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Accumulation of heavy metals in the body and nest of ants in traditional oil mining geosite Wonocolo, Bojonegoro Geopark, East Java, Indonesia

Laily Agustina Rahmawati[1*], Dwi Oktafitria[2], Nova Nevila Rodhi[3], Dyah Setyaningrum[4], Ahmad Ni'amul Abrori[1], Intan Ayu Pebiola[1], Tria Agustining Tyas[1], Santi Amanda Tiara Putri[1], Evi Cici Kumala[1], Abilsa Duanda Ramadhan Firdaus[1]

[1] Department of Environmental Science, Faculty of Science and Engineering, Bojonegoro University, Lettu Suyitno Street No. 2 Bojonegoro, East Java, Indonesia
[2] Department of Biology, Faculty of Mathematics and Natural Sciences, Ronggolawe University, Manunggal Street No. 61 Tuban, East Java, Indonesia
[3] Department of Civil Engineering, Faculty of Science and Engineering, Bojonegoro University, Lettu Suyitno Street No. 2 Bojonegoro, East Java, Indonesia
[4] Department of Chemistry, Faculty of Science and Engineering, Bojonegoro University, Lettu Suyitno Street No. 2 Bojonegoro, East Java, Indonesia
* Corresponding author's e-mail: laily.tiyangalit@gmail.com

ABSTRACT

Traditional oil mining in Wonocolo, Kedewan District, Bojonegoro Regency, East Java Province, Indonesia, causes soil contamination, also by heavy metals. On the basis of bioindicators, 63.3% of soil macrofauna are dominated by ants (Hymenoptera), which have the potential to be heavy metal bioaccumulators. This study aimed to analyze the types and amounts of heavy metal accumulation in the bodies and nests of ants found in Wonocolo. Samples were collected using exploration and hand shorting methods, while heavy metal accumulation was analyzed using XRF. The results showed that 13 types of ants collected accumulated heavy metals in their bodies and nests. Ten types of heavy metals were identified in the bodies of ants, with 3 of them found in all types of ants, namely Cu, Fe, and Zn. Thirteen types of heavy metals were also identified in the nests, with 6 of them found in all nests, namely Al, Fe, Mn, Sr, Ti, and Zn. On the basis of the number of metal type of accumulated, four types of ants have the potential to be bioaccumulators, namely: *Camponotus pennsylvanicus*, *Tetramorium bicarinatum*, *Solenopsis invicta*, and *Cerebara colobobsis*. The detection of various types of heavy metals in the bodies and nests of ants in the Wonocolo traditional oil mining area suggest that the ants in this area can potentially act as heavy metal bioaccumulators that can help the remediation process of soil contaminated by traditional oil mining activities.

Keywords: bioaccumulator; geosite, heavy metal, hymenoptera, XRF.

INTRODUCTION

The traditional oil mining area in Wonocolo Village, Kedewan District, Bojonegoro Regency, East Java has been exploited for more than 120 years (Naumi and Trilaksana, 2015; Rahmawati et al., 2021; Subadi, 2023). Oil mixed with formation water from oil wells, exploited in the traditional way causes spills from oil sprays on the ground around the well (Sartika and Fateah, 2020; Rahmawati et al., 2021; Rahmawati et al., 2024), and the residual produced water is discharged into the river through the ground surface (Rahmawati et al., 2021). Previous studies on soil pollution in Wonocolo due to traditional oil mining activities have shown contamination of the soil around the oil wells with total petroleum hydrocarbon (TPH) (Sari and Trihadiningrum, 2018; Sari et al., 2018;

Rahmawati et al., 2024), and heavy metals such as Pb, Cr, as well as Hg have been detected at concentrations of 0.10; 0.03; and 0.06 mg/g, respectively (Sari et al., 2018). The maximum threshold for Pb, Cr, and Hg in soil based on World Health Organization (WHO) standards is 0.10 m/g (Chen et al., 2014; Duressa and Leta, 2015).

The soil conditions in Wonocolo, based on the structure of the soil macrofauna community, indicate that 63.3% of the soil macrofauna is dominated by the Hymenoptera order, specifically the ant group (Formicidae) (Rahmawati et al., 2024). Hymenoptera are frequently found in the areas contaminated by petroleum refining waste (Muli et al., 2015; Kavehei et al., 2021; Mujianto et al., 2022; Khan et al., 2023). The abundance of this order is related to its ability to utilize various resources to support its survival (Vasconcellos et al., 2013), including food sources or nesting materials. Additionally, Hymenoptera can be used as an indicator of reclamation success (Andersen and Majer, 2004; Vlasakova et al., 2009; Toro et al., 2012) and can function as a heavy metal bioaccumulator (Skaldina et al., 2018; Klimek et al., 2022).

Heavy metals are naturally occurring metal elements that have a relatively high density (Ojovan and Lee, 2005) with an atomic weight five times heavier than water (Tchounwou et al., 2012). They can be released into the environment through industrial, household, agricultural, medical, and technological activities (Ojovan and Lee 2005; Tchounwou et al., 2012), raising concern about their impact on human health and the environment (Ojovan and Lee, 2005; Tchounwou et al., 2012; Liu et al., 2019). The toxicity of heavy metals depends on several factors, including dose, route of exposure, and characteristic of the exposed organism, such as age, gender, genetics, and nutritional status (Tchounwou et al., 2012). Highly toxic heavy metals include arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) (Ojovan and Lee, 2005; Tchounwou et al., 2012), as well as selenium (Se) and zinc (Zn) (Ojovan and Lee, 2005). These elements significantly impact public health because they are considered systemic toxins that can damage organs-even at low exposure levels- and are carcinogenic (Tchounwou et al., 2012).

The abundance of ants in Wonocolo is suspected to be related to the presence of heavy metals in the area (Sari et al., 2018; Sari, 2019). Ants have the ability to accumulate heavy metals in their bodies (Skaldina et al., 2018; Belskaya et al., 2019; Kavehei et al., 2021; Khan et al., 2023) as well as in their nest-building materials (Skaldina et al., 2018; Klimek et al., 2022), with the level of accumulation in the nest often being greater than in the ant bodies. Therefore, the abundant presence of ants in Wonocolo suggest that they could serve as bioaccumulators of heavy metal waste and natural bioremediators of the soil contaminated with heavy metals, making further research essential to confirm this potential.

This study aimed to analyze the type and levels of heavy metal accumulation in the bodies and nest of local ant found in the Wonocolo traditional oil mining area. The ant species with the highest accumulation and concentration of heavy metals is considered to have the potential to act as a bioaccumulator and could, in the future, be developed as a bioremediation agent for the soil contaminated with petroleum waste, especially in Wonocolo. This will support the development of sustainable tourism, as Wonocolo has currently been designated as a geosite, as part of the Bojonegoro Geopark. The use of ants as natural remediators in heavy metal-contaminated soil in Wonocolo will help solve the problem of soil pollution in the area, making it safer for tourism areas and more in line with the direction of geosite development, one of which is conservation.

MATERIALS AND METHODS

Study area

The research location is in the Woncolo Traditional Oil Mining Area (latitude 7° 20 S, longitude 111° 38 E) (Rahmawati et al., 2024), located in Wonocolo Village, Kedewan District, Bojonegoro Regency, East Java, Indonesia. This area covers approximately 50 hectares, with around 700 oil wells, both active and inactive (Rahmawati et al., 2021). The location includes a production forest area and is situated at an altitude of 200 meters above sea level (Rahmawati et al., 2024).

Procedures

The ant and ant nest samples were collected in July 2024 using the exploration method by surveying the area around each oil wells within a radius of less than 10 meters. The selection of exploration areas was based on previous research, where the Wonocolo traditional oil mining area was divided into 50 grids, measuring 100 x 100 m, and grouped into three clusters: rare (R), medium (M), and dense (D), based on oil well density (Rahmawati et al., 2024). Three areas were selected from each cluster, resulting in a total of nine exploration areas.

In this study, ant nests were categorized into two types: external nests and internal nests. External nests are nests that are located above ground level and may consist of earth mounds, sand dunes, trees or wood, litter, or grass. An internal nest is a nest located in a hole below the ground surface with varying depths. Ant nests can be easily identified by observing the presence of ant colonies around them. The sample collection process involves three stages:

a) nest identification – this process begins with measuring physical parameters, including temperature (using a thermometer), pH (using a pH meter), and humidity (using a hygrometer). Afterward, nest identification is conducted by assessing the type (internal or external); nest color; nest composition (soil, sand, wood, litter, etc.); and the size of the nest (height, hole depth, hole diameter) which are measured using wire and calipers.
b) anthill sampling – sampling of ant nests is performed with a spoon, collecting at least 1 gram of sample and storing it in a sample vial.
c) ant sampling – ant sampling is conducted using the hand-sorting method, where ants at the location are collected by hand or tweezers and placed them into a microtube. The ant samples are then stored in a box or refrigerator for preservation. Finally, ants are identified in the laboratory using a stereo microscope.
d) ant identification – ant identification is conducted through morphological characterization using the Borror key determination guidebook (Triplehorn and Johnson, 2004) (Triplehorn and Johnson, 2004) (Triplehorn and Johnson, 2004) (Triplehorn and Johnson, 2004) to minimize identification errors.

After identifying the ant and ant nests samples, the samples were taken to the laboratory for XRF (X-Ray Fluorescence) testing. XRF testing is a non-destructive analytical technique that enables the qualitative and quantitative characterization of solids, liquids, and powders to quickly determine elemental composition, including heavy metals, in a material/sample (Kohli, 2012; Tonazzini et al., 2019). This technique identifies elements by using X-ray excitation on the materials/samples, which in this case are the bodies of ants and their nests. The XRF method in this study is based on the consideration that this technique has a detection limit of up to parts per million (ppm), allows for multi-elemental, and rapid analysis, as well as provides both qualitative and quantitative results (Jamaluddin and Umar, 2018). In this study, only ant samples with an accompanying nest were tested with XRF. The ant samples without nest were not tested.

RESULTS AND DISCUSSION

Result

The study identified 13 types of ants and their nests found in the Wonocolo traditional oil mining area, as shown in Figure 1 and Table 1. The

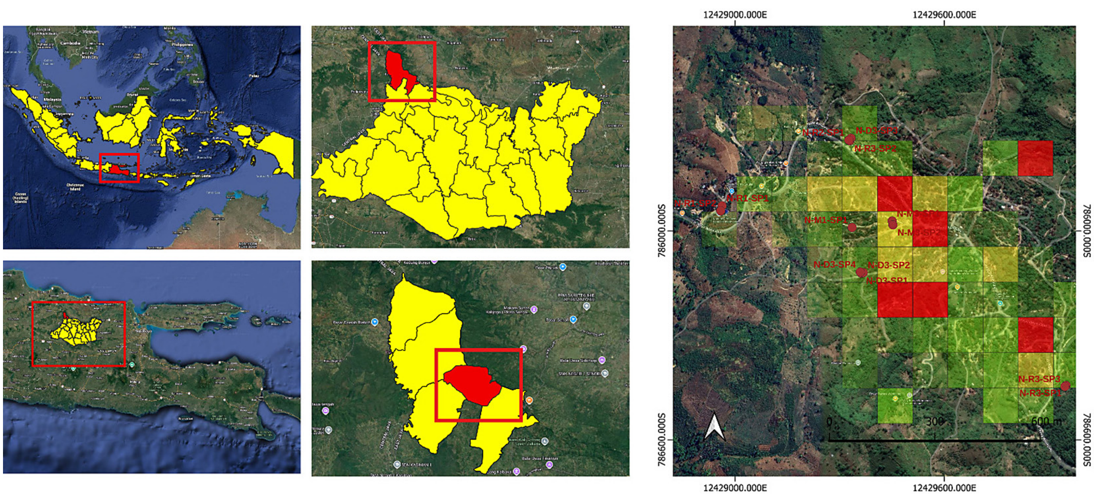

Figure 1. Selected soil macrofauna sampling points in the Wonocolo traditional oil mining area

Table 1. Ant species (Formicidae) in the Wonocolo traditional oil mining area

No.	Roaming area	Types of ants						
		Code	Class	Order	Family	Genus	Species	Sum
1	R1	R1-SP1	Insecta	Hymenoptera	Formicidae	*Solenopsis*	*Solenopsis sp*	44
2	R1	R1-SP2	Insecta	Hymenoptera	Formicidae	*Tetramorium*	*Tetramorium bicarinatum*	15
3	R2	R2-SP1	Insecta	Hymenoptera	Formicidae	*Odontoponera*	*Odontoponera denticulata*	5
4	R3	R3-SP1	Insecta	Hymenoptera	Formicidae	*Cerebara*	*Cerebara colobobsis*	9
5	R3	R3-SP2	Insecta	Hymenoptera	Formicidae	*Censored*	*Diacamma intricatum*	16
6	R3	R3-SP3	Insecta	Hymenoptera	Formicidae	*Solenopsis*	*Solenopsis invicta*	74
7	M1	M1-SP1	Insecta	Hymenoptera	Formicidae	*Myrmecocystus*	*Myrmecocystus sp*	19
8	M3	M3-SP1	Insecta	Hymenoptera	Formicidae	*Nylanderia*	*Nylanderia pubens*	6
9	M3	M3-SP2	Insecta	Hymenoptera	Formicidae	*Odontomachus*	*Odontomachus haematodus*	12
10	D3	D3-SP1	Insecta	Hymenoptera	Formicidae	*Camponatus*	*Camponotus pennsylvanicus*	37
11	D3	D3-SP2	Insecta	Hymenoptera	Formicidae	*Crematogaster*	*Crematogaster claudiae*	23
12	D3	D3-SP3	Insecta	Hymenoptera	Formicidae	*Linepithema*	*Linepithema sp*	47
13	D3	D3-SP4	Insecta	Hymenoptera	Formicidae	*Monomorium*	*Monomorium sp*	13

13 species of ants found in Wonocolo belong to the Order Hymenoptera, Family Formicidae. The ants with the highest number of individuals are *Solenopsis invicta*, with 74 individuals, and *Odontomachus haematodus*, with 5 individuals. The most diverse areas where ants were found were D3, where 4 types of ants were identified, and R3, where 3 types of ants were found. In the M2, D1 and D2 areas, no ants and ant nests were discovered. Before conducting the XRF test, the physical condition of nest was assessed, along with the identification of the type of ant. This included variables such as nest type, nest color, nest composition, size, pH, temperature, and humidity at the sample nest location, as shown in Table 2. Regarding the nest type variable, of the 13 nests found, 10 were internal nests, and the remaining were external nests. Internal nests are defined as burrows in the ground with a certain depth. In this study, the depth of the ant nest varied from 5 to over 15 cm, and the diameter of nest openings also varied from 0.3 to 5.7 cm. For the pH parameter, the ant nest had a pH in the neutral range of 7 to 8, even in acidic or alkaline soil (Jílková et al., 2011). In turn, the nest temperature parameter ranged from 25.06 to 34.01 °C, with the highest temperature recorded in area D3, with the nest code N-D3-SP1. The humidity of the ant nest was generally dry to low, ranging from 1 to 4%. The results of XRF tests on the bodies of 13 species of ants, as shown in Figure 2 and Table 3, identified a total of 10 types of heavy metals: aluminium (Al), barium (Ba), cuprum (Cu), ferrum/iron (Fe), manganese (Mn), nickel (Ni), rhenium (Re), titanium (Ti), zinc (Zn), and zirconium (Zr). Not all species of Wonocolo ants accumulate all types of heavy metals; however, heavy metals such as Cu, Fe, and Zn are found to accumulate in the bodies of all ant species. *Camponotus pennsylvanicus* and *Tetramorium bicarinatum,* are the ant species that accumulate the most diverse heavy metals in their bodies, specifically 8 types, although the percentage of each heavy metal accumulated varies. The heaviest metal accumulated by *Camponotus pennsylvanicus* is Al (17%), the percentage is higher when compared to the accumulation of Al in the body of other ants types, except for *Solenopsis sp.* which accumulates up to 18.5% Al, the highest among the ant species studied. The highest Fe accumulation was found in the bodies *of Odontomachus haematodus* and *Monomorium sp.*, reaching 12%; Cu is highest in the species *Solenopsis invicta*, at 6.85%; The highest Zn was in the species *Odontomachus haematodus* and *Monomorium sp.* at 3.55%; Ni is highest in *Camponotus pennsylvanicus species* at 4.4%; and Re was highest in *Odontomachus haematodus*, *Monomorium sp.* and *Diacama intricatum* at 3%. The highest Mn was *Camponotus pennsylvanicus,* at 2.1%; Ti was highest in *Tetramorium bicarinatum* at 0.8%; and Zr was highest in *Odontomachus haematodus* and *Monomorium*

Table 2. Physical condition of ant nests

No.	Species	Nest code	Nest parameters						
			Type	Color	Compositions	Size	Ph	Temperature (°C)	Humidity (%)
1	*Solenopsis* sp.	N-R1-SP1	Internal	Ash	Soil	Depth: 5 cm Diameter: 0.4 cm	7	29.06	Dry
2	*Tetramorium bicarinatum*	N-R1-SP2	External	White	Rock	Depth: > 15 cm Diameter: 5.7 cm	7	31.05	Dry
3	*Odontoponera denticulata*	N-R2-SP1	Internal	White	Soil	Depth: 6.8 cm Diameter: 1.5 cm	8	32.03	Dry
4	*Cerebara colobobsis*	N-R3-SP1	Internal	Brown	Soil	Depth: < 15 cm Diameter: 0.3 cm	8	25.06	2
5	*Diacamma intricatum*	N-R3-SP2	External	Brown	Soil and wood	T: 15 cm	8	26.02	1
6	*Solenopsis invicta*	N-R3-SP3	External	Brown	Soil and leaves	-	8	27.03	1
7	*Myrmecocystus* sp.	N-M1-SP1	Internal	Brown	Soil	Depth: 5.2 cm Diameter: 2.7 cm	7	26.09	1
8	*Nylanderia pubens*	N-M3-SP1	Internal	Brownish Yellow	Soil	Depth: 7.6 cm Diameter: 2 cm	7	29.06	2
9	*Odontomachus haematodus*	N-M3-SP2	Internal	White	Soil	Depth: 7.1 cm Diameter: 5.5 cm	7	26.08	4
10	*Camponotus pennsylvanicus*	N-D3-SP1	Internal	Brown	Soil	Depth: 14.5 cm Diameter: 0.3 cm	7	34.01	Dry
11	*Crematogaster claudiae*	N-D3-SP2	Internal	Brown	Soil	Depth: 11 cm Diameter: 0.7 cm	7	33.01	1
12	*Linepithema* sp.	N-D3-SP3	Internal	White chocolate	Soil	Depth: 8.9 cm Diameter: 0.5 cm	8	31	Dry
13	*Monomorium* sp.	N-D3-SP4	Internal	White	Sandy soil	Depth: 5.5 cm Diameter: 0.2 cm	7	29.07	1

sp at 0.35%. Ba was only found in the body of *Tetramorium bicarinatum,* amounting to 2%.

The accumulation of heavy metals in ant nests, as shown in Figure 3 and Table 4, identified 13 types of heavy metals, consisting of aluminum (Al), barium (Ba), cuprum (Cu), ferrum/ iron (Fe), manganese (Mn), nickel (Ni), rhenium (Re), titanium (Ti), zinc (Zn), zirconium (Zr), vanadium (V), chromium (Cr), and strontium (Sr), with 6 of them found in all nests: Al, Fe, Mn, Sr, Ti, and Zn. The anthill that accumulates the most diverse types of heavy metals is *Cerebara colobobsis* which accumulates all 13 types of heavy metals, with the highest percentage accumulated being Al (5.95%) and Fe (34%), slightly lower than *Myrmecocystus sp.* which accumulates Al and Fe at 6.4% and 34.75%, respectively. The highest accumulation of Zn, Mn, Cu, Ni and Sr was found in the nests of *Crematogaster claudiae* (5.17%, 0.55%, 1.15%, 0.58% and 2.15%); Ba was highest in *Odontoponera denticulata* nests (0.25%), and Re, V and Cr were highest in *Diacamma intricatum* nests (1%, 0.09% and 0.07%). Zr was highest in *Cerebara colobobsis nests* (0.76%). If the XRF data on ants and ant nests are combined, it shows that the types of heavy metals found in both the bodies and nests of almost all types of ants are Cu, Fe and Zn. The ant species whose bodies and nests accumulate the most diverse types of heavy metals are *Camponotus pennsylvanicus* and *Tetramorium bicarinatum*, with 8 types of heavy metals in the body and 11 types of heavy metals in the nest. The heavy metal with the highest percentage in both the body and the nest is Fe. The heavy metal Al was found in all ant nests in a fairly large percentage, but was not found in the bodies of all types of ants. Similarly, Sr, Cr, and V were only found in the nest, but not found in the bodies. The heavy metals Ti, Zr and Mn were also found in

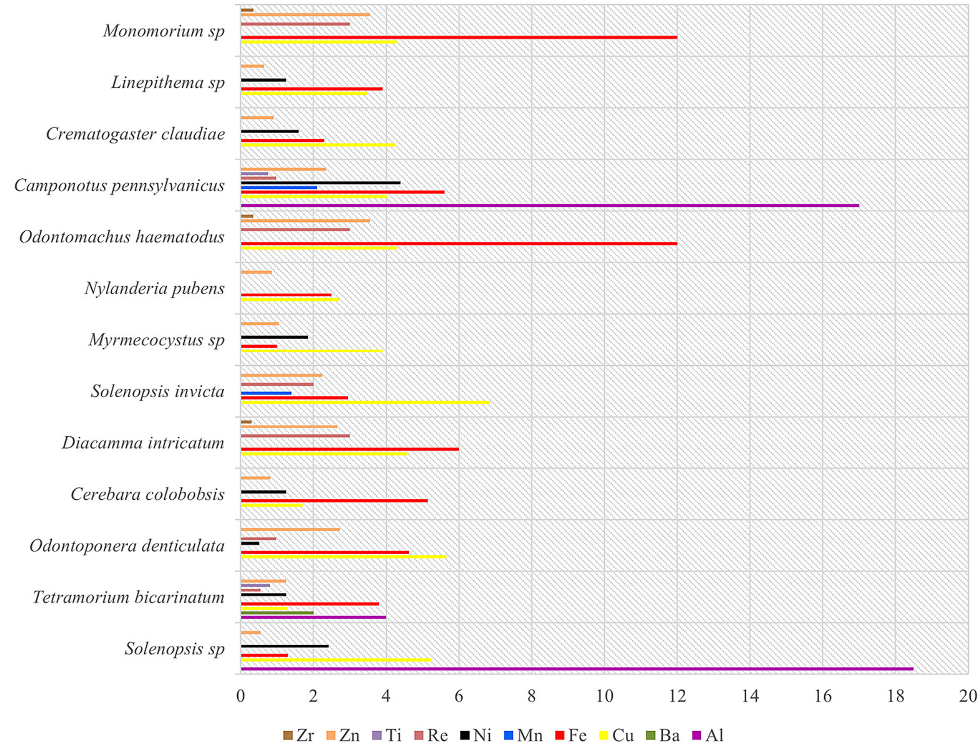

Figure 2. Accumulation of heavy metals in ant bodies in Wonocolo traditional oil mining area

Table 3. Heavy metal content in ant bodies in the Wonocolo traditional oil mining area

Species name	Species code	Compound (%)																									
		Al		Ba		Cu		Fe		Mn		Ni		Re		It		Zn		Zr		V		Cr		Sr	
		Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest	Body	Nest
Solenopsis sp	R1-SP1	18.5	3.1		0.35	5.25	0.28	1.3	18.95		0.42	2.425					2.35	0.55	0.081		0.68		0.045		0.05		0.285
Tetramorium bicarinatum	R1-SP2	4	2.55	2	0.2	1.3	0.19	3.8	12.4		0.35	1.25		0.55	0.06	0.8	1.9	1.25	0.035		0.425		0.045				0.295
Odontoponera denticulata	R2-SP1		1.7		0.25	5.65	0.16	4.625	10.9		0.27	0.515		0.975	0.08		1.05	2.725	0.07		0.13		0.035				0.6
Cerebara colobobsis	R3-SP1		5.95		0.13	1.7	0.3	5.15	34		0.43	1.25	0.11		0.095		2.86	0.82	0.155		0.765		0.085		0.067		0.515
Diacamma intricatum	R3-SP2		6.3		0.2	4.6	0.25	6	33.2		0.37			3	0.1		2.92	2.65	0.115	0.3	0.72		0.09		0.075		0.48
Solenopsis invicta	R3-SP3		5.3		0.1	6.85	0.28	2.95	32.2	1.4	0.39		0.24	2	0.065		2.5	2.25	1		0.67		0.07		0.06		0.39
Myrmecocystus sp	M1-SP1		6.4		0.1	3.9	0.32	1	34.75		0.43	1.85			0.095		2.585	1.05	0.185		0.695		0.095				0.43
Nylanderia pubens	M3-SP1		5.15		0.15	2.7	0.26	2.5	26.9		0.5				0.06		2.885	0.85	0.09		0.59		0.08		0.069		0.635
Odontomachus haematodus	M3-SP2		4.7			4.3	0.27	12	26.3		0.52			3	0.05		2.865	3.55	0.165	0.35	0.645		0.065		0.055		0.525
Camponotus pennsylvanicus	D3-SP1	17	5.3		0.08	4.02	0.25	5.6	23.5	2.1	0.27	4.4		0.975	0.095	0.75	2.305	2.35	0.09		0.325		0.06				0.57
Crematogaster claudiae	D3-SP2		2			4.25	1.15	2.3	5.97		0.55	1.6	0.58				0.45	0.9	5.175								2.15
Linepithema sp	D3-SP3		2.35		0.15	3.5		3.9	17.95		0.37	1.25			0.035		1.3	0.65	0.295		0.19		0.045		0.22		0.675
Monomorium sp	D3-SP4		3.6		0.2	4.3	0.23	12	19.25		0.33			3	0.04		2.1	3.55	0.19	0.35	0.34		0.055				0.52

greater amounts in the nest than in the bodies of the ants. Conversely, Ni was found in greater amount in the bodies of the ant than in the nests. Thus, the results of the XRF test can provide an overview of the percentage of heavy metal accumulation in the bodies and nests of ants, illustrating their potential as heavy metal bioaccumulators.

DISCUSSION

Ants act as bioaccumulators because of their unique lifestyle, such as living in the soil and being omnivores, which allows them to easily accumulate heavy metals from the food chain. This accumulation affects their morphology, behavior,

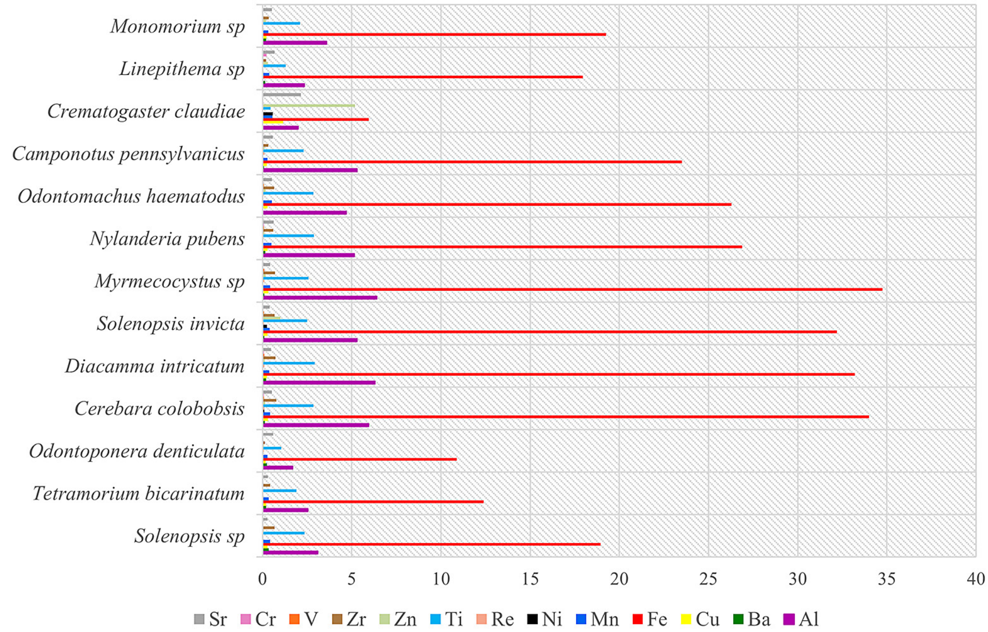

Figure 3. Accumulation of heavy metals in the ant nests in Wonocolo traditional oil mining area

Table 4. Heavy metal content in the ant nest in the Wonocolo traditional oil mining area

Species name	Species code	Compound (%)												
		Al	Ba	Cu	Fe	Mn	Ni	Re	It	Zn	Zr	V	Cr	Sr
Solenopsis sp	R1-SP1	3.1	0.35	0.28	18.95	0.42	-	-	2.35	0.081	0.68	0.045	0.05	0.285
Tetramorium bicarinatum	R1-SP2	2.55	0.2	0.19	12.4	0.35	-	0.06	1.9	0.035	0.425	0.045	-	0.295
Odontoponera denticulata	R2-SP1	1.7	0.25	0.16	10.9	0.27	-	0.08	1.05	0.07	0.13	0.035	-	0.6
Cerebara colobobsis	R3-SP1	5.95	0.13	0.3	34	0.43	0.11	0.095	2.86	0.155	0.765	0.085	0.067	0.515
Diacamma intricatum	R3-SP2	6.3	0.2	0.25	33.2	0.37	-	0.1	2.92	0.115	0.72	0.09	0.075	0.48
Solenopsis invicta	R3-SP3	5.3	0.1	0.28	32.2	0.39	0.24	0.065	2.5	1	0.67	0.07	0.06	0.39
Myrmecocystus *sp*	M1-SP1	6.4	0.1	0.32	34.75	0.43	-	0.095	2.585	0.185	0.695	0.095	-	0.43
Nylanderia pubens	M3-SP1	5.15	0.15	0.26	26.9	0.5	-	0.06	2.885	0.09	0.59	0.08	0.069	0.635
Odontomachus haematodus	M3-SP2	4.7	-	0.27	26.3	0.52	-	0.05	2.865	0.165	0.645	0.065	0.055	0.525
Camponotus pennsylvanicus	D3-SP1	5.3	0.08	0.25	23.5	0.27	-	0.095	2.305	0.09	0.325	0.06	-	0.57
Crematogaster claudiae	D3-SP2	2	-	1.15	5.97	0.55	0.58	-	0.45	5.175	-	-	-	2.15
Linepithema sp	D3-SP3	2.35	0.15	-	17.95	0.37	-	0.035	1.3	0.295	0.19	0.045	0.22	0.675
Monomorium sp	D3-SP4	3.6	0.2	0.23	19.25	0.33	-	0.04	2.1	0.19	0.34	0.055		0.52

immune defense, and community structure (Khan et al., 2023; Xiao et al., 2024). The accumulation of heavy metals in the bodies of ants is mostly in the stomach, particularly in the epithelium of the midgut (Zhang et al., 2024), although accumulation is also found in the labial (salivary) glands (Zhang et al., 2024). This study shows that all types of ants found in Wonocolo accumulate at least three types of heavy metals in their bodies, namely Cu, Fe and Zn. The heavy metals accumulated by ants in Wonocolo are most likely sourced from traditional oil exploitation that takes place in the area. However, based on previous research, the types of heavy metals detected in Wonocolo soil are Pb, Cr and Hg (Sari et al., 2018). When compared to the results of the current study, there is only one type of heavy metal that overlaps with those found in the soil, namely Cr. This study identified more types of heavy metals that accumulated both in the body and nests of ants with a total of 13 types of heavy metals. Thus, there is a possibility that these thirteen types of heavy metals are also present in Wonocolo soil.

The heavy metals found in the bodies of all types of ants in this study were Cu, Fe and Zn. Cu is considered an olfactory toxin, because it can cause dysfunction and damage chemosensory perception (Xiao et al., 2024), which is a chemical signal in the environment that is important for coordinating various social behaviours, such as foraging, recognizing conspecifics, nesting preferences, and also communication. In addition, Cu is also considered the metal that has the most significant impact on the decline in the physical condition of ants (Singh et al., 2022; Zhang et al., 2024), including dry weight, residual mass (Zhang et al., 2024), and body size of ants (Klimek et al., 2022). Zn is included in the first hazard class (Ojovan and Lee, 2005) and is a type of heavy metal that is often detected in the bodies of insects living in metal industrial areas (de Santana et al., 2023). Zinc (Zn) is an important micronutrient in the soil, which is released into the soil due to the anthropogenic processes (Lopes et al., 2021; Wang et al., 2022). The accumulation of Zn in the soil affects soil quality, because it can limit soil nutrient transport and trigger toxic reactions in the reproduction, development, and behavior of various soil organisms (Wang et al., 2022). It has the potential to become an inorganic toxic pollutant when its concentration in the soil environment is too high (You et al., 2020; Zhang et al., 2020; Wang et al., 2022). If Zn enters the human body through the food chain and reaches a certain level, it can affect oxidative stress, which triggers chronic diseases (Huang et al., 2020; Wang et al., 2022).

Fe is a type of heavy metal found with the highest composition both in the bodies and nests of ants. Iron (Fe) pollution, is an environmental problem that generally occurs in abandoned mining areas, threatening human health and the ecosystem (Zhou et al., 2024). Sources of Fe pollution come from physicochemical and biological corrosion of metal equipment, such as anchors in coal mines, as well as natural weathering of sulfidic minerals containing Fe in rock layers (Zhou et al., 2024). In the traditional Wonocolo oil mine, metal equipment that is a source of Fe pollution is likely to come from iron poles supporting wells, steel ropes to pull oil "dippers", and oil "dippers" made of iron pipes.

The heavy metals accumulated in ant nests are more diverse than in their bodies. In addition to Fe and Zn, four other types of heavy metals were also found in all ant nests: Al, Mn, Sr, and Ti. The detection of heavy metals in ant nests can provide information about the potential accumulation of heavy metals of the same type in Wonocolo soil. Ants are commonly found in metal-polluted areas and are relatively resistant to metal pollution compared to other epigeic fauna groups due to their ability to regulate their internal metal content. However, metal pollution can reduce ant colony size and survival (Grześ et al., 2019; Klimek et al., 2022). Previous studies have suggested that ants tend to prefer building their nests in locations with lower heavy metal concentrations (Khan et al., 2024). Many studies support the idea that ants are able to help reduce heavy metals by accumulating them in the soil of their nests and then removing them when they die (Shi et al., 2023). Additionally, the reduction of heavy metal content by ants also occurs through the bioturbation mechanism (Jílková et al., 2011; Khan and Al-Khedhairy, 2017; Skaldina and Sorvari, 2019; Khan et al., 2024), which is the biological weathering mechanism of soil and sediment. This results in enzymatic activity and increased soil porosity, especially in nests (Khan et al., 2024). The study found the types of ants that have the potential to accumulate heavy metals. When viewed in terms of the diversity of types of heavy metals accumulated:

a) *Camponotus pennsylvanicus*, is able to accumulate the most diverse types of heavy metals, namely 8 types of heavy metals in the body (Al, Cu, Fe, Mn, Ni, Re, Ti, Zn) and 11 in the nest (Al, Ba, Cu, Fe, Mn, Re, Ti, Zn, Zr, V, Sr). This type of ant is also able to accumulate heavy metals with the highest percentage in the body for Al (17%), Ni (4.4%), Mn (2.1%). Previous research on the genus *Camponotus sp*. showed the same results that there was also an accumulation of heavy metals in the bodies of ants, although the types of heavy metals accumulated were not exactly the same (Chandran et al., 2018; Khan et al., 2024; Zhang et al., 2024). The accumulation has a significant impact on the physiological and morphological conditions of ants (Zhang et al., 2024), but it also indicates the potential of ants as bioremidiators, because they can change the chemical properties of the soil due to ant activity (Chandran et al., 2018).

b) *Tetramorium bicarinatum*, is able to accumulate the most diverse types of heavy metals, namely 8 types of heavy metals in the body (Al, Ba, Cu, Fe, Ni, Re, Ti, Zn) and 11 in the nest (Al, Ba, Cu, Fe, Mn, Re, Ti, Zn, Zr, V, Sr). This type of ant is also able to accumulate heavy metals with the highest percentage in the body for Ti (0.8%). In addition, this type of ant is also the only species that accumulates heavy metals of the Ba type in its body (2%). In the same genus, the *Tetramorium sp.* also accumulate heavy metals in their bodies and nests, with the content of heavy metals in the nest being higher than in the surrounding soil (Jílková et al., 2011).

c) *Solenopsis invicta*, accumulates 5 types of heavy metals in its body (Cu, Fe, Mn, Re, Zn), with the highest percentage for Cu (6.85%). In addition, this ant also accumulates 13 types of heavy metals in its nest (Al, Ba, Cu, Fe, Mn, Ni, Re, Ti, Zn, Zr, V, Cr, and Sr). In previous studies, *Solenopsis invicta* was also able to accumulate heavy metals in its body, but the type of metal accumulated was Cd. The presence of Cd metal interferes with olfactory signal transduction and social behavior driven by smell (Yang et al., 2021; Xiao et al., 2024). Accumulation of Cr, Pb, Cu, and Ni, was also detected in *Solenopsis invicta* nests. This ant is able to survive in the soil contaminated with heavy metals by modifying the physicochemical properties of the nest soil, working with the bacterial community to create a suitable habitat for survival and reproduction (Shi et al., 2023).

d) *Cerebara colobobsis*, in addition to accumulating 4 types of heavy metals in its body (Cu, Fe, Ni, and Zn), this type of ant is apparently able to accumulate 13 types of heavy metals in its nest (Al, Ba, Cu, Fe, Mn, Ni, Re, Ti, Zn, Zr, V, Cr, and Sr) with the highest percentage being Zr (0.76%) and Re (0.095%). There has been no previous research that has considered this type of ant in relation to its ability to accumulate heavy metals. However, this study shows that this type has great potential as a bioaccumulator of heavy metals in its nest.

When viewed based on the high percentage of heavy metals that can be accumulated, the following ants species show potential: *Myrmecocystus sp.* for Fe (34.75%) and V (0.095%) in their nests; *Crematogaster claudiae* for Zn (5.17%) and Sr (2.15%) in their nests, but previous studies have shown that *Crematogaster sp*. are less suitable as bioindicators because this type of ant accumulates lower concentrations of metals in mining areas (Kavehei et al., 2021); *Diacamma intricatum* for Re (3%) in its body, and Ti (2.92%) and Cr (0.075%) in its nest; *Cerebara colobobsi* for Zr (0.76%) in its nest; *Solenopsis invicta* for Cu (6.85%) in its body, but based on previous studies, this type of ant is often associated with Cd (Xiao et al., 2024; Yang et al., 2021); *Solenopsis sp* for Al (18.5%) in its body; *Tetramorium bicarinatum* for Ba (2%) in its body; and *Camponotus pennsylvanicus* for Al (17%), Mn (2.1%) and Ni (4.4%) in its body.

The ant species *Camponotus pennsylvanicus* and *Solenopsis* sp. accumulate Al metal at the highest percentages in their bodies, reaching 17% and 18.6%, respectively. This percentage of Al metal is the highest compared to other heavy metals that accumulate in ants. Previous studies support this finding, revealing that Al is the most abundant heavy metal in ants of the genus *Camponotus* (Silva et al., 2006). *Camponotus* ants primarily feed on nectar and honeydew secreted by aphids or other insects that consume plant sap in a mutualistic relationship known as trophobiosis (Delabie, 2001; Eeva et al., 2004). Honeydew contains a high concentration of metals derived from plant sap, which in turn absorbs metals from the surrounding environment (Eeva et al., 2004). In addition, Fe is the heavy metal with the highest percentage in all ant nests. These findings align with previous research. There are two possible explanations for the high Fe concentration in ant nests. First, it may be related to ant foraging behavior, where ants collect food from distant locations and discard unnecessary elements, such as Fe, in their nests after consumption. Second, Fe in ant nests may originate from deeper soil layers, accumulating as ants mix the soil while using their mouthparts (Roivainen et al., 2022).

CONCLUSIONS

The conclusion of this study is that of the 13 ant species in Wonocolo that were tested, all of them accumulated heavy metals in their bodies and nests. A total of 10 types of heavy metals were identified in the bodies of ants, with 3 types of heavy metals found in all species, namely Cu, Fe, and Zn. Additionally, a total of 13 types of heavy metals were identified in the ant nests, with 6 types of heavy metals found in all ant nests: Al, Fe, Mn, Sr, Ti, and Zn. On the basis of the number of metal types accumulated, four types of ants have the potential to be bioaccumulators: *Camponotus pennsylvanicus, Tetramorium bicarinatum, Solenopsis invicta,* and *Cerebara colobobsis*.

The detection of various types of heavy metals in the bodies and nests of ants in the Wonocolo traditional oil mining area suggest that the ants in this area can potentially act as heavy metal bioaccumulators, which can help the remediation process of soil contaminated by traditional oil mining activities. Furthermore, the discovery of 13 types of heavy metals in the ant nest also indicates that the soil in Wonocolo is also contaminated by the thirteen types of heavy metals, which has not been revealed by previous research. Therefore, further studies are needed to confirm these findings.

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