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DEGRADATION OF BURIED DNA SAMPLES IN DIFFERENT TYPES OF SOIL

Abstract: Biological samples buried in different types of soil are often found in crime scenes. These samples are usually highly degraded which difficult its analysis. Several factors contribute to the degradation of biological material including temperature variation, humidity, UV light and especially the presence of microorganisms.

Blood was collected to three non related male donors and blood stains were made in fabrics such as jeans, cotton and lycra. Blood stains were dried at room temperature and buried in three different types of soil, to promote its degradation.

It was found that samples suffer a high degradation over time which difficult their analysis. The marshy soil proved to be the most aggressive, leading to rapid degradation of the different analyzed fabrics, probably because of its high percentage of moisture and microbial proliferation.

Introduction

Biological samples buried in different types of soil are often found in crime scenes. These samples are usually highly degraded which difficult its analysis. Several factors contribute to the degradation of biological material including temperature variation, humidity, UV light and especially the presence of microorganisms.

The cellular post-mortem degradation starts with the autolysis of the cell membrane. As a consequence the DNA is released to the environment and once in the soil it can 1) connect to minerals and humic substances such as humic acid (HA), 2) be degraded by bacterial DNases and used as nutrients for growth of plants and microorganisms or 3) be incorporated into the bacterial genome.

The preservation of DNA from buried samples is influenced by physical, chemical and biological properties of DNA and soil, such as pH, moisture percentage, concentration of humic substances, mineral content and cation concentration, and is dependent on its connection to certain minerals, humic substances and organomineral complexes.

The increase of humidity percentage leads to an increase in the number of microorganisms and consequently to a higher DNase activity. Another factor which affects the DNA degradation rate is temperature: whenever it rises, the half-life time of DNA decreases, as a consequence of increased activity of DNases [1, 2, 3].

Materials and Methods

18 ml of blood were collected from three non related male donors and 36 blood stains with approximately 7 cm of diameter were made in three different fabrics such as jeans, cotton and lycra, previously washed and decontaminated for 20 minutes with UV light. Blood stains were dried during 3 days at room temperature before being buried in three different types of soil (sand, marsh and clay).

Small pieces of each stain (12,5 cm^2) were collected after 15, 30 and 90 days. At this time, day, hour, place, presence of vegetation, temperature and humidity were registered. Photographic registration of the places and stains were also obtained, as well as, graphical registers from the closest meteorological stations, such as temperature, pressure, humidity and rainfall of the 31 days that precedes the collection. The different soils were also chemically characterized (Table 1).

Positive controls (blood stains of each individual) were made in all types of fabric. All fragments, as well as the control samples, were properly conditioned and frozen at – 80ºC until its analysis.

DNA extraction was performed using Chelex 100 method [4], QIAmp Investigator kit (Qiagen) and DNA IQ™ System kit (Promega). Samples were quantified with Human Quantifiler™ kit (Applied Biosystems), according to manufacturer's instructions using an ABI Prism® 7000 (real-time PCR).

Results

After ninety days jeans and cotton fabrics buried in marshy soil disappeared (Figures 1 and 3). Since only lycra remained in this type of soil for so long (Figure 2), it seems that this is a highly resistant fabric. Despite of its resistance, the DNA in lycra fabric undergoes a high degradation, not allowing its analysis.

In spite of the fabrics buried in the other types of soil didn't disappear after ninety days (Figures 4 to 9), quantification results after 15, 30 and 90 days, showed a high DNA degradation rate over time.

The greater quantity of DNA was obtained with samples buried during 15 and 30 days in sandy soil, extracted with QIAmp Investigator kit (Table 2).

Discussion

It was found that samples suffer a high degradation overtime which difficult their analysis. The marshy soil proved to be the most aggressive, leading to rapid degradation of the different analyzed fabrics, probably because of its high percentage of moisture and microbial proliferation, which are also responsible for the extensive DNA degradation verified after 15 days. The sandy soil with the highest pH showed the lowest degradation rate.

Conclusion

It is important to continue seeking for new methods of DNA extraction as well as improve the existing ones, to enable recover even the smallest amount of DNA present in degraded samples.

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Samples	SiO2 (%)	Al2O3 (%)	CaO (%)	MgO (%)	Na2O (%)	K2O (%)	Fe2O3 (%)	P2O5 (%)	TiO3 (%)	Mn (ppm)	Cu (ppm)	Zn (Ppm)	pH
Marshy soil	55,14	17,56	4,12	2,57	1,07	1,63	3,53	0,65	0,80	978	27,1	160	5,91
Sandy soil	68,32	8,66	7,51	1,05	0,89	1,12	1,24	0,09	1,23	234	12,6	71	6,84
Clay soil	54,63	20,31	2,13	1,54	0,84	1,72	4,96	0,56	0,78	563	32,1	97	5,80

Table 1 – Chemical characterization of the different soils

15 days - Extraction with QIAmp			
	Soil	Quantification	IPC
N. Denim	Sand	0.8705	31.26
N. Lycra	Sand	0.7747	32.04
N. Cotton	Sand	undetermined	31.44
A. Denim	Sand	undetermined	*
A. Lycra	Sand	2,625	30.01
A. Cotton	Sand	0.0053	*
P. Denim	Sand	0.0134	*
P. Lycra	Sand	2,282	31.04
P. Cotton	Sand	2,793	30.47
N. Denim	Clay	undetermined	39.32
N. Lycra	Clay	undetermined	*
N. Cotton	Clay	0.0101	*
A. Denim	Clay	undetermined	*
A. Lycra	Clay	0,0117	*
A. Cotton	Clay	undetermined	*
P. Denim	Clay	undetermined	*
P. Lycra	Clay	0.0065	*
P. Cotton	Clay	ind	30.89
N. Denim	Marsh	0,0112	*
N. Lycra	Marsh	undetermined	32,47
N. Cotton	Marsh	0,002	*
A. Denim	Marsh	undetermined	und
A. Lycra	Marsh	undetermined	30,47
A. Cotton	Marsh	undetermined	32,98
P. Denim	Marsh	0,0023	*
P. Lycra	Marsh	undetermined	*
P. Cotton	Marsh	0,0004	und

30 days - Extraction with QIAmp			
	Soil	Quantification	IPC
N. Denim	Sand	undetermined	und
N. Lycra	Sand	undetermined	und
N. Cotton	Sand	0.0026	*
A. Denim	Sand	0.004	*
A. Lycra	Sand	1,9384	30.35
A. Cotton	Sand	0.0038	35.02
P. Denim	Sand	0.0082	*
P. Lycra	Sand	0.0659	36.54
P. Cotton	Sand	undetermined	und
N. Denim	Clay	0.0055	*
N. Lycra	Clay	0.0129	*
N. Cotton	Clay	0.0083	*
A. Denim	Clay	0.0018	*
A. Lycra	Clay	0.0037	*
A. Cotton	Clay	undetermined	*
P. Denim	Clay	0.0113	*
P. Lycra	Clay	0.008	*
P. Cotton	Clay	0.0035	*
N. Denim	Marsh	undetermined	und
N. Lycra	Marsh	undetermined	und
N. Cotton	Marsh	undetermined	30.19
A. Denim	Marsh	undetermined	und
A. Lycra	Marsh	undetermined	38.76
A. Cotton	Marsh	undetermined	und
P. Denim	Marsh	undetermined	und
P. Lycra	Marsh	undetermined	und
P. Cotton	Marsh	undetermined	und

Table 2 – Quantification results of samples buried in the different soils for 15 and 30 days. N., A., and P. correspond to the blood donors. IPC values: und – undeterminated; * – normal (<30)

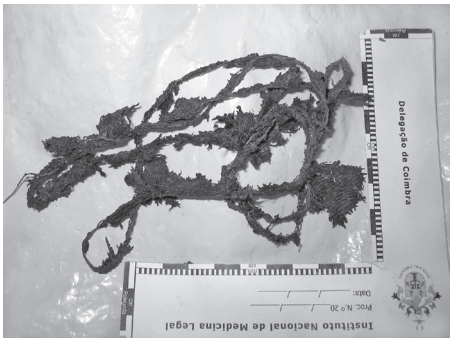

Figure 1 – Denim 90 days in marshy soil

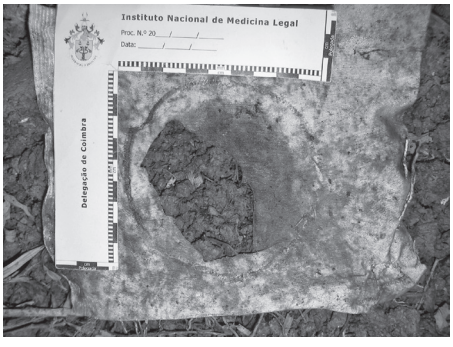

Figure 2 – Lycra 90 days in marshy soil

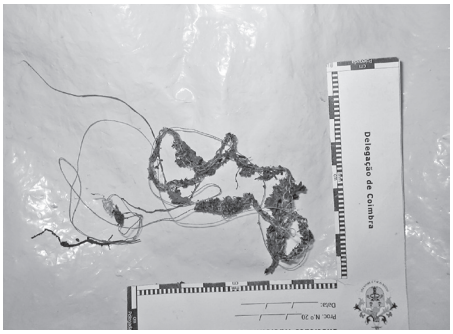

Figure 3 – Cotton 90 days in marshy soil

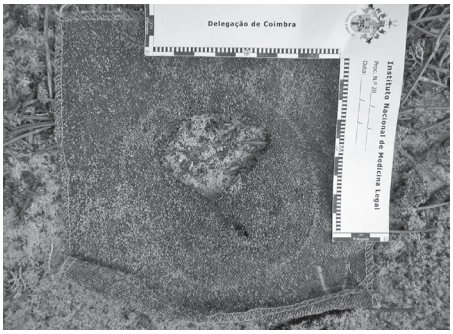

Figure 4 – Denim 90 days in sandy soil

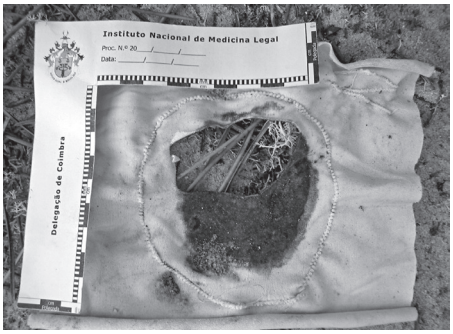

Figure 5 – Lycra 90 days in sandy soil

Figure 6 – Cotton 90 days in sandy soil

Figure 7 – Denim 90 days in clay soil

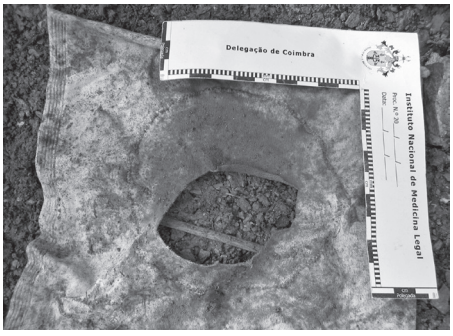

Figure 8 – Lycra 90 days in clay soil

Figure 9 – Cotton 90 days in clay soil