Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Review
Volume 15, Issue 2, 2025, 30
https://doi.org/10.33263/BRIAC152.030

Assessing the Ecotoxicological Impact of Copper-Based Nano-Pesticides: A Comprehensive Review

Dipak Kumar Mahida [1], Ankita Patel [1,*], Mahipal Singh Sankhla [2,*], Bhumit Chavda [1], Vishal M. Makwana [3], Kapil Kumar[1], Rakesh Rawal [4]

[1] Department of Biochemistry & Forensic Science, Gujarat University, Ahmedabad, India.; dipakkumar.mahida@gmail.com (D.M.); anki.patel122@gmail.com (A.P.); bhumitchavda10@gmail.com (B.C.); kkforensic@gmail.com (K.K.);
[2] School of Basic and Applied Sciences, K.R. Managlam University, Gurugram, India; mahipal4n6@gmail.com; (M.S.S.);
[3] Department of Zoology, Sir P.P. Institute of Science, M.K. Bhavnagar University, Bhavnagar, Gujarat, India; vishalmakwana98@gmail.com;
[4] Department of Life Sciences, Gujarat University, Ahmedabad, India; rakeshrawal@gujaratuniversity.ac.in;
* Correspondence: anki.patel122@gmail.com (A.P.); mahipal4n6@gmail.com (M.S.S.);
Scopus Author ID 57219964786

Received: 31.07.2024; Accepted: 6.03.2025; Published: 15.04.2025

Abstract: Copper-based nanoparticles have attracted a lot of interest in agricultural applications due to their many different qualities and usefulness. Pesticides and fertilizers are used in agrochemicals for effective delivery and regulated release. However, the growing usage of innovative pesticides using nanomaterials is seen as a viable way to lessen the impact of farming practices on the environment and the well-being of the general population. Nanopesticides may negatively affect creatures that are not their intended targets, a possibility often not adequately taken into account by those who produce them. This review paper discusses the potential harmfulness and ability to be absorbed by living organisms of copper nanoparticles to aquatic and terrestrial plants, algae, and other organisms, as well as their interactions with soil and microbial communities. The article gathers data from several research that demonstrate the influence of copper nanoparticles on the physiology, metabolism, and development of diverse plant species. Eventually, the review concludes by looking at the possible hazards associated with using copper nanoparticles in the surrounding ecosystem and outlining possible scopes of further investigation.

Keywords: ecotoxicity; Cu-based nanopesticides; accumulation; non-target organism; environmental fate.

1. Introduction

In recent years, nanotechnology has been studied for its potential applications in agriculture, specifically in crop production, as a means to use resources more efficiently. Nanotechnology can help improve crop yields, promote food safety, and reduce the use of resources. Scientists are investigating various nanotechnology-based solutions, such as delivery systems for agrochemicals (fertilizers and pesticides) and nanosensors to monitor soil and plant health [1-4]. Metal oxide nanoparticles are presently being produced in substantial quantities for industrial and domestic use, but their potential for future applications is even more significant [5]. Copper-based nanoparticles (Cu NPs) have various current and prospective applications in engineering, agriculture, and other fields. While a considerable amount of these Cu NPs might be deposited in landfills, a portion can also find their way into soils and water bodies, particularly when copper-based insecticides and biosolids are employed. It is necessary to comprehend the interactions between plants, plant-derived biomolecules, and NPs, as well as the bioavailability of the NPs [6]. The metabolic functions of plants, such as photosynthesis, electron transport, respiration, oxidative stress response, hormone signaling, cell wall metabolism, transcription signaling, protein trafficking, oxidative phosphorylation, and iron mobilization, depend on copper, an essential nutrient [7]. There are apprehensions regarding the potential impacts of these nanoparticles on the environment and human well-being. CuO NPs' toxicity, accumulation, and environmental destiny have all been the subject of much research. However, $Cu(OH)_2$ nanopesticide provides an environmental concern; however, the present extent of understanding does not offer a dependable evaluation of this potential risk. Particularly, it is unknown whether and how $Cu(OH)_2$ nanopesticide, once applied in the field, influences the degradation of organic contaminants in soil. These concerns are crucial for thoroughly evaluating the risks associated with using the nanopesticide $Cu(OH)_2$ since environmental safety and the degradation of organic pollutants are strongly intertwined. The current work aims to clarify how $Cu(OH)_2$ nanopesticide slows down insecticide breakdown in soil [8]. In agriculture, Copper oxide nanoparticles (CuO NPs) are employed as an effective alternative to conventional copper and have the potential to enter the soil. Their toxicity against cells and microorganisms is significant, whereas their toxicity for soil invertebrates is moderate [9]. Also, they are widely recognized for their well-known antimicrobial actions, which may be harmful to the health of the soil, especially to the activities of soil microorganisms, which are crucial to the operation of terrestrial and agroecosystems [10]. This review paper offers a comprehensive summary of the current understanding regarding utilizing CuO NPs as nano pesticides, their potential adverse impacts on the environment and human health, and any practical challenges that may arise.

2. Materials and Methods

A systematic search was done using electronic databases such as PubMed, Web of Science, and Google Scholar and manual searches of pertinent peer-reviewed journals to gather the scientific papers for this review. The following keywords were used in the search from August 2022 to January 2023: (i) "Cu-based nano" and "Ecotoxicity," (ii) "Cu-based nano" and "Bioaccumulation," (iii) "Cu-based nano" and "non-target organism" (iv) "Cu-based nano" and "Agrochemicals" and "Ecotoxicity" (v) "Cu-based nano" and "Bioavailability". Additional research papers were found by manually searching the references of pertinent papers. Two reviewers independently assessed the titles and abstracts of the identified papers to determine their relevance to the review topic. Disagreements were settled through discussion or consultation with another reviewer. Following the retrieval of the full texts of the chosen studies, eligibility was determined in accordance with the inclusion and exclusion criteria.

2.1. Inclusion criteria.

Studies that indicate the ecotoxicological effect of Cu-based nanomaterials such as nanopesticides, nanofertilizers, nanoparticles, nanorods or nanowires, etc., on living organisms and/or environmental factors (e.g., soil, water) are included for the review. Indication of environmental fate is also taken into consideration.

2.2. Exclusion criteria.

Studies that do not indicate direct environmental fate or negative impacts on living organisms are excluded. Specific studies on other pesticides were also excluded. In the retrieved materials, some review articles, technical notes, protocols, and articles in languages other than English were also identified and excluded from this review.

To find themes, discrepancies, and gaps in the body of research, the included publications underwent a thorough analysis and comparison. The research design, sample size, intervention/exposure, outcome measures, statistical analysis, and other elements of the publications were the main topics of the analysis. After the identified references, articles were explored for relevant information. Data of our interest were extracted in an Excel file for the further process. This procedure was created to provide a thorough and objective search for relevant research and to reduce the possibility of overlooking important articles.

3. Results and Discussion

3.1. Comprehensive background.

In an initial finding, we yielded 76 papers of our interest. After applying inclusion/exclusion criteria, 44 papers were selected for the review. These articles were classified into 3 categories according to study samples: (i) Studies that indicated effects on living organisms (Table 1), (ii) Studies that indicated effects on soil (Table 2), and (iii) Studies that indicated effects of water (Table 3). Most articles were categorized in 1st category because researchers had focused more on the ecotoxicity of pesticides on living organisms such as vertebrates, invertebrates, and crop plants. We found very few studies indicating the ecotoxic effects of Cu-based nanomaterial on water. There may not be enough study in this field since the ecotoxic effects of Cu-based nanoparticles on water have not garnered significant attention from the scientific community [11,12]. Another possible aspect may be the absence of regulatory requirements for researching the ecotoxic effects of copper-based nanoparticles on water. Although not all nations have such restrictions, some demand that nanoparticles be evaluated before being utilized in commercial goods. As a consequence, there could be less motivation for researchers in nations without such rules to investigate the ecotoxicity of nanoparticles [13]. Some of the articles indicate a combined study of soil and sample organisms. These are included in both categories and reviewed for both aspects.

The findings establish a robust foundation for further exploration, encompassing diverse methodologies, experimental subjects, and chemical compounds. In particular, including numerous test species and various chemical categories enables in-depth discussions regarding the specific toxicity of each group of organisms.

Table 1. Studies of Cu-based nano-pesticides impact different living organisms.

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[1]	Zebrafish (*Danio rerio*)	Cu NPs	According to the quantity of copper supplied, each exposure was carried out in triplicate tanks holding 10 female zebrafish, each in 10 L of facility water with a 0.45 m filter and constant aeration. The dosage was carried out by adding 10 mL of a 1000 stock that had been made in ultrapure water.	Acute toxicity: 48 hrs	Gill toxicity	Wild-type zebrafish were purchased from Ekk-Will (Gibsonton, FL) and maintained at the Aquatic Toxicology Laboratory of the University of Florida.	ICP-MS	The predominant site of acute toxicity brought on by copper nanoparticles was the gill. Interlamellar cell growth was inhibited by copper nanoparticle exposure, and interlamellar cell proliferation was induced; nevertheless, there was no histological or biochemical indication of injury to other organs.
[2]	mung bean (*P. radiatus*)	Cu NPs	Plant agar method: Following thorough rinsing with deionized water and sterilization in a 5% sodium hypochlorite solution for 10 minutes, the seeds were placed on moist cotton at a consistent temperature of 25°C (with a mean standard deviation) under dark conditions.	Acute toxicity: 24 hrs	seedling and shoot growth	The American Society for Testing and Materials (ASTM), located in West Conshohocken, Pennsylvania, USA, and the Organization for Economic Cooperation and Development both endorsed mung beans. Every seed was grown in Korea.	ICP-AES, TEM-EDS	The plant was exposed to NPs, which decreased its growth rates. The apparent toxicity was obviously caused by the Cu NPs, even though the generation of cupric ions by the Cu NPs was minimal. The test species' TEM scans demonstrated that Cu NPs may pass the cellular membrane and have the potential to accumulate within the cells.
[2]	Wheat (*T. aestivum subsp. aestivum*)	Cu NPs	Plant agar method: Following thorough rinsing with deionized water and sterilization in a 5% sodium hypochlorite solution for 10 minutes, the seeds were placed on moist cotton at a consistent temperature of 25°C (with a mean standard deviation) under dark conditions.	Acute toxicity: 24 hrs	seedling and root growth	Wheat has received endorsement from both the United States Food and Drug Administration and the Organization for Economic Cooperation and Development (based in Paris, France). Every seed was grown in Korea.	ICP-AES, TEM-EDS	The plant was exposed to NPs, which decreased its growth rates. The apparent toxicity was obviously caused by the Cu NPs, even though the generation of cupric ions by the Cu NPs was minimal. The test species' TEM scans demonstrated that Cu NPs may pass the cellular membrane and have the potential to accumulate within the cells.
[3]	*Pseudokirchneriella subcapitata*	CuO NPs	Laboratory setting: OECD, 1984	72 hrs	Growth inhibition	The study employed exponentially developing algal cultures that were subjected to different test drug concentrations under controlled circumstances. Over the span of three	Fluorometer	In comparison to the bulk form, CuO nanoparticles were unquestionably more harmful to algae. The growing medium lacked any discernible aggregates. Compared to bulk CuO, CuO

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
						days, the cell concentration in the control culture increased by a minimum of 16-fold.		nanoparticles were both more soluble and poisonous.
[4]	Algae (*Microcystis aeruginosa*)	CuO NPs	Each of the six treatments contained a concentration of 0.5 mg L1 of CuO NPs in the medium prepared using natural water, and the tested solutions were prepared by adding stock solutions of CuO NPs to the algal medium.	0-5 days	Cytological and internalization	The Chinese Academy of Sciences' Institute of Hydrobiology sold the Microcystis aeruginosa with the code M-905. Algal cells were cultivated in the whole BG11 media using the strain, which was identified from Dianchi Lake in China. The modified BG11 medium, which lowers the ionic strength by half by adding an inorganic nutrient and one-fifth by adding citrate, was used for all subsequent bioassays.	SEM, TEM	NPs smaller than 5 nm could get into M. aeruginosa intact cells congregate in certain regions, produce too much ROS, harm DNA, and ultimately compromise membrane integrity and algal development. In addition, upon internalization, CuO was converted to Cu2O.
[5]	Cucumber (*Cucumis sativus*)	Cu NPs	Prior to applying nCu to the soil (Sedgwick soil), DI water was sonicated for 30 minutes with nCu suspended in it. 0 (control) and 800 mg/kg of nCu were the final concentrations in the soil. In the first 30 days of the SRE treatment, 0 (control) and 5 mL of the SRE mixture were applied to the soil.	30 days	Interaction, transformation, and bioavailability	The cucumber plants were cultivated in a greenhouse for a period of 60 days, with a controlled temperature range of 25.5 to 30.0°C during the day and 17.7 to 18.9°C at night.	ICP-MS	The availability of free Cu significantly increased in soils exposed to nCu and SRE, impacting soil organisms such as bacteria, worms, and other invertebrates. On the other hand, plants exposed to soil containing nCu and additional SRE saw a markedly reduced amount of Cu translocation to the leaves and higher plant tissues.
[6]	Plants	$Cu(OH)_2$ nano pesticide	The Kocide suspension (6.68 mg/L in DI water) was applied to the foliage of each mesocosm using a Hudson sprayer (Model 13581, Chicago, IL, United States), resulting in an aboveground plant biomass exposure of 30 mg/m2. This was done in accordance with	0, 75, 155 days	Accumulation	The outdoor terrestrial mesocosms were located in the Duke Forest, situated in Durham, North Carolina, USA.	ICP-MS	Control and Kocide-exposed mesocosms no longer had substantially differing plant biomass Cu contents.

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
			the manufacturer's dosage and exposure mode recommendations.					
[7]	The vegetables, [kale (*Brassica oleracea, var. Acephala Lacinato*), lettuce (*Lactuca sativa var. green leaf cultivar*), and collard green (*Brassica oleracea, var. Acephala*)]	CuO NPs	n.d.	n.d.	Accumulation	To ensure freshness, three USDA-certified organic vegetables were purchased on the same day as the test, and leaf tissues were obtained from them. In Bakersfield, California, the United States, Cal-Organic Farms produced the veggies.	ICP-MS	Sp-ICP-MS is a valuable tool for understanding the interactions between engineered nanomaterials (ENMs) and plants, including processes such as absorption, accumulation, translocation, and biological effects.
[8]	Tobacco hornworm (*Manduca sexta*)	Cu(OH)2 nano pesticides	From the first and second-instar stages, *M. sexta* larvae were fed with the treated leaves.	6 days	Accumulation, Transformation	Eggs of the tobacco hornworm (*Manduca sexta*), sourced from Carolina Biological Supply and originating from North Carolina, were acquired and allowed to hatch on untreated leaves.	ICP-MS	With rising dissolved Cu_2 concentrations in the treatments, growth inhibition becomes more pronounced. Numerous species have been proven to suffer different negative consequences from prolonged exposure to Cu_2. Cu_2 is very effective in altering the redox conditions in biological systems, harming macromolecules, and interfering with cellular activity and metabolism.
[8]	Tomato (*Solanum lycopersicum cv.*)	$Cu(OH)_2$ nano pesticides	In order to spray nanomaterial suspensions onto the leaves, which were spread flat over the filter paper, A spray chamber was constructed by utilizing a 1-L polyethylene wide-mouth container and a concentric	n.d.	Accumulation, Transformation	Plants for the tomato variety Micro-Tom (*Solanum lycopersicum*) were germinated on Phytagel plates (Sigma-	ICP-MS	With rising dissolved Cu_2 concentrations in the treatments, growth inhibition becomes more pronounced. Numerous species have been proven to suffer different negative consequences from

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
			nebulizer that was operated with argon gas.			Aldrich, St. Louis, MO, USA).		prolonged exposure to Cu_2. Cu_2 is very effective in altering the redox conditions in biological systems, harming macromolecules, and interfering with cellular activity and metabolism.
[9]	Bacterial Communit y	$Cu(OH)_2$ nano pesticide and nano-$Cu(OH)_3$	To attain a moisture content of 20% (equivalent to 50% maximum water-holding capacity), soil samples weighing 10 g per glass vial (dry weight) were supplemented with homogeneous aqueous suspensions (2.0 mL) containing different concentrations of $Cu(OH)_2$ nanopesticide or nano-$Cu(OH)_2$. This procedure resulted in $Cu(OH)_2$ doses of 0.5, 5, or 50 mg of Cu per kg of dry soil.	21 days	Bacterial community analysis	A surface loamy soil sample (0-20 cm) was collected from a greensward located on the campus of Zhejiang Sci-Tech University in Hangzhou, China, which is situated at coordinates 30.3147° N and 120.3504° E.	n.d.	Slight differences in the bacterial population and enzyme activity of the soil were observed between NPF and AI-NPF treatments, suggesting that the presence of nano-$Cu(OH)_2$ was primarily responsible for the effects observed in the $Cu(OH)_2$ nanopesticides.
[10]	Barley Plant (*Hordeum sativum L*)	CuO nanopowde r	The Petri dish containing no extra nutrients was given. A quantity of 5 ml of double-distilled water was used, with the option of including nano-CuO and bulk-CuO (control, 300 mg/L, 2000 mg/L), and it was kept in a growth chamber at 28°C.	24 days	Growth, Bioaccumu lation	The *H. sativum distichum cv*. Ratnik specimens were visually inspected to assess any observable morphological harm, and 25 sterilized seeds were evenly dispersed on a circular filter paper within three sterile Petri dishes.	AAS	Nano-CuO exposure led to a significant increase in the metal content in both the shoots and roots of *H. sativum*, with concentrations rising several-fold.
[11]	Eastern mosquitof ish (*Gambusi a holbrooki*)	$Cu(OH)_2$ nano pesticide	Before beginning the NP dose, the initial concentration of Cu in the mesocosm water was 1.48 0.93 g L1. Despite the initial Cu background, a variable dosage rate was employed to make the Cu identifiable at the initial time point (T0) and during the first quarter of the sampling period.	12 months	Bioaccumu lation	The Duke Forest, an experimental site located in Durham, North Carolina, houses outdoor wetland mesocosms, which are sizable containers measuring 3.66 meters in length, 1.22 meters in width, and 0.81 meters in height. These mesocosms consist of distinct zones, including a permanently flooded area (aquatic zone), a	ICP-MS	Less than 1% of the extra NP mass was accumulated as Cu in all aquatic species (fish, snails, and macroinvertebrates).

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
						periodically flooded area (transition zone), and a rarely flooded area (upland zone).		
[12]	*F. candida*	CuO NPs	Five duplicates of each treatment were used in one independent test for the growth and bioaccumulation in each soil. At the onset of the experiment, airtight plastic containers were filled with 40 grams of soil that had been spiked, along with 30 juvenile organisms.	14 days	Growth	*F. candida* was cultivated at a temperature of 16 °C in the facilities of UFT Bremen. The initial culture of F. candida was acquired from Freie Universität Berlin during the 1990s.	AAS	enhance the absorption of CuO-NP within the cells of F. candida.
[13]	*Leptocheirus plumulosus*	$Cu(OH)_2$ nano pesticide	Sample aliquots were collected from microcosms containing nominal Cu concentrations of 0.25 and 2.5 mg L-1.	1, 4, 7 days	Mortality	Brood stocks of *Leptocheirus plumulosus* were acquired from Aquatic Biosystems located in Fort Collins, Colorado, USA. The cultures were maintained in polystyrene containers containing fine quartz and 3 liters of aerated, filtered seawater (salinity adjusted to 20 ppt with deionized water) at a temperature of 20.0°C ± 0.5°C. Illumination was provided by cool fluorescent lights following a 14-hour light and 10-hour dark photoperiod.	ICP-MS	Both the nominal concentration and the body load of copper serve as suitable dosage metrics to assess the impact of copper exposure on survival and population respiration. Copper, being an essential micronutrient, exhibited a significant no-effect body load on both survival and respiration, which is consistent with expectations.
[11]	macroinvertebrates (primarily Odonate nymphs)	$Cu(OH)_2$ nano pesticide	Before beginning the NP dose, the initial concentration of Cu in the mesocosm water was 1.48 0.93 g L1. Despite the initial Cu background, a variable dosage rate was employed to make the Cu identifiable at the initial time point (T0) and during the first quarter of the sampling period.	11 months	Bioaccumulation	Located in Durham, North Carolina, the Duke Forest is home to outdoor wetland mesocosms. These mesocosms are sizable containers measuring 3.66 meters in length, 1.22 meters in width, and 0.81 meters in height. They consist of	ICP-MS	Less than 1% of the extra NP mass was accumulated as Cu in all aquatic species (fish, snails, and macroinvertebrates).

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
						distinct zones, including a permanently flooded area known as the aquatic zone, a periodically flooded region called the transition zone, and a rarely flooded section referred to as the upland zone.		
[11]	Macrophyte (*Egeria densa*)	$Cu(OH)_2$ nano pesticide	Before beginning the NP dose, the initial concentration of Cu in the mesocosm water was 1.48 0.93 g L1. Despite the initial Cu background, a variable dosage rate was employed to make the Cu identifiable at the initial time point (T0) and during the first quarter of the sampling period.	9 months	Transformation	Located in Durham, North Carolina, the Duke Forest is home to outdoor wetland mesocosms. These mesocosms are sizable containers measuring 3.66 meters in length, 1.22 meters in width, and 0.81 meters in height. They consist of distinct zones, including a permanently flooded area known as the aquatic zone, a periodically flooded region called the transition zone, and a rarely flooded section referred to as the upland zone.	ICP-MS	Metal measurements were carried out on macrophytes without prior rinsing, including the metal present within the periphyton that forms on the surface of their leaves.
[11]	Snail species (*Physella acuta* and *Lymnaea sp.*)	$Cu(OH)_2$ nanopesticide	Before beginning the NP dose, the initial concentration of Cu in the mesocosm water was 1.48 0.93 g L1. Despite the initial Cu background, a variable dosage rate was employed to make the Cu identifiable at the initial time point (T0) and during the first quarter of the sampling period.	10 months	Bioaccumulation	Located in Durham, North Carolina, the Duke Forest is home to outdoor wetland mesocosms. These mesocosms are sizable containers measuring 3.66 meters in length, 1.22 meters in width, and 0.81 meters in height. They consist of distinct zones, including a permanently flooded area known as the aquatic	ICP-MS	Similar to naturally occurring Cu species, Cu was dispersed and converted as sulfide minerals, protein storage, or ligated with organic phases.

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
						zone, a periodically flooded region called the transition zone, and a rarely flooded section referred to as the upland zone.		
[14]	Zebrafish	$Cu(OH)_2$ nano pesticide	The CNPE exposure concentrations were 1, 0, 2, 4, 8, and 16 mg/l.	96 hrs	Genotoxicity	Atatürk University's Faculty of Aquaculture's Aquarium Fish Research Center provided the wild-type zebrafish (AB). Healthy adult fish were kept in 15-liter glass tanks for a period of 14 days, following the care guidelines outlined by Westerfield20. To collect fish embryos, the female and male fish were housed in separate 10-liter tanks for one week.	n.d.	Exposure to CNPE significantly increased the expression of the il-8 gene. In the groups exposed to 4.0 and 8.0 mg/l CNPE, there was a notable increase in the transcription level of the tlr4 gene.
[15]	Propionibacterium acidipropionici	CuO NPs	For the purpose of examining how nanomaterials affect P. acidipropionici, Four experimental conditions were selected, including the use of 5 mg/L CuO NPs, 10 mg/L MWCNTs, a combination of 5 mg/L CuO NPs and 10 mg/L MWCNTs, and a control group with no treatment (blank group).	30 min	Growth, cytotoxicity, metabolism	The American Type Culture Collection's P. acidipropionici, ATCC 39073, a typical propionic acid bacterium, was bought.	GC	CuO NPs exhibit significant inhibitory effects on microbial growth, leading to damage to cell membranes and suppression of gene expressions and activities associated with acidogenic metabolism.
[16]	*Scrobicularia plana*	CuO NPs	Simultaneously, five treatments were established in triplicate for testing: 1) a control group with uncontaminated sediment; 2) exposure to low levels of $CuCl_2$; 3) exposure to high levels of $CuCl_2$; 4) exposure to low levels of CuO NPs; 5) exposure to high levels of CuO NPs.	30 days	Accumulation	The experiment included exposing the chosen S. juveniles. plana to spiking sediments in 3 L polycarbonate tanks for 30 days. In each tank, 500 g of Rio San Pedro sediment and 2 L of water were combined in a 1:4 (w/v) ratio.	ICP-MS	*S. plana* uses various metal handling techniques based on the metal shape to reduce rising copper exposure.

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[17]	Soybean plant	$Cu(OH)_2$ nano-wires	A common handheld sprayer was used to apply Colloidal suspensions of CNWs or Kocide, $CuSO_4$ solution, or NPW prepared in aqueous form 24-day-old soybean plants for foliar exposure.	16 and 32 days	Mobility and gas exchange	The seeds were planted in seed-starter trays and, upon germination, transferred to small pots (10.2 cm × 7.6 cm) with three seedlings per pot. They were cultivated in these pots for a period of 22 days. Each healthy and uniformly grown seedling was transferred to a separate pot (20.5 cm 21.6 cm) when the first true leaf appeared. After two days, the pots were utilized for the exposure experiment.	ICP-MS, IR gas analyser, LC-MS/MS,	Medium and high levels of CNW exposure affected the tissues' accumulation of Co, Mn, Zn, and Fe and increased photosynthetic activity. Exposure to CNWs induced a dose-dependent response in soybean plants, leading to the activation of essential biological processes. Proteomic and metabolomic studies conducted on the leaves of these plants revealed significant changes in key pathways such as photosynthesis, energy generation, fatty acid metabolism, lignin biosynthesis, and carbohydrate metabolism. Kocide exposure, in contrast to CNW treatments, boosted the oxidative stress response and activated the metabolism of amino acids.
[18]	wheat (*Triticum aestivum*)	$Cu(OH)_2$ NPs	The start of the fourth week marked the onset of foliar exposure.	7 days	Accumulation, Transformation	MoO_3 and $Cu(OH)_2$ NMs of 99.94% and 99.5% purity, respectively, were bought from the United States. (Houston, USA) Research Nanomaterials Inc.	LC-MS/MS	The plant response to the low concentration of Cu ions was minimal, likely due to the limited solubility of $Cu(OH)_2$ nanomaterials in the presence of SA. In contrast, the activation of wheat plant tissues was more pronounced when exposed to $Cu(OH)_2$ nanomaterials compared to MoO_3 nanomaterials.
[19]	corn (*Z. mays*), wheat (*Triticum aestivum*)	CuO NPs	The fresh weight (FW) and dry weight (DW) of rice seedlings were affected by the presence of CuO nanoparticles (CuO NPs) at different concentrations (0, 100, 250, 450, and 600 mg L1), resulting in changes in their biomass.	5 Days	Accumulation	The University of Agriculture Faisalabad (UAF) provided the seeds for our wheat (Triticum) and maize (Zea mays) plants. The Advanced Materials Department of Physics (AMDP) and the Nanotechnology Lab collaborated to collect suitable and uniform-sized seeds for examination.	XRD	The growth parameters of *Z. Mays* and *Triticum aestivum*, including root length, shoot length, and plant biomass, exhibited enhancements when compared to the control group.

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[20]	Human	$Cu(OH)_2$ nano pesticides	Cell samples were prepared for different treatment groups, including the control, 1 g mL-1 copper treatment, 10 g mL-1 copper treatment, 25 g mL-1 copper treatment, and ions or nanopesticides. The experiment was performed with six replicates per treatment.	48 hrs	cytotoxicity	The HepG2 human liver cancer cell line used in this study was provided by the Institute of Biochemistry and Cell Biology in Shanghai, China. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) obtained from Corning Incorporated, USA, supplemented with 10% fetal bovine serum (FBS) from ExCell Bio, China, and 1% penicillin-streptomycin from HyClone, USA. The cell cultures were maintained at a temperature of 37 °C with 5% CO2 in a CO_2 incubator.	ICP-MS, HPLC-MS/MS	The metabolic and bioenergetic responses observed in HepG2 cells due to the nanopesticide were attributed to the release of copper ions. Among the components of the nanopesticide, $Cu(OH)_2$ was found to have negligible harmful effects. Both treatments resulted in a decrease in the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS), as well as an increase in the abundance of TCA cycle metabolites. These effects negatively impacted mitochondrial bioenergetics and redox homeostasis. Furthermore, the nanopesticide and copper ions at a concentration of 25 g mL1 enhanced glycolysis in the cells.
[21]	Lettus (*Lactuca sativa*)	CuO NPs		60 days	Accumulation, Enzyme activity	The greenhouse experiment took place at the Instituto Tecnologico de Torreon in Coahuila, Mexico, located at 102° west longitude, 1,120 meters above sea level, and 24°30' north latitude. Lettuce seeds (*Lactuca sativa L.*) of the Parris Island variety (Huertas®, La Huerta, Mexico) were used, and they were germinated on agricultural foam plates.	AAS	Cu-NPs treatment results in higher-grade lettuces for human consumption due to bioactive chemical buildup.
[22]	Lettus (*Lactuca sativa*)	$Cu(OH)_2$ nanowires	To create the various exposure groups mentioned above, different 100 mg/L PS MPs solutions or DI waters were combined with certain $Cu(OH)_2$ nanowires.	n.d.	Growth, accumulation, chlorophyll	n.d.	ICP-MS	$Cu(OH)_2$ nanowires' harmful effects on lettuce were caused by a non-negligible toxicity mechanism called released Cu^{2+}. The vigor index of lettuce seeds was raised, and the

Ref.	Organism	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
					measurement			development of lettuce seedlings was enhanced by PS MPs (1 mg/L).
[23]	Male rats	CuO NPs	The sixth group was given CuONSp with NCur at a dosage of 50.0 mg/kg/day over the course of 30 days.	30 days	Physiological responses	In this investigation, 36 male Wistar rats weighing 120–150 g were used. All experimental procedures were conducted in accordance with the recommendations, guidelines, and regulations set forth by the regulatory committee. Animal Care according to European Community Directive (86/609/EEC Edition 8).	TEM	When CuONSp is used, NCur has a significant impact on the toxicity reduction process.
[24]	Rice	CuO NPs	Different concentrations of CuO NPs in a nutritional solution (0, 100, 250, 450, and 600 mg L1) were applied to rice seedlings.	7 days	Growth, Physiological responses	The rice seeds (*cv. Nipponbare*) used in this study were provided by the College of Agriculture and Biotechnology at Zhejiang University in China.	SEM, TEM, UV-Vis spectrophotometer	Rice seedlings exhibited harmful effects when exposed to 450 mg L1 of CuO NPs. The elevated oxidative stress markers (O_2, H_2O_2, and MDA) were connected to the ethylene-mediated CuO NP-induced phytotoxicity.
[25]	Springtail	CuO NPs	Laboratory setting: OECD 232, 2009	4 weeks	Survival, Reproduction, Accumulation	The adult F. candida used in this study were obtained from our laboratory cultures, where they were maintained on charcoal-plaster plates in the dark at 15°C. The individuals were synchronized at room temperature. For all bioassays, the juveniles used were 10-12 days old at the beginning of the test.	AAS	The significantly decreased CAT activity in springtails suggests that the most plausible mechanism by which CuONP-montmorillonite interactions cause this toxicity is ROS production.

Table 2. Studies of Cu-based nano-pesticides impacts on soil.

Ref	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[6]	$Cu(OH)_2$ nanopesticide	The foliage of each mesocosm was sprayed using a Hudson Model 13581 sprayer from Chicago, IL, United States. The Kocide suspension, prepared at a concentration of 6.68 mg/L in DI water, was used for spraying. This resulted in an aboveground plant biomass exposure of 30 mg/m2. The application followed the recommended dosage and exposure mode guidelines provided by the manufacturer.	0, 75, 155 days	Accumulation, Microbial response	The outdoor terrestrial mesocosms were located in the Duke Forest, situated in Durham, North Carolina, USA.	ICP-MS	Six months following the last Kocide 3000 exposure, the only metrics that showed a substantial drop were the microbial enzyme activity.
[26]	$Cu(OH)_2$ nano pesticide	Experimental conditions in the laboratory involved replicating the agricultural application of $Cu(OH)_2$ nanopesticides. To achieve this, 2 mL of a freshly prepared aqueous solution containing $Cu(OH)_2$ nanopesticides at different concentrations was uniformly added to the soil in glass vials. The soil was weighed to be 10 g per vial (dry weight), resulting in a moisture content of 20% (equivalent to 50% of the maximum water-holding capacity, MWHC). The $Cu(OH)_2$ nanopesticides were applied at mass doses of 0.5, 5, and 50 mg/kg (dry weight).	21 days	Degradation	The soil samples (30.3147°N, 120.3504°E) utilized in this investigation were manually gathered from the Zhejiang Sci-Tech University campus in Hangzhou, where thiacloprid has never been used.	HPLC	The presence of $Cu(OH)_2$ nanopesticides was found to decrease the bioavailability of thiacloprid due to its adsorption. Additionally, our study revealed that $Cu(OH)_2$ nanopesticides had a noticeable impact on soil microbial communities. They resulted in a decrease in nitrile hydratase activity and downregulation of the abundance of the nth gene, which plays a crucial role in thiacloprid degradation. As a result, the breakdown of thiacloprid was significantly affected by the presence of $Cu(OH)_2$ nanopesticides.
[27]	CuO NPs	Soil spiking was carried out by spreading or causing test chemicals to dissolve in MQW. For the experimental treatments, a daily stock solution containing CuO nanoparticles (CuO-NP) was prepared with a concentration of 1.359 g of CuO.	6-7 days	Accumulation, microbial activity	Refesol test soils that had been air-dried had been acquired from Fraunhofer IME in Schmalenberg, Germany.	AAS	The amount of Cu ingested may not be the most important factor since the lowest effects were detected in soil and treatments with the greatest levels of Cu bioaccumulation, whereas impaired Cu removal was more closely associated with the harmful effects that were observed.
[28]	$Cu(OH)_2$ nano pesticides	Three treatments were used in microcosm experiments: The experimental groups consisted of a negative control (CT) with uncontaminated soil, soil contaminated with $Cu(OH)_2$ nanopesticide, and soil polluted with ionic Cu(OH).	90 days	Accumulation, microbial activities	The selected soil used in the experiment was LUFA 2.2, sourced from LUFA-Speyer in Speyer, Germany.	ICP-AEP	The presence of the soil invertebrate *P. pruinosus* appears to mitigate the impact of $Cu(OH)_2$-nano pesticide and Cu ionic form on soils, resulting in reduced effects on fungal diversity and carbon consumption at both structural and functional levels.

Ref	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[27]	$Cu(OH)_2$ nanopesticide	Repeated applications of the copper (Cu) nano pesticide were conducted over several months, although the rates of application were different in the wetland and terrestrial tests due to the distinct exposure situations that were reproduced in the two systems.	Wetland trials (every week for 9 months; 38 applications total) and terrestrial (The copper (Cu) nano pesticide was applied every 2.5 months for a period of one year, totaling three applications.)	Accumulation, microbial activity	In the Duke Forest, two outdoor mesocosm studies were conducted between 2016 and 2017.	ICP-MS	We discovered considerable changes in the eukaryotic (protists, fungi, and algae) sediment communities of the wetland mesocosms. The fungal and overall eukaryotic community compositions were significantly different between the groups exposed to nano pesticides for extended periods and the untreated groups without fertilization. We observed a total of 60 taxa that were notably affected by nanopesticide exposure, with a majority of them belonging to microeukaryotes such as cercozoans, Gastrotricha, and unicellular algal taxa.
[9]	$Cu(OH)_2$ nanopesticide and nano-$Cu(OH)_2$	To attain a moisture content of 20%, equivalent to 50% of the maximum water-holding capacity, the soil was treated with uniform additions of aqueous suspensions (2.0 mL) containing different concentrations of $Cu(OH)_2$ nanopesticide or nano-$Cu(OH)_2$. The soil-to-glass vial ratio was 10 g of soil per gram, based on dry weight. This application method resulted in $Cu(OH)_2$ doses of 0.5, 5, or 50 mg of Cu per kilogram of dry soil.	21 days	Enzyme assay	Surface loamy soil (0-20 cm) was collected from a greensward located on the campus of Zhejiang Sci-Tech University in Hangzhou, China. The coordinates of the university are approximately 30.3147° N and 120.3504° E.	n.d.	Slight differences in the soil's bacterial population and enzyme activity were observed between the NPF (non-pesticide formulation) and AI-NPF (active ingredient-free non-pesticide formulation) treatments, suggesting that the presence of nano-$Cu(OH)_2$ was the primary factor influencing the effects of the $Cu(OH)_2$ nanopesticide.
[29]	$Cu(OH)_2$ nanofertilizers, $Cu(OH)_2$ nanotube	Since aqueous solutions of copper hydroxide NFF are usually sprayed on the ground, several Cu concentrations of NFF suspensions were made for this investigation.	1, 7, 8, 14, 15, 21 Days	Accumulation, enzyme assay	In the Chinese city of Hangzhou, a location with a 2 m2 area and coordinates of 30.3147°N, 120.3504°E, was used to hand collect surface dirt (0–20 cm).	ICP-OES	Whether subjected to single or multiple applications, the presence of copper hydroxide NFF and NT or CuSO4 led to elevated levels of FDAse and NAG activity, reduced URE activity, and minimal changes in LAP activity.
[30]	CuO NPs	Irrespective of the copper source, the Cu treatments were conducted using equal	70 days	PLFA analysis by	In October 2016, soil samples were collected from the Russell Ranch	IPC-MS, XFM, X-ray	The response of soil respiration and microbial biomass to copper treatment varies across different land uses,

Ref	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
		volumes of water with a conductivity of 18.2 M cm.		microbial ID, INC	Sustainable Agriculture Facility at the University of California-Davis, including areas used for managed agriculture as well as unmanaged forest and grassland systems.	absorption spectroscopy	indicating that the previous land use has a greater influence on microbial reactions to copper amendment than the rate or type of copper treatment.
[31]	CuO NPs	The soil received applications of CuO nanoparticles (CuO NPs), copper nitrate ($Cu(NO_3)_2$), and CuO bulk particles (CuO BPs), as well as powdered CuO BPs.	n.d.	Dissolution, solubility	A soil sample was collected from the surface layer (0-20 cm) of agricultural fields in eight different provinces of China, with one sample representing each region.	AAS	The concentration of DTPA-Cu decreased over time in soils that were spiked with $Cu(NO_3)_2$, while in soils spiked with CuO NPs or CuO BPs, the concentration showed varying degrees of increase.
[25]	CuO NPs	Laboratory setting: OECD 232, 2009	4 weeks	Accumulation	The soils consisted of two distinct clay fractions, as prescribed by the OECD guidelines (OECD, 2009), with one fraction composed of kaolin sourced from Erbslöh Lohrheim GmbH KG.	AAS	The three-layer clay mineral montmorillonite is reactive and may enhance the toxicity of CuO NPs to springtails.
[32]	$Cu(OH)_2$ Nanopesticides, $Cu(OH)_2$ nanorods	Three soil samples, taken from different locations in China (Tongren City, Anji City, and Zhejiang Sci-Tech University campus in Hangzhou City), were collected from the surface layer (0-20 cm). The concentration of copper (Cu) utilized in the study was determined based on the actual application of $Cu(OH)_2$ nanopesticide, with a recommended concentration of 48 mg/L. Computational analysis considered the application rate of up to 11.8 kg/ha.	15 minutes	Accumulation, aggregation	Soil samples were manually collected from three different locations in China: Tongren City, Anji City (from an unidentified hill), and the Zhejiang Sci-Tech University campus in Hangzhou City. The samples were taken from the surface layer of the soil, specifically the 0-20 cm depth.	DLS, UV-Vis spectrophotometer	Aggregation, sedimentation, and $Cu(OH)_2$ nanopesticide dissolution were all influenced by the fundamental feature of soil solution.

Ref	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[33]	CuO NPs	The white powder was used to ensure uniform distribution, and CuO nanoparticles (CuO NPs) were introduced into 200 grams of air-dried soil and thoroughly blended using a handheld mixer.	n.d.	Nitrogenase activity	Soil samples were collected from the arable land at the Agro-Ecological Experimental Station of the Chinese Academy of Sciences, located in Fengqiu County, Henan Province, China (35°00′N, 114°24′E).	GC, UV-Vis spectrophoto meter	CuONPs were found to have a detrimental impact on the diazotrophic population in the soil. To investigate whether these effects of CuONPs on soil diazotrophs can influence the quality of maize grains, it is essential to analyze how the activity, diversity, and community composition of soil diazotrophs change throughout the growth of maize.

Table 3. Studies of Cu-based nano-pesticides impacts on water.

Ref	Compound	Exposure setting	Duration of exposure	Impact assessment	Sampling context	Quantitative method	Significant details
[4]	Cu NPs, CuO NPs, $Cu(OH)_2$ NPs	n.d.	n.d.	Accumulation, Aggregation, Dissolution, Transformation	Here, five natural and three man-made waters were employed.	ICP-AES	The lack of a strong correlation between aggregate size and sedimentation rate suggests that other variables may also play a role in sedimentation. Dissolution typically corresponded with pH, yet in salt conditions, dissolved copper might form insoluble complexes.

3.2. Bibliographic depiction of research papers.

Selected materials show that the earliest study was done in Florida in 2007. The article addressed a study that examined the impacts of copper nanoparticles on zebrafish. The findings revealed that exposure to these nanoparticles resulted in gill damage and acute mortality. The researchers exposed zebrafish to different concentrations of copper nanoparticles and monitored their survival and gill health over time [14]. Between 2007 and 2017, there was a minimal amount of published research on the topic. However, since that time, the number of publications has been consistently on the rise. In the past four years, a significant number of studies have been published on the subject (Figure 1). The quantity of published papers on the examination of toxicity of Cu-based nanomaterials varies significantly across different countries (Figure 2). The USA leads the pack with 36.36% papers (n=16) published on this topic, followed by China with 29.54% papers (n=13). Germany, Korea, and Turkey follow with 9.09% (n=4), 4.54% (n=2), and 4.54% (n=2) papers, respectively. Interestingly, some countries with a smaller presence in the global pesticide market, such as Estonia and Portugal, have also published papers on the ecotoxicity of Cu-based nanopesticides. Egypt, Florida, Mexico, Russia, and Spain each have one paper published on this topic. The number of papers published indicates varying interest levels and research funding for the topic across different countries. It also highlights the importance of collaborative research efforts across borders to gain a deeper understanding of the environmental destiny and behavior of Cu-based nanopesticides. Furthermore, a variety of analytical approaches have been used to quantify the amounts and possible impacts of Cu-based compounds on diverse species and environmental matrices (Figure 3). The method used most often to measure the concentration of Cu in materials is inductively coupled plasma-mass spectrometry (ICP-MS), which has been employed in 21 investigations. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are two additional frequently used methods that have been used to observe the shape and structure of Cu-based nanoparticles and their interactions with living things. Furthermore, techniques such as Fourier transform infrared spectroscopy (FTIR), Dynamic Light Scattering (DLS), and X-ray Diffraction (XRD) have been employed to investigate the physicochemical properties of Cu-based nanomaterials. ICP-MS is useful and efficient since it enables the simultaneous investigation of many different elements. Although other methods like AAS, XRF, and ICP-OES have benefits and drawbacks of their own, ICP-MS is favored above these methods because of its greater sensitivity, accuracy, and capacity to identify numerous elements [43] simultaneously.

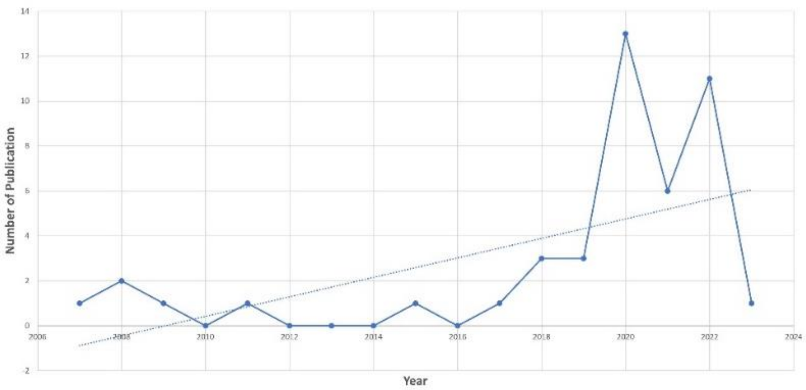

Figure 1. Year-wise, there are a number of studies related to the impacts of Cu-based nanopesticides.

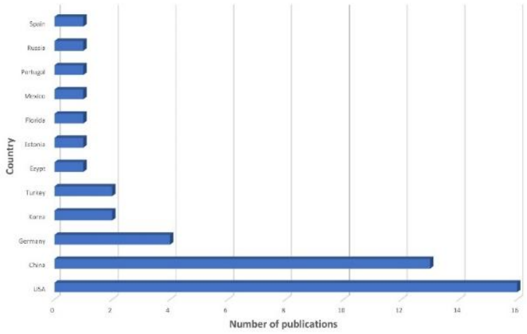

Figure 2. Country-wise, there are a number of studies related to the impacts of Cu-based nanopesticides.

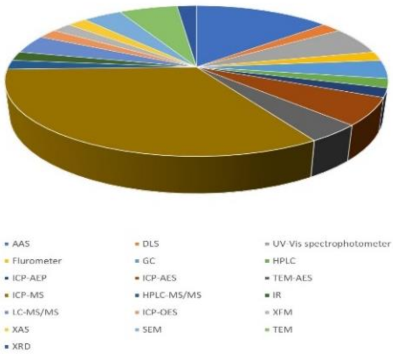

Figure 3. Different instrumental techniques were used in studies related to the impacts of Cu-based nanopesticides.

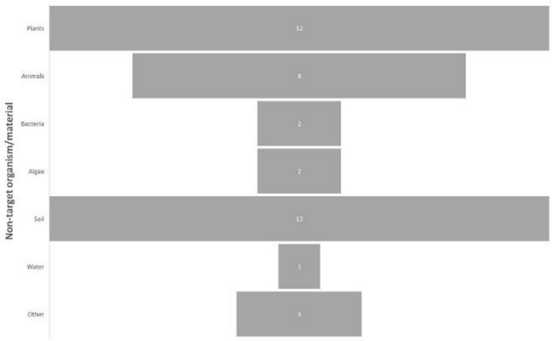

Figure 4. Different living organisms/materials used in studies related to the impacts of Cu-based nanopesticides.

3.3. Cu-based nano pesticides and their modes of action.

A variety of Cu-based nanomaterials, such as Cu nanoparticles, CuO nanoparticles, $Cu(OH)_2$ nanoparticles, $Cu(OH)_2$ nanotubes, $Cu(OH)_2$ nano pesticide, $Cu(OH)_2$ nanowires, $Cu(OH)_2$ nanofertilizers, and CuO nanopowder, have been utilized in toxicity investigations. $Cu(OH)_2$ nanopesticides have received the most attention out of them, a total of 36.73% (n=18). Studies have shown their toxicity to species other than the intended target. In 34.69% (n=17) studies, the ecotoxicity of CuO nanoparticles, another subject of regular study, was discussed. There were fewer studies of other Cu-based nanomaterials, such as CuO nanopowder, $Cu(OH)_2$ nanowires, and $Cu(OH)_2$ nanotubes. These nanoparticles have demonstrated strong pesticidal efficacy against various agricultural pests and pathogens. The exact modes of action of Cu-based nano pesticides are not fully understood. Still, it is believed that they act through multiple mechanisms, including disruption of cell membranes, generation of reactive oxygen species, and interference with metabolic processes. Cu-based nano pesticides are regarded as a favorable substitute for traditional chemical pesticides, as they are effective at lower doses and have lower environmental impact. There are numerous effects that have been studied in living organisms, depending on the research question or objective (e.g., gill toxicity, cytotoxicity, genotoxicity, bioaccumulation, motility, mortality, growth, etc.).

Furthermore, the soil was studied mostly for bioaccumulation and transformation. In water, the study examines how copper nanoparticles interact with various water components, including dissolved organic matter and minerals, and how these interactions affect the nanoparticles' aggregation, dissolution, and transformation. In fishes, the gills were the primary target of acute toxicity caused by Cu NPs, while other organs showed no histological or biochemical damage [14]. Several studies showed that exposure to Cu NPs inhibited the growth rates of plants and that TEM scans revealed that Cu NPs could cross cell membranes and agglomerate in cells [5,9,15,21,25,32,34]. Additionally, CuO nanoparticles were found to be more toxic to algae than the bulk form, and NPs less than 5 nm could enter intact cells and damage DNA [5,44]. The availability of free Cu in soils exposed to nCu and SRE exhibited a substantial increase, which has implications for soil organisms. However, plants exposed to soil with nCu had significantly less translocation of Cu to the leaves and upper plant tissues [6]. Several studies also reported shifts in microbial communities and enzyme activity due to exposure to Cu NPs [8,17, 39]. In aquatic animals, accumulation of Cu accounted for less than 1% of the total mass of added NPs [22]. Cu was distributed and transformed similarly to naturally occurring Cu species, and toxicodynamic models described the effects of copper exposure on survival and population respiration [23]. The influence of $Cu(OH)_2$ nanopesticide on soil communities was primarily attributed to the existence of nano-$Cu(OH)_2$.

3.4. Toxicity of Cu-based nanopesticides to non-target organisms.

Cu-based nano pesticides are a novel category of pesticides that have emerged recently and have been created for their improved effectiveness and reduced environmental impact compared to traditional pesticides. However, there is a worry that warrants more research about their possible toxicity to creatures other than the targets. Numerous ecotoxicity studies on diverse species have been conducted as a result of concerns about the possible environmental toxicity of Cu-based nanomaterials (Figure 4). The selection of 12 species of plants for ecotoxicity investigations of Cu-NMs is not unexpected, given that plants have been regarded as one of the most significant categories of organisms to study. Due to their role as primary producers and the foundation of the majority of ecosystems, plants are used extensively in ecotoxicity investigations. Animals, such as fish, insects, and snails, are particularly significant study subjects because of their ecological significance and ability to serve as bioindicators of environmental toxicity. Zebrafish, male rats, and tobacco hornworms were among the eight animal species whose ecotoxicity to Cu-NM was investigated. The ecotoxicity studies also included two species of bacteria and algae, which are significant players in the natural processes involved in the cycling of nutrients within the environment. The wide spectrum of creatures that may be impacted by Cu-NM exposure is highlighted by the introduction of additional organisms, such as macroinvertebrates and human cells. The wide range of creatures included in ecotoxicity experiments offers a thorough comprehension of the possible environmental impact of Cu-NMs and emphasizes the significance of taking into account the impacts of these substances on different levels of biological organization.

3.5. Terminal effects and methodological conditions.

The toxicity endpoints observed in a particular species are not only influenced by the mechanism of action (MoA) and toxicity towards the specific species. Other variables such as exposure time, chemical properties, experimental settings, toxicokinetics and toxicodynamics, formation of derivatives through biotransformation or in the environment, and specific biological characteristics can also lead to differences in effects among similar species and chemicals [45]. Acute toxicity tests are made to gauge an organism's initial negative reactions to a substance. The exposure periods for acute toxicity studies range from 24 to 72 hours, with 48 hours being the most typical amount of time. The OECD 202 guideline for fish acute toxicity testing, among others, uses the 48-hour exposure duration in many of its standardized toxicity tests [46]. However, chronic toxicity studies are carried out over a longer time frame to assess a chemical's long-term effects on an organism. Depending on the study's goals, the exposure periods for testing for chronic toxicity might be anywhere from a few days to many months. In one study, Exposures were conducted in triplicate, using 10 liters of facility water filtered through a 0.45 µm filter and containing 10 female zebrafish per tank. The exposures were based on the mass of copper added, and continuous aeration was provided throughout the experiment. Dosing was accomplished by adding 10 mL of a 1000× stock prepared in ultrapure water [14]. The plant agar method was used in another study where seeds were sterilized in a 5% sodium hypochlorite solution for 10 min, rinsed thoroughly with deionized water, and subsequently placed in wet cotton at a controlled temperature of 25±1°C in the dark [15]. The tested solutions were prepared by adding stock solutions of CuO NPs to the algal medium, and the six treatments consisted of 0.5 mg L^{-1} CuO NPs in a medium prepared using natural water. In another study, nCu was suspended in deionized (DI) water and subjected to 30 minutes of sonication before being applied to the Sedgwick soil. For the SRE treatment, 0 (control) and 5 mL of SRE mixture were added to the soil in the first 30 days [6,17,44]. In two studies, the foliage of each mesocosm was sprayed with the Kocide suspension (6.68 mg/L in DI water), so the aboveground plant biomass exposure was 30 mg/m², per the manufacturer's instructions for dosage and exposure mode [17,22]. In yet another study, 2 mL of a freshly prepared $Cu(OH)_2$ nanopesticide aqueous solution with different concentrations was homogeneously added to the soil, yielding a moisture content of 20% (corresponding to 50% maximum water-holding capacity, MWHC) and $Cu(OH)_2$ mass doses of 0.5, 5, and 50 mg/kg (dry weight) [36]. Treated leaves were then fed to M. sexta larvae, starting from the first and second-instar stages [19]. The studies used various techniques such as spraying, soil application, and testing on larvae and zebrafish to determine the toxicity of Cu-based nanopesticides on non-target organisms.

3.6. Environmental fate and behavior of Cu-based nano pesticides.

Environmental fate refers to the behavior of a chemical in the environment, including its transformation, transport, and eventual fate. Studies have shown that Cu-based nanopesticides can cross the cell membrane and agglomerate in cells and that they can cause growth inhibition in plants [15]. Cu-based nanopesticides are more soluble and toxic than bulk CuO, and they can cause acute toxicity in aquatic organisms, particularly algae [5,44]. They can also change soil microbial communities, reduce enzyme activity, and down-regulate gene abundance, leading to the mitigation of pesticide degradation. Also, they can enter cells intact, form excess ROS, damage DNA, and affect membrane integrity as well as algae growth

[5,8,17,39,44]. In addition, CuO is reduced to Cu_2O after internalization. Cu^{2+} is extremely effective in altering the redox conditions of biological systems, resulting in harm to macromolecules, disruption of cellular function, and disturbance of metabolic processes. Cu^- based nanopesticides can also accumulate in aquatic animals, although this accumulation accounts for less than 1% of the total mass of added nanoparticles [22]. Here, we represent some conclusive key points that strongly support that Cu-based nanomaterials are toxic to the environment: **(i)** Copper nanoparticles (Cu NPs) caused acute toxicity in aquatic organisms by targeting the gill and inhibiting Na^+/K^+ ATPase activity. The copper nanoparticles (Cu NPs) have the capability to traverse the cell membrane and cluster together within the cells, while the released cupric ions have negligible effects on toxicity. CuO NPs were more soluble and toxic to algae than bulk CuO [14]. (ii) NPs smaller than 5 nm could enter algae cells and affect growth by forming excess reactive oxygen species, damaging DNA and affecting membrane integrity. CuO was reduced to Cu_2O after internalization, and excessive exposure to Cu^{2+} could cause damage to macromolecules and disrupt cellular function [16]. (iii) Soil organisms showed increased bioavailability of Cu when exposed to nanoscale copper (nCu) and soil remediation enhancers (SRE). Still, less Cu was translocated to the leaves and upper plant tissues. $Cu(OH)_2$ nano pesticides could adsorb pesticides and change soil microbial communities, reducing nitrile hydratase activity and down-regulating thiacloprid degradative genes [44]. (iv) H. sativum plants accumulated several times more Cu in their shoots and roots after exposure to nano-CuO [21]. Aquatic animals accumulated less than 1% of added NPs, and metal measurements on macrophytes included metal accumulated within the periphyton [22]. (v) Cu was distributed and transformed similarly to naturally occurring Cu species, stored in proteins, or ligated with organic phases [22]. The presence of soil invertebrates could minimize the impact of $Cu(OH)_2$ nanopesticides on soil fungi diversity and carbon consumption. (vi) The impact of nano pesticides on sediment communities of wetland mesocosms was significant, with shifts in fungal and total eukaryotic community compositions. The effects of copper exposure on survival and population respiration are roughly described equally well by toxicodynamic models using either the nominal concentration or body burden as a dosage measure [23].

4. Conclusions

We provide an insightful review of the ecotoxicity of copper-based nano pesticides, which have uncovered possible hazards related to the usage of these compounds and highlighted areas that need additional investigation. The findings of the study serve as a foundation for subsequent debate and investigation of the toxicity of these nanopesticides on different species, as well as their possible environmental implications. This is crucial because copper-based nanopesticides are often used as pesticides because of their effectiveness and enduring effects. However, it is still unclear how they may affect the environment and living things. Higher-level studies, long-term exposures, exposures to several species, exposures in micro- and mesocosms, and exposures throughout generations could also present plausible exposure situations, including toxicity and destiny. The exposure concentrations had an impact on the accumulation of Cu NPs. There are issues with NPs' toxicity that need to be resolved, and when applying NPs to different items, extra caution should be used. It is necessary to conduct field monitoring of copper nano-agrochemical concentrations and effects in soil and various plant tissues while taking into account safer permissible limits. Since a broad range of nanopesticides are being created globally, along with the quick development of nanotechnology, researchers should pay greater attention to the possible environmental dangers of nanopesticides.

Funding

This review received no external funding.

Acknowledgments

None.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Griffitt, R.J.; Weil, R.; Hyndman, K.A.; Denslow, N.D.; Powers, K.; Taylor, D.; Barber, D.S. Exposure to Copper Nanoparticles Causes Gill Injury and Acute Lethality in Zebrafish (Danio rerio). *Environ. Sci. Technol.* **2007**, *41*, 8178-8186, https://doi.org/10.1021/es071235e.
2. Lee, W.-M.; An, Y.-J.; Yoon, H.; Kweon, H.-S. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): Plant agar test for water-insoluble nanoparticles. *Environ. Toxicol. Chem.* **2008**, *27*, 1915-1921, https://doi.org/10.1897/07-481.1.
3. Aruoja, V.; Dubourguier, H.-C.; Kasemets, K.; Kahru, A. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. *Sci. Total Environ.* **2009**, *407*, 1461-1468, https://doi.org/10.1016/j.scitotenv.2008.10.053.
4. Conway, J.R.; Adeleye, A.S.; Gardea-Torresdey, J.; Keller, A.A. Aggregation, Dissolution, and Transformation of Copper Nanoparticles in Natural Waters. *Environ. Sci. Technol.***2015**, *49*, 2749-2756, https://doi.org/10.1021/es504918q.
5. Huang, Y.; Zhao, L.; Keller, A.A. Interactions, Transformations, and Bioavailability of Nano-Copper Exposed to Root Exudates. *Environ. Sci. Technol.* **2017**, *51*, 9774-9783, https://doi.org/10.1021/acs.est.7b02523.
6. Simonin, M.; Colman, B.P.; Tang, W.; Judy, J.D.; Anderson, S.M.; Bergemann, C.M.; Rocca, J.D.; Unrine, J.M.; Cassar, N.; Bernhardt, E.S. Plant and Microbial Responses to Repeated $Cu(OH)_2$ Nanopesticide Exposures Under Different Fertilization Levels in an Agro-Ecosystem. *Front. Microbiol.* **2018**, *9*, 1769, https://doi.org/10.3389/fmicb.2018.01769.
7. Keller, A.A.; Huang, Y.; Nelson, J. Detection of nanoparticles in edible plant tissues exposed to nano-copper using single-particle ICP-MS. *J. Nanoparticle Res.* **2018**, *20*, 101, https://doi.org/10.1007/s11051-018-4192-8.
8. Li, J.; Rodrigues, S.; Tsyusko, O.V.; Unrine, J.M. Comparing plant–insect trophic transfer of Cu from lab-synthesised nano-$Cu(OH)_2$ with a commercial nano-$Cu(OH)_2$ fungicide formulation. *Environ. Chem.* **2019**, *16*, 411-418, https://doi.org/10.1071/EN19011.
9. Zhang, X.; Xu, Z.; Qian, X.; Lin, D.; Zeng, T.; Filser, J.; Li, L.; Kah, M. Assessing the Impacts of $Cu(OH)_2$ Nanopesticide and Ionic Copper on the Soil Enzyme Activity and Bacterial Community. *J. Agric. Food Chem.* **2020**, *68*, 3372-3381, https://doi.org/10.1021/acs.jafc.9b06325.
10. Rajput, V.; Chaplygin, V.; Gorovtsov, A.; Fedorenko, A.; Azarov, A.; Chernikova, N.; Barakhov, A.; Minkina, T.; Maksimov, A.; Mandzhieva, S.; Sushkova, S. Assessing the toxicity and accumulation of bulk- and nano-CuO in *Hordeum sativum* L. *Environ. Geochem. Health* **2021**, *43*, 2443-2454, https://doi.org/10.1007/s10653-020-00681-5.
11. Avellan, A.; Simonin, M.; Anderson, S.M.; Geitner, N.K.; Bossa, N.; Spielman-Sun, E.; Bernhardt, E.S.; Castellon, B.T.; Colman, B.P.; Cooper, J.L.; Ho, M.; Hochella, M.F., Jr.; Hsu-Kim, H.; Inoue, S.; King, R.S.; Laughton, S.; Matson, C.W.; Perrotta, B.G.; Richardson, C.J.; Unrine, J.M.; Wiesner, M.R.; Lowry, G.V. Differential Reactivity of Copper- and Gold-Based Nanomaterials Controls Their Seasonal Biogeochemical Cycling and Fate in a Freshwater Wetland Mesocosm. *Environ. Sci. Technol.* **2020**, *54*, 1533-1544, https://doi.org/10.1021/acs.est.9b05097.

12. Fischer, J.; Evlanova, A.; Philippe, A.; Filser, J. Soil properties can evoke toxicity of copper oxide nanoparticles towards springtails at low concentrations. *Environ. Pollut.* **2021**, *270*, 116084, https://doi.org/10.1016/j.envpol.2020.116084.
13. Vignardi, C.P.; Muller, E.B.; Tran, K.; Couture, J.L.; Means, J.C.; Murray, J.L.S.; Ortiz, C.; Keller, A.A.; Smith Sanchez, N.; Lenihan, H.S. Conventional and nano-copper pesticides are equally toxic to the estuarine amphipod *Leptocheirus plumulosus*. *Aquat. Toxicol.* **2020**, *224*, 105481, https://doi.org/10.1016/j.aquatox.2020.105481.
14. Aksakal, F.I.; Sisman, T. Developmental toxicity induced by $Cu(OH)_2$ nanopesticide in zebrafish embryos. *Environ. Toxicol.* **2020**, *35*, 1289–1298, https://doi.org/10.1002/tox.22993.
15. Dong, L.; Wu, Y.; Bian, Y.; Zheng, X.; Chen, L.; Chen, Y.; Zhang, X. Carbon nanotubes mitigate copper-oxide nanoparticles-induced inhibition to acidogenic metabolism of *Propionibacterium acidipropionici* by regulating carbon source utilization. *Bioresour. Technol.* **2021**, *330*, 125003, https://doi.org/10.1016/j.biortech.2021.125003.
16. 16. Scola, S.; Blasco, J.; Campana, O. “Nanosize effect” in the metal-handling strategy of the bivalve *Scrobicularia plana* exposed to CuO nanoparticles and copper ions in whole-sediment toxicity tests. *Sci. Total Environ.* **2021**, *760*, 143886, https://doi.org/10.1016/j.scitotenv.2020.143886.
17. Majumdar, S.; Long, R.W.; Kirkwood, J.S.; Minakova, A.S.; Keller, A.A. Unraveling Metabolic and Proteomic Features in Soybean Plants in Response to Copper Hydroxide Nanowires Compared to a Commercial Fertilizer. *Environ. Sci. Technol.* **2021**, *55*, 13477-13489, https://doi.org/10.1021/acs.est.1c00839.
18. Huang, X.; Keller, A.A. Metabolomic Response of Early-Stage Wheat (*Triticum aestivum*) to Surfactant-Aided Foliar Application of Copper Hydroxide and Molybdenum Trioxide Nanoparticles. *Nanomaterials* **2021**, *11*, 3073, https://doi.org/10.3390/nano11113073.
19. Haider, H.I.; Zafar, I.; Ain, Q.u.; Noreen, A.; Nazir, A.; Javed, R.; Sehgal, S.A.; Khan, A.A.; Rahman, M.M.; Rashid, S.; Garai, S.; Sharma, R. Synthesis and characterization of copper oxide nanoparticles: its influence on corn (*Z. mays*) and wheat (*Triticum aestivum*) plants by inoculation of *Bacillus subtilis*. *Environ. Sci. Pollut. Res.* **2023**, *30*, 37370-37385, https://doi.org/10.1007/s11356-022-24877-7.
20. Li, X.; Qin, Y.; Kong, L.; Yan, X.; Zhang, W.; Martyniuk, C.J.; Wang, X.; Yan, B. Metabolomic and bioenergetic responses of human hepatocellular carcinoma cells following exposure to commercial copper hydroxide nanopesticide. *Environ. Sci.: Nano* **2022**, *9*, 589-605, https://doi.org/10.1039/d1en00790d.
21. Gaucin-Delgado, J.M.; Ortiz-Campos, A.; Hernandez-Montiel, L.G.; Fortis-Hernandez, M.; Reyes-Pérez, J.J.; Gonzáles-Fuentes, J.A.; Preciado-Rangel, P. CuO-NPs Improve Biosynthesis of Bioactive Compounds in Lettuce. *Plants* **2022**, *11*, 912, https://doi.org/10.3390/plants11070912.
22. Yan, G.; Sun, Y.H.; Yang, L.; Zhang, Y.; Zhang, W. Polystyrene microplastics protect lettuce (Lactuca sativa) from the hazardous effects of $Cu(OH)_2$ nanopesticides. *Front. Plant. Sci.* **2022**, *13*, 1087754, https://doi.org/10.3389/fpls.2022.1087754.
23. Abdel-Azeem, A.M.; Abdel-Rehiem, E.S.; Farghali, A.A.; Khidr, F.K.; Abdul-Hamid, M. Ameliorative role of nanocurcumin against the toxicological effects of novel forms of Cuo as nanopesticides: a comparative study. *Environ. Sci. Pollut. Res.* **2023**, *30*, 26270-26291, https://doi.org/10.1007/s11356-022-23886-w.
24. Azhar, W.; Khan, A.R.; Salam, A.; Ulhassan, Z.; Qi, J.; shah, G.; Liu, Y.; Chunyan, Y.; Yang, S.; Gan, Y. Ethylene accelerates copper oxide nanoparticle-induced toxicity at physiological, biochemical, and ultrastructural levels in rice seedlings. *Environ. Sci. Pollut. Res.* **2023**, *30*, 26137-26149, https://doi.org/10.1007/s11356-022-23915-8.
25. Fischer, J.; Talal, G.D.A.; Schnee, L.S.; Otomo, P.V.; Filser, J. Clay Types Modulate the Toxicity of Low Concentrated Copper Oxide Nanoparticles Toward Springtails in Artificial Test Soils. *Environ. Toxicol. Chem.* **2022**, *41*, 2454–2465, https://doi.org/10.1002/etc.5440.
26. Zhang, X.; Xu, Z.; Wu, M.; Qian, X.; Lin, D.; Zhang, H.; Tang, J.; Zeng, T.; Yao, W.; Filser, J.; Li, L.; Sharma, V.K. 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, https://doi.org/10.1016/j.envint.2019.05.022.
27. Carley, L.N.; Panchagavi, R.; Song, X.; Davenport, S.; Bergemann, C.M.; McCumber, A.W.; Gunsch, C.K.; Simonin, M. Long-Term Effects of Copper Nanopesticides on Soil and Sediment Community Diversity in Two Outdoor Mesocosm Experiments. *Environ. Sci. Technol.* **2020**, *54*, 8878-8889, https://doi.org/10.1021/acs.est.0c00510.

28. Peixoto, S.; Henriques, I.; Loureiro, S. Long-term effects of $Cu(OH)_2$ nanopesticide exposure on soil microbial communities. *Environ. Pollut.* **2021**, *269*, 116113, https://doi.org/10.1016/j.envpol.2020.116113.
29. Tang, Q.; Xu, Z.; Hong, A.; Zhang, X.; Kah, M.; Li, L.; Wang, Y. Response of soil enzyme activity and bacterial community to copper hydroxide nanofertilizer and its ionic analogue under single versus repeated applications. *Sci. Total Environ.* **2021**, *796*, 148974, https://doi.org/10.1016/j.scitotenv.2021.148974.
30. Rippner, D.A.; Margenot, A.J.; Fakra, S.C.; Aguilera, L.A.; Li, C.; Sohng, J.; Dynarski, K.A.; Waterhouse, H.; McElroy, N.; Wade, J.; Hind, S.R.; Green, P.G.; Peak, D.; McElrone, A.J.; Chen, N.; Feng, R.; Scow, K.M.; Parikh, S.J. Microbial response to copper oxide nanoparticles in soils is controlled by land use rather than copper fate. *Environ. Sci.: Nano* **2021**, *8*, 3560-3576, https://doi.org/10.1039/d1en00656h.
31. Yang, Q.; Liu, Y.; Qiu, Y.; Wang, Z.; Li, H. Dissolution kinetics and solubility of copper oxide nanoparticles as affected by soil properties and aging time. *Environ. Sci. Pollut. Res.* **2022**, *29*, 40674–40685, https://doi.org/10.1007/s11356-022-18813-y.
32. Xu, Z.; Tang, Q.; Hong, A.; Li, L. Aggregation, Sedimentation and Dissolution of $Cu(OH)_2$-Nanorods-Based Nanopesticide in Soil Solutions. *Nanomaterials* **2022**, *12*, 3844, https://doi.org/10.3390/nano12213844.
33. Cao, J.; Liu, Z.; Zhao, H.; Lai, F.; Han, Y.; Lin, X. Effects of copper oxide nanoparticles on soil diazotrophic communities in maize rhizosphere. *J. Soils Sediments* **2023**, *23*, 1760-1774, https://doi.org/10.1007/s11368-023-03430-0.