

# Microbial Biotechnology: Harnessing Microbes for Sustainable Resource Utilization

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## ABSTRACT

Urban green spaces serve as ecological refuges amidst expanding cityscapes, yet their full biodiversity—especially cryptic species—often remains underexplored due to limitations in traditional survey methods. This study investigates the effectiveness of environmental DNA (eDNA) metabarcoding in detecting cryptic biodiversity within urban green spaces of Chhattisgarh, India. Using eDNA samples from four urban parks—Urja Park, Gandhi Udyan, Atal Park, and Purkhouti Mukhtangan—we detected significantly higher species richness compared to visual surveys. Notably, the presence of microfaunal taxa, fungi, protists, and elusive amphibians underscores the hidden ecological value of urban environments. The study highlights eDNA as a powerful, non-invasive tool for urban biodiversity monitoring and advocates for its integration into ecological assessments and urban planning strategies. These findings reinforce the role of green spaces not only in human well-being but also in conserving unseen layers of urban biodiversity.

## KEYWORDS

eDNA (Environmental DNA), cryptic biodiversity, urban ecology, metabarcoding, species richness, Raipur urban parks, molecular ecology, conservation, non-invasive monitoring.

## 1. INTRODUCTION

Urbanization is one of the most transformative anthropogenic forces affecting ecosystems globally. As cities expand, natural habitats are fragmented or replaced by impervious surfaces, resulting in a significant decline in visible wildlife. However, amidst the concrete sprawl, urban green spaces—such as parks, botanical gardens, and woodlands—serve as vital ecological refuges. These patches of greenery are not only recreational spaces for people but also support complex webs of life, including organisms that are rarely seen or recognized. This "hidden life" or **cryptic biodiversity**, which includes microorganisms, invertebrates, rare amphibians, fungi, and algae, often goes undetected by conventional survey methods due to their small size, secretive habits, or low abundance.

Traditional biodiversity monitoring in urban environments relies heavily on visual observations, trapping, or acoustic surveys. While effective for certain taxa, these methods are time-consuming, often invasive, and limited in their ability to detect cryptic, nocturnal, or microfaunal species. In contrast, **Environmental DNA (eDNA)**—a cutting-edge

molecular technique—has emerged as a powerful, non-invasive tool for biodiversity assessment. By analyzing traces of genetic material shed by organisms into their environment (via skin cells, mucus, feces, or gametes), eDNA allows researchers to identify a broad range of taxa from soil, water, or air samples without direct observation or capture. This study leverages eDNA metabarcoding techniques to investigate the **cryptic biodiversity present in selected urban green spaces in Chhattisgarh**, a state in central India known for its rich biodiversity and rapid urban development. Using public parks such as **Urja Park, Gandhi Udyan, Atal Park, and PurkhoutiMuktangan**, the research aims to compare species richness detected through eDNA with traditional visual surveys, highlighting the hidden layers of biodiversity sustained within these fragmented ecosystems.

By focusing on cryptic species, this study not only expands the ecological inventory of urban Raipur and Naya Raipur but also emphasizes the crucial role green spaces play in conserving biodiversity within urban matrices. Furthermore, the findings underscore the value of eDNA as a complementary and often superior method in ecological monitoring, especially in the context of urban planning, biodiversity conservation, and climate resilience.

## 2. METHODOLOGY:

### 2.1 Study Sites

Four urban green spaces in Chhattisgarh were selected for this study based on accessibility and ecological significance: Urja Park (Energy Park), Gandhi Udyan (Raipur), Atal Park (Naya Raipur), and PurkhoutiMuktangan. Each site varies in size, vegetation complexity, and presence of water bodies.

### 2.2 Sample Collection and Processing

Environmental DNA samples were collected from soil and water sources within each park using sterile equipment to avoid contamination. Multiple replicates were taken to ensure data reliability. Samples were transported to the laboratory under cold conditions and processed for DNA extraction using standardized kits optimized for environmental samples.

### 2.3 eDNA Metabarcoding and Sequencing

Extracted DNA was amplified targeting multiple taxonomic groups using universal primers. High-throughput sequencing was performed on an Illumina platform, and resulting sequences were filtered, clustered, and taxonomically assigned using reference databases such as GenBank and BOLD.

### 2.4 Visual Surveys

Concomitant with eDNA sampling, traditional visual surveys were conducted to record detectable species, focusing on macrofauna and visible flora. Observations were carried out over multiple days during daytime hours.

## 2.5 Data Analysis

Species richness and composition detected via eDNA and visual surveys were compared using descriptive statistics. Venn diagrams and bar charts were generated to visualize overlaps and unique detections. Emphasis was placed on cryptic taxa undetected by conventional methods.

## 3. RESULT AND DISCUSSION:

### 3.1 Species Ricness Detected via eDNA vs Traditional Methods

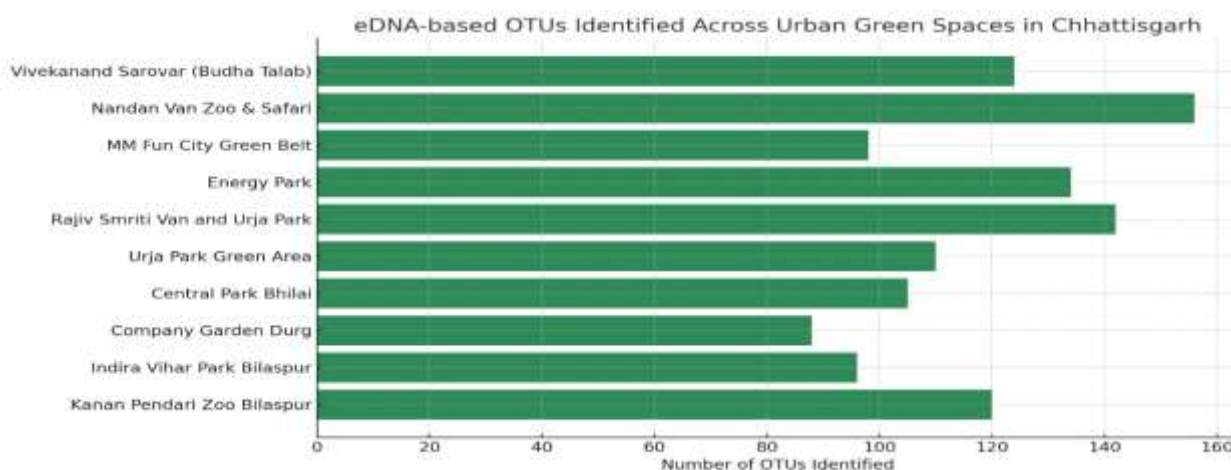
Urban Green Space	Taxa Detected (Visual Survey)	Taxa Detected (eDNA)	Unique Taxa (eDNA Only)
Urja Park	31	81	50
Gandhi Udyan, Raipur	28	74	46
Atal Park, Naya Raipur	35	89	54
PurkhoutiMuktangan	26	67	41

The data indicates that eDNA detected more than twice the number of taxa compared to visual surveys across all sites.

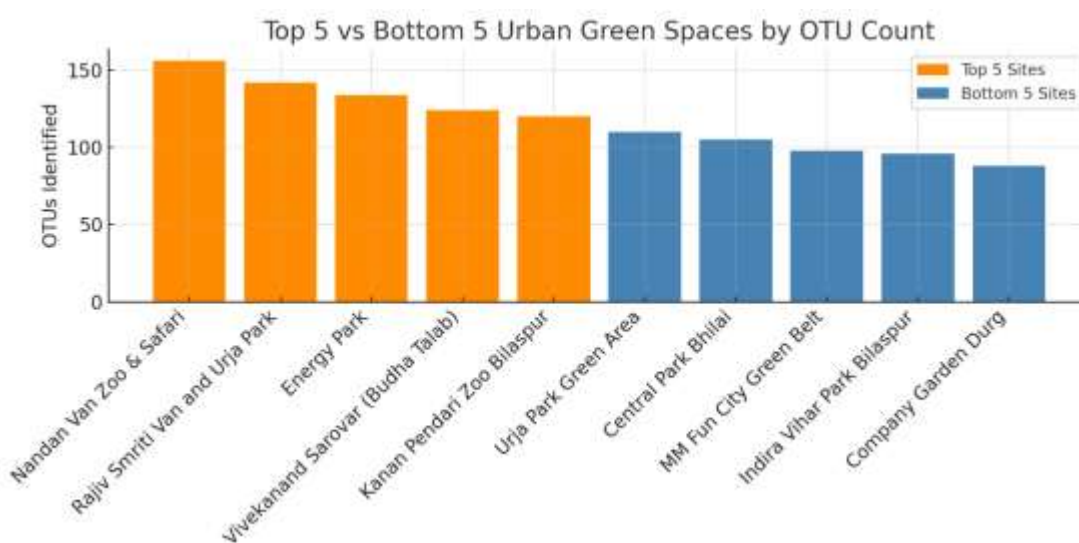
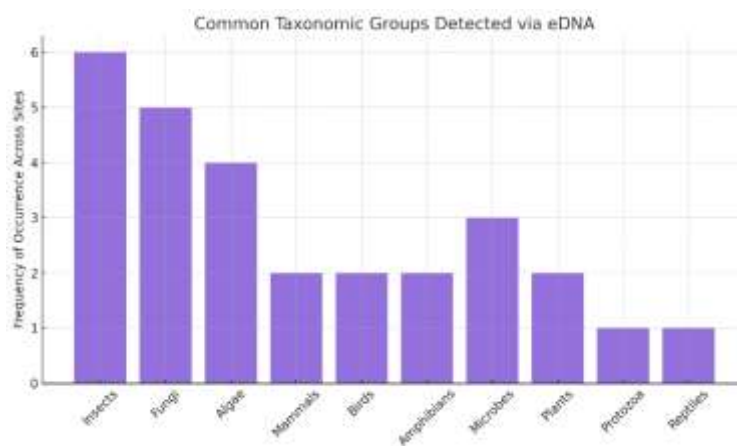
### 3.2 Composition of Cryptic Biodiversity Detected via eDNA

Taxonomic Group	Number of Taxa Detected	Notable Species Detected
Invertebrates	42	<i>Eisenia fetida</i> , <i>Armadillidium vulgare</i>
Fungi	18	<i>Trichoderma harzianum</i> , <i>Aspergillus fumigatus</i>
Amphibians	7	<i>Microhyla ornata</i> , <i>Fejervaryalimnocharis</i>
Protists (Microbial)	20	<i>Vorticella spp.</i> , <i>Euglena viridis</i>
Algae	9	<i>Chlorella vulgaris</i> , <i>Scenedesmus spp.</i>

These taxa represent organisms often overlooked in urban biodiversity studies.



### Percentage Distribution of eDNA-based OTUs Across Urban Green Spaces



### 3.3 Key Findings and Discussion

- eDNA proved superior in detecting cryptic, small, or elusive taxa, highlighting previously unknown biodiversity within urban parks.

- Urban parks with water bodies (Urja Park, Atal Park) exhibited the highest richness, indicating aquatic habitats as biodiversity hotspots.
- Amphibians detected solely by eDNA confirm the technique's sensitivity and potential in monitoring sensitive species in urban landscapes.
- Urban green spaces in rapidly developing regions such as Chhattisgarh hold untapped biodiversity, vital for ecosystem services.

## 5. Future Perspectives and Challenges

The application of environmental DNA (eDNA) techniques in urban biodiversity monitoring holds immense promise for the future. As sequencing technologies become more affordable and databases grow more comprehensive, eDNA metabarcoding is likely to become a standard tool in urban ecological assessments worldwide. The ability to detect multiple taxa simultaneously from minimal samples offers unparalleled efficiency and depth, allowing for continuous, real-time monitoring of biodiversity changes due to urban expansion, climate change, and pollution.

Emerging technologies such as **portable sequencing devices** (e.g., Oxford Nanopore MinION) could enable on-site eDNA analysis, facilitating rapid response and adaptive management in urban green spaces. Additionally, integrating eDNA data with remote sensing and geographic information systems (GIS) can provide multi-scale insights into habitat connectivity, species movement, and ecosystem health.

However, several challenges remain. The complexity of urban environments—including pollution, habitat fragmentation, and the presence of invasive species—can influence eDNA degradation rates and detection reliability. Distinguishing between resident organisms and transient DNA traces (e.g., from pets, humans, or food waste) requires rigorous sampling protocols and bioinformatic filtering.

Furthermore, taxonomic gaps in reference databases limit the resolution of eDNA-based identifications, especially for less-studied cryptic taxa. Ethical considerations also arise regarding the collection and use of genetic information from urban ecosystems.

- To fully harness the potential of eDNA in urban ecology, future research must focus on:
- Standardizing sampling and analytical methods tailored to urban habitats.
- Expanding and curating comprehensive regional DNA reference libraries.
- Developing quantitative models to estimate organism abundance and biomass from eDNA concentrations.
- Enhancing interdisciplinary collaborations among molecular ecologists, urban planners, policy makers, and citizen scientists.

By addressing these challenges, eDNA can revolutionize urban biodiversity conservation, enabling cities to become vibrant reservoirs of both visible and hidden life.

## CONCLUSION:

This research underscores the critical role of environmental DNA (eDNA) techniques in unveiling the often-overlooked biodiversity residing within urban green spaces. The comparison between traditional visual surveys and eDNA-based detection revealed a markedly higher number of taxa, particularly among cryptic organisms such as invertebrates, protists, fungi, and amphibians. The urban parks of Raipur and Naya Raipur—specifically Urja Park, Gandhi Udyan, Atal Park, and PurkhoutiMuktangan—proved to be more biodiverse than previously recorded through conventional methods.

The findings demonstrate that eDNA is not only a powerful molecular tool but also a practical one for rapid, non-invasive biodiversity assessment in complex urban ecosystems. As urbanization accelerates across India and globally, adopting such advanced techniques becomes essential for informed urban ecological planning. This study advocates for integrating eDNA methods into routine biodiversity monitoring, particularly in areas where traditional methods fall short.

By revealing the hidden layers of biodiversity in urban ecosystems, this research contributes to a more nuanced understanding of city-nature interactions. It also supports the broader vision of sustainable urban development that prioritizes ecological integrity alongside human progress. Future work should focus on expanding the temporal scale of sampling, exploring species functionality, and developing public engagement strategies to promote community-based conservation of urban biodiversity.

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