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Assessing mercury concentration in amphibians and reptiles near artisanal gold mining sites in Saweak Village, Lebong Regency, Bengkulu, Indonesia

Vestidhia Yunisya Atmaja[*1], Aldy Riau Wansyah Hasibuan[2], Deni Parlindungan[3], Eti Widya Lestari[1], Cindy Fadli Wulandari Hutabarat[1], and Desy Millenia Ramadhany[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Indonesia
[2]Graduate Student, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[3]Department of Science Education, Faculty of Teacher Training and Education, Universitas Bengkulu, Indonesia
[*]Corresponding author: vesti.atmaja@unib.ac.id

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ABSTRACT

Traditional gold mining activities are widespread in various regions of Indonesia, including Bengkulu Province. Many of these operations are unlicensed, resulting in inadequate supervision and poor waste management. Mercury is one of the most common pollutants produced by artisanal mining. This study aimed to determine the diversity of reptile species and analyze mercury accumulation in keratin samples obtained from reptiles inhabiting Saweak Mining Village, Pinang Belapis District, Lebong Regency. Sampling was conducted along river sections in Saweak Village suspected to be contaminated by mercury waste. Herpetofauna were surveyed using the Visual Encounter Survey (VES) method combined with transects during their active periods, both diurnal (08:00-11:00) and nocturnal (19:00-22:00). Collected specimens were identified and analyzed for mercury content. A total of nine herpetofaunal species were recorded, consisting of six amphibians and four reptiles. Mercury concentrations varied among species, with the highest to lowest levels observed in the following order: *Wijayarana sumatrana* (AMP LB31), *W. sumatrana* (AMP LB16), *Odorrana hosii* (AMP LB26), *Chalcorana chalconota* (AMP LB30), *Hylarana chalconota* (AMP LB12), *Phrynoidis aspera*, *Megophrys nasuta*, *O. hosii* (AMP LB11), and *Fejervarya limnocharis*. The highest mercury level (3,073.19 µg/kg) was detected in *Odorrana hosii*, while the lowest (240.40 µg/kg) was found in *Fejervarya limnocharis*. These findings indicate that mercury contamination in the study area is pervasive and has been transferred across trophic levels, affecting both aquatic and terrestrial herpetofauna. This highlights the potential ecological risk of mercury pollution and supports the use of herpetofauna as effective biomonitors of long-term environmental contamination.

Key words: artisanal gold mining, Bengkulu, bioaccumulation, herpetofauna, mercury contamination

INTRODUCTION

Artisanal and small-scale gold mining (ASGM) is recognized as one of the world's largest anthropogenic sources of mercury emissions, contributing an estimated 37% of the total mercury released into the environment (UNEP, 2018). In Indonesia, ASGM activities are widespread, occurring across 30 of the country's 34 provinces (Basri et al., 2020). The sector supports the livelihoods of nearly two million people, including miners as well as individuals involved in processing, trading, and other related activities (Ismawati et al., 2018; Basri et al., 2020). Despite its socio-economic importance, much of the sector operates informally or outside regulatory oversight. Gold extraction commonly relies on whole-ore mercury amalgamation, a simple but environmentally damaging technique that releases substantial amounts of mercury into surrounding ecosystems (Esdaile & Chomyshyn, 2020). As a result, mercury is continuously discharged into soils, rivers, and the atmosphere, creating persistent contamination and posing significant risks to both ecosystems and human health (Gafur et al., 2018).

After entering the environment, inorganic mercury can be transformed by microorganisms into methylmercury (MeHg), a highly toxic and bioavailable form that readily accumulates and biomagnifies through aquatic and terrestrial food webs. Exposure to methylmercury has been associated with adverse effects on the endocrine, immune, nervous, and reproductive systems of vertebrates, with particularly high risks for species that rely on fish or other aquatic organisms as prey. Owing to its strong affinity for sulfur-containing amino acids and sulfhydryl groups, methylmercury preferentially accumulates in skeletal muscle and keratinized tissues, making these tissues suitable for assessing long-term mercury exposure (Adel et al., 2017).

Amphibians and reptiles have therefore gained increasing attention as effective bioindicators of heavy metal contamination because of their distinct ecological roles and physiological characteristics (Schneider et al., 2013; Sumanasekara, 2015). Amphibians are especially vulnerable to environmental pollutants due to their highly permeable skin, which allows contaminants to be absorbed directly from water and soil. Reptiles, in contrast, are generally exposed through dietary intake and tend to accumulate mercury as it is transferred across trophic levels (Salice, 2011; Schneider et al., 2013). Their relatively limited dispersal and strong association with particular habitats further enhance their value as indicators of local environmental conditions. Consequently, changes in mercury concentrations within these taxa can provide an early indication of contamination in ecosystems which are affected by mining activities (Bergeron et al., 2010).

Tambang Saweak Village, situated in Pinang Belapis District, Lebong Regency, Bengkulu Province, is one of the areas where artisanal and small-scale gold mining remains an important local activity. Gold extraction and purification are typically carried out at the household level using mercury amalgamation to recover gold (Trimiska et al., 2018). This practice generates both liquid effluent and mercury vapor, much of which is released into the surrounding environment with little or no waste treatment. Previous studies have documented mercury contamination in water and sediment from several locations in Lebong (Ali et al., 2018; Mulyadi et al., 2020), yet information on mercury accumulation in local amphibian and reptile populations is still lacking. To address this knowledge gap, the present study quantified mercury concentrations in the muscle tissues of amphibians and reptiles collected from

Tambang Saweak Village. The findings provide baseline information on mercury bioaccumulation in herpetofauna inhabiting an artisanal gold mining landscape in Bengkulu Province, Indonesia.

MATERIALS AND METHODS

A. Sample Collection

The study was conducted between May and October 2022 in Tambang Saweak Village, Pinang Belapis District, Lebong Regency, Bengkulu Province, Indonesia (03°30′46″S, 102°32′53″E). The study area lies within the Bukit Barisan Mountain range and is characterized by interconnected forest ecosystems and river networks. Tambang Saweak Village covers approximately 6,400 ha, while the surrounding Pinang Belapis District extends over 35,912 ha, forming a heterogeneous landscape shaped by both natural processes and artisanal mining activities. Field sampling focused on river sections located near artisanal and small-scale gold mining (ASGM) operations, where mercury contamination was considered likely. A purposive sampling approach was adopted to select three representative sites based on their proximity to mining activities and the potential risk of mercury exposure. The sites represented upstream, midstream, and downstream sections of the river, allowing spatial variation in mercury contamination to be evaluated. Each sampling site consisted of small rivers bordered by paddy fields and forest edges, with nearby artisanal mining areas and household-scale gold processing facilities. Herpetofauna surveys were conducted using the Visual Encounter Survey (VES) method following Kusrini (2009), combined with line transects during nocturnal sampling between 19:00 and 22:00. At each site, environmental variables, including air temperature, water temperature, relative humidity, and water pH, were measured to characterize local habitat conditions.

B. Tissue Collection for Mercury Content Analysis

All field sampling and tissue collection procedures were performed in accordance with established trace-metal contamination control protocols (United States Environmental Protection Agency [EPA], 1998; U.S. Geological Survey [USGS], 2001). Each specimen was assigned a unique identification code before sampling. To minimize the risk of cross-contamination, all reusable instruments were cleaned with 10% HNO_3 and rinsed thoroughly with 18 MΩ deionized water before use and between successive samples. Disposable scalpels and powder-free nitrile gloves were used throughout the sampling process and replaced after handling each individual. Prior to tissue collection, frogs and toads were rinsed with deionized water and gently blotted dry. Small keratinized tissues, including toe tips, scales, or claws, were then excised using sterile instruments. For reptiles, keratin samples were collected from claws or tail tips with pre-cleaned scissors. Where euthanasia had been approved, small portions of liver or muscle tissue were also collected using sterile instruments. Immediately after collection, each tissue sample was transferred to a pre-cleaned polypropylene vial, wrapped in baked aluminum foil, and transported on dry ice to the laboratory. Before mercury analysis, the samples were rinsed once more with deionized water and stored at -20°C following recommended protocols for trace-metal sample handling and preservation (Ackerman et al., 2023).

C. Mercury Analysis

Mercury concentrations in muscle tissue were determined by Atomic Absorption Spectrophotometry (AAS) in accordance with the Indonesian National Standard (SNI 2354.6:2011). Prior to analysis, tissue samples were oven-dried at 60°C, homogenized, and digested in a 1:1 mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) until a clear solution was obtained (Munns & Holland, 1977; Mohammed et al., 2017). The resulting digest was then diluted to a final volume of 50 mL with deionized water before total mercury concentrations were measured at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada.

Quality assurance procedures included reagent blanks and standard reference materials in every analytical batch. Mercury concentrations were reported as milligrams per kilogram (mg/kg) on a dry-weight basis and calculated using the following equation: Hg = (Hg reading-blank) × final volume / sample weight.

RESULTS

A total of nine adult herpetofauna species were recorded, comprising five amphibian and four reptile species. The amphibians identified were *Phrynoidis aspera*, *Wijayarana sumatrana*, *Odorrana hosii*, *Hylarana chalconota*, and *Fejervarya limnocharis*, while the reptiles consisted of *Bronchocela jubata*, *Eutropis multifasciata*, *Gekko monarchus*, and *Xenopeltis unicolor* (Fig. 1). Reptile species such as *E. multifasciata* and *G. monarchus* are predominantly terrestrial and synanthropic, with minimal interaction with aquatic environments, and were therefore excluded from mercury analysis. Likewise, *B. jubata* is an arboreal species that mainly occupies vegetation above ground level, limiting its direct exposure to aquatic contaminants. These ecological characteristics suggest that terrestrial and arboreal reptiles generally experience lower mercury exposure because of their limited association with aquatic habitats and prey (Bergeron et al., 2007).

Mercury concentrations in the analyzed samples ranged from 0.24 to 18.23 mg/kg (Fig. 2). The highest concentration was found in tadpoles (18.23 mg/kg), whereas the lowest was detected in adult *Fejervarya limnocharis* (0.24 mg/kg) (Table 1). These results indicate potential mercury bioaccumulation across different developmental stages and trophic levels within herpetofauna inhabiting the river ecosystem. The elevated mercury concentration observed in tadpoles is consistent with previous findings, which have shown that larval amphibians are continuously exposed to contaminated water and sediments throughout their aquatic stage, thereby facilitating mercury uptake (Unrine et al., 2004; Bergeron et al., 2010; Bergeron et al., 2011).

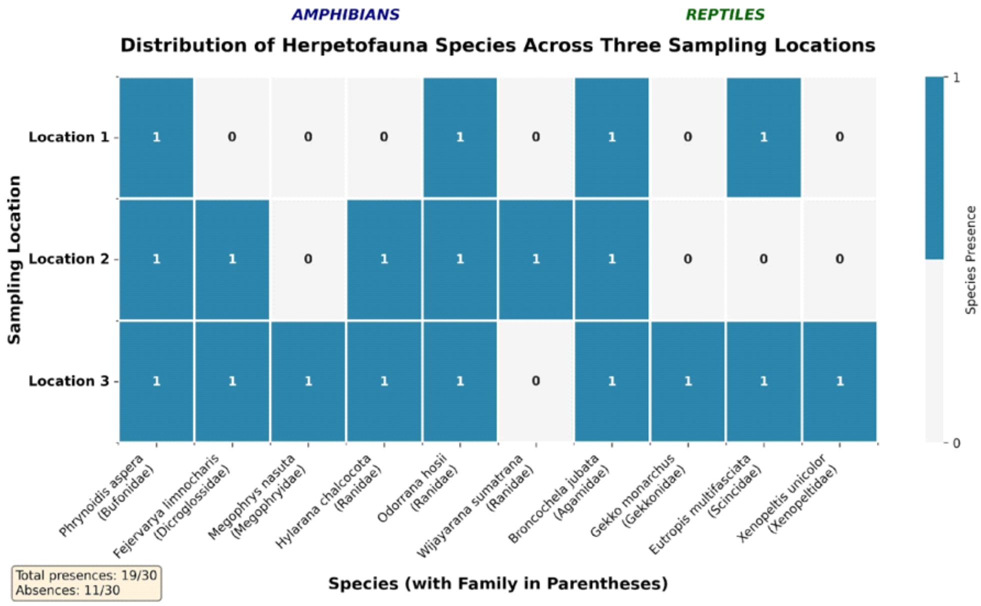

Figure 1. Species composition of adult herpetofauna identified in the Saweak Mining Village.

Table 1. Mercury levels detected in Herpetofauna samples

No (mg/Kg)	Code	Sample	Weight (gr)	Final volume (mL)	Observed Hg (µg/Kg)	Final Hg (µg/Kg)	Hg (mg/Kg)	Average Hg
1	AMP LB 008	*Phrynoidis aspera*	0.9676	50	12.030	621.12	0.62	0.62
2	AMP LB11	*Odorrana hosii*	1.2775	50	14.481	566.38	0.57	0.49
3	AMP LB12	*Hylarana chalconota*	1.0429	50	13.781	660.23	0.66	0.66
4	AMP LB14	*Fejervarya limnocharis*	1.1568	50	5.572	240.40	0.24	0.24
5	AMP LB16	*Wijayarana sumatrana*	1.3913	50	35.047	1,259.15	1.26	1.26
6	AMP LB26	*Odorrana hosii*	0.4078	50	9.079	1,111.94	1.11	1.11
7	AMP LB30	*Chalcorana chalconota*	1.2615	50	24.742	980.26	0.98	0.98
8	AMP LB31	*Wijayarana sumatrana*	0.2471	50	9.013	1,821.73	1.82	1.82
9	AMP LB32	*Megophrys nasuta*	0.4917	50	5.054	512.91	0.51	0.51
10	BLANKO			50	0.010			

Note: Observed = raw measurement; Final = concentration after dilution and analytical correction; Average = mean of replicate measurements. Mercury (Hg) concentrations were determined using Atomic Absorption Spectrophotometry (AAS) following standard procedures (United States Environmental Protection Agency [EPA], 1998; Welz & Sperling, 1999).

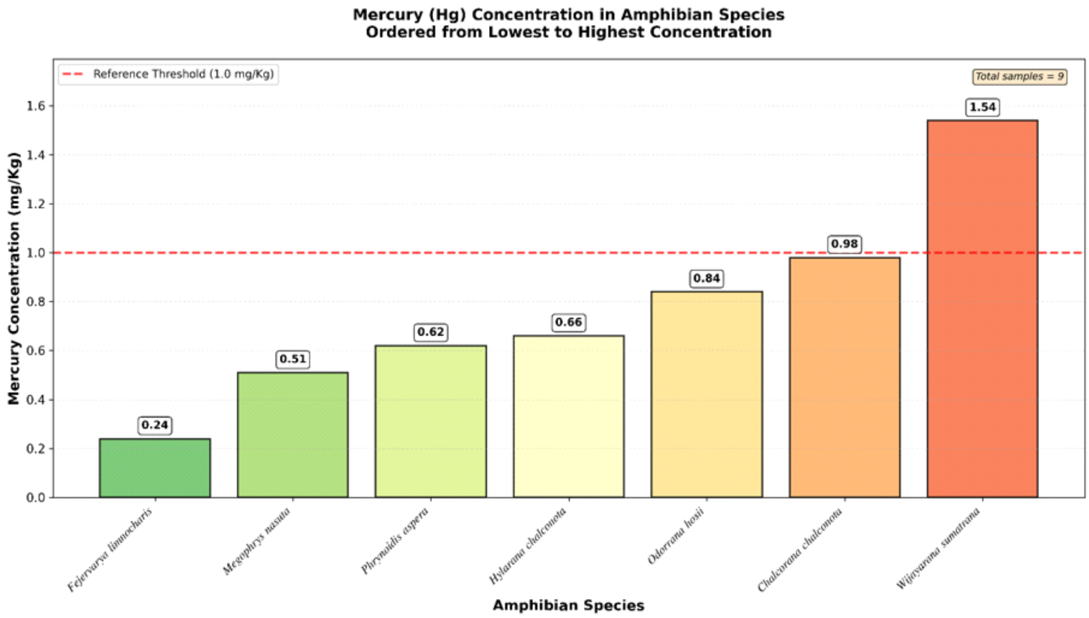

Figure 2. Interspecific variation in mercury (Hg) accumulation (mg/kg) among amphibians inhabiting the Saweak Mining Area, Lebong Regency, Bengkulu Province, Indonesia.

Measurements of water quality at Tambang Saweak Village (Table 2) showed that most physicochemical parameters, including pH (6.4 ± 0.02), temperature (27.7 ± 0.01 °C), and dissolved oxygen (7.48 ± 0.01 mg/L), complied with the Class II river water quality standards established under Government Regulation of Indonesia No. 22 of 2021. Likewise, total suspended solids (TSS), total dissolved solids (TDS), and conductivity remained below the permissible limits. Despite these acceptable physicochemical conditions, mercury (0.01 mg/L) and cyanide (0.006 mg/L) were detected, indicating localized contamination that is likely linked to nearby artisanal gold-mining activities. These findings suggest that chemical contamination may persist even when conventional water quality parameters fall within regulatory limits, particularly in environments affected by mining activities (Driscoll et al., 2013).

Table 2. Water quality parameters at the sampling sites in Tambang Saweak Village

Parameter	Result	River Quality Standard Class II (PP RI No. 22 of 2021)
pH (unit)	6.4 ± 0.02	6 – 9
Temperature (°C)	27.7 ± 0.01	26 – 30
Salinity (ppt)	1	1
DO (mg/L)	7.48 ± 0.01	> 4
TSS (mg/L)	7 ± 0.2	50
TDS (mg/L)	75.37 ± 0.3	1000
Conductivity (µS/cm)	156.3 ± 0.2	200 – 1000
Cyanide (mg/L)	0.006	0.02
Mercury (mg/L)	0.01	0.002
Cadmium (mg/L)	< 0.01	0.01

DISCUSSION

The detection of mercury (Hg) in all analyzed herpetofauna samples indicates that contamination is widespread within the aquatic ecosystems of Saweak Village. This pattern reflects the movement of mercury from abiotic components, such as water and sediment, into biological organisms. The occurrence of Hg residues in both amphibians and reptiles demonstrates its high bioavailability within the riparian environment and is further supported by the observed concentration patterns. Species with stronger associations with aquatic habitats, including tadpoles (18.23 mg/kg) and *Wijayarana sumatrana* (up to 1.82 mg/kg), contained higher mercury concentrations than more terrestrial species such as *Fejervarya limnocharis* (0.24 mg/kg). These findings indicate that mercury has entered the local food web and has been transferred across trophic levels. Such a pattern is consistent with the processes of bioaccumulation and trophic biomagnification commonly reported in ecosystems affected by ASGM, where inorganic mercury is converted into the more toxic and persistent form, methylmercury (Scheuhammer et al., 2007; Espejo et al., 2019).

Amphibians are particularly susceptible to heavy metal exposure because of their permeable skin and biphasic life cycle, which includes both aquatic larval and terrestrial adult stages. These biological characteristics facilitate contaminant uptake through direct dermal absorption from the surrounding water as well as contact with contaminated substrates (Hopkins, 2007; Pérez-Iglesias et al., 2020). During the fully aquatic larval stage, tadpoles can absorb mercury through their gills and skin while also ingesting contaminated detritus from sediments. The elevated mercury concentrations recorded in tadpoles in the present study support the view that sediments function not only as a sink but also as a secondary source of mercury contamination. This observation is in agreement with recent studies identifying amphibians as highly sensitive sentinel organisms for methylmercury contamination in wetland ecosystems, even surpassing fish at comparable trophic levels (Ackerman et al., 2023).

Mercury accumulation was also detected in *Xenopeltis unicolor* (0.59 mg/kg), a species that is primarily terrestrial and fossorial but is frequently associated with wetlands and other moist habitats (Malkmus et al., 2002; Das, 2015). This ecological flexibility likely increases its exposure to contaminants originating from both aquatic and terrestrial environments. Likewise, the other reptile species recorded in this study, namely *Eutropis multifasciata* and *Gekko monarchus*, are mainly terrestrial and synanthropic, whereas *Bronchocela jubata* is arboreal (Handziko et al., 2021). Although these reptiles have limited direct contact with aquatic habitats, mercury detected in comparable reptilian taxa suggests that indirect exposure through trophic transfer is possible. Consequently, the mercury accumulated in their tissues is likely derived from the consumption of contaminated prey, including frogs and small mammals, illustrating the biomagnification of mercury from aquatic ecosystems to secondary consumers in terrestrial food webs. These findings suggest that mercury contamination is not confined to aquatic environments but may also spread to terrestrial predators through food-web interactions (Burger et al., 2018; Yum et al., 2024).

The relationship between mercury accumulation and lifespan provides additional context for interpreting these results. Because mercury is eliminated slowly from the body, it can accumulate progressively with prolonged exposure. As a result, long-lived organisms generally carry greater mercury burdens due to continuous dietary intake over time. Reptiles, which typically have relatively long lifespans and low metabolic rates, are especially susceptible to this process. Previous studies have demonstrated that mercury concentrations increase with age in reptiles and other vertebrates, reflecting cumulative exposure throughout an individual's lifetime (Schneider et al., 2015). These observations highlight lifespan as an important factor influencing mercury accumulation.

This study has several limitations, including the relatively small sample size and the lack of age-specific information, both of which may affect the interpretation of bioaccumulation patterns among species. Nevertheless, the detection of mercury in herpetofauna suggests that the measured concentrations reflect not only recent exposure but also long-term accumulation. These findings further support the use of reptiles as effective biomonitors because contaminant levels in their tissues integrate environmental exposure over extended periods. As meso- and upper-level predators, herpetofauna in Saweak Village may also facilitate the transfer of mercury to higher trophic levels, including birds and mammals, with potential implications for long-term ecosystem stability (Lima et al., 2021).

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