Research Article

Utilizing ICP-OES for Elemental Analysis of Soil Samples in Forensic Science

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ABSTRACT

Soil is a crucial form of physical evidence in forensic investigations. Each type of soil possesses unique characteristics that serve as identifying markers, making it admissible as evidence in a court of law. The trace, major, and minor elements in the soil contribute to a distinctive elemental profile unique to each location. In this research, we aimed to classify and differentiate soil groups using ICP-OES for forensic science purposes. The study analyzed soil samples from various groups, including forest soil, orchard soil, and rice field soil. The results revealed that elements in the Ca, Mg, and Mn groups displayed high standard deviation values, while elements in the Zn, Cr, Cu, and Pb groups demonstrated low standard deviation values. Soil samples from two provinces in the central region of Thailand displayed similar elemental concentrations, whereas samples from the northeastern region showed distinct elemental concentrations compared to those from the central region. However, statistical analysis using one-way ANOVA to compare elemental concentrations across different soil types revealed no significant differences at the 0.05 level among the soil sample groups. Although ICP-OES is highly effective, its application in analyzing soil as chemical evidence may not always yield successful results in a single attempt. Repeated testing is often necessary to identify the most appropriate elements. It is recommended to examine the area's conditions and data on soil usage in the location where soil evidence is collected for comparison before selecting elements for analysis, as this helps minimize costs and save time.

Keywords: Soil, Forensic science, Element, ICP-OES

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Introduction

Soil is a complex mixture of minerals, organic matter (both living and decomposed organisms), air, and water. These components interact in various ways, making soil one of Earth's most dynamic and essential natural resources. Soil is a limited natural resource. While it is considered renewable because it is constantly forming, this process occurs at an extremely slow rate. For instance, it can take several hundred years or more to create just one inch of topsoil. The rate of soil formation differs globally: the slowest rates are found in cold, dry areas (over 1,000 years), while the fastest rates occur in hot, wet regions (several hundred years) [1].

Soil materials can change over time and across different spaces due to environmental factors such as leaching, weathering, microbial activity, and bushfires. Soil properties are typically more variable in areas influenced by human activities, such as agriculture, farming, or horticulture, compared to undisturbed natural environments like native forests or grasslands. In rural areas, crops like vegetables are commonly grown in carefully managed fields. These crops are of significant economic value, and the soil they grow in is often intensively managed through practices such as irrigation, fertilization, and pesticide application. As a result, soil materials exhibit variation at different stages of landscape development. The variability of soil materials across locations and at any given time is especially important in forensic soil science. For example, differences between undisturbed natural soil beneath native vegetation, which has a high organic matter content, and nearby disturbed, farmed soil can change gradually over a distance of about 1 meter. In contrast, soil affected by a bushfire can show sharp, distinct differences, even at a micro-scale within millimeters. This suggests that no two locations on Earth share identical Earth materials [2, 3].

Soil is frequently used as evidence in forensic investigations [4], because it can transfer and adhere to surfaces, particularly finer particles such as clay, silt, and organic matter. Larger quartz particles (e.g., those greater than 2 mm in size) tend to have poor retention on clothing, shoes, and carpets. In contrast, finer soil particles (e.g., those smaller than 50–100 μm) are more likely to be found, though typically in small quantities [5]. Forensic soil examination typically involves analyzing the color, elemental composition, and microbiological properties of soil. Color analysis is most effective when there is significant variation in the soil's color. However, microbiological analysis is susceptible to contamination, both at the crime scene due to soil transfer and in the laboratory. Additionally, delays in discovering a crime scene or submitting evidence for examination can impact the accuracy of the results. In contrast, elemental analyses of soil tend to remain stable over time due to the consistent composition of soil elements. Therefore, soil elemental analysis is a valuable tool for examining evidence in criminal cases [6].

Many elemental analysis techniques are available, including atomic absorption spectrometry (AAS), inductively coupled plasma-atomic/optical emission spectroscopy (ICP-AES/ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) [6]. However, due to the nature of soil evidence collected from a crime scene, it is often impossible to determine the minimum sample volume required for testing, and the composition of the elements cannot be predicted.

ICP-OES is particularly well-suited for forensic soil sample examination due to its wide wavelength range and superior tolerance to complex matrices compared to AAS. It offers enhanced resistance to interference and enables the simultaneous analysis of multiple elements, whereas AAS is typically limited to detect one or a few elements at a time. Furthermore, the detection limits of AAS are relatively high, restricting its effectiveness for trace analysis. In contrast, ICP-OES provides greater sensitivity across a broader range of elements and significantly lower detection limits. This capability allows for the detection and quantification of elements even at ultratrace levels. The numerous advantages of ICP-OES contribute to a more efficient workflow, saving valuable time and resources [7, 8].

Thailand's diverse topography and climate lead to a wide variety of soil resources, with more than 300 distinct soil series identified [9]. As a result, criminal activities in Thailand can take place across a broad range of environmental settings. Crimes that aim to conceal or hide evidence are often committed in secluded areas, far from populated communities, and out of sight from the public. These locations often include private spaces such as abandoned lands, forests, orchards, farms, or fields, which have been the scenes of various criminal activities.

This research focuses on analyzing the elemental composition of soils using ICP-OES from various regions with similar usage characteristics. The results aim to establish guidelines for forensic investigations, particularly in identifying the provenance of soil in evidentiary contexts.

Materials and Methods

Study sites

To ensure consistency with soil samples typically found at crime scenes in Thailand, the samples were categorized into three main groups: forest soil, orchard soil, and rice field soil. The samples were collected from three provinces: Nakhon Pathom (Area 1), Samut Sakhon (Area 2), and Nakhon Ratchasima (Area 3). The collection areas were selected from provinces with similar land-use characteristics, including two provinces from the same region and one from a different region for comparison. Nakhon Pathom and Samut Sakhon are located in the central region, while Nakhon Ratchasima is situated in the northeastern region of Thailand. The soil profile is shown in Table 1 and Figure 1.

Table 1 Locations from which soil samples were collected based on GPS coordinates.

Area	Soil Types	Soil profile	Geographical coordinates (GMS)
A	1	sandy, sandy loam, and arboretum forest	13°59'21.9"N 99°57'51.3"E
B	1	grey, clay, and mangrove forest	13°30'08.9"N 100°16'15.8"E
C	1	black, loam, and roadside forest	14°54'42.5"N 101°50'57.2"E
A	2	black, loam, and banana orchard	13°41'39.2"N 100°14'51.1"E
B	2	black, loam, and banana orchard	13°35'58.7"N 100°18'20.7"E
C	2	black, loam, and banana orchard	14°54'40.6"N 101°50'53.4"E
A	3	grey, clay, and wetland	13°58'32.9"N 99°59'14.1"E
B	3	grey, clay, and wetland	13°37'35.9"N 100°15'38.4"E
C	3	grey, clay, and wetland	14°54'51.0"N 101°51'03.7"E

A= Nakhon Pathom; B= Samut Sakhon; C= Nakhon Ratchasima

1= forest soil; 2= orchard soil; 3= rice field soil

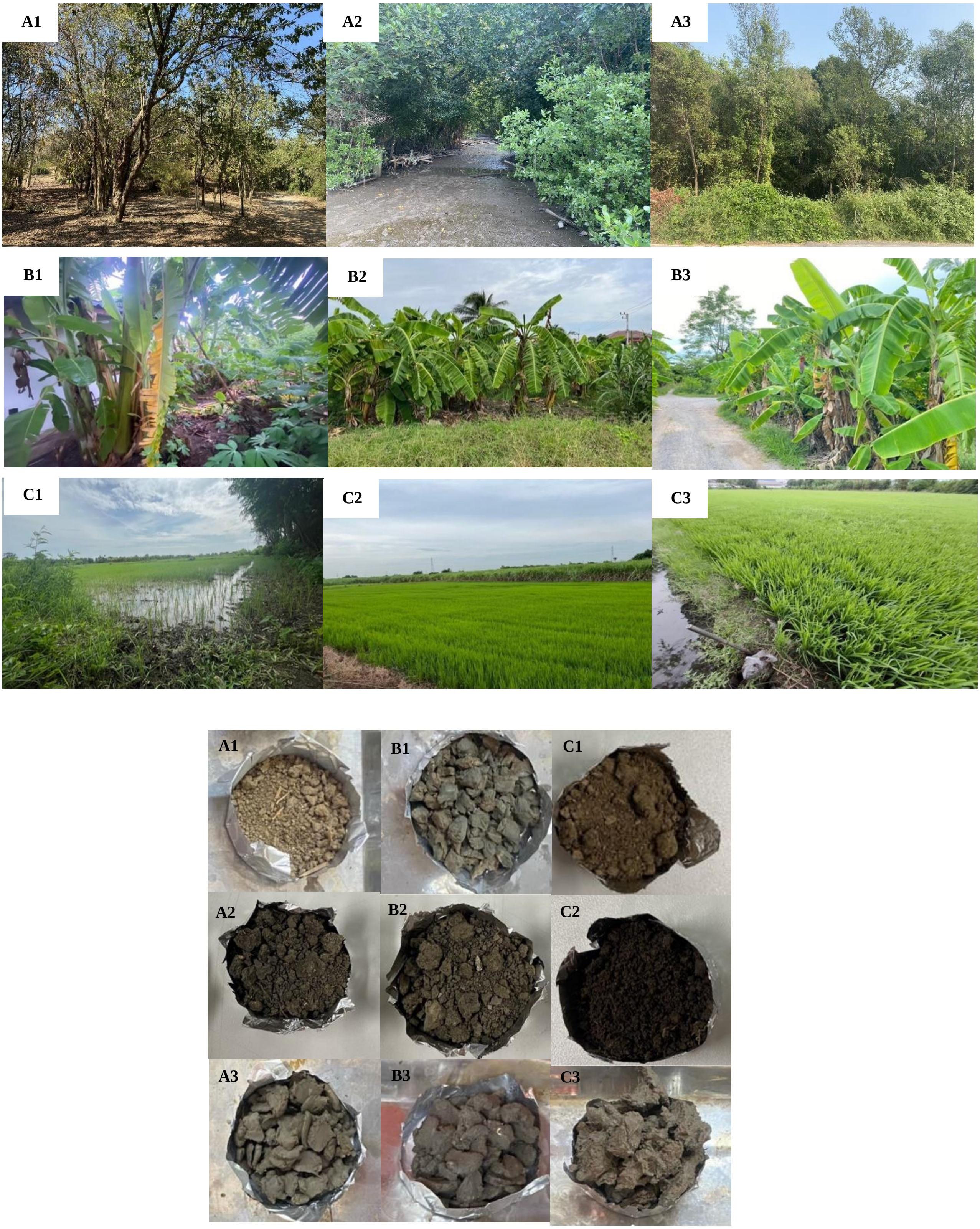

Figure 1 The soil profiles at specific sites are as follows: A1, B1, and C1 represent forest soil from Nakhon Pathom, Samut Sakhon, and Nakhon Ratchasima, respectively; A2, B2, and C2 represent orchard soil from the same provinces; and A3, B3, and C3 represent rice field soil from Nakhon Pathom, Samut Sakhon, and Nakhon Ratchasima, respectively.

Soil sampling

Soil samples were randomly collected from each sampling area. Five samples will be taken from different spots within a 1 m^2 sampling grid: one from the center and four from the corners [10]. The soil samples were collected horizontally from the top 0–5 cm of the surface layer in the fall of 2024. Approximately 500 grams of soil were collected from each location using a clean spatula, yielding a total of about 2.5 kilograms of soil per sampling area. The collected samples were placed in separate sterile plastic bags at each location and were subsequently analyzed to determine the trace element composition of the soil.

Soil sample preparation

Soil samples from each area were dried in a hot air oven at 80°C for 24 hours, ground into a fine powder, and sieved through a 2 mm sieve to remove larger particles such as plastic, leaves, twigs, and rocks [11]. After drying, five individual soil samples of 1 gram (dry weight) each were collected from different spots. The dried samples were homogenized to create a total of 5 grams (dry weight) of soil per area. This mixed soil was then reweighed, and 1 gram (dry weight) was set aside for the subsequent soil digestion process.

To prepare for digestion, 1 gram (dry weight) of soil was added to a digestion vessel. Then, 40 mL of concentrated (60–70%) HNO_3 and 35% H_2O_2 were added to the sample for digestion. The sample was placed into a Graphite Digester at 45°C for 3 hours. After digestion, the sample was cooled, and the digestate was filtered through Whatman^{TM} No. 41 filter paper. The filtrate was collected in a 50 mL volumetric flask. In total, nine samples were prepared and analyzed using ICP-OES.

Analysis of elements in the soil samples

All analyses were performed using an Avio 200 ICP-OES (PerkinElmer, Shelton, Connecticut, USA) under the conditions and parameters detailed in Table 2 [12], with the elements and their corresponding wavelengths listed in Table 3. The soil samples analyzed in this study were collected exclusively from the surface and represent a preliminary examination intended to guide further forensic soil investigations. Specific elements were selected as indicators for tracking and monitoring soil elemental concentrations. These elements were chosen based on their significance and pre-determined quantification criteria. The critical elements analyzed included calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), chromium (Cr), and lead (Pb), totaling eight elements [4, 13]. Calibration standards for these eight elements were used for both calibration and quality control (QC) of the ICP-OES. Multi-element calibration ICP-OES standards were obtained from Peak Performance (TruQ Calibration Std Kit EPA 200.7, PerkinElmer, USA). Solutions were prepared by diluting stock standard solutions, with concentration curves constructed for the ranges of 0.1, 0.5, 1, 2, and 5 mg/L. To estimate precision, each soil sample analyzed for three times. The ICP-OES is set to perform repeated analysis of each soil sample three times.

Table 2 Avio 200 ICP-OES Instrumental parameters and conditions.

Parameter	Baffled Cyclonic Spray Chamber, Low-Flow GemCone Nebulizer and 1.2 mm Injector
RF power	1500 Watts
Nebulizer flow	0.35 L/min
Auxilliary flow	0.6 L/min
Plasma flow	10 L/min
Sample flow rate	1.0 to 4.0 mL/min
Equilibration Time	15 s
Torch Position	-3

Table 3 Elements, wavelengths, view, and standards.

Element	**Wavelength (nm)**	**Plasma View**
Ca	317.933	Radial
Mg	285.213	Axial
Mn	257.610	Radial
Zn	206.200	Radial
Fe	238.204	Axial
Cr	267.716	Axial
Cu	327.393	Axial
Pb	220.353	Axial

Statistical analysis

The mean values, SD, and percent relative standard deviation (% RSD) of the selected elements were computed. A one-way ANOVA was conducted to analyze the concentration of each element across soil groups. Differences were considered significant at the 0.05 level. When significance was observed ($p < 0.05$), Tukey's post hoc test was employed for multiple comparisons. All statistical analyses were performed using SPSS software, version 29.0.2.0.

Results and Discussion

Calibrations of ICP-OES

Calibration standards for eight elements were used for calibration, with concentration ranges of 0.1, 0.5, 1, 2, and 5 mg/L, correlation coefficients (r^2) for all analytes were >0.999, and the precision for individual elements in each sample was typically less than 10% relative standard deviation (RSD), except for some Ca, Mg, and most Fe, which were detected at saturation levels, preventing the calculation of RSD. This confirms the accuracy and precision of the method.

Element analysis soil samples

The concentrations of each element in different areas, analyzed using ICP-OES, are presented in Table 4. The table shows that elevated mean levels of elements exceeding 1,000 mg/kg include Ca, Mg, Mn, Fe in certain soil types. Among these, Fe is the most abundant across all soil groups, with the highest average levels found in soil samples from rice fields in all provinces. In contrast, Cr, Pb, and some groups of Cu were measured at levels below 1,000 mg/kg. The lowest concentration of Pb was found in soil from in rice field soil in Nakhon Ratchasima Province.

The average percentage of each element across the three soil groups shows that the highest concentrations are found in the four most abundant elements: Fe (42.23%), Mg (28.26%), Ca (15.30%), and Mn (14.11%), as illustrated in the pie chart in Figure 2.

This study emphasizes notable variations in the elemental composition of soil samples from different locations and soil types. In particular, calcium (Ca), magnesium (Mg), manganese (Mn), and iron (Fe) showed substantial variability, with standard deviation (SD) values, and the %RSD for Ca, Mg, and Mn was especially high, while the lowest standard deviation was found for iron (Fe). These results align with a study on trace element detection in soil samples from plain areas in Thailand using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The study found elevated levels of elements such as Mg, Al Ca, and Fe (greater than 1,000 mg/kg), while elements like Zn, Li, V, Cr, Pb, Nd, Ce, La, As, Cu, and Co were present in moderate amounts (typically less than 1,000 mg/kg). Additionally, elements with low concentrations (less than 10 mg/kg), including Sm, Gd, Pr, and Cs, were also detected [14].

Table 4 Concentrations of the element (mean, standard deviation, mg/kg) in soil samples from different areas and soil types.

Area	Soil Types	Concentration of element (mg/kg)							
		Ca	**Mg**	**Mn**	**Zn**	**Fe**	**Cr**	**Cu**	**Pb**
A	1	4,298.19	97,608.59	48,804.29	67.84	97,608.59	20.50	79.55	9.27
B	1	97,608.59	97,608.59	48,804.29	71.74	97,608.59	22.45	76.13	8.78
C	1	4,185.94	715.96	210.35	62.47	97,608.59	16.59	270.86	3.90
	Mean	35,364.24	65,311.05	32,606.31	67.35	97,608.59	19.85	142.18	7.32
	SD	53,905.21	55,940.99	28,055.73	4.656	-	2.982	111.45	2.969
	%RSD	152.43%	85.65%	86.04%	6.91%	-	15.04%	78.39%	40.58%
A	2	97,608.59	97,608.59	48,804.29	43.92	97,608.59	21.47	81.02	10.25
B	2	4,361.64	97,608.59	48,804.29	195.71	97,608.59	29.28	357.74	6.83
C	2	97,608.59	907.76	474.38	237.19	6,027.33	14.64	21.96	7.81
	Mean	66,526.27	65,374.98	32,694.32	158.94	67,081.50	21.80	153.57	8.30
	SD	53,836.15	55,830.25	27,903.29	101.74	52,874.46	7.33	179.26	1.76
	%RSD	80.92%	85.40%	85.35%	64.02%	78.82%	33.61%	116.73%	21.23%
A	3	97,608.59	97,608.59	318.20	25.87	97,608.59	22.94	67.35	15.62
B	3	4,104.44	97,608.59	107.86	68.33	97,608.59	17.57	224.01	12.69
C	3	2,579.31	1,518.30	176.18	72.23	97,608.59	19.03	75.65	0.49
	Mean	34,764.11	65,578.49	200.75	55.47	97,608.59	19.85	122.34	9.60
	SD	54,430.26	55,477.75	107.30	25.72	-	2.775	88.15	8.02
	%RSD	156.57%	84.60%	53.45%	46.36%	-	13.98%	72.06%	83.60%
Total mean		45,551.54	65,421.51	21,833.79	93.92	87,432.89	20.50	139.36	8.41

A= Nakhon Pathom; B= Samut Sakhon; C= Nakhon Ratchasima; 1= forest soil; 2= orchard soil; 3= rice field soil

*= ICP-OES can detect saturated concentrations by using the maximum concentration per line (mg/L) for measurement [15].

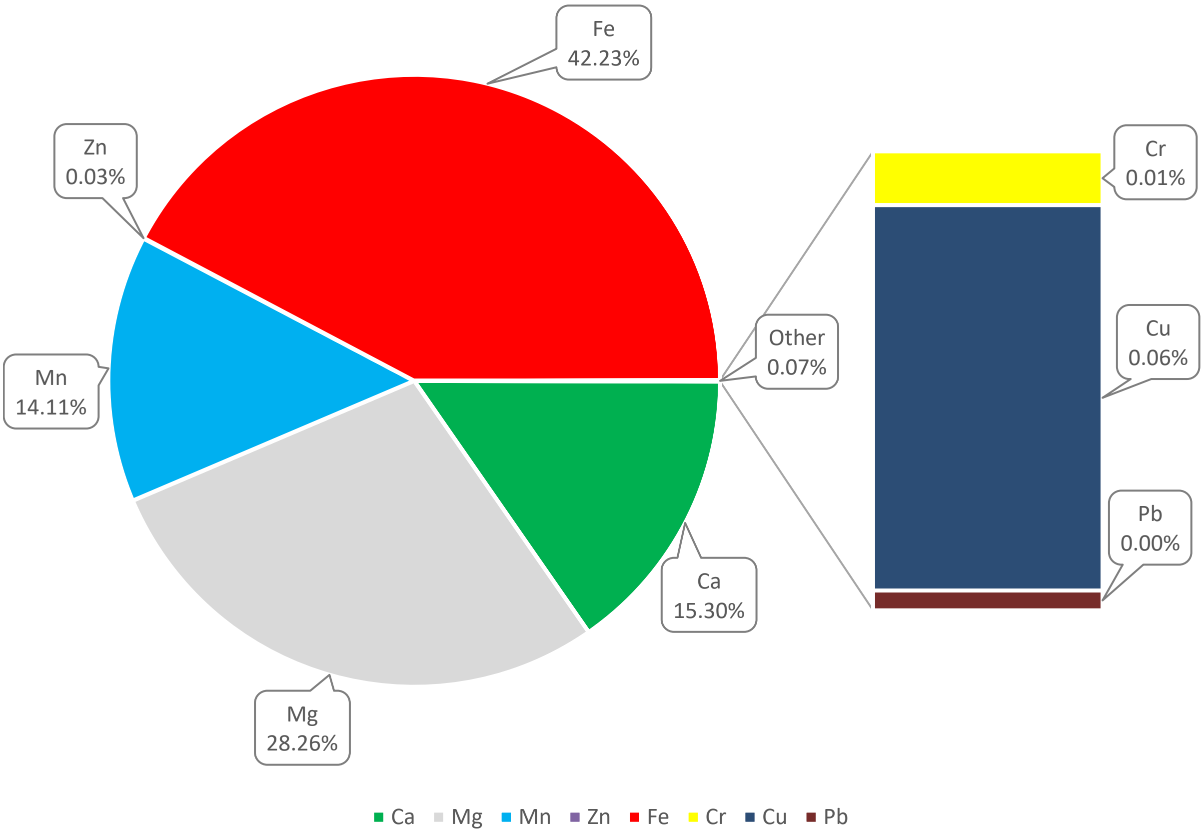

Figure 2 The average percentage of each element across the three soil groups.

The highest concentrations of iron were detected in all soil samples, except those collected from banana orchards in Nakhon Ratchasima Province. The orchard owner confirmed that no fertilizers were used, and the crops were grown naturally, as the orchards were primarily intended for the family's consumption, with only small quantities sold. In contrast, soil samples from other banana orchards had been fertilized to nourish the bananas. Iron is a naturally abundant element. Soil iron concentrations typically range from 0.2% to 55% (20,000 to 550,000 ppm) and can vary significantly, even within localized areas, depending on soil types and other contributing factors [16, 17]. A study of highly weathered tropical soils revealed that significant amounts of calcium (Ca), magnesium (Mg), and potassium (K) were sequestered in iron (hydr)oxide secondary mineral phases [18, 19]. Calcium and magnesium share similar chemical properties, as both are doubly positively charged in the soil-water phase and on soil cation exchange sites. The levels of exchangeable Ca and Mg can vary widely in soils and may closely correlate with water-soluble concentrations of these elements. This variation is strongly influenced by the amount of organic matter and the type of clay, both of which play a significant role in determining the cation exchange capacity (CEC) of the soil [20]. Analysis with Portable X-ray fluorescence (PXRF) spectrometry showed strong linear correlations for key agricultural elements in soil, such as As, Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, Si, Ti, and Zn, with particular focus on Ca, Fe, Mn, and P [21].

Although these elements are commonly detected in large quantities and are often found in soils, their concentrations can vary due to various factors, including human activities and other environmental influences. These variations make it challenging to use this group of elements to classify soils into distinct categories.

Additionally, the detection upper limitations of the ICP-OES for each element further complicate the ability to categorize soil samples based on their concentrations.

In examining soil evidence in various lawsuits, selecting the elements to analyze soil samples from evidence and crime scenes can be challenging. This is particularly difficult when the operating conditions of the soil in the incident area are unknown beforehand. As a result, the soil evidence will be analyzed according to the standards of the laboratory conducting the test. From this experiment, several elements were found to be detectable by ICP-OES at saturating concentrations, which made it difficult to differentiate between the various soil groups. The detection of elements at concentrations lower than those typically observed was particularly intriguing. A study conducted in the plain areas of Thailand using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) identified 12 elements that could be traced back to the coordinates of the soil sample collection points and the boundaries of the sampling area. Nine of these 12 elements were detectable in small quantities in the soil [14]. Corresponds to the study of a survey of soil elemental variability in Singapore parks for forensic purposes found that the profiles of the soil elemental composition (by XRF) in the seven parks revealed 15 common elements in all the surveyed soil samples. The rest of the trace elements, i.e., Rb, Sr, Ga, Cu, Pb, Br and Cr could be found in 81 to 97 % of the samples, while Hf (67 %), Ba (14 %) and As (13 %) were the rarer elements in terms of their occurrence. It is noteworthy that Ba and As were found only in soils collected from CBP and LP park, respectively, implying that comparing soils with such trace elements could give a stronger indication than the soil sample’s common origin [22]. In a study using soil organic factor analysis for crime scene discrimination in a forensic setting, the elements Ba, Ca, K, Mg, Mo, and C were found to be the most important for sample classification, while the element Si could also be used to clearly distinguish one area from another [19]. In addition to examining the elements in the soil, it could also connect the crime scene, the tools used in the crime, and the individuals involved in the case, such as in a case of excavating ancient tombs to steal antiques. The soil from the criminal tools, tombs, and the antique were collected and analyzed using SEM-EDS, XRF, and HCA. Based on color, elemental, and mineral analysis, it was determined that the antique came from tomb A, and the excavation was done using tool 3. The suspect confessed to the crime after these findings. The antique was confirmed to be from tomb A, and all three tools were used in the excavation of tomb B. Later, tool 3 was used to excavate tomb A. As a result, most of the soil on tool 3 came from tomb A, with a small amount from tomb B. This explains why the soil's color and elemental composition on tool 3 closely matched that of tomb A, and why the dendrogram distance between tool 3, tomb A, and the antique was large [23]. This case illustrates that soil analysis in criminal investigations is more complex than standard soil analysis, as it involves various efforts to conceal the crime. In such cases, the analysis may necessitate the use of multiple analytical tools and demands expertise in interpreting the results.

Statistical analysis

The results of the one-way ANOVA showed that the soil types and the concentration of each element were not significantly different ($p > 0.05$) for all elements in every soil type, as shown in Table 5

Table 5 Results One-way ANOVA at 0.05 significant level.

Element	df	F	Sig.
Ca	8	.339	.725
Mg	8	.000	1.000
Mn	8	2.018	.214
Zn	8	2.614	.153
Fe	8	1.000	.422
Cr	8	.162	.854
Cu	8	.043	.958
Pb	8	.155	.860

The statistical analysis results in Table 5 show the concentrations of major and minor elements in each soil group, with no significant differences observed. An analysis of the elemental composition in soil from forests and orchards in Nakhon Ratchasima Province revealed notable differences in various elements compared to the other two provinces. However, due to the substantial variability of each element within each soil group and the lower detectable levels of these elements in Nakhon Ratchasima Province, the data had minimal influence on the statistical analysis of variation. As a result, no statistically significant differences were identified, even for elements generally found in higher concentrations.

Including the small sample size, moderate to high levels of Ca, Mn, and Mg were detected in the samples, making it difficult to clearly differentiate the soils in each group. On the other hand, soil samples tested for minor elements (heavy metals) indicated that all samples were collected from areas where pesticides were not used and were not near any communities or factories. This could explain why the levels of these elements showed no statistically significant differences.

The ICP-OES machine used in this research is typically employed for heavy metal analysis in routine environmental work, the elements selected for analysis were chosen from various research studies and further narrowed down to those already programmed for analysis in the ICP-OES machine. As a result, no additional trace elements were available for comparison.

An experiment was conducted to compare and analyze the element concentrations in various soil groups and those found on shoe soles using t-test statistics. The results indicated no significant differences at the 0.05 significance level, suggesting that the soil on the shoes matches the soil used for testing. To ensure reliability in determining the origin of soil samples, diverse sampling should be conducted. This includes collecting soil from the crime scene, nearby areas, and unrelated or distant locations, allowing for a more accurate comparison and identification of the soil's source [24]. Several factors influence the transfer of soil to shoes, such as soil type, soil moisture, shoe tread pattern, shoe size, and the walker's weight [25]. These environmental factors collectively play a critical role in the success of forensic soil analysis.

Conclusions

Soil is a valuable yet often overlooked type of trace evidence, known for being highly distinctive, easily transferred and retained, and simple to collect and analyze. For example, identifying the origin of soil found on items such as shoes or tires can provide crucial information in a criminal investigation [26]. However, the variability in the concentration of different soil elements can make them challenging to use as reliable trace evidence in forensic science. While ICP-OES is effective for determining the elemental composition of the soil, the concentration levels measured can sometimes be too high, requiring the sample to be diluted to within the standard solution concentration range. This process can be difficult for randomly collected evidence samples and may require repeated analyses, leading to increased costs. Therefore, it is important to consider the environment and land use of the soil or nearby locations before selecting elements for analysis. This approach allows for the creation of a unique signature or profile for each soil type and location, which can then be compared to a database. When soil samples from two sources are likely of the same type, the same major and minor elements should be detected and found in largely identical or nearly identical amounts, with a low standard deviation. However, since iron measurements are largely consistent across all sample groups and show no visible differences, they are not suitable for inclusion in the analysis.

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