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Effect of Different Types of Soil and Time Intervals on Isolation and Quantification of DNA: A Forensic Management Technology Perspective

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Abstract—

DNA fingerprinting has proved valuable in the field of forensic management and criminal investigations; not only for convicting felons and exonerating the innocents but also for establishing maternity - paternity and proving family relationships. An individual's DNA is as distinct as his fingerprints except in case of monozygotic identical twins. Unlike conventional fingerprint that occurs only on the fingertips, a DNA fingerprint is the same for every cell, tissue, and organ of a person. It cannot be altered by any known treatment. Most often than not we encounter cases where bloodstained weapons, clothing and other belongings and sometimes the body of the victim is thrown away at outdoor locations where these may come in contact with soil. It is then that we require a technique to first isolate the blood mixed with soil and then extract DNA for individualisation. Different soil types (sand, silt and clay) were spiked with blood and samples were collected methodically every week for a month. The DNA extracted from different soils at different time intervals showed a remarkable decrease in yield as well as degradation with increase in time.

Keywords*— *Criminal investigation, DNA fingerprinting, Forensic, Management, Soil.

I. INTRODUCTION

The past two decades have witnessed tremendous leaps in scientific advancement of DNA typing. The advent of modern DNA technology has resulted in increased ability to perform human identification. The characteristic of certain segment of DNA (about 99.9%) is same in all human beings; it is only 0.1% which varies from person to person and forms highly individual, unique and identifiable genetic coding [1]. DNA is often relied upon to identify criminals, victims, missing persons and establish kinship between individuals. Blood stained soil may be of great interest in forensic incidents due to its ubiquitous presence at all outdoor crime scenes. Soil can provide important information to criminal investigations as transfer evidence because many criminal cases take place under circumstances such that soil transfers to a criminal or victim [2]. Thus soil can be a good source of evidence especially in murder cases when it is stained with blood. Blood stained soil samples can be obtained after some incident. The availability of the samples from such cases and places is directly affected by soil pH, soil colour, nutrients, humus and microorganism present in the soil [3]. The amplification of DNA from soil is often inhibited by co-purified contaminants. Humic and fulvic substances can covalently bind to nucleic acids or proteins causing inhibition or seriously reducing the sensitivity and specificity of PCR amplifications [4]. The efficiency and reproducibility of DNA extraction from soil was tested for variations in purification treatments and their effect on yield and purity of DNA. The extraction yield was improved by increasing the concentration of EDTA or monovalent ions in isolation buffers. Purity was improved using buffers with decreasing concentration of EDTA or by reducing the ionic strength of the buffer, and by all mechanical treatments [5]. The present study aims to reveal the effect of different types of soils and time-intervals on isolation and quantification of DNA from blood samples. The three types of soil samples collected for this study were sand, silt and clay [6].

II. METHOD AND METHODOLOGY

A. Sampling and preservation:

10g of sand, silt and clay soils were collected from bank of river Yamuna, North Delhi, construction site and garden area in dried form and stored in separate vials. The soil colour, texture, pH and particle size were noted before staining each soil type. About 250 ml of blood was obtained from mortuary and preserved with EDTA in an air tight tube. 1g of each soil type was spiked with 2ml blood sample. About 12 soil (mixed with blood) samples were stored over a period of 4 weeks in sand, silt and clay respectively.

B. DNA extraction procedure:

Soil samples were taken in 2ml centrifuge tube, mixed well with n- saline (0.9% NaCl) and vortexed. The samples were introduced to serial dilution, till third dilution was carried out. DNA extraction was performed by modifying Phenol-Chloroform-Isoamyl alcohol, Sodium Acetate, Sodium dodecyl sulphate (SDS), Proteinase K method [7]. Samples incubated at 600C for 2 hours so that blood in soil samples mixes with solution. Samples were taken from the incubator, upper phase was taken and repeated 2–3 times, centrifuged at 14,000 rpm for 4 min. The upper phase was decanted and the lower layer contained pellet was continued to process. 1 ml 70% ethanol was added in the tube, vortexed and centrifuged at 14,000 rpm for 5 min. The tube was air dried and after that 100 ml TE buffer was

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added. Samples were incubated stored at 40 C. Proteinase K was added along with 0.6 M Sodium acetate and 20% SDS solution and was incubated overnight. The DNA concentration was measured using UV-Visible spectrophotometer and 20 µl of stained DNA (stained with 5µl bromo-phenol blue dye + 5µl 20% glucose) was used to run on a 2% agarose gel electrophoresis. The concentration for double stranded DNA was calculated. 1 ng DNA was used for PCR amplification.

III. RESULT AND DISCUSSION

The samples that were run for electrophoresis were analysed for the presence and quantification of DNA which was obtained from the bloodstains that were mixed with three different types of soil (sand, silt and clay) and were preserved at 40 C over 1 week, 2 weeks, 3 weeks and 4 weeks time. DNA extraction procedure validated for blood stained soil samples was employed. The organic comparison of soils demonstrates the different types of soils (sand, silt and clay) ability to hold DNA. It was observed from the results that there was a significant effect of different types of soils and time intervals on quality and quantity of DNA. The DNA extracted was quantified at 260nm and 280nm of wavelengths in UV-Visible spectrophotometer, respectively. The concentration of DNA (µg/ml) at A260/ A280 for 1, 2, 3 and 4 weeks are shown in Table-1.

TABLE I
SHOWS THE CONCENTRATION OF DNA IN DIFFERENT SOIL TYPES AT A_{260}/ A_{280} OVER 4 WEEKS TIME PERIOD

Weeks/ Wavelengths (nm)	**Conc. (µg/ml) of DNA**		
	Sand	**Silt**	**Clay**
I 260 280	2060 1445	1895 1395	1715 1315
II 260 280	2025 1390	1640 1325	1595 1170
III 260 280	1940 1325	1525 1245	1365 1060
IV 260 280	1840 1285	1435 1120	1225 1005

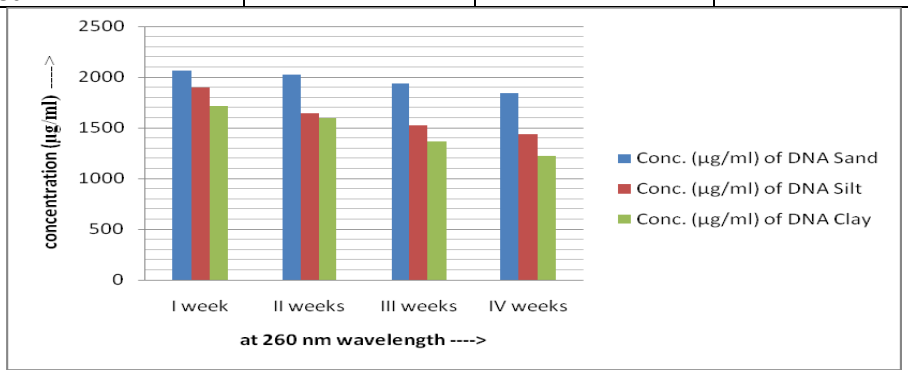

Figure-1 illustrates the DNA yield distribution at 260 nm wavelength over 4 weeks.

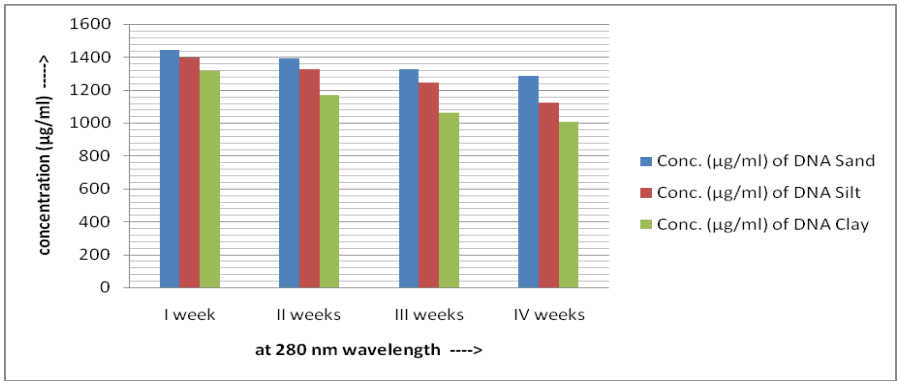

Figure-2 illustrates the DNA yield distribution at 280 nm wavelength over 4 weeks.

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From Table-1 we observed that the sand soil showed highest yield of DNA of 2060 µg/ml and 1445 µg/ml at 260 nm and 280 nm wavelengths, respectively in the first week among all groups. The sand soil sample showed gradual decrease in yield of DNA in the second week to 2025 µg/ml and 1390 µg/ml at 260 nm and 280 nm respectively. The DNA yield kept falling for sand soil in subsequent weeks with DNA concentration of 1940 µg/ml and 1840 µg/ml at 260 nm while 1325 µg/ml and 1285 µg/ml at 280 nm wavelength respectively. The same pattern of regular decrease over four weeks was observed for silt soil samples also. The silt soil showed DNA yield of 1895 µg/ml, 1640 µg/ml, 1525 µg/ml and 1435 µg/ml at 260 nm wavelength while 1395 µg/ml, 1325 µg/ml, 1245 µg/ml and 1120 µg/ml at 280 nm wavelength respectively. The similar expected reduction in the concentration of DNA was observed for clay soil as well with 1715 µg/ml, 1595 µg/ml, 1365 µg/ml and 1225 µg/ml at 260 nm wavelength while 1315 µg/ml, 1170 µg/ml, 1060 µg/ml and 1005 µg/ml at 280 nm wavelength respectively.
Figures-1 & 2 illustrate the decrease in DNA yield over a period of 4 weeks at 260 nm and 280 nm respectively.

IV. CONCLUSION

The samples that have been obtained in the first week showed highest yield as well as the quality of DNA were found to be good in all three types of soils. However, the concentration and quality of yield gradually kept decreasing over a period of one month. This could be attributed to the presence of microorganism, humic acid contents, organic matter and other contaminants in soil types that degraded the DNA.

Burgmann et al. (2001) suggested in their study that extraction of genomic DNA from soils of different texture and chemical characteristics influence the yield of DNA extracted. They further stated that there cannot be a single, general protocol for optimal DNA recovery from different soil types [8]. Krsek et al. (1999) in their study compared in situ and ex situ DNA extraction and found that DNA isolated ex situ was more contaminated by humic acids than using the in situ approach. Also the yield after ex situ method was only 60% of the yield using the in situ isolation. Hence, concluded that quality and quantity of isolated DNA relates to the extraction and purification treatments and type of soil used [5].

Thus it can be concluded from the present study that although quantification of DNA from blood is an enormous task in itself, mixing of blood with soil makes it even harder. Since, soil is evidence that is found mostly at all crime scenes, it is apparent to find blood in contamination with soil. As it is found from the present experiment that there is a significant effect of different soil types on DNA isolation so a forensic management technology perspective should be highlighted where in more researches have to be conducted in this area with wider time frames.

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