

# Diversity of soil macrofauna in traditional oil mining of Wonocolo Geosite, Bojonegoro Geopark, East Java, Indonesia

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**Abstract.** Rahmawati LA, Afiati N, Putranto TT. 2024. Diversity of soil macrofauna in traditional oil mining of Wonocolo Geosite, Bojonegoro Geopark, East Java, Indonesia. *Biodiversitas* 25: 2148-2160. Wonocolo traditional oil mining has been going on for 130 years and has led to soil pollution. This study aims to characterize the composition of macrofauna in the soil affected by Wonocolo's traditional oil mining. The existence of macrofauna is important as a bioindicator of soil quality and its role in maintaining soil fertility levels. Mining areas are grouped based on the density of the oil well (rare, medium and dense), then three sample points are selected in each group. Macrofauna were collected using quadrat-hand sorting, pitfall trap, and flannel trap methods, and then the community structure index was analyzed. The results showed that 709 individuals were collected consisting of 17 orders and 71 species, with 64.8% of individuals found in category-dense areas. Hymenoptera and *Polyrhachis* sp. as the most abundant order and genus, while *Odontomachus brunneus* Patton 1894 is the most frequently encountered species. The average value of biodiversity, dominance, and evenness index ranged  $H'=1.28-1.57$  (low),  $C=0.29-0.38$  (low), and  $E=0.78-0.90$  (high), respectively. Indications of environmental conditions in traditional oil mining areas cannot be seen solely from the value of the macrofauna biodiversity index but are apparent in the abundance of the Hymenoptera order in all areas categories and significant presence of *Polyrhachis* sp. species in the dense category.

**Keywords:** Bioindicators, Hymenoptera, polluted soil, *Polyrhachis*, Shannon-Wiener

## INTRODUCTION

Wonocolo Geosite is part of the Bojonegoro Geopark, covering an area of 140,002 m<sup>2</sup>, housing traditional oil mining activities. Currently, there are 493 oil wells scattered throughout the area (Rahmawati et al. 2021), with 227 of them being remnants from the Dutch colonial government era. The Wonocolo traditional oil mine has been in operation since 1883 (Subadi 2023), making it nearly 130 years old. Traditional oil mining is a petroleum extractive industry sector that is managed by local communities and still uses simple methods and equipment (Belvage 2016; Rahmawati et al. 2021). Oil mixed with water and sludge is extracted using an iron pipe (Timbel), which is pulled by a steel wire from the oil well (Sartika and Fateah 2020; Rahmawati et al. 2021), and then the oil is sprayed out of the pipe, some of it flows into a separator pond and some fall and pollute the soil (Rahmawati et al. 2021).

Based on the previous studies, it is evident that the soil around the oil well contains C14-36 from Total Petroleum Hydrocarbons (TPH) at a rate of 8.33%, while the soil in the refining area and transportation lines contains 12.30% and 4.35%, respectively (Sari and Trihadiningrum 2018). These values surpass the threshold set by Minister of Environment Regulation No. 128 of 2003, which is 1%, indicating high TPH pollution in the land. Total Petroleum Hydrocarbons accumulate continually in traditional oil

mining areas operating daily with a volume of 200 L per day. As per Law No. 101 (2014), soil pollution is classified as TK-A (highly polluted) since it contains more than 4% C14-36%.

Furthermore, Sari et al. (2018) revealed the detection of heavy metals such as Pb, Cr, and Hg in the soil around traditional oil mine wells, with successive concentrations of 0.10, 0.03, and 0.06 mg/g. In a general context, the assessment of soil pollution by heavy metals adheres to the standards of the World Health Organization (WHO) / Food and Agricultural Organization (FAO) (Chen et al. 2014; Sagagi et al. 2022) with the maximum permissible limit for heavy metals Pb, Cr, and Hg set at 0.10 mg/g. According to these standards, the concentration of Pb has approached the maximum threshold of the WHO/FAO standard.

Oil spills persist in the soil for decades and are toxic to soil organisms, both macrofauna and soil mesofauna (Reinecke et al. 2016; García-Segura et al. 2018). The presence, absence, abundance, density, and diversity of soil organisms have been used as indicators of environmental impacts resulting from natural or anthropogenic activities (Ardestani and Gestel 2014; Austruy et al. 2016; García-Segura et al. 2018); they also serve as bioindicators to determine disturbance or contamination (Widhiono et al. 2017; García-Segura et al. 2018; Skaldina et al. 2018; Machado et al. 2018; Majeed et al. 2021), and can even provide clues to identify organisms with the potential to decompose waste oil (García-Segura et al. 2018).

Soil macrofauna plays crucial roles in the ecosystem, including the decomposition of organic matter (Wills and Landis 2018; Hani and Suhaendah 2019), mineralization processes, soil turnover and binding with organic matter, protection against pests (Machado et al. 2018), pollination, seed dispersal, herbivores and predators (Wills and Landis 2018). Soil macrofauna are organisms visible to the naked eye with a size of  $\geq 2.0$  mm (Machado et al. 2018; Gongalsky 2021). There are more than 20 taxonomic groups of soil macrofauna, with ants, earthworms, termites, beetles, spiders, centipedes, millipedes, crickets, cockroaches, snails, scorpions, insect and others being among the most commonly found (Machado et al. 2018). However, the existence of soil macrofauna in Wonocolo is questionable, given the soil's high pollution from petroleum waste.

Based on the description above, the main objective of this study is to determine the diversity and composition of soil macrofauna orders and types based on the density level of Wonocolo traditional oil wells. Additionally, the study aims to analyze the structure of soil macrofauna communities, including biodiversity, dominance, and species uniformity index. This research is crucial to provide an overview of the condition of soil macrofauna in the Wonocolo Geosite Area influenced by traditional oil mining. Ecologically, macrofauna plays a vital role in maintaining soil fertility levels. Moreover, considering Wonocolo's current status as one of the Geosites in the Bojonegoro Geopark, the preservation of biodiversity is essential, in addition to geological heritage (Geoheritage) and cultural diversity (Cultural Diversity), as stipulated in Presidential Regulation Number 9 of 2019. The diversity of soil macrofauna species in this location has never been studied before, even though it is essential as a basis for environmental management, especially regarding soil aspects, to support the sustainable development of the Wonocolo Geosite Tourism Area.

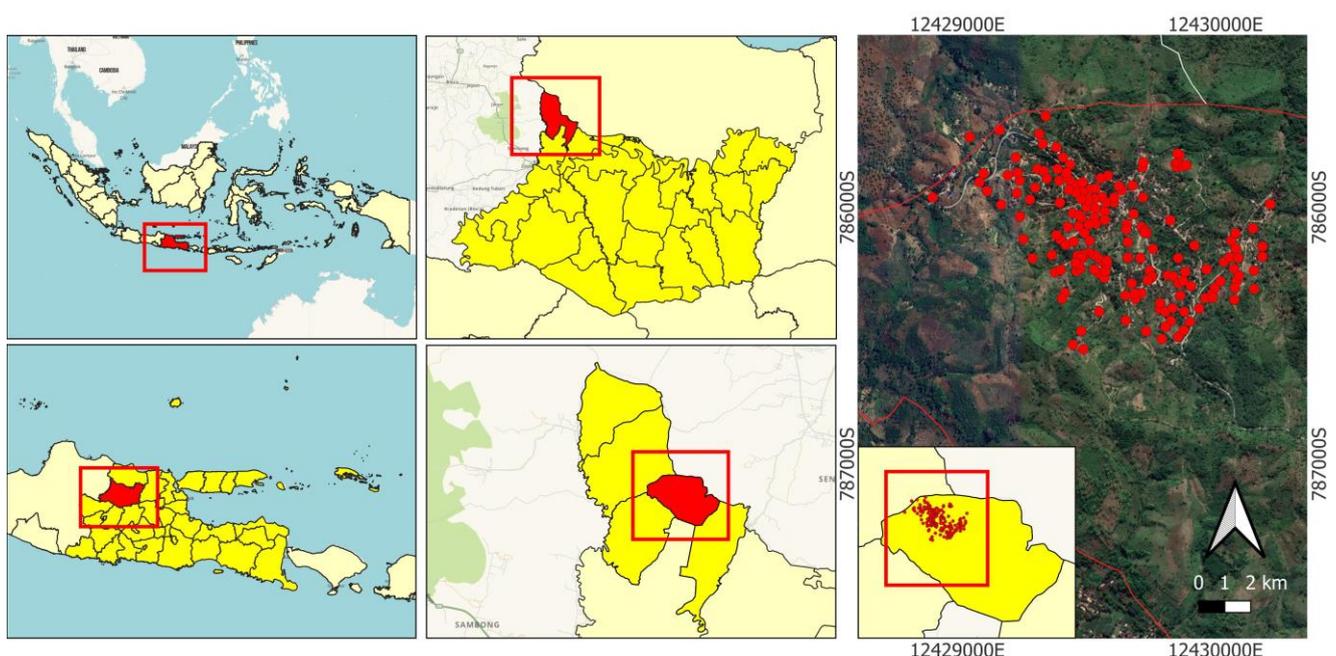
## MATERIALS AND METHODS

### Study area

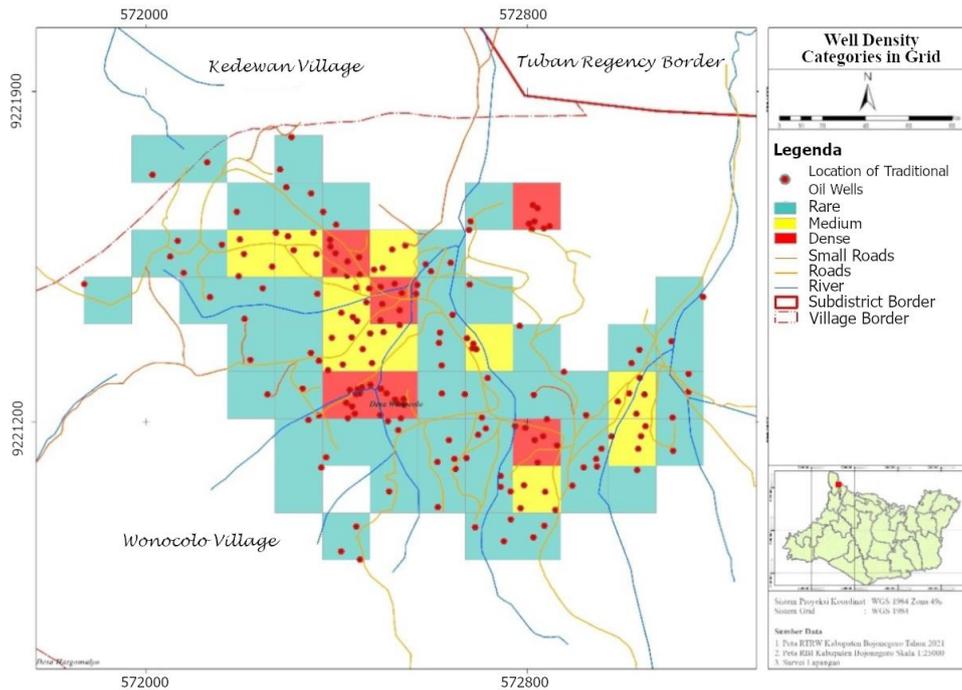
The research was conducted in the Wonocolo Traditional Oil Mining Area, Kedewan Sub-district, Bojonegoro District, East Java, Indonesia (Figure 1). This area is part of the Kendeng Mountain range (latitude  $7^{\circ} 20' S$ , longitude  $111^{\circ} 38' E$ ), with an average altitude of over 200 meters above sea level. Most of the area is covered by forests managed by the Cepu Forest Management Unit, one of the units under the State Forest General Company (Perum Perhutani) of Central Java, and some areas consist of settlements. The traditional oil wells are located within a forested area, spreading across 50 hectares. To facilitate the collection of research data, the distribution area of traditional oil wells is divided into plots (grid cells) and then grouped based on the density of oil well distribution per hectare.

### Procedures

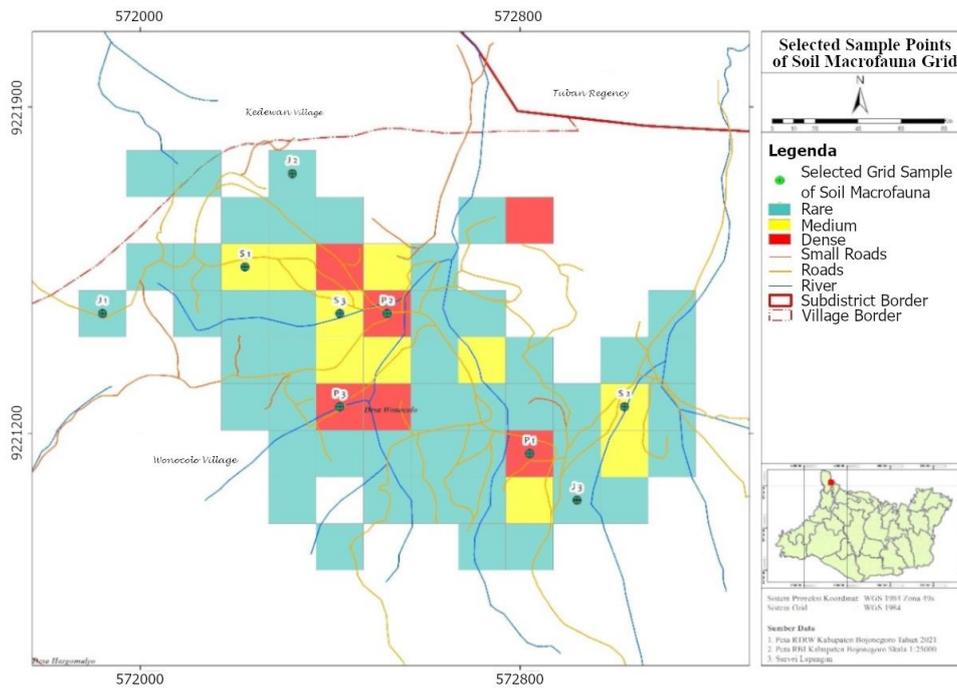
This study began with the determination of soil sampling points to gather data regarding the presence of soil macrofauna in the Wonocolo Traditional Oil Mining Area. The determination of soil sampling points utilized stratified random sampling. The Wonocolo Traditional Oil Mining Area was divided into plots (grid cells) using Geographic Information System (GIS) assistance, with a size of  $100 \times 100$  m. The results were displayed in the form of a well distribution map presented in plots (Figure 2). The plots formed were grouped into three clusters categorized based on the density of oil wells, as shown in Figure 3: rare (R) (1-3 wells/ha) is colored blue, medium (M) (4-5 wells/ha) is colored yellow, and dense (D) (6-8 wells/ha) is colored red. At each category area, three samples were taken, resulting in a total of nine sample points.



**Figure 1.** Research location Wonocolo Village, Kadewan Sub-district, Bojonegoro District, East Java, Indonesia



**Figure 2.** Wonocolo Traditional Oil Mining Area, Bojonegoro, Indonesia grid for determination of soil macrofauna sampling points



**Figure 3.** Sampling points of macrofauna in Wonocolo Traditional Oil Mining Area, Bojonegoro, Indonesia

After obtaining the soil sampling points, soil temperature and pH are measured using a thermometer, and the pH meter is inserted into the ground until the value displayed on the tool is stable. Then, soil macrofauna sampling is conducted using three methods to obtain soil macrofauna characteristics based on their specific habitat. The hand sorting method is employed to identify macrofauna that live in the soil, the pitfall trap method

captures macrofauna slithering on the ground surface, and the flannel trap method captures soil macrofauna flying over the ground surface and clinging to the grass. At each point sampling location, there are three types of traps for three sampling methods, with one repetition for each method. Sampling was carried out in July-August, coinciding with the dry season.

**Quadrat & hand sorting**

The quadratic method is used in sampling soil macrofauna that is active in the soil (Jhon et al. 2019). Soil samples were collected at each sampling point using a square monolith tool measuring 30×30 cm, reaching a depth of 30 cm. The collected soil was placed in plastic containers. Sampling was conducted between 06:00 and 09:00 am. Subsequently, soil macrofauna found in the soil was carefully extracted using the hand sorting method. The obtained soil macrofauna was then collected, cleaned with water, and placed into sample bottles according to the plot. They were preserved with 70% alcohol. Furthermore, soil macrofauna samples collected from the quadrat and hand sorting methods were taken to the laboratory for identification and analysis.

**Pitfall trap**

The pitfall trap method is used for sampling soil macrofauna that is active on the ground surface (Mhlanga et al. 2022). At each predetermined sampling point, a plastic cup with a diameter of 10 cm was placed parallel to the ground surface. Furthermore, the top of the plastic cup was covered with a plastic plate, 20 cm in diameter, with a height of 10 cm from ground level to prevent the ingress of rainwater and sunlight. The cup was filled with a 70% alcohol solution, approximately 100 ml, as a killing and preserving agent. It is mixed with a small amount of detergent solution to eliminate the surface tension of the alcohol. The pitfall trap glass is installed at 08:00 am and observed every day for three consecutive days. The soil macrofauna trapped is collected and placed into a sample bottle according to its plot, then preserved with 70% alcohol.

**Flannel trap**

The flannel trap method was developed by researchers to capture macrofauna flying above the ground surface and perching on grass. This method involves using a flannel measuring 90×100 cm, with both ends weighted along the wide side of the flannel (90 cm) for ballast when pulled. Subsequently, a rope is attached to both ends on one side of the flannel to facilitate pulling. Once prepared, the flannel is pulled, with one end sweeping across the ground surface to trap soil macrofauna flying or adhering to the grass, effectively trapping and attaching them to the flannel. The macrofauna attached to the flannel is then collected and placed into a bottle filled with 70% alcohol. Finally, the samples are taken to the laboratory for identification and analysis.

**Data analysis**

The soil macrofauna obtained were further identified and counted in the laboratory. After the macrofauna was identified, a Chi-Square test was carried out using a contingency table to determine the proportion of each order at all sample stations in each area category. Furthermore, soil quality determination was analyzed based on the community structure of soil macrofauna, including the Shannon-Wiener biodiversity index, species evenness index, and dominance index (Vasconcelos et al. 2020; Banifatemeh et al. 2023). The Shannon-Wiener biodiversity index of soil macrofauna is calculated using the formula:

$$H' = - \sum_{i=1}^s (P_i)(\ln P_i)$$

Where:

P<sub>i</sub> :  $\sum ni/N$

H' : Shannon-Wiener Diversity Index

P<sub>i</sub> : Number of individuals of a species/total number of all species

n<sub>i</sub> : Number of individuals of the i-th species

N : Total number of individuals

The species evenness index is calculated using the formula:

$$E = \frac{H'}{\ln S}$$

Where:

E : Uniformity index

H' : Diversity index

n<sub>i</sub> : Number of species

The species dominance index uses the Simpson dominance index calculated using the formula:

$$C = \sum \left(\frac{n_i}{N}\right)^2$$

Where:

C : Simpson Dominance Index

N : Number of individuals of the entire species

n<sub>i</sub> : Number of individuals of the i-th species

If the value of biodiversity index, uniformity index, and dominance index has been obtained, then Wonocolo soil quality will be determined based on community structure value criteria as Table 1.

**Table 1.** Macrofauna community structure value criteria

Index	Range	Category	Source
Biodiversity (H')	H' ≤ 2	Low diversity	Ulfah et al. (2019)
	2 < H' ≤ 3	Moderate diversity	
	H' ≥ 3	High diversity	
Evenness (E)	0.00 < E ≤ 0.50	Depressed community	Ulfah et al. (2019)
	0.50 < E ≤ 0.75	Unstable community	
	0.75 < E ≤ 1.00	Stable community	
Dominance (C)	0.00 < C ≤ 0.50	Low	Ulfah et al. (2019)
	0.50 < C ≤ 0.75	Moderate	
	0.75 < C ≤ 1.00	High	

## RESULTS AND DISCUSSION

### Environmental parameters of soil in Wonocolo Traditional Oil Mining Area

Soil macrofauna sampling was conducted in three categories of areas with different well density levels. In each category, three locations were selected for sampling, and the coordinates, temperature, and soil pH are detailed in Table 2. The soil temperature at each sampling site ranged from 24.6 to 26.7°C. The average temperature in each well density category was 25.4 to 26°C, indicating that it falls within the optimal temperature range for soil macrofauna life (18-30 °C) (Wibowo and Alby 2020). The variation in soil temperature at the sampling sites is closely related to the amount of vegetation cover present. Temperature is a crucial factor influencing the life of soil macrofauna and significantly affects the diversity of soil fauna types. Moreover, the speed of the decomposition process of soil organic matter is also determined by soil temperature (Wibowo and Alby 2020).

In addition to temperature, the soil's acidity level (pH) at the sampling site was also measured. The soil's acidity level (pH) has a significant impact on soil biogeochemical processes, influencing the biological, chemical, and physical properties of the soil (Neina 2019). The pH level also affects the distribution and density of soil macrofauna (Wibowo and Alby 2020). At the study location, the maximum to minimum pH range was 6.5-8.5, which does not align with the preferred pH range for most soil macrofauna, typically between 6-7. In the dense category area, the soil pH reached 8.5, entering the alkaline category. Soil in alkaline conditions usually contains fewer nutrients and microorganisms. However, some studies suggest that the optimal process of petroleum degradation occurs at pH levels of 7-9 (Neina 2019).

### Diversity of soil macrofauna types in Wonocolo Traditional Oil Mining Area

The results showed differences in the composition of macrofauna species and numbers found in the Wonocolo Traditional Oil Mining Area. These differences are related to the density level of oil wells and variations in macrofauna sampling methods. Based on the density level of traditional oil wells, the number of specimens found in the sparse rare category was 133 individuals, in the

medium category was 115 individuals, and in the dense category was 458 individuals. Thus, the total number of soil macrofauna specimens collected in this study was 706 individuals.

In rare category, as shown in Table 3, the abundance of soil macrofauna is 133 individuals, representing 11 orders and 37 species. The order with the highest abundance is Hymenoptera, with 63 individuals, while the order with the largest variety of species is Orthoptera, with 10 species. The species with the highest number of individuals are *Anoplolepis gracilipes* Smith 1857, with 40 individuals, and *Odontomachus brunneus* Patton 1894 with 16 individuals, both from the Order Hymenoptera. In addition to these two orders, there are also orders Araneae, Blattodea, Coleoptera, Dictyoptera, Diptera, Hemiptera, Lepidoptera, Scolopendromorpha, and Phthiraptera/Anoplura.

In the medium category area, the abundance of soil macrofauna amounted to 115 individuals, consisting of 13 orders and 36 species (Table 3). The order with the highest abundance is Hymenoptera, which has 50 individuals, while the order with the largest variety of species is Hymenoptera and Orthoptera, which each has seven species. The most abundant species are *O. brunneus*, which is 31 individuals from the order Hymenoptera, and *Lumbricus rubellus* Hoffmeister 1843, which is 22 individuals from the order Haplotaxida. In the medium category area, the order Arachnoidea, Haplotaxida, and Phasmatodea were found, which were not found in the rare category area, but the order Phthiraptera/Anoplura was found in the rare category area but was not found in the medium category area.

In the dense category area, the total abundance of soil macrofauna is 458 individuals, comprising 10 orders and 36 species, as shown in Table 3. The order with the highest abundance is Hymenoptera, with 338 individuals, while the order with the most variety of species is also Hymenoptera, consisting of seven species. The most dominant species in a dense category is *Polyrhachis* sp., with 285 individuals from the order Hymenoptera. Other species found in significant numbers are *Amplipcephalus* sp. and *Nilaparvata lugens* Stål 1854, with 26 and 23 individuals, respectively. When compared to rare and medium category areas, the three orders are exclusively found in dense category areas, namely Isopoda, Mantodea, and Uropygi.

**Table 2.** Coordinates, temperature and pH of soil macrofauna sampling site

Point number	X	Y	Temperature (°C)	Average temperature (°C)	pH	Average pH
R1	111,6514	-7,04229	25.3	25.6	7	6.3
R2	111,6543	-7,04036	25.9		7.5	
R3	111,6601	-7,04696	25.6		6.5	
M1	111,6542	-7,04268	25.7	26	7	7.5
M2	111,6612	-7,04477	26.7		7.5	
M3	111,6556	-7,0428	25.6		7.9	
D1	111,6595	-7,04509	25	25.4	7	7.6
D2	111,6562	-7,04286	26.6		8.5	
D3	111,6554	-7,04508	24.6°		7.5	







Based on the results of the chi-square test (Table 4) with the following hypothesis: (i)  $H_0$ : the proportion of each order at all sample stations is the same; (ii)  $H_a$ : the proportion of each order at all sample stations is not the same or different shows that the calculated Chi-square value of 293.52 is greater than the chi-square table of 46.194, so  $H_0$  is rejected. This means that the proportion of each order at all sample stations is not same or different.

**Soil quality based on soil macrofauna community structure**

Based on Table 5, the overall diversity of soil macrofauna in the three categories rare, medium, and dense has an average value of  $H'=1.28-1.57$ . In other words, the value is  $H'<2$ . This indicates that the biodiversity level is low across all Wonocolo traditional oil mining areas, suggesting very low ecosystem productivity. This condition signifies the significant pressure on the ecosystem, leading to an unstable state. Although the average biodiversity value in all well density categories falls into the low range, there is a noticeable decrease along with the level of oil well density, with the highest value in rare category ( $H'=1.57$ ), followed by medium category ( $H'=1.32$ ), and the lowest in dense category ( $H'=1.28$ ), as presented in Table 5.

Figure 4 illustrates the diversity values at each sampling station, with the highest diversity observed at station M1 using the FT method ( $H'=2.8$ ). Additionally, stations R1, R2, and D3 show  $H'>2$  values, also obtained through the FT method. Values of  $H'=2-3$  fall into the medium category, indicating sufficient productivity, a relatively

balanced ecosystem, and moderate ecological pressure. The lowest diversity value is at station M2 with the PT method ( $H'=0$ ). Several other stations, namely R2 and R3 with the HS method, M2 with the FT method, and D3 with HS, and D2 with PT, also exhibit  $H'<1$  values.

Comparing soil macrofauna diversity based on the method used, the average diversity values obtained with the HS and PT methods are smaller compared to the FT method. The choice of different methods is related to the objective of sampling different macrofauna populations: HS is used for macrofauna living in the soil, PT is used for macrofauna slithering on the soil surface, while FT is used for macrofauna flying or sticking to grasses above ground level. Considering this purpose, it is evident that the well density level has a more significant influence on the diversity of macrofauna types that fly or stick to grass above the ground surface. Areas in rare category have the highest average value of macrofauna diversity flying or sticking to grasses, namely  $H'=2.11$ , categorizing as moderate diversity. This is followed by medium and dense categories with diversity values of  $H'=1.77$  and  $H'=1.48$ , respectively, falling into the low diversity category.

**Table 4.** Chi-square test results for each order at all sample station

Data	X-squared	Df	p-value	Sign.
Macrofauna	293.52	32	4.924519e-44	0.05

**Table 5.** Value of biodiversity index, dominance and evenness of soil macrofauna species

Category	Method	Station	Value									
			Diversity Index (H')	$\bar{x}$ H' Per Method	$\bar{x}$ H'	Dominance Index (C)	$\bar{x}$ C Per Method	$\bar{x}$ C	Species Evenness Index (E)	$\bar{x}$ E Per Method	$\bar{x}$ E	
Rare (R)	Hand Sorting (HS)	R1	1.91	1.03	1.57	0.16	0.45	0.29	0.98	0.90		
		R2	0.50			0.68					0.72	
		R3	0.69			0.50					1.00	
	Pitfall Trap (PT)	R1	1.53	1.56		0.36	0.28		0.64		0.83	
		R2	1.67			0.22			0.93			
		R3	1.48			0.27			0.92			
	Flannel Trap (FT)	R1	2.10	2.11		0.14	0.13		0.95		0.96	
		R2	2.15			0.13			0.93			
		R3	2.08			0.13			1.00			
Medium (M)	Hand Sorting (HS)	M1	1.13	1.25	1.32	0.47	0.36	0.38	0.63	0.83	0.78	
		M2	1.51			0.27						0.84
		M3	1.10			0.33						1.00
	Pitfall Trap (PT)	M1	1.54	0.94		0.30	0.55		0.79			0.53
		M2	0			1.00			0.00			
		M3	1.27			0.35			0.79			
	Flannel Trap (FT)	M1	2.80	1.77		0.06	0.25		0.99			0.97
		M2	0.69			0.50			1.00			
		M3	1.81			0.18			0.93			
Dense (D)	Hand Sorting (HS)	D1	1.88	1.20	1.28	0.18	0.38	0.38	0.91	0.87	0.81	
		D2	1.07			0.39						0.77
		D3	0.64			0.56						0.92
	Pitfall Trap (PT)	D1	1.15	1.17		0.39	0.45		0.83			0.67
		D2	0.70			0.75			0.26			
		D3	1.64			0.22			0.92			
	Flannel Trap (FT)	D1	0.69	1.48		0.50	0.30		1.00			0.89
		D2	1.70			0.27			0.68			
		D3	2.04			0.14			0.98			

For macrofauna species residing in the soil, sampled through HS, the highest diversity is in the medium category. However, when compared to rare and dense categories, the average diversity values based on the method are not significantly different, and all three remain in the range of  $H' < 2$  values, indicating a low category. Similarly, with the PT method, the average diversity value across the three regional categories falls within the range of  $H' < 2$  (low category). However, in comparison to other regional categories, the average diversity value in the rare category is higher. Consequently, from the aspect of macrofaunal diversity, rare category areas exhibit more diverse soil macrofauna for types that fly above the ground surface and also slither on the ground surface compared to medium and dense category areas.

The average value of species dominance across all categories of oil well density is generally in the low range, with values of  $C = 0.29-0.38$  or  $C < 0.5$ . This suggests that no macrofauna species are dominating in all traditional oil mining areas. However, when comparing between categories, the rare category area exhibits the lowest dominance value compared to medium and dense. In Figure 4, the highest dominance value is observed at the M2 station with the PT method, followed by D2 and R2, both with the PT and HS methods. The lowest dominance index values are observed at M2 with the PT method, and also M1 and D3 with the PT and FT methods. Comparing species dominance values based on the sampling method within each category area, the M category area has the highest dominance value for the PT method, falling into the medium range ( $0.5 < C < 0.75$ ). This is followed by the dense category area, also with the PT method, but the species

dominance value remains in the low range ( $< 0.5$ ). This indicates that species dominance is more prevalent in macrofauna that slithers on the ground surface, especially in the medium and dense categories. A high dominance of species in a location indicates an imbalance in the ecosystem.

The average value of the species evenness index generally ranges from 0.78 to 0.90, remaining in the high category or within the range of 0.75-1, across all categories of oil well density. This indicates an even distribution of species at all points, suggesting a stable condition in the macrofauna community. However, when examining the average value of the species evenness index in each regional category, it becomes evident that the rare category area has the highest species evenness index value, while the lowest is observed in the medium category. Figure 4 illustrates the species evenness index value at each sampling station. The highest value, even reaching the maximum of 1, is found at station R3 using the HS and FT methods. Additionally, stations M1 and M2 with FT, M3 with HS, and D1 and D3 with FT methods also exhibit high values. Considering the sample collection methods in all well density categories, the highest average value of the species evenness index is obtained through HS and FT methods, while the PT method yields the lowest average value. However, in the rare category area, all average species evenness index values based on the sampling method are high ( $> 0.75$ ), including those obtained through the PT method. This indicates that macrofauna communities residing in, on the surface, and above the surface of rare category are evenly distributed in the area, indicating stable conditions.

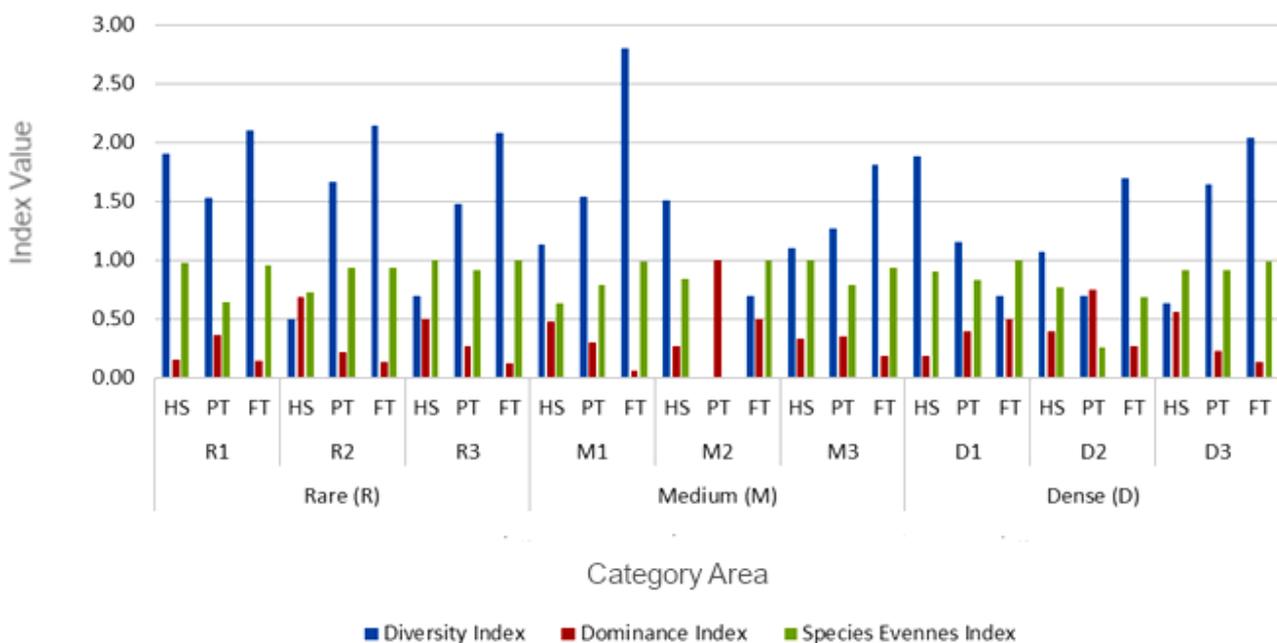


Figure 4. Community structure of soil macrofauna in oil Wonocolo Traditional Oil Mining Area, Bojonegoro, Indonesia

## Discussion

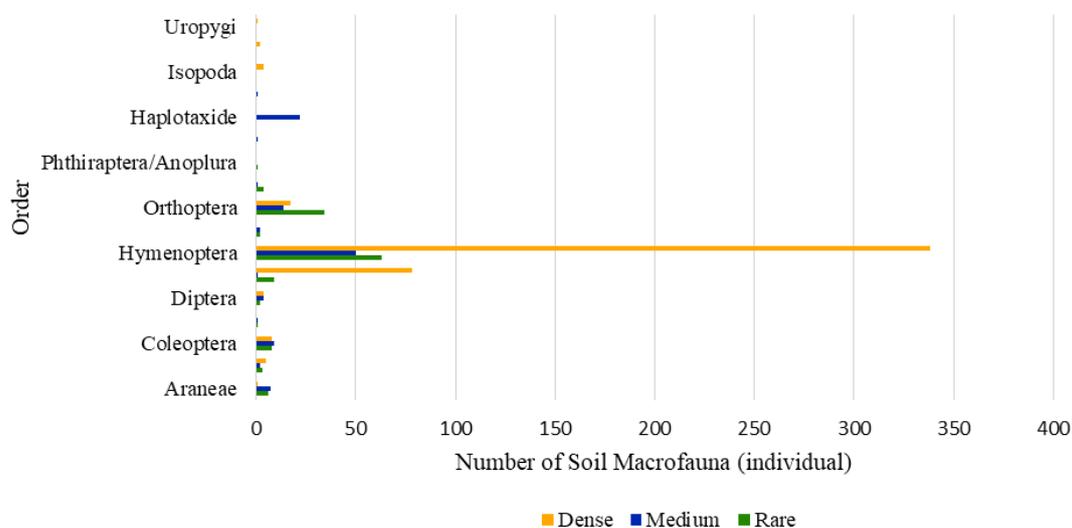
Soil macrofauna plays crucial ecological roles in terrestrial ecosystems. The community structure of macrofauna in the soil serves as a bioindicator of soil quality, as the presence of soil fauna depends significantly on the biotic and abiotic factors of the soil (Widhiono et al. 2017; García-Segura et al. 2018; Machado et al. 2018; Skaldina et al. 2018; Majeed et al. 2021). Based on the chi-square test results in Table 4, the macrofauna orders found at each sampling station in the three area categories show unequal proportions. This means that the macrofauna community structure and ecosystem conditions in the three areas categories are also different. This difference can be caused by different oil well density levels, which also have a different effect on the number of oil spills in each area category. The higher the density of oil wells, the greater the possibility of oil spills on the ground due to traditional oil mining activities. Previous studies have shown that areas contaminated with petroleum waste tend to exhibit a high abundance of macrofauna (Muli et al. 2016; García-Segura et al. 2018). Consistent with these findings, our study reveals that dense category areas, characterized by high oil well density (6-8 wells), exhibit the highest macrofauna abundance compared to medium and rare categories. The density of oil wells is presumed to be linked to the extent of traditional petroleum mining activities in Wonocolo, thereby increasing the potential for soil pollution in dense category compared to medium and rare categories.

In Figure 5, it is evident that the most abundant order of soil macrofauna in the Wonocolo Traditional Oil Mining Area is Hymenoptera. Hymenoptera is not only the most abundant but also the most frequently observed order in almost all sampling locations, particularly in dense category areas. This aligns with previous studies that identify Hymenoptera as one of the most abundant orders in areas contaminated with petroleum refining waste compared to non-contaminated areas (García-Segura et al. 2018; Machado et al. 2018; Mhlanga et al. 2022). The abundance of this order is related to its ability to utilize various

resources to support its life (Vasconcellos et al. 2013), such as their ability to digest organic particles and soil minerals, which makes its feces rich in organic matter and able to digest complex substrates such as protein-tannins, lignin and humic compounds in their surrounding environment.

The abundance of Hymenoptera, especially types of ants on land, is also influenced by the presence of the understory, which is a place for ants to find food and protection (Hood et al. 2020). Ants have a variety of food preferences. Generally they are omnivores that eat nectar and protein from carrion or dead insects, but some are specialized as pure predators or only eat gongylidia fungi (Richter and Economo 2023). Ants that eat gongylidia fungi are usually leaf-cutting ant. They cultivate fungi by collecting leaf fragments in their nests to create a humid environment so that the leaves decompose and support the growth of the fungus. In return, the ants harvest the gongylidia of their monocultural fungal garden as their food source (Moreau 2020). In polluted soil conditions, especially heavy metals, litter decomposition by microorganisms, both fungi and bacteria, cannot work optimally. The collection of leaf fragments by ants in their nests is a preferred alternative for fungi and ammonifying bacteria for decomposition, so they are found more abundantly in ant nests than in polluted soil (Grzes 2010).

Beyond their abundance, ants are often associated with environmental restoration (Vasconcellos et al. 2013). Hymenoptera, represented by ants, have been used extensively in the uranium mining industry in Australia as an indicator of rehabilitation success (Andersen and Majer 2004). The existence of ant colonies at the reclamation site of former uranium mines is considered part of the succession process to restore environmental conditions (Andersen and Majer 2004; Vlasakova et al. 2009). In areas contaminated with waste oil, ants belong to groups that are sensitive to hydrocarbon pollutants along with termites and earthworms (García-Segura et al. 2018), so the ant group is also considered an indicator of pollution (Menta and Remelli 2020).



**Figure 5.** Abundance of soil macrofauna by order in the Wonocolo Oil Traditional Mining, Bojonegoro, Indonesia

Apart from serving as bioindicators, Hymenoptera, represented by ants, can also function as bioaccumulators (Skaldina et al. 2018; Klimek et al. 2022; Khan et al. 2023). Ants are relatively resistant to heavy metal polluted environments due to their high ability to transfer metal content into their tissues (Jílková et al. 2017). The abundance of ants in the Wonocolo soil can be associated with the presence of heavy metals detected in the area, with concentrations that nearly reach the threshold for parameters such as Pb, Cr, and Hg (Sari et al. 2018). Ants accumulate heavy metals in two ways: within their bodies (Skaldina et al. 2018; Khan et al. 2023) and within nest materials (Skaldina et al. 2018; Klimek et al. 2022), with the number of heavy metals found in the nest being greater than that in the ants' bodies. Specifically, the heavy metal Pb was measured at 8 mg/g in the nest and 6 mg/g in the ant bodies. For Cr, it was only detected in the nest at 2.9 mg/g, while Hg was not found in either the nest or the ant bodies (Skaldina et al. 2018).

In addition to Hymenoptera, the order Orthoptera is also among the most common and diverse species compared to other orders in the rare, medium, and dense category areas. The majority of Orthoptera species are found in rare category areas, with 10 species, while medium and dense categories each have seven species. This suggests that Orthoptera is more prevalent in areas with low oil well densities. Orthoptera play roles within ecosystems, acting as herbivores that support trophic levels above them (Pennings et al. 2014), but some act as carnivores and omnivores (Bam 2015). Orthoptera is frequently used as a bioindicator for areas experiencing environmental changes and disturbances, particularly in the grass at ground level (Kenyeres et al. 2020; Menta and Remelli 2020; Anso et al. 2022). Its role as a bioindicator is not solely based on species richness but rather on species composition (Kenyeres et al. 2020; Menta and Remelli 2020; Anso et al. 2022). The most commonly found Orthoptera species include *Metaleptea adspersa* Blanchard 1843 and *Teleogryllus emma* Ohmachi and Matsuura 1951, especially in category R areas. Both species are grass-eaters and are commonly found in grasslands. This aligns with the conditions in the Wonocolo Traditional Oil Mine area, which is dominated by grass and shrubs. However, the specific relationship of Orthoptera to petroleum-polluted soils has not been widely discussed.

The study also found that there are species that are much more abundant than other locations in dense category areas, namely *Polyrhachis* sp. of the order Hymenoptera. The high dominance of the species in areas with high oil well density means that the species can adapt well to soil conditions polluted with oil waste and has a wide tolerance range to environmental conditions polluted with petroleum waste (John 2020). The existence of this type of ant can also be related to the ability of ants to accumulate heavy metal waste in the soil, both in the body and in the nest (Skaldina et al. 2018).

In conclusion, this study collected a total of 709 macrofauna individuals in the Wonocolo Traditional Oil Mining Area, distributed across 17 orders and 71 species, with 64.8% of individuals found in dense category areas.

The most abundant order identified was Hymenoptera, while the most abundant species was *Polyrhachis* sp.. *O. brunneus* was the species most commonly found across sampling stations. The average value of the biodiversity index (H') in all areas ranged from 1.28 to 1.57, categorizing it as low. Similarly, the species dominance index (C value) ranged from 0.29 to 0.38, also falling into the low category. The species evenness index (E) generally ranged from 0.78 to 0.90, placing it in the high category. Based on this analysis, it can be concluded that the values of the biodiversity index, dominance, and species evenness of macrofauna in the Wonocolo Traditional Oil Mining Area do not exhibit significant differences based on the level of well density. Environmental indications may be discerned from the prevalence of the Hymenoptera order in almost all categories, characterizing petroleum-polluted soils. Additionally, the substantial presence of *Polyrhachis* sp. in dense category areas can be considered a crucial indicator, given its stark abundance compared to other species.

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