Sourcing Brazilian marijuana by applying IRMS analysis to seized samples

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Abstract

The stable carbon and nitrogen isotopic ratios were measured in marijuana samples (Cannabis sativa L.) seized by the law enforcement officers in the three Brazilian production sites: Pernambuco and Bahia (the country’s Northeast known as Marijuana Polygon), Pará (North or Amazon region) and Mato Grosso do Sul (Midwest). These regions are regarded as different with respect to climate and water availability, factors which impact upon the isotope fractionations of these elements within plants. It was possible to differentiate samples from the dry regions (Marijuana Polygon) from those from Mato Grosso do Sul and Pará, that present heavier rainfall. The results were in agreement with the climatic conditions of the suspected regions of origin and this demonstrates that seized samples can be used to identify the isotopic signatures of marijuana from the main producing regions in Brazil.

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1. Introduction

1.1. Chemical fingerprint and the origin of illicit drugs

According to the United Nations Office on Drugs and Crime (UNODC), last year’s global use of illicit drugs rose from 4.3 to 4.7% in the 15-year-old population bracket, which represents an increase of 200 million-odd users, and a total value of about US$ 500 billions around the world. Although cocaine and heroin represent the most consequential problems in terms of state welfare, marijuana stands by far as the most produced and consumed drug, involving about 150 million users around the world [1]. It is estimated that the worldwide cultivation ranges from 670,000 to 1,800,000 ha with productivity from 20,000 to 30,000 tonnes a year [2].

Due to its nature of illicit trade, statistics on production, trafficking and drug dealing are mostly based on seized quantities. The UNODC mentions numerous difficulties concerning the reported figures, such as differences in the
One of the strategies adopted in order to identify and track down the origin of those materials is based on the concept of chemical fingerprint. This fingerprint is established by determining the organic, inorganic or isotopic profile of the samples, which are associated with plant growth conditions such as climate and availability of elements on the site [4–15].

The relative amounts of the main organic constituents in cannabis plants (THC, tetrahydrocannabinol; CBD, cannabidiol; CBN, cannabinol) vary widely depending on many factors, mainly the genetic variety and the environment in which the plant was grown [16,17]. These parameters are also affected by time and conditions of sample storage once THC was transformed to CBN in these plant tissues. Thus, the use of the organic profile as a chemical tracer is hindered by its complexity, requiring further information related to plant maturity and storage conditions [8,14,17,18].

In comparison to the stable isotopic ratio, the elemental profile of the plants provides further knowledge of the soil. Despite this they cannot establish explicitly their geographic origin and appear rather inconclusive [13–15,19,20]. The carbon and nitrogen isotope ratio (δ) of C and N are the most useful for sourcing the geographical origin of plant materials [21,26].

Unlike drugs such as heroin and cocaine, Cannabis sativa or marijuana is not processed for consumption and maintains its original elemental and isotopic profiles. Thus, these parameters have been used as an important indicative of its geographical origin [21,26].

Handley et al. evaluated the δ15N variation in plants and soil influenced by the following parameters: rainfall, temperature, latitude, altitude and soil pH. Their model excluded samples from regions where atmospheric N2 was a potentially major source of plant N, sites with anomalous pH values or with very high rainfall (>2500 mm annually), and regions with very low temperatures (high altitude or latitude). It was concluded that rainfall affects foliar δ15N values more strongly than whole soil δ15N, 35 and 8%, respectively [26].

Denton et al. [24] measured the δ13C and δ15N levels in marijuana samples seized in Australia, New Guinea and Thailand, with the aim of identifying their provenance. It was not possible to classify the samples according to their origin, but some important conclusions were presented: (1) δ13C and δ15N exhibit low dependence on temperature but present a direct relationship with water availability, particularly in the case of δ13C; (2) marijuana δ15N values strongly reflect the δ15N of growth substrate and fertilizer. This methodology also proved to be efficient to identify of indoor-grown sample, producing fairly characteristic δ13C values of around −31.8‰.

More recently, Galimov et al. [27] proved the potentiality of IRMS technique as a tool to source drugs using samples from different regions of Russia and Ukraine. The δ13C and δ15N results for hemp leaves showed a large variation range for δ15N (from −3.17 to 9.65‰), while for δ13C this range was narrow (from −28.38 to −26.43‰).

Although these results show the potentiality of the carbon and nitrogen isotopes ratios in assigning the geographic origin of marijuana samples, these studies are scarce in the scientific literature and still do not appear conclusive. The major difficulty reported by the authors in the development of such methodologies lies in obtaining a sufficient number of samples, particularly of recognized origin. Thus, most of these works have been achieved using samples obtained from seizures for which the original geographical origin is unclear [9,15].

The aim of this work was to verify the differences in the stable carbon and nitrogen isotopic compositions for samples seized in the main Brazilian regions of marijuana production and to evaluate the possibility of using these parameters to track the provenance of marijuana samples traded in the country. The first data for δ13C and δ15N in marijuana samples seized in South America, especially in Brazil will be presented.

1.2. Stable carbon and nitrogen isotopes in plants

The main mechanism of C fixation and fractionation in plants is photosynthesis, whereby absorption of CO2 from the atmosphere occurs. Plant tissues are deficient in 13C in relation to the atmospheric CO2, indicating that there is a discrimination against 13C absorption. This C fractionation is regulated by the plant photosynthetic pathway and is related to both stomatal limitation and enzymatic processes [23].

The three basic C pathways are Hatch–Slack–Kortchak (C4), Benson–Calvin (C3) and Crassulacean Acid Metabolism (CAM). The C3 plants reduce atmospheric CO2 to phosphoglycerate with low 13C/12C ratio, presenting δ13C average values of around −27‰. About 85% of terrestrial plants are comprehended here, including marijuana. For this plant in particular δ13C values varying from less than −30‰ to more than −24‰ have been reported according to growth conditions [24]. In general, the δ13C values decrease with
increased water availability, presenting a small dependence on temperature [24].

Nitrogen fixation occurs via incorporation of nitrated compounds from the soil. Despite the fact that the atmosphere is very abundant in N$_2$, plants only can absorb it indirectly through N-fixing bacteria. Decaying organic matter from biomass, manure and fertilizers are also important artificially introduced sources of nitrogen for plants.

In general, the global patterns of soil organic nitrogen in undisturbed ecosystems are mainly the function of annual rainfall. In the same way a strong negative correlation of foliar (leaves) $\delta^{15}$N and water availability was observed [26]. Unfortunately, for regions with N sources other than soil organic matter, the isotope ratio of this element could be more strongly associated with that source than to climactic conditions [28,29]. To summarize, we have:

(a) when N is absorbed from the atmosphere by means of N-fixing bacteria or by use of industrial fertilizers, $\delta^{15}$N values in the soil are low, around 0%o [30,31];
(b) when N is absorbed from decayed organic matter fractionation is more significant, with $\delta^{15}$N values of around 5%o, indicative of fertile soils, abundant in nutrients [31];
(c) negative values of $\delta^{15}$N are observed in soils with low ratios of organic matter, in particular dry and sandy soils [32];
(d) where manure is added as a natural fertilizer, ratios of fractionation are high, with $\delta^{15}$N above 10%o [31].

In practical terms, soil N may simultaneously originate from different sources [33]. The use of fertilizers in farming tracts, for example, may alter the soil’s isotope ratio, and as a result samples obtained from nearby locations may have significantly different isotope signatures. It is believed that this problem will be more pronounced in regions with low amounts of natural nutrients, where the use of fertilizers is necessary. However, although in many instances they may not unequivocally characterize their place of origin regarding climate, the $\delta^{15}$N values always carry strong individual indications of the planting conditions.

1.3. Marijuana in Brazil

In Brazil, figures for confiscated marijuana have risen significantly in recent years, from about 22 tonnes in 1996 to more than 150 tonnes in 2004 [1]. Those figures reflect not only a rise in production but also the efforts on the part of the Brazilian government to combat drug trafficking. In the South-American continent, Brazil leads in the number of seizures, and is second to Venezuela in terms of consumption.

Ofﬁcial data provided by the Federal Police Department (DFP) for the last years show more than 50% of the marijuana as having been seized in the country’s Midwest, which includes the State of Mato Grosso do Sul. However, eradication of plants was higher in the country’s Northeast, in the territory of the so-called “Marijuana Polygon”, traditionally a major region of production involving the States of Bahia and Pernambuco, mainly in their border region. These data are presented in Table 1. Although indicating a rising trend, seizures in the country’s North, in the Pará State, still represent a lower percentage in the national context.

<table>
<thead>
<tr>
<th>Region</th>
<th>Marijuana (g)</th>
<th>(%) Plants</th>
<th>(%) Plants (eradication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast (BA, PE)</td>
<td>16,789,563</td>
<td>22.4</td>
<td>745,534</td>
</tr>
<tr>
<td>Midwest (MS)</td>
<td>32,221,267</td>
<td>43.0</td>
<td>67,033</td>
</tr>
<tr>
<td>North (PA)</td>
<td>969,318</td>
<td>1.3</td>
<td>69,054</td>
</tr>
<tr>
<td>South</td>
<td>15,022,016</td>
<td>20.1</td>
<td>81</td>
</tr>
<tr>
<td>Southeast</td>
<td>9,876,988</td>
<td>13.2</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>74,879,152</td>
<td>100</td>
<td>881,702</td>
</tr>
</tbody>
</table>


2. Material and methods

2.1. Samples

All samples analyzed in this work were seized in the main producing States of North, Midwest and Northeast Brazilian regions by the State Police Departments (see Fig. 1 and Table 2). According to the local law enforcement officers, they were cultivated near the locales of seizures. Besides, there is no information about the existence of traffic routes between these regions.

The climatic conditions of the main producing zones are described below:

(a) The Northern region that includes the State of Pará (PA) (see Fig. 1), features equatorial climate, hot and humid with average temperatures ranging from 24 to 26 °C most of the year. With regard to precipitation, however, spatial distribution is not as homogenous as is temperature. Total annual rainfall records exceed 3000 mm along the coast and occidental areas, and drier inland regions.
strips in the state have annual rainfall averages as low as 1500–1700 mm. The annual mean rainfall is 2800 mm, with 1–3 months of dry season, depending on the region.

(b) The Northeastern States of Pernambuco (PE) and Bahia (BA) are semi-arid climates with annual average temperatures of about 25 °C. Precipitation distribution here is very complex, both in relation to occurrence (which many times may not even take place) and also in its yearly total, ranging from 200 to 2000 mm. The most rainy areas of both states are distributed along the coast. Pernambuco is drier mainly in its interior, which had around 7–9 months of dry season, with annual rainfall of up to 600 mm in the most of the state. Although Bahia has some regions with the same profile, in general its inland climate is less arid, with approximately 4–6 dry months and mean annual precipitation of 800–1000 mm. It is also important to note that Pernambuco and Bahia States include the hydrographic basin of the São Francisco River. These regions are surrounded by irrigated and fertile soils, although rainfall does not exceed 600 and 800 mm annually in PE and BA, respectively, with semi-arid climates and 7–8 months of dry season.

(c) In the Midwestern region, the State of Mato Grosso do Sul (MS), has a predominantly semi-humid tropical climate, with rainy summers and dry winters. Average temperatures are high, from 20 to 25 °C. Yearly rainfall records run from 900 to 1900 mm, a narrower variation as compared to the NE states. This state presents about 3–5 months of dry season depending on the region, with more than 70% of the precipitation occurring in the rainy season.

2.2. Samples preparation

The samples are mostly made up of leaves and a few twigs, and some may contain some seeds. In order to develop a quick and useful methodology for the police routine, the analyses were performed using the samples in their entirety. Previously to these analyses, the variability introduced by these tissues in the isotopic composition of the whole samples was evaluated for some samples from Mato Grosso do Sul. They were separated into leaves, seeds and twigs, and then the results were compared to the whole sample.
The samples were washed in sonicator for about 30 min in de-ionized water, dried at 40 °C for about 24 h, and ground using an electric mill with ceramic mortar and pestle. About 10 mg of the powdered material was used for isotope analyses.

Samples were analyzed by continuous flow isotopic ratio mass spectrometry (CF-IRMS), employing a Thermo-Finnigan Delta Plus mass spectrometer coupled to a Carlo Erba CHM 1110 elemental analyzer installed at the Isotope Ecology Laboratory, at Center for Nuclear Energy in Agriculture, CENA, University of São Paulo. In brief, organic matter is converted into gases by full combustion, generating N₂ and CO₂. These gases are chromatographically separated and carried by an ultrapure helium flow stream to the mass spectrometer. The $^{15}$N/$^{14}$N and $^{13}$C/$^{12}$C isotope ratios are evaluated after separation of molecules according to isotopic masses, and finally compared to the calibrated gases ratios using international standards as reference: $^{15}$N<sub>Amb</sub> and Pee Dee Belemnite (PDB, standard for C that presents $R = ^{13}$C/$^{12}$C = 0.0112372), respectively. Results are expressed in relative deviations of the isotope ratios as compared to the standards:

$$\delta X_{\text{sample}}(\%\text{e}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000,$$

where $R$ is the $^{15}$N/$^{14}$N or $^{13}$C/$^{12}$C.

### 2.3. Data analysis

Assessment of results followed the $k$-means cluster technique, using SPSS program, Version 10.0.5. This statistical tool is used to detect grouping in a data set and is recommended when neither the number nor the members of the groups are known [31]. It separates objects in similar groups taking into consideration two or more variables; the first $k$ cases (where $k$ is the number of clusters defined by the analyst) are used as temporary centers of the clusters. At each step, the samples are assigned in turn to the cluster with the closest center then these centers are recomputed until no further changes occur [34].

### 3. Results and discussion

In order to verify the variability introduced by the different plant tissues in the C and N isotopic composition of the samples in their entirety, five samples from Mato Grosso do Sul were separated into leaves, seeds and twigs. These results were compared to the MS group (whole samples) using $F$-test (for variance) and $t$-test (to test the equality of the means values)—see Table 3. The mean values and variance obtained for $\delta^{13}$C and $\delta^{15}$N in the different tissues and in the MS group were equivalent ($\alpha = 0.05$). Although the homogeneity of the data was lower for $\delta^{15}$N, the variability introduced by the lack of samples grooming was not significant for these studies. The results are plotted in Fig. 2 in comparison to the values obtained for MS group.

The isotopic profile of samples seized in the dry regions (BA and PE) was distinctly different from those from regions with heavier rainfall (PA and MS)—see Fig. 3.

The samples seized in BA and PE merge, with high dispersion ratios (as listed in Table 4), due to the proximity of both states and the high spatial and temporal variability.

### Table 3: Mean values and variance for $\delta^{13}$C and $\delta^{15}$N for cannabis tissues and whole samples for samples from Mato Grosso do Sul

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Mean ($%\text{e}$)</th>
<th>Variance ($%\text{e}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{13}$C ($%\text{e}$)</td>
<td>$\delta^{15}$N ($%\text{e}$)</td>
</tr>
<tr>
<td>Tissues</td>
<td>Whole sample</td>
<td>Tissues</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Mean</td>
<td>$-29.55$</td>
<td>$-29.21$</td>
</tr>
<tr>
<td>Variance</td>
<td>$0.65$</td>
<td>$0.61$</td>
</tr>
</tbody>
</table>

Fig. 2. Isotopic compositions for different cannabis tissues for five samples from Mato Grosso do Sul (leaves, twigs and seeds) in comparison to the mean value of MS group (without grooming). The bars represent 2$s$.}

Fig. 3. Isotopic profile obtained for cannabis samples from Bahia and Pernambuco (dry regions) and Mato Grosso do Sul and Pará (rainy regions).
of their precipitation records. Since PE and BA are neighboring states, many samples may have been grown in borderline areas, and those seized in PE may have been grown in BA or vice versa. Carbon and nitrogen isotopic composition cannot separate samples from these regions and then these states must be grouped as a single producing region.

Although samples from MS and PA presented overlapping, the isotopic profile obtained for Pará group pointed to heavier rainfall. The dispersion of results is slightly lower, indicating more homogenous groups particularly with respect to δ13C for PA, which may reflect a more uniform rainfall pattern in that state throughout the year.

The most significant considerations are:

(a) δ13C values of BA and PE samples ranged from −23‰ to −29‰, and δ15N values went from −3.38‰ to nearly 5‰, a high degree of variability in comparison to the other two groups.

(b) δ13C mean values of PA and MS samples were −30.5‰ and −29‰, respectively, typical values for regions with high rainfall.

(c) MS samples showed average δ15N values higher than those of PA (around 7‰ and 5‰, respectively), consistent with local rainfall values [23].

(d) These results are in agreement with the climatic condition of the locales of samples seizures.

Aiming at a better understanding of the samples behavior and in an effort to verify the existing clusters for each one of the regions, the k-means technique was employed in groups from humid and dry regions separately. In order to represent the number of the state for each cluster and to evaluate the potentiality of this statistical tool in the discrimination of the groups, k = 2 was adopted in both cases. The samples from the Northeast were classified (see Fig. 4), as follows:

**Group 1:** Samples with δ15N values in the neighborhood of 0‰. These values suggest samples from plants growing in soils not exceedingly rich in nutrients, where nitrogen may be generated by the action of N-fixing bacteria, which may also indicate the use of industrial fertilizers. Three samples from BA and one from PE showed δ15N values of less than −1.7‰, pointing to dry and sandy soils as their probable locale of growth. About 68% of these samples were seized in PE.

**Group 2:** These samples had δ15N values higher than 2.6‰ and an average value in the neighborhood of 4‰. Such values denote samples probably grown in fertile soils, with decaying organic matter and rich in nutrients. About 63% of these samples were seized in BA.

It is known by the police that marijuana in Pernambuco State is cultivated together with leguminous plants with a view to increasing the absorption of atmospheric N₂ and the availability of this nutrient in the soil. The results reinforce this theory, since δ15N values for this state were mostly around 0‰. Results for Group 2 may be associated with the São Francisco Basin, which is bordered by fertile soils and presents low annual rainfall with 7–8 months of dry season. Consistently with the semi-arid climate, some samples show extremely high δ13C values, of around −24‰.

The classification of MS and PA samples by the k-means technique is shown in Fig. 5. Group 3 basically refers to samples seized in PA (84%, but includes three samples from MS), while Group 4 basically refers to MS samples (83%, but includes four samples seized in PA). Results obtained for samples from MS presented a high degree of dispersion (see Table 3) and can be related to the local and seasonal variation of rainfall. Some samples from MS and one sample from PA presented δ13C values similar to those obtained for dry regions, and are in agreement with rainfall variation. The δ15N results for these states also are in agreement with rainfall extent, with values for PA lower than those for MS. The overlap observed between these
groups is related to similar climatic conditions and cannot be eliminated.

The behavior of samples may be more clearly observed in the histograms of Figs. 6 and 7. With the exception of Fig. 7a, the distribution of $\delta^{13}$C and $\delta^{15}$N reveal the climatic conditions of marijuana growth, allowing identification of samples from semi-arid and rainy regions. Values of $\delta^{15}$N of dry regions are more complex (see Fig. 7a), and indicate the use of fertilizers and other resources in an effort to optimize crops.

4. Conclusions

This work demonstrates the potentiality of IRMS technique as an important tool in tracking the provenance of marijuana samples seized in different Brazilian regions. The isotopic profile was in agreement with the climatic conditions of the locales of seizures, confirming the law enforcement officer information that most of them were cultivated in the same region where they were seized. As expected, due to the geographical proximity, it was observed a large overlap between samples from Pernambuco and Bahia. The overlapping of some samples from Pará and Mato Grosso do Sul can be related to the climatic similarity of these regions. In both cases, the use of complementary analytical techniques such as gas chromatography, gas chromatography mass spectrometry or even inorganic analysis, such as inductively coupled plasma atomic emission or mass spectrometry can be used to assess additional information. In spite of this, the results show a clear difference between samples from the humid and dry regions.

Based on this work, it will be possible to evaluate the possibility to create a national database of isotopic signature of Brazilian marijuana using stable isotope measurements of

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Fig. 5. Samples from Mato Grosso do Sul and Pará classified by $k$-means technique.

Fig. 6. (a and b) Histogram of $\delta^{13}$C for samples seized in semi-arid regions and in rainy regions, respectively (BA, PE, MS and PA represent Bahia, Pernambuco, Mato Grosso do Sul and Pará, respectively).

Fig. 7. (a and b) Histogram of $\delta^{15}$N for samples seized in semi-arid regions and in rainy regions, respectively (BA, PE, MS and PA represent Bahia, Pernambuco, Mato Grosso do Sul and Pará, respectively).
seized samples, which in future can also be used to source the provenance of the marijuana seized in the main centers of consumption, as the city of São Paulo.

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