

Ecotoxicological impacts of Nano-pesticides and Nano-fertilizers on non-target soil organisms and pollinators

Hema Venkatesh[1], Bhavesh Solanki[2*], Nandini Rathore[3]
[1] Associate Professor, Faculty of Environmental Science, Centre for Environmental Planning and Technology University, Ahmedabad, Gujarat, India
[2] Assistant Professor, Department of Agriculture, Centre for Environmental Planning and Technology University, Ahmedabad, Gujarat, India
[3] University of Delhi, New Delhi, Delhi, India

Corresponding Author: Bhavesh Solanki

Volume: 1, Issues: 1, Year: 2026, Page No.: 9-20

Receive Date: 24-08-2026**; Accept Date:** 09-10-2026**; Published Date:** 25-10-2026

Abstract
Nanoscale engineering has reshaped contemporary crop protection and nutrient management by introducing nano-pesticides and nano-fertilizers capable of enhancing active-ingredient dispersion, foliar adhesion, and controlled release. Despite documented gains in target pest suppression and agronomic nutrient-use efficiency, the deployment of materials engineered within the 1–100 nm domain introduces distinct ecotoxicological hazards across terrestrial food webs. Because nanoscale formulations exhibit elevated specific surface areas, altered dissolution kinetics, and prolonged environmental persistence relative to conventional bulk agrochemicals, they bypass traditional mass-based exposure assumptions. This critical mechanistic synthesis evaluates the ecotoxicological consequences of metallic, metal-oxide, carbon-based, and polymeric nano-agrochemicals across two ecologically indispensable non-target guilds: soil-dwelling organisms and insect pollinators. Evidence consolidated from mesocosm trials, high-throughput transcriptomics, 16S rRNA amplicon sequencing, and neurobehavioral bioassays reveals that inorganic metal-based formulations (e.g., nano-$Cu(OH)_2$, CuO, ZnO, Ag, and rare-earth oxides) frequently disrupt soil dehydrogenase, urease, and phosphatase activities, suppress denitrification functional genes (nosZ), and restructure rhizosphere microbial networks. Among soil invertebrates-including *Eisenia fetida*, *Enchytraeus crypticus*, and *Folsomia candida*-nanoscale copper and silver inputs can shift bioavailability and trigger oxidative stress, reproductive impairment, and gut microbiota perturbations, though toxicity varies sharply with soil pH, organic matter, and particle aging. Concurrently, systemic plant translocation and direct floral deposition expose *Apis mellifera*, *Bombus terrestris*, and solitary bees (*Osmia* spp.) to nanoscale residues in nectar, pollen, and hive wax. Sublethal ingestion induces midgut peritrophic membrane degradation, depletion of core gut symbionts (*Gilliamella*, *Snodgrassella*, *Lactobacillus*), opportunistic pathogen proliferation, brain invasion, neuronal apoptosis, and associative learning failure. Conversely, biodegradable biopolymer-encapsulated carriers and tailored nanosuspensions frequently attenuate non-target mortality compared with unformulated synthetics. Resolving this risk–benefit divergence requires transitioning regulatory frameworks from acute mass-concentration testing toward chronic, aged-matrix, and microbiome-informed ecological risk assessment.

Keywords: Nano-agrochemicals, Soil microbiome, Edaphic invertebrates, *Apis mellifera*, Pollinator cognition, Gut dysbiosis, Trophic translocation, Safe-by-design

Introduction: The Agronomic Promise Versus the Ecotoxicological Paradox

Modern intensive agriculture confronts a persistent biophysical bottleneck: conventional synthetic fertilizers and broad-spectrum pesticides suffer from low delivery efficiency, with substantial fractions lost through volatilization, photolysis, surface runoff, and leaching before reaching target biological sites. Nanotechnology has been advanced as a corrective engineering paradigm, giving rise to nano-enabled agrochemicals broadly categorized into Type 1 inorganic nanomaterials (where the nanoscale metal, metal oxide, or metalloid serves directly as the active biocidal or nutritional agent, such as Ag, CuO, $Cu(OH)_2$, ZnO, TiO_2, and Fe_2O_3) and Type 2 nanocarrier systems (where conventional organic active ingredients or nutrients are encapsulated within

polymeric, lipid, mesoporous silica, clay, or biomineral matrices such as urea–hydroxyapatite nanohybrids). Meta-analytical syntheses indicate that nano-enabled pesticides achieve approximately 31.5% higher target efficacy across hundreds of comparative trials than conventional analogues, while nano-fertilizers can substantially curb nitrogen leaching and improve crop stress resilience.

Yet the very physicochemical attributes that confer superior agronomic performance-high surface-area-to-volume ratios, tunable surface charge, enhanced cuticular penetration, and protection against premature environmental degradation-generate an ecotoxicological paradox. Once released into agroecosystems via foliar spraying, seed priming, soil drenching, or biosolid amendment, nano-agrochemicals do not behave as simple dissolved solutes. They undergo dynamic environmental transformations including homo- and heteroaggregation, sulfidation, dissolution into free metal ions, and adsorption of natural organic matter or extracellular biomolecules to form dynamic "eco-coronas". Importantly, enhanced chemical stability extends residence times in topsoil horizons and plant vascular tissues, widening the spatial and temporal window of encounter for non-target organisms.

Two non-target ecological compartments bear disproportionate exposure and functional significance: the edaphic (soil) community and insect pollinators. Soil microorganisms, symbiotic fungi, earthworms, collembolans, and nematodes underpin biogeochemical cycling, organic matter mineralization, soil aggregation, and primary productivity. Simultaneously, managed honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.), and solitary wild bees pollinate over 75% of flowering plant species and support more than 35% of global food crop production. Because terrestrial soils act as the ultimate sink for agricultural nanomaterials-with field-scale budgets showing that over 90% of foliarly applied $Cu(OH)_2$ nanopesticide can accumulate in the soil compartment-and because vascular transport or floral deposition links soil and canopy applications directly to floral rewards, these two guilds cannot be evaluated in isolation.

Conventional regulatory ecotoxicology, largely designed for soluble organic molecules and bulk metals, frequently mischaracterizes nanoscale hazards. Standardized 48-hour acute lethality tests overlook delayed ion-release kinetics, nano-enabled intracellular trafficking across epithelial barriers, non-monotonic dose responses, and sublethal disruptions to symbiotic microbiomes and insect neurobiology. This paper synthesizes mechanistic, physiological, transcriptomic, and community-level evidence to delineate how nano-pesticides and nano-fertilizers affect non-target soil biota and pollinators, contrasting inorganic nanoparticulate hazards against emerging biodegradable nanocarrier architectures.

Methodological Framework for Cross-Trophic Mechanistic Synthesis

To construct a rigorous, cross-compartment evaluation without conflating disparate experimental scales, this synthesis integrates peer-reviewed empirical studies spanning soil microcosms, multi-month outdoor mesocosms, invertebrate bioassays, and pollinator behavioral–neurochemical investigations. Literature was categorized along two orthogonal axes: (i) formulation architecture, distinguishing active metallic/metal-oxide nanomaterials, rare-earth and non-metallic oxides, macronutrient/micronutrient nano-fertilizers, and polymeric/supramolecular nanocarriers; and (ii) biological organization, spanning molecular/transcriptomic perturbations, cellular histopathology, symbiotic microbiome dysbiosis, individual fitness/cognition, and ecosystem-level biogeochemical function.

Comparative hazard interpretation requires normalizing nominal exposure metrics against matrix-specific bioavailability. Consequently, wherever empirical data permit, this synthesis contrasts nanoscale formulations directly against ionic salts and conventional bulk analogues (e.g., comparing median effective concentrations, EC_{50}, or median lethal concentrations, LC_{50}, across identical test matrices). Furthermore, because acute mortality poorly predicts ecological collapse in long-lived soil fauna and eusocial pollinators, sublethal endpoints-specifically extracellular soil enzyme inhibition, nitrogen-cycle functional gene abundance (amoA, nosZ), gut microbial beta-diversity shifts, midgut epithelial integrity, and associative olfactory learning-are weighted alongside classical survival metrics. Table 1 summarizes the major structural classes of agricultural nanomaterials and their primary environmental transformation drivers.

Table 1: Classification, Physicochemical Drivers, and Environmental Aging Dynamics of Major Nano-Pesticide and Nano-Fertilizer Classes

Agrochemical Class	Representative Formulations / Active Agents	Primary Agronomic Function	Key Physicochemical Drivers of Toxicity	Dominant Environmental Transformations in Soil & Canopy
Inorganic Metal & Metal-Oxide Nanopesticides	Nano-$Cu(OH)_2$ (e.g., Kocide 3000), CuO NPs, Ag NPs, ZnO NPs	Broad-spectrum bactericide, fungicide, and larvicide	Rapid surface dissolution (Cu^{2+}, Ag^{+}, Zn^{2+}), reactive oxygen species (ROS) generation, membrane piercing	Sulfidation (e.g., Ag_2S), complexation with humic substances, pH-driven ion release, heteroaggregation
Micronutrient & Rare-Earth Nano-Fertilizers	Nano-ZnO, Fe_2O_3/Fe_3O_4, MnO_2, TiO_2, CeO_2, La_2O_3 NPs	Micronutrient biofortification, photosynthetic enhancement, abiotic stress mitigation	Surface redox reactivity, photocatalytic ROS (TiO_2), blood–brain/cuticle translocation, ion mimicry	Root exudate-mediated dissolution, phosphate precipitation (e.g., $LaPO_4$), eco-corona protein binding
Macronutrient	Urea–hydroxyapatite (UHA)	Slow/controlled N and	High local ionic strength,	Enzymatic/hydrolytic cleavage

Nano-Fertilizers & Hybrids	nanohybrids, liquid nano-urea, nano-DAP, graphite nano-amendments	P release, reduced volatilization and leaching	osmotic/ammonia micro-gradients, pH shifts, carbon-surface sorption	of urea, gradual mineral dissolution, rhizosphere microbial mobilization
Polymeric & Supramolecular Nanocarriers (Type 2)	Chitosan/carboxymethyl chitosan (CMCS), alginate, PLGA, mesoporous silica (MSN), nanosuspensions (e.g., chlorfenapyr, chlorantraniliprole, abamectin)	Targeted pest delivery, UV shielding, sustained release, resistance management	Polymer biodegradation rate, surfactant co-formulants (e.g., Tween-80), altered cellular endocytosis	Enzymatic/hydrolytic carrier breakdown, burst vs. sustained payload release, cuticle adhesion

Environmental Fate, Soil Matrix Aging, and Plant–Pollinator Transfer Pathways

Predicting non-target ecological impacts begins with tracing how nano-agrochemicals partition across environmental compartments after application. Whether introduced via foliar misting-where off-target drift and washoff deposit substantial fractions onto topsoil-or applied directly to root zones, engineered nanoparticles enter a highly heterogeneous geochemical matrix. In an outdoor mesocosm investigation of repeated $Cu(OH)_2$ nanopesticide applications, plant tissues recovered less than 8% of total applied copper, leaving roughly 92% retained within upper soil horizons. Once embedded in soil porewater, nanoparticle fate is governed by soil pH, clay mineralogy, ionic strength, redox potential, and dissolved organic carbon (DOC). Acidic soils with low organic matter content accelerate the oxidative dissolution of CuO, $Cu(OH)_2$, and ZnO nanoparticles, elevating free bioavailable Cu^{2+} and Zn^{2+} pools and intensifying toxicity to edaphic invertebrates and bacteria. Conversely, alkaline or organic-rich soils promote surface coating by humic and fulvic acids, inducing steric stabilization or heteroaggregation with mineral colloids that slows immediate dissolution while creating long-term subsurface reservoirs.

Crucially, nanoparticles rarely act as isolated chemical stressors in agricultural soils; they actively alter the fate of co-occurring conventional agrochemicals. For example, soil amendment with $Cu(OH)_2$ nanopesticides was shown to inhibit the microbial degradation of the systemic neonicotinoid thiacloprid, extending thiacloprid persistence and amplifying combined ecological exposure risks. Similarly, co-contamination with emerging polymeric debris such as polyethylene or polylactic acid microplastics modifies soil pH and alters ZnO nanoparticle uptake and root-to-shoot translocation dynamics in maize.

From the rhizosphere and foliar canopy, nano-agrochemicals bridge the terrestrial–aerial boundary to reach pollinators through three primary vectors (Figure 1). First, root uptake and vascular translocation enable sufficiently small or surface-modified nanoparticles (typically <50 nm, or dissolved/re-precipitated ionic species) to traverse root apoplastic or symplastic pathways into xylem vessels and upward into reproductive tissues, including anthers, pollen grains, and floral nectaries. Second, direct floral and foliar deposition during spray application coats petals, extrafloral nectaries, and dehiscing pollen with nanopesticide droplets, facilitating both cuticular contact on bee tarsi and oral ingestion during nectar imbibition or pollen grooming. Third, hive-matrix bioaccumulation occurs when foragers transport nanoparticle-laden pollen pellets and water back to the colony, where lipophilic or persistent nanomaterials partition into beeswax, royal jelly, and stored bee bread, generating multi-generational larval and adult chronic exposure.

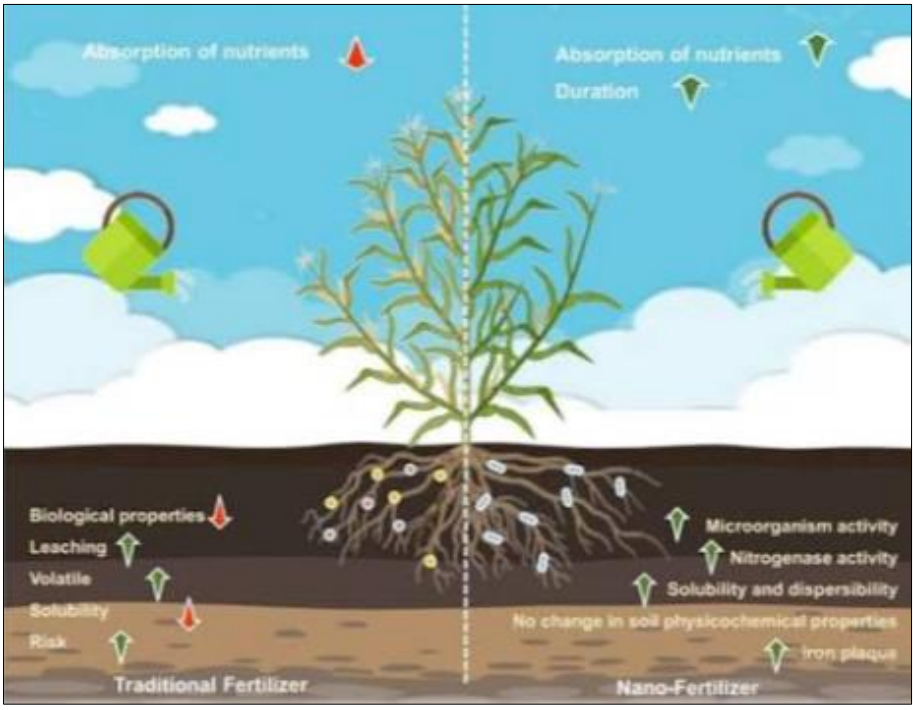

Fig 1: The environmental aging, soil–plant vascular translocation, and floral deposition routes linking agricultural nano-pesticides and nano-fertilizers to non-target soil communities and foraging pollinators.

Ecotoxicological Consequences for Non-Target Soil Microorganisms and Nutrient-Cycling Guilds

Soil microbial communities constitute the biochemical engine of terrestrial ecosystems, regulating carbon sequestration, nitrogen transformation, phosphorus solubilization, and organic pollutant mineralization. Because many metallic nano-pesticides and micronutrient nano-fertilizers are deliberately engineered for antimicrobial potency against plant pathogens, their collateral toxicity toward beneficial saprophytic and symbiotic microbes represents a first-order ecological hazard.

1. Extracellular Enzyme Suppression and Biogeochemical Disruption

Extracellular enzyme activities serve as sensitive early-warning indicators of microbial metabolic stress. In a 28-day indoor mesocosm study evaluating a recommended agricultural dose (50 mg Cu kg^{-1} soil) across nano-CuO, commercial nano-$Cu(OH)_2$ (Kocide 3000), lab-synthesized $Cu(OH)_2$ nanomaterials, and bulk $Cu(OH)_2$, every copper treatment significantly depressed core enzymatic functions: dehydrogenase activity fell by 68% to 95%, arylsulfatase by 27% to 41%, and urease by 20% to 40% relative to untreated controls. Notably, community-level physiological profiling revealed a compensatory 32% surge in carbon substrate utilization under nano-CuO exposure, indicative of metabolic reallocation toward cellular stress repair rather than balanced biomass synthesis.

Multi-season outdoor mesocosm experiments corroborate that enzyme perturbations are neither brief nor fully buffered by plant presence. When Simonin *et al.* (2018) [1] applied three sequential doses of $Cu(OH)_2$ nanopesticide (Kocide 3000) across grassland mesocosms under ambient, low, and high mineral fertilization regimes, soil microbial enzyme activities plunged by 28% to 82% in ambient plots and 25% to 47% in low-fertilization plots within 15 days of initial exposure. Although partial functional recovery occurred two months post-application, delayed legacy effects re-emerged 365 days later-six months after the final spray-with beta-glucosidase activity suppressed by 57% (ambient) and 40% (high fertilization) and phosphatase activity reduced by 47% in ambient soils. These findings demonstrate that mineral nutrient status modulates microbial resilience, yet chronic nanoparticulate copper accumulation still erodes long-term carbon and phosphorus cycling capacity.

Silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs) exhibit similarly pronounced, concentration- and texture-dependent impacts. In sandy loam soils, Ag NPs markedly reduced microbial biomass, suppressed substrate-induced respiration, and diminished bacterial taxonomic diversity. Long-term exposure to environmentally relevant Ag NP concentrations in loamy soils likewise depressed microbial biomass and altered the abundance of functional genes governing nitrogen cycling. Comparative assays with TiO_2 and ZnO NPs confirmed significant shifts in bacterial community structure and reduced overall bacterial abundance in soil microcosms, with ZnO exhibiting steeper inhibition due to partial dissolution alongside surface reactivity. Interestingly, TiO_2 NPs applied to silty-clay agricultural soils disrupted nitrification enzymatic rates in a non-linear manner lacking classical monotonic dose–response curves, complicating threshold derivation.

2. Dysbiosis in Nitrogen-Cycling Guilds and Plant–Microbe Symbioses

Functional guilds mediating nitrogen fixation, nitrification, and denitrification exhibit acute vulnerability to nanoscale metals. In copper-treated soil mesocosms, quantitative PCR tracking revealed a drastic contraction in the abundance of the nitrous oxide reductase gene (nosZ), plummeting to 0.05% across all Cu treatments relative to controls. Concurrently, 16S rRNA community profiling documented selective depletion of oligotrophic and sensitive taxa-including *Acidobacteria* and *Flavobacteriia*-paired with the opportunistic expansion of copper-tolerant classes such as *Clostridia* (under Kocide 3000) and *Gemmatimonadetes* (under synthesized nano-$Cu(OH)_2$). In peanut (*Arachis hypogaea*) rhizospheres exposed to ZnO NPs, ammonia- and nitrate-oxidizing bacteria were suppressed while denitrifying populations expanded, tilting soil nitrogen flux toward gaseous nitrogen losses. Pure-culture and microcosm assays further show that Ag NPs trigger size-dependent cytotoxicity, oxidative membrane damage, and growth arrest in free-living diazotrophs such as *Azotobacter vinelandii* and pure nitrifying bacteria.

Mutualistic symbioses between crops, rhizobia, and arbuscular mycorrhizal fungi (AMF) represent critical biological buffers against agrochemical stress, yet they face dose-dependent breakdown under excessive nano-loading. Legume-nodulating rhizobia respond to nanoscale surface stress by secreting extracellular polymeric substances (EPS) that chelate ions and physically shield cell membranes-a defense documented in *Sinorhizobium meliloti* under Ag NP and Zn^{2+} stress. However, recent nano-tracer studies warn that EPS-bound nanoparticles can hitchhike during root-hair infection, potentially interfering with nodule organogenesis and nitrogenase efficiency. Similarly, AMF colonization shows sharp concentration thresholds: while maize plants tolerated 400 mg kg^{-1} of ZnO NPs in soil microcosms, doses of 800 mg kg^{-1} and above severely inhibited both maize biomass and AMF root colonization. Notably, when AMF (*Funneliformis mosseae*) successfully established symbiosis at sub-threshold or moderate nano-stress levels (e.g., 500 mg kg^{-1} ZnO NPs or nano-CuO stress in *Canna indica*), mycorrhizal networks actively mitigated host phytotoxicity by secreting glomalin-related soil proteins and organic acids, upregulating metallothionein and antioxidant defense genes, and sequestering excess metals within fungal hyphae. Across comparative reviews, however, Ag NPs suppress mycorrhizal colonization at soil concentrations 5- to 600-fold lower than ZnO NPs, underscoring element-specific hazard hierarchies.

3. Ecological Footprints of Macronutrient Nano-Fertilizers

Relative to metal-oxide micropesticides, macronutrient nano-fertilizers generally display lower acute microbial toxicity, though their long-term biogeochemical effects remain nuanced. Urea–hydroxyapatite (UHA) nanohybrids developed by Kottegoda *et al.* (2017) [2] bond urea amine and carbonyl groups to biocompatible calcium-phosphate nanocrystals, extending nitrogen release 12-fold in column dissolution tests and sustaining rice yields with 50% less applied nitrogen. Field evaluations of liquid nano-urea in flood-irrigated Boro rice likewise demonstrated reduced ammonia volatilization and nitrate leaching losses alongside higher nitrogen-use efficiency. When integrated into balanced cropping systems with farmyard manure, nano-nutrient regimes can maintain or enhance soil microbial biomass carbon and dehydrogenase, urease, and alkaline phosphatase activities. Conversely, carbon-based nano-amendments such as graphite nanomaterials can induce complex shifts in soil enzyme profiles and functional microbial genes within soil–plant systems, and phosphate-solubilizing bacteria mobilize nano-hydroxyapatite far more effectively *in vitro* than in complex field soils where sorption and competition dominate.

Toxicity to Edaphic Fauna: Earthworms, Collembolans, and Nematodes

Soil-dwelling invertebrates physically engineer soil porosity, fragment plant litter, and stimulate microbial turnover. Their intimate dermal and oral contact with soil porewater, mineral particles, and ingested organic detritus exposes them simultaneously to dissolved metal ions and intact nanoparticles.

1. Earthworms (*Eisenia fetida*, *Lumbricus terrestris*, and *Enchytraeus crypticus*)

Earthworms serve as flagship sentinel organisms in terrestrial ecotoxicology. Bioaccumulation kinetics in *Eisenia fetida* reveal that tissue burdens and toxic outcomes depend heavily on particle dissolution rates and surface functionalization. Using bimetallic core–shell Au@Ag nanoparticles, Baccaro *et al.* (2022) [3] demonstrated that silver dissolution controls both bioavailable uptake and internal distribution within *E. fetida*, whereas recalcitrant gold cores behave primarily as particulate tracers. Similarly, hydrophobic or polymer surface coatings on Fe_3O_4 and ZnO nanoparticles modulate zeta potential, suppress reactive ion shedding, and markedly attenuate oxidative stress, tissue pathology, and metabolic disruption in earthworm tissues. Sublethal physiological and microbiome endpoints frequently reveal toxicity at doses below acute lethality thresholds. Direct soil exposure of *E. fetida* to CuO NPs (<100 nm) induced dose-dependent body weight loss over 15 days even when acute mortality lacked a classical dose-response curve. Furthermore, silver nanoparticle ingestion substantially restructures the *E. fetida* gut microbiome-an internal consortium typically enriched in *Firmicutes* (*Bacillus* spp.) and *Proteobacteria*-potentially compromising host digestion and pathogen resistance. Co-exposure with polyethylene microplastics further alters ZnO NP bioavailability and amplifies oxidative stress in *E. fetida*. Conversely, well-designed organic nanopesticide nanosuspensions can lower risk to macro-invertebrates: a 44 nm chlorfenapyr nanosuspension produced zero mortality in *Lumbricus terrestris* at concentrations up to 72 mg L^{-1}, and a chlorantraniliprole oil-in-water nanosuspension yielded ≤20% mortality at 60 mg kg^{-1} over 30 days-comparable to or milder in weight-loss impact than conventional chlorantraniliprole.

In smaller oligochaetes such as the potworm *Enchytraeus crypticus*, new approach methodologies (NAMs) have unveiled subtle molecular hazards overlooked by apical reproduction assays. A 2025 high-throughput transcriptomics investigation of a commercial nano-enabled fertilizer in *E. crypticus* revealed pronounced dysregulation of oxidative stress response, energy metabolism, and cellular homeostasis pathways, echoing subsequent transcriptomic evidence on commercial copper oxychloride nano-formulations.

2. Collembolans (*Folsomia candida*) and Nematodes (*Caenorhabditis elegans*)

Springtails (*Folsomia candida*) inhabit air-filled soil pores and feed selectively on fungal hyphae and detritus, placing them directly in the path of surface-deposited nanopesticides. Critically, nano-formulation can drastically amplify or alter chemical toxicity depending on soil properties. Neves *et al.* (2019) [4] compared conventional copper fungicides with the nanopesticide Kocide 3000 in *F. candida* across contrasting natural soils and standard LUFA 2.2 soil, recording an EC_{50} of 156 mg Cu kg^{-1} for nano-formulations versus 3030 mg Cu kg^{-1} for non-nano formulations-a nearly 19-fold increase in apparent toxicity driven by active copper speciation and soil organic matter/pH interactions. Rare-earth oxide nanoparticles (La_2O_3 and Y_2O_3) similarly impair *F. candida* reproduction and survival, with mixture exposures revealing complex interactive toxicities relative to bulk oxides.

At the microscopic trophic base, the bacterivorous nematode *Caenorhabditis elegans* exhibits acute sensitivity to both ion-shedding and redox-active nanoparticles. Chronic exposure to environmentally relevant ZnO NPs induces metabolic dysregulation and locomotive deficits in *C. elegans*, while Ag NPs suppress somatic growth, damage DNA via oxidative stress, and propagate multigenerational reproductive toxicity. Moreover, exposures to cobalt NPs, TiO_2 NPs, and multi-walled carbon nanotubes elicit neurotoxic neurotransmitter perturbations mediated by oxidative stress pathways. Figure 2 contrasts these mechanistic cascades across soil fauna and beneficial microbes, and Table 2 synthesizes representative empirical endpoints.

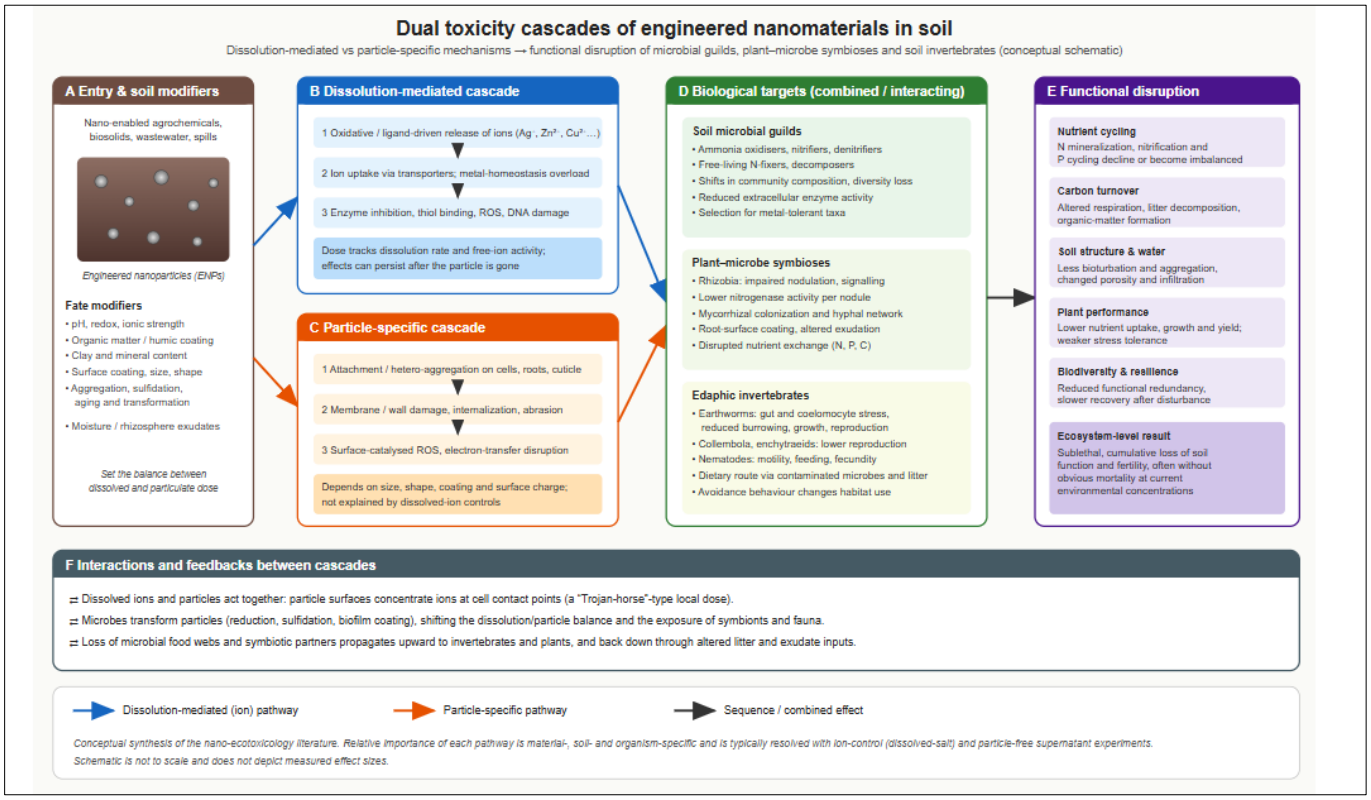

Fig 2: Dual dissolution-mediated and particle-specific toxicity cascades driving functional disruption in soil microbial guilds, plant–microbe symbioses, and edaphic invertebrates.

Table 2: Empirical Ecotoxicological Impacts of Nano-Pesticides and Nano-Fertilizers on Non-Target Soil Microorganisms and Edaphic Invertebrates

Test Organism / Guild	Nanomaterial / Formulation Tested	Exposure Matrix & Concentration	Observed Lethal / Sublethal Endpoints & Mechanisms	Reference
Soil Microbiome (Natural Community)	Nano-CuO, Kocide 3000 (nano-$Cu(OH)_2$), synthesized n$Cu(OH)_2$, bulk $Cu(OH)_2$	Indoor soil mesocosm; 50 mg Cu kg^{-1} for 28 days	Dehydrogenase (-68% to -95%), arylsulfatase (-27% to -41%), urease (-20% to -40%) inhibition; nosZ denitrification gene reduced to 0.05%; loss of *Acidobacteria* and *Flavobacteriia*; enrichment of *Clostridia*/*Gemmatimonadetes*	Peixoto et al. (2024) [5]
Pasture Soil Microbiome & Enzymatic Guilds	Kocide 3000 ($Cu(OH)_2$ nanopesticide)	1-year outdoor mesocosm; 3 sequential sprays under 3 fertilization tiers	Short-term (day 15) enzyme suppression (-28% to -82% ambient); long-term (day 365) suppression of beta-glucosidase (-40% to -57%) and phosphatase (-47% ambient); N_2 fixation and root AMF unaffected	Simonin et al. (2018) [1]
Soil Bacterial Communities	TiO_2 NPs and ZnO NPs	Soil microcosms; comparative dose gradient	Significant reductions in microbial biomass and bacterial community diversity; ZnO exerted stronger toxicity than TiO_2	Ge et al. (2011) [6]
Sandy Loam Soil Microbiota	Ag NPs	Sandy loam soil microcosm	Inhibited microbial growth, respiration, and enzymatic activity; shifted taxonomic composition	Samarajeewa et al. (2017) [7]
Maize–AMF Symbiosis	ZnO NPs + *Funneliformis mosseae*	Soil microcosm; 0, 400, 800 mg kg^{-1}	No harm at 400 mg kg^{-1}; ≥800 mg kg^{-1} suppressed maize growth and AMF root colonization; AMF inoculation alleviated Zn accumulation and phytotoxicity at sub-inhibitory levels	Wang et al. (2016) [8]
Springtail (*Folsomia candida*)	Nano-$Cu(OH)_2$ (Kocide 3000) vs. conventional Cu	LUFA 2.2 & natural soils	Markedly lower EC_{50} for nanoformulation (156 mg Cu kg^{-1}) vs. non-nano analogue (3030 mg Cu kg^{-1}); toxicity modulated by soil pH and organic matter	Neves et al. (2019) [4]
Potworm (*Enchytraeus crypticus*)	Commercial nano-enabled fertilizer	Standardized soil exposure; transcriptomics	High-throughput RNA-seq revealed disruption of stress response, metabolic pathways, and cellular homeostasis before overt mortality	Gomes et al. (2025) [9]
Earthworm (*Eisenia fetida*)	Bimetallic Au@Ag NPs	Soil exposure	Silver dissolution dictated bioavailable tissue accumulation, whereas Au cores tracked particulate uptake	Baccaro et al. (2022) [3]
Earthworm	Chlorfenapyr	Soil/contact bioassay	Zero mortality up to 72 mg L^{-1}, demonstrating	(2026)

(*Lumbricus terrestris*)	nanosuspension (CFN, 44 nm)	up to 72 mg L^{-1}	enhanced safety relative to target *Spodoptera frugiperda* toxicity	
Nematode (*Caenorhabditis elegans*)	ZnO NPs; Ag NPs; Co/TiO_2 NPs	Aqueous & soil media; chronic exposure	Metabolic and locomotive impairment (ZnO); DNA oxidative damage and multigenerational toxicity (Ag); ROS-linked neurotransmitter disruption (Co, TiO_2)	Hunt et al. (2013) [10]; Huang et al. (2017) [11]; Weishaupt et al. (2023) [12]

Lethal and Sublethal Impacts on Insect Pollinators

Pollinators encounter nano-agrochemicals across spatial scales far exceeding those of sessile soil organisms. Foraging honeybees, bumblebees, and solitary bees visit thousands of blossoms daily, collecting contaminated nectar, pollen, and surface water while carrying electrostatic charges on cuticular hairs that enhance adhesion of fine airborne or floral particulates. Unlike acute chemical poisoning that kills foragers in the field before returning to the nest, sublethal nanoscale exposure routinely enters the hive, degrading colony homeostasis over weeks or brood generations.

1. Midgut Histopathology and Epithelial Barrier Breakdown

The insect midgut-comprising a chitinous–proteinaceous peritrophic membrane (PM) enclosing a single layer of columnar epithelial cells-serves as the primary barrier against ingested xenobiotics. Ultrastructural and histological investigations demonstrate that metal-oxide nanoparticles compromise this barrier at sublethal concentrations. Dabour *et al.* (2019) [13] fed *Apis mellifera* workers sugar syrup laced with sublethal CdO NPs, PbO NPs, or their binary mixture, documenting severe cytological deterioration via transmission electron microscopy. CdO NP exposure completely ruptured the peritrophic membrane, allowing midgut contents to disperse across the alimentary lumen, thinned epithelial walls, dissolved basement membranes, and induced cytoplasmic lysis. PbO NPs thinned the PM to a single fragile layer and drove extensive vacuolization and matrix loss in columnar cells. Chronic exposure to these same CdO and PbO nanoparticles subsequently provoked widespread physiological stress and survival reductions.

Titanium dioxide (TiO_2) and silica (SiO_2) nanoparticles-widely utilized as photocatalytic pesticide carriers, anti-caking adjuvants, or UV shields-likewise inflict gut injury depending on particle size and dose. Ferrara *et al.* (2020) [14] exposed *A. mellifera* to TiO_2 NPs for 10 days across serial dilutions (1×10^{-6} to 1×10^{-3} mg per 10 mL), recording 100% lethality at the highest concentration alongside positive immunohistochemical staining for metallothionein-1 (MT1) in the honey sac and weak heat-shock protein 70 (HSP70) induction in both gut and crop tissues. By contrast, earlier short-term exposures of *Carniolan honeybees* (*A. mellifera carnica*) to carbon black and certain TiO_2 formulations showed minimal acute biochemical shifts, highlighting how crystalline phase, dispersion state, and exposure duration govern outcomes. In the buff-tailed bumblebee (*Bombus terrestris*), 7-week dietary exposure to Ludox TMA silica nanoparticles caused no acute worker mortality or foraging shifts at low concentrations (≤340 µg L^{-1}), yet doses ≥34 mg L^{-1} inflicted severe midgut epithelial damage and triggered total reproductive failure in microcolonies.

2. Gut Microbiome Dysbiosis and Compromised Xenobiotic Detoxification

Adult honeybees and bumblebees harbor a remarkably conserved, specialized core gut microbiota dominated by *Gilliamella apicola*, Snodgrassella alvi, *Lactobacillus* Firm-4/Firm-5, and *Bifidobacterium*. Far from being passive commensals, these symbionts ferment pollen wall polysaccharides, defend against opportunistic pathogens, and actively upregulate host cytochrome P450 detoxification enzymes in the digestive tract-meaning microbiome disruption directly sensitizes bees to co-occurring insecticides.

Rare-earth oxide nanoparticles such as nano-La_2O_3-used in specialized nano-enabled agricultural formulations and soil amendments-exemplify how nanomaterials destabilize this microbial shield. Liu *et al.* (2021) [15] demonstrated through 16S rRNA amplicon sequencing and multivariate dispersion analyses that oral nano-La_2O_3 exposure significantly increased *A. mellifera* mortality while inducing severe gut dysbiosis. Crucially, dysbiosis was characterized by the suppression of protective core phylotypes and the enrichment of opportunistic environmental pathogens. Parallel evidence across emerging synthetic insecticides confirms that chemical stressors erode core *Gilliamella* and *Snodgrassella* populations while allowing opportunistic pathogens such as *Serratia marcescens* and *Enterobacter hormaechei* to bloom. Similarly, chronic exposure to TiO_2 particulate matter (PM_1) alters honeybee gut microbial composition under laboratory conditions. Once the gut barrier is breached and protective symbionts are depleted, bees suffer cascading immune suppression and vulnerability to *Nosema* and viral co-infections.

3. Brain Invasion, Neuronal Apoptosis, and Cognitive Failure

Perhaps the most ecologically consequential discovery in pollinator nanotoxicology is the capacity of ingested nanoparticles to cross the gut epithelium, circulate in hemolymph, penetrate the neural sheath, and impair cognition. In a landmark 2025 investigation, Feng *et al.* (2025) [16] documented the first direct evidence of nano-La_2O_3 invasion and accumulation inside honeybee (*A. mellifera*) brains following oral exposure (>10 mg L^{-1}) via nectar/pollen-relevant syrup. Brain accumulation accelerated neuronal apoptosis and transcriptionally downregulated genes essential for memory consolidation and synaptic

plasticity-specifically cAMP-dependent protein kinase (pka), cAMP-responsive element-binding protein (creb), and N-methyl-D-aspartate receptor 1 (nmdar1). Behaviorally, these molecular lesions translated into dose-dependent failure in olfactory associative learning and memory (proboscis extension reflex assays), reduced sucrose intake, and elevated mortality. For a central-place forager relying on floral odor learning and landmark memory to return to the hive, cognitive collapse equates to ecological death.

Neurobehavioral and enzymatic disruptions are also documented for ZnO, CeO_2, and TiO_2 nanomaterials. Milivojević *et al.* (2015) [17] demonstrated that ingested ZnO nanomaterials alter acetylcholinesterase (AChE) and glutathione S-transferase (GST) biomarkers in honeybees, pointing to neurotoxic potential, while subsequent feeding trials revealed that bees detect and paradoxically alter consumption rates of ZnO-spiked sucrose. Chronic dietary exposure to cerium(IV) oxide ($nCeO_2$) nanoparticles induced sublethal physiological and enzymatic alterations across body compartments in *A. mellifera*. Furthermore, co-exposure to TiO_2 nanoparticles and the pyrethroid deltamethrin altered honeybee brain morphophysiology, illustrating how "inert" nanocarriers or adjuvants can potentiate neurotoxic synthetic pesticides.

4. Vulnerability of Bumblebees and Solitary Bees (*Osmia* spp.)

Risk assessments built exclusively on *Apis mellifera* chronically underestimate hazards to wild pollinators. Solitary mason bees (*Osmia* spp.) and ground-nesting bees lack the eusocial colony buffering of a 30,000-worker honeybee hive, where nurse bees filter contaminated food and queen replacement can offset worker attrition. Consequently, the loss of a single solitary female directly terminates her entire reproductive lineage. Moreover, ground-nesting solitary bees excavate brood cells directly within agricultural soils, facing prolonged dermal contact with soil-accumulated nanopesticides and nano-fertilizers alongside larval oral ingestion of unprocessed pollen provisions. Emerging comparative work reveals that co-exposure to pathogens and insecticides triggers synergistic mortality in solitary bee hosts where honeybees remain comparatively resilient, and larval oral exposure to neonicotinoids like thiacloprid produces pronounced developmental toxicity in *Osmia* spp. As summarized in Table 3 and illustrated in Figure 3, pollinator vulnerability arises from an interconnected gut–microbiome–brain axis.

Table 3: Lethal and Sublethal Ecotoxicological Endpoints in *Apis mellifera*, *Bombus* spp., and Solitary Bees Exposed to Nanomaterials and Nano-Agrochemicals

Pollinator Species	Material / Formulation	Exposure Route & Concentration	Primary Biological & Behavioral Findings	Reference
Apis mellifera	Nano-La_2O_3	Oral syrup (>10 mg L^{-1})	Brain invasion and neuronal apoptosis; downregulation of pka, creb, nmdar1; impaired olfactory associative learning and memory; reduced sucrose intake and survival	Feng et al. (2025) [16]
Apis mellifera	Nano-La_2O_3	Chronic oral exposure	Elevated mortality; significant gut bacterial community shift (dysbiosis) with enrichment of opportunistic pathogens	Liu et al. (2021) [15]
Apis mellifera	CdO NPs, PbO NPs, & binary mixture	Sublethal chronic oral syrup	Rupture of peritrophic membrane (CdO), epithelial thinning, loss of microvilli, cytoplasmic vacuolization, and basement membrane degeneration	Dabour et al. (2019) [13]; Al Naggar et al. (2020) [18]
Apis mellifera	TiO_2 NPs	10-day oral exposure (10^{-6} to 10^{-3} mg/10 mL)	100% mortality at 10^{-3} mg/10 mL; significant anti-MT1 induction in honey sac and weak HSP70 positivity in gut/crop	Ferrara et al. (2020) [14]
Apis mellifera	ZnO NMs; CeO_2 NPs	Dietary oral exposure	Altered AChE/GST activities and altered sucrose feeding preferences (ZnO); chronic compartment-specific sublethal biomarker shifts (CeO_2)	Milivojević et al. (2015) [17]; Kos et al. (2017) [19]
Apis mellifera	Chlorfenapyr nanosuspension (CFN, 44 nm)	48-h acute oral bioassay	48-h LC_{50} = 798.43 mg L^{-1}, demonstrating substantially improved pollinator safety relative to high target lepidopteran toxicity (LC_{50} 2.75–7.22 mg L^{-1})	(2026)
Apis mellifera	O-carboxymethyl chitosan chlorfenapyr / Abm@TPP/CMCS	Acute oral/contact assays	Sustained-release biopolymer carriers markedly reduced acute toxicity to honeybees compared with conventional technical-grade insecticides	(2023; 2026)
Bombus terrestris	Ludox TMA silica (SiO_2) NPs	7-week microcolony diet (34 µg L^{-1} to 340 mg L^{-1})	No acute worker mortality; no foraging deficit ≤340 µg L^{-1}; severe midgut epithelial injury and 100% loss of reproduction at ≥34 mg L^{-1}	Mommaerts et al. (2012) [20]
Osmia spp. & *Bombus*	Conventional vs. nano-enabled neuroactive pesticides	Larval/adult oral & contact	Solitary bees lack eusocial buffering; heightened sensitivity to combined pesticide–pathogen stress and larval neonicotinoid residues	Claus et al. (2021) [21]; (2025)

Comparative Risk Synthesis, Safe-by-Design Mitigation, and Regulatory Gaps

1. Divergent Hazard Trajectories: Inorganic Nanomaterials Versus Type 2 Biopolymer Nanocarriers

A central contribution of cross-study synthesis is resolving an apparent contradiction in the literature: why do roughly 43% of reviewed nano-enabled pesticide comparisons report reduced non-target toxicity, whereas metallic and inorganic nano-

formulations frequently trigger amplified or prolonged ecological damage? The answer lies in separating carrier-mediated dose reduction and selectivity from intrinsic nanomaterial persistence and ion-shedding.

When conventional hydrophobic insecticides are formulated into biodegradable polymeric nanocarriers-such as O-carboxymethyl chitosan cross-linked with sodium tripolyphosphate (Abm@TPP/CMCS) or stimulus-responsive pH-gated nanocapsules-or engineered as surfactant-stabilized nanosuspensions, two protective mechanisms emerge. First, high target efficacy allows dramatically lower field application rates. Second, sustained release within insect-gut-specific pH or enzymatic triggers limits rapid dermal absorption and acute spiking in non-target honeybees and earthworms. For instance, a 44 nm chlorfenapyr nanosuspension exhibited a 48-h honeybee LC_{50} of 798.43 mg L^{-1} alongside zero earthworm mortality at 72 mg L^{-1}, achieving a wide selectivity window over lepidopteran pests (LC_{50} 2.75–7.22 mg L^{-1}). Similarly, carrier-minimized prodrug self-assemblies eliminate excess synthetic surfactants and recalcitrant inorganic matrices.

By contrast, Type 1 inorganic metal and metal-oxide nanoparticles (CuO, $Cu(OH)_2$, Ag, ZnO, La_2O_3, CeO_2) do not biodegrade into benign metabolites. Their metallic cores persist across growing seasons, continually cycling between particulate and dissolved ionic states, suppressing key soil enzyme activities up to a year post-application, slowing degradation of co-applied organic insecticides, and bioaccumulating in earthworms and hive matrices. Consequently, replacing biodegradable organic agrochemicals with persistent inorganic metal-oxide nanoparticles risks trading acute organic toxicity for irreversible trace-metal soil accumulation and chronic pollinator sublethal dysfunction (Figure 4).

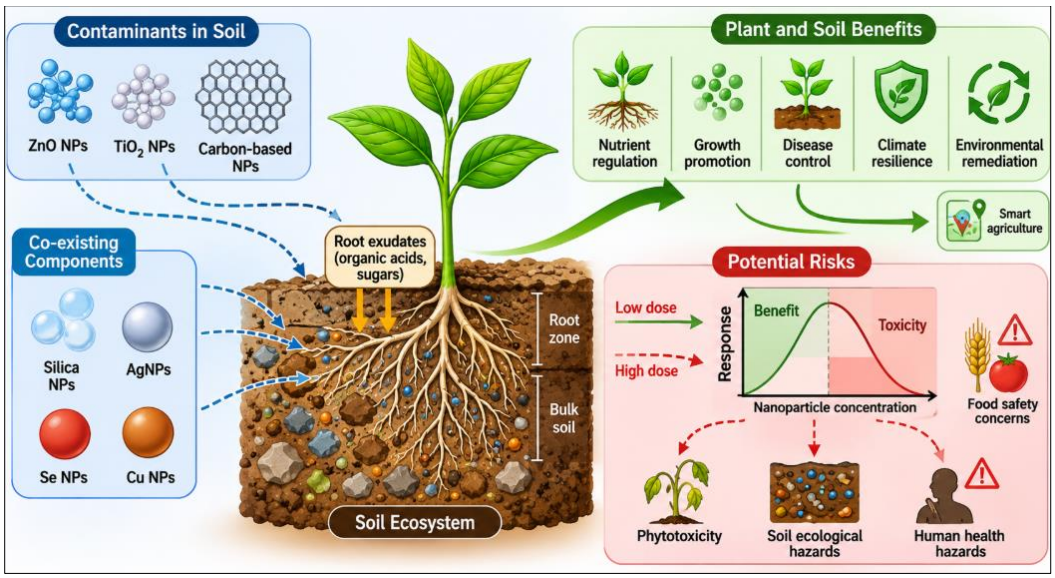

Fig 3: Comparative risk profile across agrochemical formulation architectures, illustrating how persistent inorganic nanomaterials elevate chronic terrestrial hazards whereas biodegradable, stimuli-responsive Type 2 nanocarriers offer viable safe-by-design mitigation pathways.

2. Safe-by-Design Principles and Regulatory Bottlenecks

Translating nanotechnology safely into climate-resilient farming requires embedding Safe-by-Design (SbD) principles at the molecular synthesis stage rather than relying on post-market remediation. Key engineering levers include: (1) substituting persistent inorganic cores with biodegradable polysaccharides (chitosan, alginate, cellulose, lignin) or naturally occurring biominerals (hydroxyapatite); (2) targeting release triggers to pest-specific alkaline midgut pH or digestive enzymes rather than passive diffusion, preventing premature leaching into nectar or soil porewater; (3) controlling surface chemistry and dissolution kinetics via biocompatible capping agents that minimize indiscriminate ROS bursts and ionic spikes; and (4) accounting for co-formulants, ensuring that surfactants such as Tween-80 or dispersing solvents do not independently compromise pollinator cuticular wax or microbial membranes.

At the governance level, current regulatory testing frameworks administered by agencies in North America, Europe, and Asia remain misaligned with nanoscale ecotoxicology. Standard OECD guidelines rely on short-term acute mortality (48–96 h) in surrogate species (*A. mellifera*, *E. fetida*) using pristine, unaged dispersions spiked into artificial media. These protocols fail on four critical fronts:

1. **Environmental Aging Neglect:** Toxicity in freshly spiked deionized water or artificial LUFA soil diverges markedly from aged agricultural soils where sulfidation, DOC eco-corona formation, and soil pH transform bioavailability.
2. **Blindness to Sublethal Endpoints:** Standard honeybee LD_{50} tests miss brain invasion, pka/creb suppression, olfactory memory deficits, gut dysbiosis, and bumblebee microcolony reproductive collapse.
3. **Omission of Wild and Solitary Pollinators:** Extrapolating honeybee tolerances to solitary soil-nesting bees (*Osmia*, *Andrena*) ignores distinct life-history exposure routes and the absence of colony-level buffering.
4. **Life-Cycle Risk Assessment (LCRA) Gaps:** As highlighted in recent life-cycle evaluations, manufacturing byproducts, wastewater biosolid pathways, and multi-season soil accumulation must be integrated alongside field efficacy.

Conclusion and Future Research Priorities

Nano-pesticides and nano-fertilizers represent neither a blanket ecological panacea nor a uniform hazard; rather, their ecotoxicological footprint bifurcates sharply according to material persistence, dissolution behavior, and carrier biodegradability. While controlled-release macronutrient nanohybrids (such as urea–hydroxyapatite) and biodegradable biopolymer-encapsulated insecticides demonstrate genuine potential to curb nutrient runoff and lower acute mortality in honeybees and earthworms, widespread agricultural deployment of persistent metal, metal-oxide, and rare-earth nanomaterials introduces severe, under-regulated risks. Empirical evidence demonstrates that nanoscale copper, silver, zinc, and lanthanum formulations can depress soil enzymatic resilience for up to a year, erode nitrogen-cycling nosZ gene pools, disproportionately intoxicate soil arthropods such as *Folsomia candida*, and breach pollinator gut and neural barriers-triggering microbiome dysbiosis, neuronal apoptosis, and associative learning failure.

To ensure that agricultural nanotechnology advances sustainable food security without eroding soil fertility and pollination services, four research priorities demand immediate investment:

1. **Multi-Year Field Mesocosms with Aged Formulations:** Quantifying cumulative trophic transfer from soil-aged nano-agrochemicals into crop nectar, pollen, and guttation fluid across realistic agronomic rotations.
2. **Standardized Sublethal Microbiome and Neurocognitive Assays:** Incorporating 16S/metagenomic gut dysbiosis metrics, proboscis extension reflex learning tests, and *Osmia*/*Bombus* reproductive endpoints into regulatory tier-2 testing.
3. **Mixture and Co-Stressor Ecotoxicology:** Evaluating realistic interactions between nano-agrochemicals, co-applied organic pesticides (e.g., neonicotinoid persistence shifts), microplastics, and pollinator pathogens.
4. **Biodegradable, Target-Gated Nanocarriers:** Prioritizing Safe-by-Design biopolymer and carrier-minimized formulations validated through full Life-Cycle Risk Assessment before commercial field scaling.

References

1. Adisa IO, Pullagurala VLR, Peralta-Videa JR, Dimkpa CO, Elmer WH, Gardea-Torresdey JL, et al. Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action. Environ Sci Nano. 2019;6(7):2002-30.
2. Al Naggar Y, Dabour K, Masry S, Sadek A, Naiem E, Giesy JP. Sublethal effects of chronic exposure to CdO or PbO nanoparticles or their binary mixture on the honey bee (Apis mellifera L.). Environ Sci Pollut Res Int. 2020;27:19004-15. doi:10.1007/s11356-018-3314-2
3. Asadishad B, Chahal S, Akbari A, Cianciarelli V, Azodi M, Ghoshal S, et al. Amendment of agricultural soil with metal nanoparticles: effects on soil enzyme activity and microbial community composition. Environ Sci Technol. 2018;52(4):1908-18. doi:10.1021/acs.est.7b05389
4. Asif MAA, Mahjabin F, Singha SK, Rahman Jahangir MM, Hoque SM. Application of nano-urea in conventional flood-irrigated Boro rice in Bangladesh and nitrogen losses investigation. Heliyon. 2024;10:e37150. doi:10.1016/j.heliyon.2024.e37150
5. Baccaro M, Montaño MD, Cui X, Mackevica A, Lynch I, von der Kammer F, et al. Influence of dissolution on the uptake of bimetallic nanoparticles Au@Ag-NPs in soil organism Eisenia fetida. Chemosphere. 2022;302:134909. doi:10.1016/j.chemosphere.2022.134909
6. Carley LN, Panchagavi R, Song X, Davenport S, Bergemann CM, McGee SD, et al. Long-term effects of copper nanopesticides on soil and sediment community diversity in two outdoor mesocosm experiments. Environ Sci Technol. 2020;54(14):8878-89.
7. Claus G, Pisman M, Spanoghe P, Smagghe G, Eeraerts M. Larval oral exposure to thiacloprid: dose-response toxicity testing in solitary bees, Osmia spp. (Hymenoptera: Megachilidae). Ecotoxicol Environ Saf. 2021;215:112143. doi:10.1016/j.ecoenv.2021.112143
8. Dabour K, Al Naggar Y, Masry S, Naiem E, Giesy JP. Cellular alterations in midgut cells of honey bee workers (Apis mellifera L.) exposed to sublethal concentrations of CdO or PbO nanoparticles or their binary mixture. Sci Total Environ. 2019;651:1356-67. doi:10.1016/j.scitotenv.2018.09.311
9. Das P, Barker C, Park Y, Perreault F, Westerhoff P, Penton CR. Impact of graphite nano amendments on soil enzyme activities, functional genes and microbiome composition in a soil-plant system. Soil Biol Biochem. 2025;203:109714. doi:10.1016/j.soilbio.2025.109714
10. Feng W, Ge Y, Holden PA, Zhang Y, Dai P, Liu YJ. Nano-La2O3 undermines honeybee cognition by invading the brain and accelerating neuronal apoptosis. Environ Sci Nano. 2025. doi:10.1039/D5EN00105F
11. Ferrara G, Salvaggio A, Pecoraro R, Scalisi EM, Presti AM, Impellizzeri G, et al. Toxicity assessment of nano-TiO2 in Apis mellifera L., 1758: histological and immunohistochemical assays. Microsc Res Tech. 2020;83(4):332-7. doi:10.1002/jemt.23418
12. Gao M, Chang J, Wang Z, Zhang H, Wang T. Advances in transport and toxicity of nanoparticles in plants. J Nanobiotechnology. 2023;21:75. doi:10.1186/s12951-023-01830-5
13. Ge Y, Schimel JP, Holden PA. Evidence for negative effects of TiO2 and ZnO nanoparticles on soil bacterial communities. Environ Sci Technol. 2011;45(4):1659-64. doi:10.1021/es103040t

14. Gilbertson LM, Pourzahedi L, Laughton S, Gao X, Zimmerman JB, Theis TL, et al. Guiding the design space for nanotechnology to advance sustainable crop production. Nat Nanotechnol. 2020;15(9):801-10. doi:10.1038/s41565-020-0706-5
15. Gomes SIL, Scott-Fordsmand JJ, Amorim MJB. Commercial nano-enabled fertilizer: unveiling its mechanisms of toxicity in non-target soil invertebrate species using a high-throughput transcriptomics approach. Environ Sci Nano. 2025;12(8):4096-106. doi:10.1039/D5EN00314H
16. Grün AL, Straskraba S, Schulz S, Schloter M, Emmerling C. Long-term effects of environmentally relevant concentrations of silver nanoparticles on microbial biomass, enzyme activity, and functional genes involved in the nitrogen cycle of loamy soil. J Environ Sci (China). 2018;69:12-22.
17. Huang CW, Li SW, Liao VHC. Chronic ZnO-NPs exposure at environmentally relevant concentrations results in metabolic and locomotive toxicities in Caenorhabditis elegans. Environ Pollut. 2017;220:1456-64. doi:10.1016/j.envpol.2016.10.086
18. Hunt PR, Marquis BJ, Tyner KM, Conklin S, Olejnik N, Nelson BC, et al. Nanosilver suppresses growth and induces oxidative damage to DNA in Caenorhabditis elegans. J Appl Toxicol. 2013;33(10):1131-42. doi:10.1002/jat.2872
19. Kah M, Kookana RS, Gogos A, Bucheli TD. A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. Nat Nanotechnol. 2018;13(8):677-84.
20. Kos M, Jemec Kokalj A, Glavan G, Marolt G, Zidar P, Božič J, et al. Cerium(IV) oxide nanoparticles induce sublethal changes in honeybees after chronic exposure. Environ Sci Nano. 2017;4(12):2297-310. doi:10.1039/C7EN00596B
21. Kottegoda N, Sandaruwan C, Priyadarshana G, Siriwardhana A, Rathnayake UA, Berugoda Arachchige DM, et al. Urea-hydroxyapatite nanohybrids for slow release of nitrogen. ACS Nano. 2017;11(2):1214-21. doi:10.1021/acsnano.6b07781
22. Li X, Wang X, Sun C, Wang A, An C, Li N, et al. A unimolecule nanopesticide delivery system applied in field scale for enhanced pest control. Nat Commun. 2025;16:6809.
23. Liu YJ, Jing Z, Bai XT, Diao QY, Wang J, Wu YY, et al. Nano-La2O3 induces honeybee (Apis mellifera) death and enriches for pathogens in honeybee gut bacterial communities. Front Microbiol. 2021;12:780943. doi:10.3389/fmicb.2021.780943
24. Milivojević T, Glavan G, Božič J, Sepčić K, Mesarič T, Drobne D. Neurotoxic potential of ingested ZnO nanomaterials on bees. Chemosphere. 2015;120:547-54. doi:10.1016/j.chemosphere.2014.07.054
25. Mommaerts V, Jodko K, Thomassen LCJ, Martens JA, Kirsch-Volders M, Smagghe G. Assessment of side-effects by Ludox TMA silica nanoparticles following a dietary exposure on the bumblebee Bombus terrestris. Nanotoxicology. 2012;6(5):554-61. doi:10.3109/17435390.2011.590905
26. Neves J, Cardoso DN, Malheiro C, Kah M, Soares AMVM, Wrona FJ, et al. Copper toxicity to Folsomia candida in different soils: a comparison between nano and conventional formulations. Environ Chem. 2019;16(6):419-29. doi:10.1071/EN19061
27. Peixoto S, Henriques I, Loureiro S. Long-term effects of Cu(OH)2 nanopesticide exposure on soil microbial communities. Environ Pollut. 2021;269:116113. doi:10.1016/j.envpol.2020.116113
28. Peixoto S, Morgado RG, Prodana M, Cardoso DN, Malheiro C, Neves J, et al. Responses of soil microbiome to copper-based materials (nano and bulk) for agricultural applications: an indoor-mesocosm experiment. NanoImpact. 2024;34:100506. doi:10.1016/j.impact.2024.100506
29. Read DS, Matzke M, Gweon HS, Newbold LK, Heggelund L, Ortiz MD, et al. Soil pH effects on the interactions between dissolved zinc, non-nano- and nano-ZnO with soil bacterial communities. Environ Sci Pollut Res Int. 2016;23:4120-8. doi:10.1007/s11356-015-4538-z
30. Samarajeewa AD, Velicogna JR, Princz JI, Subasinghe RM, Scroggins RP, Beaudette LA. Effect of silver nano-particles on soil microbial growth, activity and community diversity in a sandy loam soil. Environ Pollut. 2017;220:504-13. doi:10.1016/j.envpol.2016.09.094
31. Simonin M, Colman BP, Tang W, Judy JD, Anderson SM, Bergemann CM, et al. Plant and microbial responses to repeated Cu(OH)2 nanopesticide exposures under different fertilization levels in an agro-ecosystem. Front Microbiol. 2018;9:1769. doi:10.3389/fmicb.2018.01769
32. Simonin M, Martins JMF, Le Roux X, Uzu G, Calas A, Richaume A. Toxicity of TiO2 nanoparticles on soil nitrification at environmentally relevant concentrations: lack of classical dose-response relationships. Nanotoxicology. 2017;11(2):247-55.
33. Wang D, Saleh NB, Byro A, Zepp R, Sahle-Demessie E, Luxton TP, et al. Nano-enabled pesticides for sustainable agriculture and global food security. Nat Nanotechnol. 2022;17(4):347-60. doi:10.1038/s41565-022-01082-8
34. Wang F, Liu X, Shi Z, Tong R, Adams CA, Shi X. Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants: a soil microcosm experiment. Chemosphere. 2016;147:88-97. doi:10.1016/j.chemosphere.2015.12.076
35. Weishaupt AK, Kubens L, Ruecker L, Schwerdtle T, Aschner M, Bornhorst J. Evaluation of neurotoxicity and the role of oxidative stress of cobalt nanoparticles, titanium dioxide nanoparticles and multiwall carbon nanotubes in Caenorhabditis elegans. Toxicol Sci. 2023;kfad084. doi:10.1093/toxsci/kfad084

36. Zhang S, Ren S, Pei L, Sun Y, Wang F. Ecotoxicological effects of polyethylene microplastics and ZnO nanoparticles on earthworm Eisenia fetida. Appl Soil Ecol. 2022;176:104469.
37. Zhang X, Xu Z, Wu M, Qian X, Lin D, Zhang H, et al. Potential environmental risks of nanopesticides: application of Cu(OH)2 nanopesticides to soil mitigates the degradation of neonicotinoid thiacloprid. Environ Int. 2019;129:42-50. doi:10.1016/j.envint.2019.05.022