The Spatial Distribution of *Pontoscolex corethrurus* and *Haemadipsa zeylanica* in UiTM Negeri Sembilan Reserve Forest

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Abstract

Annelida, commonly found in terrestrial, freshwater, and marine environments, exhibits notable species diversity and ecological abundance. However, limited research has been conducted on Annelida in the UiTM Negeri Sembilan Forest Reserve, leading to a gap in understanding their ecological roles. This study aims to identify Annelida species and assess their diversity across four designated sites within the forest reserve. Sampling was carried out over four weeks using quadrats and hand-sorting techniques, with soil types classified through the Jar Test Method. A total of 77 Annelida specimens were collected: Site 1 (N = 14), Site 2 (N = 30), Site 3 (N = 11), and Site 4 (N = 22). Morphological identification revealed two species: *Pontoscolex corethrurus* (N = 70) and *Haemadipsa zeylanica* (N = 7). Species richness (R) was calculated at 0.23, with a diversity index (H) of 0.305 and evenness (E) of 0.44, indicating relatively low diversity. These findings contribute to the understanding and conservation of Annelida habitats in the forest reserve, highlighting the need for further research to explore the species diversity in greater depth.

1. Introduction

Annelida is a diverse phylum of organisms that includes segmented worms commonly known as annelids. They are found in various habitats, including terrestrial, freshwater, and marine environments. Annelids exhibit various forms and biological strategies, making them one of the most diverse phyla within the animal kingdom. They are second only to arthropods, vertebrates, and possibly platyhelminths regarding species richness [1]. Annelids exhibit a wide range of body forms and lifestyles, including polychaetes, such as marine worms with bristles; oligochaetes, such as earthworms and freshwater worms, and also leeches [2]. Since the early 1870s, 64 species belonging to 31 families have been reported in Malaysia, with Nereididae as the most dominant family with ten species. Polychaetes were mostly observed in Peninsular Malaysia compared with Borneo Island [3]. The previously documented species were primarily linked to intertidal and estuarine habitats, while a smaller number were identified in subtidal and freshwater environments [3]. As a bioindicator, the presence of Annelida indicates environmental health and habitat quality [4]. Changes in their distribution patterns reflect the environmental conditions, such as pollution levels or climate change impact [5].

This study was conducted in the UiTM Negeri Sembilan Reserve Forest, which features lowland forests. Despite their ecological importance, more comprehensive studies on annelids are needed in this area. Thus, this study aims to enhance the understanding of annelids by determining species occurrence and richness and evenness index in the UiTM Negeri Sembilan Reserve Forest. The data gathered will contribute to baseline knowledge of local fauna and support efforts to maintain ecological balance and conserve biodiversity.

2. Methodology

2.1 Study Site

The sampling was done from March 2024 to July 2024 in UiTM Negeri Sembilan Reserve Forest. Located in the Kuala Pilah district, Negeri Sembilan, the forest features an elevation range of 71 m to 142 m, with an average elevation of 132 m. This lowland tropical rainforest is characterized by high humidity and frequent yearly rainfall (2000mm/year), range temperature between 25°C–38°C. As a lowland rainforest, this area comprises dense forests, open spaces, and water bodies. The forest floor is covered with thick leaf litter, which supports many plants, including large trees, shrubs, and perennial plants. Hills and water reservoirs add complexity to the habitat, creating spaces for different species of plants and animals. Combining these environmental factors makes UiTM Negeri Sembilan Forest Reserve an ideal location for conducting ecological studies.

The reserve's diverse fauna, which includes both vertebrate and invertebrate species, notably black panthers and various spider species thrives in the reserve's rich biodiversity. This biodiversity is supported by the abundant vegetation and the numerous water bodies, such as lakes and ponds, which are a prominent feature of the UiTM Negeri Sembilan Reserve Forest, enhancing its hydrological features and contributing significantly to the overall ecosystem.
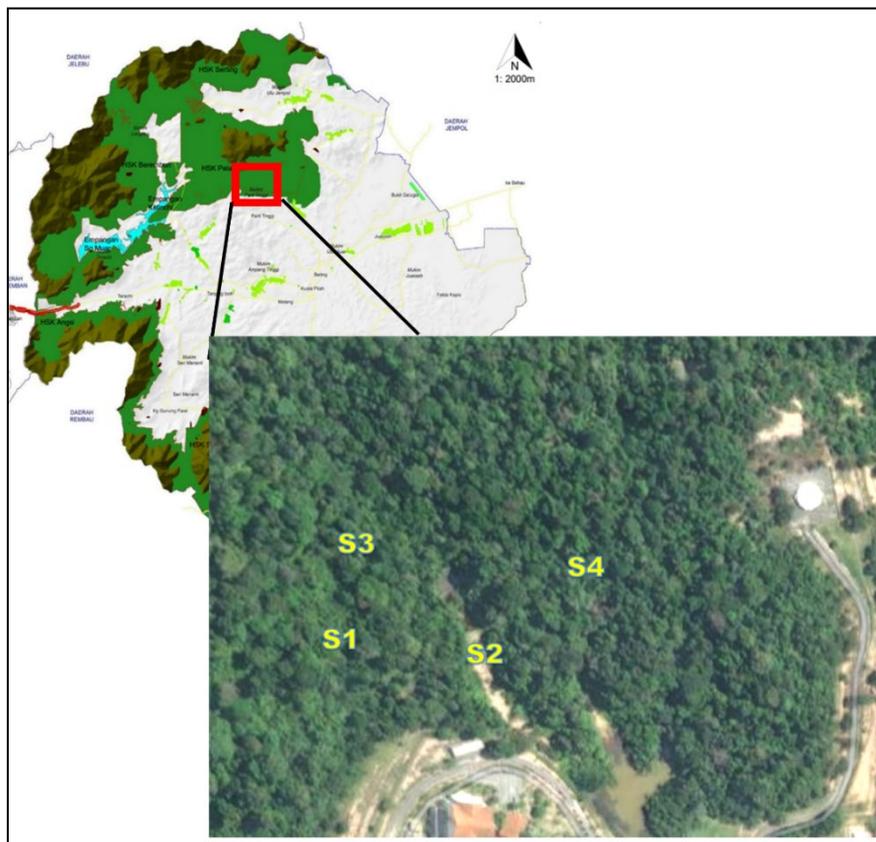

Fig. 1 *The study area (S1, S2, S3 and S4) in UITM Kuala Pilah Reserve Forest*

2.2 Field Work Setup

Earthworms were sampled by the hand-sorting method up to 30 cm deep using quadrats (30×30 cm^2 area) for each sampling site (S1, S2, S3 and S4) (Fig. 1). The obtained annelid samples were packed in polythene bags labeled with the location, date of collection, and surrounding soil biota and transported to the lab for further study [6]. The stereomicroscope application was used to aid in that identification based on their morphological traits

(tentacles, legs, and body length) [7]. The identification was conducted using the Handbook of Zoology Annelida Volume 1 [8]. The jar test method was applied to classify the soil texture by evaluating the sand, silt, and clay proportions in a soil sample collected from each site. The distinct layers of sand, silt, and clay have been measured, and the relative proportions are calculated by dividing the thickness of each layer by the total thickness of the soil column before comparing it against a USDA (United States Department of Agriculture) soil texture triangle to classify the soil type. The data values were used to determine the species composition and diversity indexes (The Margalef Richness Index, Shannon Weiner's Diversity Index, and Evenness Index) (Figure 2).
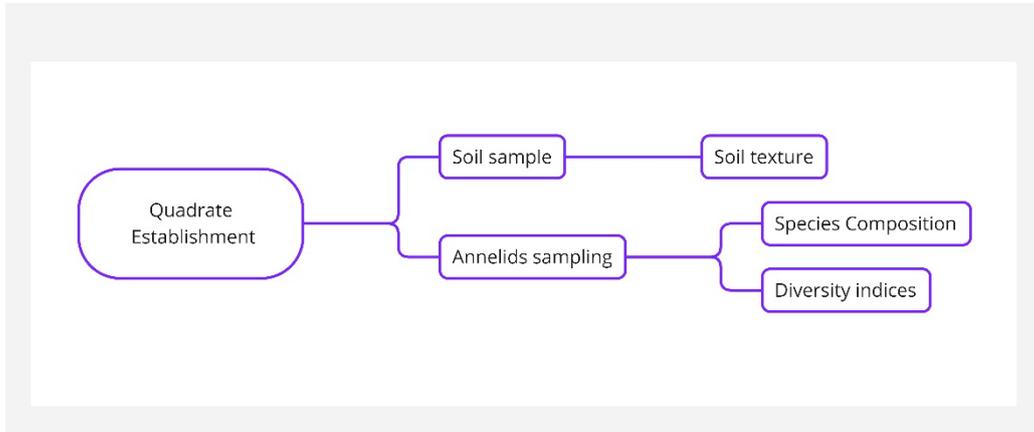
Fig. 2 *Flowchart of methodology*

3. Result and Discussion

3.1 Species Composition

The sampling was done in four weeks from March to April 2024, yielding 77 individuals. There were two distinct genera belonging to two families: Haemadipsidae and Glossoscolecidae. The species *Pontoscolex corethrurus* had the highest number of individuals collected, with 70 individuals recorded. In contrast, *Haemadipsa zeylanica* had the lowest number of individuals, with 7 (Table 1) (Figure 2).

Table 1 *Number of individuals of Annelids collected from each site in UiTM Negeri Sembilan Reserve Forest*

Sites	Soil type	*Pontoscolex corethrurus*	*Haemadipsa zeylanica*
S1 (Forest Area)	Sandy Soil	13	1
S2 (Water Bodies)	Clay Soil	27	3
S3 (Forest Area)	Sandy Soil	10	1
S4 (Forest Area)	Loamy soil	20	2
	Total	70	7

The identification of species within complex taxa of *P. corethrurus,* poses significant challenges due to the limitations of traditional morphological assessments. As highlighted by Chenuil et al. [9], cryptic species are those that are morphologically indistinguishable yet genetically distinct. This phenomenon underscores the necessity for integrating molecular techniques alongside morphological evaluations to achieve accurate species delineation. The collected Annelida samples match the description of *P.corethrurus* from the Handbook of Zoology Annelida Volume 1 [10] (Figure 3). These samples varied in length from 30mm to 150mm, reflecting the species' flexibility. Their color ranged from pale brown to brown, likely due to environmental factors, diet, and health, aligning with this species' known color range. A saddle-shaped clitellum indicates that the specimens are mature and ready for reproduction, a key characteristic of *P. corethrurus* [11]. Additionally, the samples had distinct mouth and tail ends, typical of annelids, which are essential for their movement and feeding (Table 2).
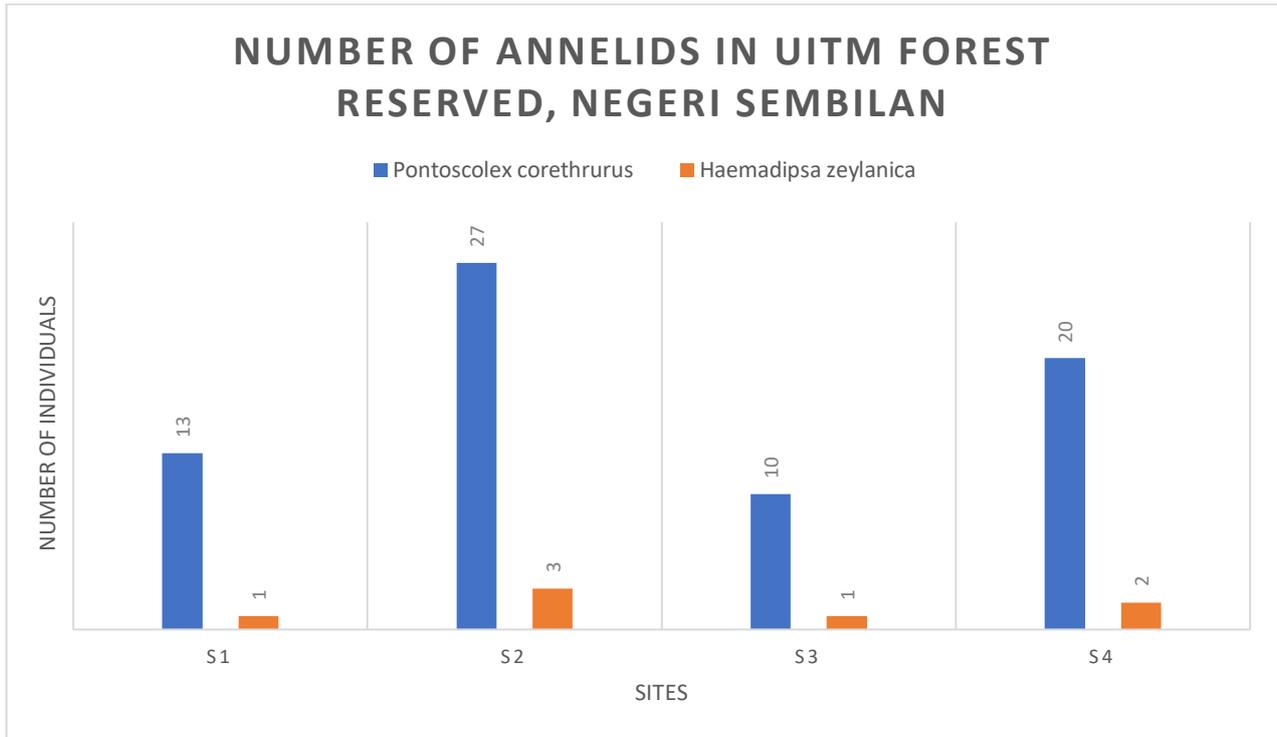
Fig. 2 *Number of Annelids in UiTM Forest Reserve, Negeri Sembilan*

Table 2 *Morphological Description of Pontoscolex corethrurus*

Morphological Traits	Description
Segment Number	Range : 30mm to 150mm
Pigmentation	Pale Brown, Brown
Clitellum	Saddle Shape
Mouth and Tail End	Present

The collected Annelida samples match the description of *Haemadipsa zeylanica* (Figure 3). They ranged in length from 10 mm to 30 mm, typical for this species, with size differences likely due to age and environment. The samples were all dark brown, helping them blend into their surroundings. The two suckers, one at each end of the body, are critical features of this species and are used for attaching to hosts and feeding. The presence of these suckers confirms the identification. Additionally, the samples had a crop, which stores and begins to digest the blood they consume [12] (Table 3).

Table 3 *Morphological Description of Haemadipsa zeylanica*[13]

Morphological Traits	Description
Stripe	A median dorsal yellowish or greenish yellow line from 1st pair of eyes to beginning of posterior sucker
Colour	Reddish brown or orange mottled with black
Body segment	98
Body length	1.2cm
Body breadth	8 mm
Breadth of anterior sucker	0.5 mm
Breadth of posterior sucker	1.5 mm
Breeding season	July - August

Fig. 3 *Sample of Annelida in UiTM Negeri Sembilan Reserve Forest*

Sites 1 and 3 comprise of sandy soil with low organic matter content and a limited water-holding capacity (Table 1). These conditions result in dry and high-temperature environments that lead to a scarcity of organic matter, a crucial food source for earthworms [14]. Moreover, Annelida requires a 20-30% moisture level, which needs to be met by sandy soil [14]. As soil dwellers, Annelida depends on the underground environment, with moisture content crucial for survival. Unlike their aquatic relatives, leeches and earthworms breathe directly through their moist skin. Sandy soil retains moisture poorly due to its large particle size and minimal pore spaces [5]. This characteristic creates a desiccating environment for earthworms. During dry periods, the thin film of water surrounding soil particles evaporates, leaving the sandy habitat dry and unsuitable for earthworm survival. Consequently, there was a significant disparity in the populations of *Pontoscolex corethrurus* and *Haemadipsa zeylanica* between Sites 1 and 3 compared to Sites 2 and 4. This variation can be attributed to the differing environmental conditions across the sites, particularly soil texture, moisture levels, and soil temperature [15].

Site 2's proximity to a water source presents a significant advantage for the populations of *Pontoscolex corethrurus* and *Haemadipsa zeylanica* (Table 1). Surface water bodies and elevated water tables act as moisture reservoirs, influencing the soil water potential in surrounding areas. This results in a more consistent and readily available moisture content within the soil profile [16]. Meanwhile, Site 4, although not near a water source, has loamy soil, which is also a favorable habitat for *Pontoscolex corethrurus* and *Haemadipsa zeylanica*. Loamy soil provides the ideal balance of moisture, structure, and organic matter content for earthworms and some leech species to thrive. It offers a suitable environment for burrowing, movement, respiration, and, in the case of earthworms, a readily available food source [17].

3.2 Diversity Indices

3.2.1 Margalef Richness Index (R)

The Margalef richness index is a metric used in ecology to estimate the species richness in a community, considering the number of species and the total number of individuals. This study's value of 0.23 suggests a relatively low species richness. The diversity of Annelida in the UiTM Forest Reserve appears low, with only two species found in the UiTM Negeri Sembilan Forest Reserve, leading to a lower Margalef index. Gamito [18] explains

that the Margalef index is susceptible to sample size. Consequently, more species in a sample area typically indicate higher species richness at that site (Table 4).

Table 4 *Diversity index of Annelida in UiTM Negeri Sembilan Reserve Forest*

Index	Value
Margalef Richness Index (R)	0.23
Shannon Weiner's Index (H)	0.305
Evennes Index (E)	0.44

3.2.2 Shannon Weiner's Index (H)

The Shannon-Weiner Index (H) calculated for the annelid community in the given study site is approximately 0.305. This value reflects the species distribution within the site, where *Pontoscolex corethrurus* significantly dominates with 70 individuals, while *Haemadipsa zeylanica* is much less prevalent, having only seven individuals. The low diversity index indicates that the community is heavily skewed towards one species, with *Pontoscolex corethrurus* being the overwhelmingly dominant species.

3.2.3 Evenness Index (E)

The evenness index (E) for the annelid community was approximately 0.44. This index reflects how evenly individuals are distributed among the species present. An evenness index close to 1 indicates a community where species are equally even, while an index closer to 0 indicates a community dominated by one or a few species [19]. The Evenness index of Annelida in UiTM Negeri Sembilan is near 0, indicating the occurrence of a dominant species (*Pontoscolex corethrurus*).

4. Conclusion

In conclusion, the study identified two Annelida species, *Pontoscolex corethrurus* and *Haemadipsa zeylanica,* highlighting their importance as valuable bioindicators of environmental health and habitat quality. Changes in their distribution can reflect shifts in environmental conditions, such as pollution levels or climate change impacts. This research successfully collected and analyzed 77 Annelida specimens, categorizing them into *Pontoscolex corethrurus* (N = 70) and *Haemadipsa zeylanica* (N = 7), indicating the dominance of *Pontoscolex corethrurus* in the study area (E=0.44). Moreover, the diversity and richness were 0.23 and 0.305, respectively, indicating this area's low diversity and richness. Various factors influence the diversity and abundance of Annelida in the UiTM Negeri Sembilan Forest Reserve, including soil moisture, soil composition, food availability, and temperature. To gain a more comprehensive understanding, increasing the spatial and temporal sampling periods could yield more specimens and potentially uncover additional Annelida species.

Acknowledgement

Conflict of Interest

The authors declare that there is no conflict of interest regarding the paper's publication.

Author Contribution

*The authors confirm contribution to the paper as follows: **study conception and design:** Nurul Nurfatehah Amir, Syazuani Mohd Shariff; **data collection:** Nurul Nurfatehah Amir; **analysis and interpretation of results:** Nurul Nurfatehah Amir, Syazuani Mohd Shariff; **draft manuscript preparation:** Nurul Nurfatehah Amir, Syazuani Mohd Shariff. All authors reviewed the results and approved the final version of the manuscript.*

References

[1]   Capa, M., & Hutchings, P. (2021). *Annelid Diversity: Historical Overview and Future Perspectives.* Diversity; Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/d13030129

[2]   Musco, L., Licciano, M., & Giangrande, A. (2021). *Diversity and Distribution of Sabellida (Annelida) under Protection Regimes.* Water: Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/w13111491

[3]   Shah, R., Ibrahim, Y. S., Villalobos-Guerrero, T. F., & Sato, M. (2023). Updated checklist of polychaete species (Annelida) recorded from Malaysia, with remarks on the research history. *Biodiversity Data Journal*. https://doi.org/10.3897/bdj.11.e110021

[4]   Kale, R. D., & Karmegam, N. (2010). The Role of Earthworms in Tropics with Emphasis on Indian Ecosystems. *Applied and Environmental Soil Science*. https://doi.org/10.1155/2010/414356

[5]   Ahmed, N., & Al-Mutairi, K. A. (2022). Earthworms Effect on Microbial Population and Soil Fertility as Well as Their Interaction with Agriculture Practices. *Sustainability, 14*(13), 7803. https://doi.org/10.3390/su14137803

[6]   Singh, S., Singh, J., & Vig, A. P. (2016). Earthworm as ecological engineers to change the physico-chemical properties of soil: Soil vs vermicast. *Ecological Engineering, 90*, 1–5. https://doi.org/10.1016/j.ecoleng.2016.01.072

[7]   Yousefi, Z., Ramezani, M., Mohamadi, S., Mohammadpo, R. A., & Nemati, A. (2019). Identification of Earthworms species in Sari Township in Northern Iran, 2007-2008. *Journal of Applied Sciences 9* (20), 3746-3751. https://doi.org/10.3923/jas.2009.3746.3751

[8]   Rizzo, A., & Magalhães, W. F. (2017) Lacydoniidae Bergstrom 1914 In *Handbook of Zoology: A Natural History of the Phyla of the Animal Kingdom*, Schmidt-Rhaesa, A. (Eds.) Walter de Gruyter GmbH.

[9]   Chenuil A., Cahill A. E., Delemontey N., du Luc E. D. S., Fanton H (2019) Problems and Questions Posed by Cryptic Species. A Framework to Guide Future Studies. In E. Casetta el al. (Eds.), From Assessing to Conserving Biodiversity (pp. 77 – 106). Springer Nature.

[10]   Purschke, G. Boggemann, M and Westheide, W (2019). *Handbook of Zoology Annelida*. De Gruyter.

[11]   Ortíz-Ceballos, A. I., Ortiz-Gamino, D., Andrade-Torres, A., Pérez-Rodríguez, P., & López-Ortega, M. (2019). *Pontoscolex corethrurus*: A homeless invasive tropical earthworm? *PLoS ONE, 14*(9), e0222337. https://doi.org/10.1371/journal.pone.0222337

[12]   Gasiorek, P., & Rozycka, H. (2017). Feeding strategies and competition between terrestrial Haemadipsa leeches (Euhirudinea: Arhynchobdellida) in Danum Valley rainforest (Borneo, Sabah). *Folia Parasitologica, 64*. https://doi.org/10.14411/fp.2017.031

[13]   Mandal, C.K., Reynolds,J.W., Nurul Hasan, Md., Deuti, K. Sinha, B. Ghosh, S and Banerjee, D. (2022). A Second New Leech *Haemadipsa zelanica dimapurensis* (Annelida: Hirudinida, Haemadipsidae) From Nagaland, India. *Megadrilogica*,27(6), 57 – 62.

[14]   Huang, J., & Hartemink, A. E. (2020). Soil and environmental issues in sandy soils. *Earth-Science Reviews*, 208, 103295. https://doi.org/10.1016/j.earscirev.2020.103295

[15]   Torppa, K. A., Castaño, C., Glimskär, A., Skånes, H., Klinth, M., Roslin, T., Taylor, A. R., Viketoft, M., Clemmensen, K. E., & Maaroufi, N. I. (2024). Soil moisture and fertility drive earthworm diversity in north temperate semi-natural grasslands. *Agriculture, Ecosystems & Environment*, 362, 108836. https://doi.org/10.1016/j.agee.2023.10883

[16]   Chen, X., & Hu, Q. (2016). Groundwater influences on soil moisture and surface evaporation. *Journal of Hydrology*, *297*(1–4), 285–300. https://doi.org/10.1016/j.jhydrol.2004.04.019

[17]   Wen, S., Shao, M., & Wang, J. (2020). Earthworm Burrowing Activity and Its Effects on Soil Hydraulic Properties under Different Soil Moisture Conditions from the Loess Plateau, China. *Sustainability, 12*(21), 9303. https://doi.org/10.3390/su12219303

[18]   Gamito, S. (2010). Caution is needed when applying Margalef diversity index. *Ecological Indicators*, 10(2), 550–551. https://doi.org/10.1016/j.ecolind.2009.07.006

[19]   Kitikidou K., Milios E., Stampoulidis A., Pipinis E., Radoglou K (2024). Using Biodiversity Indices Effectively: Considerations for Forest Management. *Ecologies* 5(1), 42-51