

LEAD CONCENTRATION IN LONG-TAILED MACAQUE (*MACACA FASCICULARIS*) HAIR IN KUALA SELANGOR, MALAYSIA

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Abstract: Long-tailed macaque (*Macaca fascicularis*) has the potential to be a good biological indicator for toxic exposure because they have an almost similar physiology and behaviour to humans. The objective of this study is to determine the concentration of lead (Pb) in hair samples of long-tailed macaques which were found in and out of the Kuala Selangor Nature Park (KSNP) area. The hypothesis is long-tailed macaques that live in the anthropogenic area (outside KSNP) may be exposed to high levels of lead compared to long-tailed macaques living in the forest area (inside KSNP). Analysis of hair samples were carried out using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The study found that the average mean of lead concentration in the anthropogenic area is 6.31 µg/g while for the forest area it is 3.16 µg/g. Lead concentration in the two areas are statistically insignificant. Nevertheless, lead concentration in the anthropogenic area recorded a slightly higher mean concentration than in the forest area. Even so, results of this study indicate that long-tailed macaques in Kuala Selangor are not exposed to high levels of lead. This study is the first in Malaysia to utilise long-tailed macaques as a biological indicator for testing the concentration of toxic substances in the environment. This study is still in its early stages; thus, future research requires improvements.

Keywords: Lead, Long-tailed macaque, *Macaca fascicularis*, Ecotoxicology, Kuala Selangor

INTRODUCTION

Heavy metals play an important role in various oxidation-reduction reactions and are important constituents of several key enzymes (WHO, 1996). Nevertheless, 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 metals tend to accumulate in the environment (Wang, 2009). Many 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, fertilisers, 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), and mercury (Hg). Commonly, human and animals may be exposed to the toxicity via routes such as contamination of drinking water, food, soil, medicines, improperly coated food containers, ingestion of lead-based paints, air or water pollution, dust, and industrial activities.

It is believed that many toxic metals exert their detrimental effects by distressing the enzyme systems of animals. Many heavy metals will bind to specific enzymes and proteins necessary for cellular function; hence, competing with other substances essential for maintenance and continued function of cells. Thus, the poisons can also induce mineral deficiencies (Korff, 2013). Additionally, many toxins appear to assist the formation of the paramagnetic anion, superoxide (O_2^-), which is toxic and seems to be widely responsible for spontaneous cell death (Davis, 2010).

Long-tailed macaques are an edge species (Gumert et al., 2009) and tend to live at forest borders in a wide range of habitats. Besides that, they can easily and quickly adapt to the environment. When they inhabit disturbed areas near human settlements, long-tailed macaques quickly learn to raid gardens or crops and beg for food from humans (Lucas & Corlett, 1991). Long-tailed macaques are omnivores whereby their primary food choice consists of fruits compared to other types of food (Berenstain, 1986). However, they are opportunistic feeders, meaning they can and will eat a wide variety of animals, plants, and other materials.

Nowadays, due to human activities, long-tailed macaques' diet may easily be exposed to heavy metal elements and their diet might be alternate to another food sources. Furthermore, they are keen to feed on food wastes in garbage bins, instead of consuming plants; this habit will affect their health. On the other hand, long-tailed macaques share important immunological and physiological similarities with humans, particularly in the way they respond to toxic exposure (Lee et al., 2012). This makes them potentially valuable as a biological indicator 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 for an organism to be a good biological indicator. These include a relatively large body size, sensitivity to the particular agents, similar physiology to 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). Long-tailed macaques satisfy all these criteria for being a good biological indicator. Based on the previous studies, it seems that there are still few researches done regarding to the heavy metal concentration in long-tailed macaques. This study is the first attempt to investigate heavy metal (lead) concentration in long-tailed macaque hair at the study area. Hence, the present study aims to determine the lead (Pb) concentration in hair samples of long-tailed macaques found inside and outside of Kuala Selangor Nature Park (KSNP).

MATERIALS AND METHODS

Study Area

This study was conducted at the habitat range of long-tailed macaques in KSNP, Selangor, Malaysia (101° 14.678'E, 03° 20.335'N) and the surrounding area including a small town, Bukit Malawati and a residential area (Fig. 1). Long-tailed macaques outside KSNP were mainly distributed along the road

at the entrance of KSNP (Hambali et al., 2012); this area is very close to the residential areas. The landform in the study area is horizontal at the road and residential area while there is also a slight slope because the study area is located near Bukit Malawati, Kuala Selangor (Hambali et al., 2014a). The ecological niche of long-tailed macaques outside KSNP was overlapping with the human population. The items offered by humans may consist of a variety of fruits such as mangoes, bananas, oranges, langsat, and apples. Besides that, junk food such as nuts, snacks, sweets, and breads are also given by humans (Hambali et al., 2014b). Based on the study by Hambali et al. (2014b), the group of long-tailed macaques outside KSNP consume food waste from garbage cans while long-tailed macaques inside KSNP mostly consume plant parts, fruits, and insects.



Figure 1: Map of study area (The black circle indicates samples of long-tailed macaque's hair that were taken).

Hair Sample Collection

Hair samples were collected from six long-tailed macaques trapped at two different locations; three macaques each from inside and outside KSNP (Table 1). The long-tailed macaques were trapped using portable aluminium cage-like trap whereby bananas and several raw chicken eggs were put in the cage as bait to attract them. Once trapped, the long-tailed macaques were hand injected with 5 mg/kg Ketamine HCl to achieve anaesthesia. A ranger from the Department of Wildlife and National Parks (DWNP) helped to make the long-tailed macaques fainted. During anaesthesia, hair specimens were taken manually using latex gloves and surgical scissors, by clipping the hair as close as possible to the skin and extracting, as much as possible, hair strands from each macaque. Moreover, hair

samples were cut from three different spots on each macaque's body. Then, each macaque's body weight, length, and sex were recorded. Hair specimens were placed into sealed and labelled plastic bags and stored at the laboratory refrigerator until processed for analysis.

Table 1: Information of captured macaques.

Location	No.	Individual	Sex	Body weight (kg)	Length (cm)	Age	Health status
Outside	1	O1	Female	1.445	74	4	Healthy/no injury
	2	O2	Male	2.830	87	4	Healthy/no injury
	3	O3	Male	5.190	101	8	Healthy/no injury
Inside	1	I1	Female	2.830	88	7	Healthy/no injury
	2	I2	Male	2.350	77	2	Healthy/no injury
	3	I3	Male	5.120	114	6	Healthy/no injury

Laboratory Analysis

The preparation of sample for lead (Pb) analysis was according to Perkin Elmer's guidebook. First, each hair sample was treated separately. Hair segments were cut about 5 to 10 mm in length and weighing at least 10 mg. In order to remove the external contamination from the macaque's hair, the pre-digestion washing technique was conducted to remove only the surface or external contamination without extracting metals from the samples or depositing metals on them. Then, the hair samples were washed in deionised water on a hot plate and then boiled. Next, the sample is transferred to a 100-mL Teflon beaker and digested with a 1:5 mixture of HClO₄:HNO₃ until a few drops of clear liquid remain. Subsequently, the sample was diluted to 1:50 with deionized water. All analysis was performed at the laboratory of Department of Biology, Faculty of Science, Universiti Putra Malaysia. An Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was used in this study to determine lead concentration.

Statistical Analysis

Data are presented as mean±SD (standard deviation). The differences in lead concentration were calculated using Student's T-test. All statistical analyses were conducted using Microsoft Excel software.

RESULTS

The average mean of Pb, by individual and trapping group, are shown in the Appendix. The results of ICP-MS analysis showed the average lead concentration obtained in $\mu\text{g/g}$ (arithmetic mean = 4.74, SD = 4.28), with a maximum and minimum concentration of 11.74 $\mu\text{g/g}$ and 1.62 $\mu\text{g/g}$, respectively, and all individual values. The average lead concentration in hair was significantly higher for outside (6.3138 \pm 5.2376 $\mu\text{g/g}$) compared to inside KSNP (3.16 \pm 2.25 $\mu\text{g/g}$). In addition, lead concentrations of individuals varied substantially (Fig. 2).

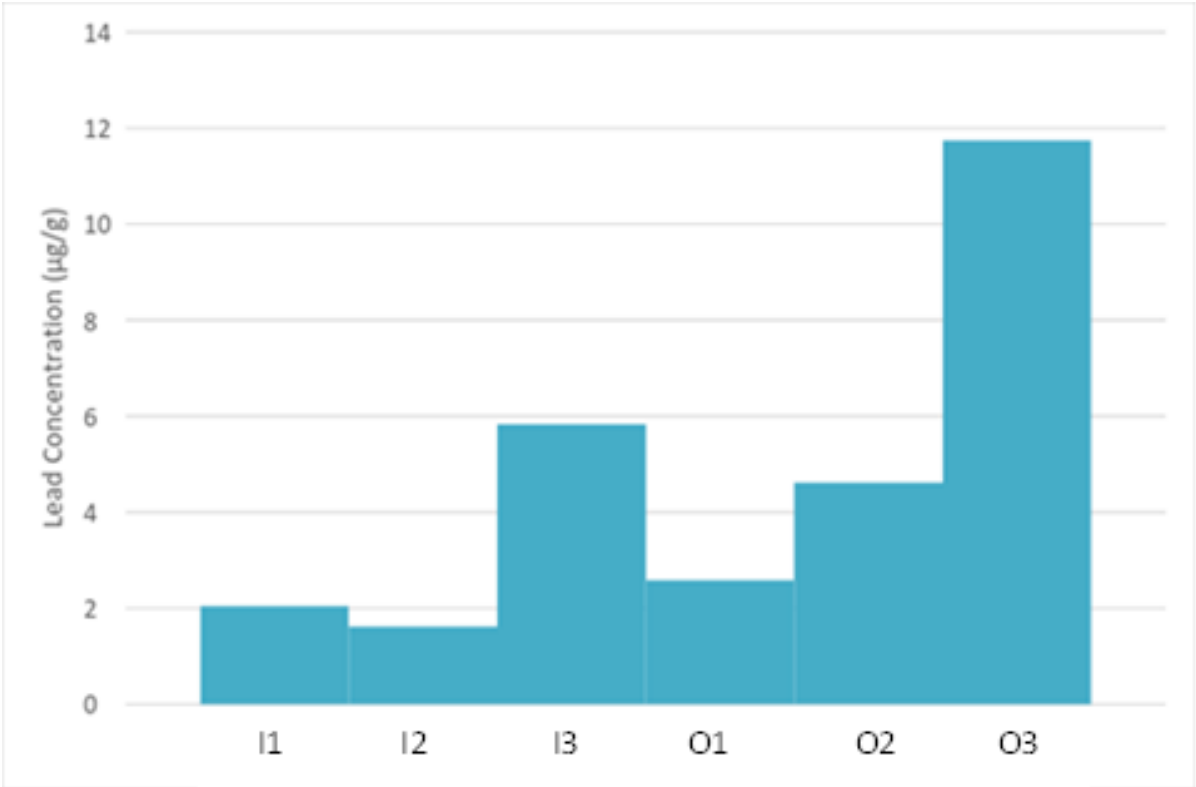


Figure 2: Average concentration of lead in different individuals determined using ICP-MS technique. *I = Inside; O = Outside

Based on Figure 2, lead concentration for outside KSNP was significantly higher between individuals with O3, scoring the highest (11.74 $\mu\text{g/g}$) and O1; scoring the lowest (2.58 $\mu\text{g/g}$). For inside KSNP, lead concentration was also significantly higher between individuals, with I3 scoring the highest with 5.83 $\mu\text{g/g}$ while I2 scored the lowest with 1.62 $\mu\text{g/g}$. Moreover, there were statistically significant differences in lead concentrations between males (5.95 \pm 2.65 $\mu\text{g/g}$) and females (2.31 \pm 0.67 $\mu\text{g/g}$) (Table 2).

Table 2: Comparison of lead concentration according to gender in the hair of long-tailed macaques ($\mu\text{g/g}$)

Element	Female (n = 2)	Male (n = 4)	Total (n = 6)
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Lead (Pb)	2.31±0.67	5.95±2.65	4.74±4.28
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In addition, the highest lead concentration in the female group was found outside KSNP, which is O1 (2.58 µg/g) while the lowest was inside KSNP, which is I1 (2.04 µg/g). For the male group, the highest lead concentration was found outside KSNP, which is O3 (11.74 µg/g) while the lowest was found inside KSNP, which is I2 (1.62 µg/g).

DISCUSSION

The data presented here imply that demographic and behavioural variables are associated with lead exposure in long-tailed macaques. Furthermore, an independent effect was seen with trapping locations. A few hypotheses could explain these observations. First, behaviour or ranging pattern may bring some animals into more frequent or intense contact with sources of lead. For example, the macaque is often seen playing in rough and impure areas, hence, coming into more frequent contact with lead-containing soil and dust compared to other animals. Moreover, lead can also be ingested when the animals clean their body. Outside KSNP, there is a source of water from a man-made pond, which the long-tailed macaques use as a place to drink and bathe (Hambali et al., 2012).

Since the long-tailed macaque is an opportunistic omnivorous animal (Bonadio & Christopher, 2000), they are likely to feed on many different types 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 anthropogenic habitats (Sha & Hanya, 2013).

In this study, the long-tailed macaque with the highest lead concentration was located outside KSNP. From personal observation, tourists visiting KSNP will feed the monkeys outside KSNP with peanuts, bananas, and long beans 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. (2014b), long-tailed macaques choose human-sourced food waste in garbage cans available at the area. Besides that, they also find food in the nearby residential area, especially in the trash. In addition, the possibility of the long-tailed macaques looking for remaining food in the trash can cause them to be exposed to polluted materials found in the trash. For example, the long-tailed macaques rummage trash that has lead-based paint cans, food and drink cans, and food waste contaminated with lead.

The Agency for Toxic Substances and Disease Registry (1999) have stated that exposure to lead primarily occurs through inhalation of lead-contaminated dust or aerosols, and ingestion of food, water, and paint contaminated with lead. 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), lead concentration in the hair of cynomolgus and rhesus monkeys were 2.51 and 6.00 µg/g, respectively. Meanwhile, lead concentration results in this study were the second highest among

these recent reports. The Pb concentration from the present study is lower than the study in Nepal but higher than those reported in Singapore. Furthermore, the Pb concentration reported in China, 0.656 µg/g (Lee et al., 2012), is the lowest Pb concentration among those reported in the aforementioned studies. All these studies determined Pb concentration using macaque species as an indicator to environmental exposure. Although this study reported only on lead concentration, further studies on measurement of other heavy metal contaminations, continuous monitoring, adverse effects, and comparison of heavy metal concentration between blood and hair are needed.

CONCLUSION

The result from this study indicates relatively low lead concentrations in long-tailed macaque hair in KSNP. Nevertheless, the highest lead concentration in long-tailed macaque hair was from outside of the KSNP, which is a tourist attraction area where long-tailed macaques frequently come in contact with the humans. Furthermore, this finding shows that long-tailed macaque could be a good biological indicator for environmental pollutants such as lead. This can be a strategy for monitoring and preparing further remedy before the lead exposure becomes a serious problem to the human population and the environment.

ACKNOWLEDGEMENT

We would like to express our gratitude towards the staffs at the Department of Wildlife and National Parks (DWNP), Sabak Bernam, Selangor branch who have helped and gave full cooperation during the hair and sample collection. The assistance from staffs at the Department of Biology, Faculty of Science, Universiti Putra Malaysia is also sincerely acknowledged for their help in running the laboratory analysis using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) to detect lead concentration.

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Sample replication and results.

				Comparison between individual (µg/g)		Comparison between location (µg/g)					
Replicate	µg/g	Average (µg/g)	SD (µg/g)	Average	SD	Average	SD				
InsideKSNP1Rep1	1.738854	1.730666	0.012289	2.0441705	0.382205157						
InsideKSNP1Rep1	1.73661										
InsideKSNP1Rep1	1.716536										
InsideKSNP1Rep2	2.442561	2.453867	0.011902								
InsideKSNP1Rep2	2.466286										
InsideKSNP1Rep2	2.452754										
InsideKSNP1Rep3	1.951256	1.947978	0.00293								
InsideKSNP1Rep3	1.945615										
InsideKSNP1Rep3	1.947064										
InsideKSNP2Rep1	1.446573										
InsideKSNP2Rep1	1.465913										

1							
InsideKSNP2Rep 1	1.453756	1.45541 4	0.00977 6				
InsideKSNP2Rep 2	1.670572						
InsideKSNP2Rep 2	1.506263						
InsideKSNP2Rep 2	1.704705	1.62718	0.10609 9				
InsideKSNP2Rep 3	1.776231						
InsideKSNP2Rep 3	1.788673					3.162801 7	2.2503225 3
InsideKSNP2Rep 3	1.754872	1.77325 8	0.01709 5	1.6186171 67	1.835282 11		
InsideKSNP3Rep 1	5.669805						
InsideKSNP3Rep 1	5.673683						
InsideKSNP3Rep 1	5.657415	5.66696 7	0.00849 7				
InsideKSNP3Rep 2	7.798856						
InsideKSNP3Rep 2	7.94674	7.90100 8	0.08862 8	5.8256174 44	2.1047164 03		
InsideKSNP3Rep 2	7.95743						
InsideKSNP3Rep 3	3.92009						
InsideKSNP3Rep 3	3.877168						
InsideKSNP3Rep	3.929373	3.90887	0.02785				

3		7					
OutsideKSNP1R ep1	2.516593	2.51295 2	0.02072 9	2.5783913 33	0.9581315 51		
OutsideKSNP1R ep1	2.490645						
OutsideKSNP1R ep1	2.5316 2						
OutsideKSNP1R ep2	2.620148	2.57732 6	0.03930 9				
OutsideKSNP1R ep2	2.568954						
OutsideKSNP1R ep2	2.542878						
OutsideKSNP1R ep3	2.642288	2.64489 6	0.01494 3				
OutsideKSNP1R ep3	2.631428						
OutsideKSNP1R ep3	2.660971						
OutsideKSNP2R ep1	4.326808						
OutsideKSNP2R ep1	5.023683						

OutsideKSNP2Re p1	4.65350 4	4.66799 8	0.34866 4	4.61870038 9	0.59050482		
OutsideKSNP2Re p2	5.08362 9	5.09549 8	0.01996				
OutsideKSNP2Re p2	5.11854 3						
OutsideKSNP2Re p2	5.08432 2						
OutsideKSNP2Re p3	4.05118 7						

OutsideKSNP2Re p3	4.08078	4.09260 5	0.04842 7			6.3138772 2	5.2376381 7
OutsideKSNP2Re p3	4.14585						
OutsideKSNP3Re p1	3.16019 9	3.93861	0.71649 6	11.744539 94	6.0681467 55		
OutsideKSNP3Re p1	4.08507 4						
OutsideKSNP3Re p1	4.57055 7						
OutsideKSNP3Re p2	13.6716 5	15.8096 1	3.01011 7				
OutsideKSNP3Re p2	19.2519 1						
OutsideKSNP3Re p2	14.5052 7						
OutsideKSNP3Re p3	14.7549 4	15.4854	0.73374 9				
OutsideKSNP3Re p3	15.4788 5						
OutsideKSNP3Re p3	16.2224						