



## Review Article

# Toxicological and Biosafety Research on Biogenic Zinc Oxide Nanoparticles: A Bibliometric and Science-Mapping Analysis

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## ABSTRACT

**Background:** Biogenic synthesis reduces reliance on hazardous reagents but does not establish the safety of zinc oxide nanoparticles (ZnO NPs). This study mapped research trends and toxicological coverage in this field .

**Methods:** English-language Scopus research articles published through 2025 were screened using predefined criteria; 272 of 821 records were analyzed using Python-based bibliometric and science-mapping procedures.

**Results:** The articles appeared in 172 sources between 2014 and 2025, and annual output increased from 1 article in 2014 to 87 in 2025. International co-authorship was 39.71%, with India, Saudi Arabia, and Egypt leading country-associated production .*In vitro* biocompatibility or cytotoxicity dominated, whereas *in vivo* systemic toxicity, hemocompatibility, and genotoxicity were less frequent.

**Conclusion:** The evidence base requires standardized characterization, normal-system comparators, exposure-relevant dosimetry, repeated-dose assessment, and cross-model validation. Green synthesis should be treated as a production route rather than evidence of safety.

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## Introduction

**Z**inc oxide nanoparticles (ZnO NPs) are used in antimicrobial materials, drug delivery, wound care, agriculture, food packaging, cosmetics, and environmental remediation [1]. Their optical activity, surface reactivity, zinc-ion release, and capacity to generate reactive oxygen species support these applications but can also produce cell injury, inflammation, DNA damage, and organism-level effects [2]. Toxicological behavior depends not only on nominal dose but also on particle size, morphology, aggregation, surface chemistry, dissolution, biological medium, and exposure duration [3].

Biogenic or green synthesis uses plants, microorganisms, algae, fungi, or biologically derived materials as reducing, stabilizing, or capping agents. This approach may reduce the use of hazardous reagents and alter nanoparticle surface chemistry [4]. However, a biologically derived coating does not guarantee biological safety. Early work already paired biosynthesis with safety evaluation [5], while later studies used cyanobacterial extracts, fruit residues, plant leaves, normal cells, blood compatibility, aquatic organisms, and animal models to evaluate different aspects of risk [6–10]. These studies vary in synthesis routes, particle characterization, biological models, dose metrics, and endpoint selection.

Existing bibliometric studies commonly emphasize green synthesis, biomedical applications, or general research on ZnO. A focused mapping of toxicological and biosafety evidence is needed to distinguish therapeutic cytotoxicity from unintended toxicity and to identify neglected safety endpoints. This study therefore assessed publication growth, leading contributors, citation patterns, conceptual structure, temporal development, and the distribution of toxicological endpoints in research on biologically synthesized ZnO NPs.

## Methods of Literature Search

### Study design, search strategy, and eligibility

We used a descriptive bibliometric and science-mapping design to characterize the development, performance, geographic distribution, conceptual structure, and toxicological scope of research on biologically synthesized ZnO NPs [11]. We selected Scopus because of its structured bibliographic metadata and suitability for bibliometric analysis [12]. The search was conducted on September 9, 2026, and

restricted to English-language research articles published through 2025. No lower publication-year limit was imposed; the eligible articles covered 2014–2025. Search terms represented three concepts: ZnO nanoparticles, biological or green synthesis, and toxicological or safety evaluation. The complete query is provided in Supplementary Table S1.

Eligible studies produced ZnO NPs through a biological, biogenic, or green synthesis route and addressed at least one evaluable toxicological or safety-related endpoint. We excluded reviews and other non-original publications, as well as records in which ZnO NPs were not central, biological synthesis was not demonstrated in the title or abstract, or safety relevance was not supported by an evaluable endpoint. We excluded cancer-directed cytotoxicity studies unless they included a normal-cell, organism-level, hemocompatibility, genotoxicity, biodistribution, or systemic-safety component. We screened titles and abstracts using these predefined criteria, and we documented record-level decisions and reasons for exclusion. We summarized the selection process using a PRISMA-style flow diagram [13]. Detailed eligibility criteria and screening decisions are provided in Supplementary Tables S2 and S3. Of 821 records identified, 272 were retained.

### Data extraction and cleaning

Extracted fields included year, source, authors, author identifiers, citations, cited references, affiliations, abstracts, and author and index keywords. Duplicates were checked using DOI, Scopus EID, and normalized titles. A controlled thesaurus standardized capitalization, punctuation, spacing, singular and plural forms, spelling variants, and unambiguous abbreviations. Conceptually equivalent terms, including “ZnO NPs,” “ZnO nanoparticles,” and “zinc oxide nanoparticles,” were consolidated, whereas distinct concepts were retained. The same cleaned author-keyword vocabulary was used for the network and temporal-overlay analyses. Scopus author and institutional names were retained; therefore, residual homonymy, profile fragmentation, and institutional-name variation may have affected productivity estimates. The complete thesaurus is provided in Supplementary Table S4.

### Bibliometric analysis and science mapping

We performed analyses using Python 3.12 with pandas 2.2, NumPy 2.3, Matplotlib 3.10, and scikit-learn 1.8. The exact package versions used in the analysis are reported in Supplementary Table S6 [14]. Descriptive indicators included annual production,

productive sources and authors, citations, country participation, international co-authorship, and highly cited articles. Author productivity was calculated from Scopus full-name entries. Country participation used non-exclusive full counting, whereby an internationally co-authored article was counted once for each represented country [15]. International co-authorship was defined as affiliations from more than one country within an article.

The author-keyword network also used full counting. Keywords occurring in at least five articles were eligible, and links were retained when two terms co-occurred in at least two articles. The weighted co-occurrence matrix was projected using spectral embedding, and six keyword communities were identified by k-means clustering with a random seed of 18 and 20 initializations. Node size represents keyword occurrence, link width represents co-occurrence frequency, cluster colors identify keyword communities, and temporal-overlay color represents mean publication year. Network parameters and underlying data are provided in Supplementary Tables S6 and S8.

### Toxicological endpoint classification

Articles were classified from titles, abstracts, and author keywords into seven non-exclusive categories: (1) *in vitro* biocompatibility or cytotoxicity; (2) hemocompatibility; (3) genotoxicity; (4) mechanistic toxicity; (5) *in vivo* systemic toxicity; (6) ecotoxicity; and (7) other safety-related endpoints. We used

predefined textual rules for classification (Supplementary Table S5), and article-level assignments are reported in Supplementary Table S9. Because categories were non-exclusive, their combined frequency could exceed the number of included articles. The resulting frequencies describe thematic emphasis rather than methodological quality, toxicological potency, effect magnitude, or quantitative risk.

## Results

The Scopus search identified 821 records. Following title and abstract screening, 549 records were excluded for not meeting the predefined eligibility criteria. The final bibliometric corpus therefore comprised 272 research articles published between 2014 and 2025 (Figure 1).

The 272 articles were published across 172 sources and involved 1,537 unique Scopus author-name entries. The corpus accumulated 7,781 citations, averaging 28.61 citations per article. Most publications were multi-authored, with only two single-authored articles. International co-authorship accounted for 39.71% of the included publications, indicating a moderate level of cross-country research collaboration. Table 1 summarizes the principal characteristics of the bibliometric corpus.

Annual production rose from one article in 2014 to 87 in 2025; 76.8% appeared during 2022–2025 (Figure 2).

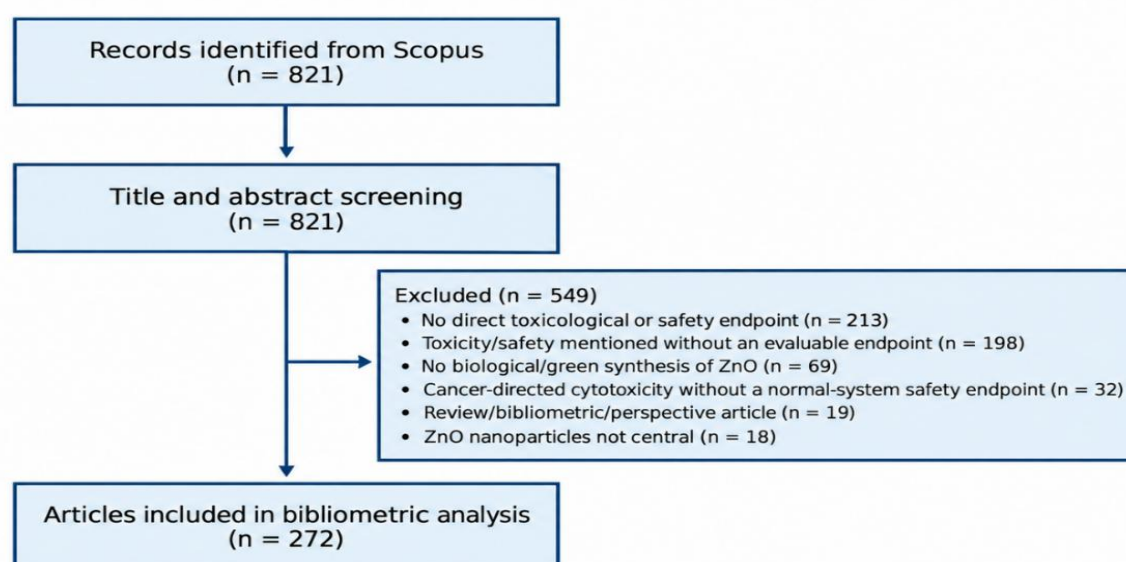
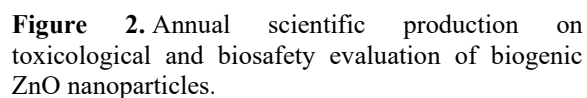


**Figure 1.** Study selection flow for the Scopus corpus.

| Indicator                     | Value     |
|-------------------------------|-----------|
| Publication period            | 2014-2025 |
| Included articles             | 272       |
| Publication sources           | 172       |
| Unique author-name entries    | 1,537     |
| Total citations               | 7,781     |
| Average citations per article | 28.61     |
| International co-authorship   | 39.71%    |
| Single-authored articles      | 2         |



| Country      | Country-associated publication frequency |
|--------------|------------------------------------------|
| India        | 114                                      |
| Saudi Arabia | 60                                       |
| Egypt        | 50                                       |
| Iran         | 30                                       |
| China        | 25                                       |
| South Korea  | 21                                       |
| Pakistan     | 18                                       |
| Iraq         | 16                                       |
| Malaysia     | 9                                        |
| Turkey       | 9                                        |

The author-keyword network contained 24 terms and 71 links. Green synthesis and ZnO NPs were central, while cytotoxicity, biocompatibility, oxidative

*In vitro* biocompatibility or cytotoxicity appeared in 190 articles, ecotoxicity in 96, mechanistic toxicity in 87, *in vivo* systemic toxicity in 52, hemocompatibility in 22, genotoxicity in 20, and other safety-related endpoints in 14. Counts were non-exclusive (Figure 6).

The rapid growth of publications after 2021 indicates increasing interest in whether environmentally preferable synthesis translates into biological safety. Nevertheless, the geographical concentration of research and the observed international co-authorship do not necessarily reflect harmonized multicountry testing, highlighting the need

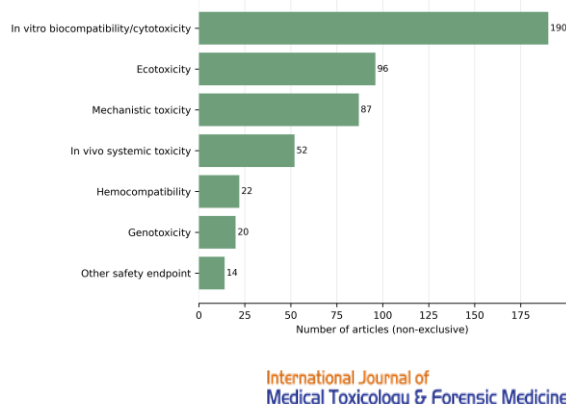


**Figure 6.** Distribution of toxicological and safety themes; counts are non-exclusive.

for shared reference materials and interlaboratory protocols. Short-term *in vitro* assays dominated the evidence base, yet nanoparticle–assay interactions can distort viability measurements. For colorimetric assays such as MTT and crystal violet, investigators should test each ZnO NP formulation across the intended concentration range in cell-free wells containing particles with and without assay reagents or stain. These controls can reveal particle absorbance, direct reagent reactions, and effects on dye recovery. Where interference is detected, results should be checked with an independent endpoint, such as direct cell counting or imaging, after confirming that the alternative method is also compatible with the particles. Assay conditions and interference-control results should be reported alongside cell-viability data [16]. Moreover, cytotoxicity against cancer cells reflects therapeutic efficacy rather than safety; appropriate evaluation requires concentration–response analysis, relevant exposure durations, non-malignant comparators, and selectivity indices. Particle-mediated effects should also be distinguished from those caused by dissolved  $\text{Zn}^{2+}$  and biologically derived surface coatings because ZnO toxicity is strongly associated with dissolution and oxidative stress [17]. Systemic, hematological, and genetic endpoints remained comparatively underrepresented. Cell-viability assays cannot reproduce processes such as aggregation, dissolution, protein adsorption, biodistribution, clearance, and organ accumulation [18]. Blood-contacting applications therefore require direct hemolysis assessment [19], whereas genotoxicity should be examined using validated DNA-damage endpoints [20]. Ecotoxicological evidence was also heterogeneous, and intended larvicidal, antimicrobial, or antiparasitic efficacy should not be equated with unintended toxicity toward non-target organisms. Environmental studies should characterize nanoparticle behavior in the exposure medium and account for agglomeration, dissolution, medium composition, and effective exposure concentrations, including dissolved  $\text{Zn}^{2+}$  where analytically feasible [21].

Biogenic synthesis should consequently be regarded as a manufacturing approach rather than a safety designation. Biological capping agents can influence nanoparticle formation, aggregation, surface charge, dissolution, and biological interactions, while differences among biological sources and synthesis conditions may affect material consistency [22]. Claims that biogenic ZnO nanoparticles are safer therefore require comparison with conventionally synthesized particles with comparable physicochemical characteristics, tested under equivalent exposure conditions. Minimum reporting should include primary and hydrodynamic particle size, size distribution, morphology, crystal phase, surface charge, chemical composition, surface-associated biomolecules, aggregation, dissolution, and stability in the relevant medium [23]. Nominal mass concentration should, where technically feasible, be complemented by  $\text{Zn}^{2+}$  release, particle number or surface area, exposure duration, and estimated delivered cellular dose [24]. Based on the identified gaps, we propose a tiered framework progressing from interference-controlled *in vitro* screening to normal-cell, blood-compatibility, oxidative-stress, and DNA-damage assays, followed by fit-for-purpose organism-level evaluation of biodistribution, clinical pathology, repeated exposure, and recovery. Environmental applications additionally require non-target organisms and realistic exposure scenarios. The present study supports these priorities by distinguishing therapeutic cytotoxicity from unintended toxicity and integrating performance indicators, science mapping, and endpoint classification. However, its findings remain subject to Scopus-only and English-language coverage, metadata-based screening, non-exclusive endpoint categories, author-name ambiguity, citation-age bias, and sensitivity of network results to data cleaning and analytical thresholds. Accordingly, the results describe the development and thematic emphasis of the literature rather than the prevalence, magnitude, or causality of ZnO nanoparticle toxicity.

## Conclusion

Research on the biosafety of biogenic ZnO NPs expanded rapidly during 2014–2025, but the literature remained dominated by short-term *in vitro* testing. Green synthesis alone cannot establish safety. Researchers should characterize nanoparticles in the relevant exposure medium, assess assay interference, distinguish particle effects from dissolved  $\text{Zn}^{2+}$ , and include normal-system comparators alongside efficacy models. Studies intended to support biomedical or environmental use should extend testing to relevant systemic, genetic, or non-target-organism endpoints. For journals, funders, and research groups, consistent reporting of material properties, exposure conditions,

and safety endpoints would make findings easier to compare and identify evidence gaps. These priorities provide a practical basis for evaluating biosafety claims; this bibliometric analysis does not, in itself, determine whether biogenic ZnO NPs are safe.

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## Conflicts of Interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript. All authors contributed to the manuscript and approved the final version.

## Ethical Considerations

Not applicable.

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