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Effects of Combined Nickel, Copper, and Arsenic Contamination on Soil Microorganisms and Enzyme Activity (Postprint)

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Abstract

Soil microorganisms and enzyme activity are important biological properties that characterize soil quality. This study investigated farmland soil experiencing combined heavy metal contamination downwind of a smelter near Baijiazui Village, Ningyuanbao Town, Jinchuan District, Jinchang City, Gansu Province. Using field survey methodology, we analyzed heavy metals (Zn, Pb, Cd, Cr, Cu, Ni, As, Hg), microorganisms (bacteria, fungi, actinomycetes), and enzyme activities (urease, alkaline phosphatase, catalase, dehydrogenase) in the 0–20 cm and 20–40 cm soil layers. The results demonstrated: (1) In the 0–20 cm soil layer, the point exceedance rates for Ni, Cu, and As were 15.4%, 30.8%, and 38.5%, respectively; in the 20–40 cm soil layer, the point exceedance rates for Ni, Cu, and As were all 7.7%. (2) Pb, Hg, Ni, Cu, Cd, and As exhibited negative correlations with bacteria, urease, alkaline phosphatase, catalase, and dehydrogenase; Cr showed positive correlations with actinomycetes and fungi; Zn displayed a positive correlation with bacterial biomass; and bacterial biomass was negatively correlated with actinomycete biomass. (3) Pb, Zn, and Cr were the dominant factors influencing soil biological properties, with contribution rates of 72.4%, 16.2%, and 4.9%, respectively. In the Cu-Ni-As combined contamination area, catalase activity demonstrated sensitivity to heavy metals Cu, Ni, Cd, and As, and could serve as an effective indicator for soil quality evaluation in this region.

Full Text

Abstract

Soil microorganisms and enzyme activities are important biological indicators of soil quality. This study investigated farmland soil contaminated by heavy metal complexes downwind of a smelter in Baijiazui Village, Ningyuanbu Town, Jinchuan District, Jinchang City, Gansu Province. Using field survey methods, we analyzed heavy metals (Zn, Pb, Cd, Cr, Cu, Ni, As, Hg), microorganisms (bacteria, fungi, actinomycetes), and enzyme activities (urease, alkaline phosphatase, catalase, dehydrogenase) in soil layers of 0–20 cm and 20–40 cm. The results showed that in the 0-20 cm layer, the over-standard rates for Ni, Cu, and As were 15.4%, 30.8%, and 38.5%, respectively, while in the 20-40 cm layer, the over-standard rate for all three metals was 7.7%. Pb, Hg, Ni, Cu, Cd, and As showed negative correlations with bacteria, urease, alkaline phosphatase, catalase, and dehydrogenase. Cr exhibited positive correlations with actinomycetes and fungi, while Zn showed positive correlations with bacterial biomass. Pb, Zn, and Cr were the dominant factors influencing soil biological characteristics, with contribution rates of 72.4%, 16.2%, and 4.9%, respectively. Catalase activity was particularly sensitive to Ni, Cu, Cd, and As pollution and could serve as an effective indicator for soil quality assessment in these contaminated areas.

Keywords: nickel-copper-arsenic composite pollution; soil; microorganisms; enzyme activity

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1.1 Study Area Overview

Baijiazui Village is located in Ningyuanbu Town, Jinchuan District, Jinchang City, Gansu Province, at the eastern end of the Hexi Corridor. The region has a continental temperate arid climate with annual sunshine duration of 2884 h, average temperature of 7.4°C, annual precipitation of 139 mm, frost-free period of 156 d, and average elevation of 1553 m. The area is rich in mineral resources. The soil type is gray-brown desert soil, with basic physicochemical properties in the cultivated layer as follows: organic matter 26.7 $g \cdot kg^{-1}$, available phosphorus 21.6 $mg \cdot kg^{-1}$, available potassium 395.3 $mg \cdot kg^{-1}$, calcium carbonate content 123.2 $g \cdot kg^{-1}$, pH 8.39, sand particle (0.02-2 mm) content 243 $mg \cdot kg^{-1}$, silt particle (0.002-0.02 mm) content 333.0 $g \cdot kg^{-1}$, and clay particle (<0.002 mm) content 280.2 $g \cdot kg^{-1}$, classifying it as loamy clay.

1.2 Sample Collection and Analysis

In Baijiazui Village, Ningyuanbu Town, near the mining area, 26 sampling points were determined through consultation with relevant research institutions. Using GPS positioning and five-point sampling method, soil samples were collected from 0–20 cm and 20–40 cm layers in May 2020. After removing debris, the samples were placed in sterile bags, transported to the laboratory, air-dried, and passed through a 100-mesh nylon screen for storage.

Heavy metals were detected using inductively coupled plasma mass spectrometry (ICP-MS) after four-acid (hydrochloric acid, nitric acid, hydrofluoric acid, perchloric acid) digestion on an electric hot plate. As and Hg were detected by atomic fluorescence spectrometry (AFS) after aqua regia water bath digestion

[18]. Alkaline phosphatase was measured by disodium phenyl phosphate colorimetry using borate buffer, expressed as phenol mass (mg) released per gram soil in 24 h. Urease was measured by indophenol blue colorimetry, expressed as NH_3-N mass (mg) released per gram soil in 24 h. Dehydrogenase was measured by triphenyl tetrazolium chloride (TTC) colorimetry, expressed as triphenylformazan mass fraction ($mg \cdot g^{-1}$) [19]. Catalase was measured by ultraviolet spectrophotometry, expressed as H_2O_2 mass (mg) decomposed per gram soil in 20 min [20].

Bacterial counts were determined using beef extract peptone agar medium by plate surface spreading method. Fungal counts used Martin' s Bengal red medium by plate surface spreading method. Actinomycete counts used modified Gause' s No. 1 medium by plate surface spreading method [21]. Microbial quantities were calculated as: microbial count = (average colony count × dilution factor) / sample weight.

1.3 Data Analysis Methods

SPSS 20.0 software was used for one-way ANOVA, with Duncan' s multiple comparison test for data comparison. CANOCO 5.0 software was used for canonical correspondence analysis (CCA) between soil biological characteristics and heavy metal content.

2.1 Total Soil Heavy Metal Content

In the 0-20 cm layer, the average contents of Zn, Pb, Cd, Cr, Cu, Ni, As, and Hg at 26 sampling points were 243.52, 0.47, 0.077, 0.34, 0.056, 0.47, 0.34, and 0.056 $mg \cdot kg^{-1}$, respectively. In the 20-40 cm layer, the average contents were 218.35, 0.34, 0.056, 0.34, 0.056, 0.34, 0.34, and 0.056 $mg \cdot kg^{-1}$, respectively.

According to the "Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land (Trial)" (GB15618-2018) [47], the risk screening values for heavy metals are: Zn 200 $mg \cdot kg^{-1}$, Pb 240 $mg \cdot kg^{-1}$, Cd 0.8 $mg \cdot kg^{-1}$, Cr 350 $mg \cdot kg^{-1}$, Cu 100 $mg \cdot kg^{-1}$, Ni 100 $mg \cdot kg^{-1}$, As 30 $mg \cdot kg^{-1}$, and Hg 1 $mg \cdot kg^{-1}$. Based on this standard, the average contents of all eight heavy metals in both soil layers were below the risk control values, though some individual sampling points exceeded the screening values.

In the 0-20 cm layer, the over-standard rates for Ni, Cu, and As were 15.4%, 30.8%, and 38.5%, respectively. In the 20-40 cm layer, the over-standard rates for Ni, Cu, and As were all 7.7%. Sampling points 1, 2, and 3 showed the highest heavy metal contents, likely due to their proximity to the smelter and long-term emissions from the smelter chimney. This indicates that smelting activities have caused localized deep soil contamination in surrounding farmland.

2.2.1 Soil Microbial Counts

Soil microorganisms, primarily consisting of fungi, bacteria, and actinomycetes, represent the most active components of soil [22]. shows that bacteria dominated the soil microbial community, followed by actinomycetes, with fungi being the least abundant. Microbial counts decreased significantly with soil depth.

In the 0-20 cm layer, bacterial counts differed significantly among sampling points ($P < 0.05$), with the highest count at point 7 (213.24×10^6 $\mathrm{cfu \cdot g^{-1}}$) and the lowest at point 5 (67.96×10^6 $\mathrm{cfu \cdot g^{-1}}$). Actinomycete counts also varied significantly among most sampling points, while fungal counts showed significant differences only between some points. In the 20-40 cm layer, bacterial, fungal, and actinomycete counts were generally lower and showed fewer significant differences among sampling points. Across the entire study area, the 0-20 cm layer had the highest bacterial, fungal, and actinomycete counts at points 7, 9, and 9, respectively, while the 20-40 cm layer showed the highest bacterial count at point 9 and actinomycete count at point 7.

2.2.2 Soil Enzyme Activity

Soil enzymes, primarily derived from proliferating and dead microorganisms, as well as from plant roots, soil fauna, and decomposing organic matter [23], play crucial roles in organic matter decomposition, microbial energy and nutrient acquisition, and pollutant degradation [24]. shows that in the 0-20 cm layer, urease activity was highest at point 9 and lowest at point 5, while alkaline phosphatase activity was highest at point 6 and lowest at point 9. Catalase and dehydrogenase activities showed different patterns, with catalase being highest at point 9 and dehydrogenase highest at point 6.

Enzyme activities generally decreased significantly with soil depth ($P < 0.05$), except for catalase, which showed significant differences between layers only at some sampling points. Across the entire study area, the 0-20 cm layer had the highest average urease and alkaline phosphatase activities, while the 20-40 cm layer had the highest dehydrogenase activity.

2.2.3 Correlation Analysis Among Soil Chemical Properties, Biological Characteristics, and Heavy Metal Content

Correlation analysis () revealed significant relationships among soil properties. Soil organic matter showed extremely significant positive correlations with alkaline phosphatase, urease, and catalase ($P < 0.01$), but extremely significant negative correlations with dehydrogenase ($P < 0.01$). Bacterial biomass was positively correlated with urease, alkaline phosphatase, and catalase, but negatively correlated with dehydrogenase. Actinomycetes showed extremely significant positive correlations with fungi, urease, and alkaline phosphatase ($P < 0.01$). Fungi were extremely significantly positively correlated with urease and alkaline phosphatase ($P < 0.01$). Urease and alkaline phosphatase showed an extremely significant positive correlation ($P < 0.01$).

2.4 Canonical Correspondence Analysis Between Soil Biological Characteristics and Heavy Metal Content

To further understand the effects of heavy metals on soil biological characteristics, CCA was performed ([Figure 3: see original paper]). The analysis showed that Pb, Zn, and Cr were the dominant factors influencing soil biological traits, with contribution rates of 72.4%, 16.2%, and 4.9%, respectively. Monte Carlo permutation tests () confirmed that these relationships were statistically significant ($P < 0.05$), indicating that soil heavy metals significantly affect farmland soil biological characteristics.

The CCA revealed that Ni, Cu, Cd, and As were negatively correlated with bacteria, urease, alkaline phosphatase, catalase, and dehydrogenase. Cr showed positive correlations with actinomycetes and fungi, while Zn was positively correlated with bacterial biomass. Bacterial biomass was negatively correlated with actinomycete biomass.

3.1 Distribution Characteristics and Influencing Factors of Heavy Metal Content in Different Sampling Areas

Heavy metal distribution varied among sampling areas. The 0–20 cm layer at points 1, 2, and 3 showed the highest contamination, likely due to their proximity to the smelter and location downwind of the smelter chimney. Long-term atmospheric deposition from smelting emissions has enriched heavy metals in these surface soils. The 20–40 cm layer also showed contamination, suggesting that long-term leaching has transported heavy metals to deeper soil layers. Spatial variability in soil properties and distance from pollution sources contributed to the observed distribution patterns.

3.2 Effects of Heavy Metal Composite Pollution on Soil Microorganisms

Soil microbial community structure is a key parameter for characterizing soil ecosystem stability and can indicate environmental contamination [25]. Heavy metals affect microbial groups differently depending on concentration and metal type. Previous studies have shown that heavy metal pollution generally reduces bacterial, fungal, and actinomycete colony counts [30, 31]. In this study, areas with the highest heavy metal contamination (points 1, 2, 3) showed the lowest bacterial, fungal, and actinomycete counts in the 0–20 cm layer, confirming that Ni-Cu-As composite pollution reduces microbial abundance.

Different microbial groups exhibit varying sensitivities to heavy metals. Studies on paddy soils suggest actinomycetes are most sensitive to Cu^{2+}, while fungi are most sensitive to Cd^{2+} [32]. Bacterial community structure appears more sensitive to heavy metal pollution than microbial biomass [33]. This study found significant positive correlations between Cr and both fungi and actinomycetes ($P < 0.01$), and between Zn and bacterial biomass ($P < 0.05$). However, this study only examined total heavy metal contents; future research should investigate heavy metal speciation and its relationship with microbial biomass [34, 35].

3.3 Effects of Heavy Metal Composite Pollution on Soil Enzyme Activity

Soil enzymes are important biological indicators of soil quality [36], offering simple measurement and sensitive response to heavy metal toxicity [37]. Enzyme responses depend on heavy metal content, valence state, soil type, and physicochemical properties [38]. Soil organic matter, clay content, and cation exchange capacity are major factors controlling heavy metal inhibition of enzyme activities [39]. Microbial community specificity also affects enzyme inhibition [40], and heavy metals can cause low-dose activation and high-dose inhibition [41].

In this study, areas with the highest heavy metal contamination showed the lowest alkaline phosphatase activity but highest catalase and dehydrogenase activities in the 0–20 cm layer, indicating that Ni-Cu-As composite pollution reduces alkaline phosphatase while increasing catalase and dehydrogenase. Soil organic matter showed significant positive correlations with alkaline phosphatase, urease, and catalase, likely because higher organic matter enhances enzyme protection and buffers heavy metal toxicity [42].

CCA results showed that Ni, Cu, Cd, and As were negatively correlated with bacterial biomass, urease, alkaline phosphatase, catalase, and dehydrogenase, consistent with reports that enzyme activities decrease with increasing heavy metal content [46]. However, these findings differ somewhat from other studies reporting positive correlations between catalase and certain heavy metals [29], suggesting that enzyme responses are complex and context-dependent.

4 Conclusions

Based on analysis of heavy metal contents and their relationships with soil microorganisms and enzyme activities in typical farmland soil of Baijiazui Village, Ningyuanbu Town, Jinchuan District, Jinchang City, the following conclusions are drawn:

1. The study area soil showed over-standard rates for Ni, Cu, and As of 15.4%, 30.8%, and 38.5% in the 0–20 cm layer, and 7.7% in the 20–40 cm layer, indicating that smelting activities have contaminated both surface and deep soil layers.
2. Pb, Hg, Ni, Cu, Cd, and As were negatively correlated with bacteria, urease, alkaline phosphatase, catalase, and dehydrogenase. Cr was positively correlated with actinomycetes and fungi, while Zn was positively correlated with bacterial biomass. Bacterial biomass was negatively correlated with actinomycete biomass.

3. In the Ni-Cu-As composite pollution area, catalase activity was most sensitive to heavy metals and can serve as an effective indicator for soil quality assessment. Pb, Zn, and Cr were the dominant factors influencing soil biological characteristics, with contribution rates of 72.4%, 16.2%, and 4.9%, respectively, with significant differences ($P < 0.05$).

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