# ABSTRACT

The rapid development and widespread use of nanotechnology has brought much advancement in human societies. However, due to growing numbers of nanoparticle-based consumer products, concerns have been raised for their potential toxicity to ecosystems and human beings during production and disposals. Nanoecotoxicology is a new subcategory of ecotoxicology that explores knowledge of hazards and safety aspects of nanoparticles (NPs). This study investigated nanoecotoxicity of two NPs that are frequently employed in compartments of soil environments and human societies. One is zero-valent iron nanoparticles ($Fe^0$NPs), the most prominent NPs in the field of environmental remediation. There is considerable concern over the potential ecotoxicity to soil ecosystems posed by $Fe^0$NPs released from *in situ* remediation. The other one is silver (Ag) NPs, one of the most frequently used NPs applied in various consumer products for human beings because of its eminent antimicrobial properties. However, soil contamination caused by AgNPs-treated sludges from sewage treatment plants is of great public concerns in these years.

This study used the nematode *Caenorhabditis elegans* (*C. elegans*) as a model organism to evaluate nanoecotoxicity of $Fe^0$NPs and AgNPs. Studies in *C. elegans* offer information about basic toxicology at molecular, cellular, and organismal levels as well as ecotoxicological effects. The *C. elegans* freely reside in soils, commonly applied in assessing ecological risks in ecosystems including pore water, soil, and sediments. *C. elegans* biomarker-based risk model developed in this study provides a new tool to assist nanoecotoxicological risk assessment of $Fe^0$NPs and AgNPs in soil environments.

To explore multigenerational reproductive toxicity of $Fe^0$NPs, toxicities of three iron species including $Fe^0$NPs, nanoscale iron oxide ($nFe_3O_4$), and ferrous ion ($Fe(II)_{aq}$) were examined and compared in *C. elegans*. Results showed that $Fe^0$NPs, $nFe_3O_4$, and

$Fe(II)_{aq}$ did not cause significant mortality after 24 h exposure in environmentally relevant concentrations. Bioassays of reproductive toxicity revealed that $Fe^0$NPs, $nFe_3O_4$, and $Fe(II)_{aq}$ significantly decreased offsprings in parental generation (F0) in accompany with increased intracellular reactive oxygen species (ROS). Furthermore, the reproductive toxicity of $Fe^0$NPs was transferrable from F0 to F1 and F2 generations, but then recovered in F3 and F4 generations. Further evidence showed that $Fe^0$NPs were accumulated in F0 and F1 generations of *C. elegans.* Results implicated that environmentally relevant concentrations of $Fe^0$NPs induce multigenerational reproductive toxicity by high production of ROS in F0 generation, toxicity of $Fe(II)_{aq}$, and iron accumulations in *C. elegans*.

Moreover, due to a lack of quantitative risk assessment of $Fe^0$NPs that has hampered the development of appropriate testing methods in environmental applications. Empirical approaches assessing external and internal $Fe^0$NPs-associated soil ecosystem health risks were developed by using *C. elegans*-based biomarkers. For risk assessments of external $Fe^0$NPs in *C. elegans*, the risk metrics of exceedance risk (ER) and risk quotient (*RQ*) of $Fe^0$NPs in various depths and distances from remediation sites were predicted. Results showed that under 50% risk probability (ER = 0.5), upper soil layer had the highest infertility risk (95% confidence interval: 13.18 – 57.40%). The margins of safety and acceptable criteria for soil ecosystems health for using $Fe^0$NPs in field scale applications were also recommended. Results showed that *RQ*s are larger than 1 in all soil layers when setting a stricter threshold of ~1.02 mg $L^{-1}$ of $Fe^0$NPs.

For risk assessments of internal $Fe^0$NPs in *C. elegans*, a toxicokinetic/toxicodynamic (TK/TD) approach was used to appraise bioaccumulation and nanoecotoxicity of $Fe^0$NPs. Built on present *C. elegans* bioassay with estimated

TK/TD parameters, probabilistic risk assessment schemes were used to assess potential ecological threats posed by environmentally relevant $Fe^0$NPs in soil ecosystems. This study found that average bioconcentration factors in *C. elegans* exposed to waterborne and foodborne $Fe^0$NPs were ~46 and ~$5\times10^{-3}$, respectively. The 10% inhibition concentrations for fertility, locomotion, and development, respectively, were 1.26 (95% CI: 0.19 – 5.20), 3.84 (0.38 – 42), and 6.78 (2.58 – 21) μg $g^{-1}$. Furthermore, most predicted 97.5%-tiles of risk quotients were larger than 1, implicating that chronic ecological risk posed by $Fe^0$NPs was alarming.

On the other hand, to rapidly screen and assess potential toxicity and risks of sewage sludge-released AgNPs in general and sludge-treated soils, a probabilistic risk assessment framework was conducted based on *C. elegans*-based bioassays. The soil environmental risks were estimated depending on characteristics of AgNPs and geographic regions. Results indicated that locomotion inhibition of *C. elegans* was depending on surface properties, diameter, and exposure time of AgNPs. The overall sewage sludge-released AgNPs-associated soil contamination risk was very low among Europe, U.S., and Switzerland. However, large production and widespread use of AgNPs are highly likely to pose a long-term ecotoxicity risk on general and sludge-treated soils, particularly for 26 nm citrate-coated AgNPs.

In conclusion, the approach of integrating probabilistic risk model and *C. elegans*-based ecological indicators provides an effective tool to screen and assess the impacts of metal NPs on soil environments. It is suggested that *C. elegans* as a proxy for estimating soil risk metrics can help develop methods of managements for mitigating the metal NPs-induced toxicity on terrestrial ecosystems.

***Keywords:*** Nanoecotoxicology; Zero-valent iron nanoparticles; Silver nanoparticles;

*Caenorhabditis elegans*; Toxicokinetic/toxicodynamic modeling; Soil ecosystems; Risk assessment

# 中文摘要

奈米科技之快速發展與廣泛應用帶給人類社會廣大之福祉。然而，由於奈米消費產品用量之日益增加，其在製造與丟棄過程中所排放至環境中之奈米物質，對於生態環境與人類健康潛在毒性之隱憂亦與日俱增。奈米生態毒理學為生態毒理學範疇之一，其欲探討之主題為奈米物質之危害與安全性。此篇論文欲探討兩種環境與人類生活常使用之奈米物質，其一為經常利用於環境復育用途之奈米零價鐵，在現地復育時所釋放之奈米零價鐵於土壤生態系所造成之生態毒性已造成許多環境上之隱憂。此外，論文所欲探討之另一奈米物質為奈米銀，由於其良好之抗菌特性，因而時常應用於多種消費產品當中。然而，奈米銀在廢水處理之過程中，亦可能因汙水處理廠所產生之汙泥於農業上之再利用，而再度將其釋放於環境中，因而在近幾年引發其對於生態環境之隱憂。

此篇論文使用秀麗隱桿線蟲為模式生物來評估奈米零價鐵與奈米銀之生態毒性，秀麗隱桿線蟲之研究可提供許多基礎毒理之資訊，從分子、細胞以至於生物體層級，亦可利用其探討生態毒理之效應。秀麗隱桿線蟲生長於於土壤當中，經常用於評估土壤孔隙水、土壤、底泥之生態毒性。因此，此篇論文利用秀麗隱桿線蟲之生物標記，可協助進行奈米零價鐵與奈米銀生態毒理於土壤環境之風險評估。

為探討奈米零價鐵於多重子代所造成之生殖毒性，本研究分別檢驗與比較三種鐵物種在秀麗隱桿線蟲中之毒性，此三種鐵物種分別為奈米零價鐵、奈米氧化鐵與鐵離子。研究結果顯示，三種鐵物種在 24 小時之環境濃度暴露之下，對秀麗隱桿線蟲之死亡率無顯著影響。然而在其生殖毒性測試下，奈米零價鐵、奈米氧化鐵與鐵離子在母代皆造成子代數目顯著下降與細胞內氧化壓力上升。此外，

奈米零價鐵之生殖毒性可從親代傳遞至 F1 與 F2 子代，但至 F3 及 F4 子代時則恢復為正常狀態。研究結果亦證明奈米零價鐵對 F0 及 F1 子代具生物累積作用。本研究結果顯示奈米零價鐵可藉由母代氧化壓力之製造、鐵離子之毒性與鐵累積而引起秀麗隱桿線蟲多重子代之生殖毒性。

再者，由於至今在奈米零價鐵尚未有量化之風險評估研究，進而可能阻礙其在環境應用之發展。此篇論文因此利用秀麗隱桿線蟲之生物指標來評估生物體外部與內部之奈米零價鐵於土壤生態系所造成之風險。在距離環境復育廠址不同深度與距離之環境濃度下，其中奈米零價鐵於秀麗隱桿線蟲體外所造成之風險，於超越風險為 0.5 條件下，土壤上層所生長之秀麗隱桿線蟲不孕之風險為最高 (95% 信賴區間: 13.18 – 57.40%)。此外，以較嚴謹與較寬鬆之標準下，奈米零價鐵於現地復育時可被接受之使用濃度，在本研究中皆進行評估與建議。研究結果顯示在較嚴謹之濃度標準下 ($1.02\ mg\ L^{-1}$)，各土壤層中之奈米零價鐵對秀麗隱桿線蟲所造成之風險商數皆大於 1。

針對奈米零價鐵於秀麗隱桿線蟲體內之風險評估方面，此篇論文亦發展毒理動力/動態之研究法來預測及探討奈米零價鐵之生物累積濃度與生態毒性。利用秀麗隱桿線蟲之生物測定法與毒理動力/動態參數，與機率風險評估之模式了解奈米零價鐵於土壤生態系中生物體內所造成之生態危害。結果顯示，透過水與食物為媒介傳遞之奈米零價鐵之生物濃縮因子分別為 46 與 $5\times10^{-3}$。針對抑制秀麗隱桿線蟲之生殖能力、運動能力與生長，所推估之奈米零價鐵之 10%抑制濃度分別為 1.26 (95% 信賴區間: 0.19 – 5.20), 3.84 (0.38 – 42), and 6.78 (2.58 – 21) $\mu g\ g^{-1}$。結果亦指出 97.5%位數之風險商數皆大於 1，顯示慢性暴露下之奈米零價鐵對秀麗隱桿線蟲具潛在危害。

另一方面，為快速檢測及評估奈米銀在一般土壤及地下水汙泥處理過土壤之潛在風險，此篇研究亦利用秀麗隱桿線蟲之生物標記進行機率風險評估。依據不同奈米銀之特性與不同地理區域之奈米銀濃度預測奈米銀在土壤之環境風險。結果顯示，根據不同表面特性、粒徑與暴露時間下，奈米銀對於秀麗隱桿線蟲運動行為上之抑制效果亦有所不同。整體而言，在歐洲、美國與瑞士地區其地下水汙泥處理過之土壤，奈米銀所造成之風險皆非常低。然而，結果亦顯示在長時間之



奈米銀製造與使用下，奈米銀很有可能對於一般土壤及地下水汙泥處理過之土壤造成長久之生態風險，尤其以 26 nm 檸檬酸包覆之奈米銀之機率為最高。

因此，利用機率風險評估與秀麗隱桿線蟲生態指標所結合之研究方法，可篩檢與評估金屬奈米對生態之衝擊。此研究建議以秀麗隱桿線蟲為生物指標所預測出之土壤環境風險，可協助減緩金屬奈米對陸地生態環境所引發之毒性與危害。

**關鍵字：** 奈米生態毒理; 奈米零價鐵; 奈米銀; 秀麗隱桿線蟲; 毒理動力/動態模擬; 土壤生態系; 機率風險評估