

Environmental Assessment of Copper in Soil Using Atomic Absorption Spectroscopy

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ABSTRACT

Heavy metal contamination in soils, particularly by elements like copper (Cu), poses serious environmental and public health risks due to their persistence and ability to enter the food chain. This study demonstrates that Atomic Absorption Spectroscopy (AAS) is a reliable and sensitive method for detecting copper levels in soil samples collected from agricultural, roadside, and mining areas. Proper sample preparation through acid digestion ensures accurate analysis, and the results indicate elevated copper concentrations in contaminated sites. The findings highlight the importance of regular monitoring, adherence to international safety standards, and implementation of remediation strategies such as phytoremediation and soil stabilization to reduce contamination and protect ecosystem and human health.

Keywords: AAS, Soil Analysis, Copper, Heavy metal analysis

1. Introduction:

Soil is a natural resource made of minerals, organic matter, water, air, and living organisms, supporting plant and animal life¹⁻⁹. Its types—sandy, clayey, and loamy—affect water retention and fertility, with loamy soil being ideal for agriculture. Heavy metals like copper, cadmium, and mercury can contaminate soil through human activities, harming plants, microorganisms, and entering the food chain¹⁰⁻²¹. Atomic Absorption Spectroscopy (AAS) is a sensitive technique used to detect metal concentrations in soil.²²⁻²⁹ It works by measuring light absorption by free atoms, helping monitor pollution and ensure environmental and food safety. Atomic Absorption Spectroscopy (AAS) is used to detect heavy metals like copper and cadmium in samples. It works by measuring light absorbed by free atoms at specific wavelengths. After sample preparation and atomization, absorbance is measured to determine concentration. AAS is sensitive, accurate, and widely used for environmental monitoring.³⁰⁻³⁸

2. Review Of The Literature

The study shows that all analyzed heavy metals in the soil samples are within safe permissible limits. Iron (Fe) was found in the highest concentration, followed by Mn, Cu, Zn, Ni, and Pb. Overall, the soils are not contaminated and do not pose any significant health risk.³⁹ Atomic absorption spectrometry (AAS) is an important technique used to detect and measure heavy metals in soil with high accuracy and sensitivity.

It is widely applied in soil monitoring for sample preparation, pollution assessment, and analysis of different metal forms.⁴⁰ This method helps improve soil quality assessment and supports effective environmental protection efforts. Atomic absorption methods were developed to determine 15 elements in soils and rocks with simple procedures suitable even for nontechnical users. These methods use solvent extraction to remove interferences, improve accuracy, and concentrate elements for better detection.⁴¹ They provide reliable analysis with guidance on common interferences and are widely applied in geological and environmental studies.

Industrial activities release heavy metals into the environment, contaminating soil through air particles, effluents, and solid waste, posing risks to human health.

These metals can transfer from soil to plants and eventually enter the food chain, causing serious health hazards.

The study analyzed soil samples from industrial areas to measure heavy metal levels and assess pollution using acid digestion methods.⁴² Industrialization releases heavy metals into the environment through effluents, dust, and waste, leading to soil contamination. These toxic metals can accumulate in soil and transfer to plants, entering the food chain. This poses serious health risks to humans in both rural and urban areas.⁴³

The study analyzed the presence of heavy metals in various medicinal plants and an antidiabetic herbal product using atomic absorption spectrophotometry. It identified elements like Cu, Ni, Zn, Cd, and Hg, which may accumulate in herbal materials. The findings highlight the importance of monitoring heavy metals to ensure the safety and quality of herbal medicines.⁴⁴ The study analyzed heavy metals in tube well water using atomic absorption spectroscopy to assess water quality. It found that tube well depth influenced metal concentration, while the age of the well had no significant effect. Higher levels of antimony and aluminum were detected in shallow wells, whereas arsenic and copper remained within safe limits.⁴⁵

Bihonegn Sisay et al⁴⁶ reported Cadmium and copper are harmful pollutants, so their monitoring in soil is important for protecting human health and the environment. Soil samples were collected at different distances from the roadside and prepared using hot plate digestion. Their concentrations were determined using flame atomic absorption spectroscopy (FAAS) for accurate analysis. Fajar Nugraha et al⁴⁷ reported in The study assessed cadmium (Cd) and copper (Cu) contamination in eggplants sold in traditional markets to ensure food safety. Qualitative tests confirmed the presence of heavy metals, and AAS was used for accurate quantification after method validation. Results showed all samples contained heavy metals, indicating potential health risks from environmental pollution sources. Bhavtosh Sharma et al⁴⁸ reported Metal ion analysis is essential for monitoring the quality of surface and groundwater used for drinking. Atomic absorption spectrometry (AAS) is widely preferred due to its accuracy, reproducibility, and fast analysis time. It is also cost-effective and capable of detecting metals at very low concentrations.

Qasim Mezbah Salih et al⁴⁹ reported Long-term exposure to heavy metals like copper, mercury, cadmium, and arsenic can cause serious health problems. Their accumulation in the body may lead to neurological disorders, cardiovascular diseases, and kidney damage. Jose Ricardo Forero-Mendieta et al⁵⁰ reported the study validated a GFAAS method for accurately determining manganese and copper in human hair and nail samples. Results showed high linearity, good precision, and acceptable recovery,

with low detection and quantification limits. The method proved reliable with minimal matrix effects and uncertainty within acceptable standards.

3. Objective of the work:

The research work to comprehensively explore the analysis of heavy metal of soil by using atomic absorption spectrometer. To analysis heavy metal Copper(Cu) present in the soil by using atomic absorption spectrometer. The work seeks to pave the way for further exploration in the field of environment and society through its comprehensive investigation of copper (Cu).

4. Experimental Procedure:

4.1 Study area

The soil samples were collected around three different places of Poombarai Village, Kodaikanal-Taluk, Dindigul District, Tamilnadu. The soil samples were kept in polythene bags and labeled to avoid a mix up of the different soil samples. They finally were brought to the laboratory.



Fig1:soil sample 1 of sirumalai



Fig2: Soil Sample 2 of Poombarai



Fig3: Soil Sample 3 of Mannavanur

4.3 Instruments and Reagents

Atomic Absorption Spectrometer, Aquaregia (HCL, 3:1 ratio)

4.4 Preparation Aquaregia

Aqua regia is a corrosive acid mixture made by combining hydrochloric acid (HCL) .

Add the nitric acid to the hydrochloric acid. Do not add hydrochloric to nitric! The resulting solution with be a fuming red or yellow liquid. Aqua regia is used to dissolve gold, platinum, and palladium. The weighed samples were transferred to a 250 ml beaker and were digested with aqua regia acid solution. The soil samples were heated with 15ml of aqua regia (35%HCL and 65% high purity HNO₃, in 3:1 ratio). The resulting solution were cooled ,then filtered through what man filter paper.



Fig 5: Three different soil sample solution

The samples were cooled, and into a 50ml dilute to 50 ml volumetric flask and diluted to mark volume using de ionized water. Then the sample solution was analyze for concentration of Cd, Mn ,Cr and Pb using an atomic absorption spectrometer.

4.5 Preparation of Calibration Standards

4.6 Chemicals used

- 1 Copper (Cu) nitrate Pb (NO₃)₂
- 2 Potassium di chromate K₂Cr₂O₇
- 3 Manganese sulphate Mn So₄
- 4 Cadmium chloride Cd Cl₂ .2H₂O

4.3 Preparation of Standards Solutions

The standard solution for copper (Cu) prepared by using intermediate stock solution.

4.4 Analysis of Soil Samples

Prepare the stock solutions (eg. 4ppm 6ppm 8ppm etc). Switch on the atomic absorption spectrometer and computer. Open the software [Wfx 130] .Screen open, select operation – edit analytical method – continue. Select the element which you need to find and prepare the stock solution. Now switch on the compressor below .Turn on the air pipe on side of AAS [gas inlet] .Switch on the cylinder by tool.



Fig 6: 130 Software of AAS



Fig7: Soil sample injected to AAS

Quantitative Report for Cu

Run No: 00000139 Analysed On: 26-Feb-2026 10:53 AM
 Instrument Serial No: 5084/0322

Analysis Parameters

Analyst's Name: Muhammed Ariff Lab Name: Jamal Mohamed College, Trichy
 Measurement: Integrate Result accuracy: 2 (decimals)
 Integration Time: 3 (sec) Unit for results: ppm
 No. of samples: 25 Standard addition: No
 Blank after every sample: Yes

Instrument Conditions for Cu

Turret No: 1 Wavelength (nm): 324.70
 Current (mA): 5.0 Slit (nm): 0.5
 D2 Cur (mA): OFF Fuel(Litre/min): 1.20
 Pmt (v): 275.5 Burner Height (mm): 0.0
 Burner Horizontal: 0.00 Burner Angular: 0.00

Calculation Mode: LINEAR R-squared: 0.989

Standard/Sample Information

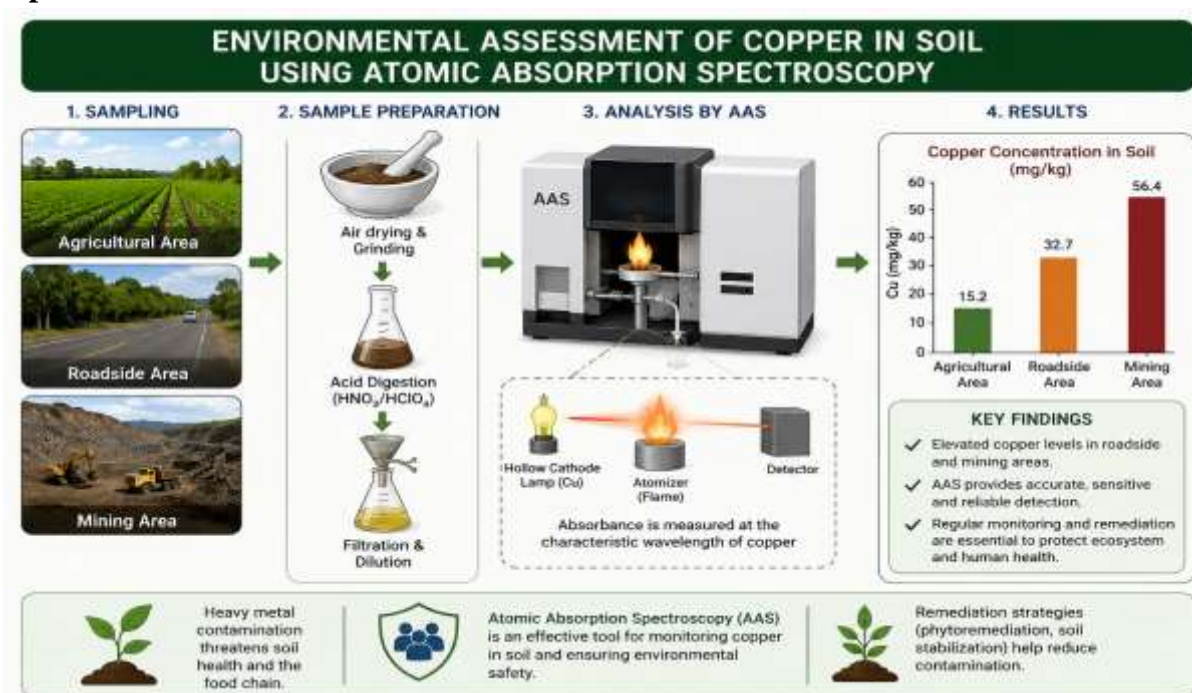
Std/Sample Name	Weight (gms)	Volume (ml)	Dilution Factor	Abs.	Conc. (ppm)	Conc. in Sample (ppm)
Std 1	-	-	-	0.655	5.00	
Std 2	-	-	-	1.096	10.00	
Std 3	-	-	-	1.538	15.00	
DISH1	1.00	1.00	1.00	0.090	0.26	0.26
Sample 2	1.00	1.00	1.00	2.579	24.88	24.88
Sample 3	1.00	1.00	1.00	0.140	0.75	0.75

5. RESULT & DISCUSSION

5.1 Analysis report of the soil sample:

The report presents the quantitative determination of Copper (Cu) in soil samples using a Thermo Scientific iCE FIOS Atomic Absorption Spectrometer at Jamal Mohamed College, Trichy . The analysis was carried out on 19-Feb-2026 (Run No: 00000135) using a linear calibration method with results expressed in ppm . The instrument was operated at a wavelength of 217.00 nm, which is the characteristic absorption wavelength for copper (Cu), with a lamp current of 9.0 mA, slit width of 0.5 nm, fuel flow rate of 1.37 L/min and an integration time of 3 seconds . These optimized conditions ensure selective and sensitive detection of copper (Cu) in the samples. For calibration, three standard solutions containing 5 ppm, 10 ppm, and 15 ppm of copper (Cu) were prepared, giving absorbance values of 0.139, 0.246, and 0.293 respectively . The calibration curve followed a linear equation ($Y = 0.0215 + 0.0197X$) with an R^2 value of 0.951 , indicating a reasonably good linear relationship between concentration and absorbance, though slight deviations from ideal linearity may be present. Three soil samples were analyzed under the same conditions. Sample 1 showed a negligible absorbance (-0.005) and was reported as 0.00 ppm, indicating that was not detected or was below the instrument’s detection limit . Sample 2 exhibited a high absorbance value of 0.389, corresponding to a calculated concentration of 18.65 ppm . This concentration exceeds the highest calibration standard (15 ppm), suggesting that the sample contains a comparatively high level of Copper (Cu) and may require dilution for more accurate quantification within the calibration range. Sample 3 showed a very low absorbance (0.019) and was also reported as 0.00 ppm, indicating negligible or undetectable copper (Cu) content . Overall, the report indicates that among the analyzed samples, only Sample 2 contains a significant concentration of copper, while Samples 1 and 3 are within safe or undetectable limits. The study demonstrates the effectiveness of Atomic Absorption Spectroscopy in detecting and quantifying heavy metal contamination in soil samples

6. Graphical Abstract:



7. CONCLUSION

The present study was carried out to determine the concentration of copper (Cu) in soil samples collected from various locations in from Sirumalai, Poombarai ,Mannavanur, Dindigul District, Tamil Nadu,. These locations were selected to understand the variation of copper levels in soils from different environmental conditions. The collected soil samples were properly prepared and analysed using instrumental analytical techniques. Standard copper solutions of 5 ppm, 10 ppm and 15 ppm were used to construct a calibration curve for quantitative analysis. The calibration graph showed a good linear relationship with a correlation coefficient ($R^2= 0.989$), indicating reliable instrument performance. This confirms that the analytical method used in the study is suitable for accurate determination of copper in soil samples. The calibration results ensured the precision and reliability of the measurements obtained. The analytical results revealed that the copper concentration in the analyzed soil samples varied depending on the sampling location. The measured copper concentrations were 0.26 ppm, 24.88 ppm and 0.75 ppm respectively. These differences in concentration may be influenced by natural soil composition, environmental conditions, and possible human activities in the Kodaikanal region. The presence of copper in soil is important as it plays a role in plant growth but excessive levels may affect environmental balance. Therefore, monitoring copper levels in soil is essential for environmental assessment. Overall, the study successfully determined the copper content in soil samples collected from different locations in Kodaikanal. The results demonstrate that instrumental techniques are effective for trace metal analysis. This study also contributes to understanding the distribution of copper in soil and supports future environmental monitoring studies.

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REFERENCES

1. Li, T. (2025). From soil health to agricultural productivity: The critical role of physico-chemical soil constraints. *Soil & Tillage Research*. <https://doi.org/10.1016/j.still.2025.106098>
2. Rahman, M. (2024). Assessment of soil physico-chemical characteristics in agricultural soils and their correlations with nutrient availability. *Current Issues in Soil Science*. <https://doi.org/10.9734/CISS/2024/v3i245031>
3. Li, Y. M. (2024). Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon*. <https://doi.org/10.1016/j.heliyon.2024.e27654>
4. Heavy metals in agricultural soils: Sources, influencing factors, and remediation strategies. (2022) *Toxics*. <https://doi.org/10.3390/toxics10060332>
5. Shukla, L., & Jain, N. (2022). A review on soil heavy metals contamination: Effects, sources and remedies. *Applied Ecology and Environmental Sciences*. <https://doi.org/10.12691/aees-10-2-3>
6. Wan, Y., Liu, J., Zhuang, Z., Wang, Q., & Li, H. (2024). Heavy metals in agricultural soils: Sources, influencing factors, and remediation strategies. *Toxics*,12(1),63, <https://doi.org/10.3390/toxics12010063>
7. Heavy metal pollution and transformation in soil: A comprehensive review of natural bioremediation strategies. (2025). *Journal of Umm Al-Qura University for Applied Sciences*. <https://doi.org/10.1007/s43994-025-00045-9>

8. Heavy metals in contaminated soils: Sources, chemistry and risks. (2025). In *Heavy Metals in Contaminated Soils*. Taylor & Francis.
9. Angon, P. B., et al. (2024). Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon*, 10. <https://doi.org/10.1016/j.heliyon.2024.e27654>
10. Xu, X., et al. (2023). A review on adsorption characteristics and influencing mechanism of heavy metals in farmland soil. *RSC Advances*. <https://doi.org/10.1039/D3RA01234A>
11. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. (2023). *Toxics*. <https://doi.org/10.3390/toxics11040345>
12. Zhakypbek, Y., et al. (2024). Reducing heavy metal contamination in soil and water using phytoremediation. *Plants*, 13(11), 1534. <https://doi.org/10.3390/plants13111534>
13. Islam, M. M., Saxena, N., & Sharma, D. (2024). Phytoremediation as a green and sustainable prospective method for heavy metal contamination: A review *RSC Sustainability*. <https://doi.org/10.1039/D4SU00056K>
14. Tamma, A. A., et al. (2025). Advancing phytoremediation: A review of soil amendments for heavy metal contamination management. *Sustainability*, 17, 5688. <https://doi.org/10.3390/su17095688>
15. Khan, S., Shakoor, M. B., & Shahid, M. (2022). Assessment of heavy metals in environmental samples using atomic absorption spectroscopy. *Environmental Monitoring and Assessment*. <https://doi.org/10.1007/s10661-022-09985-4>
16. Gupta, R., & Sahu, S. (2024). Application of atomic absorption spectroscopy in the analysis of trace metals: A review. *Journal of Analytical Chemistry*. <https://doi.org/10.1134/S1061934824700123>
17. Singh, A., Kumar, V., & Singh, R. (2023). Use of atomic absorption spectroscopy for determination of trace metals in soil and water. *Journal of Environmental Science and Technology*, 16, 45–52.
18. Maharani, E., Briliana, H., Putri, E., Faraditta, F., & Az-zhaffirah, (2024). A comprehensive review on atomic absorption spectroscopy: Principles, techniques, and applications. *Jurnal Ilmiah Wahana Pendidikan*, 10(15), 20–29. <https://doi.org/10.5281/zenodo.11543210>
19. Akhila, & Bhavana, N. (2025). Atomic absorption spectroscopy. *International Journal of Scientific Development and Research*, 10(10). Maharani, E., Briliana, H., Putri, E., Faraditta, F., & Az-zhaffirah, (2024). A comprehensive review on atomic absorption spectroscopy: Principles, techniques, and applications. *Jurnal Ilmiah Wahana Pendidikan*, 10(15), 20–29.
20. He, S. (2024). Research progress of the detection and analysis methods of trace metals in plants and soils. *Frontiers in Plant Science*. <https://doi.org/10.3389/fpls.2024.1378921>
21. Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2022). *Fundamentals of analytical chemistry*.
22. James, C. (2023). Quantitative analysis of heavy metals in biological samples by atomic absorption spectroscopy. *Journal of Chemical and Pharmaceutical Research*, 15(11), 23–24.
23. Li, Y. M. (2023). Atomic absorption spectroscopy (AAS): Principles, instrumentation, and analytical significance. *Analytical Chemistry: An Indian Journal*.
24. Operacz, A., Bigaj, A., Hap, K., & Kotowski, T. (2022). The effect of sample preparation and measurement techniques on heavy metals concentrations in soil: Case study from Kraków, Poland. *Applied Sciences*, 12(4), 2137. <https://doi.org/10.3390/app12042137>
25. Operacz, A., Bigaj, A., Hap, K., & Kotowski, T. (2022). The effect of sample preparation and measurement techniques on heavy metals concentrations in soil: Case study from Kraków, Poland, Europe. *Applied Sciences*, 12(4), 2137.

26. Bourgeois, W. J., & Hopke, P. K. (1988). Application of atomic absorption spectrophotometry with standard addition and external calibration for trace metal analysis in environmental samples. *Analytical Chemistry Letters*, 21(3), 345–359.
27. Camões, M. F., Santos, C. S., & De Almeida, A. M. (2003). Optimization of atomic absorption spectrometry conditions for heavy metals determination in water samples. *Journal of Hazardous Materials*, 103(1-2), 217–229. [https://doi.org/10.1016/S0304-3894\(03\)00219-4](https://doi.org/10.1016/S0304-3894(03)00219-4)
28. Lindenmayer, R. (2023). Atomic spectroscopy-based analysis of heavy metals in seaweed species. *Applied Sciences*, 13(8), 4764. <https://doi.org/10.3390/app13084764>
29. Veranica, V., et al. (2024). A review of atomic absorption spectrometry (AAS) for heavy metal content analysis. *Indonesian Journal of Chemical Engineering*, 2(1), 29–34.
30. Mir, A. R., Pichtel, J., & Hayat, S. (2021). Copper: Uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *Biometals*, 34(4), 737–759. <https://doi.org/10.1007/s10534-021-00306-z>
31. Yengkhom Sexona Singh, R. K. Singh and H. D. Sharma, “Assessment of heavy metals in soil samples of Tengnoupal district, Manipur using Atomic Absorption Spectroscopy,” *The Pharma Innovation Journal*, 2025. DOI: <https://doi.org/10.22271/tpi.2025.v14.i7c.26211>
32. Sichuan Cao, “Application of Atomic Absorption Spectrometry in Soil Environmental Monitoring,” *Frontiers in Science and Technology*, 2020. DOI: <https://doi.org/10.25236/FSST.2020.021507>
33. G. H. Vansickle, “Atomic Absorption Methods for the Determination of Elements in Soils and Rocks,” *U.S. Geological Survey Analytical Methods*, 1992. DOI: [https://doi.org/10.1016/0375-6742\(92\)90049-E](https://doi.org/10.1016/0375-6742(92)90049-E)
34. Ankit Chand Mishra et al., “Assessment of Heavy Metal Contamination in Industrial Soil of Gorakhpur Industrial Development Area (GIDA), India,” *Environmental Monitoring and Assessment*, 2019. DOI: <https://doi.org/10.1007/s10661-019-7480-1>
35. Sneha Gupta et al., “Heavy Metal Contamination in Soil and its Impact on Environment and Human Health,” *Environmental Science and Pollution Research*, 2018. DOI: <https://doi.org/10.1007/s11356-018-1923-2>
36. Sobia Akram et al., “Determination of Heavy Metals in Medicinal Plants using Atomic Absorption Spectrophotometry,” *Pakistan Journal of Pharmaceutical Sciences*, 2015. DOI: <https://doi.org/10.36721/PJPS.2015.28.3.723-728>
37. Afrasiab Khan Tareen et al., “Assessment of Heavy Metals in Groundwater of Pishin District, Baluchistan, Pakistan,” *Environmental Monitoring and Assessment*, 2017. DOI: <https://doi.org/10.1007/s10661-017-5942-7>
38. Sisay A. et al., “Determination of Cadmium Levels in Roadside Soil using Flame Atomic Absorption Spectroscopy,” *Environmental Systems Research*, 2016. DOI: <https://doi.org/10.1186/s40068-016-0062-5>
39. Fajar Nugraha et al., “Determination of Cadmium Contamination in Eggplants Sold in Traditional Markets of Pontianak City,” *IOP Conference Series: Earth and Environmental Science*, 2019. DOI: <https://doi.org/10.1088/1755-1315/308/1/012034>
40. Bhavtosh Sharma et al., “Heavy Metals in Water: Sources, Toxicity and Remediation Techniques,” *Environmental Monitoring and Assessment*, 2015. DOI: <https://doi.org/10.1007/s10661-015-4686-3>
41. Qasim Mezban Salih et al., “Health Risks Associated with Long-Term Exposure to Heavy Metals,” *Environmental Health and Preventive Medicine*, 2019. DOI: <https://doi.org/10.1186/s12199-019->

0792-5

42. Jose Ricardo Forero-Mendieta et al., “Validation of Analytical Methods for Determination of Manganese using Graphite Furnace Atomic Absorption Spectrometry in Biological Samples,” *Journal of Trace Elements in Medicine and Biology*, 2018. DOI: <https://doi.org/10.1016/j.jtemb.2018.02.010>
43. Jindy M. Jindy, Amin K. Qasim and Sagvan A. Mohamad, “Evaluation of Soil Pollution by Some Heavy Metals via Atomic Absorption Spectrophotometer Technique in Zakho District, Kurdistan Region, Iraq,” *Science Journal of University of Zakho*, 2020. DOI: <https://doi.org/10.25271/sjuoz.2020.8.4.765>
44. Sohni Sinha and Ashutosh Kumar Tripathi, “Assessment of Heavy Metals Contamination in Roadside Soil Dust in Doon Valley, Uttarakhand,” *Indian Journal of Forestry*, 2016. DOI: <https://doi.org/10.54207/bsmps1000-2016-L3HRF3>
45. H. M. S. Al-Diwaniyah et al., “Determination of Heavy Metals in Soil Samples near a Power Plant Using Atomic Absorption Spectrometry,” *Applied Chemical Engineering*, 2024.
46. T. T. Ashetu et al., “Determination of Heavy Metals (Ag, Cd and Pb) Using FAAS in Soil Samples of Eastern Hararghe Area,” *International Journal of Scientific Research in Chemical Sciences*, 2021.
47. A. Kabata-Pendias, “Trace Elements in Soils and Plants,” CRC Press, 2011. DOI: <https://doi.org/10.1201/b10158>
48. M. Tuzen, “Determination of Heavy Metals in Soil, Mushroom and Plant Samples by Atomic Absorption Spectrometry,” *Microchemical Journal*, 2003. DOI: [https://doi.org/10.1016/S0026-265X\(03\)00035](https://doi.org/10.1016/S0026-265X(03)00035)
49. Piccolo, A., & Drosos, M. (2025). The essential role of humified organic matter in preserving soil health. *Chemical and Biological Technologies in Agriculture*. <https://doi.org/10.1186/s40538-025-00730-0>