration of heavy metal over time (Rashed & Soltan, 2005). The objective of this study was to assess lead levels in macaque hair samples at Kuala Selangor. I hypothesized that Kuala Selangor is urbanized, its macaque population may be exposed to lead levels. One of potential advantages of measuring heavy metal levels in hair includes the ability to obtain hair noninvasively and to store and transport it easily and with little infectious risk (Engel *et al.*, 2010). Hair analysis is inexpensive and fast; it also detects and measures the content of heavy metals and minerals of the hair (Peter *et al.*, 2012).

2.6 Lead effect on animal and Long-tailed Macaques

The item like computer equipment, batteries and other toxic material are buried in the ground, or present in the stream runoff, in the garbage can, in groundwater for drinking water, or in floodwater, they are toxic to human and animal as well as plants (Govind & Madhuri, 2014). The toxic like mercury (Hg) accumulate in the aquatic system, and it will be toxic to animal when they eat fish or drink the water (Govind & Madhuri, 2014).

On the other hand, heavy metals are frequently mixed in fertilizer. However, the toxic level of such a metal are absorbed by the plants, and long-term consumption by animals may resulting into negative consequences on that animal (Govind & Madhuri, 2014). The long-tailed macaques are the omnivore's species that eat fruit or plant more than the alternate food. The long-tailed macaques may be exposed to the heavy metal when eating the contamination of fruit or vegetables.

Furthermore, some of the long-tailed macaque feed the food waste from the garbage cans that are already contaminated that are also exposed to the heavy metals. These heavy metals can cause a disease to the animal (Govind & Madhuri, 2014). The items that have been discarded in the trash may be material goods that may contain lead such as toys and cosmetics. These goods cannot be identified by macaque whether it's food or objects that may be eaten, but they will try to eat them, with the possibly of exposure to lead poisoning. The other factors that will contribute for lead poisoning are dust from manufacturing.

In addition, lead moves into and throughout ecosystems (Greene, 1993). The atmospheric lead will deposit in vegetation, ground and water surfaces (Greene, 1993). The chemical and physical properties of lead and the biogeochemical processes within ecosystems may impact the movement of lead through ecosystems (Greene, 1993). The metal can influence all components of the environment and can move through the ecosystem until it reaches an equilibrium (Greene, 1993). Lead accumulates in the environment, but in certain chemical environments it will be transformed in such a way as to increase its solubility (e.g., the formations of lead sulfate in soils), its bioavailability or its toxicity (Greene, 1993). The impacts of lead at the ecosystem level are usually seen as a form of stress (US EPA, 1986).

Generally, there are three known approach in which lead can adversely affect our ecosystems (Greene, 1993). For instance, populations of micro-organisms may be wiped out at soil lead concentrations of 1,000 parts per million (ppm) or more, cause slowing the rate of decomposition of matter (Greene, 1993). Populations of plants, micro-organisms and invertebrates may be affected by lead concentrations of 500 to 1,000 ppm, allowing more lead-tolerant populations of the same or different species to take their place (Greene, 1993). This will alter the type of ecosystem present. At all am-bient atmospheric concentrations of lead, the addition of lead to vegetation and animal surfaces can prevent the normal bio-chemical process that purifies and repurifies the calcium pool in grazing animals and decomposer organisms (UNEP, 1991).

Lead affects the central nervous system of animals and inhibits their ability to synthesize red blood cells (Greene, 1993). Lead blood concentrations of above 40 µg/dl can produce observable clinical symptoms in domestic animals. Calcium and phosphorus can reduce the intestinal absorption of lead (US EPA, 1986). The US EPA report generalizes that a regular diet of 2-8 mg of lead per kilogram of body weight per day, over an extended period of time, will cause death in most animals. Grazing animals are directly affected by the consumption of forage and feed contaminated by airborne lead and somewhat indirectly by the up-take of lead through plant roots. Invertebrates may also accumulate lead at levels toxic to their predators (Greene, 1993).

Lead shot and lead weight can severely affect individual organisms and threaten ecosystems (WHO, 1989). After three to ten days of waterfowl ingesting lead shot, the poison will reach the bloodstream and be carried to major organs, like the heart, liver and kidneys. By the 17th to 21st day the bird falls into a coma and dies. Following the ingestion of lead shot, lead toxicities has been observed in Magpie geese, Black swans, several species of duck (including Black duck and Musk duck) and Hardhead species. Organic lead is much more readily taken up by birds and fish (WHO, 1989). Aquatic organisms take up inorganic lead through a transfer of lead from water and sediments; this is a relatively slow process. Organic lead is rapidly taken up by aquatic organisms from water and sediment. Aquatic animals are affected by lead at water concentrations lower than previously thought safe for wildlife. These concentrations occur often, but the impact of atmospheric lead on specific sites with high aquatic lead levels is not clear (US EPA, 1986).

CHAPTER 3

MATERIALS AND METHODOLGY

3.1 Study Area

This study was conducted at the habitat of macaque at the Kuala Selangor Nature Park (Figure 3.1), Selangor, Malaysia (101º 14.678’E, 03º 20.335’N) (Figure 3.2) and the surrounding area including residential area and small town area especially Bukit Melawati area (Figure 3.3). The ecological niche of the macaques outside of Kuala Selangor Nature Park overlaps significantly with that of the surrounding human population.

Figure 3.2: Map of study area

Long-tailed macaque population in Kuala Selangor Nature Park is distributed mainly along the road at the entrance of Kuala Selangor Nature Park (Hambali *et al*., 2012 a) where this area is very close to urbanized areas. The landform in the study area is horizontal at road and residential area while there is a slight slope because of the study area is located near Bukit Melawati (Figure 3.3), Kuala Selangor (Hambali *et al*., 2014). The study area is so close to a residential area that is just a few meters away.

Figure 3.2: Kuala Selangor Nature Park

Source: Owned

Figure 3.3: Hair sample collection at Bukit Melawati area

Source: kuala-selangor.com

The macaques at Bukit Melawati were frequent contact with human because long-tailed macaques itself is one of the tourist's attraction there. As a result, there are varieties of human activities such as walking, jogging, exercising, riding, trading, and feeding of the monkeys. The item offered by human may consists of variety of fruits such as mangoes, bananas, oranges, langsat, and apples. Besides that, fast food such as nuts, snacks, sweets and also breads are also given by human (Hambali *et al*., 2014 a).

Based on the study by (Hambali *et al.,* 2014) stated that the group of the long-tailed macaques outside the Kuala Selangor Nature Park has consume the food waste from garbage can while, the long-tailed macaques in the Kuala Selangor Nature Park mostly consume plants parts, fruits and also insect.

3.2 Hair sample collection

Hair samples were collected from 6 long-tailed macaques (*Macaca fascicularis*) trapped at two different locations where 3 macaques in Bukit Melawati and 3 macaques in Kuala Selangor Nature Park. Monkeys were trapped with portable aluminium cage-like trap where bananas and several raw chicken eggs were put in the cage as bait to attract the monkeys for being trapped. Once trapped, monkeys were hand injected with a 5mg/kg dose of Ketamine HCI to achieve anesthesia. Wildlife Department and National Park (PERHILITAN) ranger were helping to make long-tailed macaque fainted. During anesthesia, their hair specimens were taken manually using latex gloves and surgical scissors, by clipping hair as close to the skin as possible and extracting as much as possible strands of hair from each macaque. Besides that, the hair samples were cut from 3 different spots on each macaque's body. Each macaque's body weight, length and sex were determined and measured. Then, the hair specimen was placed into a sealed plastic bag and labeled until they will be processed for analysis. The hair samples were stored at laboratory refrigerator.

Table 3.0: Information of experimented samples

Location	No.	ID	Sex	Body weight (kg)	Length (cm)	Age
Bukit Melawati	1.	BM1	Female	1.4kg	74cm	4
	2.	BM2	Male	2.8kg	87cm	4
	3.	BM3	Male	5.1kg	101cm	8
Kuala Selangor Nature Park	1.	TA1	Female	2.8kg	88cm	7
	2.	TA2	Female	2.3kg	77cm	2
	3.	TA3	Male	5.1kg	114cm	6

3.2.4 Pb sample preparation and Laboratory analysis

The preparation of the sample to analyzed for Pb were according to Perkin Elmer's AAS analysis guide. Briefly, each hair sample was treated separately. The segments of hair were cut about 5 to 10 mm in length and weighing at least 10 mg with mechanical weighing. To remove the external contamination from hair, the pre-digestion washing technique should be taken such that it will remove only the surface external contamination without extracting metals from the samples or depositing metals on them. Then, hair sample were washed in deionized water on a hot plate and then boil. Then, the sample were transferred to a 100-mL beaker and digest with a 1:5 mixtures of $HClO_4$: HNO_3 until a few drops of clear liquid remain. Next, the sample were diluted 1:50 with deionized water. All laboratory analysis was performed at the Faculty of Science Biology Laboratory at the University of Putra Malaysia. An Inductively Coupled Plasma Mass Spectrometer (ICP-MS) were used in this study to detect the lead concentrations.

3.2.5 Statistical analysis

Data are presented as mean±SD (standard deviation). The differences between heavy metal content of long-tailed macaque at the Kuala Selangor Nature Park and Bukit Melawati were calculated using the Student T-test by using Microsoft Excel 2016.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Lead concentration in macaque hair

The (Table 4.1) shows the lead concentration (µg/g) of macaque's hair in Bukit Melawati and Kuala Selangor Nature Park.

Bukit Melawati		**Kuala Selangor Nature Park**	
Individuals	Pb concentration (µg/g)	Individuals	Pb concentration (µg/g)
BM1	2.57839	TA1	2.04417
BM2	4.61870	TA2	1.61861
BM3	11.7445	TA3	5.82561
Mean (µg/g)	6.31387	Mean (µg/g)	3.16280
S.d (µg/g)	5.23763	S.d (µg/g)	2.25032
n = 6 individuals			

$t = 0.957$ $p < 0.20$

Table 4.1: Lead concentration (µg/g) in Bukit Melawati and KSNP

The average mean of Pb, by individual and trapping group are shown in Appendices. The results of ICP-MS analysis showed the average of lead concentration obtained in µg/g (Arithmetic mean = 4.7383, SD = 4.2755), with a maximum concentration 11.7445µg/g and minimum of 1.6186µg/g. as were all individual values. The average of lead concentrations in hair were slightly higher for Bukit Melawati (6.3138±5.2376µg/g) compared to KSNP (3.1628±2.2503µg/g). Lead concentrations varied substantially by individuals (Figure 4.1).

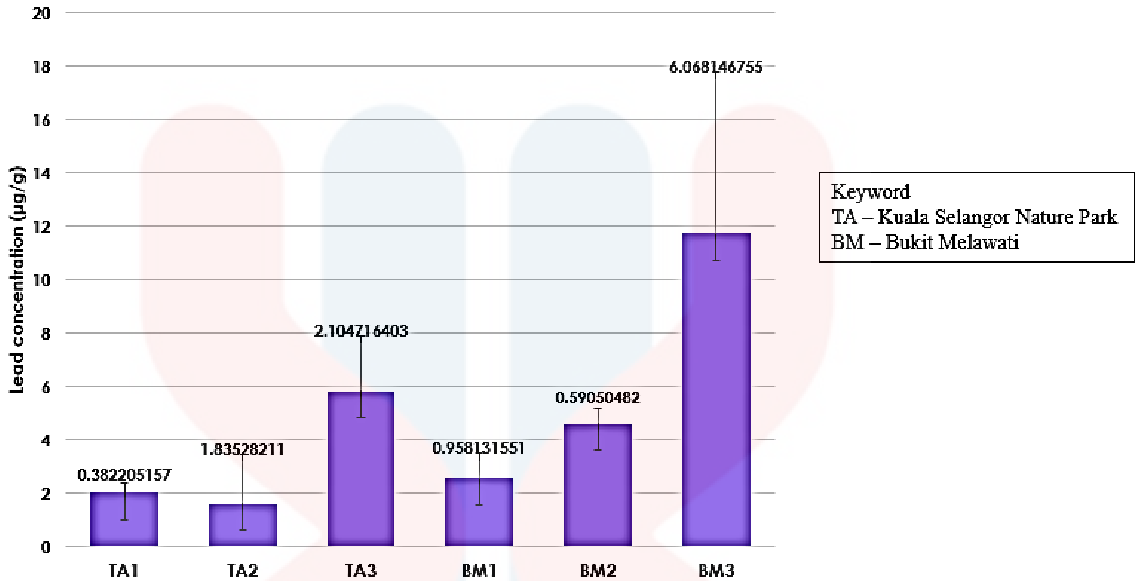

Figure 4.2: Average concentration of individual lead using ICP-MS technique.

Based on (Figure 4.2), the lead concentrations in Bukit Melawati group showed significantly higher between individuals with the highest lead concentrations which is BM3 (11.7445µg/g) while the lowest lead concentrations in the group which is BM1 (2.5783µg/g). In KSNP group, the lead concentrations also showed significantly higher between individuals with the highest lead concentrations which is TA3 (5.8256 µg/g) while the lowest lead concentrations in the group which is TA2 (1.6186µg/g).

Besides that, there were statistically significant differences in Pb concentration between males and females where; male (5.9519±2.6497µg/g) and female (2.3112±0.6701µg/g) (Table 4.2).

Table 4.2: Gender comparison of lead concentrations in hair of long-tailed macaque (µg/g)

Elements	Female (n=3)	Male (n=3)
Lead (Pb)	2.6085±0.6701	7.3963±2.6497

In addition, the highest lead concentration in female group which is from Bukit Melawati group; BM1 (2.5783µg/g) while the lowest lead concentration in the group which is from KSNP group; TA2 (1.6186µg/g). The highest lead concentrations in male group which is from Bukit Melawati group; BM3 (11.7445µg/g) while the lowest lead concentration in the group which is from KSNP group; BM1 (2.5783µg/g).

4.2 Discussion

4.2.1 Macaques as sentinels for lead exposure

The data presented here imply that demographic and/or behavioral variables are associated with lead exposure in the long-tailed macaques. Then, an independent effect was seen with trapping location. A few hypotheses might explain these observations. First, behavior or ranging patterns may bring some animals into more frequent or intense contact with sources of lead. For an example, the macaque is often seen playing in a rough and impurities area, which may come into more frequent contact with lead-containing soil and dust than other animals. Moreover, lead also can be ingested when the animals clean itself.

Since, the long-tailed macaque is an opportunistic omnivorous (Bonadio & Christopher, 2000) and they are likely to feeding a lot of different type of food if their primary food sources are unavailable. This can be attributed to the higher availability of anthropogenic food sources like human garbage and lack of natural food sources in the area of anthropogenic habitats (Sha & Hanya, 2013).

4.2.2 Environmental sources of lead

In this study, the groups with the highest lead concentrations were located at Bukit Melawati. Bukit Melawati is a hill historic where it was the administrative center and stronghold of the Selangor Sultanate in the late 18th and early 19th century but nowadays, it has become one of the most popular tourist attractions in Kuala Selangor during the day. Besides that, Bukit Melawati is also home to the Silver-Leafed Monkeys and Long-Tailed Macaques. Normally, tourists who visit the Bukit Melawati will feed the monkeys food such as peanuts, bananas and yard long bean which can be purchased from vendors around the area.

Although the real situation of exposure is unknown, ingestion of contaminated plants and water, or inhalation of dust, by the monkeys in this area may have caused the observed high exposure to lead. Based on the study by (Hambali *et al*., 2014 a), long-tailed macaques chooses human sources of food waste in the garbage cans that is available at the vicinity area. Besides the macaque roaming around Bukit Melawati to find food, they also find their food in the nearby residential area especially in the trash. In addition, the possibility of the macaques looking for remaining food in trash cans were exposed to impurities found in the trash. For example, the macaque who rummage rubbish bins where there are waste lead-based paint cans, food and drink can and food waste which has been contaminated.

Agency for Toxic Substances and Disease Registry, (1999) stated that exposure to lead primarily occurs through inhalation of lead-contaminated dust or aerosols, and lead ingestion of contaminated food, water, and paint. Common sources of lead include dust containing paint chips or lead released into the atmosphere from industrial or automotive emissions such as leaded gasoline (Goyer, 1990).

According to recent reports from Nepal (Engel *et al*., 2010) and Singapore (Schillaci *et al*., 2011), the lead concentration in the hair of cynomolgus and rhesus monkeys were 2.51 and 6.0 µg/g @ ppm, respectively. While, the lead concentrations result in this study were the second highest among these recent report (Engel *et al*., 2010) and Singapore (Schillaci *et al*., 2011). Although, in this study just report only Pb heavy metal concentration, further studies about continuous monitoring, adverse effects, and comparison between blood and hair on heavy metal concentration are needed.

4.2.3 Comparison of Pb concentration with other worldwide studies

The Pb concentration from the present study, though lower than the study that reported for Nepal (Engel *et al*., 2010) are higher than those reported in the study from Singapore (Schillaci *et al*., 2011). The Pb concentrations for China and Singapore are 6.0µg/g and 2.51µg/g respectively. The Pb concentration reported in China is 0.656µg/g (Jae-Il Lee *et al*., 2012) were the lowest Pb concentration among those reported in the study above. All of the study above determine the Pb concentration using macaque monkeys as an indicator to environmental exposure.

In addition, the study in Nigeria but the determination of Pb by using human hair reported the lowest Pb concentrations among those mentioned above. The Pb concentration in Nigeria (Peter *et al*., 2012) is 0.0738µg/g.

4.2.4 Limitation of study

This study focused on the determination of the levels of Pb only which limits the range of other heavy metal that might detect in these macaque's hair. In addition, at the beginning of the study, the analysis of the Pb was carried out using AAS machine but the AAS machine cannot detect heavy metals concentration at very lower values.

4.2.5 Further study

Further research should be needed for a better how behavior and also feeding ecology were influenced the lead exposure among the long-tailed macaque in Kuala Selangor. There should be more data acquired on feeding ecology and behavior pattern of the macaque and also precisely search for sources of lead in and around Kuala Selangor.

Besides that, a comparison lead levels in macaques to those local human populations, will help to investigate whether macaques are suitable sentinels for lead exposure for human populations. Other than that, measurement of other heavy metal contamination on environment, such as iron and mercury, could provide further insight into the ecotoxicology of these macaques.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, macaque is a good sentinel for lead exposure in human and environment. The results of this study indicated relatively low lead concentrations in hair from macaque monkeys in Kuala Selangor except for individual's BM3 which is from Bukit Melawati. Moreover, all samples were below the 11 ppm reference value used to indicate potentially unsafe exposure to lead (Lee *et al.*, 2012). The highest lead concentrations in macaque's hair were from Bukit Melawati area, which is the tourist attraction area where the macaque were frequently contact with the human and also the surrounding area. Furthermore, this finding shows that the potential utility of macaque monkeys as sentinels of environmental toxins such as lead. In addition, behavioral and/or physiologic factors contribute a role in determining the lead levels in macaque hair. If the macaque is suitable as sentinels for environmental exposure such as lead (Pb), the result of this study suggest that the environmental risk of heavy metal surround Kuala Selangor area is still minimal and safe to the human population there, so it can be monitoring and preventative strategies for further remediation before the problem of lead exposure can contribute a serious problem to the human population and also the environment.

5.2 Recommendation

For future study, refinement in the research protocol should include measurement of lead concentrations in both hair and blood, and could include other biomarkers for lead exposure. This is to acquire more data which reliable and a lot of comparison can be made for accuracy of data interpretation. Analysis of heavy metal concentration in hair were recommended using ICP-MS technique because of the ICP-MS can detect extremely low concentration or ultra-low concentration of elements compared to AAS technique.

It is recommended that further studies need to be done in evaluating the levels of other heavy metals and metalloids like arsenic, selenium, nickel, chromium and vanadium. Further, the data obtained from this study suggest that macaque hair can be used for identification and monitoring of exposure to heavy metals in Kuala Selangor.

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APPENDIX A

				Comparison between individual (µg/g)		Comparison between location (µg/g)	
Replicate	µg/g	Average (µg/g)	SD (µg/g)	Average	SD	Average	SD
TmnAlam1Rep1	1.738854						
TmnAlam1Rep1	1.73661						
TmnAlam1Rep1	1.716536	1.730666	0.012289				
TmnAlam1Rep2	2.442561						
TmnAlam1Rep2	2.466286						
TmnAlam1Rep2	2.452754	2.453867	0.011902				
TmnAlam1Rep3	1.951256						
TmnAlam1Rep3	1.945615						
TmnAlam1Rep3	1.947064	1.947978	0.00293	2.0441705	0.382205157		
TmnAlam2Rep1	1.446573						
TmnAlam2Rep1	1.465913						
TmnAlam2Rep1	1.453756	1.455414	0.009776				
TmnAlam2Rep2	1.670572						
TmnAlam2Rep2	1.506263						
TmnAlam2Rep2	1.704705	1.62718	0.106099				
TmnAlam2Rep3	1.776231						
TmnAlam2Rep3	1.788673						
TmnAlam2Rep3	1.754872	1.773258	0.017095	1.618617167	1.83528211		
TmnAlam3Rep1	5.669805						
TmnAlam3Rep1	5.673683						
TmnAlam3Rep1	5.657415	5.666967	0.008497				
TmnAlam3Rep2	7.798856						
TmnAlam3Rep2	7.94674						
TmnAlam3Rep2	7.95743	7.901008	0.088628				
TmnAlam3Rep3	3.92009						
TmnAlam3Rep3	3.877168	3.908877	0.02785	5.825617444	2.104716403	3.1628017	2.25032253

TmnAlam3Rep3	3.929373						
BktMelawati1Rep1	2.516593						
BktMelawati1Rep1	2.490645						
BktMelawati1Rep1	2.53162	2.512952	0.020729				
BktMelawati1Rep2	2.620148						
BktMelawati1Rep2	2.568954						
BktMelawati1Rep2	2.542878	2.577326	0.039309				
BktMelawati1Rep3	2.642288						
BktMelawati1Rep3	2.631428						
BktMelawati1Rep3	2.660971	2.644896	0.014943	2.578391333	0.958131551		
BktMelawati2Rep1	4.326808						
BktMelawati2Rep1	5.023683						
BktMelawati2Rep1	4.653504	4.667998	0.348664				
BktMelawati2Rep2	5.083629						
BktMelawati2Rep2	5.118543						
BktMelawati2Rep2	5.084322	5.095498	0.01996				
BktMelawati2Rep3	4.051187						
BktMelawati2Rep3	4.08078						
BktMelawati2Rep3	4.14585	4.092605	0.048427	4.618700389	0.59050482		
BktMelawati3Rep1	3.160199						
BktMelawati3Rep1	4.085074						
BktMelawati3Rep1	4.570557	3.93861	0.716496				
BktMelawati3Rep2	13.67165						
BktMelawati3Rep2	19.25191						
BktMelawati3Rep2	14.50527	15.80961	3.010117				
BktMelawati3Rep3	14.75494						
BktMelawati3Rep3	15.47885						
BktMelawati3Rep3	16.2224	15.4854	0.733749	11.74453994	6.068146755	6.31387722	5.23763817

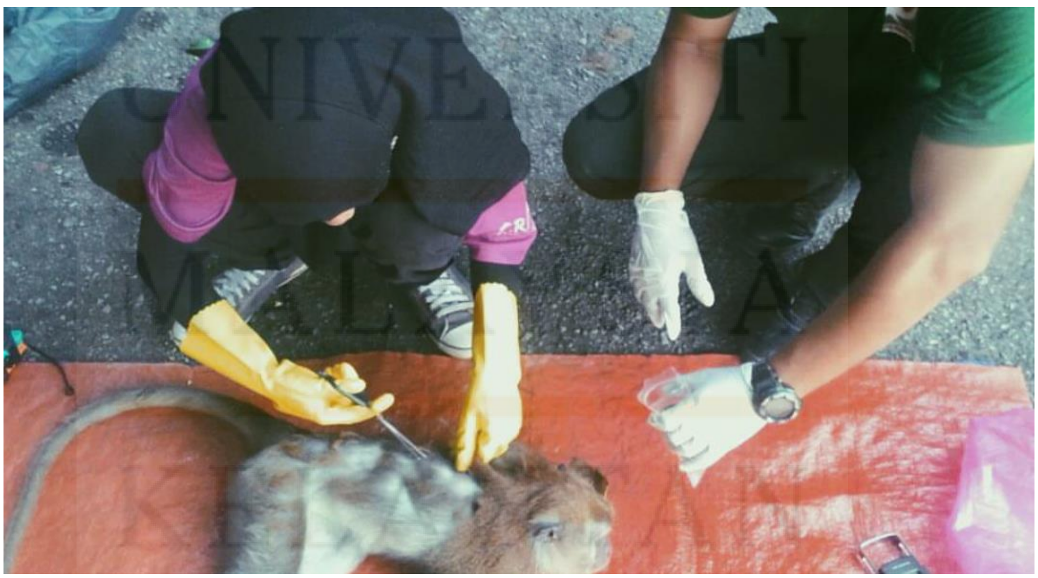
APPENDIX B

Macaque hair collection

One of macaque trapped at Bukit Melawati

Macaque trapped in portable aluminium cage at KSNP

Weighing the macaque



Cutting macaque’s hair manually with scissors

Macaque spot on the tree at KSNP

With the ranger from Sabak Bernam's Wildlife Department and National Park (PERHILITAN) in front of KSNP