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Exploration of genetic diversity of earthworms in degraded landscapes in Lagos, Nigeria using random amplified polymorphic DNA (RAPD) markers

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Abstract

Earthworms are crucial for maintaining soil ecosystems and their functions, such as improving soil structure, nutrient cycling, and decomposition of organic matter. This study aimed to assess the genetic diversity of earthworm species in three specific sites: Iwaya, Makoko, and UNILAG areas of Lagos. Random Amplified Polymorphic DNA (RAPD) analysis was employed to detect genetic variations rapidly. Thirty earthworms were collected from three dumpsites, and DNA extraction was performed following the Sambrook and Russel protocol. Four RAPD primers were utilized, resulting in the amplification of forty products. Among the primers, OPA13 produced the highest number of amplified products (22), indicating the presence of highly variable genomic regions. The UPGMA Cluster analysis revealed two distinct yet related clusters among the earthworm populations, suggesting genetic differentiation influenced by local conditions and geographic barriers. Principal Component Analysis (PCA) identified PC1 and PC2 as major contributors to the observed genetic variance using the four RAPD primers. This study unveiled a high level of genetic variation among the examined earthworms, indicating the presence of diverse genetic traits. These findings contribute to our understanding of the adaptability and resilience of earthworms to environmental changes, thereby supporting the development of effective strategies for ecological restoration and management.

Keywords: Earthworms, Ecological restoration, Environmental management, Genetic diversity, Lagos, Nigeria, RAPD analysis

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Introduction

There is a growing focus on the preservation or reintroduction of keystone species crucial for the delivery of ecosystem services within restoration initiatives (Boyer *et al.,* 2016). Restoration ecology seeks to restore functioning ecosystems, biodiversity, and communities to areas impacted by human activities (Xiao *et al.,* 2022). The significance of restoration in areas where people "live and work" is emphasized to enhance ecosystem services, foster landscape connectivity, and encourage environmental education, ultimately promoting ecological citizenship and environmental stewardship (Miller and Hobbs, 2002). Urban environments, due to

their unique nature, present distinct challenges and opportunities for ecological restoration and reclamation efforts (Kale and Karmegam, 2010).

Ecological restoration is a powerful tool in combating land degradation, enhancing biodiversity resilience, and providing essential ecosystem services (Blouin *et al.,* 2013). Despite its widespread integration into natural resource strategies at various scales, questions remain about the efficacy of restoration programs (Eitzel, 2012). This uncertainty partly stems from the relative youth of the discipline compared to the timescale required for ecological processes to fully unfold. Additionally, the lack of well-defined objectives and the absence of comprehensive monitoring protocols hinder our ability to fully comprehend the impact of restoration efforts (Akhila and Entoori, 2022). Consequently, empirical evaluations of restoration achievements are crucial for refining the practice and substantiating the incorporation of ecological restoration into natural resource management strategies (Li and Hackenberger, 2020).

In aiding the recovery of ecosystems that have suffered degradation or damage, understanding the original state of the ecosystem and the factors that led to its decline is of great importance. However, comprehensive monitoring of ecosystems, whether they are significantly degraded or relatively untouched, seldom extends beyond a few decades (Boyer and Wratten, 2010). As a result, restoration ecologists must rely on indirect methods to assess the ecological history of an area. These approaches range from examining documentary sources such as written accounts, historical photographs, maps, and paintings to utilizing paleoecological data from natural archives like tree-rings, rodent middens, and sediments from various aquatic environments like lakes, peatlands, oceans, and estuaries (Li and Hackenberger, 2020).

Fortunately, these sources can provide valuable information about environmental conditions and ecosystem characteristics across different regions of the world (Boyer and Wratten, 2010).

It is well known that earthworms play an important role in the soil macro fauna biomass (Bhadauria *et al.,* 2010; Gongalsky, *et al*.,2023). They are critical in soil formation, principally by consuming organic matter, fragmenting it, and mixing it intimately with soil mineral particles to form water-stable aggregates. In particular, the bioaccumulation ability of earthworms is essential for a bio-monitoring organism (Gongalsky, *et al.,*2023). Bio-monitoring offers an effective means to assess metal toxicity due to its sensitivity and capacity to detect unknown metabolites. Various organisms, including fish, snails, and plants, have been utilized as bio-monitors in this context. While this approach is valuable and shows promise, it is somewhat restricted as it is applicable only to certain combinations of living organisms and specific substances (Siebert *et al.,* 2019). Therefore, the identification of suitable living organisms as bio-monitors for each type of assessment is crucial (Gongalsky *et al,* 2023).

The distinctive habitat, food niches, and adaptive mechanisms of earthworms have opened up new fields for investigations into their role in organic waste management. An advantage is the use of earthworms to minimize degradable organic matter and convert it into a bioresource for organic manure production (Siebert *et al.,* 2019). The manure produced serves as a good source of soil amendment. The ecologically distinguished epigeic earthworms are used by garden lovers, agriculturists, and agro-industries to convert organic matter generated at different levels into rich, odorless, and freely flowing compost to support sustainable agriculture (Coulis,2021).

There is often a positive correlation between biodiversity and ecosystem functioning, which is referred to as the diversity-functioning relationship. In diverse

communities, species can occupy complementary ecological niches, enhance resource use, and increase the likelihood of high-functioning species being present. Therefore, biodiversity is expected to positively impact functional processes, such as primary production, decomposition, and nutrient cycling, which are crucial for energy flow in ecosystems.

Genetic diversity has been found to affect the fitness of animal populations positively and contribute to wider ecosystem functioning and resilience. Previously genetic diversity assessment had been carried out using morphological markers that are not often reliable and consistent due to environmental influences on the expression of the morphological traits. However, a more reliable tool for genetic diversity assessment are molecular markers (). Molecular markers such as Random Amplified Polymorphic DNA (RAPD), single sequence repeats (SSR), Inter-simple sequence repeats (ISSR), and Restriction fragment length polymorphism (RFLPs) provide the best estimates of genetic diversity with little DNA samples (). RAPD markers are single arbitrary markers
This study aims to provide information on the genetic diversity of earthworms present at different sites for the purpose of ecological restoration.

Materials and Methods

Study Area

Three sampling stations (landfills) University of Lagos (UNILAG) community, Iwaya and Makoko were selected for the study. The UNILAG site is a regulated landfill situated behind the new postgraduate school, while the Iwaya, situated in the Yaba local council development area of Lagos State, is one of the nine slums designated for improvement by the state government. Bounded by the Lagos Lagoon, the study site is specifically located at the waste dumpsite in Iwaya and the Makoko, a unique swamp community in Lagos, is renowned for its stilt architecture. Originally inhabited by the Ijaws and Ilajes, who are the indigenous people of Lagos and known for their fishing activities and water-based lifestyle, Makoko is characterized by a swampy environment (Figure 1). Brief description of each of the sampling sites are shown in Table 1.

Earthworm Collection

A total of thirty sexually mature earthworms, identified by the presence of the clitellum, were collected from each studied location. The earthworm cast (soil) was also collected and stored in a zip-lock bag for physico-chemical analysis. Most of earthworms were sampled during the early hours (6:30-7:00 am) of the day from underneath leaf litter and dump sites. The collection method involved digging and hand sorting. Each earthworm was washed in water, then anaesthetized in 70% ethanol as. Specimen were preserved in the Cell Biology and Genetics laboratory at the DK Olukoya Central Research Laboratory, University of Lagos, Nigeria and stored at 98% ethanol at -20°C for DNA extraction.

Physicochemical analysis

The collected soil samples were processed by air-drying, grinding, and sieving through a 2 mm sieve to prepare them for analysis. For soil samples, they were shade-dried to a constant weight and sieved through a 2 mm stainless steel sieve, and clods were broken down with a mortar and pestle to ensure homogeneity. The analysis of physicochemical properties included determining temperature, pH and Electrical Conductivity (EC) using digital meters (Hanna Digital meter).

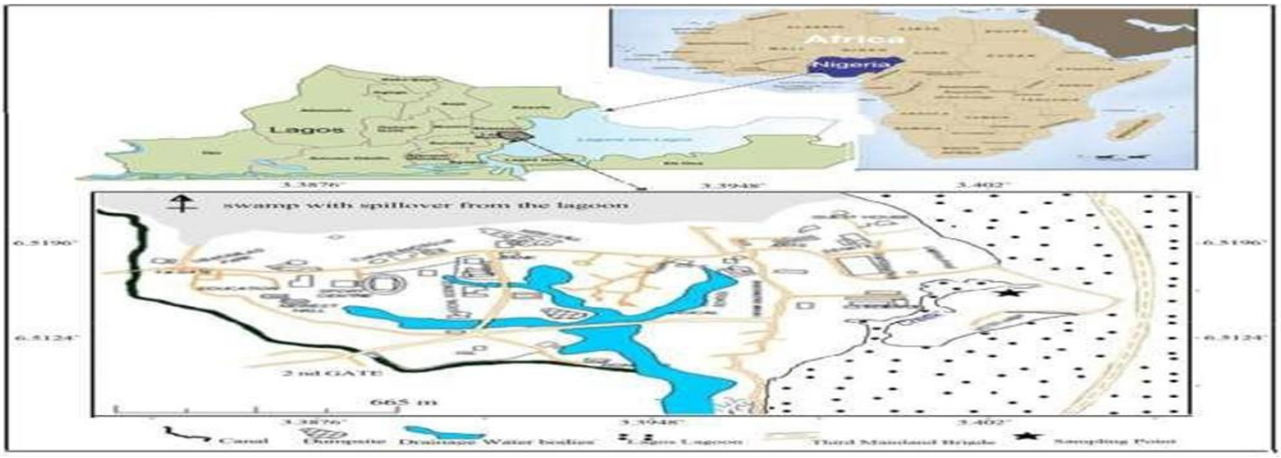

Figure 1: Map of Lagos State showing the study area

Table 1: Description of UNILAG, Iwaya, and Makoko

Study sites	Location	Description	Longitude	Latitude
1	UNILAG (Control)	A regulate Situated behind the new postgraduate school, opposite the social sciences d landfill	3.4005^0	6.5177^0
2	Iwaya	Swamp waste-dump in a slum neighborhood in Lagos State, Nigeria. Situated in the Yaba local council development area	3.3918^0	6.5057^0
3.	Makoko	Swamp dump located in a degraded environment within Makoko community	3.3880^0	6.4990^0

DNA Extraction and Polymerase Chain Reaction of RAPD Primers

Genomic DNA from earthworm muscle tissue was extracted by using modified phenol-chloroform protocol (Sambrook et al., 1989). The quantity and quality of extracted DNA were determined by measuring its absorbance value at 260 nm and estimating the ratio of absorbance values at 260 nm and 280 nm, using nanodrop spectrophotometer and 1% agarose gel electrophoresis respectively. Purified DNA was stored at -20°C till further analysis.

Initial analysis, which involved varying the DNA concentration from 10 ng to 50 ng, indicated that 25 ng of DNA yielded the highest number of reproducible bands. Consequently, 25 ng of DNA was used for all subsequent analyses. To optimize the RAPD reaction, different concentrations of Taq DNA polymerase, MgC_{l2}, and primer were tested, following the method outlined by Williams *et al.* (1990). The amplification reaction consisted of a 25 µl reaction mixture comprising AccuPower® PCR MasterMix (12 µl) containing Top DNA polymerase (1U), each dNTP (dATP, dCTP, dGTP, and dTTP) at a concentration of 250 µM, Tris-HCl (pH

9.0) at 10 mM, KCl at 30 mM, $MgCl_2$ at 1.5 mM, dH_2O at 12 µl, DNA template at 3 µl, and primer (forward and reverse) at 3 µl each. The amplification products were separated on a 2% agarose gel in a 0.5 X TBE buffer. Several optimizations were performed to determine the most suitable PCR profile. The RAPD profile consisted of an initial denaturation at 94°C for 3 minutes, followed by 45 cycles of 1 minute at 94°C, 1 minute at 35°C for annealing, and 2 minutes at 72°C for primer extension. A final extension step of 7 minutes at 72°C. The amplification products were analyzed on a 1.2% agarose gel containing ethidium bromide (0.5 µg/ml) using 1x TBE buffer. The gel was run at a constant voltage of 120 V for 2 hours, following the protocol described by Sambrook *et al.* (1989). The agarose gel was visualized and photographed under UV light using the Total Lab Gel Documentation System from New Zealand.

Table 2: RAPD Markers and Sequences utilized for PCR

s/n	NAME OF RAPD MARKER	MARKER SEQUENCE
1	OPA1	TGGCCTCACC
2	OPA7	AATCGGGCTG
3	OPA9	GAAACGGGTG
4	OPA13	GGGTAACGCC

Data Scoring and Analysis

To analyze the data, the resolved fragments on the gels were scored as 1 for presence and 0 for absence across the 30 of the earthworm genotypes. A similarity matrix was computed using the Squared Euclidean Distance (SED), which estimated all pairwise difference similarity matrices in the amplification product. The NTSYSpc version 2.02j software package (Rohlf, 1993) was used for sequential hierarchical clustering, and the obtained matrix from the gel scoring was further analyzed using Past 5.0. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram and principal component analysis (PCA) were computed using the aforementioned software

Results

Physico-chemical Parameters of Study Sites

Physicochemical parameters of samples collected from the three sites is shown on Table 1. Table 2, shows the physicochemical properties of the soils collected from the sampling sites. The analysis reveals that the UNILAG soil exhibited the highest temperature and pH (30.77^{o}C, 7.68), followed by Makoko (30.74 ^{o}C, 6.20). However, Iwaya had the highest turbidity value (86.3), followed by Makoko (51.9), and then UNILAG (41.6) having the lowest value.

Table 2: Physico-chemical parameters of study sites

Parameters	UNILAG	IWAYA	MAKOKO	WHO STANDARD	FEPA STANDARD
Temperature	30.77	28.96	30.74	20-50^{0}C	25
pH	7.68	6.04	6.20	6.5-8.5	6.5-8.5
Conductivity (m/Sc)	0.36	0.270	0.31	NS	NS
Turbidity (NTU)	41.6	86.3	51.9	NS	NS
Dissolved Oxygen (mg/L)	2.12	2.19	1.97	5	5
Total Dissolved Solids	0.22	0.16	0.25	NS	NS
Salinity	0.2	0.1	0.2	0.2	0.2

Note: NS= Not specified

Amplification Profile of the primer used

Table 3 presents the total number of DNA bands amplified from the fifteen earthworm samples obtained from the University of Lagos (UNILAG), Ilaje dumpsite, Makoko Lagos, as well as the number of polymorphic bands among them. The table shows the RAPD profile for the 30 earthworm genotypes. The number of amplification products obtained ranged from 7 to 22, with primer OPA1 producing the minimum number of bands and OPA13 producing the maximum number of bands.

Table 3: Amplification profile of RAPD primers used

S/N	Primer code	Nucleotide sequences	Number of Amplification products
1	OPA1	TGGCCTCACC	7
2	OPA7	AATCGGGCTG	9
3	OPA9	GAAACGGGTG	9
4	OPA13	GGGTAACGCC	22

Genetic relatedness among earthworms

A principal component analysis (PCA) was conducted on the four RAPD markers to provide a detailed visualization of the genetic relatedness among earthworm genotypes. In the dendrogram, E1, E2, and E3 stood out as distinct from the other genotypes, forming a separate cluster. The results obtained from the PCA were consistent with the dendrogram generated using the Nei method. Interestingly, the collections obtained from different parts of the collection site did not form well-defined distinct groups. Instead, they were interspersed with each other, indicating that there was no clear association between the RAPD pattern and the geographic origin of the accessions.

A summary of the Principal Component Analysis (PCA) for the RAPD primers is presented in Table 4. The table indicates that PC1 and PC2 contributed the most to the total percentage variance of the four RAPD primers, accounting for 60.88% of the variance. PC1 exhibited a variance of 36.54% and an Eigenvalue of 0.81, while PC2 displayed a variance of 24.34% and an Eigenvalue of 0.53. The PCA plot revealed that certain genotypes exhibited dispersion, indicating a wider genetic diversity (Figure 3). Additionally, some earthworm genotypes appeared to overlap with each other, suggesting redundancy within the genotypes.

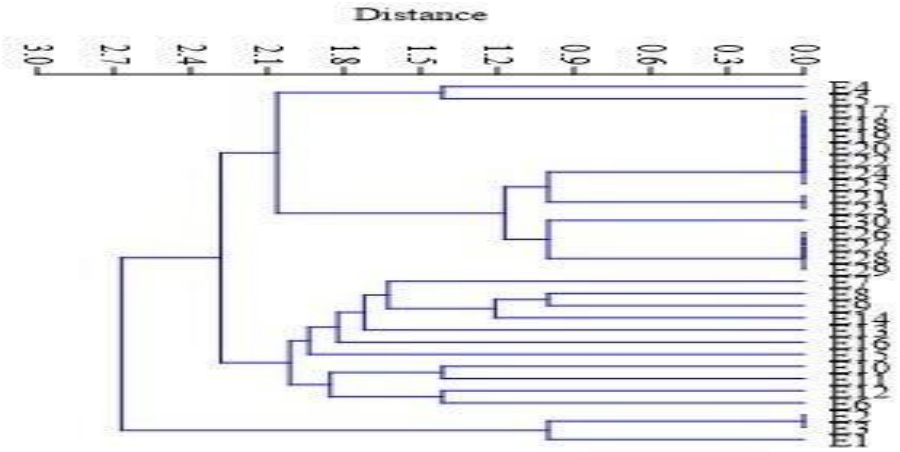

Figure 2: A UPGMA Cluster Analysis of the Earthworms using the four RAPD markers

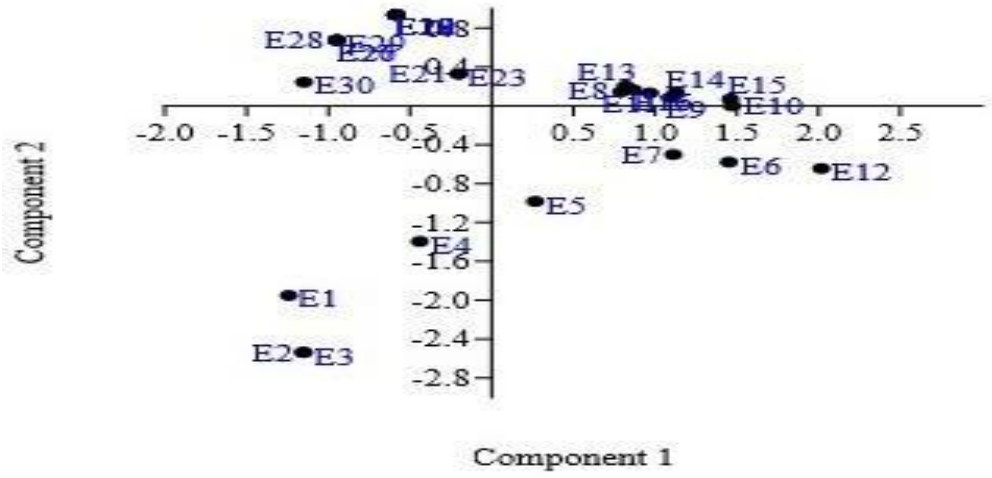

Figure 3: The scatter plot of the principal component analysis of the four RAPD primers

Table 4: Summary of the Principal Component Analysis of the RAPD primers

PC	Eigenvalue	% variance	Cumulative variance
1	0.81	36.54	36.54
2	0.53	24.34	60.88
3	0.19	8.60	69.48
4	0.15	6.98	76.46
5	0.12	5.63	82.09
6	0.11	4.97	87.06
7	0.07	3.36	90.42
8	0.05	2.43	92.85
9	0.04	2.22	95.07

Discussion

The results of this study indicate considerably high genetic diversity among the earthworms from the different dumpsites. Despite being obtained from dumpsites, which are presumed to be polluted sites, the earthworm genotypes in this study exhibited moderate diversity, indicating a broad genetic base. This was determined using the four RAPD markers employed. Distinct genotypes were observed in both the dendrogram and the PCA analysis, suggesting potential candidates for breeding earthworms or for vermiculture purposes, particularly for introducing them to polluted sites for remediation (Pavlicek *et al.,* 2006). These findings are consistent with a study by Keller *et al.* (2020), which reported genetic differentiation among earthworms across study sites in Vermont, even those located 0.6–13 km apart. These results support the idea that *L. terrestris* is a successful invasive earthworm species due to multiple introductions, which provided sufficient genetic variation for natural selection and local differentiation among locations in North America.

This study's results also agree with Sharma *et al.* (2011), who employed three primers and morphological markers to determine the level of genetic variation in individual earthworms. Sharma *et al.* (2011) reported the use of 62 molecular markers, including 10 RAPD, 10 ISSR, and 10 URPs markers for characterization, which exhibited 95.7%, 96.7%, and 98.3% polymorphism, respectively. The dendrogram generated from the DNA markers using the unweighted pair group method with arithmetic averages grouped all the isolates into two main clusters.

The abundance and diversity of earthworms in undisturbed habitats have been found to be higher compared to lands under constant use, such as cultivated land as well as dumpsites (Yadav & Mullah, 2017). Land management practices involving soil utilization are likely to positively or negatively affect earthworm abundance and diversity. These effects primarily result from changes in soil temperature, moisture, and the quantity or quality of organic matter (Yadav and Mullah, 2017). A considerably high percentage variance obtained from the PCA showed the diversity of the earthworms in the study area.

According to Singh and Gupta, (2014), factors such as tillage, single-crop cultivation, toxicants, soil acidification, and residue removal decrease earthworm abundance and diversity, while no-tillage management, crop rotation, liming, and organic amendments increase earthworm abundance and diversity all as different form of human activities have adverse effect on earthworm diversity. This is evident in the results obtained from this study

as moderately high genetic diversity was observed. In general, the greater the intensity and frequency of disturbance, the lower the population density or biomass of earthworms.

Conclusion

This study provides insights into the genetic diversity of earthworms in the study area, offering a valuable tool for ecotoxicological and ecological restoration purposes. The high diversity within the earthworm population studied has the potential to enhance vermiremediation of polluted or contaminated soil by harnessing the synergistic effects of earthworms, microorganisms, and plants to remove pollutants from the soil effectively. However, it is important to note that the population of earthworms can be adversely affected by soil contamination from organic pollutants, heavy metals, and acid precipitation. Furthermore, the high genetic diversity of earthworms can also enhance plant growth, as earthworm casts contain significantly higher levels of Phosphorus, Nitrogen, and organic Carbon compared to bulk soil (over 40-48% more).

Recommendations

Based on the findings of this study on the genetic diversity of earthworms in the study area, the following recommendations are made: The moderate genetic diversity observed in this study highlights the importance of conserving earthworm populations, especially in polluted or disturbed environments. Efforts should be made to preserve and protect the diverse range of earthworm genotypes found in the study area. The identified genotypes that showed promising characteristics for breeding or vermiculture purposes can be further explored for ecological restoration projects. These genotypes can be introduced to polluted sites to aid in the remediation of contaminated soil. Finally, continued monitoring of earthworm populations in the study area is recommended to track any changes in genetic diversity over time. Regular assessments can provide valuable information on the impact of environmental factors and land management practices on earthworm abundance and genetic variation.

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