# Effects of Sludge Application on Rhizosphere Nutrients and Various Heavy Metals in Rapeseed (Postprint)

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## Abstract

The rhizosphere is a critical factor controlling plant nutrient dynamics, which in turn influence the rhizosphere soil environment. When soils are amended with sewage sludge, the characteristics of nutrients and heavy metals in the rhizosphere soil are altered. Currently, few studies have investigated the influence of plant roots on the availability and distribution of heavy metals in the rhizosphere of sludge-amended soils. This study employed the root mat-freezing thin section method to investigate the distribution of nutrients and heavy metals in the rapeseed rhizosphere of sludge-amended soils, aiming to elucidate the activation characteristics of heavy metals in the rhizosphere of such soils. Following sludge application, DTPA-extractable Zn, Cd, Ni, Mn, available phosphorus, available potassium, and ammonium nitrogen were significantly depleted in the rhizosphere soil, whereas DTPA-extractable Cu showed no marked depletion or accumulation. When soils received high sludge application rates, rhizosphere soil pH increased with increasing distance from the root surface. Regardless of sludge treatment, exchangeable Cu in the rapeseed rhizosphere soil was significantly reduced. In soils amended with 50% sludge, carbonate-bound, Fe-Mn oxide-bound, organic-bound, and residual Cu and Zn were substantially depleted in the rapeseed rhizosphere soil at 0-2 mm from the root surface. Sludge application promoted rapeseed growth. The Cu and Zn concentrations in rapeseed shoots did not change significantly with increasing sludge application rate. In soils receiving less than 25% sludge, sludge application did not enhance heavy metal availability and mobility. Except for Cu, the depletion of DTPA-extractable Zn, Cd, and Ni in the rapeseed rhizosphere soil indicates that heavy metal activation in sludge-amended soils is very limited.

# Full Text

## Preamble

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**Effects of Sewage Sludge Application on Rhizosphere Nutrients and Different Types of Heavy Metals in Rapeseed**

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## Abstract

The rhizosphere contains important factors controlling nutrient dynamics in this zone and the mineral nutrition of plants. Nutrient dynamics also influence the rhizosphere environment. When soil is amended with sewage sludge, the nutrients and heavy metals in the rhizosphere change accordingly. However, little attention has been given to the extent to which plant roots affect the availability and distribution of heavy metals near roots in sludge-amended soils. The objectives of the present study were to investigate the distribution of heavy metals and nutrients in the rhizosphere of *Brassica campestris* grown in sludge-amended soil, and to predict the availability of metals in sludge-amended soil. The distribution of nutrients and heavy metals in the rhizosphere of sludge-amended soil was investigated using the root mat and a frozen thin slicing technique to provide indications regarding the activation of heavy metals. DTPA-extractable Zn, Cd, Ni, and Mn, available P and K, and ammonium nitrogen in the rhizosphere were markedly depleted when soil was amended with sludge. There was no conspicuous depletion or accumulation of DTPA-extractable Cu in the rhizosphere when the soil was amended with sludge. The pH value in the rhizosphere increased with distance from the roots when soil was amended with larger amounts of sludge. The exchangeable fraction of Cu in the rhizosphere was depleted whether or not the soil was treated with sludge. Carbonate, oxide, organic, and residual fractions of Cu and Zn were depleted in the rhizosphere at a distance of 0–2 mm from the roots when soil was amended with 50% sludge. Application of sewage sludge had a positive effect on *Brassica campestris* growth. With an increase in sludge amounts, the concentrations of Cu and Zn in aboveground parts of *Brassica campestris* did not change. Soil amendments with less than 25% sludge did not increase the availability or mobility of heavy metals. The depletion in rhizospheric DTPA-extractable Zn, Cd, and Ni indicated that, with the sole exception of Cu, release of metals from sludge-amended soil was very limited.

**Keywords:** rhizosphere; sludge; heavy metals; *Brassica campestris*; nutrients

## Introduction

The utilization of heavy metal-contaminated soils represents a major challenge in current soil environmental research. Direct cultivation of crops on such soils introduces heavy metals into the food chain, posing risks to human health. Many scholars have investigated how to remediate and utilize heavy metal-contaminated soils to produce safe agricultural products, achieving progress in various aspects [1-3]. In China, municipal wastewater treatment plants generate large quantities of sludge annually, much of which is landfilled or incinerated as solid waste without effective utilization [4]. Using qualified sludge from municipal wastewater treatment plants as a soil amendment to remediate heavy metal-contaminated soils represents a pathway for sludge resource utilization, simultaneously addressing the challenge of utilizing contaminated land.

Different heavy metal types exhibit substantially different chemical properties in soils, and their chemical characteristics in the plant rhizosphere significantly influence their bioavailability [5-6]. The rhizosphere refers to the soil portion adjacent to plant roots, extending from the root surface to approximately several millimeters away. Due to the influence of root life activities and exudates, its chemical and biological properties differ markedly from bulk soil, forming a micro-ecosystem that is both dependent on and relatively independent from the soil ecosystem, with highly active chemical and biochemical processes [7]. Biological processes in the rhizosphere—including root absorption and secretion, pollutant dilution and enrichment, and complexation changes—affect the migration and transformation of various substances and influence heavy metal availability [8-10]. Nutrient dynamics in the rhizosphere also affect the soil environment [11]. When soils are amended with sludge, rhizosphere nutrient and heavy metal characteristics change, yet few studies have examined these effects.

The objectives of this study were to investigate the distribution of different heavy metals and nutrients in the rhizosphere of rapeseed (*Brassica campestris*) grown in sludge-amended soil using a frozen thin-section technique, and to clarify the activation of heavy metals in sludge-amended soils. Rapeseed, a cruciferous oil crop with strong cold tolerance and certain salt and heavy metal resistance [12], was selected as the experimental plant. Its stems and leaves serve as excellent green fodder for livestock. By cultivating this oil crop on heavy metal-contaminated soil, the seeds can be processed into biodiesel, and if the straw meets relevant standards, it can be used as livestock feed. This research also aimed to determine how different sludge application rates affect rapeseed uptake of various heavy metals, providing practical guidance for soil remediation with sludge and reference data for utilizing soils contaminated with specific heavy metals.

The experimental soil was collected from the Dagu sewage irrigation area in Tianjin, where untreated industrial and domestic wastewater was historically discharged into the Bohai Sea through rivers such as the Yongding New River and Beijing Sewage River, creating decades-long sewage irrigation zones. This practice introduced substantial heavy metals into the soil [13-14]. The soil texture is heavy loam. The chemical properties of the selected soil and sludge are presented in Table 1.

The dewatered anaerobic digestion sludge was collected from the Tianjin East Suburb Wastewater Treatment Plant. According to relevant standards [15], the selected sludge met national regulations for heavy metal content in sludge application. The experimental design, based on previous work [16], involved adding 10%, 25%, and 50% dried sludge to the experimental soil and mixing thoroughly.

## Materials and Methods

The experimental apparatus was designed and improved based on previous work [16]. Rapeseed was first cultivated in a greenhouse to form root mats. The soil beneath the root mats was quickly removed and frozen in liquid nitrogen, then sectioned with a microtome to obtain thin soil slices at different distances from the root surface. This frozen thin-section technique allows investigation of how plant roots affect the distribution of various substances in the rhizosphere. The soil samples obtained through this apparatus were completely air-dried before analysis.

Heavy metals in the contaminated soil were fractionated using the Tessier sequential extraction method [17] into different forms: exchangeable, carbonate-bound, iron-manganese oxide-bound, organic-bound, and residual fractions. Heavy metals in soil and plant tissues were measured using DTPA extraction and ICP-MS [18]. Other soil chemical indicators were determined using conventional methods [19-20]. Statistical analysis was performed using Excel 2003 and SPSS 18.0 for variance analysis and multiple comparisons (Duncan' s new multiple range method).

## Results

### 1. pH in Rhizosphere Soil

Soil acidity significantly affects heavy metal and nutrient availability [21], and changes in rhizosphere pH influence root absorption rates of many cations [22]. When ammonium nitrogen serves as the nitrogen source for rapeseed, rhizosphere pH decreases near the root surface. When nitrate nitrogen is the main source, rhizosphere pH increases. In soils without sludge amendment, pH in the rapeseed rhizosphere decreases with proximity to the root surface. However, with increasing sludge application, rhizosphere pH increases with distance from the root surface. This is likely due to increased ammonium nitrogen concentration in sludge-amended soils. In soils treated with 25% or 50% sludge, ammonium nitrogen concentration in the rhizosphere is much higher than nitrate nitrogen concentration. The experimental results indicate that after sludge application, rhizosphere acidity increases relative to bulk soil with increasing sludge amounts (Table 2).

## 2. Distribution of Available Nutrients in Rhizosphere

The distribution of available nutrients in the rhizosphere is influenced by soil nutrient status, plant absorption, and soil physicochemical properties. In soils without sludge amendment, concentrations of nitrate nitrogen, available phosphorus, and available potassium show no significant differences at various distances from the root surface. However, in sludge-amended soils, concentrations of ammonium nitrogen, available phosphorus, and available potassium at 0-2 mm from the root surface are significantly lower than at 2-4 mm and 4-6 mm, indicating continuous depletion by root absorption. The influx from bulk soil cannot compensate for the absorption rate in the rhizosphere. In contrast, nitrate nitrogen shows significant accumulation in the rhizosphere regardless of sludge amendment, suggesting its influx exceeds plant uptake. The differential distribution patterns of ammonium and nitrate nitrogen indicate that rapeseed preferentially absorbs ammonium over nitrate, and these substances continuously diffuse toward the root surface in the rhizosphere (Table 3).

## 3. Heavy Metal Distribution in Rhizosphere

**3.1 Distribution of DTPA-Extractable Heavy Metals** The distribution characteristics of bioavailable heavy metals at different distances from the root surface are more important than total concentrations. DTPA extraction is an effective method for assessing bioavailable heavy metals [23]. Sludge application significantly increases concentrations of Mn, Cu, Zn, Ni, and Cd, likely due to the effects of sludge organic matter. DTPA-extractable Cu does not show obvious distance-dependent changes from the root surface, suggesting equilibrium between root uptake and influx from bulk soil. In contrast, DTPA-extractable Zn, Ni, and Cd show significant depletion near the root surface only when large amounts of sludge are applied, with concentrations increasing with distance from the root surface and sludge application rate. This indicates that in sludge-amended soils, the mobility of Zn, Ni, and Cd is relatively high. The limited mobility of Cu, Mn, Zn, Ni, and Cd in the rhizosphere reflects differential behavior among heavy metals in sludge-amended soils and suggests that rapeseed uptake of these elements is less than their influx from bulk soil (Table 4).

**3.2 Distribution Characteristics of Different Heavy Metal Fractions** The migration, phytotoxicity, and environmental impact of heavy metals depend not only on total content but also on their chemical forms in soil [24]. Cu and Zn are the primary heavy metals in the experimental soil, with relevant national standards [20]. Different chemical forms exhibit varying bioavailability and mobility [25]. The exchangeable fraction of Cu is depleted in the rhizosphere regardless of sludge treatment. Carbonate-bound Cu shows significant depletion at 0-2 mm from the root surface in sludge-amended soils, indicating that rapeseed can absorb carbonate-bound Cu after sludge amendment. Other chemical forms of Cu show no significant differences between rhizosphere and bulk soil without sludge. All Cu fractions increase with sludge amount, but only carbonate-bound Cu shows significant depletion near roots at high sludge rates (Table 5).

Similar to Cu, all Zn fractions increase significantly with sludge amount, though not correlated with distance from the root surface. Carbonate-bound, iron-manganese oxide-bound, organic-bound, and residual Zn are significantly depleted at 0-2 mm from the root surface when soil is amended with 50% sludge. The results demonstrate that at high sludge concentrations, rapeseed can absorb not only exchangeable but also carbonate-bound, organic-bound, and residual forms of Cu and Zn (Table 6).

### 4. Effects of Sludge Application on Rapeseed Growth and Heavy Metal Uptake

Sludge application positively affects rapeseed growth, with root and total dry weights increasing significantly with sludge amount. However, no further significant biomass increase occurs beyond 25% sludge application (Figure 1). The concentrations of Cu, Zn, and Cd in aboveground parts do not increase significantly with sludge application, indicating limited translocation to shoots. Although rapeseed absorbs carbonate-bound Cu and Zn in sludge-amended soils, their concentrations in aboveground parts do not increase significantly compared to non-amended soils, requiring further investigation (Table 7).

## Discussion

The results show that increasing sludge amounts raise rhizosphere pH, likely due to enhanced nitrification at the root surface and root exudates (though not measured in this study). Comprehensive analysis reveals that sludge application increases available nutrient concentrations in the rhizosphere, benefiting rapeseed growth. Available phosphorus and potassium concentrations increase with both distance from the root surface and sludge amount, showing positive correlation with sludge application rate. Nutrient diffusion and mobility in the rhizosphere depend on soil solution concentration, plant root activity, and soil properties such as cation exchange capacity, organic matter content, and texture [26]. Although sludge increases available nutrient concentrations, depletion occurs near roots due to intensive uptake [27].

Sludge amendment increases available heavy metal content in the rhizosphere, possibly due to heavy metal activation by sludge organic matter [28-29]. DTPA-extractable concentrations of Mn, Zn, Ni, and Cd increase significantly with distance from the root surface and sludge application rate, while Cu and Mn show less mobility. This reflects differential behavior among heavy metals and indicates that rapeseed uptake varies by element. Importantly, heavy metal concentrations in aboveground parts show no significant differences across sludge treatments, suggesting that rapeseed does not accumulate Cu, Zn, or Cd in shoots. These findings indicate that rapeseed grown in sludge-amended soils can be used for oil production and potentially as livestock feed, providing reference data for utilizing soils contaminated with various heavy metals.

The results demonstrate that amending heavy metal-contaminated soils with approximately 25% sludge is optimal for rapeseed cultivation. Higher application rates do not significantly promote growth but increase heavy metal content in plant tissues, limiting product utilization. The data provide practical guidance for sludge application rates in heavy metal-contaminated soils.

## Conclusion

When soils are amended with large amounts of sludge, rhizosphere pH increases with distance from the root surface. DTPA-extractable Zn, Ni, and Cd are depleted in the rhizosphere, while Cu shows no significant depletion or accumulation, indicating these heavy metals do not migrate extensively toward root surfaces. Ammonium nitrogen, available phosphorus, and available potassium are rapidly absorbed by roots. Carbonate-bound, iron-manganese oxide-bound, organic-bound, and residual forms of Cu and Zn are significantly depleted at 0-2 mm from the root surface after sludge amendment. At high sludge application rates, rapeseed can absorb not only exchangeable but also carbonate-bound forms of Cu and Zn. Sludge application rates below 25% do not significantly increase heavy metal mobility. This study examined only short-term effects; long-term impacts of increasing sludge application require further investigation.

## References

[1] Research progress on biochar remediation of soil heavy metal pollution. *Journal of Ecology and Environment*, 2015, 24(12): 2075-2081.
[2] Effects of different soil amendments on reducing Pb and Cd content in rice brown rice from heavy metal-contaminated soils. *Journal of Agro-Environment Science*, 2007, 26(2): 476-481.
[3] Effects of combined amendments on heavy metal availability in paddy soils. *China Environmental Science*, 2014, 34(2): 437-444.
[4] Current status and development of sludge treatment technology in China' s municipal wastewater plants. *Environmental Science and Management*, 2013, 38(7): 94-97.
[5] Contamination status and health risk assessment of Pb and Cd in vegetables from a mining area in southern Hunan. *Acta Ecologica Sinica*, 2014, 34(8): 2146-2154.
[6] Research on rhizosphere micro-environment. *Chinese Journal of Ecology*, 1993, (5): 225-240.
[7] Cardon ZG, Whitbeck JL. *The Rhizosphere: An Ecological Perspective*. London: Academic Press, 2007.

[8] Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytologist*, 2005, 168(2): 293-303.
[9] Keller H, Röemer W. Cu, Zn, and Cd acquisition by two spinach cultivars depending on P nutrition and root exudation. *Journal of Plant Nutrition & Soil Science*, 2001, 164: 335-342.
[10] Stratton ML, Good GL, Barker AV. The effects of nitrogen source and concentration on the growth and mineral composition of privet. *Journal of Plant Nutrition*, 2007, 24(11): 1745-1772.
[11] Potential of rapeseed as a hyperaccumulator plant for remediation of Cd-contaminated soils. *Acta Ecologica Sinica*, 2014, 34(8): 2146-2154.
[12] Soil heavy metal pollution environmental quality and environmental effects in Tianjin sewage irrigation area. *Journal of Agro-Environment Science*, 2005, 14(2): 211-213.
[13] Ministry of Urban and Rural Construction and Environmental Protection. *Control Standards for Pollutants in Agricultural Sludge* (GB 4284-1984). Beijing: China Standards Press, 1984.
[14] Tessier A, Campbell PGC, Bisson M. Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, 1979, 51(7): 844-851.
[15] Quevauviller P. Operationally defined extraction procedures for soil and sediment analysis I. Standardization. *TrAC Trends in Analytical Chemistry*, 1998, 17(5): 289-298.
[16] State Environmental Protection Administration. *Soil Agricultural Chemical Analysis Methods*. Beijing: China Agricultural Science and Technology Press, 2000.
[17] Brown G, Brinkmann K. Heavy metal tolerance in *Festuca ovina* L. from contaminated sites in the Eifel Mountains, Germany. *Plant and Soil*, 1992, 143(2): 239-247.
[18] National Bureau of Quality and Technical Supervision. *Environmental Quality Standard for Soils* (GB 15618-1995). Beijing: China Standards Press, 1995.
[19] Characteristics of salt and nutrients in rhizosphere soils of desert halophytes. *Acta Ecologica Sinica*, 2008, 29(6): 1693-1698.
[20] Determination of available elements in soil by diethylenetriaminepentaacetic acid (DTPA) and ICP-AES. *Environmental Science and Technology*, 2015, 34(8): 1578-1579.
[21] Distribution characteristics of heavy metals in profiles of suburban vegetable fields in Beijing. *Journal of Agricultural Resources and Environment*, 2015, 32(3): 282-288.
[22] Environmental behavior of heavy metal forms and dissolved organic matter in soils. *Environmental Science & Technology*, 2008, 31(7): 69-73.
[23] Effects of Fe and Cd treatments on Fe and Cd adsorption behavior in rice rhizosphere and on root surfaces. *Acta Ecologica Sinica*, 2013, 33(14): 4306-4314.
[24] Relationship between rice root aerenchyma, root oxygen release, and rhizo-

sphere nitrification. *Chinese Journal of Applied Ecology*, 2012, 32(7): 2066-2074.
[25] Plant responses to sludge and root activation of heavy metals. *Journal of Agro-Environment Science*, 2002, 11(5): 121-124.
[26] Effects of aerobic fermented municipal sludge on growth and heavy metal content of three landscaping plants. *Journal of Agro-Environment Science*, 2015, 33(S1): 1061-1064.

*Note: Figure translations are in progress. See original paper for figures.*