

Forensic Soil Analysis of Gardens from Pakistan and Turkey for Genetic Analysis and Elemental Composition using ICP-MS

Abstract

Elemental composition of the soils is the powerful mean for their forensic comparison. The potential errors in any procedure can be analyzed and quantified following some standard protocols. Such a standard protocol is put forward in this article along with the reports resulted from the instrumental measured precisions. Analysis of the soils from both locations at different intervals (1, 5, 10, 20, 30 and 60 days) were done. Results were statistically analyzed for finding mean, standard deviation and coefficient of variance. Garden soil with blood stains had all the elements (except Mg and Sr in Pakistani soil and Cr, Ba, Pb in Turkey soil) showed deviation from the mean value when compared with the none blood stained (negative control) forensic soil sample. There were high amounts of two elements that were mercury and lead that can not be satisfied by Q test and Normalization As no particular coordination or closeness exists for the presence of these two elements. Quantification values of other elements were quite close to each other. In Turkish garden soil Cd, P, Ca, Cr, Zn, As and Be deviated from the normalized values while in Pakistan garden soil there were unusual results seen in Cd, Pb, P, Cu and Be. The other elements showed the same values as the negative had have. It is recommended that during such investigations related samples should be analysed in the same sample run or atleast in sequential runs.

Keywords: Humus; Microorganisms; Phosphates; Arylsulfatase; Urease; Tripsin; Invertase

Research Article

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Introduction

Soil can provide useful information about link of persons to crime scenes because of its nature as the surface of the ground. Soils composition vary among different areas, and have some characteristics because of the natural effects and transfers made by human and other living beings in time [1]. Diversity of soil strongly depends on topography, climate, course of years, botanical, and microbiological functions, conditions of watering, and even human activities during its formation. Moreover, there are unlimited external matters such as pollen and spores and even artificial or synthetic materials. Forensic soil examination has become to very complicate because of this complexity, but at the other hand such diversity and complexity enable the examiner to discriminate soil samples with high discriminating power. The biggest problem in forensic soil examination is the limitation in the discrimination power of the standard procedures and methods. Standardization of forensic soil examination is difficult because of its diversity and non homogeneity. Forensic examination of soil is not only concerned with the analysis of naturally occurring rocks, minerals, vegetation but also with animal matter [2]. Some of the applied methods for screening, analysis for discrimination of forensic soil samples are the colour comparison of the normal air dried (dehumidified) and overheated soil samples, macroscopic observation and low power stereo-microscopic observation, determination of anionic composition by capillary electrophoresis

(CE) [3] and the elemental composition by SEM-EDS technique [4]. Inductively coupled plasma-mass spectrometry (ICP-MS) is capable of quantifying a wide range of elements at trace to ultra-trace levels [5,6] providing concentration data for epidemiological studies and quantitative source apportionment calculations. Garden soil can be a potential place for biological and other evidence material found at crime scene. The presence of blood on soil makes genetic analysis difficult due to the presence of humus, microorganisms, phosphates, arylsulfatase, urease, tripsin and invertase [7]. All these factors make extraction of DNA difficult especially if the sample remains under environmental stress.

The objective of this study was to validate and optimize microwave digestion of soil samples with 65% HNO₃ [8] and inductively coupled plasma mass spectroscopy (ICP-MS) to analyse differentiation of elemental composition of garden soil having blood stains obtained from different origin and comparing the discrimination power of the blood stained and non blood stained (negative control) forensic soil samples. Certified Reference Material (CRM) BCR 700 organic rich soil standard was used for this study. The newly developed method was applied to measure several elements (Na, Cd, Ba, Pb, Mg, P, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Be and Hg) in garden soil samples of Pakistan and Turkey The data was obtained and compared using statistical analysis like mean, standard deviation, Coefficient of variance, Q-test and normalization technique [4] (Figure 1).

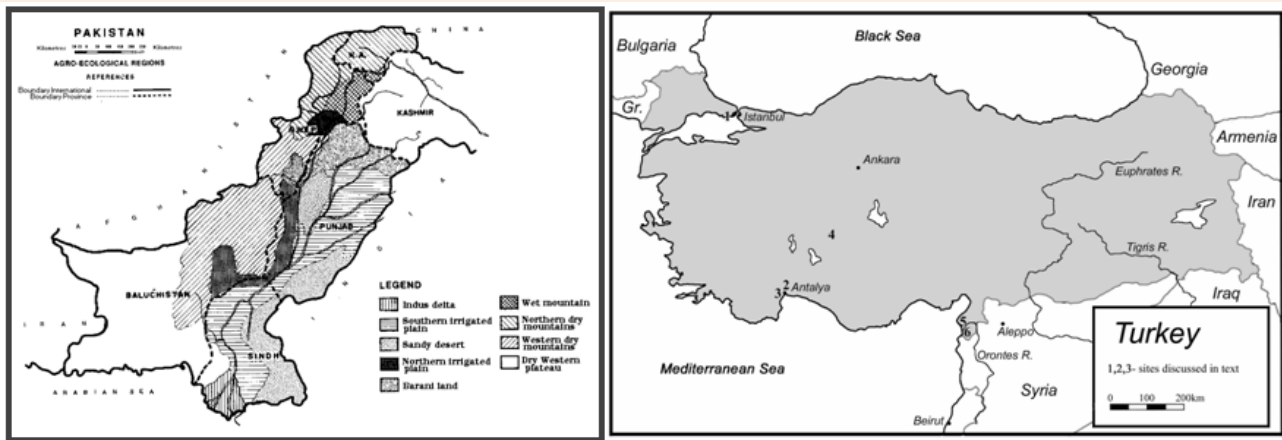


Figure 1: Map of Pakistan (Left), Map of Turkey (Right).

Material and Methods

Sample collection and preparation

Surface soil samples of gardens were sprayed with blood and the samples were collected systematically after specified interval of 1, 5, 10, 20, 30 and 60 days. In every collection the samples were taken from 3 different places in same garden for more accuracy. Negative soil samples were collected in the same way. Soil samples were taken in a 15 ml falcon tube labeled with date, sample name and location. Samples were stored at 4°C in a refrigerator. To avoid moisture content during microwave digestion, soil samples were dried in an oven at 110-120 8°C and sieved by using 0.5 mm sieve and then the under sieve fraction(<0.5 mm) according to McVicar and Graves [9].

Sample preparation for microwave digestion using 65% HNO₃

HNO₃ reacts easily with both aromatic and aliphatic organic compounds; hence the predigestion period is applied to digest organic matter with HNO₃. Gorsuch (1970) reported that predigestion with HNO₃ can be used to oxidise hydroxy compounds such as alcohols and carbohydrates [10]. Consequently, 65% HNO₃ and 1 g dried weight is recommended while working with soil sample. Keeping all these factors in consideration, 100 mg soil sample was weighted carefully and aliquots of sample were kept in Teflon vessels. 6 ml 65% HNO₃ was added. Samples were kept at room temperature for 10-15 minutes. Teflon Vessels were capped and placed in the microwave system for digestion. Microwave system from CEM Corporation “Mars5” was used for digestion. Microwave digestion was carried out by setting the parameters: Temperature was ramped to 200°C for 20 min followed by a dwell time of 20 min (with a pressure setting of 200 psi). After the process completed, Vessels were cooled for 60-90 mins then vent and opened. After cooling, the digest was diluted with ultrapure water to a final volume of 50 ml, to achieve a 4.5% concentration of HNO₃ prior to ICP-MS analysis. Undissolved soil was dried in an

oven and weighted. The difference was noted that shows the total digested contents during microwave digestion.

Soil analysis procedure for ICP-MS

Preparation of soil standard: Indium was used as internal standard. 5 ppb, 10 ppb, 20 ppb and 50 ppb concentrations were made by taking the soil standard 12.5 µl, 25 µl, 50 µl and 125 µl respectively and adding 250 µl indium. In blank sample 25 µl indium was used. Total volume was made 25 ml with 2% HNO₃. Samples were well vortexed before ICP-MS analysis.

Preparation of soil samples and CRM standards: 200ul of digested soil sample, digested CRM BCR700 was added in 100ul indium and total volume was made 10ml with 2% HNO₃ (added 9.7 ml 2% HNO₃). Samples were well vortexed before ICP-MS analysis.

Instrumentation: A LABTAM 8440M Inductively Coupled Plasma Mass Spectrometer installed in Institute of Forensic sciences, Istanbul University were used in the study. To calculate an average concentration from measured concentrations of an element in soil (The effect of a digestion parameter) 4-5 replicates were used in each treatment. Analyses of the all CRMs obtained after 60 days period were averaged, and coefficients of variation determined. Variations in the elemental concentrations reflect the long-term instrumental precision over the period of 60 days, together with variability due to sample preparation.

Results and Discussion

Sstandardizations of forensic soil examination is difficult because of diversity of soil samples [1]. Same is the case here the values obtained from ICP-MS were confusing and it was very difficult to evaluate and interpret the results. Thus the average was taken from the soils that were collected in the same day from different parts of the soils. The purpose of this practice was to maintain the data in proper form. The data obtained was then statistically analyzed for finding Mean, SD and CV (Table 1). Q-test

for identification and rejection of outliers was also calculated. This test should be used sparingly and never more than once in a data set. The data values attained after this test were normalized. Normalization is to put Distance or Similarity as Performance index into a range of 0 and 1 or [0, 1]. The process of arranging and transforming the data index from its value into a range of 0 and 1 is normalization which is used to make the data comparable. The ICP-MS data after this process gave a very positive and understanding data values which when evaluated gave good results for discriminating soils from each other. The inorganic elemental composition was evaluated for different soil samples collected from two countries. After normalization the value of Na was taken as 1 and the elemental composition was examined.

The data showed the closeness among values for the elements collected in different intervals. The values are almost same without any change when compared with negative values of soil. There were some elements which were deviated from the mean but most of the elements were within the range. The environment

and time period showed very less or no impact on the elemental composition. Only mercury and lead showed much deviation from the mean value when compared with negative samples. There were high amounts of mercury and lead unjustifiable by Q-test and normalization. There was no coordination or closeness among these two elements. The other elemental values were very close to each other. In Turkish garden soil Cd, P, Ca, Cr, Zn, As and Be deviated from normalized values (Table 2) while in Pakistan garden soil there were unusual results seen in Cd, Pb, P, Cu and Be (Table 3). The other elements showed the same values as the negative samples had. As a result we noticed, among garden soil samples 7 out of 20 elements showed deviation (overall) while 13 remained the same. Based on these results we can discriminate the forensic soil comparing their elemental composition. The garden soil samples collected from Turkey showed low discrimination among elements compared to Pakistani soil samples where the values of similar elements were high. But overall out of 20 elements, if 13 elements show the same elemental composition, we can use the garden soil as a powerful tool in identifying the soil topography and location.

Table 1: Evaluation of ICP-MS Results of Turkish/Pakistani Garden Soil Samples using CRM700 Soil Standard.

Garden Soil Pakistan CRM 700					Garden Soil Turkey CRM 700				
Elements	Negative	Mean	SD	CV	Elements	Negative	Mean	SD	CV
9Be	28.87	33.11	13.44	0.4	9Be	39.26	29.20	18.60	0.6
23Na	3.43	2.88	4.46	1.6	23Na	0.44	97.35	84.48	0.9
24Mg	0.98	0.91	0.57	0.6	24Mg	0.65	3.47	5.51	1.6
31P	2.82	4.75	2.46	0.5	31P	4.69	2.69	1.58	0.6
39K	1.65	0.91	0.36	0.4	39K	0.98	3.32	2.18	0.7
44Ca	0.70	1.54	0.49	0.3	44Ca	0.97	11.29	21.76	1.9
51V	0.35	1.95	0.71	0.4	51V	0.85	2.28	0.94	0.4
52Cr	0.91	1.63	0.81	0.5	52Cr	1.37	1.83	0.96	0.5
55Mn	1.45	0.78	0.47	0.6	55Mn	0.64	3.92	5.10	1.3
56Fe	1.66	0.88	0.63	0.7	56Fe	0.48	1.75	1.15	0.7
59Co	1.57	2.82	1.13	0.4	59Co	2.73	1.56	0.53	0.3
60Ni	2.09	3.71	1.54	0.4	60Ni	1.88	2.65	0.82	0.3
65Cu	1.28	7.90	3.90	0.5	65Cu	9.73	2.22	1.07	0.5
66Zn	16.81	2.17	2.52	1.2	66Zn	1.38	8.44	4.93	0.6
75As	3.92	7.70	4.95	0.6	75As	2.88	5.95	0.63	0.1
88Sr	0.61	0.74	0.55	0.7	88Sr	0.39	2.00	1.00	0.5
111Cd	57.09	202.20	245.58	1.2	111Cd	154.41	27.43	6.85	0.2
137Ba	0.99	1.57	0.72	0.5	137Ba	1.17	1.39	0.51	0.4
202Hg	47.64	303.19	236.37	0.8	202Hg	126.98	210.48	233.67	1.1
208Pb	0.50	2.20	1.42	0.6	208Pb	1.47	1.48	0.75	0.5

Table 2: Evaluation of ICP-MS Results of Turkish Garden Soil Samples using CRM700 Soil Standard.

Elements	Soil (1 st Day)		Soil (5 th Day)		Soil (10 th Day)		Soil (20 th Day)		Soil (30 th Day)		Soil(60 th day)	
	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values
23Na	79.11	1	78.19	1	82.85	1	79.15	1	76.64	1	73.16	1
111Cd	58.2	0.74	58.89	0.75	51.82	0.63	57.39	0.73	56.78	0.74	51.49	0.7
137Ba	1.31	0.02	1.03	0.01	1.02	0.01	0.94	0.01	1.03	0.01	0.83	0.01
208Pb	2.23	0.03	1.56	0.02	1.19	0.01	2.12	0.03	1.61	0.02	0.17	0
24Mg	1.25	0.02	1.68	0.02	1.63	0.02	1.49	0.02	1.14	0.01	0.62	0.01
31P	3.15	0.04	3.14	0.04	2.86	0.03	2.83	0.04	3.67	0.05	3.02	0.04
39K	1.84	0.02	1.01	0.01	1.68	0.02	1.26	0.02	2	0.03	1.15	0.02
44Ca	25.64	0.32	21.5	0.27	21.81	0.26	22.27	0.28	21.71	0.28	24.79	0.34
51V	0.5	0.01	0.45	0.01	0.26	0	0.84	0.01	0.41	0.01	0.22	0
52Cr	2.35	0.03	2.48	0.03	2.42	0.03	2.5	0.03	2.35	0.03	1.88	0.03
55Mn	1.4	0.02	1.93	0.02	1.34	0.02	1.81	0.02	0.92	0.01	0.49	0.01
56Fe	1.83	0.02	1.55	0.02	1.6	0.02	1.93	0.02	1.85	0.02	1.77	0.02
59Co	0.72	0.01	1.93	0.02	2.1	0.03	1.87	0.02	1.56	0.02	1.17	0.02
60Ni	2.1	0.03	2.38	0.03	2.82	0.03	2.32	0.03	3.02	0.04	2.26	0.03
65Cu	0.98	0.01	1.89	0.02	1.23	0.01	1.01	0.01	1.62	0.02	1.58	0.02
66Zn	16.79	0.21	13.23	0.17	16.4	0.2	17.16	0.22	16.88	0.22	17.17	0.23
75As	3.82	0.05	3.59	0.05	3.5	0.04	4.32	0.05	5.48	0.07	5.97	0.08
88Sr	1.55	0.02	0.3	0	0.01	0	0.51	0.01	0.65	0.01	0.99	0.01
9Be	21.65	0.27	28.72	0.37	22.87	0.28	28.89	0.36	27.03	0.35	28.56	0.39

Table 3: Evaluation of ICP-MS Results of Pakistan Garden Soil Samples using CRM700 Soil Standard.

Elements	Soil (1 st Day)		Soil (5 th Day)		Soil (10 th Day)		Soil (20 th Day)		Soil (30 th Day)		Soil(60 th day)	
	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values	Q Test Values	Normal. Values
23Na	52.56	1.00	51.63	1.00	51.98	1.00	50.80	1.00	52.88	1.00	48.84	1.00
111Cd	15.34	0.29	16.44	0.31	14.49	0.28	15.63	0.30	15.72	0.30	15.97	0.30
137Ba	1.84	0.04	0.29	0.01	1.72	0.03	1.64	0.03	1.47	0.03	1.86	0.04
208Pb	2.95	0.06	1.71	0.03	1.27	0.02	0.70	0.01	3.04	0.06	2.28	0.04
24Mg	0.87	0.02	0.97	0.02	1.05	0.02	0.47	0.01	0.78	0.01	0.73	0.01
31P	4.57	0.09	3.95	0.08	3.88	0.07	3.16	0.06	4.93	0.09	4.07	0.08
39K	0.90	0.02	0.79	0.02	0.65	0.01	0.35	0.01	0.97	0.02	0.65	0.01
44Ca	0.97	0.02	0.99	0.02	1.00	0.02	0.97	0.02	0.87	0.02	0.91	0.02
51V	0.87	0.02	0.83	0.02	0.80	0.02	1.34	0.03	0.52	0.01	0.74	0.01
52Cr	1.44	0.03	1.28	0.02	1.74	0.03	1.57	0.03	1.36	0.03	1.27	0.02
55Mn	0.59	0.01	0.57	0.01	0.93	0.02	0.61	0.01	0.72	0.01	0.94	0.02
56Fe	0.91	0.02	0.63	0.01	0.40	0.01	0.49	0.01	0.48	0.01	0.42	0.01
59Co	2.14	0.04	2.83	0.05	2.14	0.04	2.33	0.04	2.43	0.05	2.13	0.04

60Ni	1.62	0.03	1.27	0.02	1.76	0.03	1.32	0.03	1.23	0.02	1.85	0.04
65Cu	13.80	0.26	9.92	0.19	9.49	0.18	8.68	0.17	9.36	0.18	9.35	0.18
66Zn	1.83	0.03	1.63	0.03	1.14	0.02	1.72	0.03	1.81	0.03	1.65	0.03
75As	2.96	0.06	2.81	0.05	3.21	0.06	2.95	0.06	2.71	0.05	3.36	0.06
88Sr	0.95	0.02	0.41	0.01	0.81	0.02	0.55	0.01	0.52	0.01	0.54	0.01
9Be	38.32	0.73	34.36	0.65	33.05	0.63	36.01	0.69	31.29	0.60	39.49	0.75

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