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Research Article

Morphological and elemental analysis of termite mound and ant nest in agriculturally prominent area

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Abstract
Soil management is important for the farmers to improve the crop yield. In nature some invertebrates serve as bioindicators and biomonitors. Biogenic structure built by insects is important for controlling soil erosion and water reserves. Ants and termites nest architecture along with the elemental analysis was studied to evaluate soil health and possible threats imposed by heavy metals in the area. The soil samples were collected and analyzed for various parameters. Systematic study of porosity, composition, and nutritional values of soil in ant nest and termite mound were done. The Atomic Absorption Spectrophotometer and Inductively Coupled Plasma Mass Spectrophotometer studies showed that ant nest and termite mound samples were found to contain elements viz., zinc, selenium, lead, cadmium, nickel drought and chromium. Based on Scanning Electron Microscope-Energy Dispersive Analysis of X-rays, the size of soil samples collected from ant nest and termite were found to be 27.77 nm and 25.56 nm, respectively. The corrosion resistant zirconium and titanium metals were detected in 0.68 and 0.39% concentration in ant nest and termite mound samples, respectively, representing the insect house as a possible source of rich metals. The ant nest and termite mound materials contain quartz, microcline, kaolinite, and clay minerals. Ant nests and termite mounds can thus be used as hydrological indicators to address the problems of soil erosion.

***Keywords*:** Bioindicators, economical, heavy metal, minerals, soil, toxicity

Introduction
Soil functioning is important considering its role in ecosystem management (Wall et al*.,* 2012). Soil health is getting disturbed due to soil erosion and some anthropogenic activities. Termites build their mound by the combination of quartz grains with their natural secretions (humidifying agent), vegetable debris and clay minerals (plasticizer) (Echezona et al*.,* 2012). To evaluate the soil ecosystem, bioindicators and biomonitoring functioning is very important. In nature, some species of invertebrates have been recognized as bioindicators and biomonitors of ecology. Soil ecosystems can be assessed using sentinel species as bioindicators (Amiard-Triquet et al*.,* 2012). Based on the changes in the ecosystem affected by natural calamities (e.g., wasps' famine, soil erosion, heavy rainfall) or anthropogenic activities, bioindicator organisms change their communal behavior (Medhi et al*.,* 2020). Reports are available on the role of termite communities in reflecting the soil conditions including macro aggregation of soil, chemical extensive richness, biodiversity, and soil hydrological functions (Duran-Bautista et al*.,* 2020). Bioindicators include honeybees, drosophila, wasp, termites, and ants (Chowdhury et al*.,* 2023). Role of arthropods as bioindicators is attributed to their community-based structure, nature of predator and possibility of statistical analysis (Medhi et al*.,* 2020). Insects which have the capacity to serve as ecological biomarkers can be studied to find the ecotoxicity of that area (Amiard-Triquet et al*.,* 2012). The insects as bioindicators with their mechanism to combat environmental stress are represented in Table 1.

Biomonitoring is based on the finding changes in the ecosystem by using the biodiversity data of keystone species and natural inhabitants (Ma et al*.,* 2018). The wasps are chemicaresidents of rural as well urban areas and have been reported to serve as biomonitors due to their potential to accumulate metals. *Polistes dominulus* (paper wasps) larval fecal mass are found to contain lead which indicates that wasp has good heavy metal excretion mechanism (Urbini et al*.,* 2006).

Ant nests
The role of ants as ecological indicator is attributed to their nest building potential using local resources (Okrutniak and Grześ, 2021; Sorvari, 2009). Ant's nest is one of the widely studied homes in context of their composition (metal accumulation), architecture (as per the environmental factors and insects' own interest), foraging behavior and ecosystem management (Fagundes et al*.,* 2020). The way ant finds their place to build the nest or new home after the destruction or threat imposed on their nest, the journey of nest relocation, is done by scout ants which uses "one-pass" or "two pass" strategy which relies upon pheromones (Marshall et al*.,* 2003). Scouts use Buffon's needle for the evaluation of nest size. Reports are available, which suggest ant nest size and its architecture are dependent upon the local environmental

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conditions and the role of ants in their nest (Sankovitz et al., 2021). Tunnels in the ant nest indicate the competition between the social workers in ant's community. As per the pollutant concentration, ants bring about some changes in their life cycle as well as nest size. It has been reported that the red wood ant (*Formica aquilonia*) residing in the heavy metal polluted area of copper smelter in Southwest Finland, has built small size nest and showed lower reproduction rate (Eeva et al., 2004). The attributes of the ant nest are shown in Fig. 1.

Table 1 Insects as bioindicators with their mechanism to combat environmental stress

Examples of bioindicator insect	Stress factor	Mechanisms	References
Ground beetle *Parallelomorphus laevigatus*	Metals (As, Cd, Cr, Pb, Ni, and Hg)	Biomagnification	Conti et al. (2017)
New Zealand (NZ) cicada (*Amphipsalta* and *Notopsalta)*	Climate change	Fast ecological radiation	James et al. (2003); Marshall et al. (2012)
Aphids (*Acaudinum centaureae, Aphis euphorbiae, A. galiiscabri, A. helianthemi, A. klimeschi, A. leontodontis, A. molluginis, A. pilosellae, A. subnitida, Brachycaudus lucifugus, Nasonovia pilosellae, Uroleucon obscurum*	Heavy metals	Species richness	Osiadacz and Halaj (2016)
Aphid *(Aphis gossypii)*	Heavy metals (Cd, Cu, Zn, and Pb)	Bioaccumulation	Alajmi et al. (2021)
Ant *(Lasius niger)*	Heavy metals (Cd and Zn)	Bioaccumulation	Okrutniak and Grześ (2021)
Paper wasps (*Polistes dominulus)*	Lead	Bioaccumulation	Urbini et al. (2006)
Ant (*Lasius niger*)	DEHP (Diethyl hexyl phthalate)	Activation of immune state	Cuviller-Hot et al. (2014)
Red wood ant (*Formica aquilonia*)	Heavy metals (Al, Cu, Cd, Ni, Zn, As, Pb, Hg)	Lower reproduction rate	Eeva et al. (2004)

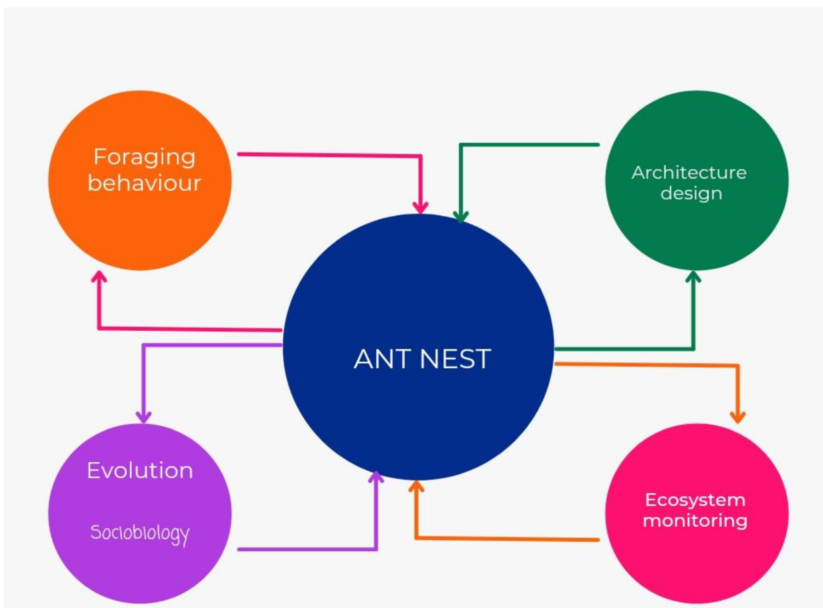

Figure 1 Attributes of the ant nest

Termite mound

Mound designed by termites have stereotypical architecture with structural changes as per the requirement of temperature, wind, and food. Mounds have tunnels, open chimneys, or vent holes for the exchange of gases (King et al., 2015). Termites build nests as shelter for protection (called as "protective trail galleries") as well as foraging tools, considering the facets of global warming induced temperature drift (Oberst et al., 2017; Oberst et al., 2020). Termite nests have been classified into three types based on their construction pattern as given in Fig. 2.

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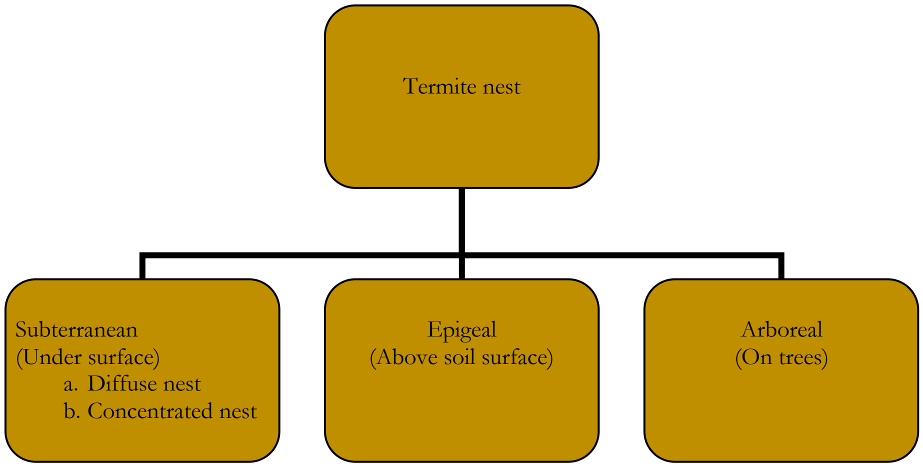

Figure 2 Types of termite nest (Genise, 2017)

As per the reports available, mounds made by termites have outgrowth of spire which is tilted as per the sunlight and internal channels. Number of internal channels in mounds depends upon the environmental conditions like soil, temperature and wind (Fagundes et al*.,* 2020). Termite *Macrotermes michaelseni* mounds in Northern Namibia are cone shaped extended spire and pediment (Turner, 2000). Based on the environmental challenges and number of species, termites create turrets in the mounds by which they can allot space (queen's royal chamber) for the residence as per the species and labor (Feltell et al*.,* 2008). Reports are available where some turrets or fungus comb when kept for the fungi; it could assist termites for the digestion of wood or old comb (Oberst et al*.,* 2020; Rouland-Lefevre, 2000). Termites although build nests with multiple compartments, they still show communication directly through vibration (biotremology) or indirectly (stigmergy) (Calovi et al*.,* 2019; Oberst et al*.,* 2017). The terminate mounds can be studied for various reasons like geochemistry, sociobiology, biotite weathering into titanium and zirconium or pedogenesis mechanism (soil formation) as given in Fig. 3.

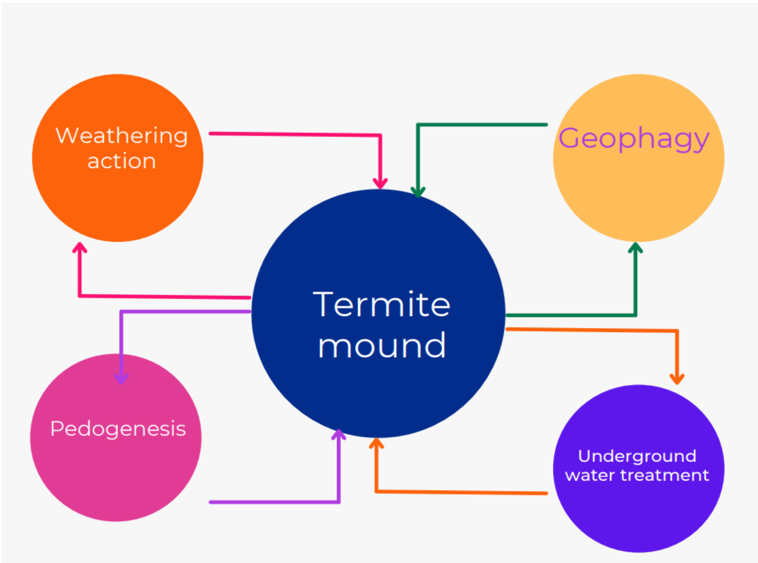

Figure 3 Attributes of termite mound

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Materials and Methods

Collection of the sample

Soil samples were collected from termite mound and ant nest, located in rural area of Pune city, Maharashtra, India. The method used for sampling was grab sampling. The soil samples collected were homogeneous. The collected samples were kept in clean sterile containers at 4 ^{0}C until use for analysis. The morphological and micro morphological studies were performed of ant nest and termite mound samples. Ant nest and termite mounds architectural pattern samples were evaluated in terms of height, diameter and number of turrets (Diehl et al*.,* 2005). The sampling points for termite mound and ant nest are shown in Fig. 4 and Fig. 5 respectively.

Figure 4 Sampling point for termite mound

Figure 5 Sampling point for ant nest

Chemical analysis of ant nest and termite mound samples

The pH and electrical conductivity (EC) parameters were measured as per the standard methods of American Public Health Association (APHA, 1998). The pH and EC were determined using pH meter and electrical conductivity meter, respectively.

Analysis of the metals

Metal contents and micromorphology of sample was determined using SEM with EDS (Model -JSM-6360, 20kV). For the estimation of extractable metals from ant nest and termite mound, samples were digested as per the protocol of Alghanmi et al. (2015). The sample (0.5 g) was mixed with acid mixture [HCl (37%): HNO_3 (70%) in the ratio 3:1]. After 12 hours the sample was digested at 120 ^{0}C for 3 hours and filtered using Whatman filter paper number 1. The metal contents were analyzed using Inductively Coupled Plasma Mass Spectrophotometer (ICP MS Spectrophotometer - EXPEC 7000) and Atomic Absorption Spectrophotometer (AAS Varian Spectra, Germany).

Determination of size of soil

The ant nest and termite mound samples were analyzed by the X-ray diffraction (XRD) techniques as per the method of Linsen et al. (2014). Advance Bruker D8 was used in this research. This instrument uses Bragg-Brentano geometry.

Results and Discussion

Considering the ecological role of termites and ants, current study was done on finding the similarities and differences in physicochemical attributes of nest prepared by ant (*Lasius niger*) and termite (*Coptotermes formosanus*). The chemical analysis of ant nest and termite mound samples is shown in Table 2. The EC is more in termite mound sample which was found to be 1.764 x10^{-3}. The element composition of ant nest and termite mound samples is represented in Table 3, where the oxygen content was found to be highest in ant nest and termite mound samples which was 57.66 and 57.21 % respectively. The carbon content in ant nest and termite mound samples was 21.24 and 13.27 % respectively (Table 3). Insects, specifically ants, serve as ecosystem engineers who affect physico-chemical and biological properties of the soil. Extensive study has been conducted by ecologists and biologists on the potential role of ants and termites as bioindicators and biomonitors. Research has been conducted on the design and ecological role of ant nests and termite mounds. Architectural designs and collective behavior of insects during construction of nests has been extensively studied in biomimetics like swarm robots' construction (Werfel et al*.,* 2014). Replacement of cement with eco-friendly material like termite mound clay soil and calcium carbide wastes as binder has been tested for the production of unfired clay bricks (Akinyemi et al*.,* 2021).

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Table 2 Morphological and chemical analysis of the ant nest and termite mound samples

Parameters	Ant nest	Termite mound
Height (m)	3.0 $\pm$ 0.01	1.0 $\pm$ 0.00
Diameter (m)	2.0 $\pm$ 0.00	2.0 $\pm$ 0.01
Number of turrets / Open channels	15.0 $\pm$ 0.00	30.0 $\pm$ 0.00
Mean diameter of turrets	0.25 $\pm$ 0.00	0.70 $\pm$ 0.01
pH	6.23	7.38
Electrical conductivity (Ohm/m)	1.112 x 10^{-3} $\pm$ 0.00	1.764 x 10^{-3} $\pm$ 0.01

The data represents average of triplicate $\pm$ standard deviation.

XRD analysis

The X-ray diffraction patterns of the ant nest and termite mound materials are represented in Fig. 6. The ant nest and termite mound materials mainly consist of quartz, microcline, kaolinite and clay minerals. The analysis shows that the peaks referable to quartz are more prominent in both the samples while microcline and kaolinite are the dominant clay mineral, which is consistent with tropical environment (Dowouna et al., 2012; Horiuchi et al., 2014; Liu et al., 2019). Moreover, the structural analysis of ant nest and termite mound samples reveals the polycrystalline nature. The average crystallite size (D) is calcmacrulated using Debye-Scherrer's equation from the full-width half maximum (FWHM) of all the obtained peaks for ant nest and termite mound sample and it is found that 28 and 26 nm respectively.

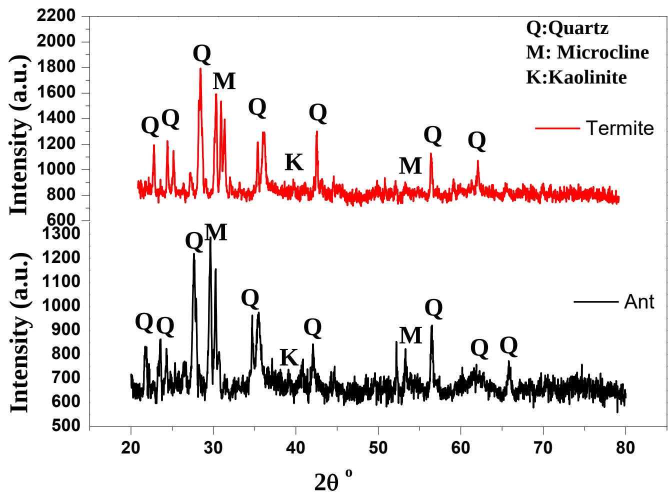

Figure 6 X-ray diffraction pattern of ant nest and termite mound sample

SEM-EDX

The SEM images of ant nest and termite mound are shown in Fig. 7 and Fig. 8, respectively at different magnification viz., x100, x500 and x1000 . SEM micrograph showed homogenous structure with micro and macropores, correlated to the structure of ant nets and termite mound.

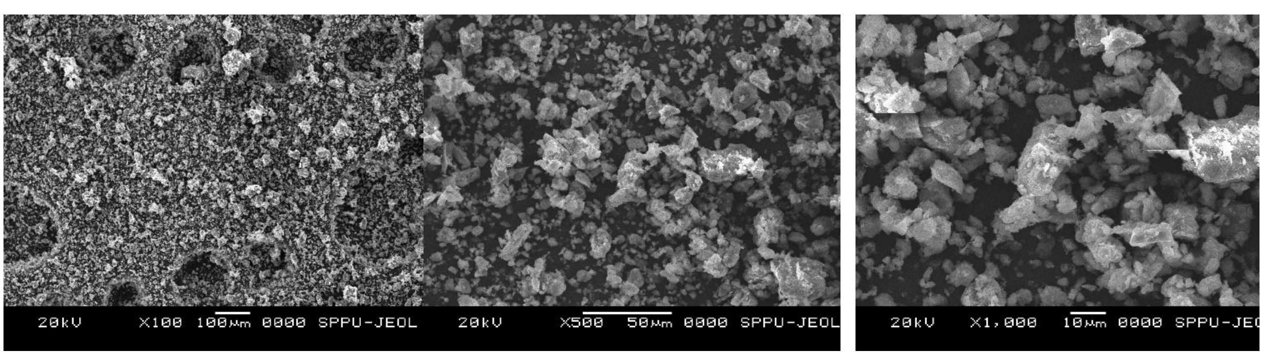

Figure 7 SEM image of ant nest sample

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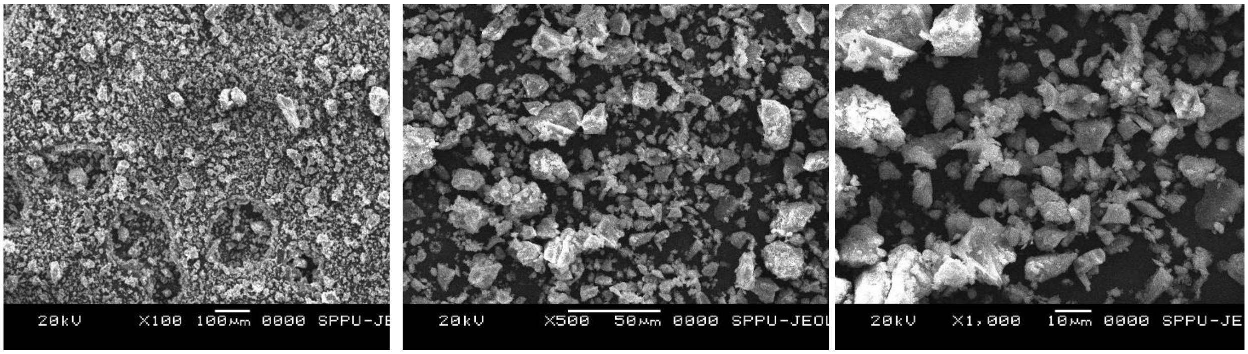

Figure 8 SEM image of termite mound sample

The SEM-EDAX images of ant nest and termite mound samples showed presence of precious metals viz., oxygen, carbon, copper, magnesium, aluminium, iron, zirconium, calcium, potassium, and silica (Fig. 9 and 10). The metals platinum and titanium were also found in termite mound samples (Fig. 10).

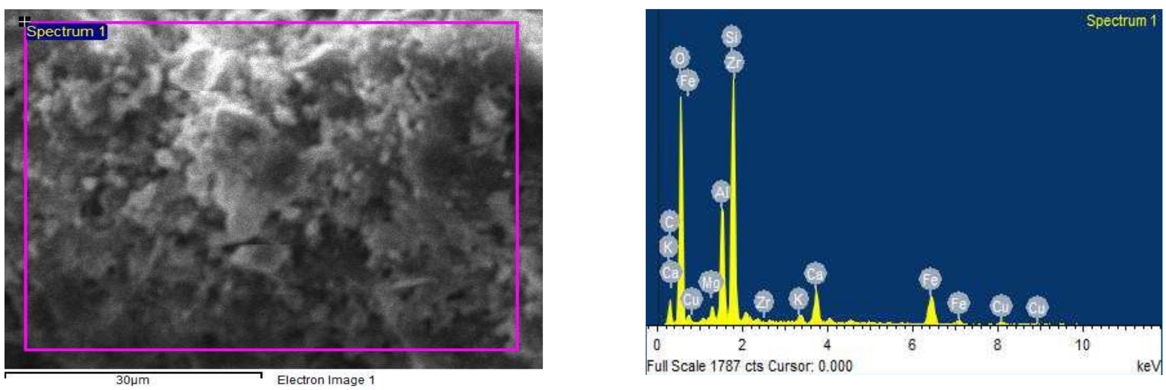

Figure 9 SEM-EDAX image of ant nest sample

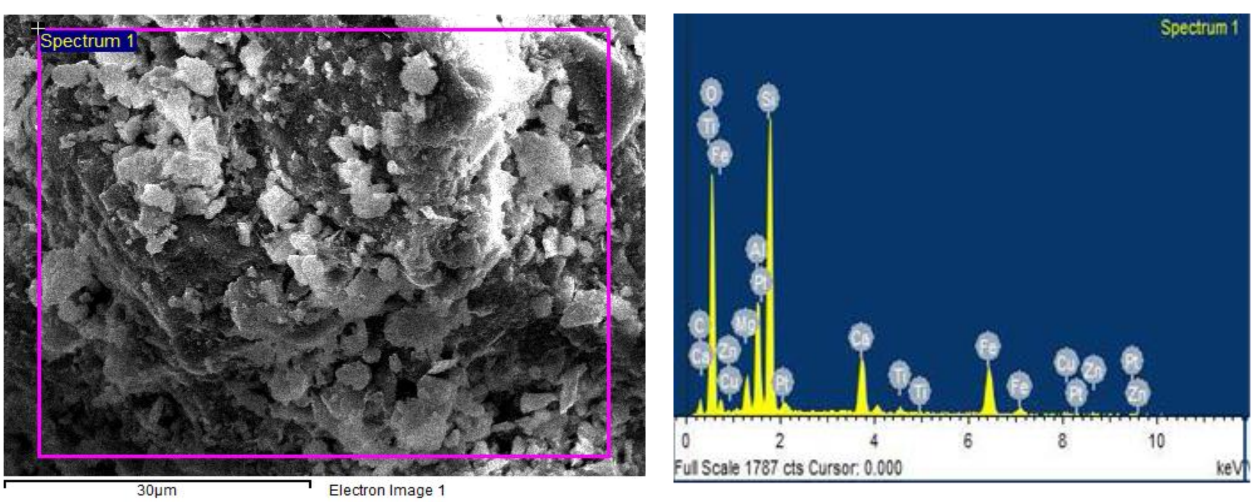

Figure 10 SEM-EDAX image of termite mound sample

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Table 3 Elemental composition in ant nest and termite mound samples as per SEM-EDS data

Elements	Ant nest sample	Termite mound sample
	Weight (%)	
Carbon	21.24 ± 0.01	13.27 ± 0.01
Oxygen	57.66 ± 0.00	57.21 ± 0.00
Magnesium	0.60 ± 0.02	1.69 ± 0.00
Aluminium	4.05 ± 0.01	4.11 ± 0.01
Silica	8.81 ± 0.02	11.29 ± 0.00
Potassium	0.42 ± 0.00	-
Calcium	1.68 ± 0.02	3.25 ± 0.01
Iron	4.24 ± 0.00	6.95 ± 0.00
Copper	0.63 ±0.00	0.63 ± 0.00
Zirconium	0.68 ±0.00	-
Zinc	-	0.90 ± 0.00
Titanium	-	0.39 ± 0.01

The data represents average of triplicate ± standard deviation.

Ants are common in metal polluted areas due to their metal resistant behavior (Klimek et al., 2022). Reports are available on the rare earth elements and trace elements in termite mound. In one study, ten termite mounds and adjacent topsoil from Central and North-Eastern Namibia was analyzed for the rare earth and mobile element. Compared to adjacent soil, *Macrotermes* termite mound has been reported to have more concentration of titanium, zirconium, selenium and Th/U ratios suggesting mineralization and chemical weathering ability of termites (Sako et al., 2009). Study conducted on ants living in meadow and forest sites in a mining and smelting region near Olkusz, Poland concluded that diversity increases with increase in metal pollution (Grześ, 2009). The metal contents (Zn, Cd, Ni, Cr, Se, and Pb) were analyzed using ICP-MS and AAS. Negligible differences were observed in the concentration of metals estimated by using AAS and ICP-MS (Figs. 11 and 12).

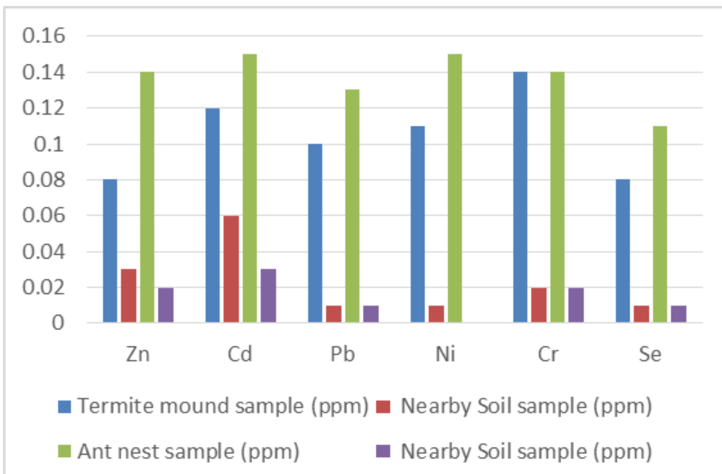

Figure 11 Metal content in samples using AAS

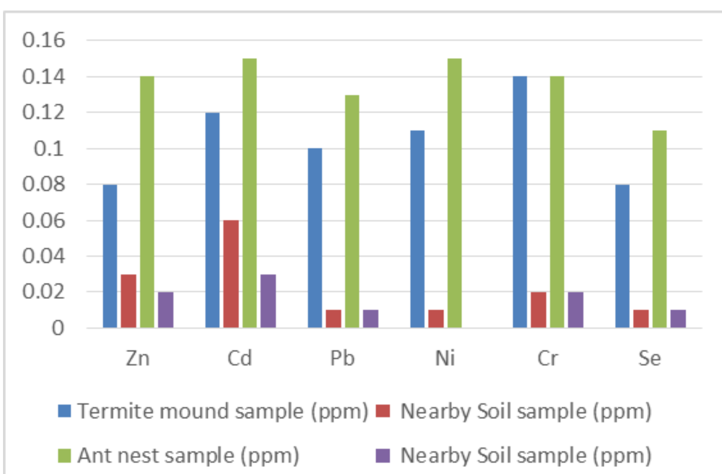

Figure 12 Metal content in samples using ICP-MS

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FTIR analysis of the ant nest and termite mound

The FTIR spectra of the ant nest and termite mound samples are shown in Fig. 13. The peak is observed at 1002.32 nm wavelength indicating the presence of presence of alkyl/aryl halides like F/Cl/I.

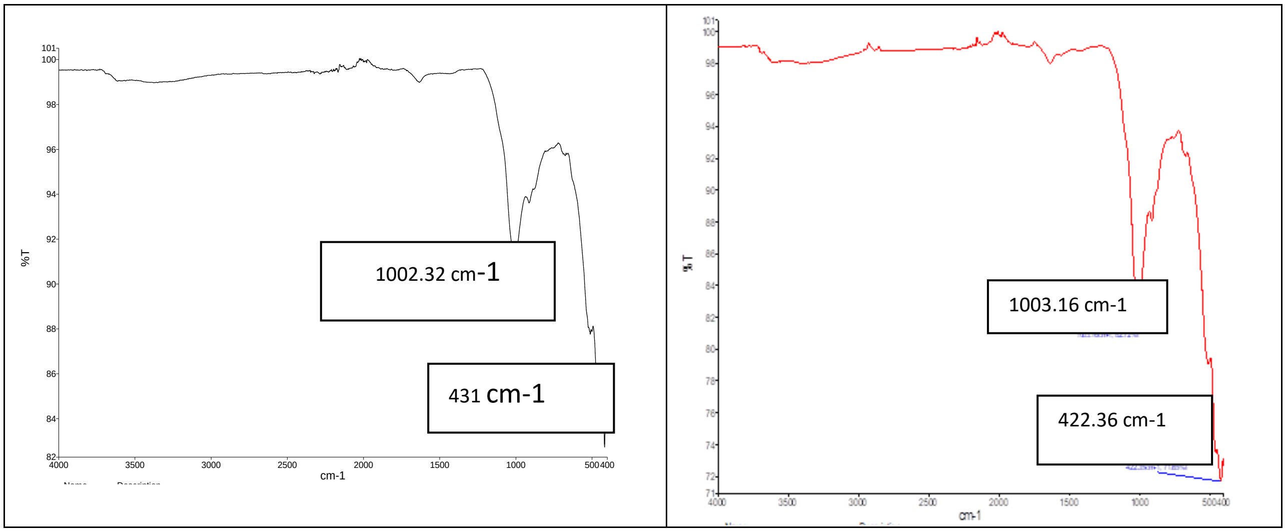

Figure 13 FTIR spectra of the ant nest and termite mound sample

Conclusions

In nature, different types of biogenic structures are observed, among which ant nest and termite mound have been studied in the biomimetic field. High mechanical resistance and ventilation pattern are two important aspects which are considered by ant and termite mound, while building their home. Nest architecture and its metallic as well as mineral contents were studied in this work. Both ant and termite nests, residing in close proximity, are found to have very much similarity in metal and mineral content, the exception was titanium and zirconium. This type of study is important for the metallurgical data on such biogenic structure. In drier areas of India such termite mound can be considered as hydrological indicators and will also prevent soil erosion. As compared to the nearby agricultural soil, ant nests and mounds showed more metallic contents.

Author Contributions: PS: Data analysis, MW: Experiments conducted and paper writing, RM: Data analysis for XRD, AG: Experiment design and paper writing, NP: paper writing, NB: FTIR analysis, UD: sample collection, SJ: sample collection, SS: SEM data analysis.

Conflicts of Interest: The author declares no conflicts of interest.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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