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Soil Studies is an international peer reviewed journal that aims to rapidly publish high-quality, novel research of studies on fertility, management, conservation, and remediation, physics, chemistry, biology, genesis, and geography of soils. In addition, the main purpose of Soil Studies is to reveal the influences of environmental and climate changes on agroecosystems and agricultural production. In this context, Soil Studies publishes international studies address these impact factors through interdisciplinary studies. In the journal, articles on hypothesis-based experimental observation of the interactions of all components of agricultural ecosystems, field trials, greenhouse or laboratory-based studies, economic impact assessments, agricultural technologies, and natural resources management will be accepted within the peer-reviewed process. Topics include, but are not limited to:

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## Editorial Information

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RESEARCH PAPER

# Genetic characterization of rhizobium bacteria isolated from bean (*Phaseolus vulgaris L.)* nodules and its effect on growth

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**Abstract**

Biological nitrogen fixation (BNF) is an important nitrogen source, providing a variety of legumes and pasture plants. Rhizobia is soil bacteria that can form nitrogen-fixing nodules on legumes. In this study, we have isolated 10 bacteria from root nodules of sugar beans from the Gembos plain Derebucak district of Konya, Turkey. The morphological and metabolic characteristics of the isolates were tested under laboratory conditions. According to molecular identification, eight bacterial isolates were identified as *Agrobacterium tumefaciens,* and two isolates (F4DC and F6DC) were identified as *Rhizobium gallicum.* Field experiments were carried out to compare the effect of one native rhizobia (*Rhizobium gallicum* F4DC), chemical fertilizer, and non-fertilizer control in SFWRRI Sarayköy Research and Application Station in Ankara. Our results show that sugar bean (*Phaseolus vulgaris L.*) inoculation with *Rhizobium gallicum* F4DC (MZ156852) induced a significant increase in the number of nodules, grain yield, number of pods, and plant height compared to the control. These results show that *Rhizobium gallicum* F4DC is a suitable choice for use in symbiotic association with beans to work as a biofertilizer.

## Introduction

It is well recognized that nitrogen is an essential element for the growth and development of plants. Chemical fertilizers are necessary for intensive agricultural methods to produce large yields, but they are also expensive and may have negative environmental effects. Biological nitrogen fixation (BNF) is an important nitrogen source, providing a variety of legume and pasture plants with about 2170–300 kg of nitrogen per hectare per year (Tu et al., 2016). Especially in regions of the world where nitrogen fertilizer imports are intense, legumes are of particular importance due to

their unique ability to fix atmospheric nitrogen and being self-sufficient in nitrogen demand, as well as their capacity to supply nitrogen to the soil. Rhizobia are alpha-proteobacteria that live in a symbiotic relationship with a wide range of leguminous plants by forming nodules in roots where these bacteria fix atmospheric nitrogen. A total of seven Alphaproteobacteria genera, including Rhizobium, Azorhizobium, Allorhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium, and Methylobacterium contain about 40 rhizobia species (Lemaire et al., 2015). Rhizobia bacteria play an important role in agro-ecosystem services as they perform biological nitrogen

fixation by establishing symbiotic relationships with a wide variety of leguminous plants (Orrell and Bennett, 2013).

$N_2$ fixing microorganisms are able to enhance the nitrogen fixation performance and also may increase the nutrient level in soil through phosphate solubilization, water uptake, and the production of substances like hormones, and siderophores. These bacteria also work to mitigate agro-environmental issues while improving soil fertility (Kumar et al., 2012). Since rhizobia-legume symbiosis is a host-specific relationship, it is necessary to identify the strains and diversity of rhizobia associated with a particular species of legume in order to better utilize the advantages of rhizobia biofertilizers (Batista et al., 2015). The symbiosis between Rhizobium bacteria and legumes is facultative and is determined by the host plant's nitrogen requirement. In this mutual symbiosis, bacteria supply nitrogen to the plant in exchange for photosynthesis products from the host (Wang et al., 2018).

The most significant grain legume for direct human consumption worldwide is the bean. Beans are an excellent supplier of protein and are also a good source of vitamins, minerals, and minerals (particularly iron and zinc). Rhizobium and beans (*P. vulgaris L*.) work together in a symbiotic relationship to create root nodules that catalyze the fixation of nitrogen from the air (Baginsky et al., 2015). The species that establish a symbiotic relationship with the bean are the generally fast-growing Rhizobiums, which include *Rhizobium phaseoli, Rhizobium leguminosarum, Rhizobium gallicum, Rhizobium tropici, Rhizobium fleshy*and and *Sinorhizobium meliloti* (Adhikari et al., 2013).

Biofertilizers containing various strains of rhizobia are being developed for use in legumes to increase soil fertility and symbiotic nitrogen fixation. A key strategy in sustainable agriculture is the use of efficient rhizobial strains as biofertilizers to increase the production of legumes. The use of native isolates in biofertilizer production; is preferred primarily due to its adaptation to environmental conditions and higher competitiveness with local strains.

The aim of this research was to isolate and identify native rhizobium bacteria and to investigate the effects of the obtained biofertilizer on bean plant growth parameters.

## Materials and methods

### Collection of nodules

The nodules were collected from ten different sugar bean (*Phaseolus vulgaris* L.) growing areas in Gembos plain Derebucak district of Konya, Turkey. The plants were carefully uprooted and transferred to the laboratory and stored at 4°C until isolation. The nodules that were healthy, unbroken, and especially pink was chosen for rhizobia isolation.

### Isolation of Rhizobia from the Root Nodules

Nodules were thoroughly washed under tap water to remove the adhering soil particles from the nodule surface and then carefully severed from the root with sterile forceps. Healthy and undamaged root nodules were subjected to surface sterilization by washing in 3-5% hydrogen peroxide solution for 4-5 minutes, followed by repeated rinsing with sterile distilled water. Subsequently, they were washed with sterile distilled water after being treated with 70% ethyl alcohol (Somasegaran and Hoben, 1994). After surface sterilization, the nodules were crushed and extracted in an environment containing a small amount of sterile distilled water. Yeast Extract Mannitol Agar (YEMA) plates were streaked with a loop full of nodule suspension and cultured for 3-5 days at 28°C. (Vincent, 1970). Following the incubation period, single colonies were selected and re-streaked on YEM agar for purity. A total of 10 isolates were obtained and all purified isolates were preserved at -80 °C in YEM containing 40 % glycerol.

### Morphological and Metabolic Characterization of the Isolates

Gram staining was carried out with 3–4 days old cultured on YEMA were smeared on clean microscope slides. According to Beck et al. (1993) instructions, the wet smears were air dried, heat fixed, and then Gram stained. On a compound light microscope, the produced slides were examined under immersion oil.

The isolates were incubated in YEMA medium containing Congo red for detection of absorption congo red (Somasegaran et al., 1994). Then, rhizobia isolates were cultured in glucose peptone agar plates supplemented with bromocresol purple, incubated at 28°C for 4 days in the dark. In YEMA medium containing bromothymol blue (YEMA-BTB), the formation of acid or alkali was determined. The plates were incubated at 28°C for 5 days in the dark and color changes of the medium were recorded. The isolates that turned YEMA-BTB from green to yellow were found to generate acid and grow fast. Slow-growing isolates that turned the YEMA-BTB medium blue were recognized as alkaline producers. Isolates were checked on the basal medium of YEMA supplemented 2% NaCl as 2% NaCl is inhibitory for most rhizobial isolates it can serve as an identification tool (Koskey et al., 2018).

### Molecular Identification of Isolates

The genomic DNA of the bacterial isolates was extracted using the CTAB (cetyltrimethylammonium bromide) technique (Wilson, 2001). A Microvolume spectrophotometer was used to measure the concentration of DNA, which was then kept at -20°C until use. With the use of the universal primers 27F (5'-AGAGTTTGATC(AC)TGGCTCAG-3') and 1492R (5'-

CGG(CT)TACCTTGTTACGACTT-3'), 16S rDNA regions of genomic DNA isolated from bacteria were amplified. (Khan et al., 2014). The 50 μl reaction volume used for the PCR amplification contained 3 μL of template genomic DNA, 5 μL of 10xTaqbuffer, 1 μL of 10 mM dNTP, 1 μL of each primer, 0.25 μL of 5 U Taq polymerase, and 4 μL of 25 mM MgCl₂. The following conditions were used for the PCR reaction: a 2-minute denaturation period, 35 cycles of temperature cycling (95°C for 45 s, 57°C for 45 s, and 72°C for 2 min), and a final 7-minute extension period at 72°C (Özdoğan, D.K. et al., 2022). Amplified fragments were checked, then purified and sequenced at BM Labosis (Ankara, Turkey). The NCBI-BLAST server is used to analyze 16S rDNA sequences. To perform molecular phylogenetic and evolutionary relationship analysis, isolates 16S rDNA gene sequences were aligned by ClustalW using four reference strains (Saitou and Nei, 1987) and the phylogenetic tree was conducted using Neighbor-Joining method with MEGA software v.10.0.5 (Kumar et al., 2016).

**Amplification of nif H gene from isolates**

polF (5'-TGCGAYCCSAARGCBGACTC-3') and polR (5'-ATSGCCATCATYTCRCCGGA -3') primers were used for application of Preserved region of the *nif H* gene fragment (Poly et al. 2001). For this reaction *Azotobacter vinelandii* was used as positive control.

**Field experiment**

Field experiment was conducted to examine the impact of the native rhizobia, chemical fertilizer, and a non-fertilizer control on nodulation, growth, and plant yield of the common bee. The experiment was carried out in SFWRRI Sarayköy Research and Application Station (Ankara, located at 36.46_N, 52.56_E, and 25 masl) in a fully randomized design trial with three replicates in 2020. The test subjects were seeds without bacterial treatments and without chemical fertilization (C0), microbial fertilizer prepared using *Rhizobium gallicum* F4DC strain (MF), and seeds without bacterial treatments but treated with optimal chemical fertilization (CF). According to the soil analysis results, triple super phosphate fertilizer (9.6 kg/da) with planting was given to microbial fertilizer and optimum fertilizer subjects, and the nitrogen requirement of CF subject was divided into two parts, half of it was applied in the form of ammonium sulfate (7 kg/da) during planting and the other half during flowering period. Each plot measured 2 × 3 m and a spacing of 2 m between the plots was left to minimize inter-plot interference. The soil of the experimental area is classified as clay soil according to the USDA texture classification (Gerakis and Baer, 1999) and the amount of organic matter is very low. The soil reaction of the area, which is medium in terms of lime content, is slightly alkaline and moderately salty.

The bacteria were grown in a yeast extract mannitol broth and added to milled peat in the ratio of 1 bacteria inoculum to 2 parts peat. The rhizobium in peat was prepared and applied as a seed treatment at one kg per 100 kg seed. During the flowering stage, three plants from each plot were selected randomly and harvested for assessment of nodulation in the first five cm and after the harvesting plant height, number of pods per plant, 100 grain weight, grain yield, and protein ratio were measured.

One-way analysis of variance (ANOVA) was performed on the experimental data using the SPSS-22 software package, and Duncan's multiple range test was used to determine the significance of treatment effects at the $p < 0.05$ level.

**Morphological and Metabolic Characterization of the Isolates**

In the present study, ten strains were isolated from root nodules of sugar beans. According to microscopic investigation all isolates are gram negative, rod shaped. Gram staining results preliminary confirmed the standard morphological characteristics of *Rhizobium* as described by Vincent (1970) and Somasegaran and Hoben (1994). All isolates grew on 2% NaCl supplemented YEMA. Rhizobium is inhibited by high salt concentrations like 2% NaCl, however, practically all of the strains we obtained grow at this concentration (Figure 1b), supporting the research done by (Dubey et al., 2010). Bromothymol blue-supplemented yeast mannitol agar (YMA) plates were used to differentiate rapid (acid-producing) growing strains from slow (non-acid-producing or alkali-producing growing rhizobia). (Somasegaran et al., 1994). Isolates F4DC, and F6DC can not absorb the Congo red dye (Figure 1a). It is known that most rhizobia can absorb the congo red only weakly whereas contaminants including Agrobacterium, can absorb strongly (Beck et al., 1993). Out of 10 isolates, 8 isolates were turned by the BTB indicator from blue to yellow and not growth on glucose peptone agar. While 8 isolates were acid producers and fast growers, two isolates (F4DC, F6DC) did not turned BTB color so they indicated as slow growers and they also were not grown on glucose peptone agar (Figure 1c, 1d). The little or no growth on the glucose peptone agar without altering the pH of the media was a clear indication of the presence of *Rhizobium*. The strains F4DC and F6DC did not grow on glucose peptone agar. It is known that contaminants like Agrobacterium, show growth on the glucose peptone agar with a distinct change in pH (Pervin et al., 2017). Ten pure isolates were placed into two distinct groups based on glucose peptone growth, BTB reaction and absorption of congo red dye (Table 1). According to the results we obtained in our study, growth in 2% NaCl is not a distinguishing feature for Agrobacterium and Rhizobium.

**Molecular Identification of Isolates**

**Table 1.** Morphological and Metabolic Characterization of the Isolates

| Isolates | F1DC | F2DC | F3DC | F4DC | F5DC | F6DC | F7DC | F8DC | F9DC | F10DC |
|---|---|---|---|---|---|---|---|---|---|---|
| Gram reaction | - | - | - | - | - | - | - | - | - | - |
| Cell shape | Rod | Rod | Rod | Rod | Rod | Rod | Rod | Rod | Rod | Rod |
| CR absorption | A | A | A | NA | A | NA | A | A | A | A |
| BTB reaction | Y | Y | Y | B | Y | B | Y | Y | Y | Y |
| Glucose peptone growth | G | G | G | NG | G | NG | G | G | G | G |
| Growth on 2% NaCl | G | G | G | NG | G | NG | G | G | G | G |

A: absorption NA: No absorption Y: Yellow B: Blue G: Growth NG: No growth CR: Congo red BTB: bromothymol blue
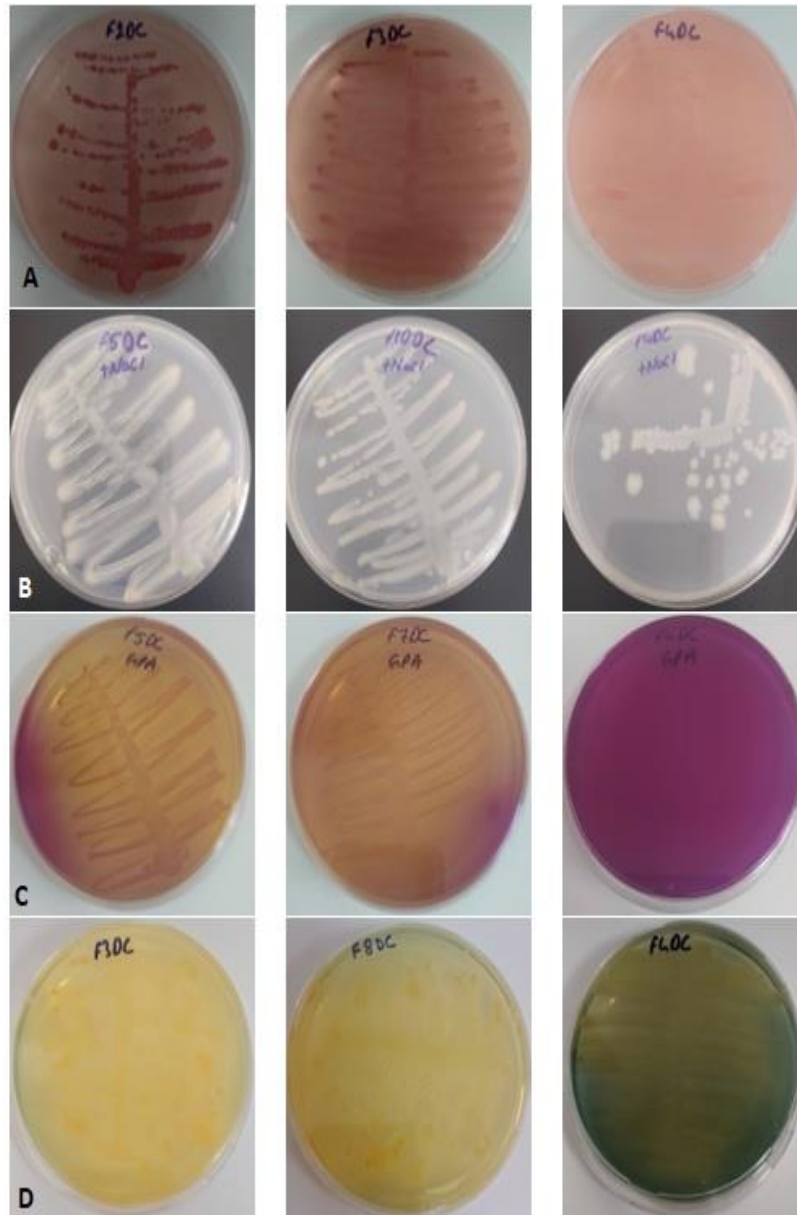


**Figure 1.** Growth of selected isolates at A. on YEMA with Congo red, B. YEMA supplemented with 2%NaCl C. Glucose Peptone Agar D. YEMA supplemented with BTB

The PCR amplification of the 16S rDNA region produced a single band with a size of roughly 1500 bp. 16S rDNA gene sequences of bacterial isolates compared with previously deposited bacterial sequences in the NCBI GenBank database. According to the BLAST results, eight of the bacterial isolates were identified as *Agrobacterium tumefaciens,* and two isolates (F4DC and F6DC) identified as *Rhizobium gallicum*. The GenBank accession numbers of *Rhizobium gallicum* F4DC (MZ156852) and *Rhizobium gallicum* F6DC (MZ156854) are given in Figure 2. The neighbor-joining method was used to create the phylogenetic tree. The phylogenetic tree was formed by the neighbor-joining method. According to the phylogeny of 16S rRNA genes, one group belongs to the genera Rhizobium, and the other one belongs to the genera *Agrobacterium.* As in our study, Agrobacterium strains have been isolated from root nodules of beans and other legumes in many previous studies (De Lajudie et al., 1999; Mhamdi et al., 2002; Hameed et al., 2004). Similar to metabolic characterization according to molecular identification, isolates are divided into two groups as Agrobacterium and Rhizobium. The ability of non-nodulating Agrobacterium to colonize the root nodules of common beans was demonstrated by Mhamdi et al. (2005). In the infected nodules, Rhizobium and Agrobacterium coexisted. The mechanism by which these isolates integrated nodules is still not known. More recently, Mrabet et al. (2006) discovered that in non-sterile soil samples and in vitro antibiosis experiments, nodulation by *Rhizobium gallicum* was selectively reduced by Agrobacterium strains derived from root nodules of common beans.

**Field experiment**
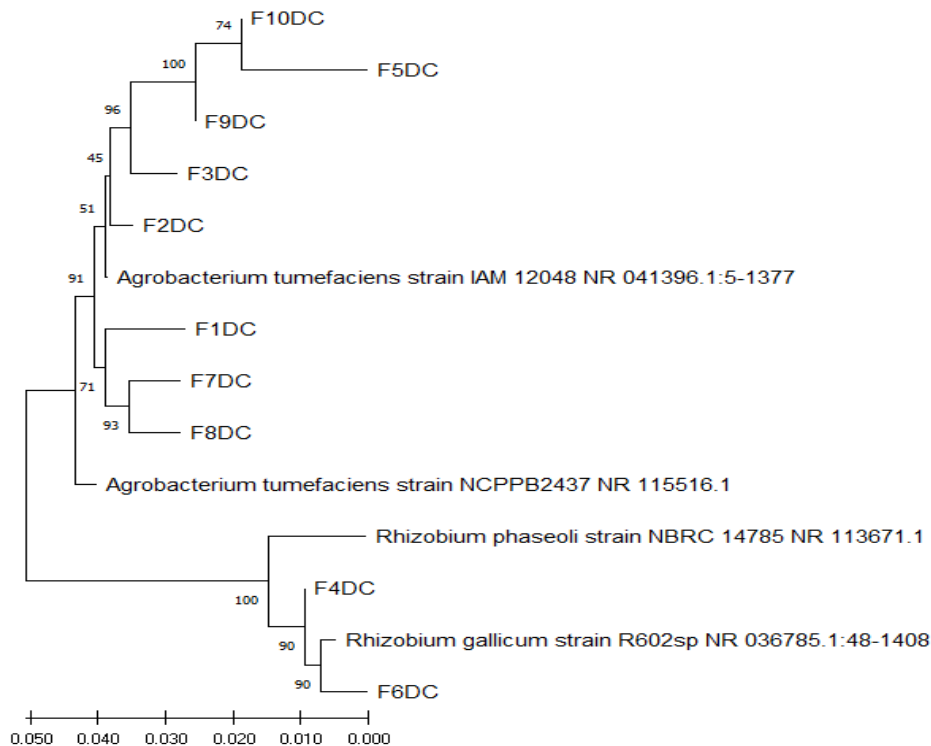


**Figure 2.** Phylogenetic tree of isolates and reference strains from GenBank database based on sequence of 16S rDNA constructed by maximum likelihood method.

Rhizobium are nitrogen-fixing bacteria that process the nif gene, responsible for the nitrogenase enzyme that converts atmospheric nitrogen (N2) to ammonia. The *nifH* gene amplification of the F4DC and F6DC bacteria resulted in an amplified segment of approximately 360 bp that resembled the positive control *Azotobacter vinelandii*. The dinitrogenase reductase subunit of the nitrogenase enzyme, which is responsible for biological nitrogen fixation, is encoded by the *nifH* gene and is employed as a biomarker to research the ecology and evolution of nitrogen-fixing bacteria. In our study, *nifH* genes of *Rhizobium gallicum* F4DC and F6DC strains were detected in the gel image shown in Figure 3. In many studies amplification of the *nifH* gene fragment was detected for Rhizobium strains (Akter et al., 2016; Khalid et al., 2020).

**Figure 3.** PCR amplification of *nifH* gene from genomic DNA of isolates. M: molecular size marker, P: positive control (*Azotobacter   vinelandii*), N: negative control (water)

In the field experiment, the effect of inoculation with *Rhizobium gallicum* F4DC (MZ156852) on sugar beans was studied. In order to control nodulation, 5 plants were randomly selected from each plot during the flowering period and the number of nodules in the first 5 cm was recorded. Plant height, number of pods per plant, 100 grain weight, grain yield, protein ratio was determined in all plots after harvest. The SPSS-22 package program was used to do a one-way analysis of variance (ANOVA) on the experimental data, and the Duncan multiple comparison test was used to determine the significance of the effects of the applications at the p < 0.05 level.

F4DC strain induced a significant increase in grain yield, number of pods and plant height compared to the control and also induced a significant increase in the number of nodules both compared to the control and chemical fertilizer treatments. (Table 2). Also, the highest protein ratio recorded in the F4DC treatment however, there was no significant increase.

It was found that *P. vulgaris* inoculated with different rhizobial isolates showed substantial changes in the shoot, root, and total dry biomass. One of the primary approaches for evaluating the symbiotic efficiency of rhizobial isolates is to look at the major changes in growth parameters, which revealed clear disparities in the ability of the isolates to fix nitrogen. (Sharma et al., 2003). *Rhizobium sp.* inoculation of seeds prior to planting has reportedly been found to be a significant impact in boosting early emergence, product viability, and high grain production. (Figueiredo et al., 2008; Otieno et al., 2009) Similar to our study Bambara and Ndakidemi (2010); also reported high sugar bean seed yield inoculation of seeds with Rhizobium bacteria compared to the uninoculated control. Koskey et al. (2018) reported that inoculating climbing beans with rhizobium dramatically increased nodule and shoot dry biomass, number of pods per plant, seed yields, and nitrogen content in shoot in the field.

**Conclusion**

**Table 2.** Effects of MF (F4DC) on yield parameters of bean under field condition

| Treatments | Plant height cm | Number of pods/plants | 100 grain weight g | Grain yield kg/da | Number of nodules | Protein ratio % |
|---|---|---|---|---|---|---|
| MF | 61,23 a | 17,87 a | 21,79 | 107.5 a | 38,67 a | 26,00 |
| CF | 61,67 a | 16,03 a | 23,47 | 112,0 a | 6,00 b | 24,88 |
| C0 | 48,27 b | 14,63 b | 21,11 | 84,75 b | 7,00 b | 24,35 |

In this study, 10 rhizobia isolates were obtained from the root nodules of sugar bean identification based on morphological, metabolic, and molecular characteristics. We found that molecular techniques are more sensitive in identifying Agrobacterium and Rhizobium strains than their morphological and metabolic characteristics. In addition, this study showed that the bacteria isolated from the root nodules of sugar beans cause a significant increase in the number of nodules, grain yield, number of pods and plant height compared to the control. Based on the results obtained, it can be concluded that *Rhizobium gallicum* F4DC could potentially be used as microbial fertilizer.

## Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

## Funding Information

## Acknowledgement

## Author Contribution

**DKO:** conducted fieldwork, collected plant and nodule specimens and prepared for analysis, drafted 17 (review and editing) **CS**: conducted fieldwork, collected plant and nodule specimens and prepared for 18 analysis, drafted (review and editing) **Vİ:** conducted fieldwork, Performed soil analysis of the trial area 19 **AP:** conducted fieldwork, Performed soil analysis of the trial area **EK**: conducted fieldwork, Performed 20 plant analysis of the trial area.

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SOIL STUDIES

# Responses of interspecific hybrid eggplant f4 inbred lines to drought and heat stress

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**Abstract**

Eggplant is grown in and near the tropic and sub-tropic zones where climate change effects can be seen dramatically in agricultural production. As a vegetable, it is considered has tolerance to some abiotic stresses compared to others. However, significant reductions in yield and marketable fruit quality were observed under stress conditions that exceeded the tolerance level. Eggplant has wild relatives which are known as tolerant to stresses. As a part of the comprehensive project this research includes drought-tolerant line development from the F4 population obtained by interspecific crossing between *Solanum incanum* L., a wild relative of eggplant naturally distributed in the Sub-Saharan zone, and a pure line with superior characteristics developed in BATEM. Qualified 50 inbred lines were selected as drought tolerant among F3 progenies in previous studies. In this experiment, 12 plants from each 50 genotypes at F4 level were exposed to drought stress, which was created by 25% watering of full irrigation. On the 25th day of treatment, tolerant plants were distinguished by using 0-5 scale. From each genotype, 4 plants were selected as drought tolerant, and they were transferred to greenhouse to identify their heat tolerance. Among the drought-tolerant group 42 F4 lines were found as heat tolerant.

## Introduction

Eggplant (*Solanum melongena* L.) as an old-world plant has been known and consumed for thousands of years in China and India where it is originally cultivated and spread around the globe. It is produced nearly 59 million tonnes on 1.9 million ha areas yearly in the World. Türkiye annually produces around 835 thousand tonnes on 17 thousand ha areas. With this production amount, it ranks fourth after China, India, and Egypt whose production annually 37, 12 and 1 million tonnes respectively. Considering the yield values obtained per hectare, Turkey ranks first among these four countries (Anonymous, 2023). Eggplant is a valuable healthy vegetable with its high fibre content, bioactive compounds (Sharma and Kaushnik, 2021) and strong antioxidant capacity (Bouhajeb et al., 2020). However, while global production has been increased steadily, slower scientific development achieved on eggplant compared to the other *Solanaceae* crops.

Average yield is a fraction of maximum or record yield obtained under optimal cultivation conditions. Decreases in the yield are mostly caused by abiotic stresses affecting the agricultural production in the fields, such as drought, heat, salt, wind, flooding, etc. (Fita et al., 2015). Drought is the most stressful factor

that limiting the crop productivity. Osmotic stress reduces some traits such as plant height, and leaf size (Nakanwagi et al., 2018). Prolonged drought periods can cause losses of yield at first, then even abandonment of agricultural lands in time. Today, with the effect of climate change, arid and semi-arid regions, which are almost 50% of the earth's land surface, are in danger of more frequent and more intense drought periods (IPCC, 2014). For this reason, it is urgent to enhance crop productivity by developing crop adaptation.

High temperature is another important environmental stress causing a heavy reduction in the yield of eggplant especially in the Mediterranean region. Eggplant optimum growth and development temperature ranges from 22 to 30 °C. Today, under the effect of global warming, temperatures in subtropical and tropical regions where eggplant is mostly grown, are observed as often above 35 °C. Although eggplant is one of the heat-loving vegetables, when the temperature rises above 35 °C, it may show defects (Wu et al., 2020). High temperature causes heat injuries in eggplant, including restricted growth, reduced productivity, and harmed quality (Li et al., 2011). Heat stress leads to abnormal flower development, low pollen viability, bud drop, reduced fruit set (Hazra et al., 2007; Santhiya et al., 2019). Lack of rain accompanying with high temperatures are contributing to the spreading of desertification therefore, it is substantial to develop new strategies for improving agricultural production (Fita et al., 2015). Adaptation success of crops to drought and heat depends on effective and combined use of modern methods and traditional breeding tools.

Drought and heat stresses beneath changing climatic conditions affect eggplant like other vegetables. Varieties used in the production are generally sensitive to abiotic stress conditions. However, tolerant local germplasm of the countries (Faiz et al., 2020) and crop wild relatives of eggplant are gaining interest in new breeding programmes (Plazas et al., 2016; Prohens et al., 2017; Afful et al., 2018; Kouassi et al., 2021). Interspecific hybridization of cultivated local heirlooms with the related species is an important step in improving tolerance to abiotic factors. Because many CWRs can thrive in harsh environmental conditions like semi-desertic or desertic areas, they represent a valuable tool for improving drought and heat tolerance in tolerant variety development studies (Knapp et al., 2013; Davidar et al., 2015; Kaushik et al., 2016; Vorontsova and Knapp, 2016).

Because eggplant production generally spreads near the tropic and subtropic climate zone regions, it is expected to tolerate drought and extreme heat.

Therefore, the development of drought and heat-tolerant varieties are a major eggplant breeding objective (Gramazio et al., 2018; Prohens et al., 2017). Although the genetic diversity of cultivated eggplant has limited, it can be expanded by interspecific hybridization which ensure a significant source of variation (Daunay and Hazra, 2012; Plazas et al., 2016; Rakha et al., 2020). Some of these eggplant wild relatives, which have tolerance to abiotic and biotic stresses also used in rootstock development studies (Gisbert et al., 2011; Sabatino et al., 2018; Rakha et al., 2020). However, in some cases, grafting incompatibility may emerge as a problem in eggplant production made by using grafted plants (Krommydas et al., 2018). The development of tolerant cultivars offers several advantages and low-cost solutions for the producers.

This study, which is a part of the breeding project on the development of drought-tolerant lines in eggplant, it was aimed to determine the heat tolerance levels of selected plants of F4 inbred lines after subjected to drought stress.

## Material and Method

### Plant material

This study, consisted of 800 plants of 50 F4 inbred lines were used as a plant material. These plants were provided by interspecific hybridization between *S. incanum* L., which is a crop wild relative naturally distributed in Sub-Saharan zone of Africa, and a pure line "BATEM-TDC47" was developed in Bati Akdeniz Agricultural Research Institute under the project "Development of Qualified Genitors (Halfway Material) for Eggplant Breeding Programs and Seed Technology" (Project number: TAGEM/BBAD/10/A09/P01/12). In this study, plants belong to parents were also tested against drought in the same method as the F4 population.

### Method

This study is a part of a comprehensive project "Development of Tolerant Lines to the Salt and Drought Stress by Interspecific Hybridization in Eggplant" (Project number: TAGEM/BBAD/B/20/A1/P1/1476). In this project, wild relative *S. incanum* L. and pure line "BATEM-TDC47" were crossed in 2020. Following years resulting 256 plants of F3 progenies were drought tested and 50 tolerant individuals were selected, and self-bred thus, F4 population was generated. In this study, 20 seeds of each of these tolerant 50 F4 progenies and parents were sown on 01.03.2022. Seedlings with 2-3 true leaves were transferred to the 3-liter pots filled with peat moss and perlite (1:1) on 21.04.2022. All seedlings were irrigated normally for

two weeks after transfer. When the seedlings reach 3-4 true leaves stage rooting was completed and drought treatment was started on 10.05.2022. The 12 plants from each 50 genotypes were drought treated and 4 plants used as control. While the group of control plants were fully irrigated (100%), drought tested plants watered with 25% of full irrigation. Drought treatment continued 25 days and irrigation was done considering control plants situation. On the 25th day all plants were evaluated using 0-5 visual scale (Kuşvuran et al., 2012; Kıran et al., 2016) where 0: No symptoms (control plants), 1: slow growth (compared to control plants) 2: start of wilting on lower leaves, 3: curling and wilting of upper leaves, 4: severe wilting, yellowing of leaves and drying of leaf margins, 5: wilting of whole plant and drying of lower leaves.

On the 25th day of the treatment 4 of 12 each 50 F4 plants showing "slow growth" (Scale = 1) were selected as "tolerant to drought" by using 0-5 scale. Selected drought tolerant 200 plants transferred to the greenhouse for the heat tolerance study. Temperature and humidity values were recorded during both drought and heat stress experiments, which were conducted in pots and in greenhouse respectively, by hobo data logger (Figure 1 and 2).
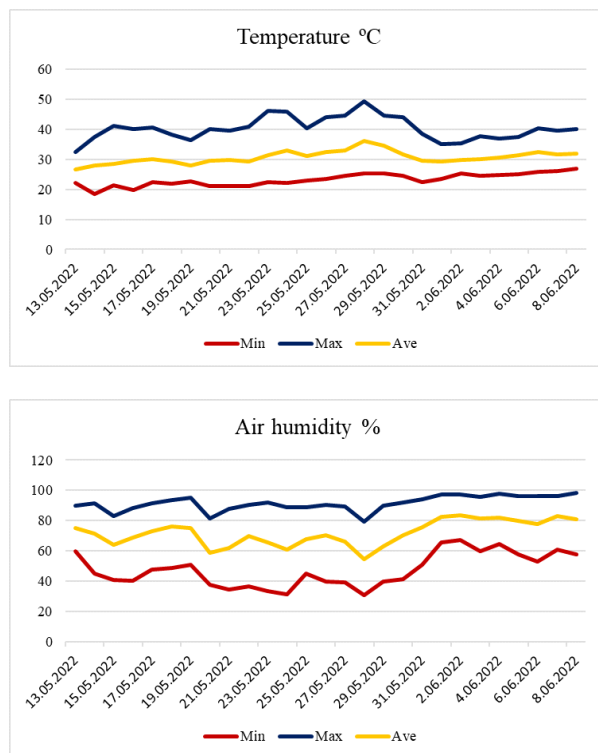


**Figure 1.** Temperature and air humidity records during drought experiment in pots.
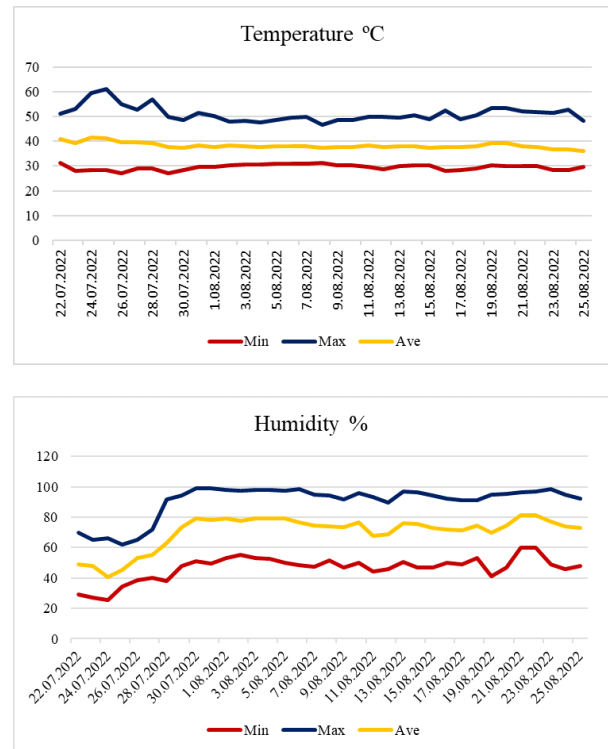


**Figure 2.** Temperature and air humidity records during heat experiment in greenhouse.

All drought tolerant plants selected by their performance in pots were transferred to the greenhouse. Heat sensitive plants were defined observing their fruit setting performance. While their heat tolerance was evaluated in greenhouse, each drought and heat tolerant plant was selfed to generate the F5 generation.

## Results and Discussion

Previous studies on eggplant proved that tolerance to abiotic stresses can be improved by interspecific hybridisation studies (Daunay and Hazra, 2012; Plazas et al., 2016; Rakha et al., 2020). Therefore, this study consists of developing drought and heat tolerant eggplant lines from F4 population, which was generated interspecific hybridization between the *S. incanum* L. and an eggplant valuable pure line BATEM TDÇ-47. Due to its location, Türkiye is under the effect of different climatic conditions and varieties that has already used in production need to develop their adaptation skills against the climate change. Because of interspecific hybridization enhances the hybrid vigour, it is a common concept that has been extensively applied for improvement of adaptation traits to changed climatic conditions in breeding (Sseremba et al., 2018).

Recent studies showed that *S. incanum* L. as a wild relative from primary genepool could provide fertile hybrids with eggplant (Knapp et al., 2013; Davidar et al., 2015; Plazas et al., 2016). In this study, hybridization between the cultivated eggplant and wild relative *S. incanum* L. which is known as drought tolerant (Gramazio et al., 2017) was achieved successfully (Figure 3). Improving crop adaptation to abiotic stresses through interspecific hybridisation can result in new cultivars with better resilience (Prohens et al., 2017; Gramazio et al., 2018). Rotino et al. (2014) has been described many tolerance sources in eggplant wild relatives to abiotic stresses but conducted studies transferring these skills to the cultivated eggplant have to develop (Toppino et al., 2008; Liu et al., 2015).



**Figure 3.** An eggplant pure line Batem TDÇ-47, *S. incanum* L. and their F4 progeny.

Along with the drought treatment in pots temperatures were recorded as minimum 18.5 °C, maximum 49.4 °C, and average 36.1 °C. Humidity was also recorded as minimum 30.9%maximum 98% and average 83.6% (Figure 1). Eggplant optimum growth and development temperature ranges from 22 to 30 °C. Average temperature was recorded during the drought treatment as 36.1 °C in the study. It was clear that the created environment for the drought experiment for this study was successful. In addition to this, irrigation with 25% of the full watering provided drought conditions to seedling in the pots filled with peat moss and perlite (Figure 4).
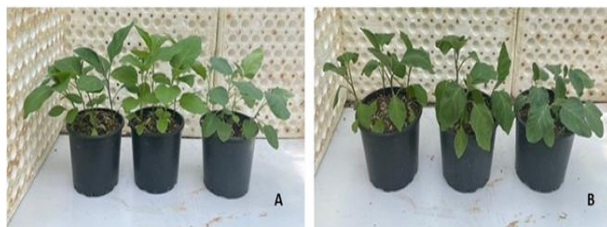


**Figure 4.** From left to right: BATEM TDÇ-47, BATEM TDÇ-47 × *S. incanum* and *S. incanum* plants in A: normally irrigated control conditions, B: drought treated conditions.

After hybridisation, population was tested against drought stress. During drought treatment, tested plants were selected in terms of their growing capacity under stress conditions. While selection was making among the drought treated individuals, beside drought tolerance capacity, spineless, hairless, and strong individuals were preferred for the desired line development. Because it is known that, under some stress conditions plants can give responses by increasing their spine and hair however, these characters undesirable attributes in cultivars. The cross ability among the *S. melongena* genotypes with their wild relatives showed wide range of variations in morphological characters. Definition of character traits of breeding objects is a fundamental step for the efficient utilization of them in breeding studies (Prohens et al., 2013; Kaushik et al., 2016).

During drought treatment in pots, obtained results showed that F4 progenies have better tolerance under drought and heat stress (Figure 5). As shown in figure 5, leaves of parents' plants have shrunk more than F4 plants. In a study, eggplant, its close wild relatives *S. insanum* and *S. incanum* and their interspecific hybrids with *S. melongena* were subjected to drought for 18 days. Their results which were support our results, showed that the hybrid between *S. melongena* and *S. incanum* displayed a better response than the other hybrids and even its parents (González-Orenga et al., 2023).
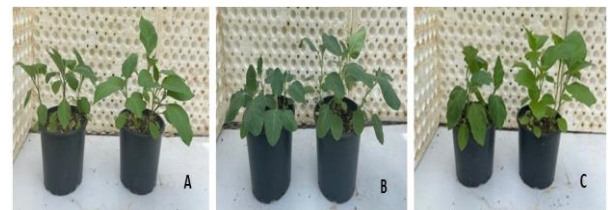


**Figure 5.** Control plants (right), drought treated plants (left): A- BATEM TDÇ-47, B- *S. incanum* L., C- BATEM TDÇ-47 × *S. incanum* L.

On the 25th day of the treatment 4 of 12 each 50 F4 plants showing "slow growth" (Scale = 1) were selected as "tolerant to drought" by using 0-5 scale. Selected drought tolerant 200 plants transferred to the greenhouse for the heat tolerance study. Greenhouse temperatures were recorded as maximum 61 °C, minimum 27.1 °C and average 38.8 °C and air humidity was also recorded as minimum 26%, maximum 98.9 %and average 70.3 % for 2022 summer season (Figure 2).

Previous studies reported that *S. incanum* is highly tolerant to drought and to some fungal diseases (Knapp et al., 2013; Plazas et al., 2016), but its tolerance to other abiotic stresses have not been performed so far

(Gramazio et al., 2019). In this study, drought tested F4 lines developed with introgressed genetic material from the wild species *S. incanum*, were also subjected to heat stress. Heat stress leads to abnormal flower development, low pollen viability, bud drop, reduced fruit set (Hazra et al., 2007; Santhiya et al., 2019). Because of pollen and ovaries can be damaged above the optimum growing temperature and this led to embryo abortion, flower and fruit shedding, heat sensitive plants were defined observing their fruit setting performance in this study. Some of heat tolerance performance of some F4 individuals are presented in figure 6. According to this, in terms of fruit setting capacity, picture A and B represents relatively heat tolerant plants compared to the picture C. Because it was observed that, end of the season eggplant in picture C has only one fruit.



**Figure 6.** Plants under heat stress in greenhouse, A and B: heat tolerant, C: heat intermediate tolerant.

Beside this, total 32 individual plants from eight genotypes could not set any fruit. These plants were determined as drought tolerant but their performance under heat found insufficient and they were categorized as heat sensitive in the study. Beside this, 18 genotypes were found heat intermediate tolerant and the other 24 genotypes were found heat tolerant. Ansari et al. (2011) reported that when the day and night temperature exceeds 35 °C there are dramatic decreases in fruit set and temperatures above and low of the limits also causes decrease in fruit weight.

## Conclusion

Climate change induced drought and heat stress have already influenced many parts of the world. Especially, Mediterranean region most effected of these abiotic stresses firstly. Therefore, it is urgent to develop varieties that tolerant to extreme weather conditions. Crop wild relatives and landraces which has tolerance to abiotic stresses are valuable sources could be used in breeding programs. This study is a part of the comprehensive project aimed to improve tolerant

eggplant lines to the drought and heat stresses using interspecific hybridization technique. Previously, hybridisation between eggplant and crop wild relative was produced successfully and F3 population were tested against drought conditions. Derived 50 genotypes from F3 population selfed and F4 population was generated. In this study, these 50 genotypes (800 plants) were tested under drought conditions, 200 plants were selected as drought tolerant. These 200 plants responses under high heat stress tested in greenhouse and 32 plants from 8 genotypes defined as heat sensitive in terms of their fruit set capacity.

Further research in progressing years heat and drought tolerant pure breeding lines with desired characteristics will be developed by using these inbred lines. Produced lines could also be used in rootstock development studies.

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## Author Contribution

Conceptualization: ŞŞE, Investigation: EC and HFB, Methodology: HFB, SK, Field work: EC and HFB, Writing -original draft: EC, Writing -review and editing: ŞŞE, SK, HFB, EC.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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RESEARCH PAPER

SOIL
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# Assessment of ecological risk potential in metal-contaminated soils of Baghdad city, Iraq

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**Abstract**

Metal pollution in the soil leads to the deterioration of agricultural production by negatively affecting the all properties of the soil. Because the more than 7 million people living in and the old military camp was used as residence by poor families after the war of 2003, Baghdad city has a risk factor from metal pollution. With this aim, metal pollution in the soil of Baghdad city was studied. Three different sites (farm, camp, and park) were selected. Ten soil samples were taken randomly from 0-15 cm depth on each of the sites. Concentrations of metals [calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), cobalt (Co), chromium (Cr), lead (Pb), cadmium (Cd), and nickel (Ni)] of soil samples were measured using atomic absorption spectrometer (AAS). Basic soil properties such as texture class and pH were measured on the disturbed samples. Concentrations increased Cr<Ni<Fe<Co<Mg<Cd<Cu<Ca<Zn<Pb<Mn respectively. The highest coefficient of variation value is Mg (90.76%) in camp and Fe in farm (77.81%) and park (68.66%) soil samples. The lowest values were found in Ca (farm 6.12%, camp 12.51%, park 22.37%). Metal concentrations were evaluated for soil quality by Pollution (CF) and Ecological Risk Factor ($E_r^i$), Potential Ecological Risk Index (PERI), and Geoaccumulation Index (Igeo). For CF, only Cd was found as slightly contaminated in farm (1.89) and park (1.35) soils, and moderately contaminated in camp (2.11) soils. According to $E_r^i$ values, a serious risk of Pb was found in farm and camp soils in two samples each and 3 samples in park soils. According to the PERI results, no risk was determined in all soils except for the extreme (21 samples) and high (6 samples) Cd risk. Similarly, Igeo values of Pb increased in the farm (1.23), camp (1.44) and park (2.11), while Cd increased in the park (5.22), farm (5.71), and camp (5.87). High concentrations in all soils of Cd (4.74, 5.29, and 3.37) and Pb (35.36, 40.71, and 64.97) were attributed to anthropogenic activities such as the population, household waste, car exhausts, and the results of 2003 war.

## Introduction

The soil that makes up our terrestrial ecosystem is a natural resource that can renew itself. However, most of the main sources of soil pollution are anthropogenic (Wei & Yang, 2011) and can cause the accumulation of pollutants in large quantities beyond the regenerative capacity of the soil (Cachada et al., 2018). These accumulations originate from wrong use of fertilizer and agricultural pesticides and accumulation of chemical and biological household and factory wastes in soil that include toxic metals. On the

other hand, anthropogenic sources of toxic elements in urban soils include emissions from vehicles, industrial waste, atmospheric deposition of dust and aerosols, domestic emissions, and incinerators (Lee et al., 2006; Luo et al., 2012). Studies confirmed that potentially toxic element pollution in soils causes the degradation of soil quality (Hu et al., 2020), threatens the living organisms in the soil, and reduces productivity (Yang et al., 2018a; Tseng et al., 2021). To remediation measures for metal pollution, it is thought that a comprehensive study of metal pollution from possible sources and their spatial distribution should be known. According to Salman et al., (2019), pollution indices are a powerful tool for environmental quality assessment. Generally, in soil pollution studies three single indices, namely index of geoaccumulation ($I_{geo}$), contamination factor (CF) ecological risk factor ($E_r^i$), and potential ecological risk index (PRI) are used. Studies on heavy metals at different scales have been conducted in the urban soils of Iraq (Ismail, 2010; Al Obaidy & Al Mashhadi, 2013). However, studies conducted on mapping metals in urban soils and using pollution indices for evaluating metal pollution were rare. The aims of this study were 1) to examine pollution levels of some metals in soil samples taken from 3 different regions in Baghdad city, 2) to investigate the environmental factors that caused pollution and their effects and the spatial distribution of metals, and 3) to calculate the Potential Ecological Risk Index (PERI) and Geo-Accumulation Index ($I_{geo}$) to assess soil quality in Baghdad urban soils.

## Materials and Methods

The study was carried out in 3 different locations in Baghdad city, located in the center of Iraq (33° 15' 5165'' North Latitude and 44° 28' 0976'' East Longitude) (Figure 1). Soil samples were selected from different areas where different factors such as dense population and traffic, limited agricultural practice, and military camps were effective. The first sampling area is an abandoned farm that has more than 3000 m$^2$ area. It has desert plants such as thorns and saplings growing naturally and an apartment complex just a few meters away. The second is an old camp turned into a random residential area that does not contain municipal services or service facilities. The number of housing ranges from 200 to 300 housing approximately 2.000 people.

The third is a public park where samples were taken from the side of the street and in the park. A total of 30 sample points were determined by the Global Positioning System, 10 samples from each different area (Figure 2). After cleaning the topsoil, undisturbed samples were taken with a Kopecky steel cylinder (V=100 cm$^3$) (Holpp et al., 2010). Disturbed soil



**Figure 1**. (a) Abandoned farm (b) Residental area (c) City Park
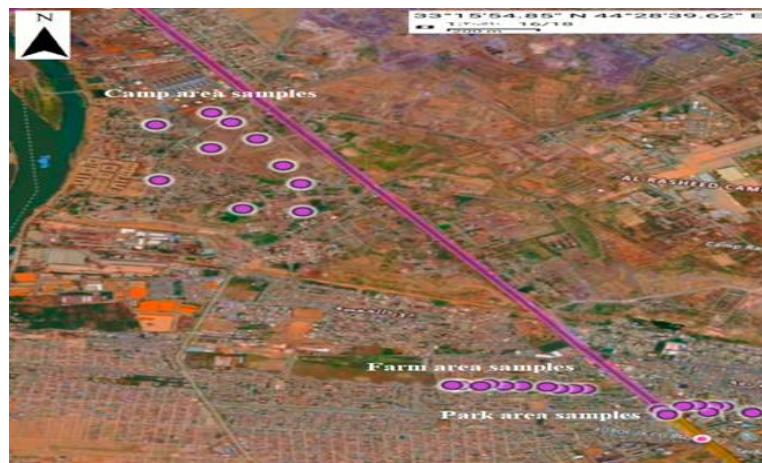


**Figure 2.** Sampling points for studied locations (The map was downloaded from Google Earth, and sampling points marked according to their coordinates)

samples were taken from 0-15 cm depth for basic soil analyses from the same points.

Soil parametric characteristics, texture class (Gee & Bouder, 1986), soil reaction (pH) (Page et al., 1982), electrical conductivity (EC), (Rhoades, 1982), total CaCO$_3$ and organic matter (OM) (Page et al., 1982), FC and WP (Klute, 1986), bulk density (D$_b$) (Blake & Hardge, 1986) were measured. For metal (Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Pb, Cd, and Ni) analyses, the standards were read to the device in certain calibrations, a calibration curve was drawn, samples were burned with different solvents and made ready for the device (Izol & Inik, 2022). Jackson and Watson (1977) were used for analyzing heavy metals in soils

with Atomic Absorption Spectrometry. SPSS 23 package program was used for descriptive statistical values of soil properties and heavy metal contents. Kabata Pendias (2001), Rose et al., (1981), and Lindsay (1979) were used for the limit values of metals in the soils. Pollution factor (CF) was calculated by Hakanson (1980) (Equation 1) and grouped into four grades. Ecological risk factor ($E_r^i$) (Equation 2) is the ecological risk values from anthropogenic factors (Hakanson, 1980) and grouped into five grades (Brady et al., 2015). $T_r^i$ is the toxicological response factor of heavy metals (Hakanson, 1980; Şen and Yakupoğlu, 2022). The Potential Ecological Risk Index (PERI) proposed by Hakanson (1980) was used to assess the contamination

**Table 1.** Thresholds for soil quality classification for metal indices

| Class | CF | Qualification | $E_r^i$ | Qualification | PERI | Qualification |
|---|---|---|---|---|---|---|
| 0 | CF<1 | Unpolluted | $E_r^i$<1 | Unpolluted | PERI<150 | Low ecological risk |
| 1 | | Slightly | | Slightly | | |
| 2 | 1<CF<3 | Moderately | 2<$E_r^i$<5 | Moderately | 150≤ PERI<300 | Modarete ecological risk |
| 3 | | Heavily | | Heavily | | |
| 4 | 3<CF<6 | Severely | 5<$E_r^i$<20 | Severely | 30≤ PERI<600 | Significant ecological risk |
| 5 | | High | 20<$E_r^i$<40 | High | | |
| 6 | CF>6 | Extreme | $E_r^i$>40 | Extreme | PERI>600 | Very high ecological risk |

CF. Pollution factor, $E_r^i$: Ecological risk factor, PERI: Potential Ecological Risk Index

risk of metals (Equation 3) and grouped into four grades (Krisha and Mohan, 2016) (Table 1).

$$CF = \frac{C_s}{C_b} \tag{1}$$

$$E_r^i = T_r^i \times CF \tag{2}$$

$$PERI = \sum_{i=1}^{n} E_r^i \tag{3}$$

Where C$_s$ is the concentration in soil samples, C$_b$ is the background value of metals.

The geoaccumulation index (I$_{geo}$) value is used to assess the intensity (Kowalska et al., 2018) and degree of anthropogenic pollution as a source of metal pollution and is also used to understand whether a metal comes from natural or anthropogenic sources (Xu et al., 2021). I$_{geo}$ was calculated to assess the level of metal accumulation in urban soil (Muller, 1969) (Equation 4).

$$I_{geo} = \log_2 \left( \frac{C_n}{1.5B_n} \right) \tag{4}$$

Where, C$_n$ is the measured concentration of

element n, B$_n$ is the background value of the element in the studied soil, the constant 1.5 compensates for natural fluctuations of a given metal and minor anthropogenic impacts (Taylor & Mclennan, 1995). I$_{geo}$ index values of metals are classified according to Yaqin et al. (2008). Horizontal distributions of metals in soils were mapped by Geostatistics Software, GS$^+$ program (Robertson et al., 2001).

**Results and Discussion**

Descriptive statistics of some soil variables (texture class, pH, EC, total CaCO$_3$, OM, FC, WP, D$_b$) and metals (Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Pb, Cd, Ni) were given in Table 2 and 3. In general, all soils were sandy in texture, very strongly calcareous, and high in organic matter content. The bulk density values (1.32-1.59 gr cm$^{-3}$) are in agreement with the given limit values (1.10-1.78 gr cm$^{-3}$) given for sandy soils (Bruand et al., 2005). Sandy soils often have low FC and permanent WP (Huang & Hartemink, 2020). Compared with the FC and WP limit values for sandy soils (Yost & Hartemink (2019), FC was slightly high although WP was quite high in farm and camp soils.

**Table 2.** Descriptive statistics of some soil variables in the farm, camp, and park soils

| | Soil variables | Min | Max | Mean | SD | Skewness | Kurtosis | CV(%) |
|---|---|---|---|---|---|---|---|---|
| **Farm (n:10)** | Sand (%) | 37.20 | 79.20 | 51.69 | 15.36 | 1.28 | 0.51 | 29.00 |
| | EC(dSm$^{-1}$) | 2.40 | 47.10 | 23.48 | 16.40 | 0.19 | -1.35 | 69.00 |
| | pH (1:2.5) | 7.11 | 7.44 | 7.28 | 0.12 | -0.15 | -1.68 | 2.00 |
| | CaCO$_3$ (%) | 25.64 | 36.51 | 29.77 | 3.52 | 0.73 | -0.44 | 12.00 |
| | FC(%) | 0.16 | 0.27 | 0.21 | 0.03 | 0.15 | -1.20 | 16.00 |
| | WP (%) | 0.13 | 0.22 | 0.17 | 0.03 | 0.66 | -1.20 | 19.00 |
| | Bulk density (gr cm$^{-3}$) | 1.32 | 1.55 | 1.44 | 0.10 | -0.05 | -2.40 | 7.00 |
| | OM (%) | 3.20 | 5.90 | 4.79 | 0.95 | -0.43 | -1.33 | 19.00 |
| **Camp (n:10)** | Sand (%) | 36.00 | 69.20 | 55.40 | 9.37 | -0.40 | 1.60 | 16.00 |
| | EC(dSm$^{-1}$) | 3.20 | 133.80 | 48.38 | 47.72 | 0.86 | -0.35 | 98.00 |
| | pH (1:2.5) | 7.19 | 8.10 | 7.54 | 0.28 | 0.63 | 0.15 | 3.00 |
| | Calcium carbonate (%) | 24.11 | 37.49 | 30.41 | 4.71 | 0.02 | -1.50 | 15.00 |
| | FC(%) | 0.21 | 0.28 | 0.25 | 0.02 | -0.54 | -1.02 | 9.00 |
| | WP (%) | 0.18 | 0.23 | 0.21 | 0.01 | -0.06 | -1.79 | 8.00 |
| | D$_b$(gr cm$^{-3}$) | 1.35 | 1.54 | 1.49 | 0.05 | -1.95 | 4.06 | 4.00 |
| | OM (%) | 4.90 | 7.10 | 5.82 | 0.77 | 0.41 | -0.97 | 13.00 |
| **Park (n:10)** | Sand (%) | 36.00 | 69.20 | 55.40 | 9.37 | -0.40 | 1.60 | 16.00 |
| | Electrical conductivity (dSm$^{-1}$) | 3.20 | 133.80 | 48.38 | 47.72 | 0.86 | -0.35 | 98.00 |
| | Soil reaction, pH (1:2.5) | 7.19 | 8.10 | 7.54 | 0.28 | 0.63 | 0.15 | 3.00 |
| | CaCO$_3$ (%) | 24.11 | 37.49 | 30.41 | 4.71 | 0.02 | -1.50 | 15.00 |
| | Field capacity(%) | 0.21 | 0.28 | 0.25 | 0.02 | -0.54 | -1.02 | 9.00 |
| | Wilting point (%) | 0.18 | 0.23 | 0.21 | 0.01 | -0.06 | -1.79 | 8.00 |
| | D$_b$(gr cm$^{-3}$) | 1.35 | 1.54 | 1.49 | 0.05 | -1.95 | 4.06 | 4.00 |
| | Organic matter (%) | 4.90 | 7.10 | 5.82 | 0.77 | 0.41 | -0.97 | 13.00 |

n: Number of soil samples, Min: Minimum, Max: Maximum, SD: Standard deviation, CV: Coefficient of variation

Due to their proximity to the Tigris River, the groundwater level is high in some sampling sites. These soils comprise a high OM, especially on the first site, and have high values of FC. Al-Adari (2020) Indicated that the rise in groundwater leads to an increase in its moisture by filling soils' pores with water, and thus the increase in the field capacity of the soil. It was reported that higher temperatures in areas with semi-arid climates cause a rise in the water in their soils, due to the high rates of evaporation, which increases the value of water losses. This increases the ability of soil to absorb water. Soil pH ranged from 7.11 to 8.10 and had the lowest variability (1, 2, 3%). Same results were reported in other studies (Erşahin, 1999; Mulla & McBratney, 2001). EC has the highest variability (69, 98, 116%) in farm, camp, and park soil samples. It can be explained by the rather high EC values (120.6 and 133.8 dSm$^{-1}$), especially in a few samples in the camp soils.

**Table 3.** Descriptive statistics of metal values in the farm, camp, and park soils

| | Metals (mg kg$^{-1}$) | Min | Max | Mean | SD | Skewness | Kurtosis | CV (%) |
|---|---|---|---|---|---|---|---|---|
| **Farm (n:10)** | Manganese, Mn | 58.54 | 99.18 | 79.10 | 14.76 | -0.20 | -1.57 | 18.66 |
| | Copper, Cu | 14.62 | 31.52 | 23.02 | 6.22 | 0.05 | -1.63 | 27.03 |
| | Zinc, Zn | 18.45 | 54.29 | 34.90 | 12.12 | 0.15 | -0.83 | 34.73 |
| | Cobalt, Co | 2.11 | 5.01 | 3.59 | 0.96 | 0.04 | -0.64 | 26.92 |
| | Chrome, Cr | 0.98 | 1.51 | 1.31 | 0.16 | -1.01 | 0.52 | 12.54 |
| | Lead, Pb | 13.47 | 77.25 | 35.36 | 25.46 | 0.88 | -0.92 | 71.98 |
| | Cadmium, Cd | 2.27 | 7.12 | 4.74 | 1.80 | -0.23 | -1.72 | 37.98 |
| | Nickel, Ni | 1.00 | 3.46 | 1.84 | 0.69 | 1.44 | 2.78 | 37.35 |
| | Iron, Fe | 0.90 | 9.20 | 3.22 | 2.51 | 1.64 | 2.99 | 77.81 |
| | Calcium, Ca | 23.4 | 29.7 | 27.37 | 1.67 | -1.37 | 3.53 | 6.12 |
| | Magnesium, Mg | 1.1 | 9.00 | 4.28 | 2.87 | 0.89 | -0.67 | 67.10 |
| **Camp (n:10)** | Manganese, Mn | 61.58 | 125.44 | 97.72 | 19.61 | -0.16 | -0.07 | 20.07 |
| | Copper, Cu | 11.25 | 33.87 | 23.92 | 8.461 | -0.26 | -1.28 | 35.36 |
| | Zinc, Zn | 27.99 | 58.14 | 39.08 | 10.69 | 0.60 | -1.12 | 27.36 |
| | Cobalt, Co | 1.54 | 9.32 | 5.00 | 2.43 | 0.41 | -0.53 | 48.63 |
| | Chrome, Cr | 0.88 | 1.69 | 1.19 | 0.31 | 0.65 | -0.78 | 26.23 |
| | Lead, Pb | 2.74 | 83.96 | 40.71 | 26.56 | 0.59 | -0.78 | 65.24 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cadmium, Cd | 3.12 | 7.41 | 5.29 | 1.56 | -0.01 | -1.57 | 29.61 |
| | Nickel, Ni | 1.99 | 4.33 | 3.41 | 0.88 | -0.58 | -1.59 | 26.03 |
| | Iron, Fe | 3.00 | 15.10 | 8.32 | 3.57 | 0.55 | 0.14 | 42.87 |
| | Calcium, Ca | 21.12 | 30.00 | 26.06 | 3.26 | -0.140 | -1.44 | 12.51 |
| | Magnesium, Mg | 1.00 | 8.49 | 2.39 | 2.17 | 2.99 | 9.24 | 90.76 |
| Park (n:10) | Manganese, Mn | 61.55 | 129.32 | 100.85 | 22.10 | -0.06 | -0.47 | 21.91 |
| | Copper, Cu | 13.56 | 29.54 | 18.00 | 4.82 | 1.58 | 3.24 | 26.81 |
| | Zinc, Zn | 20.29 | 55.87 | 36.12 | 10.68 | 0.64 | 0.01 | 29.56 |
| | Cobalt, Co | 3.11 | 7.32 | 5.03 | 1.53 | 0.48 | -1.03 | 30.47 |
| | Chrome, Cr | 0.55 | 1.98 | 1.15 | 0.54 | 0.61 | -1.38 | 47.22 |
| | Lead, Pb | 36.99 | 89.32 | 64.97 | 15.83 | -0.24 | -0.46 | 24.36 |
| | Cadmium, Cd | 1.24 | 6.39 | 3.37 | 1.92 | 0.32 | -1.58 | 57.15 |
| | Nickel, Ni | 1.10 | 5.00 | 2.83 | 1.36 | 0.42 | -0.66 | 48.13 |
| | Iron, Fe | 0.10 | 8.82 | 4.38 | 3.01 | -0.07 | -1.19 | 68.66 |
| | Calcium, Ca | 15.00 | 29.12 | 24.30 | 5.43 | -0.96 | -0.97 | 22.37 |
| | Magnesium, Mg | 1.21 | 5.00 | 2.58 | 1.26 | 0.74 | -0.30 | 48.94 |

n: Number of soil samples, Min: Minimum, Max: Maximum, SD: Standard deviation, CV: Coefficient of variation

**Correlations between Soil Variables and Metals:** Linear correlations between soil properties and total metal contents were carried out to assess which soil properties affected metal distribution in Tables 4, 5, and 6 for the farm, camp, and park soils, respectively. In the farm soils, the highest correlation coefficients between soil variables were found between $D_b$ and clay content (-0.83) and $D_b$ and sand content (0.77). In addition, in the park soils, $D_b$-sand content (0.68) and $D_b$-pH (-0.66). It was mentioned that there is a strong relation between soil bulk density and soil texture components (Chaudhari et al., 2013). As related to soil moisture properties and metals, we found correlations between WP and Co (0.95), and WP and Mn (-0.86) in farm soils. In a similar study that investigated the effects of soil water status on metals in serpentine soil (Gunarathne et al., 2019), there was a positive relation between WP and Mn and Co. They attributed it to the microbial reduction that occurs by water deficiency below the WP. Ittihad (1989) found a negative (-0.90) relation between Mn and WP on the outskirts of Baghdad and he attributed this result to the soil's properties. However, the microbial reduction caused by the wilting point did not adversely affect, on the contrary, a positive effect was observed on the amount of Co. In camp soils, there were correlations between FC and Cu (-0.68), FC and silt content (-0.71), and WP and EC (0.65). In addition, it was found that there were significant correlations between WP and CaCO₃ (0.80), WP and FC (0.72), and WP and Zn (-0.75) in park soils. Zheng and Zhang (2011) reported that soil moisture dynamics such as field capacity and wilting point directly affect many physicochemical properties of soils, including organic matter content, pH, and electrical conductivity, and are indirectly involved with metals in soils. Therefore they have drawn attention to the current soil water status such as field capacity and wilting point as well as the dynamics of soil moisture content should be taken into account to assess the status of metals in soils. There was a positive correlation between Cu and EC (0.80) in the park soils.

Peris et al. (2008) investigated the heavy metal content and sources of agricultural soils in Castellón province (Spain), a representative area of the European Mediterranean region, and found that Cu and Pb concentrations were found to be positively correlated with EC (r=0.200; p≤0.05). Correlations between sand content and Pb (0.68) and silt content and Ni (0.78) were significant in the farm soils. The effect of texture on metal accumulation in the soil has been reported, for example, Brümmer et al., (1986), Ma and Rao (1997), Selim & Sparks (2001) noted that the severity of pollution depends not only on the total heavy metal content of the soil but also on the proportion of their mobile and bioavailable forms, which are generally controlled by the texture and other physicochemical properties of soils.

However, no studied metals were correlated with soil pH except Fe metal in the farm soils. The pH values of all soil samples taken from all three regions are between 7.11 and 8.10, and the studied soils are characterized by alkaline. pH has a moderate level significant correlation (-0.65) with Fe at 0.05 level negatively in the farm soils (Table 4). Alloway (2012) reported that the movement and content of heavy metals in the soil can be controlled by pedogenic processes, soil management, and various anthropogenic and soil factors such as soil pH, organic matter, clay, carbonates, and salt content. In addition, studies are reporting the accumulation of Cd and Pb in alkaline soils. It was stated by Metin (2010) that lead can dissolve at a very low level in acidic and alkaline conditions. Ismail (2010) studied heavy metal concentration in neutral soils (pH=7.35) of A-Tala village in Maysan Governorate and reported the lead concentration to be approximately the same (35 mg kg⁻¹) as our study and exceeding the limit of Cd (8 mg kg⁻¹). However, she concluded that besides alkaline properties, the main factor in the development of high cadmium concentrations was pollution resulting from human activities. In our farm soil samples, the mean Cd concentration exceeding the limit value (1.5 mg kg⁻¹ for

**Table 4.** Corelations between soil variables and metals in farm soils (n:10)

| | Db | Sand | Silt | Clay | EC | pH | CaCO₃ | FC | WP | OM | Mn | Cu | Zn | Co | Cr | Pb | Cd | Ni | Fe | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Db(g cm³) | 1 | | | | | | | | | | | | | | | | | | | | |
| Sand (%) | **0.77**\*\* | 1 | | | | | | | | | | | | | | | | | | | |
| Silt (%) | 0.49 | 0.03 | 1 | | | | | | | | | | | | | | | | | | |
| Clay (%) | **-0.83**\*\* | -0.47 | **-0.75**\* | 1 | | | | | | | | | | | | | | | | | |
| EC | -0.11 | -0.50 | 0.11 | -0.19 | 1 | | | | | | | | | | | | | | | | |
| pH | -0.30 | 0.01 | **-0.66**\* | 0.49 | 0.02 | 1 | | | | | | | | | | | | | | | |
| CaCO₃ | 0.55 | 0.28 | 0.01 | -0.50 | 0.49 | 0.22 | 1 | | | | | | | | | | | | | | |
| FC (%) | 0.54 | 0.32 | 0.28 | -0.45 | 0.19 | -0.20 | 0.34 | 1 | | | | | | | | | | | | | |
| WP (%) | 0.17 | 0.55 | -0.34 | -0.11 | -0.02 | 0.33 | 0.14 | 0.37 | 1 | | | | | | | | | | | | |
| OM (%) | -0.37 | -0.02 | -0.18 | 0.39 | -0.57 | -0.23 | **-0.93**\*\* | -0.36 | 0.04 | 1 | | | | | | | | | | | |
| Manganese | -0.36 | -0.59 | 0.01 | 0.35 | 0.02 | -0.19 | -0.42 | -0.52 | **-0.86**\*\* | 0.30 | 1 | | | | | | | | | | |
| Copper | 0.42 | 0.46 | 0.04 | -0.32 | -0.07 | 0.48 | 0.49 | 0.55 | 0.51 | -0.49 | **-0.70**\* | 1 | | | | | | | | | |
| Zinc | -0.11 | 0.01 | -0.22 | 0.12 | 0.10 | -0.15 | -0.33 | 0.59 | 0.53 | 0.34 | -0.31 | 0.05 | 1 | | | | | | | | |
| Cobalt | 0.01 | 0.42 | -0.48 | 0.02 | 0.05 | 0.52 | 0.17 | 0.22 | **0.95**\*\* | 0.02 | **-0.76**\* | 0.51 | 0.45 | 1 | | | | | | | |
| Chrome | 0.19 | 0.21 | 0.35 | -0.24 | -0.04 | -0.27 | -0.19 | -0.31 | -0.01 | 0.31 | 0.25 | -0.43 | -0.22 | -0.11 | 1 | | | | | | |
| Lead | 0.56 | **0.68**\* | 0.28 | -0.49 | -0.44 | 0.15 | 0.04 | 0.10 | 0.37 | 0.12 | -0.33 | 0.57 | -0.14 | 0.33 | 0.28 | 1 | | | | | |
| Cadmium | 0.54 | 0.57 | 0.07 | -0.43 | -0.03 | -0.45 | 0.29 | 0.58 | 0.48 | -0.13 | -0.61 | 0.09 | 0.41 | 0.248 | -0.02 | -0.06 | 1 | | | | |
| Nickel | 0.20 | -0.14 | **0.78**\*\* | -0.42 | 0.01 | -0.31 | 0.11 | 0.29 | -0.34 | -0.35 | -0.05 | 0.34 | -0.28 | -0.43 | -0.12 | 0.15 | -0.16 | 1 | | | |
| Iron | 0.20 | 0.06 | 0.49 | -0.38 | 0.29 | **-0.65**\* | 0.03 | 0.23 | 0.11 | 0.01 | -0.11 | -0.38 | 0.19 | -0.08 | 0.59 | -0.22 | 0.52 | 0.13 | 1 | | |
| Calcium | 0.14 | 0.06 | 0.58 | -0.25 | 0.00 | -0.33 | -0.01 | 0.24 | -0.01 | -0.04 | -0.12 | 0.02 | -0.02 | -0.08 | 0.33 | 0.04 | 0.09 | 0.53 | **0.64**\* | 1 | |
| Magnesium | -0.31 | -0.19 | 0.07 | 0.19 | 0.22 | -0.06 | -0.18 | 0.12 | 0.23 | 0.15 | -0.10 | -0.18 | 0.33 | 0.25 | 0.28 | -0.27 | 0.02 | 0.04 | 0.60 | **0.78**\*\* | 1 |

\*\* Significant at P<0.01 \*Significant at P<0.05. n: Number of samples, Db: Bulk density, pH: Soil reaction, EC: Electrical Conductivity, CaCO₃: Calcium carbonate content, OM: Organic Matter, FC: Field capacity, WP: Wilting point

**Table 5.** Corelations between soil variables and metals in camp soils (n:10)

| | $D_b$ | Sand | Silt | Clay | EC | pH | CaCO$_3$ | FC | WP | OM | Mn | Cu | Zn | Co | Cr | Pb | Cd | Ni | Fe | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $D_b$ (g cm$^{-3}$) | 1 | | | | | | | | | | | | | | | | | | | | |
| Sand (%) | 0.43 | 1 | | | | | | | | | | | | | | | | | | | |
| Silt (%) | -0.52 | -0.34 | 1 | | | | | | | | | | | | | | | | | | |
| Clay (%) | -0.24 | 0.24 | -0.06 | 1 | | | | | | | | | | | | | | | | | |
| EC | -0.09 | 0.63 | -0.43 | 0.28 | 1 | | | | | | | | | | | | | | | | |
| pH | 0.15 | 0.01 | 0.45 | 0.12 | -0.54 | 1 | | | | | | | | | | | | | | | |
| CaCO$_3$ | -0.27 | 0.13 | 0.32 | -0.02 | 0.17 | -0.45 | 1 | | | | | | | | | | | | | | |
| FC (%) | 0.32 | 0.27 | **-0.71*** | -0.16 | 0.42 | -0.39 | -0.07 | 1 | | | | | | | | | | | | | |
| WP (%) | 0.03 | 0.51 | -0.19 | 0.01 | **0.65*** | -0.38 | 0.18 | 0.42 | 1 | | | | | | | | | | | | |
| OM (%) | 0.43 | -0.03 | -0.01 | 0.14 | -0.59 | 0.57 | -0.41 | -0.47 | -0.44 | 1 | | | | | | | | | | | |
| Manganese | 0.14 | -0.10 | 0.10 | -0.58 | -0.15 | -0.48 | 0.53 | -0.13 | -0.02 | -0.16 | 1 | | | | | | | | | | |
| Copper | -0.22 | 0.05 | 0.52 | -0.21 | -0.29 | 0.35 | 0.15 | **-0.68*** | -0.36 | 0.33 | 0.27 | 1 | | | | | | | | | |
| Zinc | -0.63 | -0.16 | 0.10 | 0.47 | -0.01 | 0.12 | -0.09 | -0.11 | -0.36 | -0.08 | -0.36 | 0.25 | 1 | | | | | | | | |
| Cobalt | 0.02 | -0.10 | -0.26 | -0.16 | -0.12 | -0.52 | 0.43 | -0.08 | -0.32 | 0.15 | 0.62 | 0.31 | 0.13 | 1 | | | | | | | |
| Chrome | 0.36 | 0.29 | -0.15 | -0.12 | -0.15 | 0.46 | -0.22 | 0.20 | -0.48 | 0.16 | -0.12 | 0.30 | 0.30 | 0.04 | 1 | | | | | | |
| Lead | 0.28 | 0.21 | -0.23 | -0.21 | -0.11 | 0.23 | -0.35 | -0.15 | -0.49 | 0.37 | 0.15 | 0.60 | 0.28 | 0.37 | **0.71*** | 1 | | | | | |
| Cadmium | -0.41 | 0.15 | 0.03 | 0.43 | 0.50 | -0.39 | 0.08 | -0.29 | 0.51 | -0.16 | 0.03 | -0.01 | 0.11 | -0.01 | **-0.68*** | -0.22 | 1 | | | | |
| Nickel | 0.14 | 0.38 | -0.23 | 0.03 | 0.53 | -0.51 | 0.23 | 0.30 | 0.54 | -0.54 | 0.44 | -0.33 | -0.18 | -0.01 | -0.19 | -0.12 | 0.47 | 1 | | | |
| Iron | 0.47 | -0.28 | -0.21 | -0.42 | -0.22 | -0.29 | -0.06 | 0.04 | -0.23 | -0.02 | **0.63*** | -0.26 | -0.48 | 0.23 | -0.03 | 0.11 | -0.14 | 0.43 | 1 | | |
| Calcium | -0.03 | -0.29 | 0.26 | -0.26 | -0.11 | -0.16 | 0.01 | -0.51 | -0.25 | -0.06 | 0.59 | 0.23 | -0.21 | 0.14 | -0.19 | 0.22 | 0.28 | 0.38 | **0.71*** | 1 | |
| Magnesium | 0.38 | 0.12 | 0.18 | -0.08 | -0.24 | 0.08 | 0.01 | -0.38 | 0.20 | 0.35 | 0.49 | 0.12 | -0.45 | 0.03 | -0.30 | -0.01 | 0.36 | 0.46 | 0.46 | 0.50 | 1 |

*Significant at P<0.05. n: Number of samples, $D_b$: Bulk density, pH: Soil reaction, EC: Electrical Conductivity, CaCO$_3$: Calcium carbonate content, OM: Organic Matter, FC: Field capacity, WP: Wilting point

**Table 6.** Corelations between soil variables and metals in the park soils (n:10)

| | $D_b$ | Sand | Silt | Clay | EC | pH | CaCO₃ | FC | WP | OM | Mn | Cu | Zn | Co | Cr | Pb | Cd | Ni | Fe | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $D_b$(g cm³) | 1 | | | | | | | | | | | | | | | | | | | | |
| Sand (%) | **0.68*** | 1 | | | | | | | | | | | | | | | | | | | |
| Silt (%) | -0.11 | -0.38 | 1 | | | | | | | | | | | | | | | | | | |
| Clay (%) | -0.06 | 0.57 | **-0.73*** | 1 | | | | | | | | | | | | | | | | | |
| EC | -0.18 | 0.37 | -0.18 | **0.66*** | 1 | | | | | | | | | | | | | | | | |
| pH | **-0.66*** | **-0.67*** | 0.24 | -0.27 | -0.13 | 1 | | | | | | | | | | | | | | | |
| CaCO₃ | 0.08 | 0.53 | -0.13 | **0.67*** | **0.69*** | -0.35 | 1 | | | | | | | | | | | | | | |
| FC (%) | -0.16 | 0.19 | 0.00 | 0.48 | 0.37 | 0.01 | 0.62 | 1 | | | | | | | | | | | | | |
| WP (%) | 0.14 | 0.22 | 0.09 | 0.33 | 0.26 | -0.22 | **0.80**** | **0.72*** | 1 | | | | | | | | | | | | |
| OM (%) | 0.01 | -0.14 | -0.12 | -0.01 | -0.47 | 0.41 | -0.11 | 0.10 | 0.23 | 1 | | | | | | | | | | | |
| Manganese | -0.18 | 0.17 | -0.21 | 0.22 | 0.02 | -0.08 | 0.13 | -0.16 | -0.19 | -0.29 | 1 | | | | | | | | | | |
| Copper | **-0.68*** | -0.09 | -0.22 | 0.59 | **0.80**** | 0.30 | 0.42 | 0.37 | 0.07 | -0.34 | 0.22 | 1 | | | | | | | | | |
| Zinc | 0.04 | -0.03 | 0.26 | -0.43 | -0.08 | -0.05 | -0.51 | -0.56 | **-0.75*** | **-0.65*** | 0.40 | -0.06 | 1 | | | | | | | | |
| Cobalt | **-0.64*** | -0.36 | -0.22 | 0.43 | 0.50 | 0.54 | 0.33 | 0.36 | 0.30 | 0.24 | -0.21 | **0.74*** | -0.56 | 1 | | | | | | | |
| Chrome | -0.19 | 0.28 | -0.40 | 0.58 | 0.19 | -0.00 | 0.58 | 0.52 | 0.41 | 0.08 | 0.54 | 0.34 | -0.39 | 0.24 | 1 | | | | | | |
| Lead | -0.17 | 0.046 | -0.32 | 0.26 | 0.17 | -0.18 | -0.06 | 0.13 | -0.22 | -0.54 | 0.54 | 0.39 | 0.44 | -0.10 | 0.18 | 1 | | | | | |
| Cadmium | -0.10 | 0.088 | -0.27 | 0.50 | **0.65*** | 0.24 | 0.47 | 0.43 | 0.42 | 0.06 | -0.23 | 0.62 | -0.40 | **0.73*** | 0.12 | 0.11 | 1 | | | | |
| Nickel | 0.24 | 0.31 | 0.11 | 0.01 | 0.29 | -0.15 | 0.06 | 0.17 | -0.08 | -0.25 | -0.4 | -0.03 | 0.03 | -0.11 | -0.12 | -0.34 | -0.06 | 1 | | | |
| Iron | 0.25 | 0.28 | 0.35 | -0.20 | 0.21 | -0.06 | -0.11 | 0.12 | -0.24 | -0.40 | -0.34 | -0.05 | 0.41 | -0.33 | -0.25 | -0.09 | -0.10 | **0.86**** | 1 | | |
| Calcium | 0.14 | 0.06 | 0.25 | -0.18 | 0.03 | -0.24 | 0.01 | 0.14 | 0.03 | -0.23 | -0.54 | -0.21 | -0.07 | -0.18 | -0.10 | -0.40 | -0.34 | **0.87**** | **0.65*** | 1 | |
| Magnesium | 0.26 | 0.21 | 0.13 | -0.08 | 0.25 | -0.20 | -0.01 | 0.15 | -0.08 | -0.34 | -0.60 | -0.05 | 0.07 | -0.12 | -0.22 | -0.24 | -0.02 | **0.97**** | **0.85**** | **0.88**** | 1 |

** Significant at P<0.01, *Significant at P<0.05. n: Number of samples, $D_b$: Bulk density, pH: Soil reaction, EC: Electrical Conductivity, CaCO₃: Calcium carbonate content, OM: Organic Matter, FC: Field capacity, WP: Wilting point

pH>7 soils) was found to be 4.74 mg kg$^{-1}$ although there was no human activity. However, there is an apartment complex just a few meters away near the abandoned farm. According to the results of such research, we can say that the higher Cd and Pb concentration in the park soils is of anthropogenic origin. Weaver and Pollard (2011) reported that the higher the percentage of organic matter in the soil, the higher the cadmium concentration with it is adsorbed on the particles of the organic matter, and when organic matter decomposes, cadmium is released into the soil. Since there were not adequate plants in the sampling areas, soil organic matter would be highly stabilized.

**The Potential Ecological Risk Index (PERI) of Metals:** According to CF, only Cd was found as slightly (1) in farm (1.89) and in park (1.35) soils, and moderately (2) contaminated in camp (2.11) soils. For $E_r^i$ values, a serious risk of Pb was found in farm (5.51 and 5.40) and camp (5.99 and 5.45) soils in two samples each and 3 samples (5.59, 6.38, and 5.71) in park soils. In addition, it was determined the extreme (7 in farm, 9 in camp, 5 in park) and high (3 in farm, 1 in camp, 2 in park) ecological risk for Cd in soils. The highest $E_r^i$ value for Cd was found in the camp sample, and for Pb in the park sample. According to the PERI results, no risk was determined in all soils except for Pb and Cd. However, the results of $E_r^i$ showed that 70% of samples were at extreme ecological risk with Cd and 30% of samples showed severe ecological risks with Pb (Table 7). Similarly, I$_{geo}$ values of Pb increased in the farm (1.23), camp (1.44), and park (2.11), while Cd increased in the park (5.22), farm (5.71), and camp (5.87). High concentrations in farm, camp, and park soils of Cd (4.74, 5.29, and 3.37) and Pb (35.36, 40.71, and 64.97) and high ecological risk of Cd above the unpolluted range of <40 could be related to anthropogenic

activities such as household waste, burning of urban wastes (Pan et al., 2010), car exhausts, and the results of 2003. The increase in human activities with the increasing population increases the amount of metal that creates pollution (Kumar et al., 2015).

**The Geoaccumulation Index (I$_{geo}$) of Metals:** The Geoaccumulation index was calculated according to Muller's (1969) equation and classified using the Table that was given by Yaqin et al. (2008) and He et al. (2022) in Table 8. The I$_{geo}$ values for the metals Cd > Pb > Cu > Zn > Co > Mn > Cr > Ni > Mg > Ca > Fe in the farm soils; Cd > Pb > Zn > Cu > Co > Ni > Cr > Fe > Mn > Ca > Mg in the camp soils; and Cd > Pb > Cu > Zn > Co > Mn > Cr > Ni > Fe = Ca > Mg in the park soils.

All of the I$_{geo}$ values of metals except Cd and Pb were lower than 0. Therefore, it was concluded that the soils of Baghdad were not contaminated with these metals. I$_{geo}$ values of Cd in the farm, camp, and park soils were larger than 5, and they were calculated to exceed the extremely contaminated class. I$_{geo}$ values of Pb in the farm and camp soils were larger than 1, taking place in the moderately contaminated class. However, in park soils, it was larger than 2, and in the moderately to heavily contaminated class. Farm and camping areas are quite uncrowded places. The higher Pb content in the park soils than in the farms and camp was attributed to the low density of people and therefore traffic since the park is located in the center of the city. Some studies associate metal accumulations in city park soils with anthropogenic factors due to high population and traffic. He et al. (2022) stated that Cd and Pb, along with Zn and Cu, were the most studied metals in urban soils, as their concentrations are generally high. Chen et al. (2005) reported that the high metal accumulation index values in China's cities

**Table 7.** Ecological Risk Index (E$_r^i$) values of metals in farm, camp and park soils

| o | Ca | Mg | Fe | Zn | Mn | Cu | Co | Cr | Pb | Cd | Ni |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **F1** | 0.99 | 0.45 | 0.19 | 0.31 | 0.10 | 2.21 | 0.24 | 0.02 | 0.96 | 62.52 | 0.08 |
| **F2** | 0.78 | 0.25 | 0.08 | 0.35 | 0.11 | 1.84 | 0.17 | 0.03 | 1.15 | 40.32 | 0.07 |
| **F3** | 0.90 | 0.10 | 0.04 | 0.57 | 0.07 | 2.94 | 0.36 | 0.02 | 5.51 | 76.32 | 0.05 |
| **F4** | 0.90 | 0.13 | 0.03 | 0.19 | 0.11 | 1.53 | 0.25 | 0.03 | 3.33 | 27.24 | 0.07 |
| **F5** | 0.96 | 0.06 | 0.02 | 0.41 | 0.07 | 2.97 | 0.31 | 0.02 | 1.42 | 66.48 | 0.10 |
| **F6** | 0.94 | 0.13 | 0.02 | 0.26 | 0.09 | 3.15 | 0.24 | 0.03 | 5.40 | 30.12 | 0.18 |
| **F7** | 0.94 | 0.31 | 0.04 | 0.41 | 0.10 | 2.19 | 0.24 | 0.02 | 0.99 | 35.52 | 0.08 |
| **F8** | 0.89 | 0.45 | 0.09 | 0.53 | 0.08 | 1.92 | 0.36 | 0.03 | 1.20 | 66.24 | 0.11 |
| **F9** | 0.90 | 0.14 | 0.01 | 0.41 | 0.10 | 1.46 | 0.15 | 0.03 | 1.58 | 85.44 | 0.12 |
| **F10** | 0.90 | 0.14 | 0.04 | 0.20 | 0.07 | 2.78 | 0.28 | 0.03 | 3.69 | 78.60 | 0.09 |
| **C1** | 1.00 | 0.09 | 0.21 | 0.49 | 0.09 | 1.12 | 0.38 | 0.02 | 0.19 | 88.92 | 0.22 |
| **C2** | 0.73 | 0.10 | 0.32 | 0.51 | 0.11 | 3.32 | 0.68 | 0.03 | 5.99 | 40.20 | 0.21 |
| **C3** | 0.96 | 0.07 | 0.17 | 0.31 | 0.07 | 1.74 | 0.11 | 0.02 | 2.14 | 63.48 | 0.13 |
| **C4** | 0.97 | 0.10 | 0.20 | 0.38 | 0.11 | 2.42 | 0.19 | 0.03 | 1.69 | 37.44 | 0.12 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **C5** | 0.70 | 0.05 | 0.06 | 0.61 | 0.10 | 3.35 | 0.35 | 0.02 | 2.51 | 76.20 | 0.21 |
| **C6** | 1.00 | 0.10 | 0.10 | 0.36 | 0.14 | 3.38 | 0.54 | 0.02 | 4.95 | 83.88 | 0.21 |
| **C7** | 0.84 | 0.10 | 0.26 | 0.30 | 0.14 | 1.23 | 0.54 | 0.02 | 1.57 | 51.00 | 0.15 |
| **C8** | 0.81 | 0.11 | 0.16 | 0.51 | 0.10 | 2.01 | 0.21 | 0.03 | 5.45 | 61.44 | 0.22 |
| **C9** | 0.87 | 0.07 | 0.13 | 0.32 | 0.11 | 2.51 | 0.30 | 0.03 | 2.43 | 49.68 | 0.10 |
| **C10** | 0.80 | 0.42 | 0.12 | 0.29 | 0.13 | 2.78 | 0.34 | 0.02 | 2.12 | 83.28 | 0.19 |
| **P1** | 0.97 | 0.25 | 0.01 | 0.35 | 0.15 | 2.95 | 0.53 | 0.04 | 5.59 | 64.92 | 0.25 |
| **P2** | 0.50 | 0.20 | 0.10 | 0.58 | 0.15 | 1.41 | 0.24 | 0.01 | 4.87 | 15.00 | 0.26 |
| **P3** | 0.92 | 0.18 | 0.08 | 0.37 | 0.15 | 1.76 | 0.31 | 0.04 | 6.38 | 52.32 | 0.05 |
| **P4** | 0.57 | 0.07 | 0.09 | 0.31 | 0.10 | 1.95 | 0.53 | 0.02 | 3.73 | 64.68 | 0.10 |
| **P5** | 0.60 | 0.11 | 0.14 | 0.29 | 0.10 | 1.35 | 0.32 | 0.01 | 4.89 | 43.80 | 0.16 |
| **P6** | 0.94 | 0.15 | 0.11 | 0.32 | 0.07 | 2.0 | 0.47 | 0.01 | 3.95 | 76.68 | 0.17 |
| **P7** | 0.87 | 0.12 | 0.18 | 0.52 | 0.12 | 1.95 | 0.30 | 0.02 | 5.71 | 26.76 | 0.17 |
| **P8** | 0.87 | 0.09 | 0.16 | 0.45 | 0.11 | 1.44 | 0.21 | 0.01 | 3.67 | 27.00 | 0.13 |
| **P9** | 0.97 | 0.06 | 0.02 | 0.37 | 0.10 | 1.75 | 0.38 | 0.02 | 4.94 | 14.88 | 0.06 |
| **P10** | 0.91 | 0.06 | 0.02 | 0.21 | 0.12 | 1.35 | 0.36 | 0.04 | 2.64 | 18.36 | 0.11 |
| **PERI** | Low | Low | Low | Low | Low | Low | Low | Low | Severely | Extreme | Low |

F: Farm, C: Camp, P: Park, PERI: Potential Ecological Risk Index

**Table 8.** I$_{geo}$ index classes of metals in the farm, camp, and park soils

| | Farm soils | | | | |
|---|---|---|---|---|---|
| | Metals | Igeo | Class | Urban soil quality | Data sources |
| **Farm soils** | Manganese, Mn | -4.01 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Copper, Cu | -0.38 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Zinc, Zn | -1.1 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Cobalt, Co | -1.73 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Chrome, Cr | -4.18 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Lead, Pb | 1.23 | 2 | Moderately contaminated | Yaqin et al. 2008; He et al. 2022 |
| | Cadmium, Cd | 5.71 | >5 | Extremely contaminated | Yaqin et al. 2008; He et al. 2022 |
| | Nickel, Ni | -5.01 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Iron, Fe | -7.96 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Calcium, Ca | -7.64 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Magnesium, Mg | -7.38 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| **Camp soils** | Metals | Igeo | Class | Urban soil quality | Data sources |
| | Manganese, Mn | -7.38 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Copper, Cu | -0.33 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Zinc, Zn | -0.09 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Cobalt, Co | -1.26 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Chrome, Cr | -4.32 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Lead, Pb | 1.44 | 2 | Moderately contaminated | Yaqin et al. 2008; He et al. 2022 |
| | Cadmium, Cd | 5.87 | >5 | Extremely contaminated | Yaqin et al. 2008; He et al. 2022 |
| | Nickel, Ni | -4.13 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Iron, Fe | -6.64 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Calcium, Ca | -7.64 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Magnesium, Mg | -8.38 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| **Park soils** | Metals | Igeo | Class | Urban soil quality | Data sources |
| | Manganese, Mn | -3.66 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Copper, Cu | -0.74 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Zinc, Zn | -1.05 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Cobalt, Co | -1.25 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Chrome, Cr | -4.38 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Lead, Pb | 2.11 | 3 | Moderately to heavily contaminated | Yaqin et al. 2008; He et al. 2022 |
| | Cadmium, Cd | 5.22 | >5 | Extremely contaminated | Yaqin et al. 2008; He et al. 2022 |
| | Nickel, Ni | -4.41 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Iron, Fe | -7.64 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Calcium, Ca | -7.64 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |
| | Magnesium, Mg | -7.96 | 0 | Practically uncontaminated | Yaqin et al. 2008; He et al. 2022 |

I$_{geo}$: The Geoaccumulation index

were higher in old park soils than in new parks. They noted that it was related to the exposure time. Likewise, Peng et al. (2013) reported that the concentrations of metals in residential soil was increasing with the age of the residential buildings. These reports confirm that metal concentrations were related to the increasing population in urban soils. These results agree to that there was more contamination with Pb and Cd metals in the park soils of Baghdad city. On the other hand, extremely high $I_{geo}$ values of Cd in the farm, camp, and park soils (larger than 5) show that Cd pollution was at a very significant level in this city. Anthropogenic sources such as traffic emissions can be suspected as the main reason for the high $I_{geo}$ values for Cd in Baghdad urban soils in Table 8.

**Spatial Distribution of Metals:** The kriging maps were built for depicting spatial distribution pattern of metals in farm, camp, and park soils (Figures 3, 4, and 5). He et al. (2022) reported that heavy metal contents in soils have strong spatial variability, and their statistical distributions are useful indicators of the long-term characteristics of urban pollution. Concentrations of Mn, Fe, and Ca have an increasing trends in the same direction while Mg has trended in the opposite

direction on the farm site. These trends in farm soils can be attributed to the distribution of household and farm wastes since the site was used as a farm before. Concentrations of Zn and Mg showed a patchy distribution. Similarly, the concentration of Cd showed an increasing trend in the opposite direction with Cu, Pb, and Ca trends in the camp soils, and Zn and Ni showed patchy distribution. Fe is high in the camp soils compared to farm and park soils and is mostly evenly distributed. The high concentration of Fe may be due to the long-term use of the campsite as a military area. As related to the park soils, Cu, Co, Cd, and Ca had a trend in the opposite direction with Mn and Pb. Zn, Co, Pb, and Ca showed patchy distribution in the park soils. Ca Co, Zn, Cu, Pb, and Mn concentrations were greater in park soils than in farms and camps. It may be attributed to the fact that the mostly higher concentration of metals are in the park soils that it is in the center of the city, and that human-related factors such as traffic are effective. Besides human-induced pollution, dry climatic conditions, low rainfall, and vegetation can be important. Imperato et al. (2003) reported that urban soil pollution would result from the accumulation of nonsoil originated pollutants as



**Figure 3.** The spatial distributions of the Mn, Cu, and Zn metals in the farm (left), camp (middle), and park(right) soils

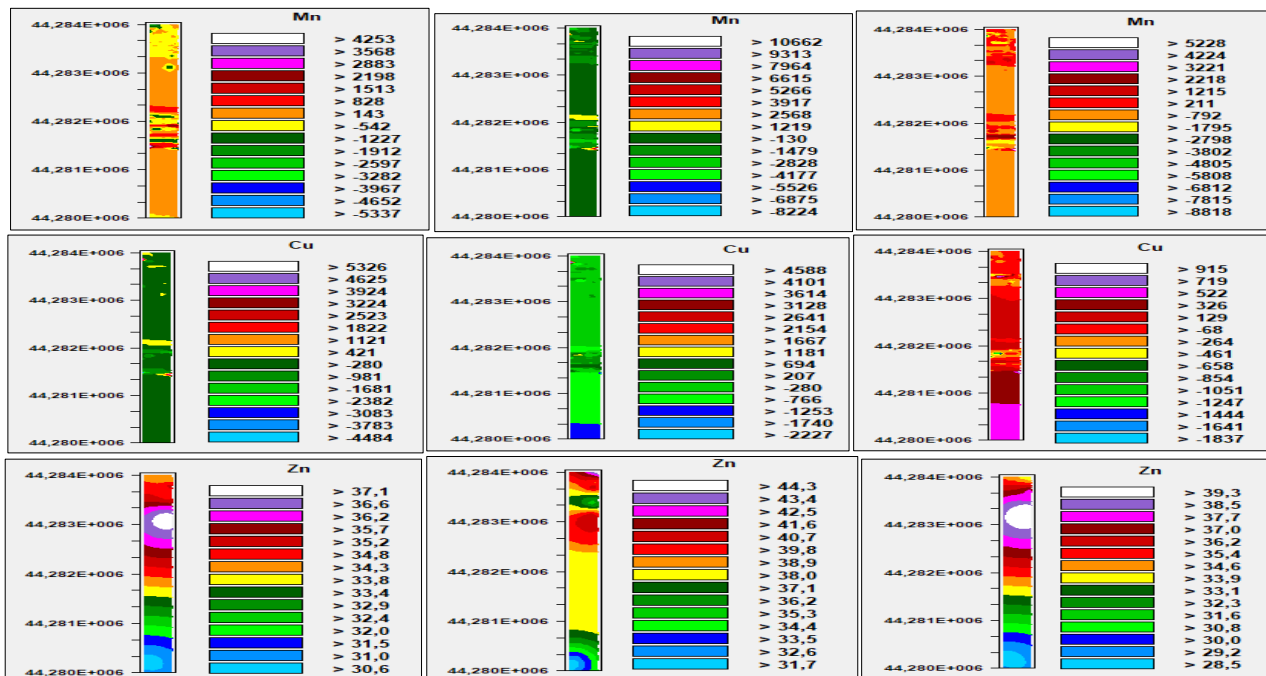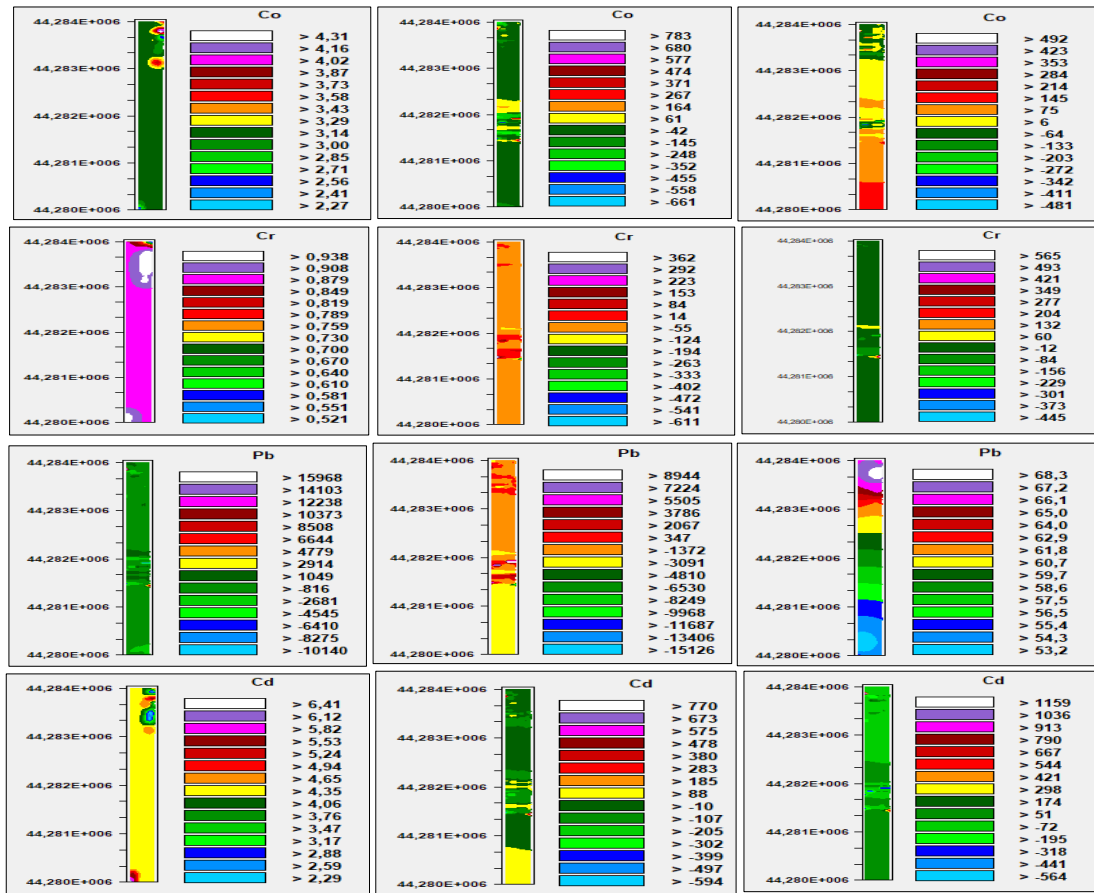Soil Studies 12(2), 77-91



**Figure 4.** The spatial distributions of the Co, Cr, Pb, and Cd metals in the farm (left), camp (middle), and park (right) soils
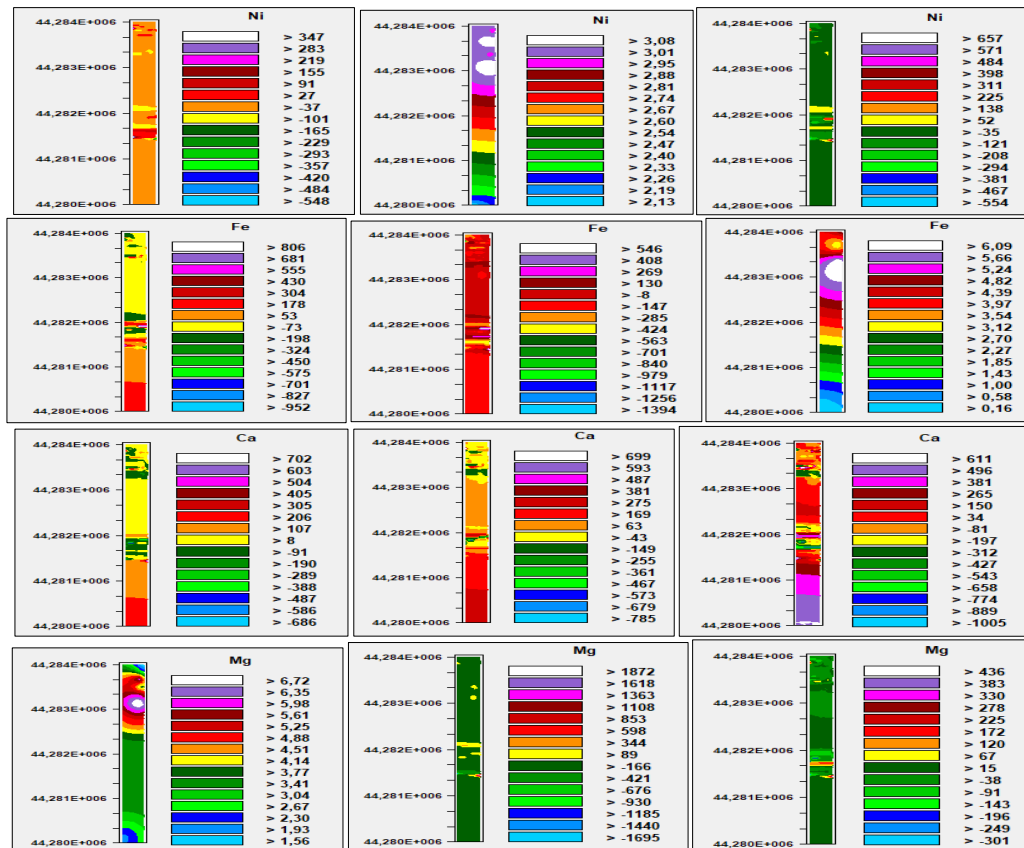


**Figure 5.** The spatial distributions of the Ni, Fe, Ca, and Mg metals in the farm (left), camp (middle), and park (right) soils (continue)

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well as where was little vegetation cover or dry conditions subsist.

## Conclusion

In this study, metal (Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Pb, Cd, and Ni) pollution levels in soils of Baghdad city were evaluated under three different land uses (farm, camp, and city park site). The results revealed that urban soils in Baghdad city were polluted by Cd and Pb. Cd was the most seriously polluting metal with 3.37%, 4.74%, and 5.29% of the pollution in the park, farm, and camp soil, respevtively. Pb was significant with 35.36%, 40.71%, and 64.97% of the pollution in the farm, camp, and park soils. The high rates of soil pollution with Cd and Pb resulted from the accumulation of household waste, sewage water, and gases emitted from cars all triggered by increased population. In addition, the accumulation of industrial waste from factories, blacksmithing workshops, and car repairs in soil and its failure to decompose are the reasons. Therefore, human activity played an important role in the high levels of Cd and Pb besides natural factors such as soil texture components and soil dust in Baghdad. Since urban soil is considered a sink for metals, these metals are an increasingly serious problem for human health besides soil quality. Especially metals, which are chemical warfare agents such as lead, have toxic properties on the nervous system and organs. These results must lead us to assess the health risk caused by exposure to metals in the urban soils, especially park soils in Baghdad City since children and adults spend a substantial time on those sites. The recommendations are to impose environmental and health control over factories, power stations, blacksmiths, and car repair workshops and provide to dispose of industrial waste correctly, reduce the use of pesticides and chemical fertilizers, and use of especially plant that reduces pollution with heavy metals. Along with these practices, more attention should be paid to metal pollution in city soils with dense populations and traffic.

## Authors contributions

**GK:** Conceptualization, Data Curation, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing. **HAHA:** Data Curation, Investigation, Resources, Writing-review.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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S O I L
S T U D I E S

RESEARCH PAPER

# Investigation of the relationships between selenium fractions and soil properties by canonical correlation

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**Abstract**

The availability and distribution of an element are determined by soil conditions and the geochemical fractions. In this study, canonical correlation (CCA) was used to examine the associations between the soil parameters (X set) of the Amik plain and the fractions of selenium (Se) (Y set). A statistically significant correlation (P < 0.01) of 0.391 ± 0.06 was observed between the first canonical variable pair (FCVP). The clay, sand, and silt variables exhibited the greatest standardized canonical coefficients, with values of 74.55, 75.83, and 94.77, respectively. Additionally, the B3 percent had a standardized canonical coefficient of 1.029. The analysis of the FCVP showed that the original variables of the X set and/or its canonical variable (U1) had the strongest linear connection (0.770) with organic matter (OM) and the B3 percentage in the Y set (0.603). The OM (0.3012) exhibited the most substantial linear structural correlations between the canonical variables of the Y set and the original variables of the X set. However, the canonical redundancy analysis revealed that in the FCVP, the variance of the X set was described by its canonical variables to the extent of 20.97%, whereas the Y set was explained by 21.96%. Hence, it can be inferred that the use of canonical correlation is effective in elucidating the relationship between soil parameters and various components of plant nutrition.

## Introduction

Selenium (Se) is one of the trace elements that are absolutely needed for humans and animals (Rayman, 2000), owing to its key roles in the defence system as an antioxidant (Tapiero et al., 2003) and its crucial functions in cancer prevention (Reid et al., 2008). Hence, it is imperative for both animals and humans to consume sufficient quantities of this essential element for the maintenance of good health. Nevertheless, it is worth noting that in several nations, the average daily selenium consumption per person

falls below the World Health Organization's suggested threshold of 40 µg (Navarro-Alarcon and Cabrera-Vique, 2008). One of the primary factors contributing to this circumstance is the inadequate uptake of this element by plants, which fails to satisfy the dietary needs of animals and people via consumption. The extent of this phenomenon is contingent upon the aggregate quantity of selenium (Se) present in the soil, as well as the chemical and geochemical proportions of various selenium species. However, it is important to

note that the characteristics, accessibility, conversions, and absorption of selenium (Se) by plants exhibit variability in response to both biotic and abiotic variables during the process of soil development, therefore being influenced by soil qualities and agricultural methodologies.

The bioavailability and geochemical fractionation of selenium in soils are influenced by many continuous processes, including sorption/desorption, precipitation-dissolution, and ligand synthesis (Alfthan et al., 2015). The overall concentration of selenium (Se) in a given soil is also influenced by the various transport mechanisms taking place inside the solid or solution phase. The transformations between different geochemical fractions in the field of interest are primarily influenced by factors such as pH and redox potential (Nakamaru and Altansuvd, 2014; Xing et al., 2015; Fan et al., 2018), organic matter (Xing et al., 2015), soil texture and mineralogy (Antoniadis et al., 2017; Munier-Lamy et al., 2007), Fe/Al oxides extractable with dithionite-citrate-bicarbonate (Araujo et al., 2019; Fan and Zhao, 2018; Hyun et al., 2006), microbial characteristics (Munier-Lamy et al., 2007), Se content (Winkel et al., 2015; Saha et al., 2017), topographic features (Xu et al., 2018), and the presence of competitive anions such as sulphates and phosphates (Lee et al., 2011). The impact of competitive anions is particularly apparent in soils that receive excessive amounts of fertilizer, particularly phosphorus. In the soil environment, the presence of inorganic Se is seen in several forms, including selenite ($Se^{-2}$), elemental selenium [Se (0)], and selenate ($Se^{4, 6+}$), which are determined by factors such as pH and redox potential. Selenium is present in well-drained soils in the forms of $SeO_4^{-2}$, $SeO_3^{-2}$, and $HSeO3-$. In soils with neutral and alkaline pH levels, the major species of Se is $Se^{6+}$. This particular form of Se is often highly soluble, mobile, and readily available to plants (Mikkelsen et al., 1989). In contrast, previous studies have shown that the application of humic acid and the presence of inadequate drainage in neutral soils might induce inter-fractional transitions (Fan et al., 2019). According to many studies (Tolu et al., 2014; Eswayah et al., 2017; Li et al., 2017; Wang et al., 2019), it has been shown that the presence of organic matter in soils, either naturally rich or organically amended, leads to the reduction of hazardous Se-oxyanions into the elemental fraction that exhibits limited bioavailability. In contrast, the adsorption characteristics of Se are influenced by several factors such as the presence of different iron/aluminum oxides, organic matter, and pH levels. Nevertheless, the allocation of Se across different fractions is subject to variation, which is contingent upon the elemental form of Se and the duration of time that Se ion has been adsorbed, as shown by Fan and Zhao (2018).

The comprehension of Se concentration and geochemical fractions in the soil system cannot be achieved by simplistic one-dimensional interactions between dependent and independent variables. The lack of clear differentiation between dependent and independent variables is typically seen while analysing the connections between nutrient availability, soil fractions, and soil attributes within the soil system. Hence, in order to conduct a more comprehensive analysis of the connections, it is advisable to use multivariate statistical methods that do not differentiate between dependent and independent variables (Shin, 1996).

Canonical correlation analysis (CCA) is a statistical technique that assesses the degree of association between two groups of variables, which may be either dependent or independent (Sharma, 1996; Ozsoy, 2019). Canonical correlation analysis is a multivariate statistical approach that has similarities with principal component analysis (PCA) and factor analysis. However, CCA distinguishes itself as the most suitable and robust method in situations when there are several sets of dependent and/or independent variables.

The objective of this research was to examine the impact of soil characteristics on the alterations in selenium (Se) fractions in soils within the Amik plain region. Since Amik plain has key properties for accumulation/leaching of Se and partitioning to different Se fraction it may be regarded as good data sets to test the relations by multivariate statistical approach. This investigation, therefore, used canonical correlation analysis, using two distinct sets of variables: soil properties and Se fractions to extract the coherence between assigned two data set.

## Materials and Methods

**Sampling and descriptive analysis:** A total of 179 soil samples were collected from the Amik plain, specifically at the intersections of designated transects of 2 x 2 kilometres. Assigned sampling coordinates and the drainage-web map were merged in ArcMAP environment (Figure 1). The soil parameters examined in this study included textural components by hydrometer method (sand, silt, and clay), pH and electrical conductivity (EC) in 1:2.5 soil: distilled water suspension, organic matter content by wet oxidation method of Walkley-Black, and calcium carbonate equivalent by a manometric method. These parameters were analysed using the conventional procedures commonly used in Türkiye, as described by Kacar (2009) and site properties of the Amik plain were given elsewhere (Özkan et al., 2022). Table 1 presents the descriptive statistics for the aforementioned attributes.

**Selenium fractionation method:** The sequential extraction technique is a method used to separate and isolate different components or fractions of a sample in a step-by-step manner. Martens and Suarez (1997) conducted sequential extraction, which was described in the following manner.
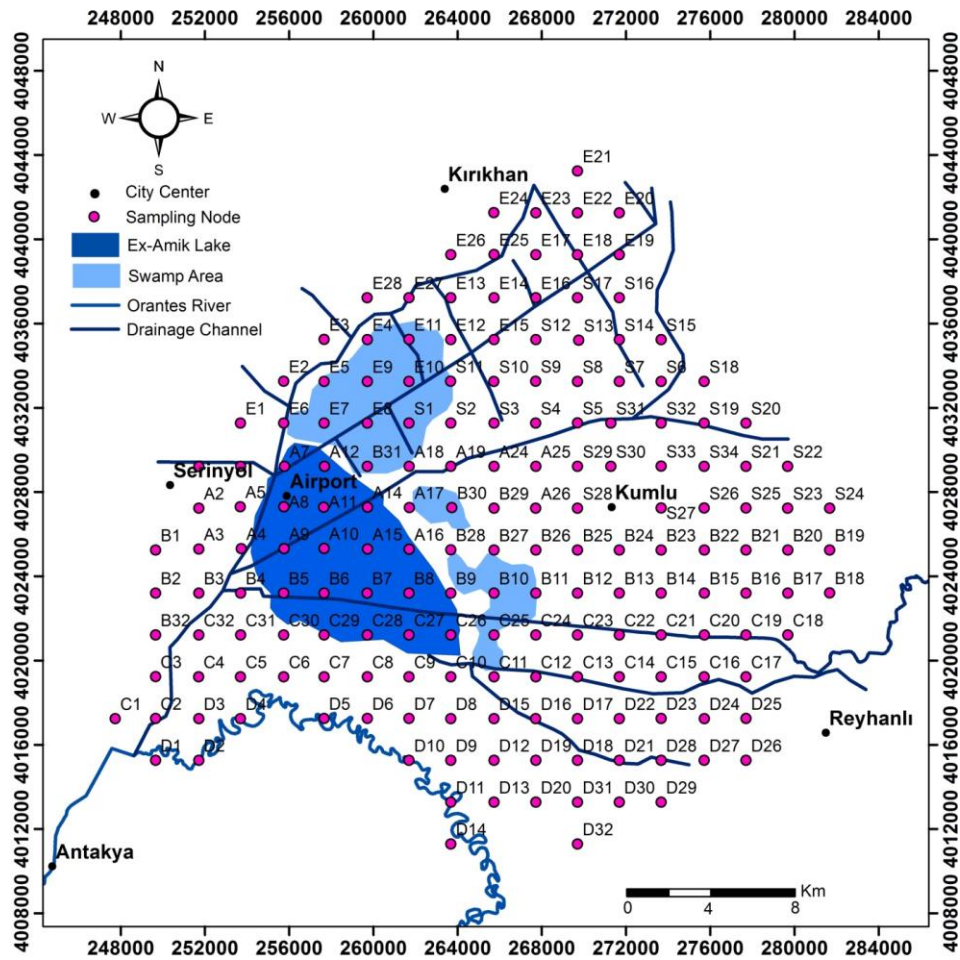
**Figure 1.** Sampling nodes with drainage-web, former Amik Lake and related swamp areas

**Table 1.** Descriptive statistics for measured soil properties and selenium fractions

| Parameters | Min. | Max. | Mean | Std. err. | Std. dev. | Variance | Skewness* | Kurtosis** |
|---|---|---|---|---|---|---|---|---|
| OM (%) | 0.10 | 4.035 | 1.509 | 0.062 | 0.824 | 0.679 | 0.610 | 0.138 |
| pH (1:2.5) | 7.02 | 8.930 | 7.935 | 0.020 | 0.274 | 0.075 | 0.187 | 0.843 |
| EC (dS cm$^{-1}$) | 78.00 | 1189 | 274.0 | 12.23 | 163.6 | 26774 | 2.154 | 6.641 |
| CCE (%) | 0.00 | 43.95 | 19.31 | 0.738 | 9.87 | 97.51 | -0.025 | -0.649 |
| Clay (%) | 0.00 | 58.85 | 13.56 | 0.912 | 12.20 | 148.9 | 1.263 | 1.009 |
| Sand (%) | 4.58 | 70.88 | 26.49 | 0.932 | 12.47 | 155.4 | 1.210 | 1.518 |
| Silt (%) | 15.58 | 88.76 | 59.95 | 1.164 | 15.57 | 242.3 | -0.653 | -0.168 |
| Se1 (µg kg$^{-1}$) | 6.43 | 177.3 | 62.68 | 2.370 | 31.70 | 1005 | 1.185 | 1.584 |
| Se2 (µg kg$^{-1}$) | 13.10 | 3591 | 246.8 | 21.32 | 285.3 | 81372 | 9.320 | 107.2 |
| Se3 (µg kg$^{-1}$) | 34.80 | 1226 | 189.4 | 12.39 | 165.7 | 27461 | 3.211 | 13.58 |
| ΣSe (µg kg$^{-1}$) | 150.5 | 3802 | 498.9 | 27.22 | 364.2 | 132670 | 5.134 | 39.47 |

*Standard error of skewness 0.182, ** Standard error of kurtosis 0.361 (N= 179), ΣSe is the total of Se1-3 fractions. CCE calcium carbonate equivalent.

The extraction of phosphate buffer, Se1, involves the determination of the combined presence of exchangeable and soluble ionic species of selenium, including $Se^{4+}$, $Se^{6+}$, and $Se^{2-}$. A soil sample weighing 1 gram was passed through a sieve with a mesh size of 150 μm. The resulting sieved soil sample was then subjected to an extraction process using 25 mL of a phosphate buffer solution with a concentration of 0.1 M and a pH value of 7. The extraction was carried out by shaking the mixture at a speed of 130 revolutions per minute at room temperature for a duration of 1 hour. The liquid phase was prepared for examination by centrifugation and filtering techniques. The sample was maintained at a temperature of +4℃ until it was subjected to analysis. In this work, the total concentration of distinct ion species of Se was used, despite the fact that they were analyzed individually.

Persulfate oxidation involving the proportion of Se2 that is either bound or adsorbed to organic matter was conducted as follows: Following a first stage whereby soil samples were subjected to the addition of 25 mL of a 0.1 M persulfate solution, the mixture was heated in a sand bath at a temperature of 90℃ for a duration of 2 hours. Subsequently, the liquid phase was separated and subjected to analysis using the same procedure as outlined in Step 1. The introduction of persulfate resulted in the oxidation of Se (II-) present in the medium to Se (VI). Next, a volume of 0.5-2 mL of the extract was selected and supplemented with 6 M HCl until the total volume reached 25 mL. The resulting mixture was then subjected to heating in a sand bath at a temperature of 90℃ for a duration of 30 minutes. This process aimed to facilitate the reduction of Se (VI) to Se (IV). The quantification of the $Se^{4+}$ ion species in the solution phase was conducted.

The process of oxidation of nitric acid on Se3, refers to the residual selenium fraction associated with the mineral structure. A volume of 2.5 mL of concentrated $HNO_3$ was introduced to the remaining samples obtained from step 2 and then subjected to heating using a sand bath.

The sample was subjected to a temperature of 90℃ for a duration of 30 minutes. Subsequently, it was allowed to cool down before adding 20 mL of distilled water. The resulting mixture was then placed in a sand bath and maintained at a temperature of 90℃ for a period of 1.5 hours. After routinely separating the liquid phase, the Se (0) concentration in the solution was measured by HRC-AAS. Ozkan et al. (2022) provided a comprehensive account of the analytical techniques involved in verifying the analysis using reference material (BCR-176 fly ash, the Sigma-Aldrich, Belgium) and optimizing High-Resolution Continuum Source Atomic Absorption Spectrometer, HRC-AAS (Analytic Jena ConrtAA 700). Table 1 displays the descriptive data according to the fractions and total Se parameters.

**Statistical analysis:** In the context of statistical analysis, the observation vector Y represents the observed characteristics. The symbol μ denotes the overall mean, while a represents the random additive genetic influence of the individual soil. Additionally, e represents the random error connected with the observation vector Y. Fixed effects were not included in the analysis due to the inherent difficulty in discerning and quantifying the precise impact of soil forming elements at individual sample locations, even if these components do vary. The estimation of variance components and genetic factors arising from soil formation was conducted using the constrained maximum likelihood method (Madsen and Jenson, 2010). Canonical correlations were computed using genetic correlations resulting from the soil formation processes. The study used canonical correlation analysis to elucidate and quantify the association between two qualities, using the SAS 9.0 PROC CANCORR software package (SAS Institute, 2002).

Canonical correlation analysis is based on the correlation of the linear combination between two sets of variables (*Xp*) (*Yq*) (Johnson and Wichern, 1986). In this case, linear combinations of variables' sets can be defined as follows:

$$U_i = a_{i1}X_1 + a_{i2}X_2 + \cdots + a_{ip}X_p$$
$$V_i = b_{i1}Y_1 + b_{i2}Y_2 + \cdots + b_{ip}Y_p$$

Where $a_{i2}$ and $b_{i2}$ are the canonical coefficients, $U_i$ and $V_i$ , *i* are the $i^{th}$ pair of canonical variables. $U_i$ and $V_i$ are the first pair of canonical variables associated with the first canonical correlation that is formed as follows:

$$r_i = \frac{C\hat{o}v(U_1, V_1)}{\sqrt{V\hat{a}r(U_1)V\hat{a}r(V_1)}}$$

The percentage of variance explained by the first canonical variables $U_{x_i}^2$ and $V_{w_i}^2$ was calculated as follow:

$$U_{x_i}^2 = \frac{\Sigma_{j=1}^p a_{ij}^2}{p} \text{ and } V_{w_i}^2 = \frac{\Sigma_{j=1}^q b_{ij}^2}{q}$$

*p* and *q* are the variable numbers of *X* and *Y*, respectively. The smallest value between p and q defines the number of pairs of canonical variables.

Two sets of features were established: The first included the descriptive soil properties, and the second consisted of Se fractions, including total Se.

## Results and Discussion

The examination of correlation coefficients within the soil characteristics dataset may provide insights into the collective impact of soil-forming variables and agricultural activities within the designated research region. The variables included in the analysis of soil

attributes show a strong negative association between silt content and clay and sand content, with correlation coefficients of -0.554 and -0.600, respectively. This relationship is derived from the meticulous measurement of textural fractions and the underlying processes of soil formation. The inverse correlation between EC and CCE suggests that the soil has developed from lime-based parent material or is mostly composed of secondary lime formations (Usta, 1995), with deposition playing a minor role. According to Usta (1995), the presence of clay is associated with a negative association, suggesting that the adsorption process effectively decreases the concentration of soluble salts when adequate drainage conditions are present. In contrast, it has been shown that soils containing a significant proportion of sand exhibit elevated electrical conductivity (EC) values when subjected to comparable environmental circumstances. This phenomenon might be attributed to the existence of a clay barrier located near the surface within the plain, particularly in areas of low elevation or former lake beds (Akis, 2015). The inverse correlation between lime and sand is characterized by the absence of lime buildup in areas with high sand content, often seen in the elevated regions of the research area. This lack of lime accumulation may be attributed to the limited water retention capacity of sandy soils, resulting in rapid drainage of incoming water from the soil profile. However, it is important to note that due to the bowl-shaped physiography of the plain, these particular places also serve as the primary locations for the deposition of coarse materials that have been eroded from higher elevations. The inclusion of the former lake bottom within the research area suggests that the clay fraction, which is transported by erosion, tends to collect more prominently in this particular region. This accumulation signifies the potential development of secondary carbonate formations over time at the bottom of the lake.

The presence of selenium (Se) in the Amik plain exhibits a significant and inverse correlation with the organic-bound (Se2) and residual-Se (Se3) fractions, while demonstrating a comparatively weaker association with the available-Se fraction, as shown in Table 2. These findings suggest that the distribution of selenium in the region may be influenced by the processes of solute and solid transport. This method is substantiated by the observed positive relationship between the sum of selenium (ΣSe) and sand content, which represents the indigenous fraction in water with moderate erosion, as well as the electrical conductivity (EC), which is indicative of solute transport as influenced by the physiography of the plain. Nevertheless, the proportion of accessible selenium (Se1) was dependent on the concentration of S3, which is likewise closely associated with the levels of S2 components. Hence, organic matter plays a pivotal part in this process. The aforementioned findings were substantiated by the statistically significant correlations

**Table 2**. Correlations within and between sets of variables for soil properties and selenium content

| Correlation coefficients for within soil properties set | | | | | |
|---|---|---|---|---|---|
| | OM | pH | EC | CCE | Clay | Sand |
| pH | -0.406[**] | | | | | |
| EC | -0.249[**] | 0.174[*] | | | | |
| CCE | -0.033 | 0.061 | -0.275[**] | | | |
| Clay | -0.004 | 0.150[*] | -0.144[*] | 0.149[*] | | |
| Sand | -0.058 | -0.033 | 0.358[**] | -0.387[**] | -0.211[**] | |
| Silt | 0.068 | -0.114 | -0.173[*] | 0.201[**] | -0.554[**] | -0.600[**] |

| Correlation coefficients for within selenium fractions set | | |
|---|---|---|
| | Se1 | Se2 | Se3 |
| Se2 | -0.086 | | |
| Se3 | 0.441[**] | 0.397[**] | |
| ΣSe | -0.299[**] | -0.818[**] | -0.811[**] |

| Correlation coefficients for between the sets | | | |
|---|---|---|---|
| | Se1 | Se2 | Se3 | ΣSe |
| OM | -0.116 | 0.299[**] | 0.139 | -0.238[**] |
| pH | 0.187[*] | -0.095 | -0.116 | 0.087 |
| EC | -0.117 | -0.225[**] | -0.266[**] | 0.305[**] |
| CCE | -0.051 | 0.121 | 0.172[*] | -0.189[*] |
| Clay | 0.026 | 0.149[*] | 0.137 | -0.164 |
| Sand | 0.008 | -0.214[**] | -0.218[**] | 0.269[**] |
| Silt | -0.059 | 0.083 | 0.082 | -0.110 |

OM organic matter, CCE calcium carbonate equivalent

observed between organic matter and S2 (r = 0.299, p < 0.01) as well as ΣSe (r = -0.238, p < 0.01). The pH (0.186*) most likely had an impact on the regulation of the Se1 fraction in the soils. According to Usta (1995), there is a positive correlation between the Se2 percentage and the presence of organic matter and clay content. This relationship is attributed to the ability of organic matter and clay to protect organic matter from degradation by generating organo-mineral complexes. In contrast, it has been shown that sand (-0.214**) and electrical conductivity (EC) (-0.225**) exhibit a negative correlation with the ability of organic matter (OM) content to grow. The observed strong association between Se3, sand, and EC underscores the importance of solute and solid transport mechanisms. The lithological composition of the studied region mostly consists of Quaternary alluvium and colluvium, as shown by Korkmaz et al. (2011). However, it is possible to interpret the positive correlation of CCE as the result of primary and/or secondary carbonate minerals playing a role in enhancing this percentage via adsorption processes. Another notable observation is that carbonated rocks have a greater Se concentration (0.8 mg/kg) compared to metamorphic and

sedimentary rocks (0.6 mg/kg) as well as igneous rocks (0.5 mg/kg) (Aubert and Pinta, 1977), providing an explanation for the correlation.

Table 3 presents the results of the canonical correlations, adjusted canonical correlations, standard errors, and squared canonical correlations computed between the variable sets of soil characteristics and Se fractions. The statistical results for evaluating the hypothesis of the canonical structure are also shown in Table 3. The first canonical correlation, which represents the correlation between the first pair of canonical variables, for soil characteristics and Se fractions sets is reported to be 0.466 ± 0.059 (Table 3). The first canonical correlation has statistical significance and is distinguishable from the next three canonical correlations. The observed significance of the first canonical correlation suggests a strong link between linear combinations of soil characteristics variables and linear combinations of Se fractions variables. In this particular instance, hypothesis H1 is deemed valid, hence refuting the null hypothesis H0 which posits that all canonical correlations derived from the study are equal to zero (Pr>F; 0.001). Nevertheless, this assertion holds true just for the first

**Table 3**. Canonical correlations and their significance test

| Canonical variables | Canonical correlations | Adjusted canonical correlations | St. error | Squared canonical correlations | Eigen values | Differences | Percentage (%) | Cumulative percentage (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.466 | 0.418 | 0.059 | 0.217 | 0.279 | 0.180 | 63.0 | 63.0 |
| 2 | 0.300 | 0.232 | 0.068 | 0.090 | 0.099 | 0.053 | 22.4 | 85.4 |
| 3 | 0.211 | 0.159 | 0.072 | 0.045 | 0.047 | 0.029 | 10.5 | 95.9 |
| 4 | 0.133 | 0.110 | 0.074 | 0.018 | 0.018 | | 4.1 | 100.0 |

| Canonical variables | Probability ratio | F values | DF | Pr > F | Wilks-Lamda value |
|---|---|---|---|---|---|
| 1 | 0.668 | 2.57 | 28 | <0.001 | <0.0001 |
| 2 | 0.854 | 1.53 | 18 | 0.076 | - |
| 3 | 0.939 | 1.10 | 10 | 0.365 | - |
| 4 | 0.982 | 0.77 | 4 | 0.544 | - |

canonical correlation that is substantiated by the Wilks-Lambda test statistic.

According to Keskin and Ozsoy (2004), when the variables in the data sets have different units of measurement and unequal variances, it is more appropriate to interpret standardised canonical correlation coefficients rather than canonical correlations in research. Standardized coefficients have significance due to their ability to demonstrate the magnitudes of the effects of the original variables within a given set on the occurrence of a canonical variable within the same set (Keskin and Ozsoy, 2004). Hence, Table 4 presents the standardized canonical

correlation coefficients pertaining to the first pair of canonical variables that has been shown to possess statistical significance.

The critical questions for Se in soils are related to i) soil properties can be responsible for the total Se, and ii) the availability of Se as a function of soil properties and Se fractions. Using the standardised canonical coefficients given in Table 4, the equations for the first canonical variable pair of soil properties (Soil-1) and Se fractions (Se1-1) can be calculated as follows:

Soil-1 = 0.438 OM- 0.352 pH – 0.063 EC + 0.349 CCE +

0.565 clay – 0.020 sand + 0.481 silt
Sel-1 =-0.733 Se1- 0.263 Se2 + 0.213 Se3 – 0.950 ΣSe

**Table 4.** Standardized coefficients of the first canonical variable

| Soil properties set | | Se fractions set | |
|---|---|---|---|
| | Soil-1 | | Sel-1 |
| OM | 0.438 | Se1 | -0.733 |
| pH | -0.352 | Se2 | -0.263 |
| EC | -0.063 | Se3 | 0.213 |
| CCE | 0.349 | ΣSe | -0.950 |
| Clay | 0.565 | | |
| Sand | -0.020 | | |
| Silt | 0.481 | | |

The loadings for the organic matter (OM), cation exchange capacity (CCE), clay, and silt variables are 0.438, 0.349, 0.565, and 0.481, respectively. These loadings exhibit a positive relationship in the formation of the Soil-1 canonical variable. The clay variable exhibits the greatest loading (0.565) among the given variables. In contrast, the variables of pH, EC, and sand exhibit negative and minor contributions, with corresponding coefficients of -0.352, -0.063, and -0.020, except for pH. The presence of selenium (Se) in a particular soil is influenced by several factors, including the parent material, adsorption, and desorption processes, precipitation, and dissolution processes, as well as solute and solid deposition processes. The susceptibility of these processes is influenced by various soil properties, including pH, redox potential (Fang and Zhao, 2018; Xing et al., 2015; Nakamaru et al., 2014), organic matter (Smazikova et al., 2019; Xing et al., 2015), soil texture and mineralogy (Antoniadis et al., 2017; Munier-Lamy et al., 2007), sesquioxide content (Araujo et al., 2020; Fan et al., 2019), and topographic parameters (Xu et al., 2018). The role of topography may be considered a significant factor influencing the occurrence of short and long-range transport phenomena. The presence of organic matter (OM), clay, silt, and sand are strongly influenced by topography under certain climatic circumstances at a localized level, as shown in this research. In a similar vein, Xu et al (2018) found that the concentration of selenium (Se) in soil is predominantly influenced by topography and the presence of iron oxide ($Fe_2O_3$). On the other hand, the availability of Se in soil is strongly correlated with factors such as pH, weathering processes, and eluviation. The drainage processes of the old Amik Lake likely had a significant effect on the transport phenomena that led to the buildup of substances before drainage and facilitated their leaching during the post-drainage era. The accumulation of organic debris, clay, silt, and secondary carbonate has been seen in the shallow ex-lake. In their study, Fan et al. (2019) documented the movement of selenium (Se) in soils as a result of humic acid and waterlogging. The waterlogging phase is associated with a fall in pH, as shown by Oren et al. (2018). In contrast, Lindsay (2001) found that a rise in pH characterizes the post-drainage period. This is supported by the negative impact of pH (-0.352) on the Soil-1 function. In the derivation of the canonical variable for the Se fraction (Sel-1), it was seen that the variables Se1, Se2, and ΣSe had negative contributions (-0.733, -0.263, and -0.950, respectively), but the variable B3 had a positive contribution (+0.213). The ΣSe variable has the greatest effect on the establishment of the canonical correlation of Se fractions. Hence, the rationale provided for the function of Soil-1 might similarly apply in this context. Furthermore, within the research region, there is a process of organic matter decomposition after drainage as well as intermittent occurrences of waterlogging, particularly in the lake-bed. These factors together contribute to the accelerated depletion of Se. In general, it can be said that leaching processes are active inside the research region.

Another correlation that is derived from the canonical correlation analysis is the correlation between the pairs of canonical variables and the original variables in both their own set and the counter set. Table 5 presents the multiple correlation coefficients between the initial canonical variables derived from both sets, as well as the original variables inside their respective sets and the counter set. The significance of these correlation coefficients is in their ability to quantify the amount of variation accounted for by the canonical variable in both the original variables within its own set and the complementary set. This is often referred to as the coefficient of determination ($R^2$). Among these correlations given in Table 5, the highest correlation (+0.617) between the first canonical variable (Soil-1) of the soil properties set and the original variables in its own set is between organic matter content. Conversely, the canonical variable (Sel-1) representing the initial fraction of selenium (Se) had the strongest connection (-0.795) with the aggregate sum of Se across all original variables. Furthermore, the other correlations that were identified exhibited statistical significance and were determined to be in close proximity to the aforementioned values. This observation indicates that the initial set of canonical variables, namely Soil-1 and Sel-1, effectively capture the variability within their respective variable sets. Nevertheless, the first pair of canonical variables exhibits a lesser degree of effectiveness compared to their respective variable sets in elucidating the variability inside the opposing variable set. The strongest association (r = +0.288) is seen between the first canonical variable of selenium fractionation (Sel-1) and the original variables representing soil characteristics, namely organic matter. Simultaneously, it is seen that 28.8% of the variance in the organic matter variable can be accounted for by the first canonical variable of Se fractions. Similarly, the Soil-1 canonical variable explains 37.1% and 35.0% of the variances in the ΣSe

**Table 5.** Correlations between canonical variables of soil properties and selenium fractions sets and the variables in these sets

| Variables | Soil-1 | Sel-1 | Variables | Sel-1 | Soil-1 |
|---|---|---|---|---|---|
| OM | 0.617 | 0.288 | Se1 | -0.301 | -0.141 |
| pH | -0.489 | -0.228 | Se2 | 0.749 | 0.350 |
| EC | -0.501 | -0.234 | Se3 | 0.642 | 0.300 |
| CCE | 0.518 | 0.242 | ΣSe | -0.795 | -0.371 |
| Clay | 0.310 | 0.145 | | | |
| Sand | -0.599 | -0.280 | | | |
| Silt | 0.331 | 0.154 | | | |

OM organic matter, CCE calcium carbonate equivalent

and Se2 variables, respectively. This suggests a strong correlation between Se's overall quantities and proportions and soil formation processes.

The redundancy analysis assesses the degree to which the standardized variances of the canonical variable pairs explain the variability in both their own set and the counter set. Table 6 presents the proportion of the overall standardized variation in the soil characteristics dataset that is accounted for by its respective canonical factors, as well as the proportion explained by the canonical variables of the Se fractions dataset. The first canonical variable in the soil characteristics set accounts for 24.3% of the overall variation. The explanatory percentages for the second and subsequent canonical variables were 11.8%, 17.5%, and 12.5%, respectively. The first canonical variable had the greatest coefficient of determination ($R^2$) at 21.8%. The cumulative proportions of the canonical variables were determined to be 66% in the end. The first canonical variable in the soil characteristics set accounted for 9.2% of the standardized variation in the

**Table 6**. Explained percentages of standardised variance in the set of soil properties and selenium fractions by canonical variables

| | Amounts of total variance in the set of soil properties explained by their canonical variables | | | Amounts of total variance in the set of selenium fractions by their canonical variables | |
|---|---|---|---|---|---|
| Canonical variable | Percentage | Cumulative percentage | Canonical $R^2$ | Percentage | Cumulative percentage |
| 1 | 24.3 | 24.3 | 0.218 | 9.2 | 9.2 |
| 2 | 11.8 | 36.1 | 0.090 | 2.9 | 12.1 |
| 3 | 17.5 | 53.4 | 0.045 | 0.6 | 12.7 |
| 4 | 12.5 | 66.0 | 0.018 | 0.2 | 12.9 |

Se fractions set. The aggregate proportion of four canonical variables within this dataset was found to be 12.9%.

## Conclusions

In recent decades, there has been a significant increase in the availability of research data, facilitated by the advancement and cost reduction of current technology. The use of multivariate analysis methodologies enables the extraction of novel and valuable insights while assessing the interconnections across extensive data sets. The present work effectively employs canonical correlation, a multivariate statistical method that provides a fresh viewpoint on elucidating the interrelationship between Se fractions and soil parameters. This research investigates the distribution of Se fractions in the soils of Amik Plain. The first canonical correlation demonstrates a substantial link between the soil characteristics and Se fraction data sets. The findings indicated that soil characteristics and

the transit of soluble and solid fractions of Se had a significant influence. The contributions of organic matter, clay, and silt characteristics to the fractionation of Se and its overall concentration were shown to be statistically significant among the various soil parameters. The data presented indicates that the first canonical variable has a significant capability for accurately predicting the occurrence of Se. In this regard, the soil parameters may be regarded as the independent variables used to elucidate the variability in the Se fractions, which serve as the dependent variables. The assessment of the associations between created data sets via the use of multivariate analytic techniques, such as canonical correlation analysis, yields novel and valuable insights. Nevertheless, it is important to highlight that the suitability of the canonical correlation technique for various ecosystems characterized by varying scales and plant nutrient compositions has to be empirically evaluated. Another factor to consider is the heterogeneity of the research region, which may provide limitations on the ability to

extract more information. Hence, it is essential to thoroughly evaluate the whole process in order to address the constraints associated with data screening methodologies.

## Authors contributions

**AÖ:** Contributed substantially to the conception and design of the study, soil sampling, the acquisition of data or the analysis and interpretation. Provided final approval of the version to publish. **ANÖ:** Statistical analysis, interpretation of statistical data, drafted the statistical part of the manuscript and approved the final version. **VU:** Contributed substantially to the conception and design of the study, drafted and approved the final version of the manuscript.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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SOIL STUDIES

# Heat pre-treatment as an initial step in vermicomposting significantly influences worm population and cocoon production

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**How to cite**

**Abstract**

Heat treatment of vermicompost and cow manure is mandated before their use as fertilizers to address the risk of pathogenicity in vermicompost derived from cow manure. While vermicomposting under mesophilic conditions does not significantly reduce microorganisms or degrade enzymes and proteins, organic matter passing through the earthworm's digestive system is effectively composted and sterilized. This study focused on cow manure and subjected it to three heat treatments: 25 °C (HT1), 70 °C for 1 hour (HT2), and 121 °C at 1.5 atm for 15 minutes (HT3). We assessed these treatments over five incubation periods (0, 7, 15, 30, and 45 days) on earthworm (*Eisenia fetida* L.) biomass, juvenile counts, and cocoon production. The results showed that all earthworms in HT3 died at the second incubation, while the HT1 and HT2 groups survived. Cocoon counts in HT1 and HT2 increased with each period ($P<0.01$). Remarkably, the number of cocoons in the control group exceeded that of the HT2 group throughout the study ($P<0.01$), highlighting the significant impact of heat treatment on vermicompost quality and earthworm productivity. Earthworm productivity was significantly affected by the carbon-to-nitrogen ratio (C/N), organic carbon (%), total nitrogen (%), $NH_4^+$-N, and $NO_3^-$-N. Elevated $NH_4^+$-N from heat treatments had a negative effect on earthworm activity.

## Introduction

Vermicompost is a product derived from composting crop residues and animal waste with the help of earthworms under aerobic and mesophilic conditions. This method offers an alternative to traditional composting techniques for recycling organic material. During vermicomposting, organic materials are rapidly humified, detoxified, and disinfected, aided by beneficial microorganisms and earthworms (Kızılkaya and Hepşen, 2014; Rakıcıoğlu and Kızılkaya, 2021). The epigeic earthworm species, *Eisenia foetida* L., is preferred for vermicomposting due to its outstanding traits, such as a high reproductive rate, adaptability, and salt tolerance (Dominguez et al., 2001; Edwards et al., 2011; Garg et al., 2005; Namlı et al., 2014). Vermicompost has a higher concentration of beneficial microbial populations, readily available plant nutrients, and plant growth regulators compared to conventional compost. It also effectively suppresses plant diseases without issues related to odor or proliferation.

Vermicompost is a finely divided, peat-like substance with a low C: N ratio, superior structure, porosity, aeration, drainage, and water-holding capacity (Demir, 2019; 2020; Yılmaz and Uğur, 2022). It provides a balanced mineral content, enhances the availability of plant nutrients, and can act as a complex nutrient granule (Tomati and Galli, 1995; Tomati et al., 1987; 1988; Türkay and Öztürk, 2023). These qualities endow vermicompost with significant potential economic value as a soil conditioner or plant nutrient source. However, there is some risk of pathogenicity in vermicomposting since it employs animal waste (typically cow manure) and operates under mesophilic conditions, unlike thermophilic composting. Consequently, vermicompost is categorized as an animal by-product. As such, a heat treatment at 70 °C for 1 hour is mandated for vermicompost, as outlined by Regulation (EC) No. 1774/2002 (Animal By-Products Regulation) in the European Union and the Regulation on Animal By-Products not Intended for Human Consumption (dated 24/12/2011 with reference number 28152) in Turkey. The efficiency of vermicomposting depends on the biomass of earthworms and the proportion of adults and juveniles (population structure) in the population (Rajapaksha et al., 2013). The main element of population structure is biological reproduction and biomass is related to growth rate, both of which depend on environmental conditions such as light, temperature, and humidity (Auerswald et al., 1996; Sarwar et al., 2006).

Studies have shown that vermicomposting inherently serves as a sanitizing process. As organic materials traverse the intestinal system of earthworms, pathogenic microorganisms are suppressed by the intestinal flora due to antagonistic mechanisms (Eastman et al., 2001). Consequently, the final product does not necessitate additional heat treatment. Such treatments can also eradicate beneficial microorganisms, which are among the most valuable features of vermicompost (Boran et al., 2017; Brown and Mitchell, 1981). One suggested approach involves heat-treating the primary material, which is animal organic waste, instead of the end product, vermicompost. This strategy remains under discussion. An alternative method that avoids heat treatment, while adhering to legal stipulations, might be worth investigating. The necessity of heat-treating primary material for earthworm consumption is debated, as it can be unnecessary and detrimental to vermicompost quality.

This study aimed to develop a different heat treatment technique in the production of vermicompost. Given that heat treatment of the pre-product already reduces the risk of pathogenicity of vermicompost, and considering the sanitization of the precursor as it traverses the worm's digestive system,

such treatment seems both redundant and harmful. Moreover, the heat treatment of both the vermicompost and the pre-product imposes additional energy costs for the producers. In this study, we investigated the effects of different heat treatments on the primary product (cow manure) and recorded the number of worm cocoons in each heat treatment. The chemical properties of organic wastes determine their suitability for vermicomposting. The evaluation of organic wastes is usually based on their initial physicochemical properties before the addition of earthworms, so the results should be evaluated by comparing them with the differences in the initial physicochemical properties of the organic wastes. (Zhou et. al., 2021).

The objective of this study was to evaluate the influence of the pre-treatments on the reproductive abilities of earthworms during the vermicomposting process across the four incubation periods. In particular, we assessed the impacts in terms of cocoon count, juvenile count, and overall earthworm biomass.

## Materials and Methods

We examined the effects of three different heat treatments (HT1, control 25 °C; HT2, 70 °C 1 h; HT3, 121 °C, 1.5 atm, 15 min) over four incubation periods (7, 15, 30, 45 days) on cocoon count, juvenile count, and the overall biomass of earthworms. To prepare the feed for the earthworms, the cow manure (the primary product) was sieved and pre-composted for a duration of 45 days. The cow manure used in the experiment was rinsed with water using a separator device. The study was structured as a randomized block design, executed with three replicates under laboratory conditions. We conducted an analysis of the cow manure to determine various chemical properties, including pH, EC, total N%, organic C%, organic matter content, mineral N, $NH_4^+$-N, $NO_3^-$-N, C/N ratio, and plant nutrient contents. At the beginning of the experiment, different heat treatments were administered to 1000 grams of pre-composted cow manure. Subsequently, 80 adult *Eisenia fetida* L. earthworms were introduced into each pot. Table 1 lists some biological characteristics of *Eisenia fetida*.

Throughout the vermicomposting period, the moisture content of the cow manure was maintained at approximately 60% of its maximum water-holding capacity through daily weighing of the pots. We counted the number of earthworms, juveniles, and cocoons during each incubation period, and collected vermicompost samples. At the experiment's conclusion, we analyzed the chemical properties of three distinct vermicomposts, each subjected to a unique pre-heat treatment. These properties included pH, EC, total N%, organic C%, organic matter content,

**Table 1.** Some characteristics of worms belonging to the species *Eisenia fetida* L.

| Attributes of *Eisenia fetida* | Specific Details |
|---|---|
| Color | Brown-red |
| Life Expectancy | 45-51 days |
| Temperature Limits of Media | 0-35 ˚C |
| Optimum Temperature Requirement | 25 ˚C |
| Humidity Limits of Media | 70-90% |
| Optimum Humidity Requirement | 80-85% |
| Time Until the Adolescent Phase | 21-28 day |
| Number of Cocoons Produced Per Day | 0.35-0.50 |
| Cocoon Sizes | 4.8 mm × 2.82 mm |
| Survival Rate of Fry Emerging from Cocoons | 20% |

mineral N, $NH_4^+$-N, $NO_3^-$-N, C/N ratio, and plant nutrient contents.

This study was conducted as an incubation experiment in the laboratories of Kırşehir Ahi Evran University's Faculty of Agriculture, following a randomized block experimental design. For the laboratory experiment, we used *E. fetida* worms, which are known for their high adaptability and resistance to conditions such as salinity and drought. They are among the species with the highest reproductive capacity. Notably, *E. fetida* is the only species used for vermicompost production in our country and is considered the most widely used earthworm species worldwide. The experiment consisted of four distinct incubation periods (0, 7, 15, 30, 45 days), with sampling and counting scheduled for each period, culminating at 45 days. The duration of the experiment was determined based on the reproductive cycles of *E. fetida*, the number of earthworms, the volume of the pots, and the quantity of vermicompost. We selected pots with a height of 30 cm to allow the worms to replicate their natural vertical movements in the burrows. Each pot was populated with a total of 80 adult *E. fetida* worms, weighing between 22-25 g collectively, and was provided with 1000 g of earthworm food (sourced from cow manure) based on its oven-dry weight for the 45-day period. Before the introduction to the pots, the worm food underwent various heat treatment processes in line with the study's objectives. The HT2 heat treatment was carried

out in an oven, while the HT3 sterilization occurred in an autoclave, typically used for sterilizing materials like soil. Table 2 gives an overview of the chemical composition of the cow manure utilized in the experiment.

The experiment was conducted in a randomized block design and involved 36 pots. This accounted for 4 different incubation periods, 3 distinct heat treatments, and 3 replications for each combination. After setting up the pots, the ambient moisture content was adjusted to 80% of the worm food's maximum water holding capacity using distilled water. Throughout the experiment, this moisture level was sustained by adding earthworms and offsetting the daily water deficit with pure water. Furthermore, the ambient temperature was kept constant under laboratory conditions.

At the end of each incubation period, each worm, juvenile, and cocoon in the pots was carefully counted and documented using laboratory tweezers. We also collected samples from the medium to assess their chemical properties. By the end of the experiment, we had examined the variations in earthworm biomass, juvenile count, cocoon count, and the chemical composition of the medium at different temperatures. To determine the chemical properties of the three types of cow manure (each of which was subjected to a unique heat treatment and utilized as primary materials for the experiment), we performed analyses for of pH, EC, total nitrogen, and organic matter

**Table 2.** Methods used to determine the nutrient content and chemical properties of cow manure (Kacar, 1972, 1995; Bayraklı, 1986; Ryan et al., 2001).

| Analysis | Methods |
|---|---|
| Organic matter | Dry burning (adding 1 mL of 5% H2SO4 dissolved in ethyl alcohol to each 1 g of material and burning at 550 °C in porcelain crucibles) |
| Total Nitrogen | Kjeldahl method |
| pH | 1:10 (w/v), pH-meter in soil: organic waste mixture |
| EC | 1:10 (w/v), EC-meter in soil: organic waste mixture |
| Total Phosphorus | The extract obtained by dry digestion was analyzed spectrophotometrically |
| Total Potassium | Flame photometry of the extract obtained by dry combustion |
| Total Ca, Mg, Zn, Cu, Fe, Mn | The extract obtained by dry digestion was analyzed by Atomic Absorption Spectrophotometer |

content, following the methods described by Kacar (1995).

**Data analysis**

In this study, we utilized Pearson correlation coefficients to ascertain the relationships between heat treatments, physicochemical properties, and the measured biological parameters. For each physicochemical property, the mean values of the three heat treatments (HT1, HT2, and HT3) were correlated with the mean values of the biological parameters (such as biomass increase %, cocoon count, and juvenile count) for each specific time interval (7th, 15th, 30th, and 45th day). The significance of these

correlations was assessed at two levels: $P<0.05$ and $P<0.01$. Statistical analyses were performed using SPSS version 15.

**Results and Discussion**

In this study, cow manure was used as food for the worms. To reduce the presence of pathogens, the manure was subjected to heat treatment before being provided to the earthworms. To evaluate the effect of heat treatment on the worms' essential activities at different temperatures, we implemented two specific temperature treatments. These were compared to a control group that did not undergo any heat treatment.

**Table 3.** Physicochemical properties of cow manure and heat-treated cow manure used in this trial (Kacar, 1972, 1995; Ryan et al., 2001).

| Physicochemical parameters | HT1 (Control, 25 °C) | HT2 (70 °C, 1 h) | HT3 (121 °C, 1.5 atm, 15 min) |
|---|---|---|---|
| pH | 7.78 | 7.36 | 6.92 |
| EC, dS/m | 3.95 | 4.25 | 4.1 |
| Organic matter, % | 39.25 | 38.98 | 40.04 |
| Organic Carbon (C), % | 22.76 | 22.61 | 23.22 |
| Total Nitrogen (N), % | 1.685 | 1.619 | 1.712 |
| Mineral N, % | 0.247 | 0.250 | 0.256 |
| $NH_4^+$-N, mg kg$^{-1}$ | 188.320 | 580.36 | 1141.29 |
| $NO_3^-$-N, mg kg$^{-1}$ | 2287.82 | 1595.64 | 1364.73 |
| Organic N, % | 1.686 | 1.624 | 1.618 |
| C/N | 13.507 | 13.965 | 13.567 |
| Total Phosphorus (P), % | 2.035 | 2.036 | 2.021 |
| Total Potassium (K), % | 3.432 | 3.487 | 3.447 |
| Total Calcium (Ca), % | 1.726 | 1.794 | 1.755 |
| Total Magnesium (Mg), % | 6.212 | 6.317 | 6.294 |
| Fe, mg kg$^{-1}$ | 41634.11 | 41867.96 | 41992.84 |
| Cu, mg kg$^{-1}$ | 322.69 | 323.17 | 323.11 |
| Zn, mg kg$^{-1}$ | 17721.58 | 17801.32 | 17814.57 |
| Mn, mg kg$^{-1}$ | 1723.80 | 1743.28 | 1749.46 |
| Cr, mg kg$^{-1}$ | 701,11 | 701,96 | 702,13 |

After applying the three heat treatments to the cow manure prepared for vermicomposting, we analyzed certain chemical properties of the manures, which are listed in Table 3.

During the experiment, the changes in the worm biota, which initially consisted of adult individuals, were determined according to the differences in heat treatment. In the pots subjected to the HT3 treatment, all earthworms died by the second incubation period (7th day) across all replicates. In contrast, in both the control and HT2 treatments, the earthworms survived

throughout all incubation periods. Although the number of earthworm cocoons increased in each incubation period in both control and HT2-treated pots, the control group consistently recorded higher cocoon counts than the HT2-treated group across all periods.

When the data on cocoon numbers were evaluated, as depicted in Figure 1, the cocoon numbers in the HT1, HT2, and HT3 heat treatments decreased, respectively, compared to the HT1-control group without heat treatment. Notably, there was no cocoon production in the HT3-sterilisation treatment. As the
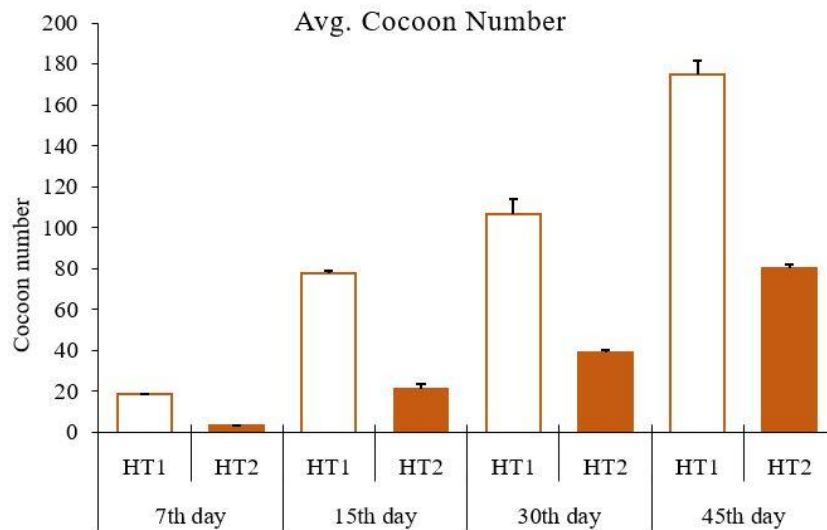
Figure 1. Variations in the average number of cocoons in pots during the incubation period. HT1: Control, 25 °C; HT2: 70 °C 1h treatment. No cocoons were found in pots for HT3 (121 °C for 15 min at 1.5 atm), and thus they are not shown in the diagram.

Upon analyzing the data on earthworm biomass from the experiment, as depicted in Figure 2, it was observed that the earthworm biomass decreased in the HT1, HT2, and HT3-sterilisation treatments compared to the HT1-control group without heat treatment. In the HT3-sterilisation treatment, all worms perished, leaving no biomass behind. With respect to the increasing incubation periods, there was no significant growth in biomass during the first three incubation periods. However, a significant increase in earthworm biomass was observed during the last incubation period. During the 4th incubation period, the juveniles emerging from the cocoons were also accounted for in the biomass. While the number of adults remained unchanged compared to the beginning of the experiment, the weight of the adults increased noticeably.
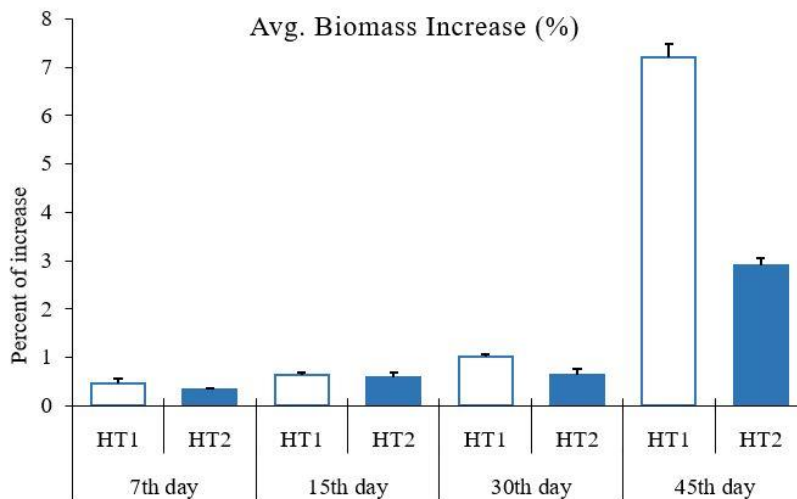


Figure 2. Variations in the average biomass increase in pots during the incubation period. HT1: Control, 25 °C; HT2: 70 °C 1h treatment. No biomass increase was observed in pots for HT3 (121 °C for 15 min at 1.5 atm), and thus they are not shown in the diagram.

After analyzing the data on the number of juveniles from the experiment, as depicted in Figure 3, it was observed that the number of juveniles in the HT1, HT2, and HT3 heat treatments decreased, respectively, compared to the HT1-control group without heat treatment. There were no juveniles in the HT3-sterilisation due to the death of all worms. As the incubation period progressed, there was no significant increase in juvenile numbers in the first three periods. However, in the last incubation period, a significant increase was observed. During the 4th incubation period, the surge in juvenile numbers corresponded to the growth of earthworm biomass. During the initial incubation period, there were no juveniles because the cocoons had not yet completed their maturity cycle.
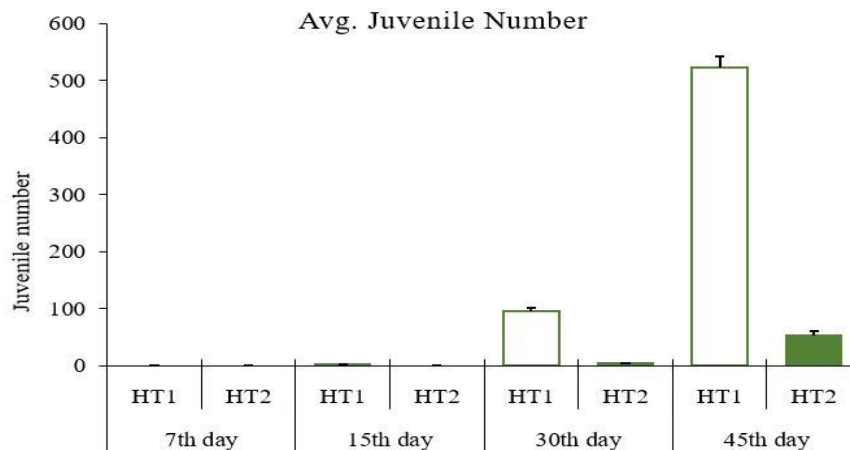


Figure 3. Variations in the average number of juveniles in pots during the incubation period. HT1: Control, 25 °C; HT2: 70 °C 1h treatment. No juveniles were observed in pots for HT3 (121 °C for 15 min at 1.5 atm), and thus they are not shown in the diagram.

incubation periods increased, and cocoon numbers in all treatments rose compared to the initial incubation period.

Both the HT2 and HT3 heat treatments resulted in a decrease in earthworm biomass and cocoon numbers compared to the control group. Especially in the HT3 treatment, the earthworms did not survive (Figure 1). An analysis of the chemical content of the treated materials showed variations in pH, EC, and $NH_4^+$-N levels, as listed in Table 1. In treatments with higher temperatures than the control, these crucial limiting factors increased to potentially hazardous levels. The HT2 treatment was carried out in an oven with dry air conditions, whereas the HT3 treatment was performed in an autoclave under sterilization conditions.

The mortality of the worms in our study was associated with the ammonia released during the heat treatment of the preproduct under the sterilization condition (HT3). Earthworms are very sensitive to ammonia and have difficulty surviving in organic waste with a high content of this cation, such as fresh poultry litter. They also cannot endure in wastes rich in inorganic salts. Both ammonia and inorganic salts have specific thresholds that delineate toxic from non-toxic levels (<1 mg $g^{-1}$ for ammonia and >0.5% for salts), as highlighted by Edwards (1988). The optimal breeding conditions for *E. foetida* and *E. andrei* closely resemble those favorable for other species (Edwards, 1988). In addition to environmental factors, population density

also plays a role in influencing the growth and reproduction rate of earthworms. Even if the physical and chemical conditions are ideal for vermicomposting, overcrowding of worms can still present problems. The findings of Reinecke and Viljoen (1990) and Domínguez (1997) indicate that when *E. foetida* was fed cow manure and *E. andrei* was given pig manure, earthworms in densely populated containers exhibited slower growth and reached a reduced final body weight. Nonetheless, the overall weight of earthworm biomass produced per unit of waste was greater when both species were cultivated at different population densities. The maturation rate, as indicated by the development of the clitellum, also fluctuated based on population density, resulting in earthworms of the same age reaching maturity at different intervals.

Statistical analysis indicated that earthworm biomass was primarily influenced by the C/N ratio, Organic C, Total N, $NH_4^+$-N, and $NO_3^-$-N of cow manure subjected to various heat treatments. The total biomass of earthworms, cocoon numbers, and juvenile counts were correlated with the C/N ratio, OC, and TN (Table 4). The biological parameters of earthworms were strongly influenced by the C/N ratio, OC, TN, $NH_4^+$-N, and $NO_3^-$-N. Increases in $NH_4^+$-N concentrations resulting from heat treatments were negatively associated with earthworm biological activity (Table 4).

According to a study by Zhou et al. (2021),

**Table 4.** Correlation coefficients between physicochemical properties and biological parameters following heat treatments. Values without asterisks are not statistically significant. '*' indicates *P<0.05*, and '**' indicates *P<0.01*.

| Physicochemical Parameters | Biomass increase | Cocoon number | Juvenile number |
|---|---|---|---|
| pH | -0.874 | -0.837 | -0.895 |
| EC, dS/m | 0.512* | 0.487 | 0.503* |
| Organic matter, % | 0.634** | 0.621** | 0.642** |
| Organic Carbon (OC), % | 0.598** | 0.587** | 0.605** |
| Total Nitrogen (TN), % | 0.512* | 0.503* | 0.517* |
| Mineral N, % | -0.312 | -0.298 | -0.307 |
| $NH_4^+$-N, mg kg$^{-1}$ | -0.874** | -0.865** | -0.881** |
| $NO_3^-$-N, mg kg$^{-1}$ | 0.742** | 0.731** | 0.748** |
| Organic N, % | 0.513* | 0.504* | 0.518* |
| C/N | -0.612** | -0.601** | -0.618** |
| Total Phosphorus (P), % | 0.287 | 0.276 | 0.293 |
| Total Potassium (K), % | 0.315 | 0.304 | 0.322 |
| Total Calcium (Ca), % | -0.287 | -0.276 | -0.293 |
| Total Magnesium (Mg), % | 0.298 | 0.289 | 0.305 |
| Fe, mg kg$^{-1}$ | 0.276 | 0.268 | 0.282 |
| Cu, mg kg$^{-1}$ | -0.265 | -0.257 | -0.270 |
| Zn, mg kg$^{-1}$ | 0.257 | 0.249 | 0.262 |
| Mn, mg kg$^{-1}$ | -0.248 | -0.240 | -0.253 |
| Cr, mg kg$^{-1}$ | 0.240 | 0.233 | 0.245 |

increases in $NH_4^+$-N and $NO_3^-$-N concentrations, coupled with decreases in total organic carbon content, adversely impacted earthworm growth and reproduction. The chemical composition of organic residues, particularly the C/N ratio, plays a pivotal role in determining earthworm abundance and population dynamics (Lavelle et al., 2006). Organic residues that possess a C/N ratio ranging between 20 and 30 are deemed optimal for earthworm development (Fusilero et al., 2013). This is attributed to the fact that the activity and community structure of decomposer microbes are influenced by the C/N ratio of the organic residues (Sarathchandra et al., 2006; Pang et al., 2009). In addition to the C/N ratio, earthworms are also sensitive to the pH and salinity of organic residues, parameters that can be determined by measuring the electrical conductivity.

Alterations in the physicochemical properties of organic residues during the vermicomposting process influence both earthworm biomass and population structure. Nitrogen concentration has an impact on earthworm reproduction, with the C/N ratio, $NH_4^+$-N, and $NO_3^-$-N emerging as predominant factors negatively affecting the adult population. Research indicates that the number of offspring depends largely on total organic C, whereas the number of cocoons is influenced by total N, the C/N ratio, and $NH_4^+$-N (Zhou et al., 2021). Consequently, lower N concentrations combined with a favorable C/N ratio are viewed as conducive for earthworm growth and reproduction. In

contrast, elevated N concentrations (TN, $NH_4^+$-N, and $NO_3^-$-N) lead to a decline in earthworm abundance and biomass.

It was noted that the heat treatment of the initial product could be an alternative to treating the final product. However, this preproduct heat treatment had a detrimental effect on the reproductive and survival capacities of the worms compared to the control group. The application of heat treatment to products that are inherently at a low lisk of pathogenicity and therefore do not require such treatment has also been found to be counterproductive. While heat-treating the pre-product hampers worm reproduction and survival during vermicomposting, treating the end-product can undermine its beneficial attributes.

## Conclusion

To mitigate the risk of pathogenic contamination in vermicompost, we proposed and implemented heat treatment of the initial product, using vermicompost prepared from cow manure, as an alternative to treating the end product. The results of the study showed that all heat treatment methods, applied to the initial material at different temperatures, had a significant negative impact on the cocoon counts of earthworms, thereby affecting their reproductive capacity and rate. Among the three heat treatments, including the control group, cocoon numbers

diminished with increasing treatment temperatures. The control group registered the highest cocoon count, while the HT3 sterilization condition, being the most intense treatment, yielded the lowest. Notably, all worms that consumed the initial product treated under the HT3 sterilization conditions did not survive. The chemical analysis for the HT1 treatment showed a smaller increase in ammonia compared to HT3. The decline in biological parameters could be linked to the HT1 treatment carried out at 70 °C in an oven, which likely diminished the activity of microorganisms, crucial nutrients for the earthworms. Earthworms are sensitive to particular environmental conditions in waste, especially substances such as ammonia, salts, and other chemicals. However, these can be effectively removed or reduced through thermophilic pre-composting processes. While heat-treating the product has its drawbacks, applying this treatment to the initial product also presents several adverse effects. These include a reduction in beneficial microorganisms, degradation of proteins and enzymes, and hindrance to the biology and reproduction of earthworms. Nevertheless, the pathogen load in the initial product naturally decreases during the vermicomposting process. This makes vermicomposting a suitable method to counter the negative impacts of heat treatment arising from concerns about pathogenicity. Additionally, heat-treating the initial product affects the essential activities of earthworms by rapidly converting nitrogen to ammonia in the organic material. Higher temperatures can lead to an increased release of ammonia, which can lead to the death of earthworms. It is understood that pathogenic microorganisms in worm food, which probably originate from the digestive systems of warm-blooded animals, are neutralized by the microorganisms in the digestive tract of the worms. Therefore, based on the findings of this study, we conclude that heat-treating the initial product, which has a negative effect on earthworm viability and reproduction, is both unwarranted and impractical. Instead, we recommend regular sampling and pathogen load tests to keep track of any unexpected pathogen presence in the final vermicompost.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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SOIL STUDIES

**R E V İ E W   P A P E R**

# The dynamic interplay of root exudates and rhizosphere microbiome

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**Abstract**

The rhizosphere microbiome plays a vital role in plant growth, health, and nutrient acquisition. One of the key factors that shape the composition and function of the rhizosphere microbiome is root exudates, the complex mixture of organic compounds released by plant roots. Root exudates serve as a source of energy and nutrients for the rhizosphere microbiome, as well as a means of communication between plants and microbes. The dynamic interplay between root exudates and rhizosphere microbiome is a complex and highly regulated process that involves multiple feedback loops and interactions. Recent studies have revealed that the composition and quantity of root exudates are modulated by a range of biotic and abiotic factors, including plant genotype, soil type, nutrient availability, and microbial community structure. In turn, the rhizosphere microbiome can influence the production and composition of root exudates, through processes such as nutrient cycling, plant hormone synthesis, and modulation of plant defense responses. Understanding the dynamics of root exudates and rhizosphere microbiomes is crucial for developing effective strategies for microbiome engineering, plant-microbe symbiosis, and sustainable agriculture. This review provides an overview of the current state of knowledge on the dynamic interplay between root exudates and rhizosphere microbiomes, highlighting the key factors and mechanisms that govern this complex relationship.

## Introduction

The rhizosphere microbiome is a key determinant of plant growth, health, and nutrient acquisition. The rhizosphere, which is the soil surrounding plant roots, harbors a diverse community of microorganisms that interact with plant roots and influence their growth and development (Garcia and Kao-Kniffin, 2018). These microorganisms include bacteria, fungi, and other microbes that perform a range of functions, such as nutrient cycling, disease suppression, and symbiotic interactions. One of the key functions of the rhizosphere microbiome is to facilitate nutrient uptake by plants. Microorganisms in the rhizosphere can

solubilize nutrients in the soil, such as phosphorus and nitrogen, making them more available to plants. In addition, some microorganisms can fix atmospheric nitrogen, providing a source of nitrogen for plant growth.

The rhizosphere microbiome also plays a key role in plant health and disease resistance. Some microorganisms in the rhizosphere can produce antibiotics and other compounds that protect plants from pathogens and pests. In addition, some microorganisms can induce systemic resistance in plants, priming them to better defend against future

pathogen attacks. The rhizosphere microbiome is a critical component of plant-microbe interactions and plays a key role in plant growth, health, and nutrient acquisition. Understanding the dynamics of the rhizosphere microbiome and its interactions with plants is important for developing sustainable agricultural practices and improving plant health and productivity (Kumawat et al., 2022).

The rhizosphere microbiome also plays a key role in soil health and ecosystem functioning. Microorganisms in the rhizosphere are involved in processes such as nutrient cycling, organic matter decomposition, and carbon sequestration, which are critical for maintaining soil fertility and productivity. Some microbes in the rhizosphere can also detoxify soil pollutants and contaminants, making them valuable for environmental remediation (Khan, 2005). In addition, the composition and diversity of the rhizosphere microbiome can be influenced by a range of factors, including soil type, plant genotype, and environmental conditions. By understanding these factors, we can develop strategies for manipulating the rhizosphere microbiome to promote desirable plant-microbe interactions and improve soil health. The rhizosphere microbiome represents a fascinating and important area of research with significant implications for agriculture, soil science, and environmental management (Mendes et al., 2013).

Root exudates are compounds released by plant roots into the soil, which can have a significant impact on the composition and function of the rhizosphere microbiome. These compounds can provide a source of energy and nutrients for microorganisms in the rhizosphere, shaping their composition and diversity. In addition, root exudates can also influence the behavior and function of microorganisms in the rhizosphere, such as by modulating gene expression or inducing chemotaxis. Root exudates are highly diverse and can include compounds such as sugars, amino acids, organic acids, and secondary metabolites (Carvalhais et al., 2011). The composition of root exudates can vary depending on a range of factors, such as plant genotype, soil type, and nutrient availability. As such, the composition and quantity of root exudates can have a profound impact on the rhizosphere microbiome and the ecosystem processes it supports.

Furthermore, the dynamic interplay between root exudates and the rhizosphere microbiome is a complex and dynamic process. Microorganisms in the rhizosphere can modulate the production and composition of root exudates by influencing plant gene expression or by altering the soil environment. In turn, changes in root exudates can feedback on the composition and function of the rhizosphere microbiome, influencing ecosystem processes such as nutrient cycling and carbon sequestration. Root exudates play a key role in shaping the composition

and function of the rhizosphere microbiome. Understanding the dynamics of this relationship is essential for developing strategies to manipulate plant-microbe interactions for sustainable agriculture and environmental management (Choudhary et al., 2016).

In addition to their role in shaping the rhizosphere microbiome, root exudates can also play a crucial role in plant growth and development. Some root exudates can act as growth promoters, stimulating root elongation and branching, while others can act as signaling molecules, mediating plant-microbe interactions or inducing systemic resistance (Narula et al., 2012). Furthermore, the production and composition of root exudates can be influenced by a range of environmental factors, such as drought, nutrient availability, and soil pH. For example, plants grown under drought stress may produce root exudates that promote water uptake and drought tolerance, while plants grown in nitrogen-limited soils may produce exudates that enhance nitrogen acquisition.

The ability to manipulate root exudates represents an exciting area of research with significant implications for sustainable agriculture and environmental management. By developing strategies to enhance the production of beneficial root exudates or to target specific microorganisms in the rhizosphere, we can improve plant growth and health, enhance nutrient acquisition, and promote ecosystem functioning. The dynamic interplay between root exudates and the rhizosphere microbiome represents a fascinating area of research with broad implications for plant-microbe interactions, soil science, and environmental management.

**The Composition and Function of Root Exudates**

Root exudates are a complex mixture of organic compounds released by plant roots into the soil. These compounds are secreted by specialized cells in the root and can be actively transported across the plasma membrane and into the rhizosphere. Root exudates can include a wide range of compounds, such as sugars, amino acids, organic acids, enzymes, and secondary metabolites. The production and composition of root exudates can vary depending on a range of factors, such as plant species and genotype, soil type, nutrient availability, and biotic and abiotic stress (Badri and Vivanco, 2009). For example, some plant species may produce specific exudates that are toxic to certain soil pathogens or that attract beneficial microbes, while others may produce exudates that enhance nutrient acquisition or water uptake.

Root exudates are also highly dynamic and can change over time in response to changing environmental conditions or interactions with microbes in the rhizosphere. Microbes in the rhizosphere can

influence the production and composition of root exudates through a range of mechanisms, such as by inducing changes in plant gene expression or by modulating the soil environment (Vives-Peris et al., 2020). Root exudates are a complex and dynamic mixture of compounds that play a crucial role in shaping the rhizosphere microbiome and mediating plant-microbe interactions. Understanding the production and function of root exudates is essential for developing strategies to promote sustainable agriculture and environmental management.

Root exudates can serve a wide range of functions in the rhizosphere, such as providing a source of energy and nutrients for microbes, mediating plant-microbe interactions, and modulating soil chemistry and structure. For example, some root exudates can promote the growth and activity of beneficial microbes in the rhizosphere, such as nitrogen-fixing bacteria or mycorrhizal fungi, which can enhance nutrient availability and plant growth (Igiehon and Babalola, 2018). Other exudates can act as signaling molecules, mediating interactions between plants and pathogens or promoting systemic resistance to stress. Root exudates can also influence the physicochemical

properties of the soil, such as by altering soil pH or promoting the formation of soil aggregates, which can impact nutrient cycling, water availability, and soil structure. Some root exudates may also have allelopathic effects, inhibiting the growth of competing plant species or serving as chemical cues for plant defense.

The composition and quantity of root exudates can be influenced by a range of factors, such as plant genetics, nutrient availability, and environmental stressors. Furthermore, the production and function of root exudates can be influenced by the diversity and activity of microorganisms in the rhizosphere. For example, certain microbes can induce changes in root exudate production or composition by modulating plant gene expression or by competing for resources in the rhizosphere (Berendsen et al., 2012). The dynamic interplay between root exudates and the rhizosphere microbiome is a complex and multifaceted process that plays a crucial role in plant-microbe interactions, soil ecology, and ecosystem functioning. Understanding the production and function of root exudates is essential for developing sustainable agricultural practices, managing soil health, and promoting ecosystem

**Table 1.** Types of compounds found in root exudates.

| Type of Compound | Examples | Function | Reference |
|---|---|---|---|
| Sugars | Glucose, fructose, sucrose | Source of energy for microbes, mediate plant-microbe interactions | Franzino et al., 2022 |
| Amino Acids | Glutamate, aspartate, proline | Source of nitrogen for microbes, mediate plant-microbe interactions | Carvalhais et al., 2013 |
| Organic Acids | Citrate, malate, oxalate | Mobilize nutrients in soil, modulate soil pH | Lopez-Bucio et al., 2000 |
| Enzymes | Phosphatases, cellulases, proteases | Break down complex organic compounds in soil, release nutrients | Pritsch and Garbaye, 2011 |
| Secondary Metabolites | Phenolics, flavonoids, terpenes | Defense against pathogens, allelopathy, signaling molecules | Wang et al., 2018 |
| Hormones | Auxins, cytokinins, gibberellins | Regulation of plant growth and development, mediate plant-microbe interactions | Giron et al., 2013 |

resilience.

Root exudates play a crucial role in providing energy and nutrients for the rhizosphere microbiome. The exudates released by plant roots contain a diverse range of compounds, including sugars, amino acids, and organic acids, which serve as a primary source of energy and nutrients for microbes in the rhizosphere (Table 1). Microbes in the rhizosphere can use root exudates as a source of carbon and energy for their growth and metabolism. For example, some bacteria can metabolize sugars such as glucose or fructose, while others can break down amino acids or organic acids such as citrate or malate (Zaunmüller et al., 2006). By utilizing root exudates, microbes in the rhizosphere can increase their population size and activity, which can have a range of positive effects on plant health and growth.

In addition to providing a source of energy, root

exudates can also serve as a source of nutrients such as nitrogen and phosphorus. Some microbes in the rhizosphere, such as nitrogen-fixing bacteria or phosphate-solubilizing bacteria, can utilize root exudates to access these nutrients, which can enhance nutrient availability for plant growth. Furthermore, the release of enzymes by microbes in the rhizosphere can break down complex organic compounds in soil, releasing nutrients such as nitrogen and phosphorus that can be taken up by plant roots. Root exudates play a critical role in shaping the composition and function of the rhizosphere microbiome by providing energy and nutrients for microbial growth and metabolism (Yue et al., 2023). Understanding the production and function of root exudates is essential for developing strategies to promote sustainable agriculture and soil health.

Root exudates also play a key role in promoting plant-microbe interactions in the rhizosphere. The

release of specific compounds in root exudates can attract beneficial microbes to the rhizosphere, such as those that can promote plant growth or provide protection against pathogens. For example, some plants can release flavonoids in their root exudates, which can attract rhizobial bacteria that can form a symbiotic relationship with the plant, resulting in the formation of nitrogen-fixing nodules on the roots (Narula et al., 2012; Mahmud et al., 2020). Other plants can release terpenes in their root exudates, which can attract beneficial microbes that can provide protection against pathogens.

Furthermore, the production of root exudates can be regulated by plant hormones, such as auxins, cytokinins, and gibberellins, which can influence plant growth and development (Kurepin et al., 2014). These hormones can also mediate plant-microbe interactions in the rhizosphere by regulating the production of root exudates and their effects on microbial communities. Root exudates are essential for the functioning of the rhizosphere microbiome and play a vital role in plant growth, health, and nutrient acquisition. By providing a source of energy and nutrients, promoting plant-microbe interactions, and regulating microbial communities, root exudates contribute to sustainable agriculture and soil health.

## The Composition and Function of Rhizosphere Microbiome

The rhizosphere microbiome is the complex community of microorganisms that live in the zone of soil surrounding plant roots, known as the rhizosphere. The rhizosphere microbiome is a dynamic and diverse community, consisting of bacteria, fungi, archaea, viruses, and other microorganisms. The characteristics of the rhizosphere microbiome are influenced by a range of factors, including plant species, soil type,

environmental conditions, and microbial interactions. The microbial community in the rhizosphere is distinct from that in bulk soil, as the release of root exudates and other organic compounds from plant roots creates a unique microenvironment that supports the growth and activity of certain microbial taxa (Hartmann et al., 2009).

The rhizosphere microbiome can have a range of positive effects on plant growth and health. For example, some microbes in the rhizosphere can solubilize nutrients such as phosphorus, making them more available for plant uptake. Other microbes can produce plant growth-promoting hormones or stimulate plant defense mechanisms against pathogens (Van Loon, 2007; Glick, 2012). The composition and function of the rhizosphere microbiome can be influenced by management practices such as tillage, fertilization, and crop rotation. Understanding the dynamics of the rhizosphere microbiome is essential for developing strategies to promote sustainable agriculture and soil health.

The rhizosphere microbiome is a complex and dynamic community, with microbial populations that can change over time in response to various biotic and abiotic factors. Microbial interactions within the rhizosphere can be competitive, cooperative, or neutral, and can impact the composition and activity of the microbiome (Hassani et al., 2018). The rhizosphere microbiome is also influenced by the root exudates released by plants. Root exudates can provide a source of energy and nutrients for microbial growth and activity and can also influence the composition and function of the rhizosphere microbiome.

Recent research has shown that the rhizosphere microbiome can have significant effects on plant growth, health, and productivity. For example, certain microbes in the rhizosphere have been shown to promote plant growth by producing hormones or

**Table 2.** Types of microbes found in rhizosphere microbiome.

| Microbe Type | Examples | Function in Rhizosphere | Mode of Interaction | Reference |
|---|---|---|---|---|
| Bacteria | Pseudomonas, Rhizobium, Bacillus | Nutrient cycling, plant growth promotion, pathogen suppression | Competitive, cooperative, neutral | Hayat et al., 2010 |
| Fungi | Mycorrhizae, Trichoderma, Fusarium | Nutrient cycling, plant growth promotion, pathogen suppression | Mutualistic, parasitic, saprophytic | Azcón-Aguilar and Barea, 1992 |
| Archaea | Methanogens, halophiles, thermophiles | Nitrogen cycling, methane production | Cooperative, neutral | Naitam and Kaushik, 2021 |
| Viruses | Bacteriophages | Regulation of microbial populations, horizontal gene transfer | Parasitic, neutral | Haudiquet et al., 2022 |
| Protozoa | Ciliates, amoebae | Predation on bacteria and fungi, nutrient cycling | Predatory, neutral | Alphei et al., 1996 |
| Nematodes | Root-knot nematodes, predatory nematodes | Pathogenic or beneficial effects on plants and microbes | Parasitic, predatory | Tapia-Vázquez et al., 2022 |

facilitating nutrient uptake, while others can protect plants from pathogens or other stresses (Hayat et al., 2010). The study of the rhizosphere microbiome is an important area of research, with significant implications for agriculture and soil health. By understanding the interactions between plants and their associated microbiomes, researchers can develop strategies to promote sustainable agriculture and soil management practices that optimize plant-microbe interactions for enhanced plant growth, health, and productivity.

The rhizosphere microbiome plays a critical role in plant-microbe interactions and soil health (Table 2). One of the primary functions of the rhizosphere microbiome is to facilitate nutrient cycling and uptake by the plant. Microbes in the rhizosphere can solubilize and mineralize nutrients, such as nitrogen and phosphorus, making them available for plant uptake. Additionally, certain microbes can produce plant growth-promoting hormones, such as auxins and cytokinins, which can stimulate root growth and enhance plant growth. The rhizosphere microbiome also plays an important role in plant defense against pathogens. Some microbes in the rhizosphere have been shown to produce antimicrobial compounds that can inhibit the growth of plant pathogens, while others can induce systemic resistance in the plant (Beneduzi et al., 2012). This can lead to increased plant resistance to a variety of biotic and abiotic stressors.

In addition to their role in plant-microbe interactions, the rhizosphere microbiome can also have a significant impact on soil health. Microbes in the rhizosphere can secrete extracellular enzymes that break down organic matter, releasing nutrients into the soil and improving soil structure (Frey, 2019). This can lead to increased soil fertility and water-holding capacity, as well as reduced erosion and nutrient leaching. The rhizosphere microbiome is a complex and dynamic ecosystem that plays a vital role in plant health, soil health, and ecosystem function. Understanding the interactions between plants and their associated microbiomes can lead to more sustainable and efficient agricultural practices, as well as improved soil and plant health.

**The Dynamic Interplay between Root Exudates and Rhizosphere Microbiome**

The production and composition of root exudates are influenced by a variety of factors, including plant genotype, soil type, and nutrient availability (Singh and Mukerji, 2006; Rengel and Marschner, 2005). These factors can impact the quantity and quality of exudates produced by the plant, as well as the types and abundance of microbes in the rhizosphere. Plant genotype plays a significant role in determining the composition of root exudates. Different plant species and cultivars produce unique profiles of exudates, which can influence the composition and function of the rhizosphere microbiome (Tiziani et al., 2022). For example, some plant species produce exudates that are rich in organic acids and sugars, while others produce exudates that are rich in amino acids and proteins. Soil type can also influence the production and composition of root exudates. Soil properties such as texture, pH, and nutrient availability can impact the types and abundance of microbes in the rhizosphere, which in turn can influence the types of exudates produced by the plant. For example, plants grown in nutrient-poor soils may produce exudates that are rich in organic acids and sugars, which can help to solubilize and mobilize nutrients in the soil (Ström, 1997).

Nutrient availability is another important factor that can influence the production and composition of root exudates. Plants grown under nutrient-rich conditions may produce fewer exudates overall, but those exudates may be of higher quality and contain a greater diversity of compounds (Oburger et al., 2018; Badri and Vivanco, 2009). Conversely, plants grown under nutrient-poor conditions may produce more exudates overall, but those exudates may be of lower quality and contain a narrower range of compounds. The production and composition of root exudates are complex processes that are influenced by a variety of factors. Understanding the factors that influence exudate production and composition can help optimize plant-microbe interactions and improve soil health and productivity in agricultural systems.

In addition to the factors mentioned above, other environmental factors such as temperature, moisture, and light can also impact root exudate production and composition. For example, plants grown under high temperatures may produce more volatile organic compounds in their exudates, which can influence the types of microbes present in the rhizosphere. Similarly, plants grown under drought conditions may produce exudates that are more concentrated, as a mechanism to conserve water. It is also important to note that the composition of the rhizosphere microbiome can influence the production and composition of root exudates. For example, certain microbial taxa in the rhizosphere have been shown to promote the production of specific compounds in root exudates, which can in turn influence the types of microbes present in the rhizosphere (Singh and Mukerji, 2006; Berendsen et al., 2012). The complex interplay between plant genotype, soil type, nutrient availability, and environmental factors all contribute to the production and composition of root exudates, which in turn influence the types and abundance of microbes in the rhizosphere. Understanding these complex interactions is essential for developing sustainable agriculture practices that optimize plant-microbe interactions and promote soil health and productivity

(Mandal et al., 2021; Choudhary et al., 2016).

Oppenheimer-Shaanan et al., (2022) used advanced approaches in microbiology, plant physiology, and organic chemistry to study the dynamic rhizosphere interplay between tree roots and soil bacteria under drought stress. They collected root exudates and analyzed them for metabolites using metabolic profiling. They found that 44 metabolites in exudates were significantly different in concentration between irrigated and drought trees, including phenolic acid compounds and quinate. When adding these metabolites as carbon and nitrogen sources to bacterial cultures of both bacterial species, eight of nine metabolites stimulated bacterial growth. The researchers also found that soil phosphorous bioavailability was maintained only in inoculated trees, mitigating drought-induced decrease in leaf phosphorus and iron.

The rhizosphere microbiome can modulate root exudate production and composition through a variety of mechanisms, including nutrient cycling, hormone synthesis, and modulation of plant defense responses. These interactions can be beneficial for both the plant and the microbes, as they can help to improve nutrient acquisition and promote plant growth and health (Pettit, 2004). One important mechanism by which the rhizosphere microbiome modulates root exudate production and composition is through nutrient cycling. Many microbes in the rhizosphere are capable of breaking down complex organic compounds in the soil, such as lignin and cellulose, into simpler compounds that can be taken up by plants. This process, known as mineralization, releases nutrients such as nitrogen, phosphorus, and sulfur into the soil, which can then be taken up by the plant and incorporated into its exudates (Etesami and Adl, 2020). In turn, these exudates can promote the growth of beneficial microbes in the rhizosphere, which can further enhance nutrient cycling and improve plant growth and health.

Another mechanism by which the rhizosphere microbiome can modulate root exudate production and composition is through hormone synthesis. Some microbes in the rhizosphere are capable of synthesizing plant hormones, such as auxins and cytokinins, which can influence the growth and development of the plant (Hayat et al., 2010; Arshad and Frankenberger, 1991). These hormones can also influence root exudate production and composition, as they can promote the release of specific compounds that are beneficial for the microbes. For example, some microbes have been shown to promote the production of amino acids and organic acids in root exudates, which can provide a source of carbon and nitrogen for the microbes. The rhizosphere microbiome can modulate root exudate production and composition by influencing plant defense responses. Some microbes in the rhizosphere can produce compounds that can stimulate the plant's immune system, which can in turn influence the types and amounts of exudates produced by the plant. For example, some microbes produce compounds that can trigger the production of phytohormones and other defense-related compounds in the plant, which can help to protect the plant against pathogenic microbes and other stresses (Zehra et al., 2021). The rhizosphere microbiome can modulate root exudate production and composition through a variety of mechanisms, which can help to improve nutrient cycling, hormone synthesis, and plant defense responses. These interactions are essential for promoting plant growth and health, and for maintaining a healthy and productive soil ecosystem.

In addition to the mechanisms mentioned above, the composition of the rhizosphere microbiome itself can also play a role in modulating root exudate production and composition. For example, studies have shown that the presence of specific microbial taxa in the rhizosphere can influence the types and amounts of exudates produced by the plant (White et al., 2017). This may be due to the fact that different microbes have different metabolic pathways and nutrient requirements, which can influence the types of compounds they can utilize in root exudates. Furthermore, environmental factors such as soil type and nutrient availability can also play a role in modulating root exudate production and composition. For example, plants growing in nutrient-poor soils may produce more exudates in an effort to attract beneficial microbes that can help to improve nutrient availability. Similarly, plants growing in soils with high levels of pathogens may produce exudates that contain compounds with antimicrobial properties, which can help to protect the plant against infection. The dynamic interplay between root exudates and the rhizosphere microbiome is a complex and multifaceted process that is influenced by a variety of biotic and abiotic factors (Kotoky et al., 2018). Understanding these interactions is essential for developing strategies to promote plant growth and health, improve soil fertility, and maintain a healthy and productive soil ecosystem.

The relationship between root exudates and the rhizosphere microbiome is bidirectional, meaning that they can both influence each other in a feedback loop. For example, the composition of the rhizosphere microbiome can affect the types and amounts of exudates produced by the plant, which in turn can influence the growth and activity of the microbiome (Bakker et al., 2012). Similarly, the composition and activity of the microbiome can influence the types and amounts of exudates produced by the plant. One example of this feedback loop is the role of plant hormones in modulating both root exudate production and the composition of the rhizosphere microbiome. Studies have shown that plants can produce hormones such as auxins and cytokinins in response to specific microbial signals, which can then stimulate the

production of specific exudates. These exudates, in turn, can attract specific microbial communities that are capable of metabolizing the exudates and promoting plant growth.

Another example of this feedback loop is the role of nutrient cycling in the rhizosphere. Microbes in the rhizosphere are involved in the cycling of nutrients such as nitrogen, phosphorus, and sulfur, which can then be taken up by the plant (Osorio Vega, 2007). In turn, the plant can modulate the production of specific exudates that attract microbes capable of mobilizing these nutrients. The feedback loops and interactions between root exudates and the rhizosphere microbiome are complex and dynamic. Understanding these interactions is essential for developing strategies to manipulate the microbiome and promote plant growth and health.

**Implications for Microbiome Engineering and Sustainable Agriculture**

The manipulation of root exudates and the rhizosphere microbiome has enormous potential for applications in sustainable agriculture and environmental management. By promoting healthy soil microbial communities and optimizing nutrient cycling, these strategies can enhance plant growth and reduce the need for synthetic fertilizers and other inputs. This can lead to improved soil health, reduced greenhouse gas emissions, and increased carbon sequestration in soils. In addition, manipulating root exudates and the rhizosphere microbiome can have important implications for environmental management beyond agriculture (Bakker et al., 2012; Tiziani et al., 2022). For example, it can help to promote the restoration of degraded soils and ecosystems, enhance carbon sequestration in soils, and improve water quality by reducing nutrient runoff. However, it is important to recognize that any manipulation of the rhizosphere microbiome and root exudates must be carefully evaluated for potential risks and unintended consequences. This includes potential impacts on non-target species, such as soil organisms and beneficial insects, as well as potential effects on soil and water quality. Therefore, it is essential that these strategies be developed and implemented in a responsible and sustainable manner, with a focus on minimizing negative impacts on the environment and maximizing the benefits for both agriculture and the broader ecosystem.

To achieve the full potential of these strategies, more research is needed to better understand the complex interactions between plants, root exudates, and the rhizosphere microbiome. This includes developing a deeper understanding of the molecular mechanisms that underlie these interactions and how they can be manipulated to achieve specific outcomes. It also involves understanding the role of the broader soil and environmental context in shaping these interactions, including the influence of soil type, climate, and other environmental factors. Furthermore, there is a need for greater collaboration and knowledge-sharing across different scientific disciplines, as well as between scientists, farmers, and other stakeholders in the agriculture and environmental sectors. This will help to ensure that the latest scientific findings are translated into practical and effective strategies that can be implemented on the ground. The manipulation of root exudates and the rhizosphere microbiome represents a promising approach for achieving sustainable agriculture and environmental management (Bano et al., 2021). By promoting healthy soils and ecosystems, these strategies have the potential to enhance food security, reduce the environmental footprint of agriculture, and contribute to broader efforts to address global environmental challenges such as climate change and biodiversity loss.

**Future Directions**

Research on the dynamic interplay between root exudates and the rhizosphere microbiome is essential for several reasons. It will help to deepen our

**Table 3.** Strategies for manipulating root exudates and rhizosphere microbiome.

| Strategy | Description | Reference |
|---|---|---|
| Plant breeding | Breeding plants with specific exudate profiles that can selectively recruit beneficial microbes, such as nitrogen-fixing bacteria or mycorrhizal fungi | Jacoby et al., 2017 |
| Soil amendments | Adding amendments such as organic matter or biochar can enhance microbial diversity and activity in the rhizosphere, leading to changes in exudate production and composition | Li et al., 2021 |
| Microbial inoculants | Adding specific microbial inoculants to the soil can selectively promote the growth of beneficial microbes, leading to changes in exudate production and composition | Saad et al., 2020 |
| Inter-cropping | Planting two or more crops together can lead to changes in the composition of the rhizosphere microbiome, which can in turn affect exudate production | Bennett et al., 2012 |
| Nutrient management | Managing soil nutrient availability can affect the composition of the rhizosphere microbiome and thus impact exudate production | Kumar and Dubey, 2020 |
| Biostimulants | Biostimulants, such as humic acids or seaweed extracts, can promote plant growth and affect exudate production by stimulating microbial activity | Calvo et al., 2014 |

understanding of the complex and multifaceted interactions between plants and microbes in the soil, which are essential for the health and productivity of agricultural systems and natural ecosystems. It will enable the development of more effective and sustainable strategies for managing plant-microbe interactions in the soil (Choudhary et al., 2016; Morgan et al., 2005). By better understanding the mechanisms that underlie these interactions, researchers will be able to develop new approaches for manipulating root exudates and the rhizosphere microbiome to achieve specific outcomes, such as enhancing nutrient acquisition, improving plant health and resilience, and promoting sustainable agriculture and environmental management.

Continued research in this area is necessary to address some of the pressing challenges facing agriculture and the environment today, including climate change, soil degradation, and biodiversity loss. By promoting healthy soils and ecosystems through the manipulation of root exudates and the rhizosphere microbiome, we can contribute to broader efforts to address these global challenges. Continued research on the dynamic interplay between root exudates and the rhizosphere microbiome is crucial for advancing our understanding of plant-microbe interactions in the soil, developing new strategies for managing these interactions, and addressing some of the most pressing challenges facing agriculture and the environment today (Bakker et al., 2012; Munoz-Ucros et al., 2021).

Additionally, continued research on the dynamic interplay between root exudates and the rhizosphere microbiome can also provide valuable insights into fundamental ecological and evolutionary processes. For example, studying the mechanisms by which plants and microbes co-evolve in the soil can shed light on the origins and maintenance of biodiversity, as well as the mechanisms that underlie the emergence and spread of plant-microbe mutualisms (Nadarajah et al., 2021). Furthermore, understanding the complex and dynamic nature of root exudates and the rhizosphere microbiome can also have implications for human health. For example, some studies suggest that the composition of the gut microbiome, which plays a critical role in human health, may be influenced by the composition of the rhizosphere microbiome in the foods we eat (Goulet et al., 2019). Therefore, a better understanding of the interactions between plants and microbes in the soil may have broader implications for human health and wellness. The continued research on the dynamic interplay between root exudates and the rhizosphere microbiome is crucial for advancing our understanding of the ecological and evolutionary processes that shape plant-microbe interactions, developing sustainable strategies for managing these interactions and addressing global challenges such as climate change, soil degradation, and biodiversity loss.

## Conclusion

The dynamic interplay between root exudates and the rhizosphere microbiome is a complex and tightly regulated process that is influenced by a range of biotic and abiotic factors. Root exudates serve as a critical source of energy and nutrients for the rhizosphere microbiome, while also shaping the composition and function of microbial communities. Conversely, the rhizosphere microbiome can modulate the production and composition of root exudates, through a range of mechanisms that include nutrient cycling, hormone synthesis, and plant defense modulation. Understanding the complex relationship between root exudates and rhizosphere microbiome is of great importance for agriculture and environmental management. By manipulating root exudates and rhizosphere microbiomes, we can develop strategies for sustainable agriculture, plant-microbe symbiosis, and soil remediation. Further research is needed to fully understand the mechanisms underlying the dynamic interplay between root exudates and rhizosphere microbiome and to develop effective strategies for microbiome engineering and plant-microbe interactions. The dynamic interplay between root exudates and rhizosphere microbiome represents a fascinating and important area of research with significant implications for agriculture, soil science, and environmental management.

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