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Determination of Iron (Fe) and Lead (Pb) in Larvae using Atomic Absorption Spectroscopy (AAS)

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GRAPHICAL ABSTRACT

The flower and fruits of *P. macrocarpa*

ABSTRACT

The effect of rapid population causes the source of food to decrease especially in animal feed which makes the consumers need to purchase higher in order to get the best quality food for their pet. Some of animals feed may contain heavy metals that come from the production process in order to reduce cost. In this study, larvae of black soldier flies were used, as it is believed could be economical, low cost and acting as essential decomposers in breaking down organic substrates and returning nutrients to soil. The eggs of black soldier fly were divided into a beaker that contained chicken livers. 5-, 10- and 15-days age of larvae were collected to determine Pb and Fe contents. The larvae were heated with sulphuric acid (H_2SO_4) and nitric acid (HNO_3) until they were totally digested. Lead (Pb) and iron (Fe) were analysed using atomic absorption spectroscopy (AAS). Chicken livers contained (0.426 ± 0.029) mg/g of Fe and (0.029 ± 0.015) mg/g of Pb. After testing and comparing the results, the concentration of lead in the larvae decreased while concentration of iron increased after eating the chicken livers. The larvae contained (0.135 ± 0.007) mg/g of Fe with 31.7% of Fe per 1 g of chicken liver in 5 days, (0.202 ± 0.009) mg/g of Fe with 47.3% of Fe per 1 g of chicken liver in day 10 and (0.247 ± 0.016) mg/g of Fe with 57.9% of Fe per 1 g of chicken liver in day 15 respectively. The larvae contained (0.027 ± 0.010) mg/g of Pb with 94.6% of Pb per 1 g of chicken liver in day 5, (0.012 ± 0.008) mg/g of Pb with 43.3% of Pb per 1 g of chicken liver in day 10 and (0.011 ± 0.005) mg/g of Pb with 38.5% of Pb per 1 g of chicken liver in day 15 respectively. In conclusion, the bigger the size of the larvae of black soldier fly, the higher the concentration of Fe and the lower the concentration of Pb in the larvae.

Keywords: digested, chicken liver, iron, lead.

1. INTRODUCTION

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least five times greater than that of water [1]. There are many kinds of industries related to heavy metals that distribute to increase the potential effect on either human or animal's health and environment such as agricultural, domestic, medical and technological application. There are two types of heavy metals; biological essential and non-biological essential metals. Biological essential heavy metals are the types of heavy metals which is good for animals in a low concentration where it is naturally consist in their body. It is known as micronutrients. Several heavy metals are essential elements and added to animal feed to balance the micro minerals. For examples are copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and cobalt (Co). Meanwhile non-biological essential heavy metals are the metals that can cause toxicity. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals [1]. Examples of non-biological essential heavy metals are lead (Pb), mercury (Hg), cadmium (Cd) and tin (Sn).

In the era of globalization, various kind of food have been marketed either in a can, plastic or even bottles. Preservatives is a must to prevent the food from damage. Preservatives are food additives which can prevent or inhibit the growth of bacteria, so there is no fermentation (putrefaction), acidification or decomposition of food due to the activity of microorganisms (bacteria) [2]. When the use of food and the dose is not regulated and supervised, there are likely to cause damage to the wearer either directly, such as poisoning or non-directly or cumulatively, for example preservatives which are carcinogenic that contain heavy metals. Vanadium pentoxide (V_2O_5) is carcinogenic in animals and, when inhaled, causes DNA damage [15]. There is no exception to livestock that their food is contaminated with heavy metals which it will be harmful to health.

Some of the animals feed may consist of heavy metals that comes either from environment or/and production process. Several heavy metals are essential elements and added to animal feed to balance the micro minerals. There are researches that showed the amount of heavy metals in animal food. Due to the research from the farm of northeast China, the heavy metal of highest contents in cattle manure was Zn and Cu [2].

In this research, larvae of black soldier fly were used as it not only contains a lot of benefits but also can reduce the cost because it is easy for breeding in high amount and can be breed in a short of time. The larvae of black soldier fly were used to detect the heavy metals. Chicken liver was chosen to be the diet of the larvae. The content of heavy metals will be detected from the larvae which it will determine whether the larvae absorb more heavy metals or vice versa after being consumed with chicken liver.

Due to rapid growth of world population, which is predicted to reach 9 billion people in 2050, the global food demand will rise by nearly 100% from 2005 to 2050 [8,9], whereas animal feed and human food production through agriculture is expected to increase by 60% [10]. Future shortage for maize, rice, wheat and soybean was estimated approximately 67%, 42%, 38%, and 55% respectively [11]. The undernourished population increased by approximately 805 million in the developing countries which could lead to greater risk of local, national and global diseases outbreaks [10].

The effect of rapid population causes the source of food to be decreases especially in animal feed. In order to solve this kind of problems, larvae is used as it contain so many benefits include low cost to process. Plus, there is less research about larvae to prove that this kind of larvae is suitable for animal feed which it actually contains several nutrients.

Furthermore, animal's foods nowadays are contaminating with heavy metals. Due to research, all feed samples contained Cu, Zn and As, indicating that these additives were widely applied in animal production in Northeast China. Chromium (Cr) in cattle feeds was almost below the detection limit; while Cr was detected in over 13% of pig feeds and 20% of chicken feeds. Although Cd is not necessary for animal growth, over 60.6% of feed samples were detected to contain this toxic metal in the survey [12].

Therefore, in order to increase the food for animals which is affordable and eco-friendly, the content of the animal feed using these larvae also being emphasized.

2. EXPERIMENTAL

Reagent and chemicals used for the laboratory procedure were all analytical grade. The solvents used in this study were sulfuric acid, H_2SO_4 98% and nitric acid, HNO_3 65% that purchased from QReC (Asia) Sdn.Bhd. The standard stock solution of 1000 ppm for Fe and Pb were used. The apparatus used in this study were 100 mL beakers, 50 mL volumetric flasks, spatula, filter funnel, filter paper and glass rod. The entire glassware is soaked in 10% nitric acid for 24 hours after washed with detergent to ensure that all metal ions and other interferences at the surfaces of the glassware are removed. Then, followed by rinsing with distilled water for three times before drying in the oven at 70 °C. The instruments used in this study were analytical balance, drying oven, Ultrasonic Cleaner Thermo-6D for extraction process and a Perkin Elmer (USA) with deuterium background corrector that was used to determine the concentration of lead and iron in the larvae of black soldier fly samples by using air/acetylene flame.

The experiment is split into three phases. The first phase was breeding segment where the black soldier fly breed from the eggs until it turns into larvae. In order to produce the eggs, the process was started with prepupal stage of black soldier fly. The prepupal of the larvae were collected and put into a medium beaker and covered with dry leaves. After 7 days, it proceeds to next stage which is pupal stage which took 10 days to produce black soldier fly. This black soldier flies were mating and produced eggs inside the corrugated sheets that clipped near the food. The odor and smells from the waste attracted the female BSF and laid eggs in the corrugated sheets that clipped over the beakers contained wastes. After 4 days, the larvae of black soldier fly hatched out from eggs. The larvae started to feed the wastes which contain chicken liver.

The second phase was feeding segment which the larvae were given the chicken liver as their source of food. The chicken livers were given in solid fractions. In order to ensure the moisture content for growth of the larvae, the water was added to the chicken liver. About 100 of 0 to 2 days old BSF larvae are placed in the plastic beaker contained chicken livers. After 5, 10 and 15 days, the BSF larvae were collected and washed using tap water until the entire residue totally removed. Then, the BSF larvae were divided into three new beakers equally and ready for the next step.

Final phase was the digestion of the chicken liver and the larvae of black soldier fly. Before the heavy metals in the larvae of black soldier fly were detected, the heavy metals in the chicken liver should be detected first. The amount and concentration of heavy metals in the chicken liver can be related to the amount and concentration of heavy metals in the larvae of black soldier fly after consumed the chicken liver. 1 g of chicken liver was weighed and put into three 100 mL beaker in triplicate. Then, the chicken liver was mixed with 5 mL of sulfuric acid (H_2SO_4) each and heated on the hot plate for 10 minutes at the temperature 85 °C until the chicken liver was totally digested. The solutions changed it color to dark purple when the chicken liver was totally digested. 5 mL of nitric acid (HNO_3) was added into the each of the mixture and further heated for another 5 minutes. The solutions turned it color from dark purple to yellowish and left in the fume hood until it cools. Next, some amount of deionized water was added into each of the solutions and mixed well. The solutions were filtered and put into three 50 mL of volumetric flask. The deionized water was added until reached the line of 50 mL. The volumetric flask was labeled as H1, H2 and H3. The heavy metals such as iron (Fe) and lead (Pb) were detected using atomic absorption spectroscopy (AAS).

In order to determine the heavy metal content in larvae of the black soldier fly, further experiment need to be done using wet ashing technique. After 5 days feed with chicken liver, 40 larvae of black soldier fly were collected and put into three 100 mL beaker in triplicate. Then, the larvae of black soldier fly were mixed with 5 mL of sulphuric acid (H_2SO_4) each and heated on the hot plate for 10 minutes at the temperature 85 °C until the larvae were totally digested. The solutions changed it color to dark purple when the larvae were totally digested. 5 mL of nitric acid (HNO_3) was added into the each of the mixture and further heated for another 5 minutes. The solutions turned it color from dark purple to yellowish and left in the fume hood until it cooled. Next, some amount of deionized water was added into each of the solutions and mixed well. The solutions were filtered and put into three 50 mL of volumetric flask. The deionized water was added until reached the line marked of 50 mL. The procedure was repeated for 10- and 15- days of BSF larvae. The heavy metals such as iron (Fe) and lead (Pb) were detected using atomic absorption spectroscopy (AAS).

3. RESULTS AND DISCUSSION

The first phase is breeding segment where the black soldier fly will be breed from the eggs until it turns into larvae. During the egg phase, the corrugated sheet was put near the chicken liver as the female of black soldier fly will not laying eggs on the chicken liver. The chicken liver was used to attract the female of black soldier fly so that they will laying eggs on the corrugated sheet. After 2 days, around 200 eggs of black soldier fly were laid on the corrugated sheet. The eggs were left near the chicken liver in the corrugated sheets until it hatched out. After within 4 days, the larvae of black soldier fly were hatched out from the eggs, the larvae were directly feed with chicken liver. Then, the age of 5-, 10- and 15-days of larvae were collected and digested. Fe and Pb were detected using atomic absorption spectroscopy (AAS).

Before the digestion of the larvae, the chicken liver needs to be digested first to determine the concentration of iron (Fe) and lead (Pb) inside the chicken liver. This is important to compare the concentration of heavy metals that has been absorbed by the larvae of black soldier fly after consumed the chicken liver for age 5-, 10- and 15-days. From the result, the pattern of the concentration of iron and lead content in larvae can be determine either increase, decrease or remain constant. Sulfuric acid (H_2SO_4) and nitric acid (HNO_3) were used for the digestion because HNO_3 acts as both acid and oxidizing agent. When H_2SO_4 was added, the solutions turned it color to dark purple. After 10 minutes, HNO_3 was added and the solution turned it color to yellow. HNO_3 as it act an oxidizing agent. HNO_3 can oxidize zero valence inorganic metals and non-metals into ions. Plus, the digestion is needed before using AAS because it can destroy the matrix, which otherwise interfere during atomization.

That is why when the HNO_3 was added into the solution, the solution turned to yellow in color as it showed the presence of the Fe^{3+}. But HNO_3 does not form any insoluble compounds with metals and non-metals, whereas H_2SO_4. That is why acid mixture is needed depend on the matter inside the samples. The correlation coefficient of the calibration curve for Pb standard was 0.9972. The y-intercept was 0.9853x + 0.0503. The correlation coefficient of the calibration curve for Fe standard was 0.9999. The y-intercept was 0.9983x + 0.006.

Table 1. The mean concentration of Fe and Pb (mg/g) in sample

Samples	Mean Concentration of Fe (mg/g)	Mean Concentration of Pb (mg/g)
Chicken Liver	0.426 ± 0.029	0.029 ± 0.015
5 days	0.135 ± 0.007	0.027 ± 0.010
10 days	0.202 ± 0.009	0.012 ± 0.008
15 days	0.247 ± 0.016	0.011 ± 0.005

From the Table 1, the mean concentration of Fe in the chicken liver was 0.426 ± 0.029 mg/g while the mean concentration of Pb in the chicken liver was 0.029 ± 0.015 mg/g. This shows that the chicken liver contained higher amount of Fe than Pb. The mean concentration of Fe (mg/g) in the sample for day 5 was 0.135 ± 0.007, day 10 was 0.202

± 0.009 and day 15 was 0.247 ± 0.016 while the mean concentration of Pb (mg/g) in the sample for day 5 was 0.027 ± 0.010, day 10 was 0.012 ± 0.008 and day 15 was 0.011 ± 0.005.

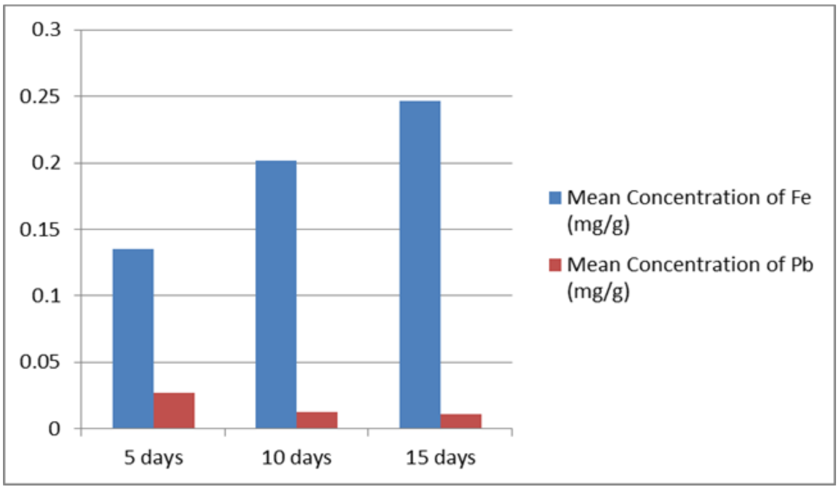

Figure 1. Comparison of mean concentration of Fe (mg/g) and Pb (mg/g) in the samples.

From Figure 1, the mean concentration of Fe (mg/g) in the samples were increased from day 5 to day 15 meanwhile, the concentration of Pb (mg/g) in the samples were decreased. From the data, the percentage of the mean concentration of Fe (mg/g) in the samples after consumed chicken liver for day 5 was 31.7%, day 10 was 47.3% and day 15 was 57.9% per 1 g of chicken liver while the percentage of the mean concentration of Pb (mg/g) in the samples after consumed chicken liver for day 5 was 94.6%, day 10 was 43.3% and day 15 was 38.5%.

There is some international/ national standard that available to be referred such as Codex Alimentarius Commission (CODEX), Mainland, US Food and Drug Administration (FDA), European Commission (EU) and Australia.

Table 2. Maximum permitted concentration of lead (Pb)

Second schedule: Maximum permitted concentration of certain metals in specified foods.		
Metal	**Description of food**	**Maximum permitted concentration in parts per million**
Chromium (Cr)	Cereals and vegetables	1
	Fish, crab-meat, prawn and shrimps	1
	Meat of animals and poultry	1
Lead (Pb)	All foods in solid form	6
	All foods in liquid form	1
Mercury (Hg)	All food in solid form	0.5
	All foods in liquid form	0.5
Tin (Sn)	All food in solid form	230
	All foods in liquid form	230

From Table 2, it shows that the maximum permitted concentration of lead (Pb) in parts per million is 6 for all foods in solid form. The result from this study showed that the concentration of Pb in larvae of black soldier fly were below the maximum permitted after consumed the chicken liver.

Table 3. Maximum permitted total Fe content

Animal species/categories	**Maximum total iron content**	
	Content feed (mg/kg)	**Water for drinking (mg/L)**
Ovine	500	100
Pet animals	1250	625
Pig:		
Piglets up to one week before weaning	250 mg/day	2273
Others pig	750	375
Other Species	750	375

From Table 3, the maximum permitted total of iron (Fe) in content feed for pet animals was 1250 mg/kg. The result obtained from the study showed that the mean concentration of Fe in the larvae of black soldier fly were below the maximum permitted total of iron in content feed for pet animals after consumed with chicken liver.

4. CONCLUSION

The result obtained from this study shows that there are two types of heavy metals in the larvae of black soldier flies after being consumed the chicken livers. These were iron (Fe) and lead (Pb). The concentration of Fe (mg/g) in the chicken liver was higher than the concentration of Pb (mg/g). Thus, affect the concentration of heavy metals in larvae content of black soldier fly. The percentage for concentration of Fe (mg/g) in the larvae increased from day 5 to day 15 whereas the percentage for concentration of Pb (mg/g) in the larvae decreased from day 5 to day 15. This shows that the bigger the size of the larvae causes the concentration of Fe to increase and Pb to decrease. So that, the larvae are suitable to make as animal's feed as the concentration of Fe and Pb in the larvae do not exceed the limit value.

REFERENCES

[1] Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). "Heavy metal toxicity and the environment." *In Molecular, clinical and environmental toxicology*, Springer, Basel, 7(1),133-164.

[2] Zhang, F., Li, Y., Yang, M., & Li, W. (2012). "Content of heavy metals in animal feeds and manures from farms of different scales in northeast China." *International journal of environmental research and public health,* 9(8), 2658-2668.

[3] Barragan-Fonseca, K. B., Dicke, M., & van Loon, J. J. (2017). "Nutritional value of the black soldier fly (Hermetia illucens L.) and its suitability as animal feed–a review." *Journal of Insects as Food and Feed*, 3(2), 105-120.

[4] Taylor, G. D. A. (2018). U.S. Patent Application No. 15/847,596-596.

[5] Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., & Vinneås, B. (2013). "Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)—From a hygiene aspect." *Science of the Total Environment,* 458, 312-318.

[6] Diclaro II, Joseph W., and Phillip E. Kaufman. "Black Soldier Fly - Hermetia Illucens." *Black Soldier Fly - Hermetia Illucens*. University of Florida, 22(5), 214-222.

[7] Park, H. H. (2016). "Black Soldier Fly Larvae." *Black Soldier Fly Manual,* 75-80.

[8] Makkar, H. P., Tran, G., Heuzé, V., & Ankers, P. (2014). "State-of-the-art on use of insects as animal feed." *Animal Feed Science and Technology,* 197, 1-33.

[9] Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). "Global food demand and the sustainable intensification of agriculture." *Proceedings of the National Academy of Sciences*, 108(50), 20260-20264.

[10] Tomberlin, J. K., Van Huis, A., Benbow, M. E., Jordan, H., Astuti, D. A., Azzollini, D. & Chapkin, R. S. (2015). "Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed." *Journal of Insects as Food and Feed*, 1(4), 307-309.

[11] Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). "Yield trends are insufficient to double global crop production by 2050." *PloS one,* 8(6), 66428.

[12] Zhang, F., Li, Y., Yang, M., & Li, W. (2012). "Content of heavy metals in animal feeds and manures from farms of different scales in northeast China." *International journal of environmental research and public health,* 9(8), 2658-2668.