

RESEARCH ARTICLE

COMPARATIVE STUDY OF FIVE DIGESTION METHODS FOR ELEMENTAL ANALYSIS OF SOIL USING ATOMIC ABSORPTION SPECTROPHOTOMETER

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ABSTRACT

The study evaluates the efficiency of different digestion methods for the recovery of heavy metals (Ni, Pb, Cu, Cd and Zn) from soil samples using Atomic Absorption Spectrophotometry (AAS) method. Various acid mixtures including aqua regia (HCl + HNO₃), aqua regia with sulfuric acid (HCl + HNO₃ + H₂SO₄), hydrofluoric acid with perchloric acid (HF + HClO₃), perchloric acid with sulfuric acid (HClO₃ + H₂SO₄), and a combination of perchloric acid, nitric acid, and hydrofluoric acid (HClO₃ + HNO₃ + HF) were evaluated for effective recovery after spiking with 10, 20 and 50 ppm of studied metals. The percentage recovery of each metal were evaluated to assess the effectiveness of these digestion methods. At 10 ppm spike levels, aqua regia demonstrated high recoveries for Zn, and Cd, while HF + HClO₃ showed moderate efficiency across metals. At higher spike levels (20 ppm and 50 ppm), aqua regia consistently showed high recovery rates for Zn, Cd and Pb, but moderate recoveries for Cu, and Ni. The addition of sulfuric acid to aqua regia generally reduced recovery rates slightly. HF + HClO₃ and HClO₃ + H₂SO₄ exhibited lower recoveries compared to aqua regia, indicating potential limitations in extracting metals at higher concentrations. The results suggest that aqua regia remains a robust choice for extracting a wide range of metals from soil samples, particularly at lower spike levels. However, the choice of digestion method should consider specific metal recovery requirements and the soil matrix characteristics to ensure accurate environmental and geochemical analyses.

KEYWORDS

Digestion methods, Heavy metal recovery, Aqua regia, Soil samples, Environmental analysis, Geochemical analysis.

1. INTRODUCTION

The determination of trace elements in environmental and biological samples requires precise and accurate analytical methods. Among these, the digestion process plays a crucial role in breaking down complex matrices to release the trace elements into a measurable form. Acid digestion involves the use of strong acids, such as nitric acid or a combination of acids, to decompose samples (Jones, 2021). Several acid digestion methods for the determination of heavy metals in soil and other environmental samples have been studied. So many studies have reported the use aqua regia in open system and also the use of hydrofluoric acid in closed system, which is seen as total digestion for breaking silicate matrices (Chen and Ma, 1998). Due to the large variation in metal content obtained by different methods, the digestion of samples is the principal factor contributing to uncertainty of analytical results (Al-Harashseh et al., 2009).

There are different methods of ashing samples, dry ashing involves the combustion of samples at high temperatures to remove organic matter, leaving behind an ash residue containing the trace elements (Johnson et al., 2023). This method is simple but can lead to loss of volatile elements. Wet ashing combines acid digestion with heating to achieve complete digestion of samples, often providing better recoveries of certain elements (Kumar and Singh, 2022). Enzyme digestion uses specific enzymes to

break down organic matter, which can be particularly useful for biological samples but may be limited by the availability of suitable enzymes for different sample types (Brown et al., 2024).

Acids in combination are preferred for certain inorganic matrices and are generally more advantageous for decomposition of organic. Digestion is important because it shows the length of exposure of the matrix to the oxidizing acid. The length of exposure can increase exothermic processes which will increase the extent of solubilization of the metal of interest from the biological matrix as well as the loss of these metals through volatilization (Gavriloaei, 2008).

Numerous instruments have been employed for element detection and quantitation, including inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-optical emission spectrometry (ICP-OES), instrumental neutron activation analysis (INAA), electrothermal-atomic absorption spectrometry (ET-AAS), and flame atomic absorption spectrometry (FAAS) (Lakshmi Priya and Geetha, 2011; Molina-Villalba et al. 2015; Grassini-Delyle et al., 2019; Kucera and Kofronova, 2021; Izydorczyk et al., 2021).

This study evaluates the efficiency of five different digestion methods for the recovery of heavy metals (Ni, Pb, Cu, Cd, Zn) from soil samples spiked with different concentration using Atomic Absorption Spectrophotometer

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(AAS). Various acid mixtures, including aqua regia ($\text{HCl} + \text{HNO}_3$), aqua regia with sulfuric acid ($\text{HCl} + \text{HNO}_3 + \text{H}_2\text{SO}_4$), hydrofluoric acid with perchloric acid ($\text{HF} + \text{HClO}_3$), perchloric acid with sulfuric acid ($\text{HClO}_3 + \text{H}_2\text{SO}_4$), and a combination of perchloric acid, nitric acid, and hydrofluoric acid ($\text{HClO}_3 + \text{HNO}_3 + \text{HF}$) were used and the percentage recovery of each metals were studied to assess the effectiveness of these digestion methods.

2. MATERIALS AND METHODS

The evaluation of digestion methods for the recovery of heavy metals from soil samples involves several key steps, including sample preparation, spiking, digestion, and analysis.

2.1 Sample Preparation

Soil samples were collected from Agricultural farm in Ekiti State University, Ado-Ekiti at various locations and homogenized to ensure uniformity. Soil samples obtained were air dried and crushed to pass through 2mm sieve. In each of the digestion methods, the inner walls of the beakers were washed with 2ml of deionized water to prevent the loss of the sample and dried using lab dryer.

2.2 Digestion Methods

2.2.1 Wet acid digestion using Aqua Regia ($\text{HCl} + \text{HNO}_3$) (1:3)

Aqua regia, a mixture of hydrochloric acid and nitric acid in a 3:1 ratio, was used to digest the soil samples. This method is known for its effectiveness in dissolving metals from soil matrices (Smith and Lee, 2022). 1g each of the grinded samples was weighed into clean Teflon beaker. 20ml of freshly prepared aqua regia (1:3 $\text{HCl} + \text{HNO}_3$) was carefully added. The beaker was gently shaken to allow the sample dissolve in the acid mixture and then heated in the fume hood until the sample was digested. After about an hour, heating was removed and the beaker allowed to cool, this was filtered and the filtrate made up to mark in a 25ml standard flask and the then subjected to AAS analysis.

2.2.2 Wet acid digestion using Aqua Regia with Sulfuric Acid ($\text{HCl} + \text{HNO}_3 + \text{H}_2\text{SO}_4$) (1: 3:1)

The addition of sulfuric acid to aqua regia was tested to assess any improvements in metal recovery. This combination aims to enhance the digestion of refractory minerals (Johnson et al., 2023). The procedure in 2.2.1 was repeated with $\text{HCl} + \text{HNO}_3 + \text{H}_2\text{SO}_4$ in ratio 1:3:1.

2.2.3 Wet acid digestion using Hydrofluoric Acid with Perchloric Acid ($\text{HF} + \text{HClO}_3$) (1: 3)

This method involves using hydrofluoric acid, known for breaking down silicate minerals, combined with perchloric acid to ensure complete digestion of the soil matrix (Brown et al., 2024). The procedure in 2.2.1 was repeated with $\text{HF} + \text{HClO}_3$ in ratio 1:3.

2.2.4 Wet acid digestion using Perchloric Acid with Sulfuric Acid ($\text{HClO}_3 + \text{H}_2\text{SO}_4$) (3: 1)

The mixture of perchloric acid and sulfuric acid was tested for its ability to digest organic matter and release heavy metals from soil samples (Kumar and Singh, 2022). The procedure in 2.2.1 was repeated with $\text{HClO}_3 + \text{H}_2\text{SO}_4$ in ratio 3:1.

2.2.5 Wet acid digestion using Perchloric Acid, Nitric Acid, and Hydrofluoric Acid ($\text{HClO}_3 + \text{HNO}_3 + \text{HF}$) (3: 1: 1)

This method combines three powerful acids to achieve comprehensive digestion of complex soil matrices, aiming for maximum recovery of heavy metals (Jones, 2021). The procedure in 2.2.1 was repeated with $\text{HClO}_3 + \text{HNO}_3 + \text{HF}$ in ratio 3:1:1.

2.3 Analytical procedures

After digestion, the samples were diluted appropriately and analyzed for heavy metal content using Atomic Absorption Spectrophotometer at Centre for Energy Research and Development (CERD) at Obafemi Awolowo University, Ile-Ife, Nigeria.

2.4 Percentage recovery

To demonstrate the validity of the studied methods, the precision and accuracy, the percentage of recovery were carried out. The percentage of recovery is a crucial parameter for method validation. 1g of already analysed soil samples were spiked with 10, 20 and 50 ppm of heavy metals (Ni, Pb, Cu, Cd and Zn) solution, and thus analysed with the five different digestion methods as earlier discussed. Spiking was performed to simulate contamination levels and to evaluate the recovery efficiency of different digestion methods (Jones, 2021).

3. RESULTS AND DISCUSSION

Aqua regia, a mixture of hydrochloric acid (HCl) and nitric acid (HNO_3), is well-known for its strong oxidizing properties, making it highly effective for metal extraction. Aqua regia is particularly efficient for extracting Cd, Zn and Pb as evident in the results (Figure 1-3) obtained from the present study. They established Aqua regia to dissolve most base metals in geochemical and environmental studies (Tessier, 1979; Kingston and Jassie, 1988).

The tri-acid mixture combines the oxidizing power of nitric acid, the complexing action of hydrochloric acid, and the dehydrating effect of sulfuric acid ($\text{HCl} + \text{HNO}_3 + \text{H}_2\text{SO}_4$) notably revealed effective for Zn and Cd extraction, most importantly with 20ppm spiking. Zinc with slightly lower values compared to aqua regia but still significant for $\text{HCl} + \text{HNO}_3 + \text{H}_2\text{SO}_4$. The combination of these three acids enhances the breakdown of complex matrices (Allen et al., 1986; Kalemekiewicz and Sočo, 2004).

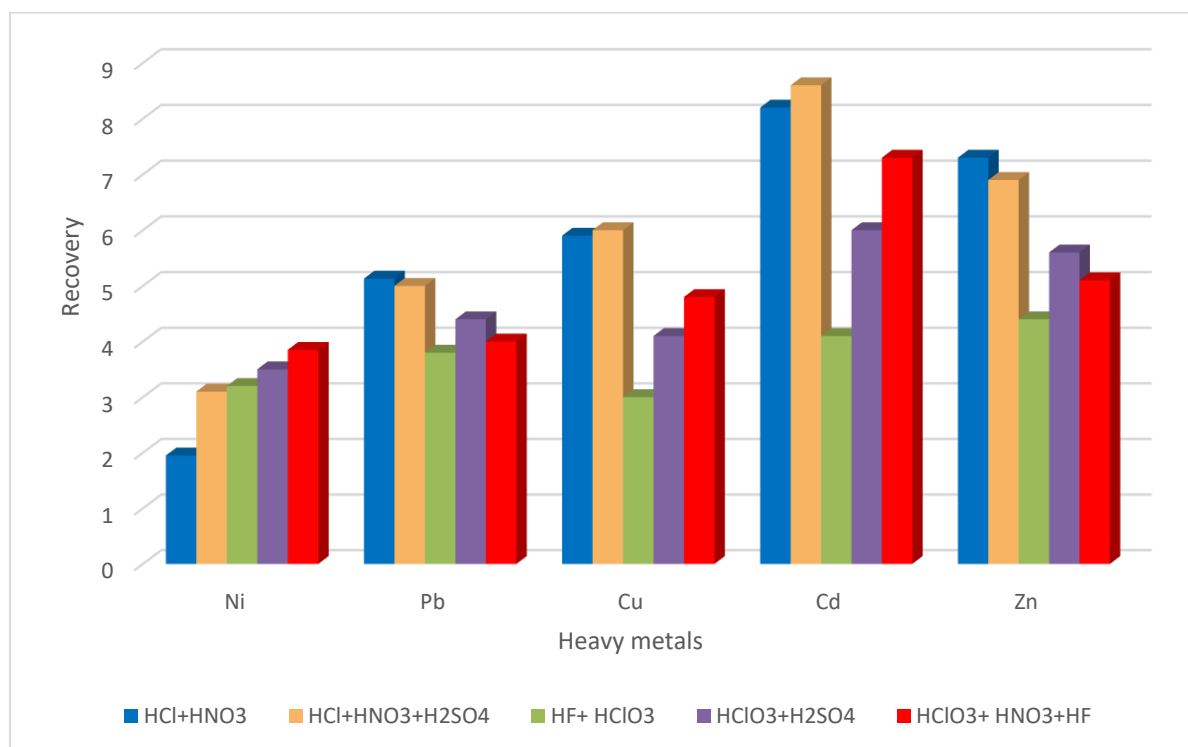


Figure 1: Recovery rate of heavy metals after 10ppm spiking using the five digestion methods

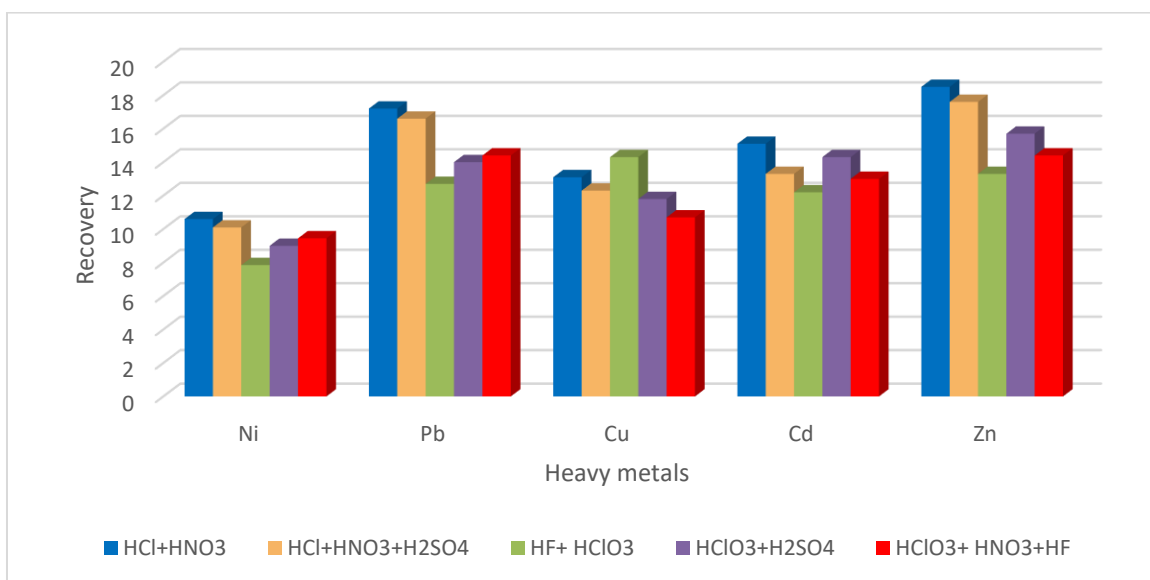


Figure 2: Recovery rate of heavy metals after 20ppm spiking using the five digestion methods

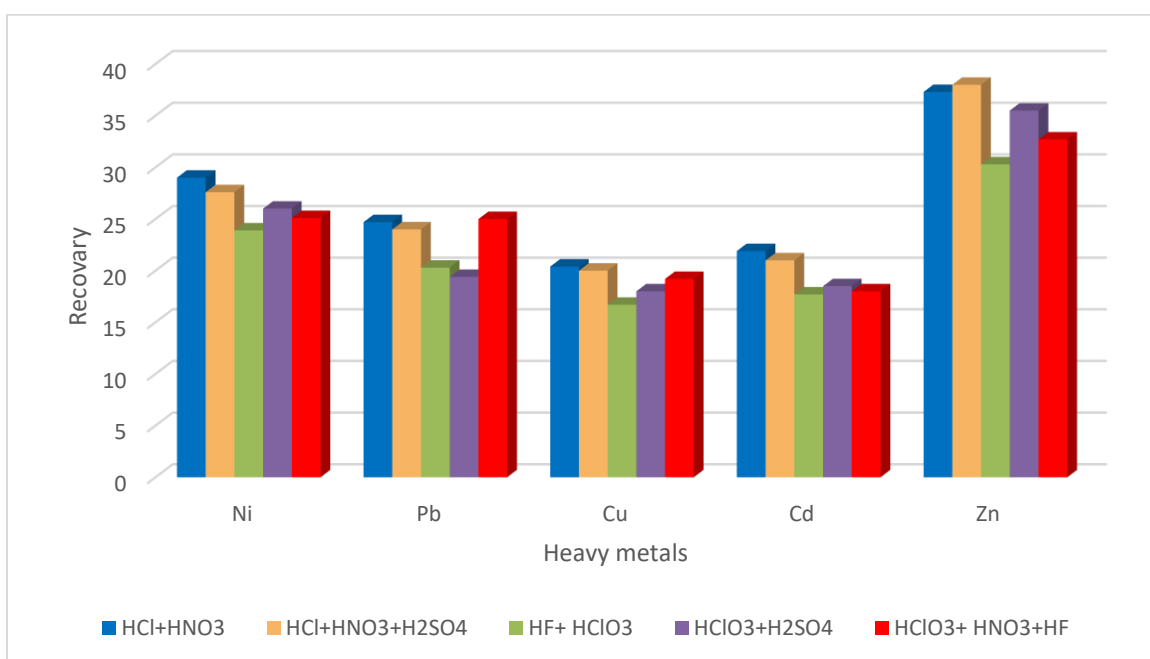


Figure 3: Recovery rate of heavy metals after 50ppm spiking using the five digestion methods

Hydrofluoric acid (HF) and perchloric acid (HClO₃) demonstrates moderate effectiveness for Pb, but yields lower concentrations for Cd, Cu and Zn in cases low spiking concentration. HF is highly efficient in dissolving silicates, making it valuable for geochemical analysis of minerals and soils with significant silicate content (Parry and Hobbs, 1993; Kılıç and Özer, 2006).

The combination of perchloric acid (HClO₃) and sulfuric acid (H₂SO₄) was effective for Zn in most cases, but shows lower extraction efficiency for Pb and Ni. The combination is particularly useful for samples where strong oxidizing conditions required to decompose organic matter and minerals (Anderson, 1966; Simsek and Kartal, 2001). The tri-acid mixture of perchloric acid (HClO₃), nitric acid (HNO₃), and hydrofluoric acid (HF) is highly effective for a variety of sample. The results of HClO₃+ HNO₃+HF revealed effective removal for Cd, Pb and Zn, with high variability with other studied metals. The variability in the levels of the studied metals across the five digestion processes is illustrated in Figure 1- 3, showing the following order: HCl+HNO₃ > HCl+HNO₃+H₂SO₄ > HClO₃+H₂SO₄ > HClO₃+HNO₃+HF > HF+HClO₃ in most cases.

Figure 4(a-e) showed the percent recovery of heavy metals (Ni, Pb, Cu, Cd, and Zn) after spiking with 10 ppm (parts per million) using five different acid digestion methods. Aqua regia revealed moderate to high recovery rates across all metals. Particularly high recoveries are observed for Cd (81.8%) and Zn (72.4%), indicating strong effectiveness in extracting these metals from the soil matrix (Allen et al., 1986). Ni (19.2%) and Pb (52.0%) exhibit lower recoveries compared to other methods, suggesting

potential limitations in extracting these metals efficiently (Kingston and Jassie, 1988). The addition of sulfuric acid (H₂SO₄) to aqua regia enhances the recovery rates for Ni (30.6%), Cu (59.4%), and Cd (85.8%) compared to aqua regia alone (Tessier et al., 1979). Pb (49.9%) and Zn (68.6%) show slightly lower recoveries compared to aqua regia. This method improves overall recovery efficiency across most metals, particularly noticeable for Ni and Cd.

This combination (HF + HClO₃) shows varied recovery rates with moderate efficiency overall. Ni (31.7%), Cu (29.6%), and Zn (43.8%) exhibit moderate recoveries, while Pb (21.0%) and Cd (40.8%) show lower recoveries compared to other methods (Kalembkiewicz and Sočo, 2004). HF + HClO₃ may not be as effective for Pb extraction, indicating limitations in recovering this metal from soil samples.

Perchloric acid (HClO₃) combined with sulfuric acid (H₂SO₄) shows improved recovery rates across all metals compared to aqua regia and HF + HClO₃. Notable improvements are observed for Ni (34.7%) and Cd (59.8%), indicating better extraction efficiency for these metals (Kılıç and Özer, 2006). Pb (43.9%) and Zn (55.6%) also show moderate to high recovery rates, demonstrating the effectiveness of this method in extracting a range of metals from soil samples.

The combination of perchloric acid (HClO₃), nitric acid (HNO₃), and hydrofluoric acid (HF) shows the highest overall recovery rates for Cd (72.3%) and Zn (50.7%) among all methods (Allen et al., 1986). Pb (39.9%) and Ni (38.2%) also exhibit moderate to high recovery rates, indicating comprehensive extraction capabilities of this method.

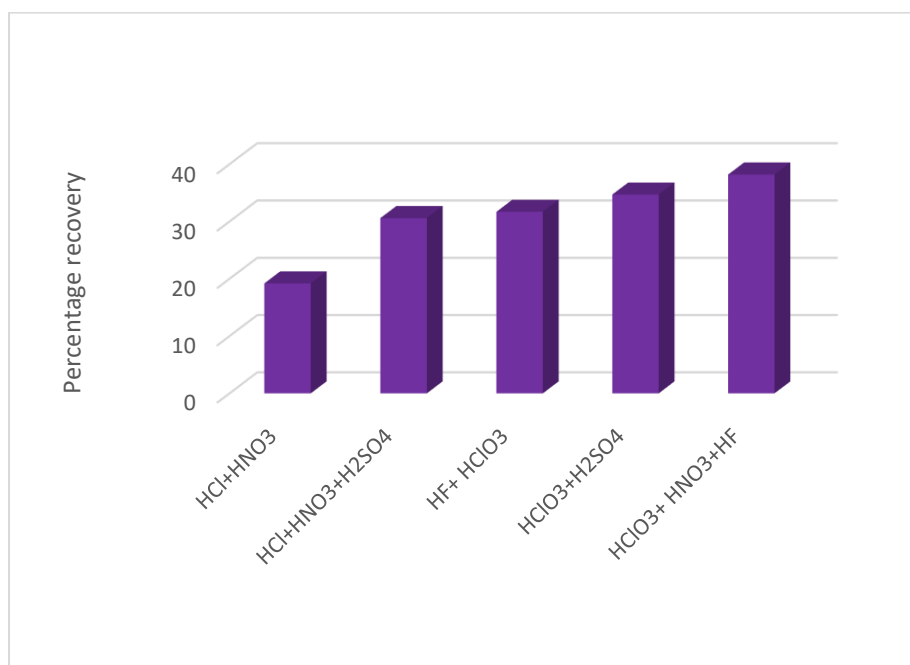


Figure 4(a): % recovery of Ni after 10ppm Spiked

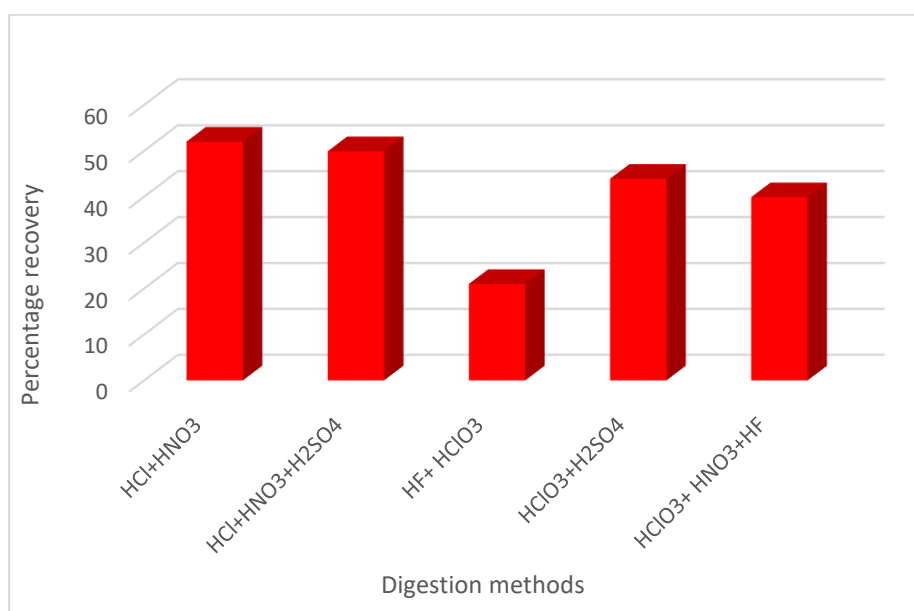


Figure. 4(b): % recovery of Pb after 10ppm Spiked

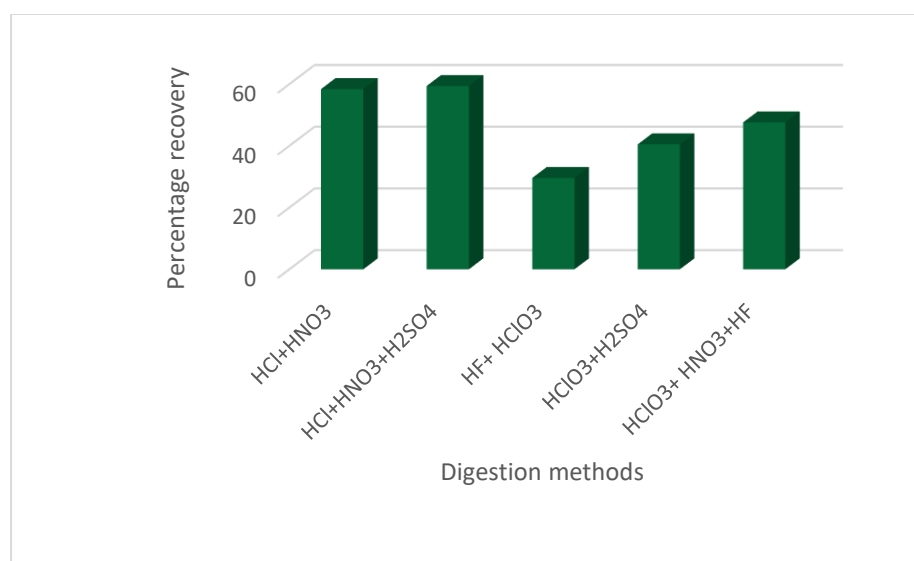


Figure 4(c): % recovery of Cu after 10ppm Spiked

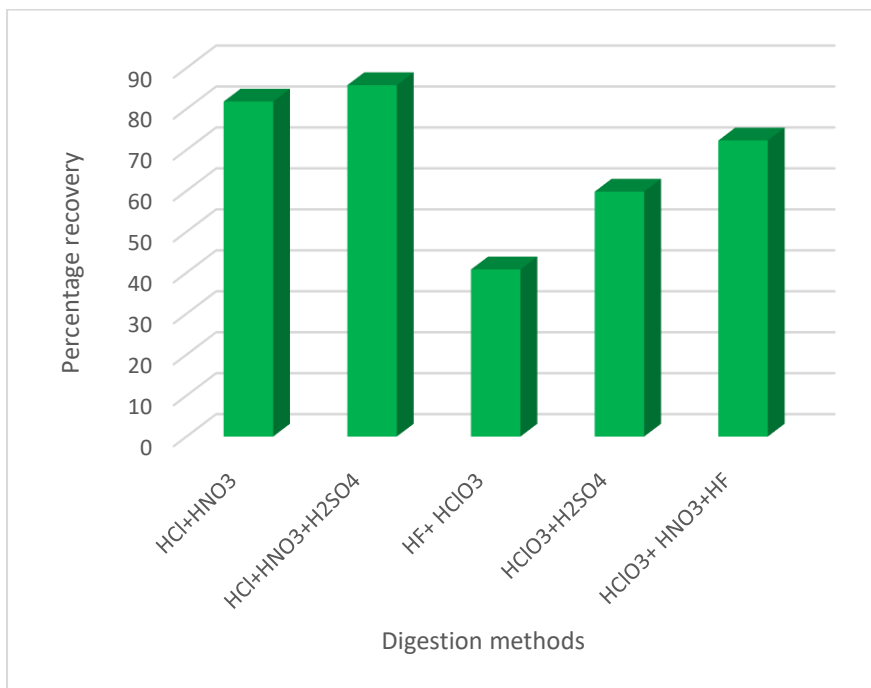


Figure. 4(d): % recovery of Cd after 10ppm Spiked

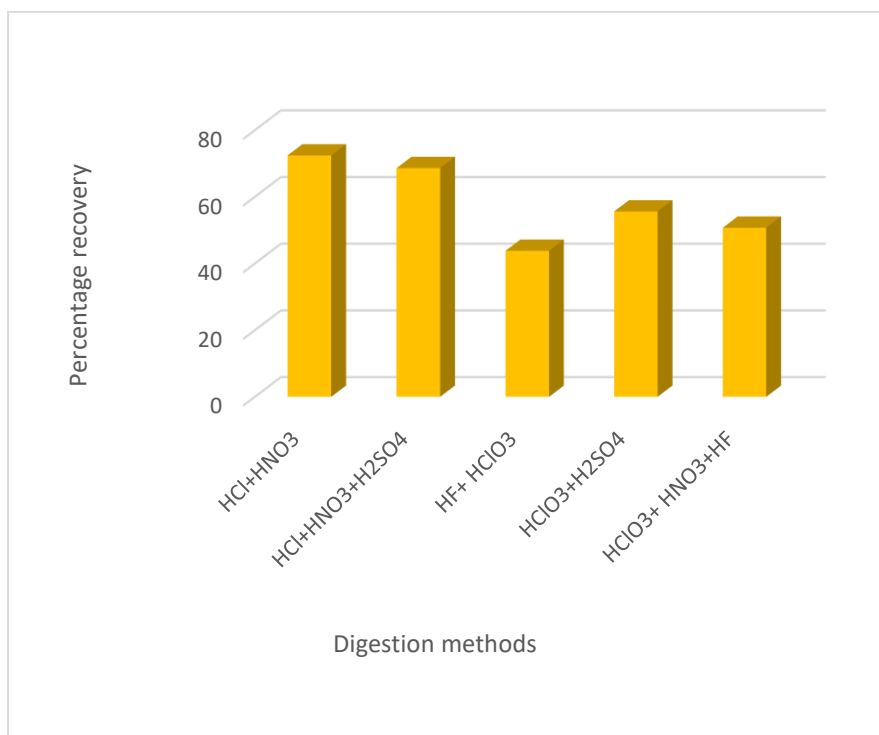


Figure 4(e): % recovery of Zn after 10ppm Spiked

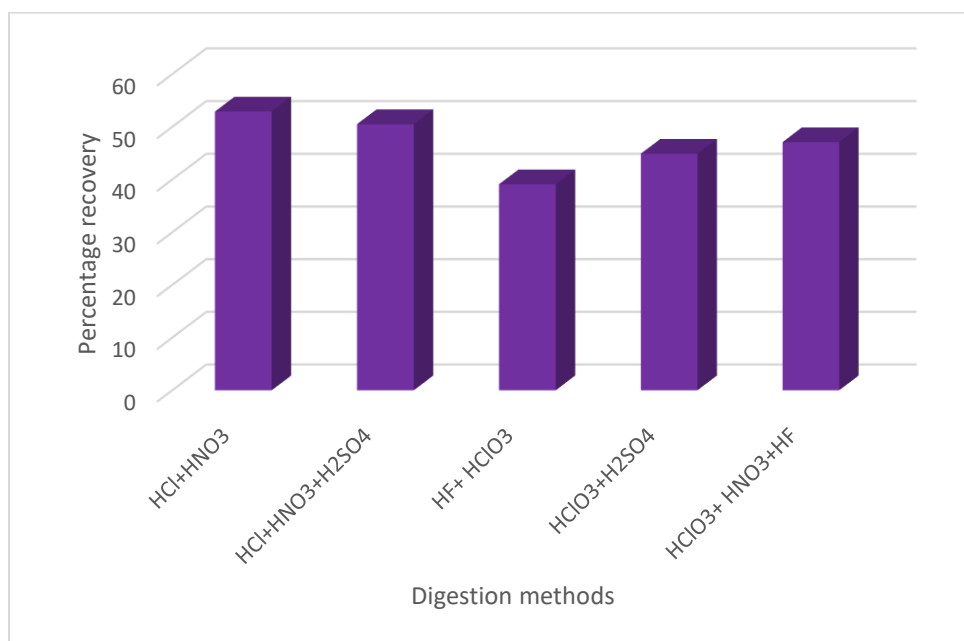
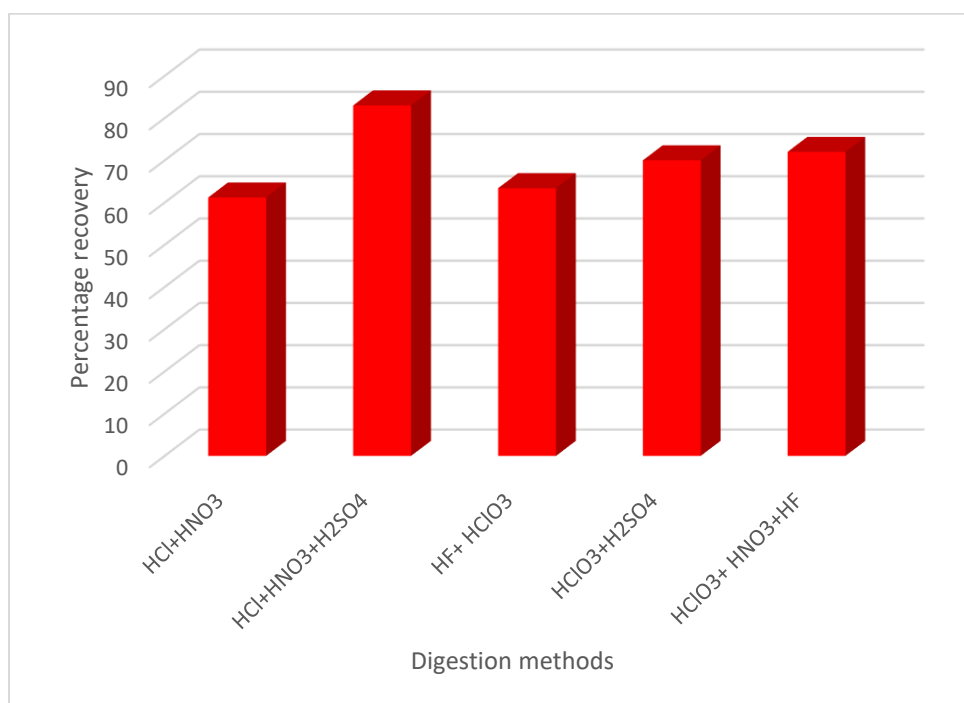
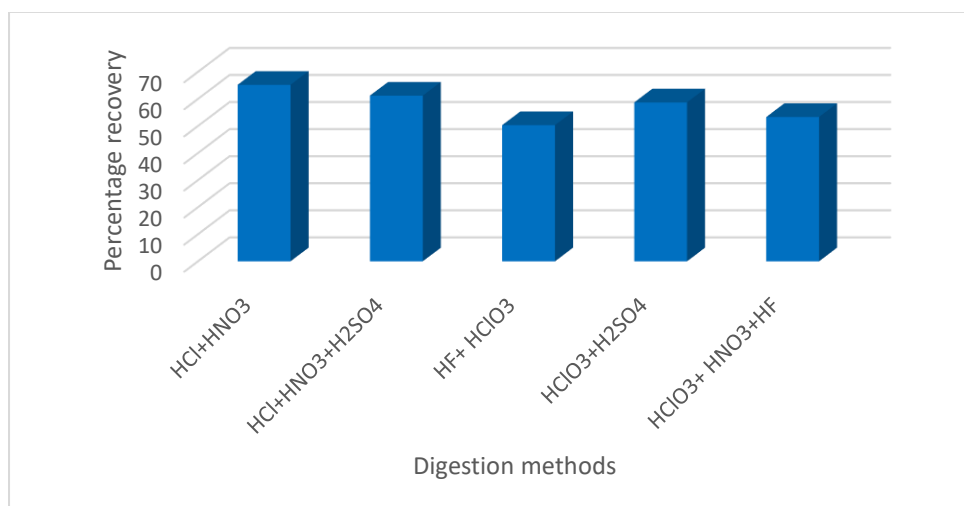
Figure 4(a-e): Percentage recoveries of heavy metals in the soil sample after spiking with 10ppm

The concentration of heavy metals in the soil samples after spiking with 20 ppm varied with the digestion method (Figure 5(a-e)). The variability could be the as a result of minerals used in the digestion and most importantly, the composition ratio of the digested acids. Spiking with 20 ppm significantly favours the Cd concentration as compared with 20 and 50 ppm. Comparative study of this methods and spiking revealed that Cd is significantly favoured as compared with other metals when the concentrations are low. Aqua regia (HCl+HNO₃) demonstrated high recovery rates for most metals, particularly notable for Zn (92.2%), Pb (85.9%), Cd (75.4%) and Cu (65.2%). It revealed effective extraction capabilities for these metals even at higher concentrations (Kingston and Jassie, 1988). Ni (52%) also showed good recovery, indicating the robustness of aqua regia in extracting a wide range of metals from soil samples.

The addition of sulfuric acid (H₂SO₄) to aqua regia slightly reduced the recovery rates for most metals compared to aqua regia alone. However, it still maintained high recoveries for Zn (68.8%), Pb (83%), Cd (85.6%) and

Cu (61.2%) (Tessier et al., 1979). Ni (50.5%) showed moderate recovery rates, indicating effective extraction but slightly lower compared to aqua regia. This combination (HF+ HClO₃) exhibited moderate to good recovery rates across metals. HF+ HClO₃ showed effectiveness but may have limitations in achieving high recoveries compared to aqua regia and other combinations.

Perchloric acid (HClO₃) combined with sulfuric acid (H₂SO₄) demonstrated moderate to good recovery rates across all metals. Pb (70%), Cd (59.8%) and Cu (58.7%) showed the highest recoveries, followed by Zn (55.6%). Ni (45%) revealed moderate recovery, indicating effective extraction but with slightly lower rates compared to aqua regia. The combination of perchloric acid (HClO₃), nitric acid (HNO₃), and hydrofluoric acid (HF) showed moderate to good recovery rates across metals. Pb (72%) exhibited the highest recoveries. Ni (47.1%) showed moderate recovery, indicating effective extraction but with slightly lower rates compared to aqua regia and other methods.

**Figure 5(a):** % recovery of Ni after 20ppm Spiked**Figure. 5(b):** % recovery of Pb after 20ppm Spiked**Figure 5(c):** % recovery of Cu after 20ppm Spiked

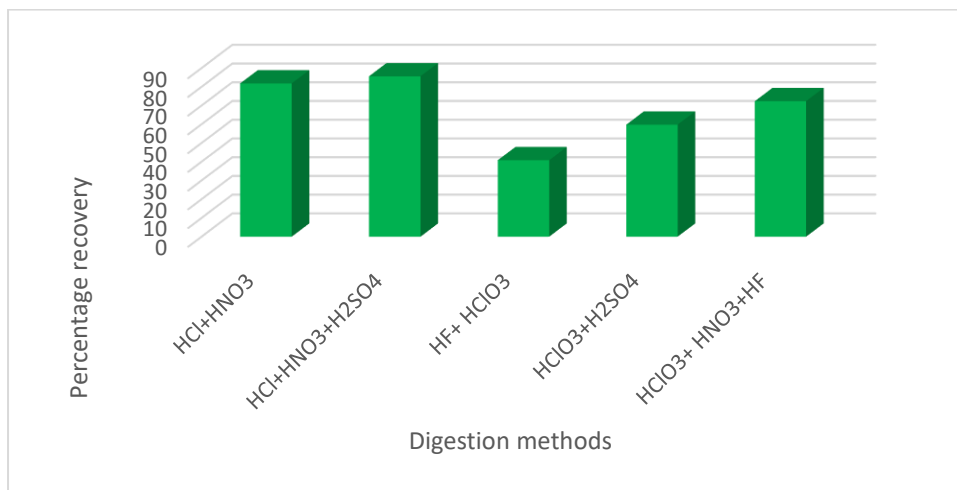


Figure 5(d): % recovery of Cd after 20ppm Spiked

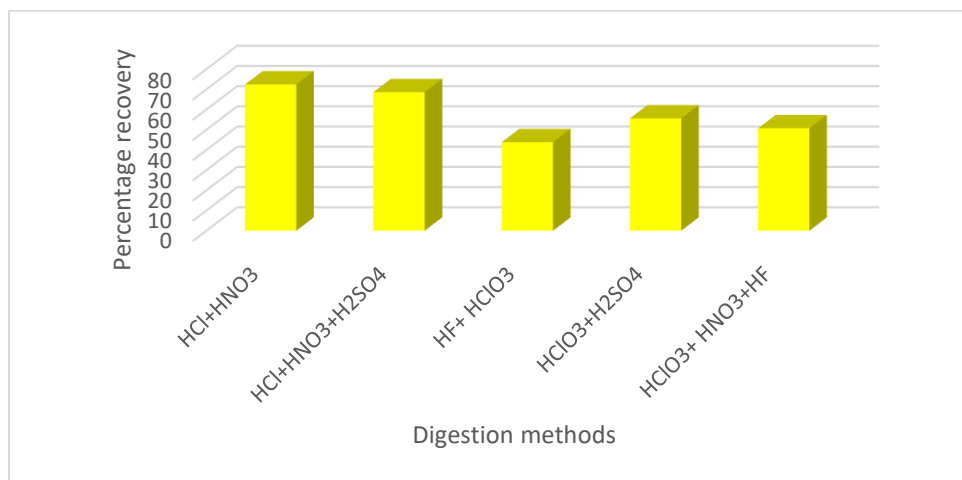


Figure 5(e): % recovery of Zn after 20ppm Spiked

Figure 5(a-e): Percentage recoveries of heavy metals in the soil sample after spiking with 20ppm

Figure 6 (a-e) showed the percentage recovery of the metals after 50ppm spiking. Aqua regia (HCl+HNO₃) shows moderate to high recovery rates for Zn (74.5%) and Ni (57.9%) at 50 ppm spike levels. However, Pb (49.4%), Cu (40.7%), and Cd (43.8%) exhibit lower recovery rates compared to lower spike levels, indicating potential saturation or matrix effects at higher concentrations.

The addition of sulfuric acid (H₂SO₄) to aqua regia slightly, reduces the recovery rates for most metals compared to aqua regia alone, with exception of Zn. Zn (75.9%) shows the highest recovery, followed by Ni (55.1%). Pb (48.0%), Cd (42.0%), and Cu (40.0%) exhibit lower recovery rates. This method maintains effectiveness but shows limitations in achieving high recoveries for all metals at higher spike levels. HF + HClO₃ exhibits lower recovery rates across all metals compared to aqua regia and other combinations. Zn (60.1%) showed the highest recovery, followed by

Ni (47.1%). This method may not be as effective for extracting metals at higher spike levels due to lower recovery rates observed.

Perchloric acid (HClO₃) combined with sulfuric acid (H₂SO₄) revealed moderate recovery rates for Zn (70.9%) and Ni (51.9%). However, Pb (38.7%), Cu (35.9%), and Cd (37.0%) exhibit lower recovery rates compared to aqua regia and other combinations (Kılıç and Özer, 2006). This method demonstrates effective extraction capabilities but with reduced recovery rates at higher spike levels. The combination of perchloric acid (HClO₃), nitric acid (HNO₃), and hydrofluoric acid (HF) shows varied recovery rates across metals. Zn (65.3%) exhibited the highest recovery, followed by Ni (50.1%). This method demonstrates effectiveness but may have limitations in achieving consistent high recoveries across all metals at higher spike levels.

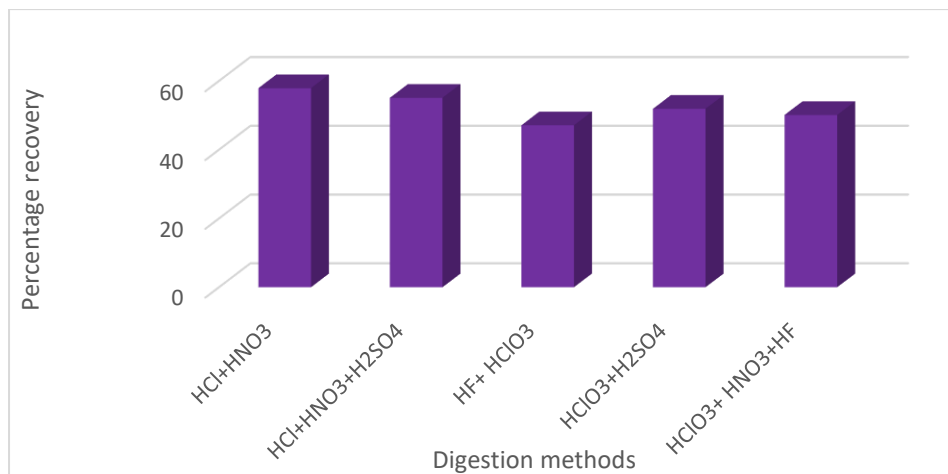


Figure 6(a): % recovery of Ni after 50ppm Spiked

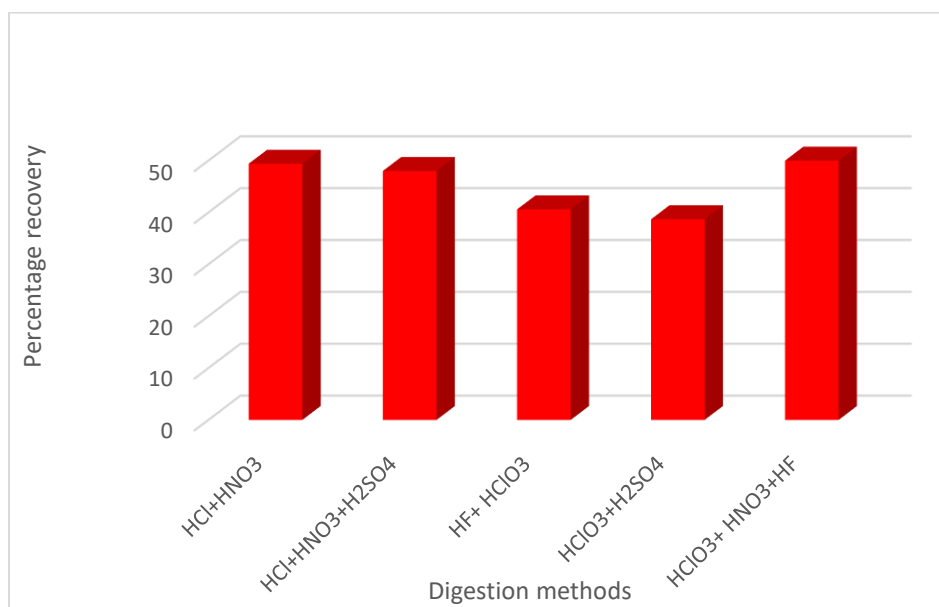


Figure. 6(b): % recovery of Pb after 50ppm Spiked

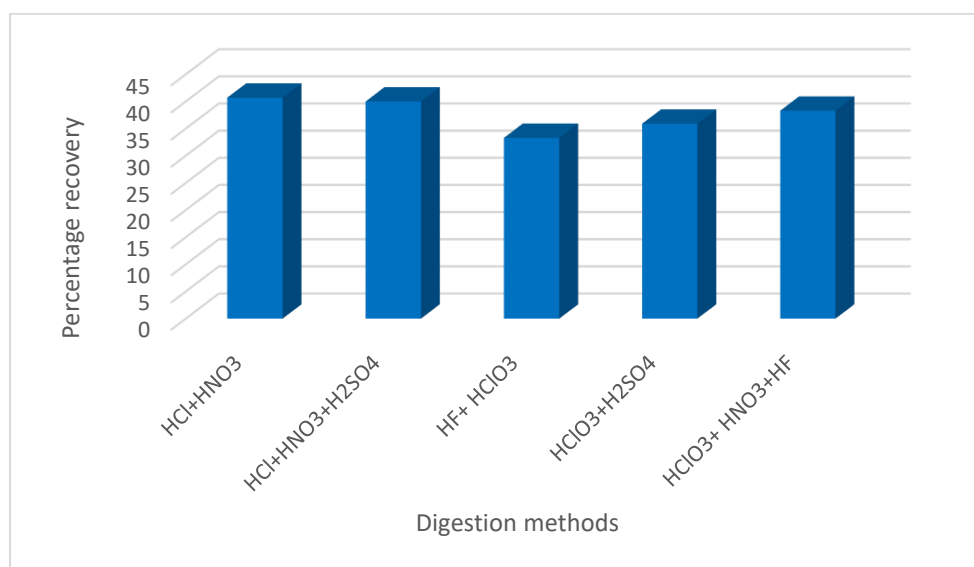


Figure 6(c): % recovery of Cu after 50ppm Spiked

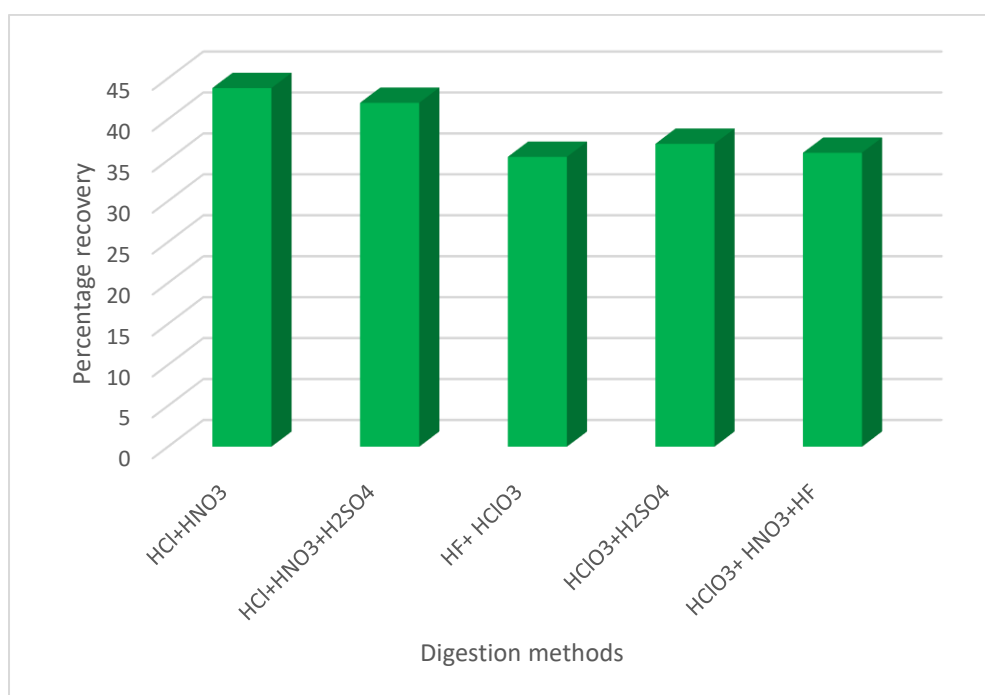


Figure. 6(d): % recovery of Cd after 50ppm Spiked

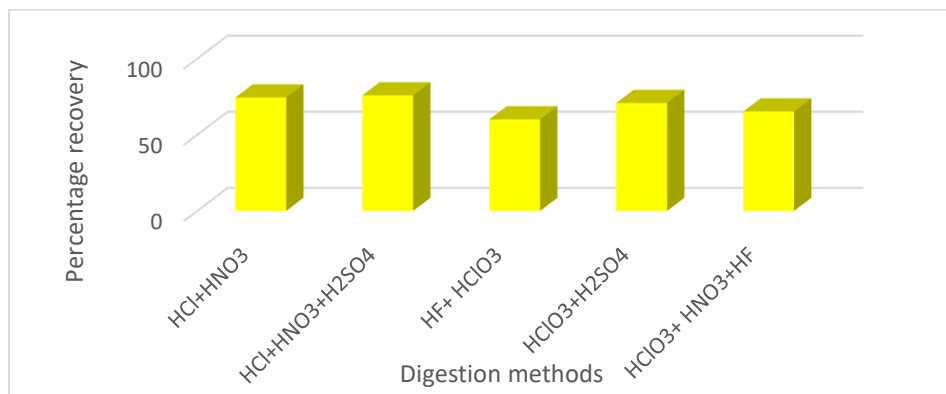


Figure 6(e): % recovery of Zn after 50ppm Spiked

Figure 6(a-e): Percentage recoveries of heavy metals in the soil sample after spiking with 50ppm

4. CONCLUSION

At 10 ppm spike levels, aqua regia demonstrated high recoveries for Cd, Zn, and Cu, while HF + HClO₃ showed moderate efficiency across metals. At higher spike levels (20 ppm and 50 ppm), aqua regia consistently showed moderate to high recovery rates, but lower recoveries for Ni and Cu. The addition of sulfuric acid to aqua regia generally reduced recovery rates slightly at higher spiked levels. The HF + HClO₃ and HClO₃ + H₂SO₄ exhibited lower recoveries compared to aqua regia, indicating potential limitations in extracting metals at higher concentrations. The findings suggest that aqua regia remains a robust choice for extracting a wide range of metals from soil samples, particularly at lower spike levels. However, the choice of digestion method should consider specific metal recovery requirements and the soil matrix characteristics to ensure accurate environmental and geochemical analyses of toxic metals.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

- Al-Harashsheh, M., Kingman, S., Somerfield, C., and Ababneh F., 2009. Microwave-assisted total digestion of sulfide ores for multi-element analysis, *Analytica Chimica. Acta*, 638, Pp. 101.
- Allen, S. E., Grimshaw, H. M., Rowland, A. P., and Moore, P. D., 1986. Chemical analysis. In Moore, P. D., and Chapman, S. B. (Eds.), *Methods in plant ecology* (2nd ed., Pp. 285-344). Blackwell Scientific Publications.
- Anderson, J. M., 1966. *Biochemical and microbial mineralization of organic matter*. John Wiley and Sons.
- Brown, P., Davis, T., and Miller, L., 2024. Advances in enzyme digestion methods for trace element analysis. *Journal of Analytical Chemistry*, 76(2), Pp. 123-134.
- Chen, M. and L.Q. Ma. 1998. Comparison of four EPA digestion methods for metal analysis using certified and Florida soils. *J. Environ. Qual.*, 27, Pp. 1294-1300.
- Gavriloaiei, T., 2008. Considerations of microwave- assisted digestion: towards a conceptual model. *Analele Stiintifice De Universitatii A.I. Cuza Din Iasi. Sect.2, Geologie*, 54, Pp. 19-30.
- Grassin-Delyle S., Martin M., Hamzaoui O., 2019. A high-resolution ICP-MS method for the determination of 38 inorganic elements in human whole blood, urine, hair and tissues after microwave digestion. *Talanta*, 199, Pp. 228-237.
- Izydorczyk G., Mironiuk M., Basladyńska S., Mikulewicz M., and Chojnacka K., 2021. Hair mineral analysis in the population of students living in the Lower Silesia region (Poland) in 2019: comparison with biomonitoring study in 2009 and literature data. *Environmental Research*, Pp. 196 doi: 10.1016/j.envres.2020.110441.110441
- Johnson, R., White, S., and Black, J., 2023. Comparative study of dry ashing and wet ashing methods for trace metal determination. *Analytical Methods*, 15(8), Pp. 567-579.
- Jones, A., 2021. Acid digestion techniques in environmental sample preparation. *Environmental Chemistry Reviews* 22(4), Pp. 245-258.
- Kalembkiewicz, J., and Sočo, E., 2004. Investigations of chemical extractability of heavy metals from soil. *Polish Journal of Environmental Studies*, 13(1), Pp. 37-42.
- Kılıç, E., and Özer, M., 2006. The use of hydrofluoric acid in the determination of heavy metals in sediments and soils by atomic absorption spectrometry. *Turkish Journal of Chemistry*, 30(6), Pp. 707-715.
- Kingston, H. M., and Jassie, L. B., 1988. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. *Analytical Chemistry*, 60(15), Pp. 1473-1476.
- Kingston, H.M., and Jassie, L.B., 1986. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. *Anal. Chem.*, 58, Pp. 2534 -2541
- Kucera J., and Kofronova K., 2021. Determination of as by instrumental neutron activation analysis in sectioned hair samples for forensic purposes: chronic or acute poisoning. *Journal of Radioanalytical and Nuclear Chemistry*, 287(3), Pp. 769-772.
- Kumar, R., and Singh, M., 2022. Optimization of wet ashing protocols for accurate trace metal analysis. *International Journal of Environmental Analytical Chemistry*, 102(9), Pp. 789-801.
- Lakshmi Priya M. D., and Geetha A., 2011. Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. *Biological Trace Element Research*, 142(2), Pp.148-158.
- Molina-Villalba I., Lacasana M., Rodriguez-Barranco M., 2015. Biomonitoring of arsenic, cadmium, lead, manganese and mercury in urine and hair of children living near mining and industrial areas. *Chemosphere*, 124, Pp. 83-91.
- Parry, S. J., and Hobbs, L., 1993. Advances in atomic spectrometry and analytical techniques in earth sciences. *Journal of Analytical Atomic Spectrometry*, 8(5), Pp. 651 - 656.
- Simsek, S., and Kartal, S., 2001. Digestion procedures for the determination of trace metal contents in environmental samples. *Analytica Chimica Acta*, 450(1-2), Pp. 87-94.
- Tessier, A., Campbell, P. G. C., and Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, 51(7), Pp. 844-851