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Diversity and potential of herbaceous plants as mercury (Hg) hyperaccumulators in small-scale gold mining sites in Pancurendang, Banyumas, Indonesia

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Abstract. *Muryani E, Sajidan, Budiastuti MTS, Pranoto. 2023. Diversity and potential of herbaceous plants as mercury (Hg) hyperaccumulators in small-scale gold mining sites in Pancurendang, Banyumas, Indonesia. Biodiversitas 24: 3364-3372*. Plants resistant to mercury can be used as an alternative to processing gold mining waste with the phytoremediation method. The study aimed to inventory herbaceous plant species in artisanal and small-scale gold mining (ASGM) sites in Pancurendang Village (Banyumas, Central Java, Indonesia), measure mercury concentrations in plants and root zones, and analyze the potential of herbaceous plants as mercury (Hg) hyperaccumulators. Herbaceous plants were cataloged by purposive sampling with multiple quadrats of 2m x 2m in 14 locations and then analyzed to obtain density, frequency, dominance, importance value, and diversity index. Biomass and Hg concentrations were measured from selected herbaceous plants and soil samples of the root zone. The Biological Accumulation Coefficient (BAC) was calculated to determine the plants' potential as Hg hyperaccumulators. Plant inventory identified 54 herbaceous plant species belonging to 26 families, with *Paspalum conjugatum* P.J.Bergius being the dominant species. In the root zones, Hg was found at 11-73 ppm and 7.6-85.36 ppm in 19 species. *Ipomea aquatica* Forssk. accumulated the highest concentration (85.36 ppm) and absorbed the greatest amount of Hg (5.25 mg). Based on their BACs, the nineteen species were categorized into moderate to high (hyper) accumulators, with *Plectranthus* sp. having the highest BAC of 4.54.

Keywords: Diversity, herbaceous plant, mercury hyperaccumulator, Pancurendang, small-scale gold mining

Abbreviations: AMD: Acid Mine Drainage; ASGM: Artisanal and Small-Scale Gold Mining; BAC: Biological Accumulation Coefficient; Cd: Cadmium; Zn: Zinc; Cu: Copper; H': Shannon-Wiener Diversity Index; Hg: Mercury; IVI: Importance Value Index; MeHg: methylmercury; pH: potential hydrogen; ppm: part per million; RC: Relative Coverage; RD: Relative Density; RF: Relative Frequency; RZS: Root Zone Soil; T-Hg: Total mercury; WHO: World Health Organization

INTRODUCTION

Artisanal and small-scale gold mining (ASGM) in Pancurendang (Banyumas, Indonesia) has operated since 2014. Currently, mining is conducted in about 60 holes along the banks of the Tajur and Datar Rivers. Gold is processed in more than 70 residential houses in the highlands by amalgamation, i.e., the method of separating gold from ores using water and the heavy metal mercury (Hg) with a cylindrical tool called a ball mill. Tailings and wastes from small-scale gold processing in Pancurendang and Indonesia generally are discharged into the environment without prior treatments (Meutia et al. 2022). Tailings containing mercury are prone to form acid mine drainage (AMD) if the source rocks comprise sulfide minerals (Hatar et al. 2013).

Initial observations and measurements by the authors revealed that the liquid waste collected as composite samples from two gold processing sites in Pancurendang contained $3x10^{-4}$ ppm mercury, with a potential hydrogen (pH) range of 2.2-3 (highly acidic). Also, the mercury level of tailings in one of the sacks stacked on the pool of cyanidation slurry reached 4.13 ppm with a pH of 4. In 2019, mercury was found at $3.8x10^{-2}$-$2.34x10^{-1}$ ppm in acidic soils (pH = 4-6.1) in plantations, rice fields, and settlements around gold processing areas and $8x10^{-5}$-$4.2x10^{-4}$ ppm in groundwater. Further, Prasetya et al. (2021) found that 2020 mercury was detected in the range of $8x10^{-5}$-$4.2x10^{-4}$ ppm in river water and $1.4x10^{-2}$-7.74 ppm in river sediments. Even though the mercury concentrations of the river water did not exceed the standard water quality, the soil and river water's pH values at several measurements were below 6. These findings indicate that Pancurendang is experiencing environmental contamination due to gold processing waste, particularly mercury.

Accordingly, these gold processing activities require specific technologies to treat tailings (solid waste) and acidic amalgam (liquid waste) to mitigate, control, and reverse environmental impacts (i.e., pollution) due to mercury exposure. Phytoremediation, an alternative technology to processing gold waste, is rapidly developing because of its proven cost-effectiveness and safety compared to other methods (Razzaq 2017; Khan et al. 2023). Phytoremediation draws upon the ability of plants or certain parts of a plant to remove, change, or render contaminants from water, sediments, soil, and the atmosphere harmless (Jaskulak et al. 2020; Khan et al. 2023). Plants selected for this method should be resistant to mercury and acidic environments and, in the case of Indonesia, preferably local because the high biodiversity offers myriad options of plants with less explored potential for environmental remediation.

Several herbaceous plants grow in artisanal and small-scale gold mining (ASGM) areas and absorb mercury in the environment (soil, groundwater, river water, river sediments) around mining and processing sites. For instance, cover crops such as *Paspalum conjugatum* P.J.Bergius, *Cyperus kyllingia* Endl., and *Lindernia crustacea* (L.) F.Muell. have been used to absorb mercury in the tailings disposal area in Sekotong Tengah (West Lombok, Indonesia) (Muddarisna et al. 2013). However, the potential of plant species as phytoremediation agents may differ across regions. Besides, little is known about herbaceous plants that are also effective mercury hyper-accumulators under acidic conditions. New herbaceous plant species are likely identified as hyperaccumulator plants for the research area. Therefore, this study aimed to inventory species of herbaceous plants in ASGM sites in Pancurendang, measure mercury concentration in plants and soils in the root zones, and analyze the potential of herbaceous plants as mercury (Hg) hyperaccumulators.

MATERIALS AND METHODS

Study area

The research was conducted in gold mining and processing areas in Pancurendang Village, Ajibarang Sub-district, Banyumas District, Central Java Province, Indonesia. These areas spanned from 7º 38' 42" to 7º 59' 03" S and from 110º 01' 37" to 110º 16' 26" E. Figure 1 shows maps of the research area and sampling points for the observation and measurement of herbaceous plants in the gold mining and processing areas.

Procedures

Plant survey and sampling

Data on herbaceous plants in the ASGM area in Pancurendang, Banyumas, Indonesia, were collected using a purposive sampling method with multiple quadrats to build the inventory. The position of each plot was determined purposively based on the presence of piles or stacks of tailings. Sample plots sized 2m x 2m were made with raffia strings at a 50-100 m distance from each other. Plant observations and measurements were conducted in 14 sample plots, which, according to the map presented in Figure 1, are denoted by the numbers 1 to 14. These samples consisted of six plots in gold mining areas and riverbanks (as indicated by samples 1, 2, 3, 4, 11, and 14) and eight plots in gold processing areas that included amalgamation and cyanidation sites (5, 6, 7, 8, 9, 10, 12, and 13). Figure 2 shows some photos of the condition of sampling areas during plant data collection.

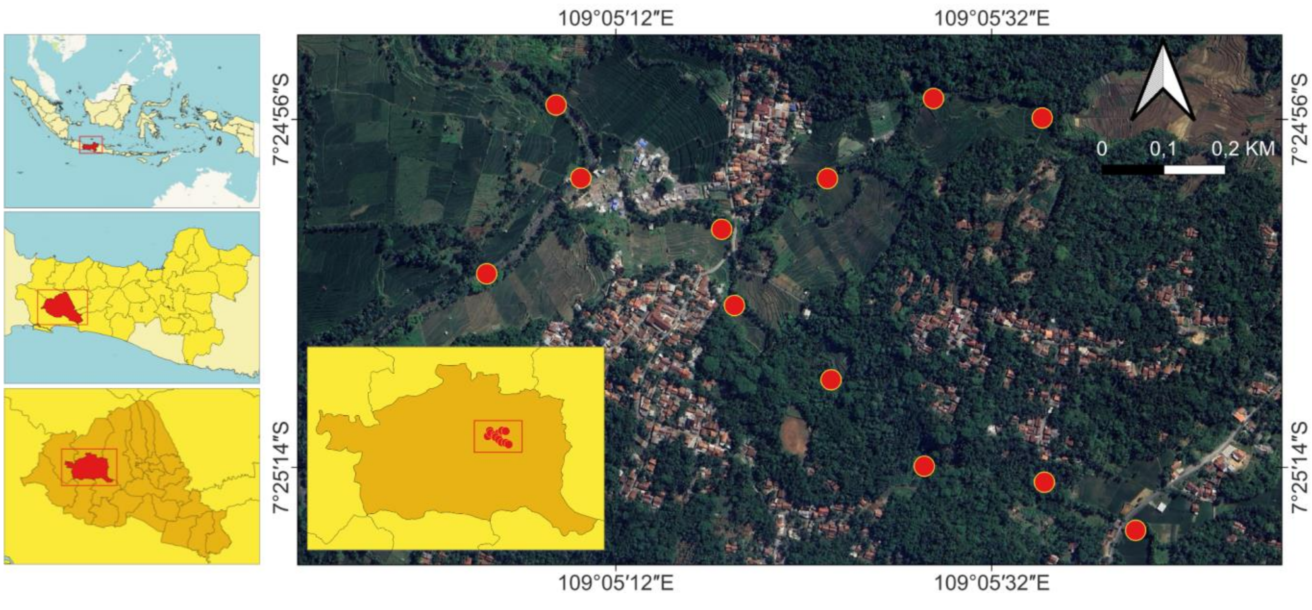

Figure 1. Maps of the research area and the observation and measurement points of herbaceous plants in gold mining and processing sites in Pancurendang Village (Banyumas, Indonesia)

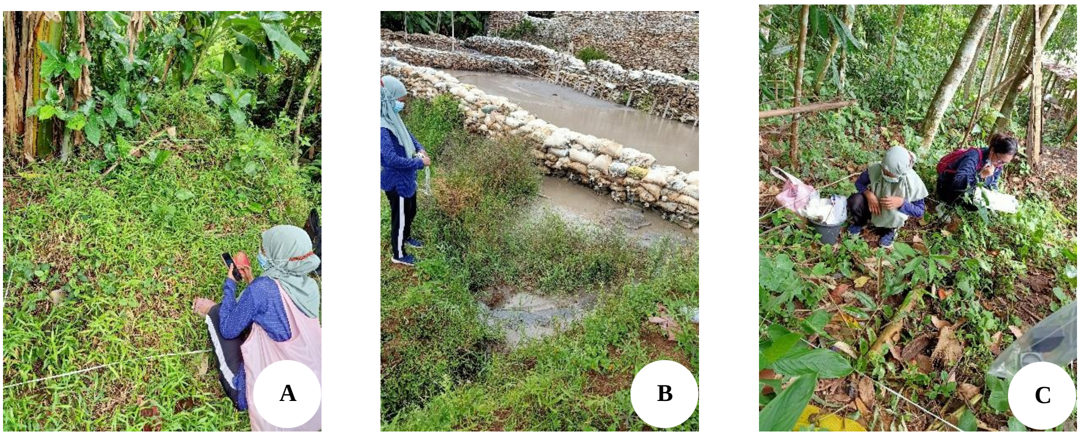

Figure 2. Photos of A. Plant observation and measurement in the mining area at sample 1; B. The pool of cyanidation slurry was made by stacking sacks of tailings at sample 6; and C. The location of the gold amalgamation process at sample 7

Soil data collection and sample preparation

Several soil characteristics were measured directly in each plot using a soil tester to read pH and other environmental parameters, like temperature, soil moisture, light intensity, and fertility. Temperature data were expressed in the normal range of the degree Celsius. Soil moisture, light intensity, and fertility were described generally as either wet or dry, light or normal, and ideal or slightly ideal.

Soil samples of the root zone or the plant growth media in the sample plots were collected at a depth of 10-30 cm and then air-dried to constant weight (7-10 days). In addition, several selected herbaceous plants in the sampling area were uprooted and dried in an oven at 60°C to constant weight (5-7 days). Afterward, the dried plants were weighed (recorded as biomass) and ground into powders with a blender. Air-dried soils and plant powders were transported to the Assessment Institute for Agricultural Technology (AIAT) Yogyakarta Laboratory for Hg content analysis using the hybrid-system atomic absorption spectrometry (AAS).

In the laboratory, samples were prepared by wet digestion for 8-12 hours using the acid mixture of HNO_3 and HCl_4. Samples were dissolved in 65% HNO_3, concentrated 95% HCl_4 in an Erlenmeyer flask, and then heated on a hot plate until completely dissolved. Steam would form as a result of the digestion. In this case, concentrated HNO_3 solution was used as a destructor, and HCl_4 solution was a catalyst to speed up the reaction of Hg dissolution from organic compounds in the sample. Afterward, the solution mixture was diluted with H_2O_2 as a heavy metal binder (Krupnova et al. 2021).

Data analysis

Vegetation data analysis

Three quantitative parameters were measured to describe the vegetation condition in the research area: density, frequency, and dominance (coverage area) (Solikhatun et al. 2020).

Density. Density was calculated from the number of individuals of a plant species in a certain area; for example, ten individuals per square meter. One individual plant was defined as one shoot (not one stem or leaf) for herbaceous plants.

Frequency. Frequency refers to the number of sample plots where a certain plant species was found.

Dominance or coverage. Dominance is the proportion of soil surface covered by prevailing plant canopy expressed in percent individuals. Dominance also implies a basal area projection on a land surface area. The graph paper method measures the area, and the derived percent coverage indicates dominance.

Afterward, vegetation analysis was conducted by calculating eight parameters: density, relative density, frequency, relative frequency, coverage, relative coverage, importance value index (IVI), and species diversity index (H'), using the equations below (Ismail et al. 2017; Solikhatun et al. 2020).

$$\text{Density (indv./m}^2\text{)} = \frac{\text{Number of unit of a species}}{\text{Total plot area}} \quad (1)$$

$$\text{Relative Density (RD)} = \frac{\text{Density of a species}}{\text{Total plant density}} \times 100\% \quad (2)$$

$$\text{Frequency} = \frac{\text{Number of plots with a certain species}}{\text{Total plots}} \quad (3)$$

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency of a species}}{\text{Total plant frequency}} \times 100\% \quad (4)$$

$$\text{Coverage} = \frac{\text{Area covered by a species}}{\text{Total area}} \quad (5)$$

$$\text{Relative Coverage (RC)} = \frac{\text{Coverage of a species}}{\text{Total plant coverage}} \times 100\% \quad (6)$$

Importance Value Index (IVI) = Relative frequency+Relative density+Relative coverage (7)

The species diversity index was analyzed using the Shannon-Wiener equation:

$$H' = Pi \log Pi; \text{ with } Pi = n/N \quad (8)$$

Where:
H' : Shannon-Wiener species diversity index
n : number of individual plants of a species
N : total number of individuals

Determination of potential mercury hyperaccumulators

The amount of heavy metal, i.e., mercury (Hg), absorbed into the plant tissue was calculated by multiplying heavy metal concentration and plant biomass (dry weight) (Schück and Greger 2020).

$$\text{Hg absorbed in plant tissues} = [\text{Hg}] \times \text{biomass (dry weight)} \quad (9)$$

Plants belonging to the phytoextraction, hyper-accumulator, large biomass, and fast-growing/easy-to-propagate categories have the potential as heavy metal phytoremediators (Krupnova et al. 2021). Moreover, it is highly recommended that these plants are not food crops that are widely consumed, non-medicinal plants, and rare or protected species. The Biological Accumulation Coefficient (BAC) measures the ability of plants to absorb metals from the substrate (Azeez 2021). BAC was calculated using the equation below (Tangahu et al. 2011; Idris et al. 2016).

Biological Accumulation Coefficient (BAC)

$$= \frac{[\text{Hg}] \text{ in plants biomass (ppm)}}{[\text{Hg}] \text{ in soil in the root zone (ppm)}} \quad (10)$$

The derived BAC was categorized according to Table 1 to determine if the plant species observed was a potential hyperaccumulator (Idris et al. 2016). Afterward, correlation analysis and multiple linear regression were performed to identify the direction and strength of the relationship between the plant and soil variables (Meléndez-Ackerman et al. 2016).

RESULTS AND DISCUSSION

Diversity of herbaceous plant species in gold mining and processing areas in Pancurendang

Plant inventory in the gold mining and processing areas in Pancurendang identified 54 species of herbaceous plants belonging to 26 families. Among these, *P. conjugatum* was the dominant species, with a relative dominance (RD) of 31.873% and an importance value index (IVI) of 92.152%. *P. conjugatum* also had the highest frequency and density compared to the other plant species. Several previous studies proved that *P. conjugatum* could adapt to gold mining environments in Indonesia and had the potential as a phytoremediation agent for Hg (Hidayati et al. 2009; Fiqri et al. 2017; Zhang et al. 2020). Plants that thrive in the disposal sites of gold processing waste may be hyper tolerant or resistant to mercury due to their ability as metal hyperaccumulators (Zakaria et al. 2021).

Table 1. Mercury accumulator categories based on biological accumulation coefficient (BAC)

Plant category	BAC
High accumulator (Hyperaccumulator)	1-10
Moderate accumulator	0.1-1
Low accumulator	0.01-0.1
Non-hyperaccumulator	<0.01

Calculation results in Table 2 also show a Shannon-Wiener diversity index (H') of 1.365, meaning that the herbaceous plants sampled in the study area had moderate density (Jianshuang et al. 2019). Therefore, small-scale gold mining activities can reduce the diversity of riparian plants in the vicinity. Takarina et al. (2021) confirmed that areas near gold mining only contributed 26.1% of the 42 riparian species on the Cikidang River in Lebak (Banten, Indonesia). In Pancurendang, Banyumas, Indonesia several species of herbaceous plants were found nearby gold tailing deposits at location number 6: *I. aquatica*, *L. perennis*, *C. esculenta*, *D. longiflora*, *M. vaginalis*, and *L. flava*, which were categorized as resistant to the heavy metals found in the tailings.

Nine out of 54 identified species (16.67%) are members of Asteraceae, and eight are of Poaceae (14.81%), making them the dominant plant families in the study area. The species with the highest IVI was *P. conjugatum*, which belongs to the family Poaceae, followed by *D. longiflora* (Poaceae), *P. repens* (Poaceae), *S. nodiflora* (Asteraceae), and then *A. conyzoides* (Asteraceae). Despite not having the highest IVI, Asteraceae is dominated by the two families. Asteraceae, known as the sunflower family, is the largest family of flowering plants comprising more than 1,600 genera and 25,000 species worldwide (Rolnik and Olas 2021). Well-known members of Asteraceae include chicory, sunflower, lettuce, dahlia, and daisy. Chamba et al. (2017) stated that *Erato polymnioides* from this family, which is associated with mycorrhizae, is a potential Hg hyperaccumulator plant. Grasses, which make up Poacea (Aitken et al. 2014), are known to survive in unfavorable soil conditions, be resistant and adaptable to heavy metals, and have deep roots (Khodijah et al. 2019; Takarina et al. 2021).

Mercury concentrations in soil and herbaceous plant biomass

Mercury concentrations were measured from root zones at ten sampling points across the gold mining and processing areas. Results showed that the Hg concentrations of the ten soil samples ranged from 11.01 to 73 ppm. Based on provisions in Appendix IX of Indonesia Government Regulation No. 22 of 2021 on the Implementation of Environmental Protection and Management, soils with Hg concentrations <75 ppm can be managed with procedures for non-toxic hazardous materials (Peraturan Pemerintah Republik Indonesia 2021). Several previous studies reported mercury contaminations in areas used for or adjacent to gold mines, industrial waste disposal sites, and agricultural lands (Betancur-Corredor et al. 2018; Esdaile and Chalker 2018). For instance, total mercury (T-Hg) and methylmercury (MeHg) found in the soil and water around the ASGM areas along Cikaniki River (West Java, Indonesia) is in the range of 0.07-16.7 ppm and <0.07-2.0 ppm, respectively (Tomiyasu et al. 2017). Small-scale gold mining waste in Pongkor (Bogor, Indonesia) contains up to 240 ppm of Hg and 0.1 ppm of CN^- ions; this waste is disposed of directly into the environment without proper waste treatment (Hidayati et al. 2009). Tailings from gold mines in Tambang Sawah

(Lebong, Indonesia) have a critically high mercury level of up to 265.4301 ppm (Irwan and Rukmini 2021). Furthermore, 0.07-77.44 ppm Hg levels are detected in soils at ASGM sites in Indonesia, Myanmar, the Philippines, and Thailand (Soe et al. 2022). These studies suggest that mercury can be present in widely varied concentrations in the soil around ASGM locations.

Table 2. Herbaceous plant analysis results in gold mining and processing areas in Pancurendang, Banyumas, Indonesia

Family	Species	RF (%)	RD (%)	RC (%)	IVI (%)
Poaceae	*Paspalum conjugatum*	5.882	54.397	31.873	92.152*
Poaceae	*Imperata cylindrica*	1.961	0.729	0.344	3.034
Asteraceae	*Mikaina micrantha*	3.922	0.472	0.430	4.823
Convolvulaceae	*Ipomea aquatica*	1.961	0.215	0.515	2.691
Oxalidaceae	*Oxalis barrelieri*	1.961	0.300	0.172	2.433
Poaceae	*Panicum repens*	0.980	7.550	7.732	16.263*
Fabaceae	*Centrosema pubescens*	3.922	0.987	1.375	6.283
Poaceae	*Oplismenus hirtellus*	0.980	0.172	0.086	1.238
Asteraceae	*Chromolaena odorata*	2.941	0.300	1.031	4.272
Acanthaceae	*Ruellia tuberosa*	0.980	0.429	0.430	1.839
Asteraceae	*Conyza sumatrensis*	0.980	0.129	0.086	1.195
Asteraceae	*Ageratum conyzoides*	5.882	2.188	2.491	10.562*
Fabaceae	*Calopogonium mucunoides*	0.980	0.472	3.436	4.889
Phyllanthaceae	*Phyllanthus niruri*	3.922	2.145	0.945	7.012
Asteraceae	*Emilia sonchifolia*	1.961	0.172	0.172	2.304
Asteraceae	*Synedrella nodiflora*	4.902	1.459	4.639	11.000*
Araceae	*Colocasia* sp.	0.980	0.300	0.859	2.140
Fabaceae	*Mimosa pudica*	1.961	0.300	0.430	2.691
Araceae	*Syngonium podophyllum*	0.980	0.686	4.296	5.962
Dryopteridaceae	*Dryopteris* sp.	2.941	0.429	0.344	3.714
Piperaceae	*Paperomia pellucida*	3.922	1.544	1.117	6.583
Onagraceae	*Ludwigia perennis*	2.941	1.716	5.241	9.898
Poaceae	*Digitaria longiflora*	1.961	8.323	6.443	16.727*
Asteraceae	*Acmella panniculata*	0.980	0.472	0.172	1.624
Asteraceae	*Eclipta prostata*	0.980	0.815	0.601	2.397
Poaceae	*Oryza sativa*	0.980	0.129	0.086	1.195
Cyperaceae	*Actinoscirpus grossus*	1.961	2.617	1.375	5.952
Apiaceae	*Centella asiatica*	0.980	0.043	0.086	1.109
Rubiaceae	*Oldenlandia diffusa*	0.980	0.086	0.086	1.152
Poaceae	*Eragrostis amabilis*	0.980	0.172	0.086	1.238
Pontederiaceae	*Monochoria vaginalis*	0.980	0.215	0.086	1.281
Alismataceae	*Limnocharis flava*	1.961	0.129	0.515	2.605
Araceae	*Colocasia esculenta*	2.941	0.858	4.038	7.837
Zingiberaceae	*Zingiber* sp.	1.961	0.429	2.148	4.538
Lamiaceae	*Plectranthus* sp.	1.961	1.502	3.007	6.469
Araceae	*Colocasia* sp.	1.961	0.515	1.718	4.194
Solanaceae	*Solanum erianthum*	0.980	0.129	0.258	1.367
Asteraceae	*Vernonia cinerea*	1.961	0.129	0.515	2.605
Plantaginaceae	*Scoparia dulcis*	0.980	0.043	0.086	1.109
Rubiacea	*Spermacoce latifolia*	0.980	0.515	0.859	2.354
Moraceae	*Ficus septica*	1.961	0.300	1.289	3.550
Melastomataceae	*Melastoma affine*	0.980	0.043	0.258	1.281
Malvaceae	*Hibiscus macrophyllus*	0.980	0.086	0.859	1.925
Cyperaceae	*Kyllinga monocephala*	1.961	0.601	0.430	2.991
Polygonaceae	*Persicaria barbata*	0.980	2.960	3.436	7.377
Convolvulaceae	*Ipomoea batatas*	0.980	0.086	0.430	1.496
Rubiaceae	*Spermacoce remota*	0.980	0.300	0.258	1.538
Fabaceae	*Flemingia macrophylla*	0.980	0.172	0.859	2.011
Vitacea	*Leea indica*	0.980	0.043	0.430	1.453
Acanthaceae	*Asystasia gangetica*	0.980	0.815	0.430	2.225
Capparaceae	*Cleome rutidosperma*	0.980	0.086	0.086	1.152
Phyllanthaceae	*Phyllantus reticulatus*	0.980	0.129	0.344	1.453
Malvaceae	*Triumfetta rhomboidea*	0.980	0.086	0.601	1.668
Poaceae	*Echinochloa colona*	0.980	0.086	0.086	1.152
Total: 26 families, 54 species		H' = 1.365			

Note: RF: Relative Frequency; RD: Relative Density; RC: Relative Coverage; IVI: Importance Value Index; H': Shannon-Wiener Diversity Index; *: Species at the top five highest IVI

The absorption of heavy metal plants is affected by soil pH, organic matter, and phosphorus content (Tangahu et al. 2011). Based on pH measurements, the gold mining areas in Pancurendang had acidic soil, pH 3.5-4.5, and the gold processing areas had varying soil pH between 4 to 9, with an average of 5.3. Further analysis revealed a correlation between soil pH and Hg concentrations in the soil (soil [Hg]), with a significance value of 0.023 (<0.05). In addition, plant absorption depends on heavy metal bioavailability in soil, which decreases at pH above 5.5-6 (Tangahu et al. 2011). Relatively acidic soil conditions in the ASGM sites in Pancurendang (Table 3) could indicate a high Hg bioavailability. From the remediation perspective, acidic soil pH increases heavy metal uptake by plants (Jaskulak et al. 2020). In this environment, agronomic practices have been developed to increase remediation by adjusting pH and adding ameliorants like chelates, fertilizers, and composts (Iqbal and Baig 2016; Khodijah et al. 2019; Utami et al. 2020). For example, the phytoremediation of mercury-contaminated soils using *Cyperus kyllingia* increased maize production to 126% higher than on untreated land (Muddarisna and Siahaan 2014). Soil pH and other agronomic factors should be considered as the determinants of Hg bioavailability before applying phytoremediation technology to deal with mercury released by ASGM practices in Pancurendang, Banyumas, Indonesia.

Therefore, 19 of the 34 plant samples collected in gold mining and processing areas in Pancurendang were selected for the Hg analysis because they weighed more than 5 g after drying. Based on the test results presented in Table 4, the Hg concentrations detected in herbaceous plant biomass were classified as high because they exceeded the upper limit for Hg content in plant organs, 0.5 ppm (FAO and WHO 2019). This corresponds to Soe et al. 2022, which found that plants in Indonesia, Myanmar, the Philippines, and Thailand contained more Hg than their maximum allowable presence per the World Health Organization (WHO) guideline (0.5 ppm). This standard is higher than the one issued in Indonesia, 0.03 ppm, as stated in the Indonesian National Standard (SNI) 7387:2009 (Badan Standardisasi Nasional 2009). Based on Table 2 (see figures with the asterisk * symbol), herbaceous plants with the five highest Hg concentrations in the study area were *I. aquatica*, *S. podophyllum*, *I. cylindrica*, *Plectranthus* sp., and *L. flava*. Meanwhile, Table 4 (figure with the asterisk * symbol) shows that *I. aquatica* had the highest Hg content in its biomass (85.36 ppm) and absorbed the greatest amount of Hg (5.25 mg) at sampling location number 6. *I. aquatica* is a proven hyperaccumulator of several heavy metals, including mercury (Göthberg et al. 2002), cadmium (Cd), zinc (Zn), and copper (Cu) (Hisam et al. 2022). *Limnocharis flava* can remove mercury from gold mining waste in an artificial wetland system (Marrugo-Negrete et al. 2015).

Table 3. Mercury concentrations and soil pH of the root zones in gold mining and processing areas in Pancurendang, Banyumas, Indonesia

Location	Sampling point	[Hg] (ppm)	Soil pH
Mining area	1	20.4	3.5
	2	12.6	4.5
	3	59.9	3.5
Processing area	5	73	5
	6	67	6.2
	7	24	5
	8	14.8	4.1
	9	35.8	9
	12	11.01	4.2
	13	19.1	4

Table 4. Mercury concentrations in plant biomass and mercury absorption by herbaceous plants in gold mining and processing areas in Pancurendang, Banyumas District, Indonesia

Species	Sample code	Fresh weight (g)	Dry weight (g)	[Hg] in plant biomass (ppm)	Hg absorbed by plant (mg)
Paspalum conjugatum	1_Pc	69	28.9	11.23	0.32
Imperata cylindrica	1_Ic	61	29.7	73.57	2.19
Panicum repens	2_Pr	34	24	25.27	0.61
Centrosema pubescens	2_Cp	25	8.4	22.46	0.19
Chromolaena odorata	2_Co	14	7	18.53	0.13
Calopogonium mucunoides	3_Cm	64	28.6	30.89	0.88
Synedrella nodiflora	5_Sn	22	15.7	7.86	0.12
Syngonium podophyllum	5_Sp	39	7.5	74.13	0.56
Digitania longiflora	6_Dl	20	6.7	30.32	0.20
Ludwigia perennis	6_Lp	21	8.3	29.75	0.25
Colocasia esculenta	6_Ce	45	6.4	16.85	0.11
Limnocharis flava	6_Lf	58	8.2	44.93	0.37
Ipomoea aquatica	S6_Ia	179	61.5	85.36*	5.25*
Zingiber sp.	7_Zsp	44	10.8	12.92	0.14
Spermacoce latifolia	9_Sl	50	34	35.94	1.22
Asystasia gangetica	11_Ag	43	18.2	11.79	0.21
Plectranthus sp.	12_Psp	69	14.5	49.96	0.72
Triumfetta rhomboidea	13_Tr	10	6.4	33.13	0.21
Vernonia cinerea	13_Vc	21	18.3	27.52	0.50

Hyperaccumulator plants can store at least 10 mg of mercury (Hg) per kg dry weight (Mariwy et al. 2021). Table 4 shows mercury was found at more than 10 mg/kg (ppm) dry weight in 18 species. The soil's mercury concentration, pH, and complex chemical elements (compounds) influence plant heavy metal accumulation. Lower pH allows for higher mobility of heavy metals in the soil; thus, more heavy metals are absorbed into plant organs. Complex compounds in the soil can trigger the accumulation and absorption of heavy metals in plants (Mariwy et al. 2021).

Heavy metal uptake (absorption) by individual plants is determined by plant biomass (Schück and Greger 2020), plant growth media (plant species and root zone), environmental factors, chemical properties or chelating agents (Zakaria et al. 2021), plant age, the amount of mercury in the soil/media, and the duration of contact between the plant and the heavy metal-containing media (Ashraf et al. 2010). The greater the plant biomass and the Hg concentration, the greater the Hg absorption by plants. In addition, the amount of Hg in plants can be used to determine if they are potential Hg hyperaccumulators. Despite being grown on the same soils, interspecific variations in heavy metal absorption likely occur because the ability to absorb Hg from gold processing waste depends on plant traits. This can be used to select suitable plant species for biomonitoring and phytoremediation (Krupnova et al. 2020). Further, because of plant biomass factors, high mercury concentrations do not necessarily mean high mercury absorption.

Multiple linear regression between soil pH, soil [Hg], and plant [Hg]

The regression analysis showed that soil [Hg] and pH positively relate to plants [Hg]. The regression equation was $Y= 22.22+ 0.211X_1 + 0.638X_2$, which means that plant [Hg] will be 22.22 units for 0 unit of soil [Hg] and pH and will increase by 0.211 for every increase in soil [Hg] and by 0.638 for every increase in soil pH. Plant [Hg] can be higher than soil [Hg] for plants that have been growing in the area for a long time and have roots reaching deeper soil where Hg most likely accumulates (Tomiyasu et al. 2017). In contrast, plant [Hg] can be lower than soil [Hg] because the mercury-containing soil has been eroded and washed away by surface runoff, diminishing Hg concentration in the root zone.

Herbaceous plants as potential mercury (Hg) hyperaccumulators

The potential of herbaceous plant species as mercury phytoremediators can be determined from biological accumulation coefficients (BACs). Table 5 shows the BACs of Hg in 19 herbaceous plants in gold mining areas in Pancurendang, which varied from 0.11 to 4.54. Even though *P. conjugatum* was the dominant species according to its relative dominance, importance value index, and frequency, it did not meet the minimum BAC of a hyperaccumulator plant, i.e., >1 (Koleli et al. 2015). Furthermore, 9 of the 19 species were categorized as moderate accumulators, while the remaining 10 were potential hyperaccumulator plants. The 10 species were *I. cylindrica*, *I. aquatica*, *P. repens*, *C. pubescens*, *C. odorata*, *S. podophyllum*, *S. latifolia*, *Plectranthus* sp., *T. rhomboidea*, and *V.cinerea*. *Plectranthus* sp. had the highest BAC of 4.54. It is one of the plant species reported by Marrugo-Negrete et al. (2016) and Liu et al. (2020) that have higher bioaccumulated mercury concentrations in the shoots than in the roots.

Table 5. Biological accumulation coefficients (BACs) of mercury (Hg) in 19 herbaceous plants in gold mining areas in Pancurendang

Species	Sample code	Plant [Hg] (ppm)	Soil [Hg] (ppm)	BAC	Category
Paspalum conjugatum	1_Pc	11.23	20.40	0.55	Moderate accumulator
Imperata cylindrica	1_Ic	73.57	20.40	3.61	Hyperaccumulator
Panicum repens	2_Pr	25.27	12.60	2.01	Hyperaccumulator
Centrosema pubescens	2_Cp	22.46	12.60	1.78	Hyperaccumulator
Chromolaena odorata	2_Co	18.53	12.60	1.47	Hyperaccumulator
Calopogonium mucunoides	3_Cm	30.89	59.90	0.52	Moderate accumulator
Synedrella nodiflora	5_Sn	7.86	73.00	0.11	Moderate accumulator
Syngonium podophyllum	5_Sp	74.13	73.00	1.02	Hyperaccumulator
Digitania longiflora	6_Dl	30.32	67.00	0.45	Moderate accumulator
Ludwigia perennis	6_Lp	29.75	67.00	0.44	Moderate accumulator
Colocasia esculenta	6_Ce	16.85	67.00	0.25	Moderate accumulator
Limnocharis flava	6_Lf	44.93	67.00	0.67	Moderate accumulator
Ipomoea aquatica	S6_Ia	85.36	67.00	1.27	Hyperaccumulator
Zingiber sp.	7_Zsp	12.92	24.00	0.54	Moderate accumulator
Spermacoce latifolia	9_Sl	35.94	35.80	1.00	Hyperaccumulator
Asystasia gangetica	11_Ag	11.79	12.60	0.94	Moderate accumulator
Plectranthus sp.	12_Psp	49.96	11.01	4.54	Hyperaccumulator
Triumfetta rhomboidea	13_Tr	33.13	19.10	1.73	Hyperaccumulator
Vernonia cinerea	13_Vc	27.52	19.10	1.44	Hyperaccumulator

According to Juhaeti et al. (2009), a plant species can be a potential heavy metal hyperaccumulator by the following criteria: (i) it has high concentrations of heavy metals in the roots and canopy tissues (indicating resistance); (ii) it absorbs heavy metal element(s) in the soil at higher rates than other plants; (iii) it can translocate and accumulate heavy metal elements from the roots to the shoots at high rates; and (iv) it has a high potential for biomass production. Plant selection for mercury phytoremediators should consider Hg concentrations in plants, high Hg accumulation in/absorption by plants, plant biomass, non-food crops usage (Handayani et al. 2019), and ease of use dissemination and replication. Ideally, plants used for phytoremediation should be native to the locations polluted by heavy metals (Sunariyati 2018), for they would have adapted to and thus developed the mechanisms to survive in extreme environments. Moreover, using non-native or invasive species can reduce the diversity of local plants (Handayanto et al. 2017). Herbaceous plants in Pancurendang can be used in the phytoremediation of gold processing waste and environment contaminated by mercury because their BACs indicate moderate and high (hyper) accumulators.

Therefore, 54 herbaceous plant species belong to 26 families in gold mining and processing areas in Pancurendang. According to the Shannon-Wiener H' index, these plants have a moderate diversity. *Paspalum conjugatum* from the family Poaceae is the dominant species with the highest importance value in the ecosystem. Mercury (Hg) is found at the highest concentration in root zones with acidic soil (pH 5). Furthermore, *I. aquatica* accumulates and absorbs the most substantial amount of mercury compared to the other species. In addition, herbaceous plants in Pancurendang can be used in the phytoremediation of gold processing waste and environment contaminated by mercury because their biological accumulation coefficients (BACs) indicate moderate and high (hyper) accumulators.

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