Morphometric characteristics and heavy metal bioaccumulation in edible freshwater gastropod (*Filopaludina javanica*)

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**Abstract.** An edible gastropod, *Filopaludina javanica*, is widely distributed in freshwater habitats. It was the most abundant gastropod species with variation on size at some lakes in Bogor. However, the relation between morphological size and heavy metal concentration in *F. javanica* is barely understood although gastropods are well known to accumulate heavy metal. The aim of the research is to measure the impact of concentration of lead (Pb) and cadmium (Cd) concentration to the morphological size of *F. javanica* in Situ Gede, Bogor. Sampling of the species was carried out in February and April 2017 in Situ Gede (SG), Situ Panjag (SP), Situ Burung (SB) and Situ LSI (SL) while samples for heavy metal accumulation analysis were collected in January and February 2018 in SG. The morphological characteristics of the samples’ shell were measured. Body mass samples were powdered and analyzed using *Atomic Absorption Spectrophotometry* (AAS). The average range of length and wide shells (mm) were 8.89-30.25 and 7.40-24.80, respectively. The average concentration of Pb and Cd were 0.82 and 0.11 ppm, respectively. The morphometric variability of the species varied among lakes while the concentration of Pb and Cd in this study was generally lower than the acceptable limit.

**Keywords:** Cadmium, *Filopaludina javanica*, gastropod, lead, morphology, situ gede,

1. Introduction

*Filopaludina javanica*, well known as *tutut* by Sundanese tribe, is an and edible freshwater gastropod, is an abundant and widespread freshwater gastropod in Bogor [1]. The abundance of this gastropod in Situ Gede (SG) is widely foraged by the surrounding community as food The gastropod contain 7.97% of protein and 2.33% fat [2], which could be an alternative food protein sources. However, there is a threat of heavy metal accumulation in the tissues which is well known for gastropods.

Tindjani et al. [3] revealed that macrozoobenthos especially gastropods that inhabit aquatic ecosystems can accumulate heavy metals (Cu, Cr, Fe, Mn, Pb, Zn, etc) passively. Heavy metals accumulate through diffusion through body membrane and into cells [4]. Examples of gastropods that can accumulate heavy metals are the riverine gastropods *Viviparus mamillatus* [5], *F. javanica* in Waung rivers and Saguling reservoir [6, 7], the intertidal gastropods, *Hemifusus pugilinus* & *Bursa spinosa* [8] and marine edible gastropod, *Thais carinifera* [9].
Lead (Pb) and cadmium (Cd) are dangerous heavy metals in the ecosystem [10, 11]. High concentrations of those heavy metals are mainly due to human sources such as residential waste, paint industry, textiles, agriculture, land transportation, cultivation, and other activities [3]. Residential and agricultural areas around SG, might contribute to the contamination of the heavy metals in lakes. The presence of heavy metals has been found in samples of gravestones in Situ Gede waters [12]. This discovery is may also be an indication of the content of heavy metals that accumulate in the gastropod in SG waters.

Morphometric analysis has been conducted in some species of gastropods, but has not been carried out for *F. javanica*. Some morphometrics studies of gastropods in Indonesia have been described for *Turbo sparverius* and *Turbo bruneus* (Turbinidae) [13], *Nerita* (Neritidae) [14]. Six species of *Filopaludina* characteristics also has been reported [15], although there is no detailed information on their morphometric.

So far, the research on morphometrics and bioaccumulation of Pb and Cd heavy metals in *F. javanica* in SG waters has never been specifically studied. This study aims to measure the correlation between morphological characteristics to the concentrations of Pb and Cd heavy metals in *F. javanica* and sediments in their habitat.

2. Study sites and methods

2.1. Study sites

*F. javanica* sample were collected from Situ Gede (SG), Situ Panjang (SP), Situ Burung (SB) and Situ Information Resource Services (SL) for morphometric analysis (February and April 2017). Sediment samples for heavy metal analysis were collected in SG (January and February 2018). Both samples were taken from the littoral area of each site. The detail information of all site (SG, SP, SB, and SL) can be found in Priawandiputra et al. [1]. The identification of *F. javanica* shell measurement, powdering process and heavy metal analysis were carried out in the Laboratory of Animal Biosistematics and Ecology (Department of Biology), the Laboratory of Animal Feed Nutrition Science (Department of Animal Nutrition and Feed Technology), and the Laboratory of Analytical Chemistry (Department of Chemistry), IPB University, respectively.

2.2. Sampling techniques

Six sample points were determined at each site for morphometric analysis while ten sample points were selected in SG for heavy metal analysis. Sampling of sediment and *F. javanica* on the benthic zone was carried out using an echman grab. All samples were sorted and put into labeled plastic containers. The number of individuals were counted in each sample points and all individuals were measured for morphological characteristic of shell. Ten gram of sediment and the twenty individuals of large gastropods were selected for heavy metal analysis.

2.3. Shell measurements

Measurements of morphometric parameters in *F. javanica* include shell height (h), shell width (w), aperture height (aph), aperture width (apw) and shell thickness (d) (figure 1). The gastropod samples for heavy metal analysis were also measured for h, w, d, perimeter of operculum and weighed for both body mass and shell and only for body mass. All measurements were taken using calipers.

2.4. Heavy metal analysis

Sediments samples were dried to remove the water content and powdering using a blender. The body mass of the gastropod samples were removed from the shell, put into erlenmeyer and dried in an oven at 60°C for 24 hours. The dry body mass samples were weighed to measure the dry weight and calculate water content. The dry body mass and sediment samples were then put into erlenmeyer and 10 ml nitric acid was added. The samples were stirred until homogenous under a fume hood for 24 hours. The samples were then heated on a hotplate at 100°C for 1 hour 30 minutes. The temperature
was steeply increased to 130°C for 1 hour, followed by an increase to 150°C for 2 hours 30 minutes, 170°C for 1 hour and 200°C for 1 hour. The extracts were filtered and put it into a volumetric flask, and diluted with 50 ml deionized water. The extracts were shaken until homogenous and stored in labeled sample bottles. Heavy metal accumulation on sediments and body mass was analyzed using the Atomic Absorption Spectrophotometer (AAS) after being reconstructed.

![Figure 1. Measurement of gastropod shell. Note: shell height (h), shell width (w), aperture height (aph), aperture width (aph), shell thickness (d) (Picture from Preston and Robert [16]).](image)

2.5. **Data analysis**

Bioconcentration factor (BCF) was calculated to compare the accumulation ability of freshwater gastropod on Pb and Cd. BCF is the ratio of chemical compound concentration in organism to chemical compound concentration in surrounding environments[17]. BCF was calculated as follows:

\[
    C_{b} = \frac{K_{b}}{C_{w}}
\]

Note

- \( C_{b} \) = Bioconcentration factor
- \( K_{b} \) = Heavy metal concentration in gastropod
- \( C_{w} \) = Heavy metal concentration in sediments

The United States Environmental Protection Agency (US EPA) [18] use the maximum consumption limit (CR\textsubscript{lim}) as parameter for maximum limit of feeding. CR\textsubscript{lim} is calculated by:

\[
    C_{\text{lim}} = \frac{\text{RfD} \times \text{BW}}{C_{m}}
\]

Remarks:

- \( \text{RfD} \) = a reference dose
- \( C_{m} \) = measured heavy metal concentration
- \( \text{BW} \) = people's body weight (usually 70 kg)

The comparison of *F. javanica* on morphometric parameters among SG, SB, SL and SP and the correlation between abundance and morphometric parameters were analyzed using Kruskal-Wallis test and Pearson correlation coefficient. Spearman rank correlation was used for calculate the correlation between morphometric parameters with concentration of Pb and Cd, and between Pb and Cd concentrations in sediment and body mass. A t-test was also conducted to check significance in differences of the bioconcentration factors between Pb and Cd. All analyses were carried out using PAST software [19].

3. **Results**

3.1. **Morphometric analysis of gastropods**

Abundance of gastropod was higher in SG (525 individuals), followed with SP (19), SB (37) and SL (37). The average size shell height and width ranged 8.89-30.30 and 7.40-24.80 mm, respectively. There were also various average size of aperture height (5.05-17.65) and width (4.35-14.9 mm). The
shells have different level of thickness (0.13-1 mm). The morphological size averages of *F. javanica* shells (shell height, shell width, aperture height, aperture width and thickness of the shell) in SG were smaller than those in other sites (figure 2). The highest average shell height, shell width, aperture height and aperture width was found in SL, while the highest thickness of the shell was found in SB. The shell morphometrics of gastropod population in SG were more homogenous than those from other sites (figure 3), described by clumped formation of SG points. Meanwhile, no clear separation on shell morphometrics of gastropod population was illustrated in other sites.

**Figure 2.** Comparison of *F. javanica* population on morphometric parameters such as a) shell height (h), b) shell width (w), c) aperture height (aph), d) aperture width (aph) and e) shell thickness (d) among sites (SG, SP, SB and SL).
Figure 3. Non-Metric Dimensional Analysis (NMDS) of morphometric parameters on *F. javanica* population in all sites with stress value = 0.011.

*F. javanica* in SG which were used for heavy metal analysis, variously differ on morphometric parameters such as shell height, shell width, shell thickness, perimeter of operculum, total weight and body mass (table 1). The largest shell height and width in sampling point 9 did not represent the highest of total weight and weight of body mass, but it correlated with the perimeter of operculum.

Table 1. The morphometrical characteristics of *F. javanica* at each point in SG (the average ± standard error).

| Sampling points | Shell height (cm) | Shell width (cm) | Shell thickness (cm) | Perimeter of operculum (cm) | Total weight (gr) | Weight of body mass (gr) |
|-----------------|-------------------|------------------|----------------------|-----------------------------|-------------------|--------------------------|
| 1               | 2.55±0.05         | 1.99±0.03        | 0.01±0.01            | 4.30±0.08                   | 3.12±0.19         | 1.25±0.08                |
| 2               | 2.72±0.07         | 2.07±0.04        | 0.03±0.01            | 4.74±0.11                   | 4.02±0.30         | 1.44±0.14                |
| 3               | 2.73±0.06         | 2.12±0.04        | 0.02±0.01            | 4.69±0.09                   | 3.77±0.30         | 1.33±0.12                |
| 4               | 2.68±0.04         | 2.10±0.02        | 0.02±0.01            | 4.63±0.06                   | 3.42±0.19         | 1.20±0.09                |
| 5               | 2.60±0.04         | 1.87±0.03        | 0.02±0.01            | 4.32±0.07                   | 3.31±0.19         | 1.26±0.07                |
| 6               | 2.74±0.03         | 2.16±0.03        | 0.02±0.01            | 4.91±0.05                   | 3.70±0.16         | 1.42±0.08                |
| 7               | 2.79±0.06         | 2.18±0.03        | 0.02±0.01            | 4.81±0.06                   | 3.67±0.30         | 1.27±0.16                |
| 8               | 2.85±0.04         | 2.23±0.03        | 0.01±0.01            | 4.98±0.08                   | 3.96±0.29         | 1.62±0.14                |
| 9               | 2.91±0.04         | 2.27±0.03        | 0.01±0.01            | 5.07±0.06                   | 3.90±0.23         | 1.51±0.11                |
| 10              | 2.80±0.05         | 2.04±0.05        | 0.01±0.01            | 4.76±0.08                   | 4.50±0.21         | 1.82±0.10                |

3.2. Effect of density on morphological size of dominant gastropods

The abundance of *F. javanica* negatively correlated with the size of all morphometric parameters (shell height, shell width, aperture height, aperture width and thickness of the shell) (figure 4). The
Figure 4. The correlation between abundance of *F. javanica* and morphometric parameters such as a) shell height (h), b) shell width (w), c) aperture height (aph), d) aperture width (aph) and e) shell thickness (d) in all sites (SG, SP, SB and SL).
3.4. The maximum consumption of F. javanica

The maximum safe consumption of F. javanica containing Pb was 0.83 kg/week, while those of F. javanica containing Cd metal was 2.70 kg/week (table 3). As F. javanica contains both heavy metals, the maximum safe limit for weekly consumption is limited by the smallest value (0.83 kg/week).

Table 2. The average concentration of heavy metals (Pb and Cd) in sediment and body mass of gastropod and Bioconcentration Factors (BCF) of gastropod.

| Parameters       | Heavy metals |
|------------------|--------------|
|                  | Pb           | Cd           |
| Sediment (ppm)   | 18.62 ± 1.6  | 0.06 ± 0.01  |
| Body Mass (ppm)  | 0.82 ± 0.29  | 0.11 ± 0.01  |
| BCF              | 0.04 ± 0.04  | 1.68 ± 1.09  |

Table 3. The calculation of the maximum consumption of F. javanica.

| Heavy metals | RFD (mg/kg-week) | Cm (mg/kg) | Human Weight | Cr\text{lim} (Kg/week) |
|--------------|------------------|------------|--------------|------------------------|
| Pb           | 0.025            | 2.09       | 70           | 0.83                   |
| Cd           | 0.007            | 0.18       | 70           | 2.70                   |

4. Discussion
The morphometric parameters in our study were variously different among situ. Based on Siregar [6], F. javanica in Saguling also has vary size on shell height between 15 and 35 mm and shell width between 12 and 25 mm. The higher nutrition in these all situ and Saguling could support the development and size of F. javanica shell. Marwoto and Nurinsyah [15] also measured the morphometric of Filopaludina, although the data were not published in their manuscript.

Our result also showed the high abundance of F. javanica decreased all morphometric parameters. The shell size (height and width) in SG was smaller than in other situ because the density of gastropods in SG was very high that intraspecific competition for food availability between individuals occurred. Some research also show that the high density decrease growing rate due to competition in food resources [20-22]. According to Nybakken [23], the development and survival of gastropods was influenced by food stability in the form of detritus and macro algae and environmental conditions.

The presence of heavy metals in the environment can accumulate in biota [3]. According to Amrani [24], heavy metals in sediments are higher due to accumulation, while heavy metals still in water can move along with the current. Heavy metals generally enter the environment through two sources: natural and anthropogenic [25]. Naturally heavy metals enter the environment through sediment due to weather, erosion, and volcanic activity while the heavy metals can be also occurred in the environment by anthropogenic of human activities such as industrial activities, agriculture, mining,
and household waste [26]. Agricultural waste could be the source of Pb and Cd in SG where SG, SB and SP have the main function as an irrigation system for the surrounding agriculture. The long-term practice of fertilizer application in surrounding farms increased the concentration of Pb and Cd in the soil [27].

Beside shell height and width measurement of *F. javanica*, Siregar [6] also measured the concentration of Pb and Cd in Saguling reservoir. He showed that the range of Pb and Cd concentration in body mass of *F. javanica* was 12.99-15.62 and 0.18-1.83 ppm, respectively. In other study, Pb concentration of *F. javanica* in Waung river, Lamongan was 4.48 ppm [7]. Both studies reported higher concentration of Pb compared with present study. It means that the heavy metal (Pb and Cd) pollution in SG was lower than large reservoir and river.

According to Dellinger [28], there are metallothioneins proteins in invertebrates, which are proteins that play a role in protecting cells and organisms from metal poisoning. Metallothioneins have a high affinity for copper, cadmium and zinc [17, 28]. The presences of protein explained the high BCF value of Cd in Situ Gede (1.68). Connell and Miller [17] suggested that the BCF value >1 indicates that aquatic organisms have high ability to accumulate pollutants, whereas the value of BCF <1 indicates that aquatic organisms have the ability to accumulate low pollutants. The processes of excretion and detoxification of organism with BCF >1 seem to be more effective [29, 30].

According to the Ontario Minstry of Environment [31] Sediment Quality Standard, the Pb content in sediments was divided into the lowest effect level (LEL) and severe effect level (SEL) with a concentration value of 31 ppm and 250 ppm, respectively while the concentration of Cd into LEL and SEL levels was 0.6 ppm and 10 ppm, respectively. If the concentration of Pb or Cd is at the level of LEL, it indicates that contamination in the sediment still can be tolerated by organisms. Our data in the present study was still below the level of LEL, which shows that the concentrations are still harmless.

The safe limit of SNI quality standards for Pb and Cd heavy metal content in mollusk-based foods based on the 2009 National Standardization Agency are 1.5 and 1 ppm, respectively [32]. The results show that Pb concentration in six of the ten sampling points were below the SNI quality standard safe limit value. Meanwhile, concentration of Cd in all sampling points were lower than SNI quality standard value.

The protein content in *F. javanica* can provide alternative food and ingredients in Indonesia. However, to prevent negative effects on human health, we need to know the consumption limits of the gastropod due to the its ability to accumulate Pb and Cd. According to US EPA [18] formula, the gastropod in SG can be consumed with less than 0.83 kg/week for adult with 70 kg weight body.

5. Conclusion
The morphological sizes averages of *F. javanica* shells were different among sites. The average size of shell height and width ranged 8.89-30.30 and 7.40-24.80 mm, respectively. The concentrations of Pb and Cd in SG were found in sediment and body mass of gastropod with values below Sediment Quality Standards and the SNI quality standard. The BCF showed that the gastropod has ability to accumulate more Cd than Pb. The maximum limit to consume *F. javanica* in SG is 0.83 kg/week for adults.

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