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A study of seasonal and locational variations of heavy metals in soil and earthworms (Annelida: Oligochaeta) in Al-Muthanna Governorate

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Abstract:

This study was conducted in Al-Muthanna Governorate from October 2024 to September 2025, over four quarters. Samples were collected from several stations: Station 1 (Al-Faz'iyah sub-district), Station 2 (Al-Hilal district), Station 3 (Al-Najmi district), Station 4 (Al-Majd district), Station 5 (Abu Shuraysh sub-district), Station 6 (Al-Rumaytha district), Station 7 (Al-Warka district), and Station 8 (Governorate Center/Al-Samawah district). The study indicated that both the station and the season affect the levels of heavy metals in the soil. Zinc recorded its highest level in the soil at station two during winter and its lowest at station four during spring. Lead recorded its highest concentration at station one during winter and its lowest at station four during spring. Chromium had its highest concentration at station four during winter and its lowest at station seven during autumn. Cadmium had its highest concentration at station six during winter and its lowest at station seven during autumn. Nickel had its highest concentration at station seven during spring and its lowest at station four during autumn. The study also indicated that both the station and the season affect the concentration of heavy metals in earthworms. The results showed that the highest concentration of zinc in earthworms was at station 1 in autumn and the lowest at station 7 in winter. Lead recorded its highest concentration at station 2 in spring and its lowest at the same station in autumn. Chromium had its highest concentration at station 5 in summer and its lowest at station 2 in winter. Cadmium had its highest concentration at station 8 in winter and its lowest at station 5 in autumn. Nickel had its highest concentration at station 2 in summer and its lowest at station 6 in spring.

Keywords: Heavy Elements, Soil, Earthworms (Annelida: Oligochaeta), Al-Muthanna.

دراسة التغيرات الفصلية والموقعية للعناصر الثقيلة في التربة وديدان الارض (Annelida: Oligochaeta) في محافظة المثنى

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الخلاصة

اجريت هذه الدراسة في محافظة المثنى للمدة من تشرين الاول 2024 لغاية ايلول 2025، لدراسة تشخيصية وبيئية، وتقدير تركيزات بعض العناصر الثقيلة في ديدان الأرض (Annelida: Oligochaeta) في محافظة المثنى / جنوب العراق. حيث اخذت العينات من عدة محطات وهي المحطة الأولى (ناحية الفزاعية)، المحطة الثانية (قضاء الهلال)، المحطة الثالثة (قضاء النجمي)، المحطة الرابعة (قضاء المجد)، المحطة الخامسة (ناحية أبو شريش)، المحطة السادسة (قضاء الرميثة)، المحطة السابعة (قضاء الوركاء)، و المحطة الثامنة (مركز المحافظة / قضاء السماوة). واشارت الدراسة الى ان لكل من المحطة والفصل له تاثير في مستويات العناصر الثقيلة في التربة، اذ سجل عنصر الخارصين اعلى مستوى له بالتربة بالمحطة الثانية شتاءا واقلها، وسجل الرصاص بالمحطة الاولى شتاءا اعلى تركيز واقلها بالمحطة الرابعة ربيعا، والكروم كان اعلى تركيز له في المحطة الرابعة شتاءا واقلها بالمحطة السابعة خريفا، والكادميوم كان اعلى تركيزا بالمحطة السادسة شتاءا واقلها بالسابعة خريفا، اما النيكل فكان اعلى تركيز له بالمحطة السابعة ربيعا واقلها بالمحطة الرابعة خريفا. واشارت الدراسة ايضا الى ان كل من المحطة والفصل تاثير في تركيز العناصر الثقيلة في ديدان الارض، اذ بينت النتائج بان اعلى تركيز للخارصين في ديدان الارض بالمحطة الاولى خريفا وادناها بالمحطة السابعة شتاءا، وسجل الرصاص اعلى تركيز بالمحطة الثانية ربيعا وادناها بنفس المحطة بالخريف، والكروم كان اعلى تركيزا بالمحطة الخامسة صيفا واقلها بالمحطة الثانية شتاءا، والكادميوم كان اعلى تركيزا بالمحطة الثامنة شتاءا واقلها بالمحطة الخامسة خريفا، اما النيكل فكان اعلى تركيزا بالمحطة الثانية صيفا وادناها بالمحطة السادسة ربيعا.

كلمات افتتاحية: العناصر الثقيلة، التربة، ديدان الارض، محافظة المثنى.

Introduction

Soil pollution with heavy metals has widespread negative effects on the organisms that live, which may be acute or chronic. Earthworms are important components of the soil, as they have a positive influence on the physical, chemical, and biological properties of the soil (Shi *et al*., 2017). Sivakumar (2015) indicated that earthworms are characterized by their close connection to the soil, and have been studied in numerous soil toxicology studies.

Earthworms consume and decompose organic matter, then mix it with the soil to produce stable clumps in water.

Prolonged exposure to heavy metals can negatively affect earthworm survival, growth, sexual development, pupal production, behavior, and population density. Heavy metals such as cadmium, copper, mercury, zinc, and lead accumulate primarily in earthworm tissues, negatively impacting them and making them suitable bioindicators for monitoring soil heavy metal pollution (Uwizeyimana *et al*., 2017).

Among the most common negative effects of heavy metals on earthworms are reduced reproduction, decreased biomass, alterations in cell division and antioxidant enzyme activity, kidney cell damage, and DNA damage (Liang *et al.*, 2011). The gradual loss of earthworms due to soil pollution can negatively impact soil quality and traditional agricultural practices in Iraq. These worms are important bioindicators of soil pollution, providing valuable information about environmental hazards. There is a relationship between the concentration of certain pollutants in the soil and its composition (Al-Mohammad, 2023).

Because heavy metals are stable in the environment, they enter living organisms from the environment and accumulate within them (Ali *et al.*, 2019). Heavy metals present in the soil accumulate in earthworms primarily through direct skin contact and ingestion (Wijayawardena *et al.*, 2017). The accumulation of heavy metals in earthworms is influenced by various factors, such as the species of earthworm, the physical and chemical properties of the soil, the level of pollution, and other environmental conditions such as soil moisture and temperature (Xiao *et al.*, 2022).

This study aims to demonstrate the seasonal and local variations in heavy metals in soil and worms in Al-Muthanna Governorate, which is the first field study in Al-Muthanna Governorate.

Materials and Methods

This study was conducted in Al-Muthanna Governorate from October 2024 to September 2025, over four quarters. Samples were collected from several stations: Station 1 (Al-Fuzai'iyah sub-district), Station 2 (Al-Hilal district), Station 3 (Al-Najmi district), Station 4 (Al-Majd district), Station 5 (Abu Sharish sub-district), Station 6 (Al-Rumaytha district), Station 7 (Al-Warka district), and Station 8 (Al-Samawah district).

The rationale for selecting these stations is that their distribution along the main course of the Euphrates River across the governorate is approximately one of eight. This allows for the study of the impact of human activity at these stations, ranging from rural areas (such as Al-Fuzai'iyah and Abu Sharish) to semi-urban areas (such as Al-Hilal and Al-Najmi), and finally to the main urban center (Al-Samawah). Furthermore, the impact of agricultural practices is considered, as most of these districts and sub-districts rely primarily on agriculture. A comparison can also be made between less densely populated areas and areas with higher levels of human activity to assess the extent to which these activities affect the accumulation of heavy metals in earthworms.

Sample Collection:

Samples were collected monthly from October 2024 to September 2025. Three replicates were performed for the worm sample at each of the eight stations. Soil samples were collected once a month to measure heavy metal concentrations.

Earthworm Sampling:

Earthworm samples were collected monthly using the quadrant method (1 added slowly to each sample, holding the tube at an angle and shaking it well. The tubes were then left to stand for 24 hours to complete the initial digestion process. The tubes were placed in a block digester at approximately 75°C for up to 3 hours. This time was extended if digestion was not complete. The samples were carefully transferred to a 50 ml volumetric flask. The tubes were washed several times with deionized water, and the wash water was added to the sample. 6. The flasks were heated to 70°C until the sample volume was reduced to approximately 2 mL, taking care to prevent the sample from drying out. The sample was dissolved in deionized water and transferred to a 50 mL volumetric flask (reservation bottles). The flask was washed several times with deionized water, and the sample was added to bring the total volume to 50 ml. The sample was centrifuged at 3500 rpm using plastic tubes for 30 minutes. The filtrate was collected and returned to the same storage bottles, and the sample was refrigerated until measurement.

Heavy Metal Extraction from Soil:

The samples were spread out in the laboratory to dry, then placed in an oven at 70°C and ground in a ceramic mill. They were then sieved through a 2 mm sieve and stored in plastic bottles until the heavy metals were extracted. The ISO (1995) method was used to extract heavy metals from soil samples as follows; 0.5–1 gm of dried and sieved soil (less than 2 mm) was taken. The sample was placed in a Teflon x 1 m) for earthworm extraction. Samples were collected in triplicate using a hand sieve with a 0.4 mm mesh size. The samples were washed with water to remove suspended particles. The samples were then placed in labeled beakers inside a box until they arrived at the laboratory. The earthworms were then thoroughly washed with water to remove mud and suspended particles. The samples were subsequently stored in labeled plastic beakers until the heavy metal extraction process could be performed.

Soil sampling:

Soil samples were collected after removing the surface layer by hand. A quantity of soil was taken and placed in labeled plastic bags and kept in a box until it arrived at the laboratory.

Heavy Metal Extraction:

Samples were digested using acid digestion for earthworm (biological tissue) and soil samples to analyze their heavy metal content, according to commonly used procedures such as the EPA (U.S. Environmental Protection Agency) method or U.S. EPA 3050B/3051A.

Heavy Metal Extraction from Earthworms:

Samples were digested according to the ROPME (2000) method, following these steps; 1 gm of the sample powder was weighed for three replicates and placed in 150 ml digestion tubes. The tube openings were covered with glass plugs during digestion. 4.5 ml of concentrated nitric acid and 1.5 ml of concentrated perchloric acid were paper. The solution was diluted to 50 ml with demineralized distilled water.

Results and Discussion:

Zinc:

The lowest zinc concentration in the soil (23.73 µg/g) was observed at station 4 during autumn, while the highest concentration (89.36 µg/g) was recorded at station 2 during winter (Table 1).

flask for digestion. The mixture was heated, and then the following were added sequentially; 10 ml of concentrated HNO_3 and 5 mL of HCl (concentrated hydrochloric acid). H_2O_2 was also used to assist in the oxidation of organic matter. The mixture was heated on a hot plate at a temperature of ~95–120°C until the liquid volume decreased and the sample was thoroughly digested. 3. The sample was filtered using mineral-free filter

Table (1) Concentration of zinc in the soil (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	44.40d±0.00	40.51d±3.33	49.97e±8.01	49.97e±5.01
Station2	46.23c±0.00	89.36a±5.09	78.23a±10.20	78.23a±7.08
Station3	39.74d±0.00	40.24e±4.88	40.601f±9.33	40.601f±6.60
Station4	23.73g±0.01	38.84f±3.01	24.75h±3.22	38.28g±4.09
Station5	37.28f±0.00	44.17c±5.03	38.28g±6.31	45.40d±7.03
Station6	48.97b±0.00	50.9b±5.02	45.402d±9.01	39.601f±5.90
Station7	77.23a±0.00	35.56h±4.01	47.23c±7.33	47.236c±4.08
Station8	39.6e±3.8	33.59g±2.01	49.97e±7.11	49.97e±8.01
Sig.	0.0001>	0.0001>	0.0001>	0.0001>
L.S.D	0.007	0.00754	0.00733	0.00724

and 3, respectively. Significant differences were found between all stations, and in spring, the lowest average concentration (80.54 µg/gm dry weight) and the highest average concentration (428.4 µg/gm dry weight) were recorded at stations 4 and 6, respectively. Differences were recorded Significant differences were observed across all stations at a probability level of 0.0003 ($P < 0.0003$). During the summer, the lowest average concentration (101.9 µg/g dry weight) and the highest average concentration (428.4 µg/g dry weight) were recorded at stations 3 and 5, respectively. Significant differences were observed across all stations at a

The results generally showed the highest zinc concentration in the worms (427.403 µg/gm) at station 1 in autumn. The lowest concentration was recorded at station 7 in winter, reaching 49.9 µg/gm. The results also showed the lowest average concentration (79.54 µg/gm dry weight) at station 4 and the highest average concentration (427.403 µg/gm dry weight) at station 1 in autumn. Significant differences were found between all stations at a probability level of 0.0001 ($P < 0.0001$). In winter, the lowest average concentration (49.9 µg/gm dry weight) and the highest average concentration (241.3 µg/gm dry weight) were recorded at stations 7 probability level of 0.0004 (P < 0.0004) (Table 2).

Table (2) Concentration of zinc in earthworms (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	427.403a±18.05	91.01f±10.3	297.9c±10.1	297.9b±9.1
Station2	127.21e±10.1	241.3a±18.0	303.5b±11.03	303.5b±10.2
Station3	172.77e±11.01	181.3b±10.10	101.97e±9.01	101.9c±6.66
Station4	79.54g±7.09	128.04c±12	80.54f±5.01	305.1b±11.2
Station5	304.14b±15.09	79.5g±8.10	305.14b±10.33	428.4a±13.4
Station6	296.96d±14.33	105.2d±11.0	428.4a±12.10	319.5a±10.11
Station7	302.36c±17.66	49.9h±6.04	128.21d±9.99	128.21c±9.97
Station8	100.97f±10.17	95.0e±9.03	297.9c±10.2	297.9b±12.12
Sig.	0.0001>	0.0002	0.0003	0.0004
L.S.D	0.93	3.25	3.01	115.8

Zinc affects earthworms by accumulating in their tissues, which can hinder their growth, and reduce their reproduction, fertility, and productivity. It also lowers their survival rate at high concentrations. The effects vary depending on the form of zinc (ions versus nanoparticles) and soil conditions. Earthworms may sometimes exhibit activation at low levels or rapid recovery in clean soil (Dai *et al.*, 2004). Although earthworms can accumulate large amounts of zinc, their ability to regulate its concentration in their bodies may mitigate some of the toxic effects. However, chronic exposure, especially to dissolved ions, remains harmful, affecting cellular function and overall soil health (Mahmood and Mohammed, 2023).

Lead:

Its lowest concentrations in the soil of Station Four were in the spring, 7.083±1.20 µg/gm, and its highest concentration was in Station One in the winter, reaching a value of 19.91±1.03 µg/gm, (Table 3).

Table (3) Concentration of lead in the soil (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	12.74f±1.1	19.91a±1.03	17.94b±2.05	17.94b±1.01
Station2	11.33g±0.9	14.93d±2.10	13.22e±1.05	13.22e±1.2
Station3	14.63d±1.04	11.805e±1.03	15.58c±2.01	15.58c±1.9
Station4	7.08h±0.99	10.86f±1.9	7.083h±1.20	18.41a±2.01
Station5	18.41a±1.9	16.05b±2. 01	18.41a±0.00	12.74f±0.90
Station6	17.94b±1.7	15.58c±2.11	12.74f±1.99	15.58c±1.40
Station7	13.22e±1.10	13.69d±2.10	11.33g±1.89	11.33g±1.10
Station8	15.58c±1.13	15.58c±0.00	17.94b±2.01	17.94b±1.09
Sig.	0.0001>	0.0001>	0.0001>	0.0001>
L.S.D	0.0001	0.00017	0.00016	0.00015

The results showed the highest lead concentration in the worms at 32.16 µg/gm at station 2 in the spring. The lowest concentration was recorded at highest concentration, 32.16 µg/gm dry weight, and the lowest concentration, 1.75 µg/gm dry weight, were recorded at stations 2 and 3, respectively. Significant differences were found. Among all stations at a probability level of 0.0005 ($P < 0.0005$). During the summer, the highest average concentration (32.16 µg/g dry weight) and the lowest average concentration (11.75 µg/g dry weight) were recorded at stations two and three, respectively. Significant differences were found among all stations at a probability level of 0.001 ($P < 0.001$) (Table 4).

station 8 in the autumn, at 0.75 µg/gm. In autumn, the highest lead concentration in the worms was 31.16 µg/gm at station 7, and the lowest was 0.75 µg/gm at station 8. Significant differences were found between all stations at a probability level of $p < 0.0001$. The lowest concentration, 3.90 µg/gm dry weight, was recorded in winter. The highest concentration, 21.35 µg/gm dry weight, was recorded at stations 2 and 7, respectively. Significant differences were found between all stations at a probability level of $p > 0.0004$. In spring, the

Table (4) Concentration of lead in earthworms (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	15.11c±3.01	8.17d±1.60	12.33d±2.10	12.33c±2.0
Station2	11.63d±3.00	3.90f±1.06	32.16a±3.50	32.16a±2.33
Station3	5.10e±0.5	4.18e±1.11	1.75f±0.06	11.75c±1.5
Station4	5.92b±0.9	9.52d±1.10	5.92e±0.70	19.88b±3.16
Station5	18.88b±2.00	6.88c±0.99	19.88b±1.40	16.11b±1.2
Station6	11.33c±1.05	14.08b±1.50	16.11c±1.50	11.32c±3.6
Station7	31.16a±5.01	21.35a±1.90	12.63d±2.00	12.63c±2.1
Station8	0.75h±0.01	11.90c±1.02	12.33d±2.05	12.33c±3.3
Sig.	0.0001>	0.0004>	0.0005>	0.001>
L.S.D	0.612	1.4	1.34	5.53

sometimes appear in naturally exposed populations (Qi *et al*., 2025).

Chromium:

The results recorded in the soil showed that its lowest value was at station seven in the autumn, 2.56, and its highest concentration was at station four in the winter, reaching a value of 23.64 µg/g (Table 5).

Lead (Pb) negatively affects earthworms by reducing their survival, growth, and reproduction. It causes tissue damage (such as intestinal and body wall) and inhibits enzymes. It leads to DNA damage, primarily through ingestion and accumulation in chlorogenic tissues. Although the specific effects vary depending on the lead concentration, form, and duration of exposure, tolerance mechanisms

Table (5) Concentration of chromium in the soil (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	13.37b±3.01	14.13h±1.06	10.46f±0.99	10.46f±1.00
Station2	12.39d±3.33	16.39g±3.0	3.56i±0.50	3.56i±0.02
Station3	13.51a±2.11	20.08c±4.04	13.81c±1.10	13.81b±1.55
Station4	4.10g±0.5	23.64a±4.17	5.101h±0.70	10.74e±1.03
Station5	9.74e±0.99	17.64d±2.03	10.74e±1.03	14.37a±1.99
Station6	9.46f±0.98	22.80b±4. 01	14.37b±1.55	12.81d±1.85
Station7	2.56h±0.01	17.37e±2.09	13.39c±1.73	13.39c±2.00
Station8	12.8c±1.3	17.09f±2.19	10.46f±1.94	10.46f±1.70
Sig.	0.0001>	0.0001>	0.0001>	0.0001>
L.S.D	0.00038	0.00045	0.00041	0.00039

The results showed that the highest chromium concentration in the worms was recorded at station five during the summer, reaching 82.52 µg/gm. The lowest concentration was recorded at station two during the winter, at 11.62 µg/gm. In the autumn, the highest average concentration was recorded at station one, at 81.52 µg/gm, while the lowest was recorded at station four, at 16.69 µg/gm. Significant differences were found among all stations at a probability level of $p<0.0001$. During the winter, the lowest average concentration was recorded at 11.62 µg/gm dry weight, and the highest average concentration was recorded at stations two and seven, at 24.43 µg/gm dry weight. Significant differences were found among all stations at a probability level of $p>0.0026$. During the spring, the highest average concentration was recorded at station six, at 82.52 µg/gm dry weight, and the lowest at station seven, at 17.69 µg/gm dry weight. Dry at Station 4. Significant differences were recorded between all stations at a probability level of 0.0019 ($P<0.0019$). During the summer, the highest average concentration (82.52 µg/gm dry weight) was recorded at Station 5. The lowest average concentration (26.46 µg/gm dry weight) was recorded at Station 3. Significant differences were recorded between all stations at a probability level of 0.001 ($P < 0.001$) (Table 6).

Table (6) Concentration of chromium in earthworms (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	81.52a±13.01	12.83d±1.88	53.62c±5.03	53.62b±4.1
Station2	57.34c±7.01	11.62d±1.73	58.30b±4.88	58.30b±6.1
Station3	43.87e±6.21	18.24b±2.01	26.48d±2.19	26.46c±3.22
Station4	16.69g±3.00	13.04b±2.08	17.69e±1.50	73.95a±8.6
Station5	72.95b±8.05	19.87b±3.30	73.95a±8.01	82.52a±5.6
Station6	52.62d±7.30	14.18c±1.92	82.52a±6.02	63.8a±9.97
Station7	57.30c±6.08	24.43a±2.30	58.34b±5.06	58.34b±7.33
Station8	25.48f±4.71	18.57b±2.60	53.62c±5.10	53.62b±6.67
Sig.	0.0001>	0.0026	0.0019	**0.001**

L.S.D	0.612	1.4	1.34	5.53

mechanisms, interactions with other pollutants (such as microplastics), and mitigation strategies using biochar. The results highlight the detrimental effects of chromium on growth, reproduction, and survival, confirming its role as an early warning sign of soil ecosystem threats (Li *et al*., 2026).

Cadmium:

Its concentrations in the soil were at their lowest value in station six in the autumn, 0.01, and their highest concentration was in station seven in the winter, reaching 1.71 µg /gm (Table 7).

Chromium (Cr) in the environment is highly detrimental to earthworms. Hexavalent chromium (Cr(VI)) is more toxic than trivalent chromium (Cr(III)), causing oxidative stress, DNA damage, enzyme inhibition, growth retardation, and impaired immune and reproductive functions, even at low levels. This impacts soil health by altering microbial communities and mineral cycling (Liu *et al*., 2023). Earthworms exhibit the ability to bioaccumulate chromium, and some bacterial groups are capable of conferring resistance to it or enhancing its activity. Recent studies have focused on these

Table (7) Concentration of cadmium in the soil (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	0.079f±0.002	0.039e±0.00	0.012g±0.00	0.012h±0.00
Station2	0.041g±0.001	0.05f±0.004	1.407a±0.01	1.41a±0.001
Station3	0.607c±0.001	0.08c±0.004	1.29b±0.01	1.29b±0.001
Station4	0.15e±0.00	0.028d±0.001	0.15e±0.001	0.11f±0.001
Station5	0.11d±0.001	0.41g±0.001	0.11f±0.001	0.07g±0.00
Station6	0.01h±0.001	0.083h±0.002	0.079h±0.00	1.29b±0.001
Station7	1.405a±0.004	1.71a±0.001	0.041i±0.001	0.041i±0.00
Station8	1.29b0±.01	0.049b±0.001	0.012g±0.00	0.012h±0.00
Sig.	0.0001>	0.0001>	0.0001>	0.0001>
L.S.D	0.0025	0.00284	0.00262	0.00257

the lowest average concentration (1.04 µg/gm dry weight) and the highest average concentration (7.08 µg/gm dry weight) were recorded at stations six and eight, respectively. Significant differences were found between all stations at a probability level of 0.0003. In spring, the lowest average concentration (0.43 µg/gm dry weight) and the highest average concentration (2.98 µg/gm dry weight) were recorded at stations two and four, respectively. Significant differences were found

The results showed the highest cadmium concentration in the worms at 7.08 µg/g in station eight during winter, and the lowest concentration at 0.01 µg/gm in station five during autumn. Autumn also saw the lowest average concentration (0.01 µg/g dry weight) at station five, while the highest average concentration (1.98 µg/gm dry weight) was recorded at station four. Significant differences were found between all stations at a probability level of 0.0001. In winter, dry weight at stations two and eight respectively, and there were significant differences between all stations at a probability level of 0.05>P (Table 8).

between all stations at a probability level of 0.0003. In the summer, the lowest average concentration was 0.43 µg/gm dry weight and the highest average concentration was 2.53 µg/gm

Table (8) Concentration of cadmium in earthworms (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	0.098e±0.00	1.78c±1.10	2.51a±0.77	2.51a±0.92
Station2	1.27c±0.01	5.82b±1.20	0.43b±0.02	0.43b±0.001
Station3	0.012g±0.001	2.45c±1.05	2.51a±0.97	2.52a±0.99
Station4	1.98a±0.09	3.02c±1.60	2.98a±0.55	1.30a±0.86
Station5	0.01f±0.00	4.21b±1.09	1.0b±0.83	1.09a±0.68
Station6	1.51b±0.01	1.04d±0.66	1.10b±0.10	1.56a±0.50
Station7	0.43d±0.01	2.51c±0.98	2.27a±0.80	2.27a±0.91
Station8	1.51b±0.3	7.08a±1.20	2.51a±0.98	2.53a±0.88
Sig.	0.0001>	0.0003	0.0002	**0.05**
L.S.D	0.0014	1.25	1.18	**1.53**

31.94 µg/gm at station four in the autumn, and the highest concentration was 137.35 µg/gm at station seven in the spring and summer (Table 9).

Nickel:

Its concentrations in the soil were as follows: the lowest concentration was

Table (9) Concentration of nickel in the soil (Mean± SD).

Stations	Seasons			
	Autumn	Winter	Spring	Summer
Station1	112.76c±12.01	77.36f±3.66	101.002f±10.01	101.02f±10.10
Station2	136.34a±14.05	84.5e±5.60	103.73e±9.98	103.73e±11.1
Station3	120.70b±10.03	112.80c±10.10	112.72c±8.01	112.72c±10.01
Station4	31.94f±3.31	118.25b±11.01	32.94h±5.03	122.27b±12.1
Station5	120.89b±11.32	85.81e±6.02	122.27b1±0.5	113.76d±11.9
Station6	100.91e±10.51	121.54a±7.91	113.7d±11.01	111.72c±13.0
Station7	102.72d±9.15	100.12d±8.02	137.35a±10.04	137.35a±14.1
Station8	112.3c±9.61	100.84d±7.05	101.002f±9.68	101.002f±10.20
Sig.	0.0001>	0.0001>	0.0001>	0.0001>
L.S.D	1.05	1.025	1.045	1.034

weight) was recorded at station 5, and the highest average concentration (75.21 µg/gm dry weight) was recorded at station 7. Significant differences were found between all stations at the $p < 0.0001$. In the winter, the lowest average

The highest nickel concentration in the worms (76.21 µg/gm) was recorded at station 2 in the summer, while the lowest concentration (7.16 µg/gm) was recorded at station 6 in the spring. In the autumn, the lowest average concentration (30.81 µg/gm dry concentration (17.91 µg/gm dry weight) and the highest average concentration (44.84 µg/gm dry weight) were recorded at stations 2 and 3, respectively. Significant differences were found between all stations at the p < 0.001. In the spring, the lowest average concentration (7.16 µg/gm dry weight) was recorded at station 6, and the highest average concentration (76.21 µg/gm dry weight) was recorded at station 2. Significant differences were found between all stations at the p<0.001. In the summer, the highest average concentration was 75.21 µg/gm dry weight and the lowest average concentration was 10.16 µg/g dry weight at stations two and five respectively, and there were significant differences between all stations at a probability level of 0.001>P (Table 10).

Table (10) Concentration of nickel in earthworms (Mean± SD).

Stations	**Seasons**			
	Autumn	**Winter**	**Spring**	**Summer**
Station1	61.6d±0.04	34.0c±2.0	37.11c±3.10	37.11b±3.55
Station2	62.20c±9.06	17.91e±1.90	76.21a±5.04	76.21a±6.13
Station3	50.56e±10.50	44.84a±9.30	63.46b±6.01	63.46a±4.5
Station4	55.58e±8.01	20.38d±3.08	56.58c±5.02	31.81b±3.89
Station5	30.81g±9.01	36.53b±5.04	31.81d±4.05	10.16c±0.97
Station6	36.11f±7.03	29.23d±2.06	7.16h±1.55	25.9b±6.2
Station7	75.21a±11.1	18.29e±1.99	63.20b±6.30	63.20a±8.44
Station8	62.46b±10.12	37.62b±3.20	37.51c±6.02	37.11b±5.55
Sig.	0.0001>	0.001	0.0008	**<0.001**
L.S.D	0.64	2.87	2.55	**19.43**

Exposure to nickel (Ni) in earthworms causes significant damage, as it disrupts their antioxidant systems (increasing reactive oxygen species and damaging cells), impairs their ability to survive, grow, and reproduce (especially cocoon production), leading to oxidative stress, and alters the activity of enzymes (such as CAT, SOD, and GST). These effects are concentration- and duration-dependent, and often exhibit synergistic toxicity with other pollutants such as plastic nanoparticles or herbicides, but may sometimes be reduced upon co-exposure with certain nanoparticles (Gao *et al*., 2024).

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