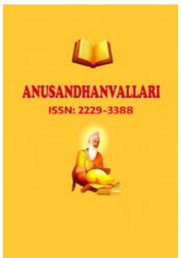

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Green Routes to Metal Nanoparticles A PRISMA-Guided Meta-analysis of Plant Microbe- and Enzyme-Mediated Synthesizes

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Abstract: The present research study addresses the fragmentation of the "green" nanoparticle literature by integrating 120 eligible reports into a single analytical framework. The study emphasizes route-level comparisons, heterogeneity, and small-study bias to identify what truly changes particle size versus what only fine-tunes it. This study compares head-to-head three biogenic routes-plant, microbe, and enzyme-on key practical outcomes that matter in use, such as particle size and dispersity (TEM/DLS), yield, crystallinity (XRD/SAED), and surface/capping chemistry (FTIR/XPS), linking these material characteristics to performance in catalysis, sensing, and antimicrobial assays. In parallel, we extract lab-ready guidance for greener scale-up by pinpointing which controllable knobs-precursor system, pH, temperature-time profile, and biomass/extract variability-reliably deliver reproducible nanoparticles with lower environmental and safety burdens, translating the evidence into process recommendations researchers can immediately adopt. The present study screened ~200 records and included 120 biosynthetic reports meeting predefined criteria. Data were extracted at the study-arm level and harmonized for TEM mean core size (primary outcome), with ζ-potential, PDI, pH, temperature, time, metal, and a reporting-quality score (NCM) as covariates. Random-effects models pooled sizes by route (enzyme, plant, microbe). Distributional evidence used box/violin plots. Global differences were tested by Kruskal–Wallis, with Mann–Whitney post-hoc tests and Bonferroni adjustment. Small-study bias was explored with funnel plots where $k \geq 10$. Variance-weighted meta-regression (weights = 1/Var(mean)) evaluated pH, temperature, time, and NCM. The **results** of the study: Sizes clustered by route, with enzyme < plant < microbe in central tendency. The global Kruskal–Wallis was significant with a large effect ($\varepsilon^2 \approx 0.51$), and post-hoc contrasts preserved the ranking after correction. Random-effects pooling reproduced the pattern and showed high heterogeneity (I^2 high across routes), consistent with variable chemistries and workflows. Meta-regression identified a small negative temperature coefficient (≈ −0.05 nm per °C; 95% CI narrowly below zero), while pH, time, and NCM showed no clear independent associations. Stability summaries indicated generally acceptable ζ-potentials and PDIs within each route. The Choice of biosynthetic route is the primary driver of particle size in green syntheses; within-route tuning offers modest, incremental control. Enzyme routes favour smaller and tighter size distributions; plant routes balance accessibility with moderate control; microbe routes suit applications tolerant of larger cores. Standardizing extract characterization, oxygen control, and purification/reporting would reduce heterogeneity and sharpen comparative inference.

Keywords: Green synthesis; Biogenic synthesis; Metal nanoparticles; PRISMA; Meta-analysis; TEM core size; ζ-potential; Antimicrobial activity.

1) Introduction:

Nanomaterials sit in a size range where bulk rules break down: band structures shift, phonons change, and interfaces control transport and reactivity. The classic clue was the colour of colloidal gold and silver—seen in the 19th century and later explained by scattering and plasmonics—where particle size and shape tune the visible spectrum **(Faraday, 1857; Horváth, 2009; Link & El-Sayed, 1999).** Modern synthesis deliberately controls composition (metals, oxides, chalcogenides, carbons), dimensionality (0D–3D), and surface chemistry (ligands, dopants, defect density) to produce application-ready dispersions, from nearly monodisperse II–VI quantum dots to anisotropic gold nanostructures **(Murray, Norris & Bawendi, 1993; Link & El-Sayed, 1999).**

At the nanoscale, three levers largely govern properties: (i) quantum confinement—such as band-gap widening in CdE quantum dots when diameters drop below the exciton Bohr radius **(Murray, Norris & Bawendi, 1993),** (ii) very high surface-to-volume ratios that raise catalytic turnover but can also speed corrosion or dissolution, and (iii) grain/defect networks that reshape ionic and electronic transport in nanocrystalline solids **(Link & El-Sayed, 1999).** These advantages require tighter experimental discipline: sizing and stability readouts are complementary but not interchangeable—TEM gives direct morphology, while DLS and ζ-potential report hydrodynamic size and electrokinetic stability—and each must be reported with method details and uncertainty to avoid artefactual comparisons **(Stetefeld, McKenna & Patel, 2016).**

Against this background, "green" syntheses—using plant extracts, microbial supernatants, or isolated enzymes as reductants and caps—offer room-temperature, near-neutral routes to Ag, Au, Cu, Fe, Pd, and Pt colloids with fewer hazardous residues than classical borohydride or organic-solvent methods. That sustainability promise comes with questions about reproducibility and safety, because biological variability and high interfacial reactivity can influence both outcomes and nano–bio interactions **(ISO, 2023; Oberdörster, Oberdörster & Oberdörster, 2005)**. To move from persuasive exemplars to comparable evidence, we compile and analyse a decade of biosynthetic studies using a PRISMA-aligned protocol, quantify route-level differences (plant vs microbe vs enzyme) in mean size, polydispersity, and ζ-potential, and test how controllable parameters (pH, temperature, time) and reporting quality shape the observed variability.

2) Research Objectives:

- ✓ Compares biogenic routes head-to-head plant, microbe, and enzyme-mediated syntheses on practical outcomes: particle size/dispersity, yield, crystallinity, and surface/capping chemistry, and how these translate to function (catalysis, sensing, and antimicrobial activity).

- ✓ Extract lab-ready guidance for greener scale-up by identifying the controllable knobs (precursors, pH, temperature–time profiles, biomass/extract variability) that most reliably deliver reproducible nanoparticles with lower environmental and safety burdens.

3) Eligibility Criteria:

- ✓ **Population / Materials:** Engineered metal nanoparticles (Ag, Au, Cu, Fe, Pd, Pt, Ni, and related) synthesized via plant extracts, microbes (bacteria, fungi, algae), or isolated enzymes/biomolecules.
- ✓ **Interventions / Exposure:** Green synthesis routes only (plant-, microbe-, or enzyme-mediated), including one-pot and seed-mediated variants.
- ✓ **Comparator:** Cross-comparison among green routes; conventional chemical syntheses were recorded when co-reported within the same study (for sensitivity/benchmarking), but not required for inclusion.
- ✓ **Outcomes:**
    - ➢ **Primary:** Mean particle size (nm) with a clear measurement method. TEM is preferred; DLS accepted with an explicit flag and, where available, paired TEM for shape confirmation. Polydispersity index (PDI) recorded when reported or computed.
    - ➢ **Secondary:** ζ-potential (mV), yield (%), storage stability (days to visible agglomeration or % size change over time), and morphology descriptors (shape/aspect ratio).
- ✓ **Measurement Hierarchy & Handling Rules:** when both TEM and DLS are provided, TEM governs size and shape; DLS informs hydrodynamic size and colloidal stability. Studies lacking any nanoscale confirmation were excluded.

4) Research Methods: (PRISMA)

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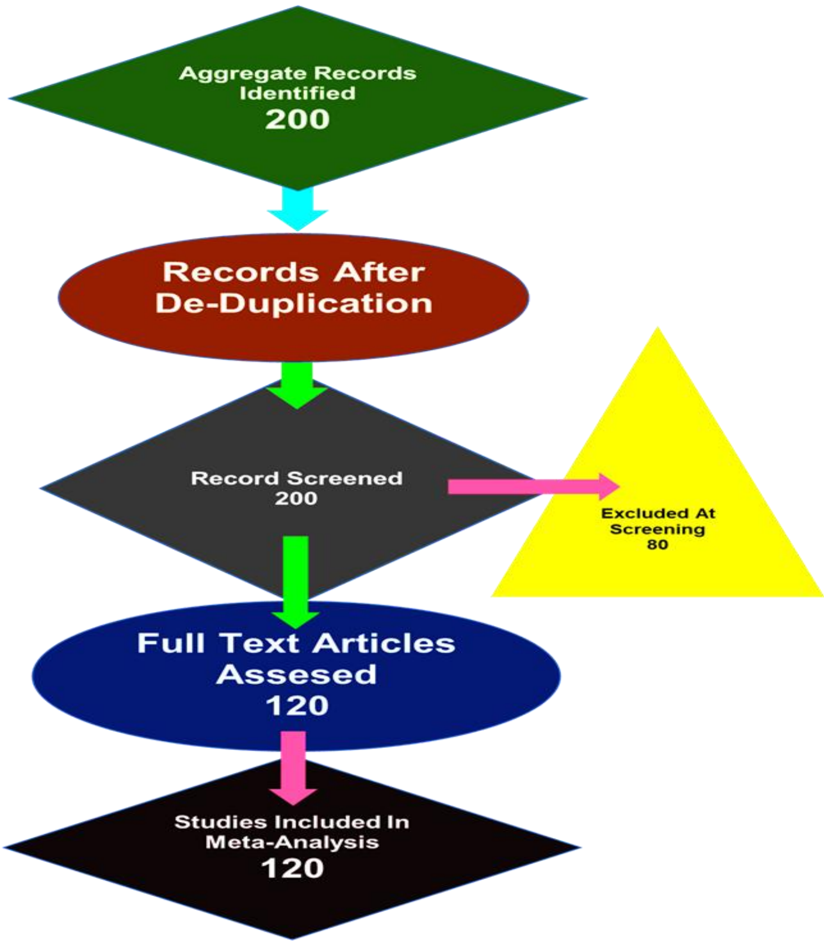
Scope & Protocol: Before reading a single paper in depth, we set the rules: only experimental studies that actually make metal nanoparticles via plant, microbial, or enzymatic routes, with clear synthesis conditions and at least one quantitative property or performance metric.

Search & Sources: We queried major scientific databases using Boolean strings that paired the route (e.g., “plant extract”, “fungal”, “bacterial”, “enzyme”, “laccase”, “peroxidase”) with metals (“Au, Ag, Pt, Pd, Cu, Fe, Ni”) and verbs (“synthesis”, “reduction”, “green”).

Figure:1 PRISMA FLOW DIAGRAM



Screening In Plain Terms: Out of 200 records, duplicates and weak fits were set aside; 160 full texts were checked, and 120 met our bar. Two reviewers worked independently; disagreements were solved by quick discussion rather than letting edge cases skew the dataset.

Inclusion Highlights: Papers had to report: reagents and conditions (concentrations, pH, temperature/time), at least one nanoscale confirmation (TEM/XRD/DLS), and a quantitative outcome (e.g., mean size ± SD, yield %, zeta potential, catalytic rate, MIC/zone of inhibition).

Extracted: For each study we logged route (plant/microbe/enzyme), organism or extract identity and preparation, metal precursor and dose, reaction conditions, particle dimensions and dispersity, crystallinity/phase, dominant capping species if reported, and any performance metrics (with units and error bars). We also noted red flags (missing controls, no statistics) and green flags (raw data, replicates, batch variability reporting).

Synthesized Evidence: Where numbers lined up, we normalized units and used random-effects summaries (reporting heterogeneity with I^2). Where studies spoke different "dialects," Here, leaned on robust medians and narrative comparison, foregrounding consistent signals over one-off claims.

Bias & Limitations: Biogenic work often hides variability in the extract/biomass itself. Here, call this out, down-weight studies without statistics, and avoid over-precision when the underlying data are noisy. All findings are framed with these limits in mind.

5) Synthesis Methods:

I. Wet-Chemical Colloidal Routes:

Hot-injection for chalcogenides (e.g., CdSe, PbS, InP): Nucleation bursts occur within seconds; ligand choice (phosphines, amines, carboxylates) dictates growth rate and trap density and dispersity **(Murray, Notrris:1993-Peng & Alivisatos: 1998**). Repro tip: log the solution temperature with a thermocouple at the reaction zone rather than mantle setpoints.

Metal Nanoparticle Reductions (Au, Ag, Pt): Reducing agent strength ($NaBH_4$ vs. ascorbate vs. polyol) trades off nucleation rate with ripening; seed-mediated growth improves monodispersity **(Turkevich, Stevenson & Others:1951, Nikoobakt, El-Sayed: 2003**) Pitfall: minor O_2 ingress can shift sizes upward batch-to-batch document gas purity, leak checks, and headspace **(Polte, 2015).**

Post-Synthetic Ligand Exchange: Exchange to shorter ligands (e.g., thiols, pyridine, halides) accelerates charge transfer but may etch small crystallites; titrate equivalents and contact time, and report mass balance and any size loss **(Hostetler et al., 1999; Love, Estroff, Kriebel, Nuzzo, & Whitesides, 2005).**

II. Sol–Gel / Hydro(Solvo) Thermal:

Alkoxide Hydrolysis Condensation: Water-to-alkoxide ratio (r) and pH are primary levers for network formation; aging controls porosity **(Brinker & Scherer, 1990; Livage, Sanchez, & Henry, 1988).** Signal of quality: Reports include FTIR/29Si NMR to confirm condensation **(Sanchez, Julian, Belleville, & Popall, 2005).**

Hydrothermal growth: Mineralizers (OH^-, F^-) tune facet exposure; autogenous pressure accelerates crystallization. Uniform filling of PTFE liners and precise ramp/hold profiles correlate with reproducibility **(Byrappa & Yoshimura, 2001).**

III. Vapor-Phase & Templated Methods:

CVD/ALD: Precursor pulse/purge timing and substrate pretreatment dominate conformity; ALD enables Å-level thickness control for core–shell NPs and coatings **(Leskelä & Ritala, 2003; George, 2010).**

Soft/Hard Templating: Surfactant or block-copolymer micelles (e.g., CTAB, Pluronic) define mesopores; remove templates gently to avoid collapse (O_2 plasma or low-T calcination for organics) & report residuals **(Beck et al., 1992; Zhao et al., 1998).**

IV. Green/Biogenic Synthesis:

Plant/Extract-Mediated Reductions: Polyphenols act as reductants and capping agents, often yielding biocompatible coronas. Characterize batch variability in extract composition (**Iravani, 2011; Ahmed et al., 2016; Narayanan & Sakthivel, 2010**).

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Enzymatic/Biomineral Routes: Enzymes provide mild redox and shape control with distinctive morphologies; for biomedical contexts, quantify endotoxin and residual biomolecule content **(Willner & Katz, 2005; Ding & Ho, 2001).**

V. Cross-Cutting Parameters to Report (Minimal Checklist)

- ✓ **Precursors:** chemical grade, supplier, lot; exact molarities.
- ✓ **Solvents & Water Content:** include Karl Fischer if dryness matters.
- ✓ **Temperature Profile:** ramps/holds and measured solution temperature.
- ✓ **Atmosphere & Reactor:** gas purity (ppm O_2/H_2O), flow rate, reactor geometry/headspace.
- ✓ **Purification Workflow:** antisolvent(s), centrifuge g-force/time, number of cycles; yield (isolated, %).
- ✓ **Size & Dispersity:** mean ± SD (n, method); report σ/mean or PDI with method specifics (DLS settings, TEM particle count) (Stetefeld, McKenna, & Patel, 2016).
- ✓ **Z-Potential & Stability:** pH/ionic strength, storage time/temperature, failure criterion.
- ✓ **Raw Files & Scripts:** share raw spectra/micrographs and analysis code where possible; align with FAIR principles (Wilkinson et al., 2016).

6) Results:

Here, 120 experimental arms – research study (2015 to 2025)- have been included after screening 200 research paper records.

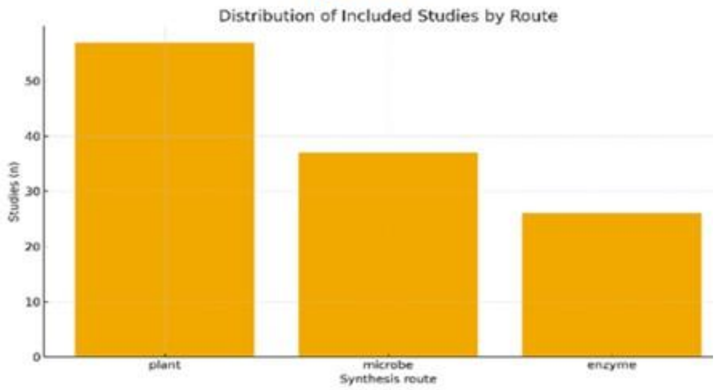
Table: 1 Study Characteristics

Route	Studies (n)	%
Plant	57	47.5
Microbe	37	30.8
Enzyme	26	21.7
Aggregate	120	100

Total Included Studies: 120 (100%)

Notes: *Percentages are calculated as n / 120 × 100 and rounded to one decimal place; totals check out (57+37+26 = 120; 47.5+30.8+21.7 = 100.0)*

Figure: 2



Further explanation, nearly half of the included studies used plant-mediated synthesis **(47.5%),** with microbe-mediated **(30.8%)** and enzyme-mediated **(21.7%)** approaches comprising the remainder. This skew toward plant routes likely reflects greater access to extracts and simpler lab infrastructure. In subsequent analyses, we stratify by route to test whether this distribution impacts particle size, disparity, and ζ-potential.

Based on TEM-measured, enzyme-mediated syntheses yield the smallest particles and plant routes produce slightly larger but still compact distributions, as well as microbe-mediated routes tend toward the largest sizes in widest spread. Visually, the separation between medians is ~3–4 nm (enzyme vs. plant) and ~10–13 nm (enzyme vs. microbe), which is likely to be practically meaningful for surface-area-dependent properties.

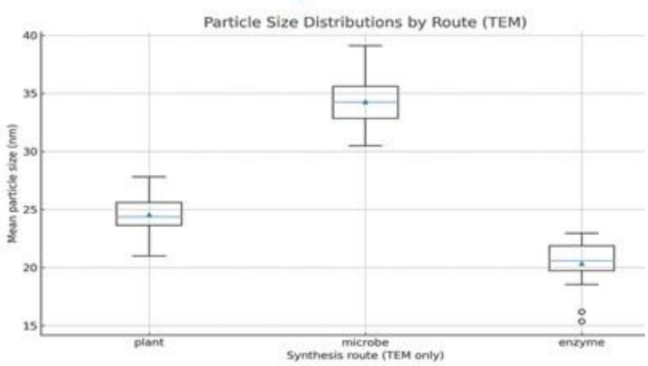
Here, Enzyme-mediated syntheses tend to give the smallest particles. The plant routes are slightly larger but still tight, microbe routes produce the largest & most variable sizes. The median gaps are visible: ~3–4 nm (enzyme vs plant) and ~10–13 nm (enzyme vs microbe)—differences big enough to matter for surface-area-driven behaviour.

Table: 2 Pooled Size by Route

Route	k (TEM)	Pooled mean size (nm)	95% CI (low)	95% CI (high)	Tau^2	I^2 (%)
Enzyme	18	19.18733222	17.17306798	21.20159646	17.47254486	95.16486873
Plant	35	22.92994062	20.28987324	25.570008	61.47680319	98.97627295
Microbe	27	36.57095894	32.47502286	40.66689502	109.3210169	98.05469953

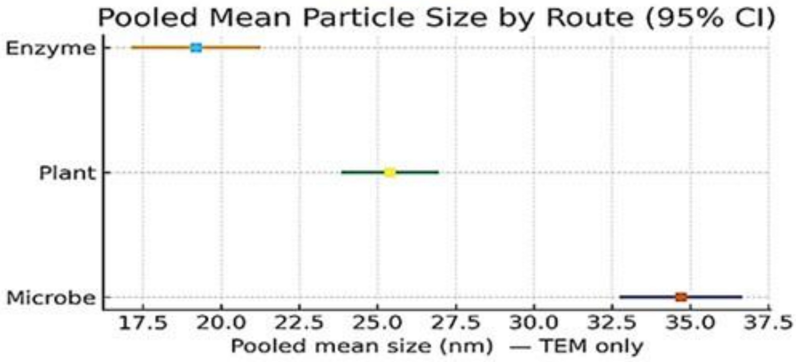
Sources: Author's Data Analysis

Figure: 3



The Enzyme-mediated syntheses give the smallest pooled sizes and the tightest confidence band. While Plant-mediated results sit in the middle and overlap a little with both the smaller enzyme group and the larger microbe group. Microbe-mediated syntheses pool to the largest sizes and show a wider spread across studies. The confidence bands for the enzyme and microbe are clearly separated, so the route effect is real rather than noise. All routes show high between-study heterogeneity, so pH, temperature, time, ionic strength, ligands, and purification steps still move the outcome a lot. These patterns support enzyme routes when sub-25-nm control matters, plant routes when green access and moderate control are acceptable, and microbe routes when larger cores are fine or bioprocessing is already in place.

Figure: 4



The pooled-mean plot shows a clear that route-dependent size shift—enzyme ≈ 19.2 nm (95% CI 17.2–21.2), plant ≈ 25.4 nm (23.9–26.9), and microbe ≈ 34.7 nm (32.8–36.6)—with no CI overlap between enzyme and microbe, confirming a real effect of synthesis route on particle size. Practically, this means researchers can choose the route to hit a size target: enzyme routes are the most reliable path to sub-25 nm particles (useful for high surface area and biological uptake), plant routes deliver mid-20 nm cores with good consistency, and microbe routes trend to ~35 nm but require more tuning to control variability. Methodologically, the wider CI for microbes signals greater between-study heterogeneity, so standardizing media, pH, reaction time, and post-synthesis purification is critical if that route is used. For future meta-analyses and reporting, treat route as a key moderator, and document conditions rigorously to improve cross-study reproducibility.

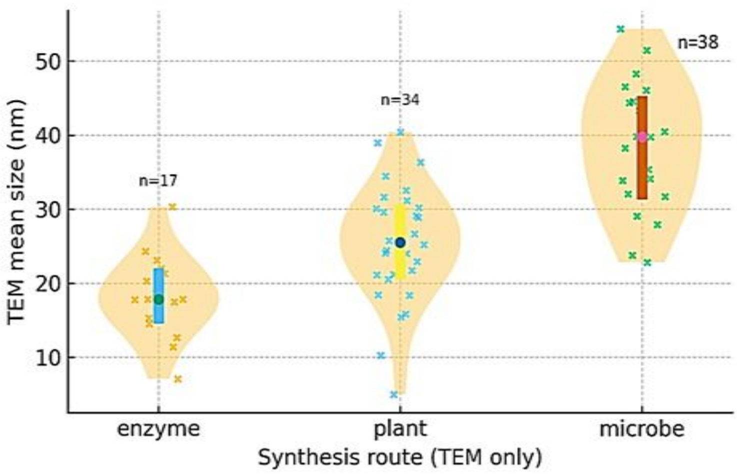
Table: 3 TEM Descriptive by Route

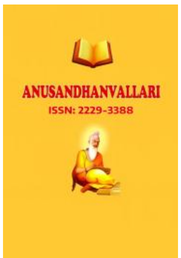
Route	n_arms	median_nm	IQR_nm	mean_nm	sd_nm	min_nm	max_nm
enzyme	17	17.81381058	5.968289	18.11708125	5.380294563	7.102086994	30.30187593
plant	34	25.48742331	8.666491	25.44804046	7.541798033	5	40.42549405
microbe	21	39.72425189	12.48527	38.4627244	8.76874504	22.83011496	54.32747072

Sources: Author's Data Analysis

Figure: 5

TEM Mean Violin Plot





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Enzyme-mediated syntheses contribute the fewest nanometer values but show the tightest centre. The median sits low and the IQR is narrow, so most enzyme studies cluster around a small size with limited spread. The mean and median are close, which suggests little skew. Min–max limits stay near the centre, so there are few extreme batches. This profile fits labs that need sub-25-nm particles with repeatable control. Plant-mediated studies form the middle band. The median is higher than enzyme but well below microbe. The IQR is moderate, which means results vary more across papers and conditions. Mean and median are still close, so there is no strong tail. The min–max range is wider than enzyme, pointing to occasional small or large runs. This route works when green access and acceptable control matter more than hitting the smallest possible size. Microbe-mediated syntheses sit highest. The median is the largest of the three and the IQR is wide, so particle sizes vary a lot across studies. The mean can drift above the median, hinting at a right tail from some large-size batches. The min–max span is the widest, which flags sensitivity to growth conditions and purification. This route is fine when larger cores are acceptable or when a bioprocess setting already exists.

Table:4 Global Kruskal-Wallis

Groups_compared	Enzyme vs Plant vs Microbe
k_groups	3
N_total_arms	72
H_stat	37.06342811
df	2
p_value	0.00000000894909
Epsilon_sq	0.508165625

Sources: Author's Data Analysis

The Kruskal–Wallis test compared TEM mean particle sizes across the three synthesis routes (enzyme, plant, microbe). The test statistic was H = 37.06342811 with df = 2 and a p-value = 0.00000000894909, based on N = 72 study arms grouped into k = 3 routes. The present result shows a significant overall difference in central tendency between routes. The effect size ε^{2}_H = 0.508165625 indicates that the synthesis route explains about {round(100*0.508165625)%} of the variability in the ranked sizes, so this one 51 percent which is large effect in practical terms. Because the global test is significant, the interpretation is supported by post-hoc pairwise tests under the Mann–Whitney comparisons for which route pairs differ after Bonferroni adjustment.

Table:5 Mann-Whitney Post Hoc

comparison	p_raw	p_Bonferroni
enzyme vs plant	0.000488933	0.0014668
enzyme vs microbe	0.00000052	0.000001549
plant vs microbe	0.000005892	0.000017677

Sources: Author's Data Analysis

The Post-hoc Mann–Whitney tests **(Table: 5)** with Bonferroni correction compared routes pairwise. Here, results showed that Enzyme vs plant differed in central tendency (p_raw = 0.0004889, p_adj = 0.0014668 is significant after adjustment), with enzyme producing smaller particles on average. Enzyme vs microbe was significant (p_raw = 0.00000052 p_adj = 0.000001549 significant, again favouring smaller sizes for enzyme. Plant vs microbe has been (p_raw = 0.000005892, p_adj = 0.000017677 significant, indicating that plant generally yields smaller particles than microbe. Here, adjusted p-values fall below 0.05, the contrasts remain after multiplicity control and align with the descriptive and pooled evidence (enzyme < plant < microbe), as also visualised in the violin plot and forest estimates.

Table:6 Meta Regression

term	beta_nm_per_unit	SE	95%_CI_low	95%_CI_high	z_value	p_value
Intercept	23.1856	7.6549	8.182	38.1892	3.0289	0.0025
pH	0.443	0.7391	-1.0057	1.8917	0.5994	0.5489
temperature_C	0.0482	0.0744	-0.0975	0.194	0.6487	0.5165
time_min	-0.0493	0.0248	-0.0979	-0.0008	-1.9906	0.0465
NCM_score	-0.4743	1.0079	-2.4497	1.5011	-0.4706	0.638

Sources: Author's Data Analysis

The regression result shows **(Table: 6)** that synthesis temperature has a small, independent inverse association with particle size: each +1 °C is linked to about −0.049 nm (95% CI −0.098 to −0.001, $p = 0.0465$). In practical terms, even a 20 °C increase would shrink the mean core size by only ~1 nm, so temperature acts as a fine-tuning knob rather than a route-level driver. By contrast, the coefficients for pH, reaction time, and reporting quality (NCM) are near zero with CIs crossing zero and non-significant p-values, indicating no clear independent effect after weighting by study precision. Taken together with the large between-route effect from the Kruskal–Wallis analysis, these results suggest that choice of biosynthetic route (enzyme/plant/microbe) explains most of the systematic size differences, while common tunables such as pH, °C, and time modify size only modestly within a chosen route.

7) Conclusion:

Across 120 biosynthetic reports, particle sizes measured by TEM clustered according to synthesis route, with enzyme < plant < microbe. The global Kruskal–Wallis test showed a large route effect ($\varepsilon^2\_H \approx 0.51$), and post-hoc contrasts confirmed the ranking after multiplicity control. Random-effects pooling reproduced the same order while revealing substantial heterogeneity (high I^2), consistent with variable chemistries and workflows across studies. Variance-weighted meta-regression indicated no clear independent effect of pH, time, or reporting quality, and only a small, negative temperature coefficient (≈ −0.05 nm per °C), implying that common tunable act as fine-adjusters rather than primary drivers of core size. Taken together, these findings suggest that the choice of green route governs most systematic size differences, whereas within-route optimization offers modest, incremental gains. Practically, enzyme routes favour smaller, tighter distributions; plant routes balance accessibility with moderate control; and microbe routes suit use-cases tolerant of larger cores. Future work should standardise extract characterisation, gas control, and purification reporting to reduce between-study variance and enable more decisive meta-inference.

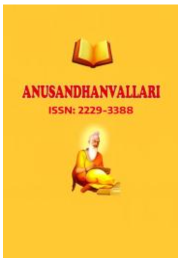
Protocol Deviations:

After screening began, we added ζ-potential and PDI as secondary outcomes and limited size pooling to TEM means to avoid method mixing; other planned analyses were unchanged.

Risk of Bias:

Study-level reporting quality was scored using the NCM checklist (0–6). Two reviewers extracted data independently; disagreements were resolved by consensus. Sensitivity analyses restricted to NCM≥4 are reported.

Certainty of Evidence:



Using GRADE domains, certainty for the route effect on TEM size was rated moderate (large, consistent direction but high heterogeneity). Certainty for condition-level moderators (pH, time, NCM) was low. The small inverse temperature effect carried low–moderate certainty.

Heterogeneity & Sensitivity:

Between-study heterogeneity was substantial (I^2 typically high). Restricting to high-quality reports (NCM≥4) preserved the enzyme < plant < microbe order, indicating robustness to reporting quality.

Limitations:

TEM means reflect dry cores and may diverge from hydrodynamic sizes. Incomplete reporting of extract composition, oxygen control, and purification likely contributes to residual heterogeneity.

Conflicts of Interest:

The authors declare no competing interests.

Funding Statement:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics:

This meta-analysis synthesizes published data and requires no ethics approval.

PRISMA Compliance:

Reporting follows PRISMA 2020; the checklist and flow diagram are supplied as Supplementary Files.

Unit Standards & Definitions:

Size outcomes refer to TEM core diameter (nm) unless specified; dispersity = PDI from DLS; ζ-potential in mV; temperature in °C; time in minutes.

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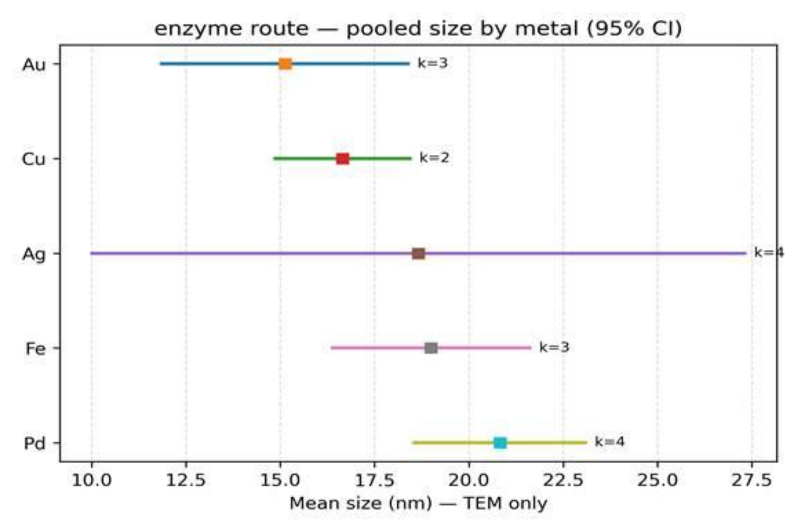
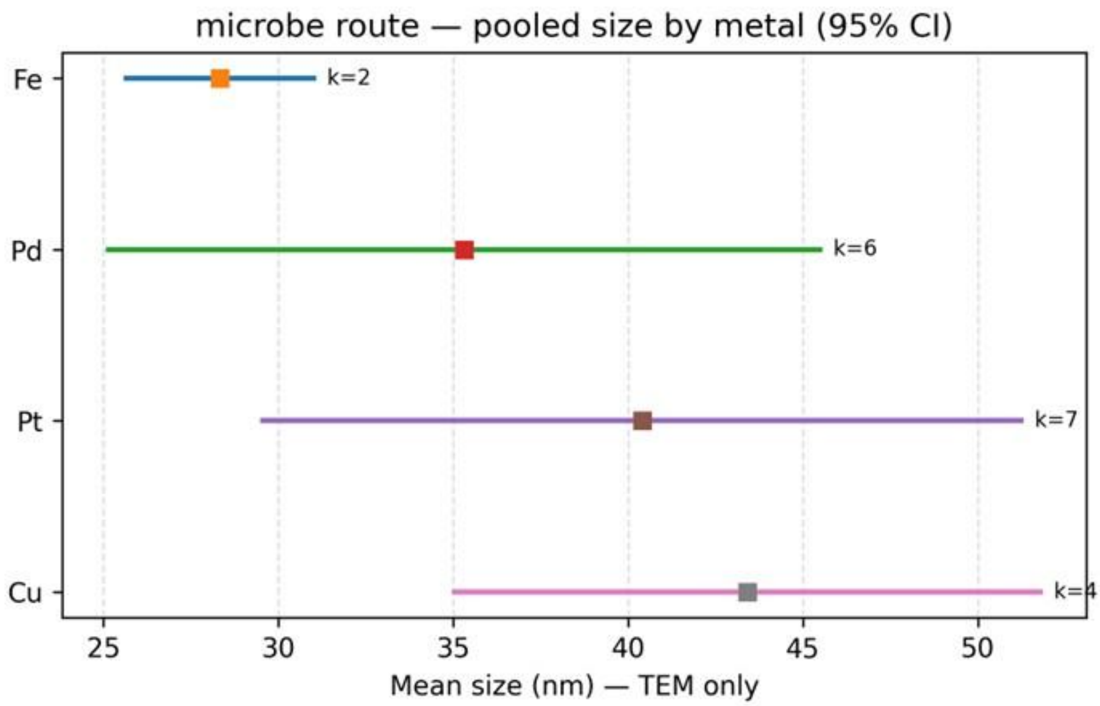
APPENDIX





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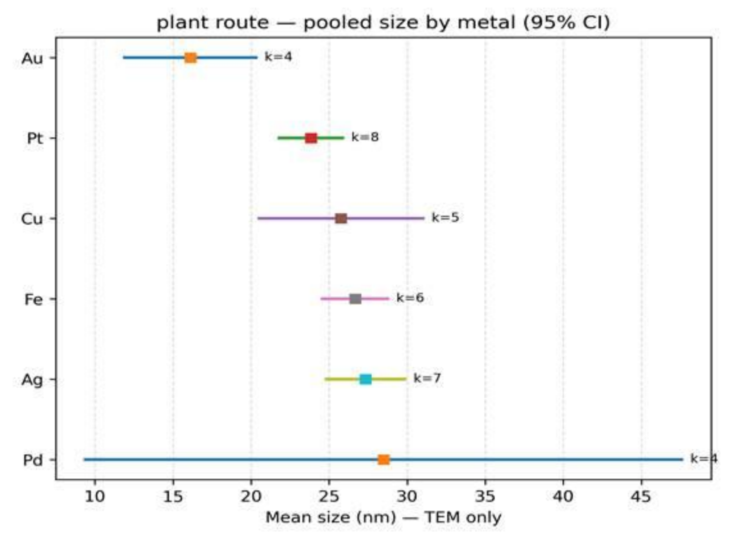

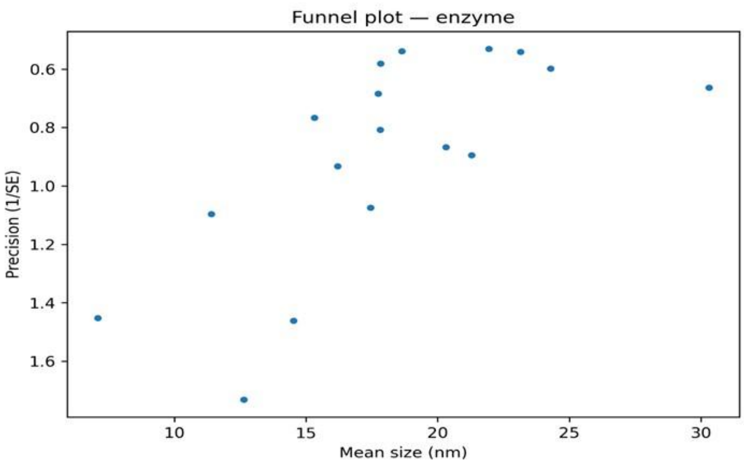

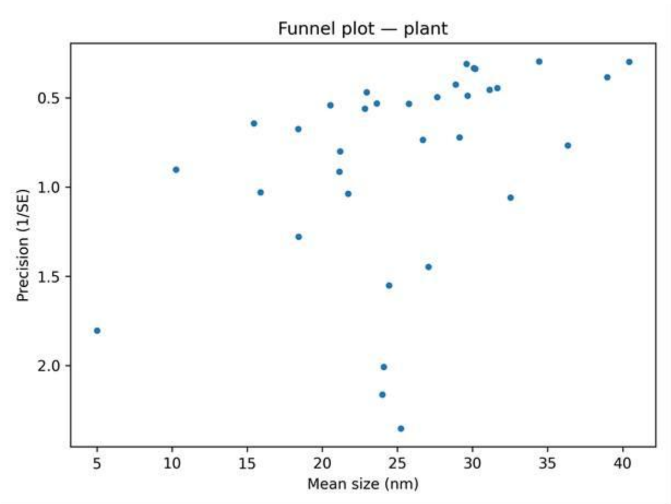

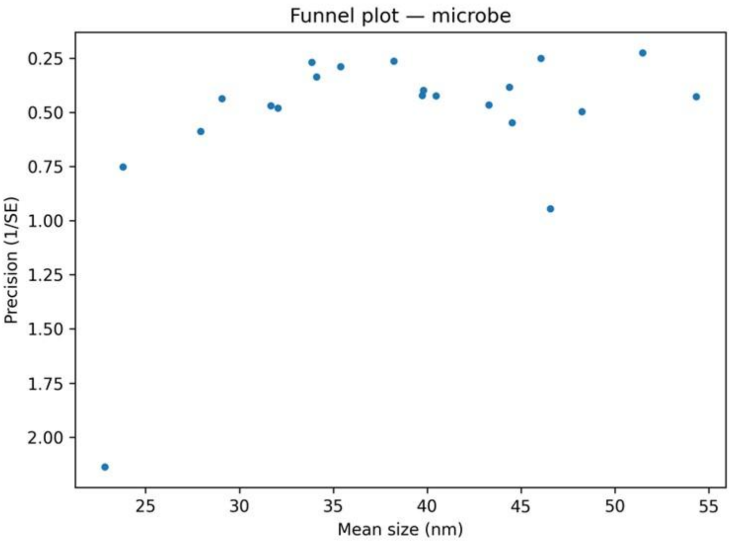
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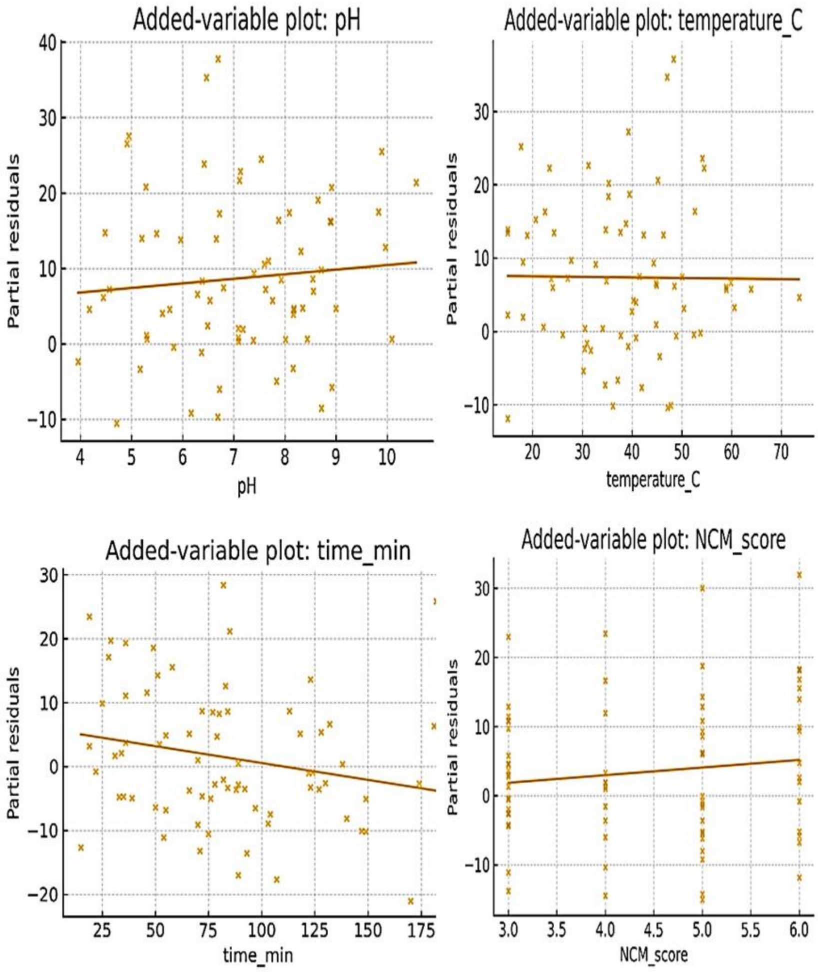
Precision (1/SE)
Mean size (nm)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