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PRESENCE OF LEAD (PB) FROM RODENTS AND FROM ITS PARASITE, *HYMENOLEPIS* SP.

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Abstract

The *Muridae* or the murid rodents have known to exist in agroecosystems as pests and are widely used for the determination of the level of environmental contamination based on the concentration of heavy metals in their different organs, tissues or in whole bodies. Helminthic parasites from its host, are also known to accumulate a high amount of lead concentration from their surroundings. This study addresses the Sustainable Development Goals (SDG 15) – Life on Land which is to protect, restore and promote sustainable use of the ecosystem and to halt the biodiversity loss by determining the presence of lead from rodents and its *Hymenolepis* species in an agroecosystem site. From a total of 41 individuals collected rodents namely, *Rattus norvegicus* and *Rattus tanezumi,* 73.17% of these were positive with *Hymenolepis* sp. A representative sample of infected rodents from each sampling station was analyzed for lead analysis. The concentration of lead in the kidney of collected rats as well as their parasite *Hymenolepis* sp. were determined and found to be negative for the presence of lead excluding the helminth parasite from *Rattus norvegicus* gathered from residential areas with 0.0086 ppm. The low level and not detectable results provide evidence that the areas in the study including commercial, agricultural and residentials have a low risk in heavy metal contamination which means that the possible lead exposure were absent to the habitat of the rodents. Possible factors influencing the low lead exposure in host tissue and its helminth parasite should be further determined as well as the factors affecting the abundance of *R. tanezmi* with regards to its behavior in agricultural habitat.

Keywords: rodents; lead; parasite; *Hymenolepis* sp.

INTRODUCTION

This study aimed to provide information on the presence of lead concentration of rodents and its associated *Hymenolepis* species in Central Luzon State University. Lead contamination in an agroecosystem was determined according to the levels of lead present in rodents and its

Hymenolepis sp. In addition, this information serves as reference data in conducting new research for those who are interested in heavy metal accumulation.

Rodents belonging to the family *Muridae* have the most speciose and diverse mammals in terms of morphology and ecology with over 30% of the mammalian fauna, comprising 22 genera and 56 native species (Me-Htwe, Singleton, Hinds, Propper & Sluydts, 2012). Murid rodents that are known to exist in the agroecosystems are pest species including Brown rat *(Rattus norvegicus)* and Oriental house rat *(Rattus tanezumi)* (Stuart, Presscott & Singleton, 2008). More than being pestiferous, rodent species are highly successful in adapting to a variety of environments which makes them extremely abundant. They have known reservoirs of various diseases as they have a small body size that can be caught easily, occupy a restricted territory, and are characterized by a relatively short lifespan (Yang, Zhao, Zhang & Liu, 2017).

Rodents are also widely used for the determination of the level of environmental contamination based on the concentration of heavy metals in tissues, and in different detoxifying organs including kidneys, liver, muscles, bones, fur, and brain (Zakrzewska et al., 2010). The kidney is known as one of the main accumulation of organs for metals in wild rats due to its highest lead content compared with other tissues which contained lead levels below the detection limit (Sures, Grube & Taraschewski, 2002).

Additionally, rodents have been reported with various infected and the greatest range harbor a higher diversity of helminth parasites (Chaisiri et al., 2010). These helminth parasites have been known to accumulate a high concentration of heavy metals from their surroundings and their appearance is a promising tool in environmental monitoring. It also has the capability to accumulate a high amount of lead content as well as the tissues of the host (Sures et al., 2002). Hence, the presence of lead in rodents and its identified *Hymenolepis* species were essential to determine as well as to indicate the level of pollutants in the environment.

MATERIALS AND METHODS

Study Area

Central Luzon State University is one of the state-institutions of higher learning in the country which is located in the Municipality of Science City of Muñoz, Nueva Ecija, Philippines (15.7305° N, 120.9297° E) that covers 658-hectare campus and with an elevation of 79 meters (259 feet). It is the lead agency of Muñoz Science Community and the seat of the Central Luzon Agriculture, Aquatic and Resources Research and Development Consortium (CLAARRDEC). CLSU has been recognized due to its agencies promoting sustainable strategies for agricultural crop production and quality livestock. Aside from the leading agencies of the university, commercial areas and residential areas can be found in CLSU where apartments, dormitories, and households are included.

Collection and Identification of Rodents

Prior to the sample processing, gratuitous permit approval from the Department of Environmental and Natural Resources (DENR) was secured pursuant to Republic Act no. 8745 or "The Animal Welfare Act of 1998". The collection of rodents was conducted from April to November 2019. Three (3) stations were established in the study; residential, commercial, and agricultural areas with the presence of crop fields including rice and vegetables. Live traps were used for trapping rodents and baited with roasted coconut and soaked in peanut butter. For houses, dormitories, and apartments, the traps were set near the kitchen sink and inside the rooms whereas, in the outdoor areas, the traps were set in the garden and nearby garbage bin. The setting of traps was conducted in the afternoon and trapped species were collected immediately in the early morning of the following day. During the handling of the captured specimens, leather or fabric gloves were worn to minimize the chances of being bitten or scratched (Salibay & Luyon, 2008).

Samples were anesthetized with diethyl ether and euthanized via cervical dislocation. Euthanized rats were examined and morphometric measurements including sex, tail, body, ear, hindfoot, and weight were taken, together with records of fur color to assist in distinguishing among rodent species. Photo documentation was also done for further distinguished characteristics of rodents.

Isolation of Tissues and Identification of *Hymenolepis* sp.

Rodents were dissected and kidney organs were separated for heavy metal analysis. *Hymenolepis* species were isolated by carefully opening the intestine lengthwise with scissors along its entire length and were identified up to the genus level through a light microscope with the examination of the scolex, eggs, and proglottid as its distinguishing features (Bordado & Paller, 2017).

Preservation of Kidney and *Hymenolepis* sp.

The harvested kidney was washed with distilled water, placed in a zip-lock plastic bag, properly labeled, and stored at -20°C while the whole segment of *Hymenolepis* species was preserved in 70% ethyl alcohol and stored in vials until processing for the analysis of heavy metals (Bordado & Paller, 2017).

Digestion of Sample

Aqua regia was used for the acid digestion of the sample. For the preparation of the solution, a mixture of concentrated hydrochloric acid and nitric acid in a ratio of 3:1 were obtained. About 0.50 gram of kidney sample from rodent and 0.5 gram of the isolated *Hymenolepis* species was mixed separately in aqua regia and the mixture was digested in a hotplate for 3 hours at 60°C. The digestion was done after the sample was fully dissolved. After digestion, the sample was set aside to cool. After cooling, the digested sample was filtered through Whatman #1 filter paper, and distilled water was added up to the mark of the 100ml volumetric flask. Subsequently, the solution was exposed to Microwave Plasma – Atomic Emission Spectroscopy 4200 (Agent technologies) for the determination of lead concentration.

Digested samples were submitted to the Chemistry Research Laboratory (CRL), CLSU for the analysis of lead. Samples were analyzed with the average concentration of metal determined as ppm (parts per million) (Bordado & Paller, 2017).

Data Analysis

The data was analyzed using the percentage occurrence of the parasites to determine the infestation of *Hymenolepis* species in its host. Bioaccumulation capacity between metal levels in the parasites and selected host tissue and ratios of the metal concentration in the parasites to the host were computed using the bioaccumulation factor (BAF) formula as $C_{[parasites]}/C_{[host\ tissue]}$ (Sures,Siddall & Taraschewski, 1999).

RESULTS AND DISCUSSION

A total of forty-one (41) individuals of rodents were collected within the sampling period. Two species belonged to the genus *Rattus (Figure 1)* namely, *Rattus norvegicus* (Brown Rat) and *Rattus tanezumi* (Asian House Rat).

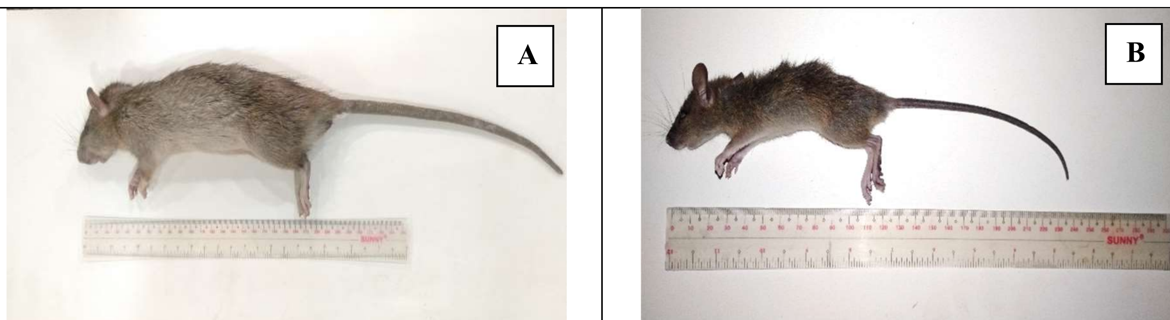

Figure 1. The two species of Genus *Rattus* collected.

Amongst the rats collected, *R. norvegicus* were the most abundant consisting of twenty-four (24) females and fourteen (14) males, followed by *R. tanezumi* consisting of two (2) females and one (1) male. The total number of rats collected in various sampling sites is shown in Table 1.

Table 1. Total number of rats collected in various sampling sites.

SAMPLING SITES	*Rattus norvegicus*		*Rattus tanezumi*		TOTAL NO. OF RATS
	Sex		Sex		
	Male	Female	Male	Female	
Residential	8	13	-	1	22
Old market	5	6	1	1	13
College of Agriculture	1	5	-	-	6
Total	**14**	**24**	**1**	**2**	**41**

Helminth Species Associated with Rodents

Helminth parasites found in the intestinal tracts of collected rats were identified up to the genus level of the species with the examination of its scolex and proglottid. The study reports the presence of cestode *Hymenolepis* sp. in *R. norvegicus* and *R. tanezumi*. Cestode of the family Hymenolepididae, *Hymenolepis* sp. were recovered in 73.175 of the rats examined. Figure 2 shows the collected *Hymenolepis* sp. from the intestine of *Rattus* species (A&B) showing its small pear-shaped scolex with suckers and hooked rostellum (C).

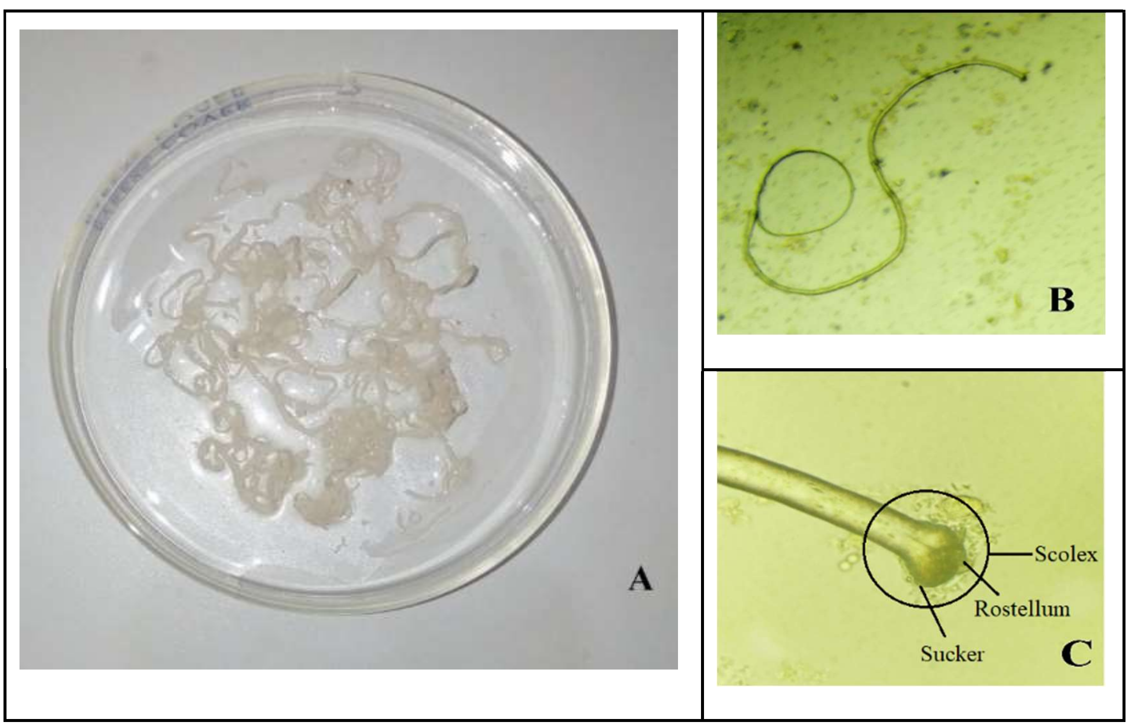

Figure 2. *Hymenolepis* sp. collected from the intestine of *Rattus norvegicus.*

The gastrointestinal helminth, *Hymenolepis* was observed in each commensal rat with an occurrence of 76.32% in *R. norvegicus* and 33.33% in *R. tanezumi* (Table 2.). Out of 38 rats that have been examined in *R. norvegicus*, 29 of these were positive for *Hymenolepis* spp. and only 1 out of 3 in *R. tanezumi*. The infected rats in *R. norvegicus* consisted of 10 males and 19 females with 19 adults, 9 subadults, and 1 juvenile. On the other hand*,* *R. tanezumi* has 1 adult and 1 female.

Table 2. Occurrence of *Hymenolepis* species in *Rattus norvegicus* collected from Central Luzon State University.

STAGES OF RAT	NO. OF RATS EXAMINED		NO. OF RATS INFECTED WITH PARASITES		% OCCURRENCE	
	R. norvegicus	*R. tanezumi*	*R. norvegicus*	*R. tanezumi*	*R. norvegicus*	*R. tanezumi*
Juvenile	3	1	1	0	33.33	0
Subadult	9	-	9	-	100.00	-
Adult	26	2	19	1	73.08	50.00
Total	**38**	**3**	**29**	**1**	**76.32**	**33.33**

The infective stage of *Hymenolepis* sp. started with an embryonated egg in feces when transmitted with the stool and cannot survive more than ten days in the external environment. According to Centers for Disease Control and Prevention (2017), the life cycle of *Hymenolepis* occurs when the eggs are consumed by an arthropod as an intermediate host including beetles and fleas, they are starting to develop into cysticercoids that can infect rats and develop into adults in the small intestine. Once the eggs are transmitted in contaminated food and water by an intermediate host, the oncosphere hatches. The eggs egg contained in oncosphere hatches and develop into cysticercoid larvae. The cysticercoids return to the intestinal lumen and eversion of their scoleces occurs then develop into adult attaches in the portion of the small intestine producing gravid proglottids. The gravid proglottids can disintegrate releasing eggs from adult worms and are expelled to the environment in the rat host's feces. Autoinfection can occur if the eggs remain in the intestine and release the oncospheres that penetrate the intestinal villus continuing the cycle. In line with this, the *Hymenolepis* species can be found wherever rodents and humans inhabit, and it also occurs in almost all types of terrestrial biomes.

Presence of Lead in Host Tissue and *Hymenolepis* species

One sample of infected *Rattus norvegicus* collected from each sampling station, College of Agriculture, Old Market, and Residential areas was analyzed for the concentration of lead (Pb). Amongst the three analyzed rats infected with helminth, only the helminth parasite of *R. norvegicus* from the residential area was positive for the presence of lead while the remaining samples from the College of Agriculture and Old market were not detectable for lead concentration. Furthermore, *Rattus norvegicus* was the only species that has been examined since *R. tanezumi* contained a very low weight of helminth parasite with less than 0.5 grams.
The amount of lead concentration from the tissue of *Rattus norvegicus* and its *Hymenolepis* species are shown in Table 3. The helminth parasite contained a very low concentration of 0.0086 ppm whereas, the kidney and parasite of two infected rats were found to be non-detectable (ND) for heavy metal analysis due to its value being less than the lowest calibration data. The results imply that the instrument used in the analysis cannot measure tissue samples with less than 0.05ppm due to its very low lead content. However, the detected value is not as reliable or acceptable since it is enclosed with the lowest calibration data, and to make the results more reliable, a higher-end instrument should be used to determine the actual lead content. Also, the data must be between the second to fifth data points.

Table 3. Lead (Pb) concentration (ppm) in the tissue of infected *Rattus norvegicus* and tits parasite.

SITE	LEAD (ppm)	CONCENTRATON
	Parasite	Kidney
College of Agriculture	ND	ND
Old Market	ND	ND
Residential Areas	0.0086	ND

*ND = Non-Detectable

The present study reports Pb concentrations in terrestrial mammals, *Rattus norvegicus* showed non-detectable although the kidney is known as one of the accumulation organs in metals and can exhibit the highest lead content compared with the other host tissues which contained lead levels below the detection limit (Sures et al., 2002). But considering also the parasites based on the study, helminth species have the capability to accumulate concentration of lead compared to their host tissue since they are mainly endoparasites without direct contact with the ambient environment, they have access to pollutants through their host. As stated by Sures et al. (2017), the dietary uptake route of metals is more important than the direct accumulation from the ambient environment. In this way, as specified by Sures & Siddall (1999) the heavy metals will become present in the endoparasites located in the intestine and other microhabitats within the host.

The *R. norvegicus* as the host of *Hymenolepis* sp. with a concentration of 0.0086 ppm was caught in a residential area that contains lead contamination based on the result of the analysis. The lead contained in the sampling area occurs in older houses which are more likely to have been coated with lead-based paint. As mentioned by Stehouwer (2010), the paint can deposit in the soil in the form of chalking, flaking, and scraping of lead paint and turns into chips or dust. The lead paint deposits in the soil near the base of the house, producing a halo of lead contamination. Therefore, built homes in the sampling area are more likely to contain lead-based paint. However, due to the presence of low lead concentration, the homes could be renovated and were considered to have a low risk of containing lead-based paint.

The low-level and non-detectable results of lead concentration among rat tissue and its helminth parasite provide evidence that rats are widely distributed all over the different ecosystems and are known to accumulate a higher concentration of heavy metals. The present study reports the low capability of rats to exhibit metal concentrations and is found to be negative for lead. This result was in accordance with the factors affecting the metal concentration in *Rattus norvegicus*. The sources of lead contamination might not necessarily be present in the habitat and the absence of usage of lead from activities in residential and agricultural areas could be the possible factor resulting in low levels of Pb concentration.

The analyzed samples for lead concentration retrieved from Old Market and the College of Agriculture were found to be non-detectable in the study. The old market is considered a commercial and residential area. No exposure to lead has been found in the area since human activities including mining, smelting, and manufacturing which lead can usually expose do not occur in either commercial or residential areas. Besides, other lead sources such as paint, lead-acid batteries, and pigments could be disposed of by the residents resulting in no lead contamination. On the other hand, fertilizers used for crop production in the College of Agriculture, an area comprised of food crops have no agrochemicals used in soils and organic fertilizers were used in fields resulting also in no lead contamination found in *Rattus norvegicus.* The study of Sumbera, Barus, & Tenora, (2003) was in line with the result of the study that the mean concentrations of lead (Pb) in the several organs of silvery mole-rat were found to be very low, near the lower limit of values known for rodents. The report suggests low contamination of the areas and this fact is probably caused by the low level of industry and low vehicular traffic in life.

Additionally, the factors affecting the exposure of lead concentration do not correspond with the sampling stations such as Old Market and College of Agriculture since the said areas have no possible sources of lead. Previous studies confirmed the different exposure of leads and its sources. As mentioned, Mattaloni, De Giovanni, & Virgolini, (2014) that higher exposure to lead sources depending on its near locations who have service pipes, peeling paint and industry. The said factors corroborate with Ceruti et al. (2022) that high exposure of wild rats was found near a busy highway where atmospheric and soil lead levels are highest that the main source of contamination in urban areas is the gasoline which is used in car and heating systems combustible. A similar result with He, Yang, & Stofella (2005) where concentrations of lead occur in the emission from automobiles that used PB-enriched gasoline and caused a significant increase of lead in the soil along old highways. The above-mentioned factors contradict to the said sampling stations in the study since the areas have no possible sources of lead contamination including nearby industries and busy highways that can be found in populated area, thus, no exposure of lead was found in the stations of College of Agriculture and Old Market.

Meanwhile, the bioaccumulation factor (BAF) between metal levels in the parasites and selected host tissue and ratios of the metal concentration couldn't meet since the tissues of *Rattus norvegicus* were found to be negative and only helminth parasite has a concentration.

CONCLUSIONS

The occurrence of *Hymenolepis* sp. associated with *R. norvegicus* and *R. tanezumi* collected in Central Luzon State University which is 73.17% implies that rodent species has a great harborage of helminth parasite. The presence of lead in helminthic parasite suggests that *Hymenolepis* species can accumulate lead contamination compared to its host, *R. norvegicus* since they are endoparasites without direct contact with the ambient environment. *Hymenoleis* species still have the capabilities in environmental impact studies, even the lowest metal concentrations have been detected due to their ability to exhibit bioavailable concentrations. On the other hand, the low level and non-detectable results provide evidence that the areas in the study including commercial, agricultural and residentials have a low risk in heavy metal contamination since anthropogenic activities wherein sources of lead pollution can be exposed were absent to the habitat of rodents.

RECOMMENDATION

In relation to the abundance of captured *Rattus tanezumi* in the study, the species has a very low population compared to *Rattus norvegicus*. Therefore, future research is required to investigate the possible factors affecting the abundance of *R. tanezumi* with regards to its behavior in agricultural habitat. Moreover, the concentration of lead appears to be limited, thus, further field studies are recommended to determine the factors that influence the low lead exposure in host tissue and its helminth parasite.

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