

Forensic toxicological analyses reveal the use of cannabis in Milano (Italy) in the 1600's

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ABSTRACT

In this paper, we reported the results obtained from toxicological investigations on bone samples collected from human remains of the 17th century in Milano (Italy). The aim of this study was to search for analytical signs of the administration of medical or recreative plants in the population of Milano during the 17th century.

Nine femoral bone samples were extracted via Solid-Phase Extraction and analyzed via Thermo Scientific™ TSQ Fortis™ II Triple-Quadrupole Mass Spectrometer.

As a result, archeotoxicological analyses revealed the presence of two cannabinoids (Delta-9-tetrahydrocannabinol and cannabidiol) of the cannabis plant present in two out of nine bone samples (that correspond to 22% of the biological samples analyzed). The presence of these two alkaloids evidences the use of the cannabis plant in the Italian population during the 17th century. The documentation related to the pharmacopeia used to treat patients in the Ca' Granda is still present in the archives of the hospital. However, this plant was not listed inside the pharmacopeia, suggesting that the plant was not administered as medical treatment at the time in the hospital.

Thus, we hypothesize that the subjects under investigation used cannabis as a recreational substance, although other sources of exposure, such as self-medication, administration as a medical plant by other doctors outside of the Ca' Granda, occupational and involuntary exposure, cannot be excluded.

In conclusion, this study reports the first physical evidence of cannabis use in Modern Age in Italy but also in Europe.

1. Introduction

Cannabis is a plant widely used since ancient times. This plant was primarily utilized as a textile fiber since Greco-Roman times, but it was also administered, as a medical plant, to the population due to its analgesic effects. However, although the plant was very commonly utilized in the Greek and Roman ages, during the Middle Age, in particular from the 12th century onward, the interest for this plant

decreased even though we know that it was still administered to treat gout, urinary infections, birthing problems, weight loss and in general as an anesthetic and analgesic medicine (Pisanti, 2019). In 1484, a *Bolla Papale* (papal edict or bull) and a strong ecclesiastical repression banned hemp from Europe. Despite the restrictions, the plant was widely spread in Northern and Eastern Europe. Furthermore, in the 16th century travelers from Africa and Asia imported hemp into Europe for medicinal and therapeutic uses (Parrella, 2014). However, in Europe, throughout

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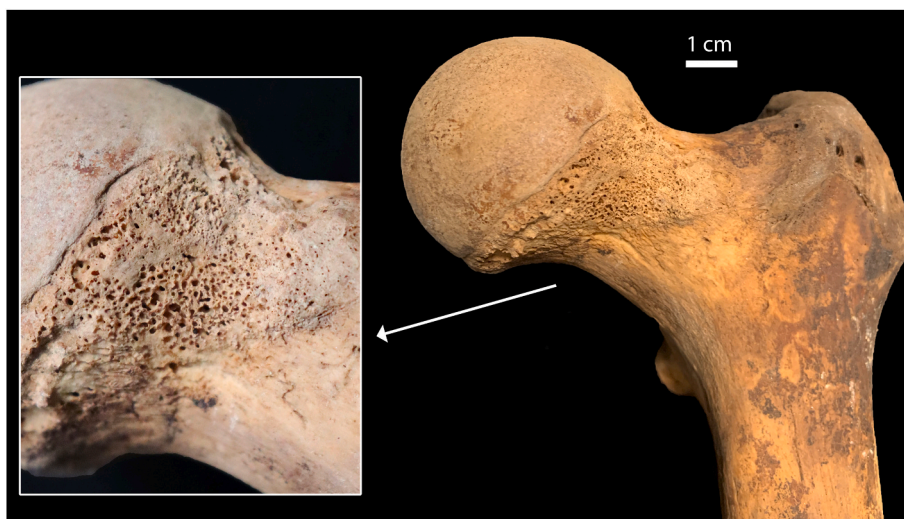


Fig. 1. Anterior view of the proximal left femur (F6) with a porotic lesion on the antero-superior region of the anatomical neck.

Table 1

Results of anthropological and toxicological analyses performed on the samples under investigation.

Sample no.	Sex	Age (years)	Pathological signs	Trauma	Stature	Toxicological results
F1	Male	55–64	Osteoarthritis	/	175.6 ± 3.27 cm	/
F2	Male	35–49	Osteochondrosis dissecans and marked enthesal changes on left femur; enthesopathies left tibia;	/	167.3 ± 3.27 cm	/
F3	Male	31–45	Osteoarthritis on vertebrae; enthesal changes on clavicles and femora	/	166.1 ± 3.27 cm	/
F4	Male	16–19	/	/	/	/
F5	Male	17–19	/	Ante-mortem trauma on left innominate	173.5 ± 3.27 cm	/
F6	Male	16–20	Porotic lesion on the left proximal femur	/	/	Delta-9-THC CBD
F7	Female	45–54	/	/	/	Delta-9-THC CBD
F8	Female	>25	/	/	/	/
F9	Male	40–54	Osteoarthritis on vertebrae	/	/	/

Table 2

Identification criteria for the molecules under investigation.

Molecule	Parent ion (m/z)	Product ion (m/z)			Retention time
CBD	315.250	123.00	193.00	259.10	3.41
DELTA-9-THC	315.250	123.00	193.00	259.10	3.60

the Modern Age, large quantities of *C. sativa* were advertised for the manufacturing commerce exclusively (Bonini et al., 2018; Zuardi, 2006).

Toxicological investigations on historical and archaeological remains are rare in literature but constitute a different and potent tool for reconstructing the past, and in particular for better understanding remedies and habits of past populations. Archeotoxicological analyses have been performed on hair samples collected from pre-Columbian Peruvian mummies revealing the presence of cocaine (Cartmell et al., 1991a, 1991b; Indriati and Buikstra, 2001; Springfield et al., 1993) or nicotine (Musshoff et al., 2009). Some other studies detected cocaine, nicotine and cannabis in hair, bone, and soft tissues of Peruvian and Egyptian mummies but the reliability of the results of these works (Balabanova et al., 1992; Parsche et al., 1993; Parsche and Nerlich, 1995) has been questioned (Musshoff et al., 2009). Therefore, to the best

of our knowledge, cannabis has never been detected or reported in archaeological human remains, in particular in bones, previous to the 21st century (Giordano et al., 2021).

For this study, biological samples from the crypt of the *Ospedale Maggiore* of Milano (Italy), one of the most innovative hospitals in Europe between the 16th and 17th century, were examined. The Ca' Granda crypt, annexed to the church of the hospital, was the place of burial of the deceased patients of this facility, from 1638 to 1697 (Agosti, 2017; Biehler-Gomez et al., 2021; Carlessi and Kluzer, 2013; Mattia et al., 2022; Vaglianti and Cattaneo, 2013). After the closure of the crypt, the human remains were preserved inside the sepulcher chambers until the beginning of the archaeological excavations. Our previous study reported the presence of opium in cranial bone samples and well-preserved brain tissues on human remains of the crypt (Giordano et al., 2023). Therefore, we decided to extend the research to long bones with a pilot study on femora. Femoral bone samples were thus collected from the human remains of the crypt with the aim to search, through archeotoxicological investigations, the presence of substances that could be associated to the administration or intake of medical or recreative plants within the population.

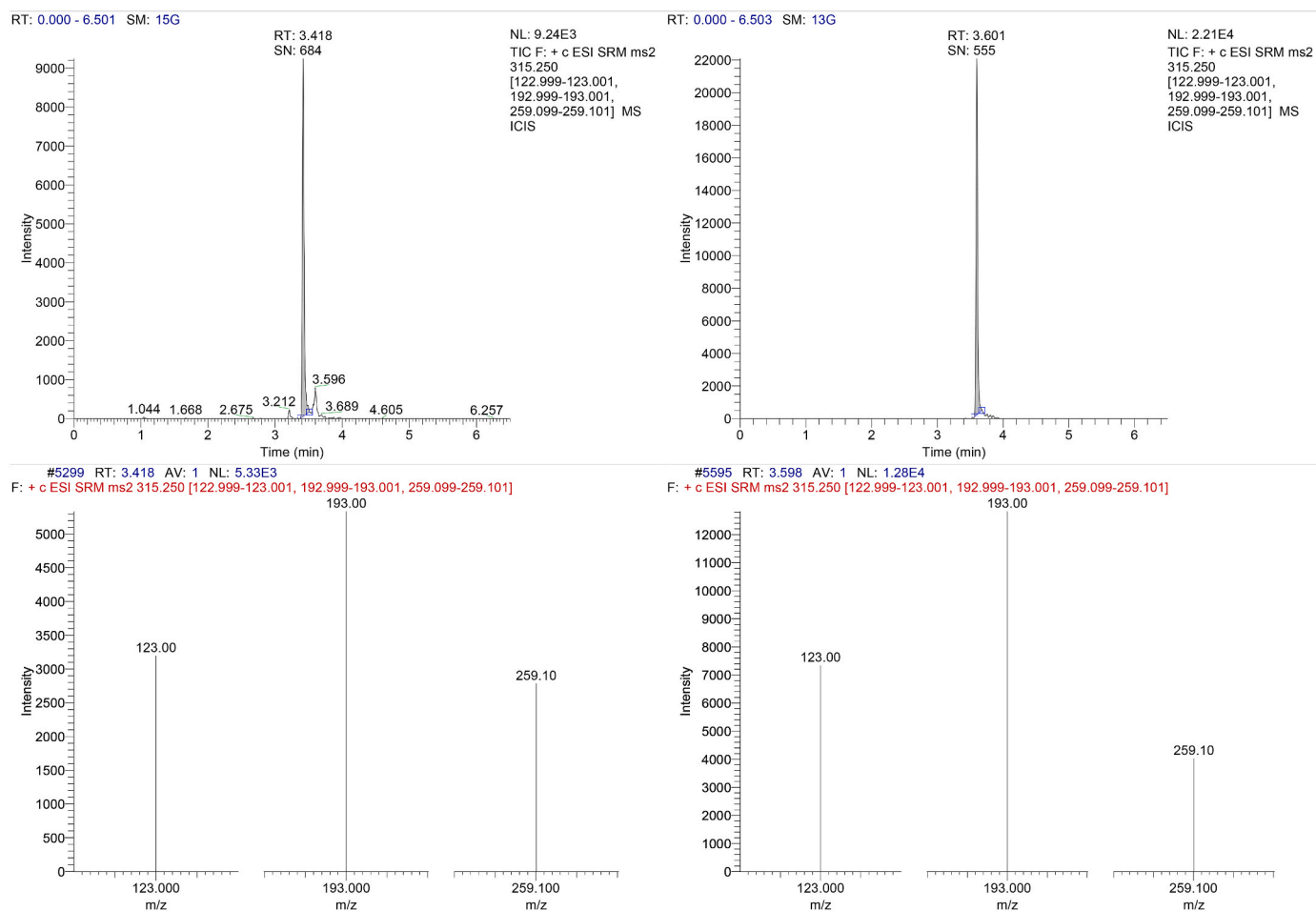


Fig. 2. Chromatographic spectrum (upper lane) and mass spectral ion ratio (lower lane) of CBD, left, and Delta-9-THC, right, detected in F6.

2. Materials and methods

2.1. Sample collection and storage

For this study, nine femoral bone samples were collected from nine individuals of the Ca' Granda crypt (the samples were named from F1 to F9). Femoral bone samples were chosen from different Stratigraphic Units (SU) of Chamber O of the crypt. Three of these subjects were excavated from SU 1, one from SU 5, and the last five cases were collected from SU 4. The individuals were placed in new plastic boxes with air inlets and left inside the Ca' Granda crypt to maintain the same environmental conditions of the sepulcher chamber until the time of analysis. Sampling was carried out inside the crypt for the same environmental reasons and to avoid its transport to the laboratory, which has different environmental conditions from the excavation site. Only the powdered samples were moved to the laboratory for analysis.

For toxicological investigation, we selected the proximal femoral epiphysis considering that the sampling site was already validated in literature (McIntyre et al., 2000; Orfanidis et al., 2018; Raikos et al., 2001), and is highly vascularized (Bairati, 1975; Watson and Adams, 2018). Moreover, the bone was present in all the individuals selected for this study, with no taphonomic alterations, allowing for equal representativity within the sample. Only one sampling site was chosen *per* skeleton in order to maintain and preserve the integrity of the cultural heritage.

Anthropological (Alqahtani et al., 2014; Brooks and Suchey, 1990; Hefner, 2009; Iscan et al., 1984, 1985; Lovejoy et al., 1985; Phenice, 1969; Rougé-Maillart et al., 2009; Scheuer and Black, 2000; Spradley and Jantz, 2011; Ubelaker, 1978; Walker, 2005, 2008) and

paleopathological (Biehler-Gomez and Cattaneo, 2021; Buikstra, 2019; Ortner, 2003; Waldron, 2008) analyses were performed, leading to the distinction of seven males and two females. Signs of osteoarthritis, osteochondritis dissecans, porotic lesions (Fig. 1) and marked enthesal changes were detected. Some bone bending indicative of possible residual rickets was noted on the left tibia of an individual and two antemortem calluses were reported on a left innominate bone and a left fibula of two different subjects. All data obtained from anthropological and paleopathological investigations are presented on Table 1. The human remains of this chamber were radiocarbon dated, as reported in Giordano et al. (2023) confirming that the human remains present inside the crypt were from the 17th century.

Radiographic imaging was performed for radiological assessment to compare and confirm the paleopathological data and potentially reveal some pathologies not seen from a macroscopic evaluation. This allowed for thorough investigation of the skeletal elements before destructive toxicological analyses. The femurs were imaged in frontal, lateral, superior, and inferior views using a Poskom PXM-40BT and an X-DR L WiFi with the following technical parameters: 73–77 kV and 4 mAs. Examion® software was used to elaborate the images acquired.

2.2. Toxicological analyses

Toxicological analyses were carried out on the proximal epiphysis of nine femoral bone samples selected for this study. The samplings were performed with a sterilized drill tip to collect the powdered bone in micro-lab tubes. Samples of 0.5 g of bone powder were weighted and decalcified in a 12.5% EDTA water solution for 48 h to remove the calcium compound and solubilize eventual molecules of toxicological

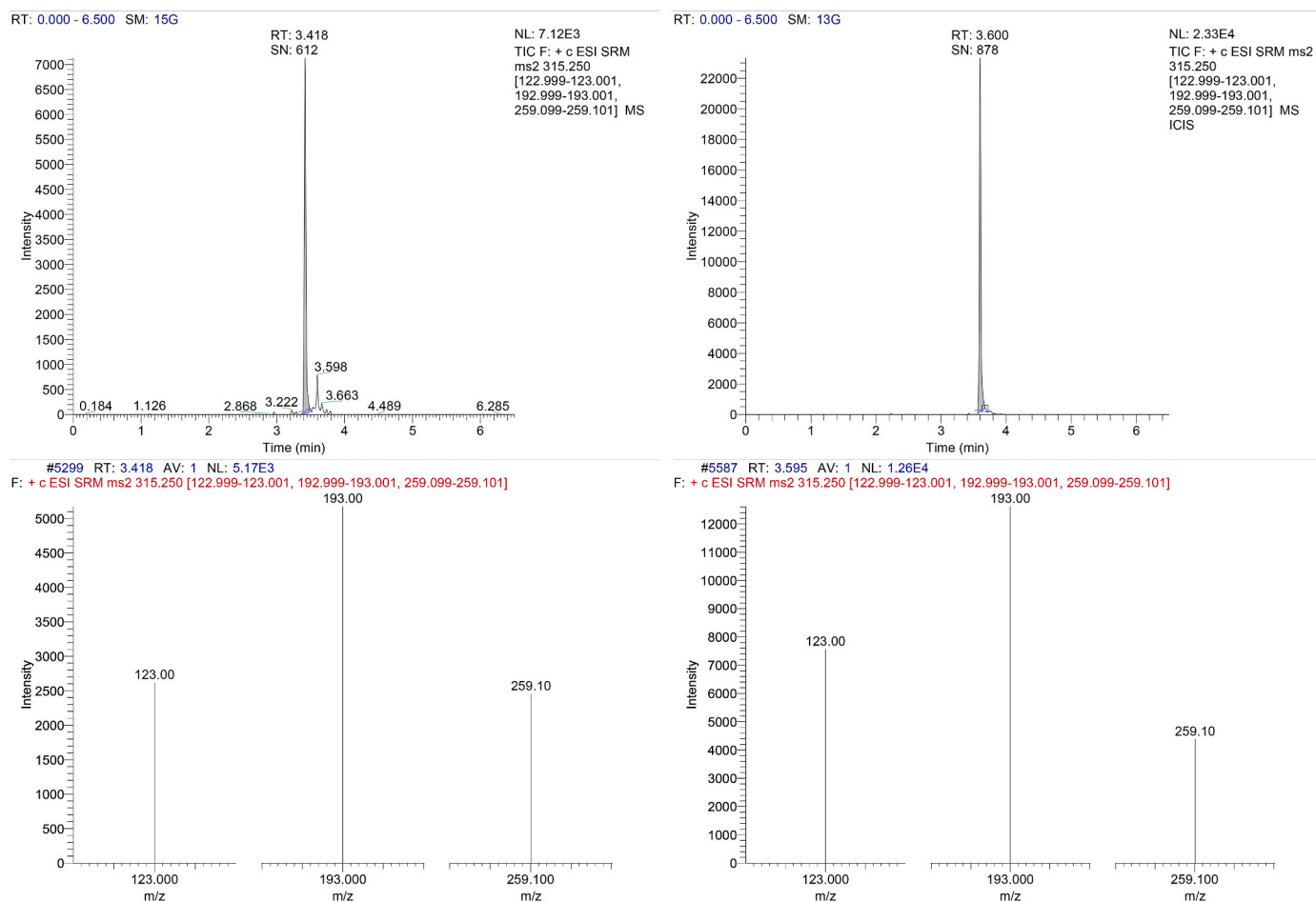


Fig. 3. Chromatographic spectrum (upper lane) and mass spectral ion ratio (lower lane) of CBD, left, and Delta-9-THC, right, detected in F7.

interest. After centrifugation, the solution was extracted with Solid-Phase Extraction technique, using a standard 12-port vacuum manifold and Bond Elut™ Certify cartridges 130 mg (Agilent). Analyses were performed with a Thermo Scientific™ TSQ Fortis™ II Triple-Quadrupole Mass Spectrometer. All details about the instrumental condition and validation procedure are reported in Di Candia et al. (2022), Giordano et al. (2023) and in the supplementary material section of this paper (Appendix A).

The molecules under investigation were confirmed following the international standard guidelines for forensic toxicology (American Academy of Forensic Science, 2019). Therefore, the analytes were identified considering the parent ion together with the characteristic fragmentation of each molecule. The signal-to-noise ratio was above 3 for all the molecules, permitting the qualitative confirmation. Then, the confirmation was assessed via reference material (analytical standards) comparing all the identification criteria.

3. Results

3.1. Toxicological results

Delta-9-tetrahydrocannabinol (Delta-9-THC) and cannabidiol (CBD), two active principles present in the cannabis plant, were discovered in two out of nine femoral samples (about 22% of the total samples analyzed), as reported in Table 1. The identification criteria used for the identification of the analytes are reported in Table 2 and the chromatographic spectra of each positive case are reported in Fig. 2 and Fig. 3.

The absence of the main metabolite of Delta-9-THC, 11-Nor-9-

carboxy- Δ 9-tetrahydrocannabinol (THCCOOH) was expected since this analyte is mainly urinary (Baselt R. C., 2004) and concentrations in different matrices, such as other storage matrices, are expected to be extremely low or absent (Muschhoff and Madea, 2006).

Any external contamination of the bone samples was excluded considering the strict sampling protocol adopted by the archaeologists, the storage of the biological samples in sterile boxes until the time of toxicological analyses and the observance of the laboratory protocol for the toxicological analyses. Furthermore, the cortical compact bone, which came into contact with the surrounding environment, was excluded during the sampling of the bone specimens.

4. Discussion

Toxicology applied to historical and archaeological human remains has not been exploited to its fullest extent. Based on the paucity of papers present in literature this discipline has been limited to studies on few biological matrices and small clusters of molecules. The present paper aims to contribute to this field of research by searching analytical signs of the administration of medical plants using long bones of individuals of the 17th century that lived in Milano. The individuals chosen for this research represent a cross-section of the poor social class of Milano cured at the Ca' Granda hospital.

The results obtained on bone samples showed the presence of two molecules, Delta-9-THC and CBD, highlighting the administration of cannabis. These results, to the best of our knowledge, constitute the first report on the detection of cannabis in historical and archaeological human osteological remains. Indeed, according to the literature, this plant has never been detected in ancient bone samples.

There is no consensus in the literature as to the main cause of the presence of substances in bone tissue, with the only interpretation being that the subject was exposed to cannabis plant from a short time to years prior to the time of death (Franceschetti et al., 2020; Horak and Jenkins, 2005; McGrath and Jenkins, 2009; Rubin, 2018; Rubin et al., 2020; Vandenbosch et al., 2020; Watterson et al., 2012; Watterson and Botan, 2009; Watterson and Desrosiers, 2011; Watterson and Vanden-Boer, 2008). However, considering the discordant sources in the literature regarding the possibility of the use of cannabis plant in the Modern Age in Europe and the discordance regarding how and for what the plant was used, it was decided to evaluate the historical source closest to the subjects under investigation: the very detailed hospital pharmacopeia. Indeed, we know that the cannabis plant was notably absent from the very detailed pharmacopeia of the hospital, suggesting that the plant was not administered as a medical treatment at that time in Milano. In fact, the hospital pharmacopeia inventoried all the plants, remedies and potions used in the various hospitals of the city, hence reflecting the medications utilized in the city of Milano during the 1600s and allowing us to discern between therapeutic and recreational plants used in that historical period by the Milanese population. If not as a medical treatment, it may be possible to hypothesize that the plant was used for recreational purposes. Indeed, we know that the cannabis plant was used for recreational aims since Greco-Roman times and administered as a medicine throughout the centuries (Mercuri et al., 2002; Pisanti, 2019), although during the Middle Ages its medical usage decreased (Pisanti, 2019). Thus, the individuals that resulted positive to cannabis may have used it for its recreational properties instead of its therapeutic ones. However, other causes of exposure to cannabis, such as self-medication, administration of the plant for therapeutic purposes by other doctors outside of the Ca' Granda, occupational or involuntary exposure, cannot be excluded.

5. Conclusion

In this paper, we presented the data obtained from toxicological investigations on long bone samples collected from the deceased patients of the Ca' Granda hospital, one of the most important hospitals in Europe of the 17th century.

The toxicological analyses performed on bone samples revealed the presence of the cannabis plant in femoral samples of two individuals (22% of the total bone samples analyzed). The analytical data obtained shed a new light on the habits of the population under investigation, demonstrating an exposure to the plant in the city of Milano during the Modern era, probably for recreational purposes given written sources, although self-medication, occupational or accidental exposure, or administration by healers not practicing in Ca' Granda are alternative possibilities that cannot be excluded.

Ethical approval

Approval to conduct this research was issued by the *Soprintendenza Archeologia, Belle Arti e Paesaggio per la città metropolitana di Milano*, an institution of the Italian Ministry of Cultural Heritage, following the ethical protocol of the agreement itself.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jas.2023.105873>.

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