FORENSIC EXAMINATION OF PLANTS AND SOIL EVIDENCE IN BAGHDAD CITY, IRAQ

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Abstract

This study was conducted in the areas that were exposed to the events of explosions, terrorism and cemeteries for the purpose of identifying the forensic science environmental changes in criminal locations selected from two areas in Tikrit and Anbar in western Iraq. Soil samples and two types of surrounding plants were collected in the region *(Ziziphus spina-christi, Eucalyptus camaldulensis)* in August 2018 and dried in the open air where the samples were divided on the basis of the necessary tests (digestion and concentration). For the purpose of obtaining the chemical composition of the X-ray XRF to quantify traceable elements, the results showed the most important elements highlighted during this study (Sr, Ti). Ti and Sr were the highest in areas exposed to explosions and explosive materials at site (S1, S2) (Tikrit and Anbar), Respectively. The samples were analyzed using ICPES for qualitative detection of these elements after soil and vegetation samples were digested for the control factor of the PTL measured by the relative proximity (RP) produced by dividing the ICPES value on the XRF results of the sample. Its plant is 50% where the highest value is recorded. The pollution threshold limit for the Ti element was 53% and the biological availability of this element was assessed in regions 1, 2, respectively.

*Keywords***:** Forensic environmental, elements, Soil, Plants.

Introduction

A relatively new activity in forensic science newly defined as a strong method orientated mostly a technique-driven activity in the multidisciplinary soil areas and surrounding plants of survey soil after the incidents that occur mineralogy soil elements, plants surrounding it, especially that involve soil morphology soil data, chemistry, mineralogy, geophysics, and biology to answer legal questions and big problems of hypotheses (Petraco *et al.,* 2008). All soil scientists depending on the soils as being made up of different size mineral particles such as sand, plants, silt, clay, elements, and organic matter and have complex biological, physical, chemical, mineralogical, and hydrological properties that are changing with time. Soil materials are routinely encountered as evidence by investigators forensic staff depending on the events (Bull *et al.,* 2008). The mineralogical examination is essential in forensic soil identification and selects an appropriate combination of methods by considering the context of the soil samples, few reports identification analysis of soil components and of closely related materials such as plant fragments, pollen and spores, and diatoms, with emphasis on the importance of screening tests consisting of several simple techniques. The soil formation process involves parent materials, temperature, water condition, vegetation, time, and the chemical processes of solution, oxidation, reduction, and even human activities. The history of a soil's development as the results of such complex soil formation process is strongly resected in soil color. Additionally, the systematic observation of multiple soil colors is especially useful for screening the soil and plants (Chan, 2016).

In the last years, the forensic science technology has advanced dramatically and have been very specialized are generally only performed in investigations of remarkable crime and usually where human DNA analyses or analyses of other more commonly used types of trace evidence. Consequently, there is an opportunity for the application of analysis in the forensic examination of plants and soil from a wider spectrum of forensic investigations (Murray RC, 2004). The cemeteries pose environmental risks for the population due to the levels of contaminants released and cemeteries represent a source of environmental liability due to their potential to accumulate and release large quantities of contaminants generated by the decomposition of corpses. Effluents can carry microorganisms and heavy metals through the soil to water resources this is what researchers refer to (Oliveira, and Pampulha, 2006).

Forensic in plants sciences consider the application to criminal investigations discipline, forensic botany incorporates several sub-disciplines palynology specialized the study of pollens, dendrochronology specialized the study of tree rings. Also, botanical evidence can be used to identify when the soil is disturbed, certain plants quickly invade the fresh surface other species follow in succession until the area recovers. However, the composition and distribution of the new assemblage are never exactly the same as the original community. In addition, the presence of a buried body may chemically change the soil and either promote or inhibit growth. Either way, the disturbed area will be at a different stage than its surroundings. These differences may be visible for decades or clandestine graves, in aquatic species can be helpful such as for algae and diatoms can be used to diagnose death by drowning in freshwater (Bandara, *et al.,* 2010). Trees and roots are useful for determining elapsed, due to woody plants and trees grow in annual cycles that vary with environmental conditions, growth rings can be counted to provide the timing of an event, sometimes centuries later decomposition processes may delay the growth of a plant and cause a plant younger (Chan, 2016).

As a new study of its kind in the role of plants and soil in the forensic investigation, It was possible to examine soil and some plant available in accident area that have occurred explosions or terrorism and place cemetery to understand the concept, the important aims that identify the changes that had happened in the environmental areas from various sites were chosen

from (Tikrit, Anbar) with the following: Identification these elements in soil samples by ICPES then calculate infrared (IR) is regarded as the most reliable method for monitor the component qualitatively and quantified and bioavailable and Properties Titanium and Strontium Isotope Ratios in plants and soils samples.

Materials and Methods

Description and samples collection of the study area

Two sites were selected on the basis needed for full coverage affected areas to come from differently exposed to terrorism and explosions as follows:

1- Al-Anbar is a rocket attack (Soil, *Eucalyptus camaldulensis*).

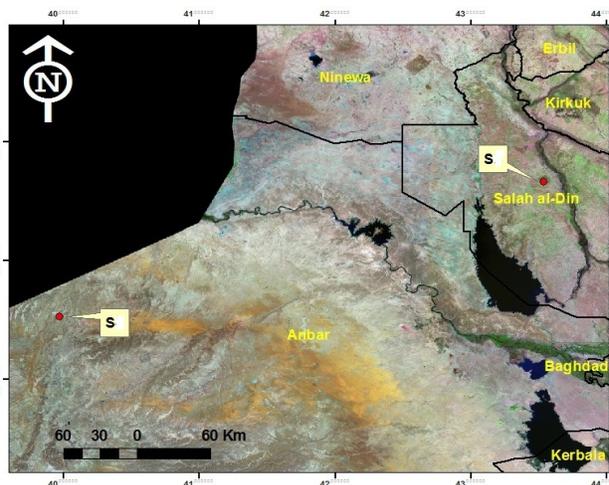2- Iraqi Air Force COB Speicher Mass graves in the presidential of Tikrit. (Soil, *Eucalyptus camaldulensis*).

Fig. 1 : General Map to Shows study areas

Collection samples

The samples were collected from the study areas during July in 2018. Five samples of each plant leaf were collected, then rinsed thoroughly with deionized water and dried outdoors at room temperature for 3-5 days, then grinded with a mill and sifted with a 1 mm diameter sieve to be ready for analysis. The total samples of the sites were 10 plant samples. Five replicates soil samples were collected using clean polyethylene bags from a depth of (10-15) cm from random locations within each studied area and dried in the open air at room temperature for 5-7 days and then grinded by the mill (Planetary Ball Mill) and sifted with a 1 mm diameter sieve to be ready for image analysis.

X-Ray Florescence (XRF)

Drying Soil samples were prepared by breaking them down into aggregates and spreading them evenly on polyethylene sheets in the open air, to ensure no contamination from an external source . To reduce soil matrix effects, the soil samples were thoroughly homogenized and sieved to fine particle sizes of about 75 μm with Retsch aluminium test-sieves with vibratory electronic sieve shaker. According to Kodom *et al.* (2010) method XRF spectrometers only analyze a sample's surface layer, which must be representative of the whole sample, each samples were carefully and homogeneously prepared into pellets with smooth surfaces of equal density. This was achieved by

milling or pulverizing the loose powdered samples (75 μm) to further reduce the particle size to about 60 μm and below. Before mixing procedure, 0.9 g of powdery binder (Hoechst wax) containing cellulose, starch, polyvinyl alcohol, or other organics (Buhrke *et al.,* 1998) was weighed into 4 g of each sample using an electronic balance. The resulting mixture (sample and binding material), having a total mass of 4.9 g, was put into deformable aluminium cups (screw-top grinding jars) for the process of thorough milling and homogenization using the RETSCH Mixer Mills (MM 301), which also aided the further pulverization of the sample. By using a SPECAC hydraulic press with a maximum pressure limit of 15 tons (or 15000 kg), each pulverized sample was manually pressed into pellets with the uniform diameter (32 mm) and thickness (3 mm).

Sample Preparation for Analysis by Top Wave

According to (Ataro *et al.,* 2008 and Chang *et al.,* 2008) all samples (soil and plant) digest preparation to determine element concentration. This method identifies the concentration of (Sr, Ti) in soil and plants, before that all samples must be converted to liquid by specific method of regulation and melting and used method validation was used as reference material with preparation of samples for analysis by Top wave analytic Jena type.

In soil samples weigh 0.5 g of the samples to the digestion vessel. Then add 8 ml of HNO_3 65% and 2 ml of HF to the samples. (The sample was then filtered with 2 filter paper (1 mm) and the sample diluted with 100 mL deionized water) and used Method of dilution to analyses of water samples.

In plant samples, weight 0.3g of samples and place them in the digestion vessels and add 7.5 ml of nitric acid HNO_3 65%, after that shake the mixture carefully or stir with clean glass bar. necessary wait at least (20 min) before the vessel is closed, Heat in the Microwave oven with the following program to avoid foaming and splashing wait until the vessels have cooled the same room temperature about (20 min). The digestion vessel is carefully opened in fume hood wearing hand Eye and body protection since a large amount of gas would be produced during the digestion process, then they were quantitatively transferred to Falcon tubes and diluted to 15 ml with deionized water. For the quality control analysis, 0.250 g is transferred into a Teflon vessel, reconstituted with 2 mL of deionized water, followed by the addition of 4 ml of HNO_3 and wavelengths of each element were examined by the Inductively Coupled Plasma Emission Spectrometry (ICP-ES). For all samples digestions, three replicates have been performed, calibration blanks of 2.0 mL deionized water are taken through the same digestion process. Detection limits for heavy metals (Sr, Ti) in this study have calculated based on three times the standard deviation of the average of 5 blank measurements to one test depending methods.

Inductively Coupled Plasma Emission Spectrometry (ICP-ES)

(A) Reagents and solutions

Standard solutions were prepared by dilution of each pure element standards. Analytical grade nitric

acid (65% Merck) was used for the mineralization of the samples. All aqueous solutions and dilutions were prepared with ultrapure water (APHA, 2008).

Analysis of mineral elements prior to analysis, the samples were thoroughly mixed and analyzed directly, using ICP-ES. The samples were diluted 1:1 with 0.2% (v/v) HNO_3 and centrifuged for 20 min at 2 000 rpm. All sample vials, sample cups, and glass-ware were cleaned by soaking in 10% (v/v) HNO_3 and raised with de-ionized 65water prior to use. The appropriate standards for each element were made within the concentration range of the elements in the samples. The results were obtained from triplicate measurements.

(B) ICP-ES works

An ICP-ES works by injecting a nebulized mist from a liquid into the center of an argon plasma. A plasma is created from a flow of gas within a high energy filed which ionizes the gas and causes intense heating. Temperatures inside ICP plasma reach 10000K. When the mist of the sample enters the plasma, the intense heat causes the dissociation of most chemical compounds, and the energy that the component atoms absorb causes them to undergo excitation and ionization energy transitions. These transitions produce spectral emissions characteristic of the elements being excited. The spectra produced by the plasma are broken down into individual spectral lines by the ICP-ES spectrometer, and the ICP-ES computer translates the spectral lines into concentrations for a specified suite of elements. In contrast to flame emission spectroscopy, the plasma is not an oxidizing environment (like a flame) and there are fewer chemical reactions to confound the analysis. Also ICP-ES has a large linear range– around 4-6 orders of magnitude for most elements. This means that fewer dilutions are required to accommodate samples with a wild range of concentrations. The evaluation of analytical results take place in one step by a computer program called Smart Analyzer. Results are given in unit mg/dm^3 (ppm). Calculate the mg/kg concentrations. From the samples element concentrations are determined 3 times successively by ICP-ES. Calculate mean, standard deviation (SD) and relative standard deviation (RSD%) of the parallel results.

Pollution Threshold Limit (PTL)

To comprehensively evaluate the raw data of XRF versus ICP-ES for these various elements, relative proximity (RP) was used. RP considers only the samples with values over the controlled threshold limit (i.e., over the pollution threshold limit (PTL), thus requiring monitoring). Therefore, the number of detected field samples of the ICP-ES results over the PTL divided by the number of detected XRF results over the PTL determines the RP, as shown in Equation (1):

$$RP = No.ICP-ES$$

$$No.XRF$$

Then determination of the elemental composition of soil and plants samples coupled argon plasma is a spectroscopic source, which can be used a wide range of major and trace elements in a single, short an integration period.

Results and Discussion

The two elements was measured and obtained chemical composition on X-ray fluorescence to detected of traceable elements and For quantitative detection, results showed the most important elements that were highlighted during this study (Sr, Ti) of quantify the precision of the elemental ratio and have been determined on uniform compositionally, results for the soil samples existence of the following are given in Table (1) Ti element in the affected areas that were exposed to the explosion and explosive materials recorded high proportions in S1, S2 sites, all the values being greater than the alert level in the less sensitive area for Sr and Ti.

Table 1 : Mean ± SD of chemical composition of (Sr, Ti) elements in Soil (ppm) measured in XRF with limited factor WHO (2008)

Elements	Sampled location		
	S4	S5	limited factor WHO 2008
Sr	901.77±42.75	895.43 ±39.52	30 ppm
Ti	6200.82±77.39	3982.92±53.93	100 ppm

Table 2 : Mean ± SD of chemical composition of (Sr, Ti) elements in Plants (ppm) measured in XRF with limited factor WHO (2008)

Elements	Sampled location		
	S4 *Eucalyptus camaldulensis*	S5 *Eucalyptus camaldulensis*	limited factor WHO 2008 in leaf plants
Sr	101.07±11.75	215.43±13.52	20 ppm
Ti	6200.82±77.39	3982.92±53.93	100 ppm

In the Table (2) the elements were measured and chemically obtained on X-ray fluorescence to For quantitative detection and traceable elements in the selected plant species. The results showed the most important elements that were combined with soil elements for the purpose of detecting the pathway of these elements (Sr, Ti) by determining the accuracy of the percentage and determined uniformly from the structural point of view. This indicates the absorption of this mineral from the soil from the soil on a daily basis. In quantities Ti, Sr, in the other sites that have exceeded the limited factors, Therefore, the next step was the re-analysis for qualitative detection of these elements using ICPES After digestion of soil samples and plants to calculate the monitoring factor PTL during the accumulation process in the soil. As well as the testing of surrounding plants to determine ability to absorb these elements although of events Table (3) showed the concentrations of elements in soil and plants using ICPES.

Table 3 : Concentration of (Sr, Ti) elements in Soil and Plants (ppm) measured In the ICPE spectrometry

Elements	Sampled location	
	S4 Mean soil Mean plants	S5 Mean soil Mean plants
Sr	201.77, 14.75	495.43, 39.52
Ti	208,17.39	382.02, 23.93

In the Table (2) the elements were measured and chemically obtained on X-ray fluorescence to For quantitative detection and traceable elements in the selected plant species. The results showed the most important elements that were combined with soil elements for the purpose of detecting the pathway of these elements (Sr, Ti) by determining the accuracy of the percentage and determined uniformly from the structural point of view. This indicates the absorption of this mineral from the soil from the soil on a daily basis. In quantities Ti, Sr, in the other sites that have exceeded the limited factors, Therefore, the next step was the re-analysis for qualitative detection of these elements using ICPES After digestion of soil samples and plants to calculate the monitoring factor PTL during the accumulation process in the soil. As well as the testing of surrounding plants to determine ability to absorb these elements although of events Table (3) showed the concentrations of elements in soil and plants using ICPES.

Individual Elements Interpretation comparisons between the XRF and ICPES qualitative and quantitative in soil and two type plants samples as one of the most important indicators for the tracking of explosive materials and the decomposition of bodies and the extent of its accumulation for limited periods and long-term, results elements (Sr Ti) by PTL the permissible exposure level for pollutants in Table (4). In contrast, Figure represents the XRF value versus ICP ES value with their regression lines for these five elements. The regression lines describe the minimized distance from the line to the data points of the individual method that show how well the slope of regression means that the XRF measurements and ICPES experiments are exactly the same comparisons between the results for each element are analyzed in the following table, the screening test of high values can be used as a reference for selecting sampling points the interpretation of the monitoring results of the elements and their accumulation during the explosion and terrorist incidents Have been a comprehensively evaluate of relative proximity (RP) was used. RP considers only the samples with values over the controlled threshold limit (i.e., over the pollution threshold limit (PTL), thus requiring monitoring). Therefore, the number of detected field samples of the ICP-ES results over the PTL divided by the number of detected XRF results over the PTL to determines the RP as following in table. The comprehensive interpretation was carried out by overall evaluations of both the raw data of XRF versus ICPES and their regression lines. Table (4) presents the summary of five elements measured by the XRF method and the results obtained from the ICPES experiments for soil and two types of plants samples from the sites under the study areas

Table 4 : PTL for elements concentration of all Soil samples.

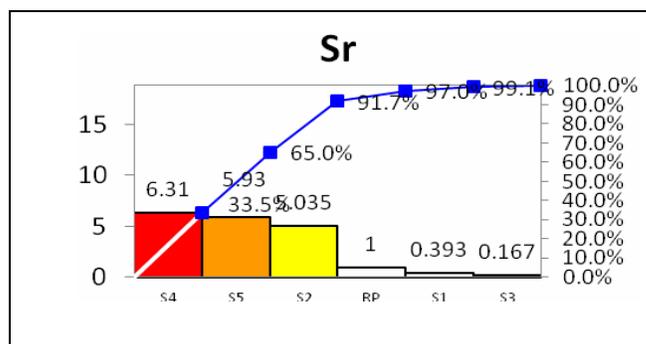| Elements	PTL for elements concentration of all Soil samples with *P value* < 0.05				
	Sampled location (S)				
	S4	S5	Mean	RP	P value P < 0.05
Sr	6.31	5.93	3.02	1.0	0.012
Ti	2.00	1.92	1.32	0.5	0.016

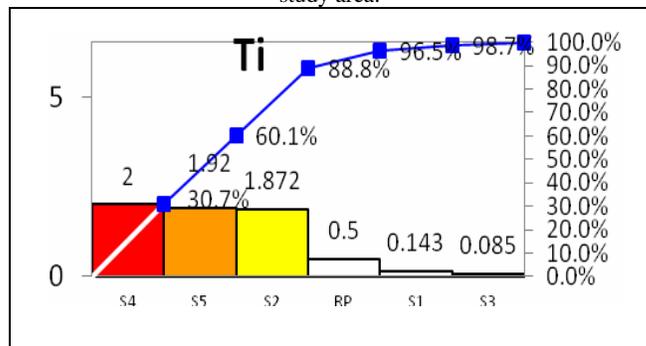Fig. 1 : Concentration PTL Sr of Soil samples in all sites under study area.

Fig. 2 : Concentration PTL Ti of Soil samples in all sites under study area.

Table 5 : PTL for elements concentration of all plant samples.

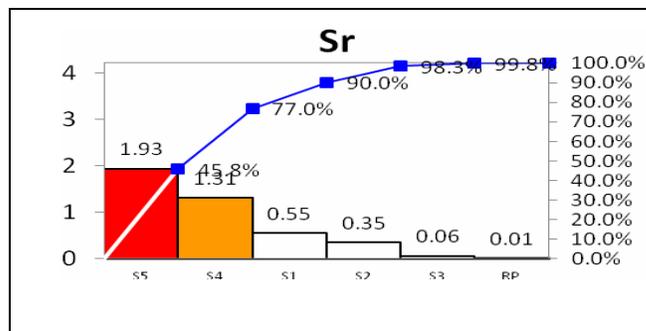| Elements	PTL for elements concentration of plants samples with *P value* < 0.05				
	Sampled location (S)				
	S4	S5	Mean	RP	P value P < 0.05
Sr	1.91	1.33	0.92	0.01	0.012
Ti	2.00	1.92	1.32	0.02	0.016

Fig. 3 : Concentration PTL Sr of types plants samples in all sites under study area.
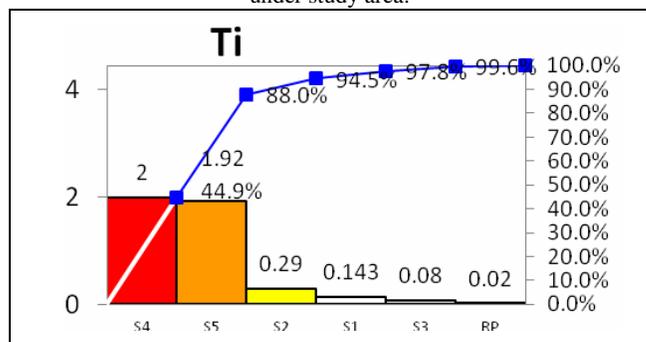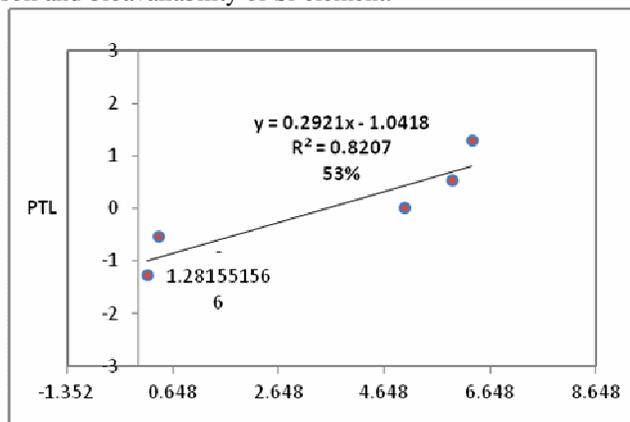

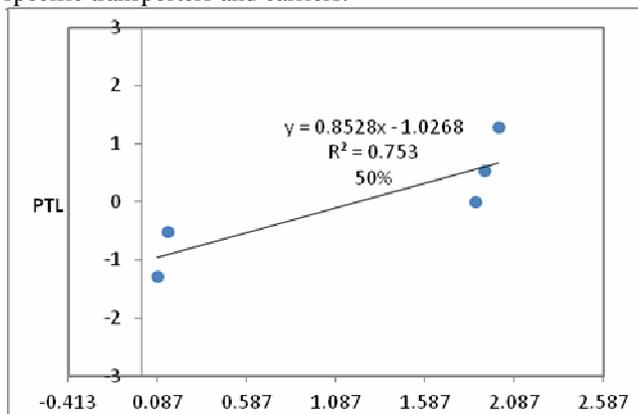Fig. 4 : Concentration PTL Ti of types plants samples in all sites under study area

The comprehensive for strontium trend measured Pollution threshold limit (PTL) show a High quality and quantity parallel with the values percentage of 53 % compared with controlling, the RP could be interpreted as only half of the soil pollution was captured by the XRF site screening (R2 value) of Eucalyptus camaldulensis (0.8207) of element Sr is higher than all other elements in most sites soil samples especially in the cities of events (1, 2). The following figure shows the relationship between plant, soil and bioavailability of Sr element.

$R^2 = 0.821$. The percentage of plant absorption is very abundant due to the type of soil area and the most bioavailability of pollution threshold limit, percentage relative proximity values 53 %.

Fig. 5 : The percentage Sr of bioavailability of pollution threshold limit.

Element (Ti): The overall trends of Ti extremely high values above the PTL were detected by both the XRF method and ICPES. Plant uptake of ions Titanium through roots or leaves involves both passive absorption and active transport. Passive absorption is facilitated by concentration gradients of an ions, while active transport is driven by the electrochemical gradient generated by H^+-ATPase to allow selective ions to move across the plasma membrane through specific transporters and carriers.

$R^2 = 0.753$. The percentage of plant absorption is very abundant due to the type of soil area and the most bioavailability of pollution threshold limit, percentage relative proximity values 50%.

Fig. 6 : The percentage Ti of bioavailability of pollution threshold limit .

Conclusion

The traceability of this measurements is Ti> Sr. The two elements (Sr and Ti) measured values have a good correlation (RP ranges from 50%–53%) Respectively.

These values instrument is a powerful tool that can be very effective in the validation of both the absence and presence of certain elements enter into the composition of explosives or residue the deceased. In principle, this instrument could be employed to provide rapid in situ detection of the presence of toxic metals and it can be traced in cultivated plant species near the place of the event as has been done before from (Radu and Diamond, 2009) have been used as a screening technique and are highly reliable in detecting samples and be applied with the rest elements contained in the C4 66% effective methodology needs to be verified and calibrated according to (Shrivastava *et al.*, 2005). Therefore, it was the next step that identified and confirmed the availability of the elements Sr and Ti concentrations of sites (1 , 2) Respectively.

References

Petraco, N.; Kubic, T.A. and Petraco, N.D.K. (2008). Case studies in forensic soil examinations. Forensic Science International, 178(2-3): 23-27.

Bull, P.A.; Morgan, R.M. and Freudiger-Bonzon, J. (2008). A critique of the present use of some geochemical techniques in geoforensic analysis. Forensic Science International. 178(2-3): e35-e40.

Murray, R.C. (2004). Evidence from the Earth Forensic Geology and Criminal Investigation. Missoula, Montana: Mountain Press Publishing Company.

Oliveira, A. and Pampulha, M.E. (2006). Effects of longterm heavy metalcontamination on soil microbial characteristics.Journal of bioscience and bioengineering. 102(3): 157-161.

Bandara, V.; Weinstein, S.A.; White, J. and Eddleston, M. (2010). A review of the natural history, toxinology, diagnosis and clinical management of Nerium oleander (common oleander) and Thevetia peruviana (yellow oleander) poisoning. Toxicon, 56(3): 273-281.

Chan, T.Y. (2016). Aconitum Alkaloid Poisoning Because of Contamination of Herbs by Aconite Roots. Phytother Res., 30(1): 3-8.

Kodom, K; Wiafe-Akenten, J. and Boamah, D. (2010). Soil heavy metal pollution along Subin River in Kumasi, Ghana; Using X-ray fluorescence (XRF) analysis. 20th International Congress on X-Ray Optics and Microanalysis, X-ray Optics and Microanalysis, Proceedings Book Series. In AIP Conference Proceedings. 1221(1): 101–108.

Buhrke, V.E.; Jenkins, R. and Smith, D.K. (1998). A Practical Guide for the Preparation of Specimens for X-ray Fluorescence and X-ray Diffraction Analysis. John Wiley and Sons, Inc., New York, 1–7.

Ataro, A.; McCrindle, R.I.; Botha, B.M.; McCrindle, C.M.E. and Ndibewu, P.P. (2008). Quantification of trace elements in raw cow's milk by inductively coupled plasma mass spectrometry (ICP-MS). Food chemistry, 111(1): 243-248.

Chang, R.; Piló-Veloso, D.; Morais, S.A. and Nascimento, E.A. (2008). Analysis of a Brazilian green propolis from Baccharis dracunculifolia by HPLC-APCI-MS and GC-MS. Revista Brasileira de Farmacognosia

Brazilian Journal of Pharmacognosy. 18(4): 549-556.

APHA, A. (2005). WEF, 2005. Standard methods for the examination of water and wastewater, 21: 258-259.

Radu, T. and Diamond, D. (2009). Comparison of soil pollutions determined using AAS and portable XRF techniques. Journal of Hazardous Materials, 171: 1168–1171.

Shrivastava, P.; O'Connell, S. and Whitley, A. (2005). Handheld x-ray fluorescence: practical application as a screening tool to detect the presence of environmentally-sensitive substances in electronic equipment. In Proceedings of the 2005 IEEE International Symposium on Electronics and the Environment, IEEE, 157-162.