

Carbon and nitrogen stable isotopes as indicative of geographical origin of marijuana samples seized in the city of São Paulo (Brazil)

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Abstract

The drug trafficking is one of the most serious problems related to the Public Safety in Brazil, especially in the most populous areas of the country, as is the case of the city of São Paulo. In this work, it was developed a methodology that can help tracking the traffic routes of marijuana samples seized in the city of São Paulo, based on stable carbon and nitrogen isotopes, which are related to the climate and plant growth conditions. A model to classify the origin of unknown samples was built using linear discriminant analysis based on about 150 samples apprehended in the main producing regions of the country. Results for 76 samples seized in the city of São Paulo showed that most of them were cultivated in a humid region with the same origin as those from Mato Grosso do Sul. The provenance of 13 outliers samples from Northeast region (an important producing region) also were evaluated and some of them presented same profile of those from Mato Grosso do Sul, pointing to the existence of the traffic routes between the Northeast and Midwest region, probably as a consequence of the intensive field raids by Brazilian Federal Government since 1999.

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1. Introduction

Nowadays, illicit drug trade affects almost all countries, despite of existing differences in its extension and characteristics related to economic, social and cultural factors. According to the United Nations, this illegal sector activities use resources, labor and so complex infrastructure that represents a huge impact in the world economy moving about US\$ 500 billions annually [1].

The United Nations Educational, Scientific and Cultural Organization (UNESCO) report mentions that the 1980s and 1990s were strongly marked by the surge of activities related to the production and drug trade [2]. During this period, the major

criminal organizations also emerged and now they are spread in all the major regions of the world [2]. The globalization that increases the licit commercial routes makes also all countries vulnerable to these criminal activities. In this period, Brazil began to be an important transit country to the traffic of cocaine and marijuana (*Cannabis sativa* L.) produced in Colombia, Bolivia and Paraguay for United States and European countries, which continuous nowadays together with national production, that has suffered a huge increase since the 1980s [2,3]. Despite of the importance of cocaine consumption, marijuana is the most popular drug in the country, following the worldwide trend [1].

It is known that the drug trade represents a serious problem for the society, as pointed by the statistical indexes [4] and the São Paulo State Government has been demonstrating deep concerns regarding drug dealing making the combat to this social problem one of their priorities in Public Safety's area. The major problem against these activities in the State is the one

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related to the lack of information regarding their origin and distribution routes. As known, São Paulo is not an important *Cannabis* producer but is certainly the major consumer market in the country [3–5]. Thus, the point is “where does the marijuana of the State come from?” By the end of the 1990s, Marijuana Polygon located in Brazilian Northeast region and Paraguay appear as the main *Cannabis* suppliers to São Paulo State [3,5]. However, the intensive and sustained field raids promoted by Brazilian Federal Police between 1999 and 2003 in this region eradicated about 10 millions of *Cannabis* plants, reducing the local productivity, forcing the producers to migrate to other States, mainly those located in country’s North region such as Pará and Maranhão which could cause changes in the traffic routes throughout the country [5,6]. Nowadays, the apprehensions accomplished by the São Paulo Police suggest that most of these drugs are coming from Paraguay, via Mato Grosso do Sul route (a neighboring State) or from producing regions located along this Brazil–Paraguayan border [3,5,7]. Recent information also indicates the rising of traffic between Paraguay and Ceará State, although by now there are no reliable tools to verify its truthfulness [5].

A peculiar feature in our country lies in the fact that *Cannabis* plants are cultivated together with normal crops, aiming to hinder the identification by aerial monitoring. It is also known that the soil management practices and highly irrigated areas frequently occur in these farms (personal communication).

The establishment of patterns to classify the geographical origin of drug samples that is based on chemical profile or chemical fingerprint has been escalated in the last years [8–21]. The stable carbon and nitrogen isotopic ratios are related to the plants growth climatic conditions, mainly water and nutrient availability besides light intensity and temperature, and can be useful as indicatives of their origin, providing tools to delimit their potential cultivation areas if these conditions are significantly different [14–24]. Despite of the potentiality, the effective use of this technique by the Law Enforcement Officers to trace the origin of drug samples seized in the streets has not yet been performed.

In a previous work, marijuana seized from dealers and users by the State Law Enforcement Officers within the main producing zones of Brazil was analyzed. The carbon and nitrogen isotopic compositions were determined in samples from Mato Grosso do Sul (Midwest), Pernambuco and Bahia (Northeast) and Pará (North) [15]. The purpose of this strategy was to verify the existing differences in the carbon and nitrogen isotopic composition of samples seized in the main Brazilian regions of marijuana production. The results were in agreement to the climatic conditions of the locations of samples seizures being possible to clearly separate samples from the humid regions (North and Midwest) from those produced in the drier Northeast region of Brazil and confirming the information that most of them were cultivated in the same region where they were seized [15].

The aim of this work was to use these stable isotopic compositions database to source the geographical origin of marijuana samples apprehended by the State Police Department

in the streets of the São Paulo city. The evaluation of the data was performed using linear discriminant analysis (LDA). In order to increase the database used as reference, additional samples from Mato Grosso do Sul and Pernambuco were analyzed and it was included two new States in the study: Maranhão and Ceará. The former is located close to Marijuana Polygon but not appears as an important producing zone, supplying only the local market. The Maranhão State, on the other hand, has being pointed by specialists as the main *Cannabis* producer in the country, a recently acquired status that can be a consequence of intense monitoring by the Federal Police since 1999.

2. The stable isotope methodology

Stable isotopes are generally expressed as the isotopic ratio R , which represents the ratio between the heavy (^{13}C , and ^{15}N) and the light isotopes (^{12}C , and ^{14}N). For carbon the isotopic ratio is the molar ratio of $^{13}\text{C}/^{12}\text{C}$ and for nitrogen the molar ratio of $^{15}\text{N}/^{14}\text{N}$. The isotopic compositions are expressed by the “ δ ” notation as follows:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right] \times 1000 \quad (1)$$

where R_{sample} is the isotopic ratio of the samples, and R_{std} is the isotopic ratio of the standard. For carbon, the international standard is CO_2 from Pee Dee Belemnite limestone. For nitrogen, the standard is the atmospheric air. The δ values are conveniently presented in parts per thousand (‰).

The stable carbon isotopic composition of plants is regulated by their photosynthetic pathway. There are three basics pathways called: Hatch–Slack–Kortschak, Benson–Calvin and Crassulacean Acid Metabolism (CAM). The *Cannabis sativa* L. is a C3 plant and follows the Benson–Calvin photosynthetic pathway, and as most plants shows a range of $\delta^{13}\text{C}$ values from -24 to -35 ‰. Tropical grasses like corn, sugar cane and grasses used as forage for livestock generally follow the C4 pathway, and only a small proportion of plants in tropical regions follows the CAM pathway.

The stable isotopic composition of C3 plants is mainly controlled by the relationship between the CO_2 concentrations inside the stomata (c_i) and of the atmospheric air (c_a) following the equation below [22]:

$$\delta^{13}\text{C}_{\text{C}_3} = \delta^{13}\text{C}_{\text{atm}} - a - (b - a) \frac{c_i}{c_a} \quad (2)$$

where $\delta^{13}\text{C}_{\text{atm}}$ is the carbon isotopic composition of atmospheric CO_2 , a the physical fractionation of the CO_2 from the air to the stomatal chamber (4.4‰) and b is the fractionation involved in the Benson–Calvin photosynthetic pathway (27.5‰).

The ratio c_i/c_a is controlled by the rate between photosynthetic activity and stomata conductance, that in turn is affected by environmental conditions. Therefore, a smaller c_i/c_a ratio will determine a higher $\delta^{13}\text{C}$ value for a C3 plant tissue and, conversely a higher c_i/c_a ratio will be related to a smaller $\delta^{13}\text{C}$.

The nitrogen stable isotopic composition of plants is not as easily explained as the carbon composition. Innumerable factors

may interfere with this composition [25]. The sources of N to plants are wet and dry deposition, N fixation from the air through association with N-fixing bacteria, the soil, and in the case of crops, the type of fertilizer used. All these sources may have distinct N stable isotopic compositions acquired by plants when using different sources. For instance, some plants from the Legume family fix nitrogen from the air through association with N-fixing bacteria; the $\delta^{15}\text{N}$ values of fixing plants tend to be lower than non-fixing plants, because the $\delta^{15}\text{N}$ value of the atmospheric N is 0‰. Then, the cultivation of non-fixing plant like marijuana, associated with N-fixer plants may lower its $\delta^{15}\text{N}$ [26,27]. Mineral nitrogen fertilizers have also a $\delta^{15}\text{N}$ near 0‰, while the $\delta^{15}\text{N}$ of animal manure is generally much higher (G.B. Nardoto, non-published data). Therefore, the preferential use of one of these N-sources will affect the $\delta^{15}\text{N}$ of plants making use of them. The form of N that plants take up from the soil and their rooting depth also influence the nitrogen isotopic composition. Plants may take up either NH_4^+ and NO_3^- , and also organic N under special conditions. Generally, the N isotopic composition of these different forms of N is different and consequently, plants absorbing NH_4 will tend to have a different N isotopic composition from plants absorbing NO_3 . Water stress and N limitation also may affect the N isotopic composition of plants. The general rule is that a drier region would have higher $\delta^{15}\text{N}$ values than a wetter region [28], and N limited sites would have a lower $\delta^{13}\text{C}$ than N rich sites [29]. Also, a high variability in the $\delta^{15}\text{N}$ values for plants cultivated in regions where N severely limits plant growth [30] is expected.

3. Samples

In a previous work 90 samples seized in the North (Pará-PA), Midwest (Mato Grosso do Sul-MS) and Northeast (Bahia and Pernambuco-BA and PE) regions of Brazil were analyzed for C and N isotopic compositions using isotope ratio mass spectrometry technique [15]. Besides the 76 samples of marijuana seized in São Paulo city, new samples from Pernambuco and Mato Grosso do Sul and new samples from other States of the Northeast (Maranhão-MA, and Ceará-CE), were included in this study, making a total of 230 samples of marijuana (Table 1). With the exception of 76 samples seized in the city of São Paulo, all other samples analyzed in this work were seized by the State Police Departments within the main producing States in the North, Midwest and Northeast. With the exception of five

samples from Mato Grosso do Sul and three from São Paulo apprehended in 2004, all seizures were performed between 1999 and 2002.

3.1. Climatic conditions of marijuana-producing regions

The climatological conditions of the regions under investigation relevant to the carbon and nitrogen isotope compositions are briefly described as follows.

Brazil's Northern region where the State of Pará (PA) is located features equatorial climate, hot and humid with average temperatures ranging from 24 to 26 °C most of the year. However, spatial distribution of precipitation is not as homogenous as temperature: total annual rainfall records exceed 3000 mm along the coast and occidental areas, and drier inland strips in the state have annual rainfall averages as low as 1800 mm. The annual mean rainfall is 2800 mm, with 1–3 months of dry season, depending on the region (Fig. 1). Although the State of Maranhão is not part of Brazil's North, its western portion has a climate and vegetation similar to the Northern region, while its eastern portion has a semi-arid climate. Due to this difference, the samples from this State need to be grouped in two categories: those with isotopic values similar to samples from the North (MA-w) and those with isotopic values similar to samples from the Northeast (MA-d).

The country's Northeastern region encompasses the States of Pernambuco (PE), Bahia (BA), Ceará (CE) and the eastern portion of Maranhão (MA) which have 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. Rainfall is generally higher on the coast (1700–2000 mm) and drops dramatically inland (0–800 mm), causing severe drought problems for agriculture and public water supply (Fig. 1). The length of the dry season inland varies from 7 to 8 months.

Finally, located in the Midwest, 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 in comparison to the NE states (Fig. 1). It is important to observe that the climatic conditions of the Eastern region of Paraguay, where *Cannabis* farms are located, are similar to this Brazilian State.

Table 1
Number of marijuana samples analyzed in this work seized in different States of Brazil

Provenance	Shibuya et al. [15]	This study	Apprehension year (number of samples)	Total
Pernambuco (PE)	27	20	2000 (15), 2002 (9), ND (23)	47
Bahia (BA)	20	–	2001 (15), ND (5)	20
Maranhão (MA)	–	12	2002	12
Ceará (CE)	–	25	2002	25
Pará (PA)	20	–	2002	20
Mato Grosso do Sul (MS)	23	7	1999 (5), 2000 (7), 2001 (9), 2002 (4), 2004 (5)	30
São Paulo (SP)	–	76	1999 (3), 2001 (36), 2003 (16), 2004 (3), ND (18)	76
Total	90	140		230

ND: not determined.

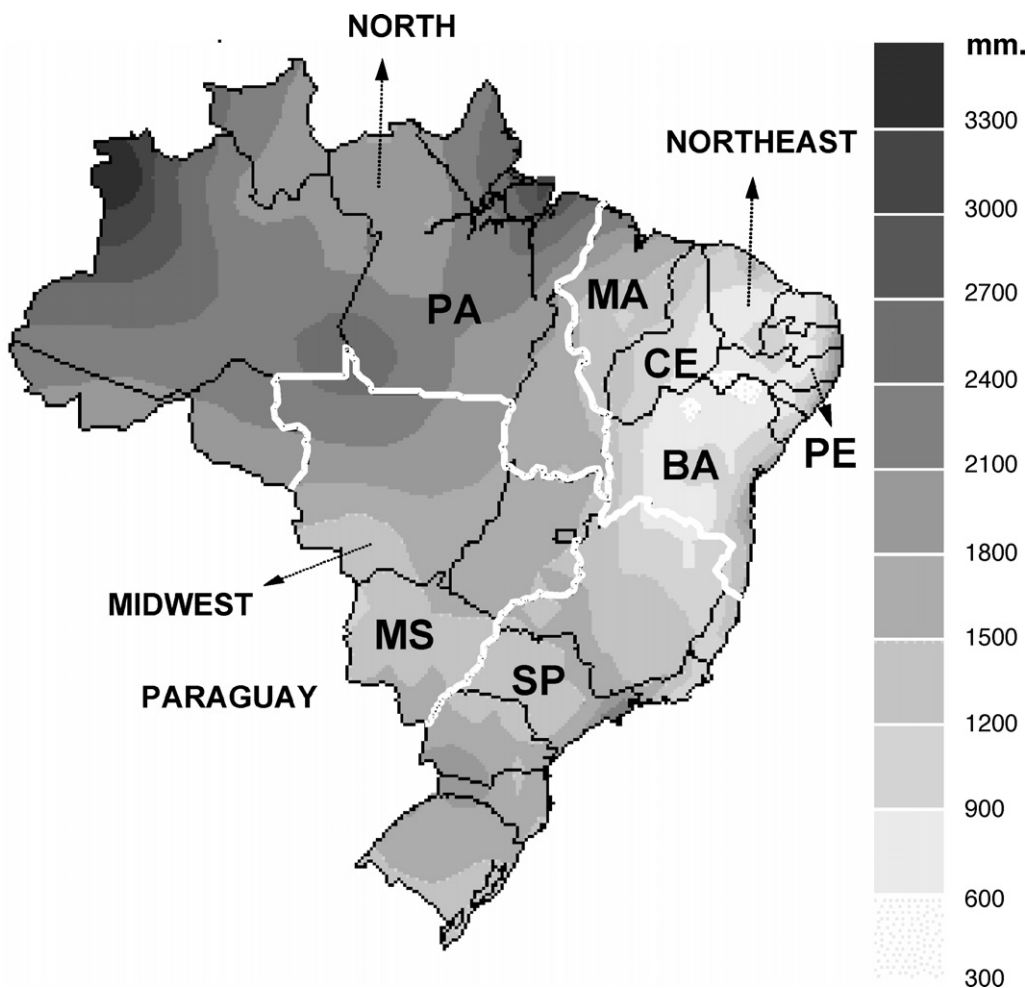


Fig. 1. Map of Brazil showing the main geographical regions (white line), State divisions (black line), and the average annual precipitation from www.ibge.org.br. North Region-State of Pará (PA); Northeast Region-States of Bahia (BA), Ceará (CE), Maranhão (MA), and Pernambuco (PE); Midwest Region-State of Mato Grosso do Sul (MS).

3.2. Sample preparation and data analysis

According to the methodology presented in Shibuya et al., about 2 g of each samples were decompressed, washed in ultrasonic cleaner for about 30 min in deionized water, dried at 40 °C for about 24 h, and ground in an electric mill with ceramic mortar and pestle. Finally, about 10 mg of material were set aside for isotope analyses.

Samples were analyzed by continuous flow isotopic ratio mass spectrometry (CF-IRMS), employing a Carlo Erba CHM 1110 elemental analyzer coupled to a Thermo-Finnigan Delta Plus mass spectrometer 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 in the elemental analyzer, generating N₂ and CO₂. These gases are chromatographically separated and carried by an ultrapure helium flow stream to the mass spectrometer for isotopic and concentration analysis. The ¹⁵N/¹⁴N and ¹³C/¹²C isotope ratios are evaluated after separation of molecules according to isotopic masses, and expressed as δ (parts per thousand) according to Eq. (1).

The assessment of the results followed the *k*-means cluster and linear discriminant analysis, using Statistical Package for Social Science (SPSS) program, version 10.0.5. Both methods separate objects into similar groups taking into consideration two or more variables [31].

In the *k*-means technique, 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, and then these centers are recomputed until no further changes occur [32]. This technique was used to group together samples from Maranhão into wet Maranhão (MA-w) and dry Maranhão (MA-d).

The linear discriminant analysis identifies a linear combination of quantitative predictor variables that best characterizes the difference among known groups (called calibration set) [32]. This methodology enhances the separation of the groups, allows a classification of unknown samples and lists the group in which each case is most likely a member, and the probability for belonging to this group (*P*-value). The first canonical variable account for a large proportion of the variability within the original data and the plot is defined, so that the most

significant differences among the groups are displayed along the horizontal axis. The second canonical variable represents the maximum dispersion in a perpendicular direction to the first one, and so forth. Before this evaluation, the boxplot (box-and-whisker plot) was constructed in order to identify the existence of outliers (cases that have values more than 1.5 box-lengths from 25th or from 75th percentile) [31]. This method was used to build a model based on samples seized in the producing zones, which was used to classify samples from the city of São Paulo.

4. Results and discussion

In comparison to the previous works, it can be seen that the same trends [15] still persists with the larger database analyzed in this study. According to Eq. (2) contrasting climatic conditions yield a distinct carbon isotopic composition of marijuana samples in different regions of the country. The $\delta^{13}\text{C}$ values for samples from NE were in a range from -30 to -24‰ , with most of samples ranging from -28 to -25‰ , typical of dry regions, while for samples from humid regions the results varied from -32 to -25‰ . The $\delta^{15}\text{N}$ values for humid regions varied from 2 to 10‰, with most of samples between 4 and 8‰. For dry regions the results ranges from -4 to 11‰, with most of samples between -1 and 5‰, a high dispersion in comparison to the other group.

The State of Maranhão has a peculiar geographical situation, where the western portion of the State lies on the Amazon region and the eastern portion on the drier Northeast (Fig. 1). The $\delta^{13}\text{C}$ values of marijuana samples seized in this State reflect these distinct climatic conditions with values ranging from -31.5 to -26.2‰ . The k -means technique divided this group into wet Maranhão and dry Maranhão with $\delta^{13}\text{C}$ values ranging from -31.5 to -29.7‰ and from -28.7 to -26.2‰ , respectively.

The $\delta^{13}\text{C}$ of marijuana samples from the North were similar to natural shrubs and trees of the region. The average foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of almost 800 trees collected in the Amazon region were equal to -32.3 ± 2.5 and $5.8 \pm 1.6\text{‰}$, respectively [33]. Values of nitrogen and carbon stable isotopic compositions of the natural vegetation of the Northeast region are not available in the literature. Thus the stable isotopic compositions of marijuana samples seized in the Northeast were compared to plants of the tropical savanna located in Brazil's central region, locally called Cerrado (Fig. 2). The average precipitation in the Cerrado area (1500 mm) is higher than the precipitation in the semi-arid region of the Northeast, however more than 90% of the precipitation falls in the rainy season, leading to a severe water stress during the dry season [30], which is a similar condition observed in Brazil's Northeast. However, the $\delta^{13}\text{C}$ values of Cerrado plants are approximately 2‰ lower than the marijuana samples seized in the Northeast. For $\delta^{15}\text{N}$, marijuana samples seized in the Northeast presented values similar to the Cerrado and much lower than samples seized in the Amazon.

Samples from Ceará also presented highly dispersed results, with $\delta^{13}\text{C}$ values between -29.5 and -25.5‰ and $\delta^{15}\text{N}$ between -0.8 and 7.8‰. Values for $\delta^{13}\text{C}$ about -29‰ and $\delta^{15}\text{N}$ above 6‰ were observed for nine samples from these groups, a non-expected profile considering the local climatic

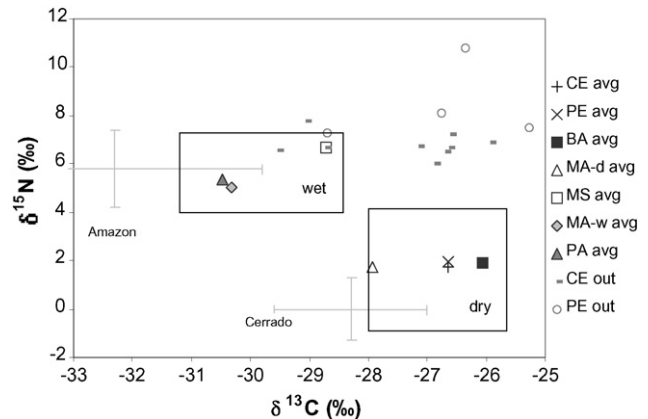


Fig. 2. Plot of averages $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values of marijuana samples seized in Brazilian States. Dots represent the average of each State grouped as humid (North and Center-West) and dry region (Northeast). The dotted boxes represent one standard deviation of all samples from the humid and dry regions. The average values with bars (1 standard deviation) represent the natural vegetation of the Amazon region [33]; and the natural vegetation of the Brazilian cerrado located in the Central part of the country, near the city of Brasília [30]. “CE out” and “PE out” represent the outliers samples from each State.

conditions. These results can be related to the existence of highly irrigated regions with management practices in the soil. Another feature is the possibility of these samples to be originated in other locations, once Ceará is not considered an important producer. For these reasons, these nine samples were considered outliers (Fig. 2), while the rest of them presented isotopic profile within the expected range ($n = 16$). The results for each State in comparison to native plants for Cerrado and Amazon region can be observed in Fig. 2.

Isotopic values of marijuana samples seized in the city of São Paulo were plotted against the values obtained for the main Brazilian growing regions. Most of the samples seem to be from humid regions (North or Midwest) and not from the dry region (Northeast) (Fig. 3). Despite the overlapping between samples from Pará and Mato Grosso do Sul, it appears that the bulk of the samples seized in São Paulo presents the same origin of samples from Mato Grosso do Sul (Fig. 3). Exceptions to this general trend were samples SP2 and SP3, which had isotopic

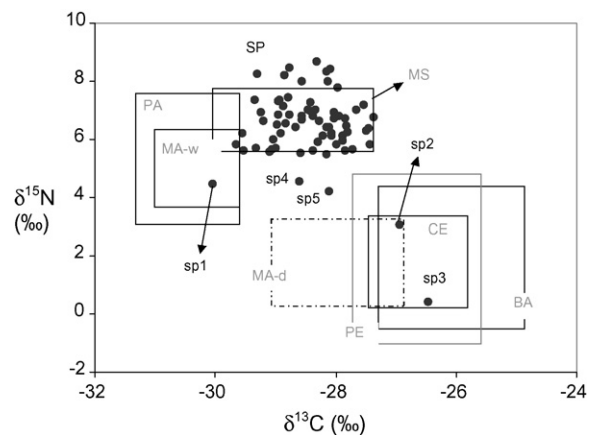


Fig. 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of samples seized in São Paulo city (full circles). Dotted boxes represent 1 standard deviation of each State.

profile similar to the Northeastern group, and sample SP1 that seems to originate from the Amazon region (Fig. 3).

In order to verify these visual observations, to delimit the distribution of the groups in the canonical discriminant function space and establish a territorial map, the linear discriminant analysis was applied to the data set. In this model the most important regions of marijuana production were considered as follows: Region 1-Mato Grosso do Sul, Region 2-Northeastern States (Bahia, Pernambuco, Maranhão-dry, and Ceará-dry), and Region 3-Amazon Region (Pará and Maranhão-wet). Four samples from Pernambuco were outliers presenting $\delta^{15}\text{N}$ values higher than 7‰, and were not considered in the building of the classification model (Fig. 2).

An overlapping of around 16% between Mato Grosso do Sul and the Amazon region was observed and can be explained by the similarity in their climatic conditions. Samples from the Northeast were successfully separated (100%) from those seized in locations with high water availability. This model classified correctly about 93% of the calibration set. Regarding samples seized in São Paulo, most of them appears to present the same origin as those from the State of Mato Grosso do Sul (Fig. 4a).

In this two-dimensional case, the LDA only performs an axis rotation. For this reason, it is not possible to improve the separation between groups, as can be observed. However, this methodology delimits the distributions of the groups in the canonical space, identifies outliers and allows classifying unknown samples, providing their probabilities for belonging to this group, which is very useful in this problem. As expected,

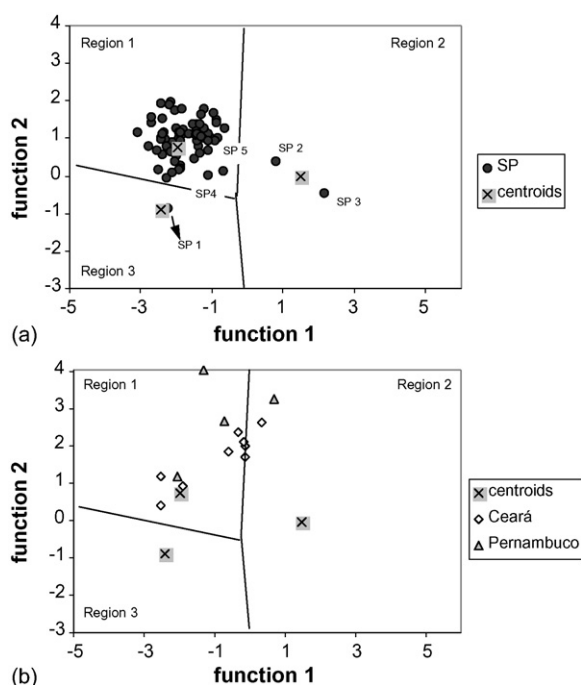


Fig. 4. (a) Territorial map and classification of samples seized in São Paulo according to the model built using linear discriminant analysis considering the following groups-Regions 1 (MS), 2 (PE, BA, MA-d, CE) and 3 (PA, MA-w). (b) Outliers samples from Pernambuco and Ceará classified according to the model. Four samples seem to present the same origin of those from Mato Grosso do Sul, while the rest of them could not be clearly classified.

it was possible to identify the Amazon region as the most probable locale of origin of sample SP1 ($P = 0.759$) (Fig. 4a). Samples SP2 and SP3 were probably cultivated in the Northeastern region ($P = 0.985$ and 1.000 , respectively) and the samples SP4 and SP5 appear to be cultivated also in the same locations of samples seized in the State of Mato Grosso do Sul ($P = 0.645$ and 0.609 , respectively) (Fig. 4a). Once LDA does not recognize cases where a set of samples do not belong to any of the studied classes, the possibility that samples SP4 and SP5 may have been cultivated in another region cannot be discarded.

According to Mingardi, most of marijuana consumed in the city between 1996 and 1999 was originating from Paraguay and Marijuana Polygon, especially Pernambuco State. The existence of this traffic routes was sustained by the Brazilian Federal Police until 2002, however the samples analyzed here which are seized between 1999 and 2004 showed no significant traffic from Northeast Region to São Paulo. This result can be explained, firstly by the low number of samples analyzed in this work, which could result in a non-representative sampling. However, this apparent contradiction could also be related to the continuous changes in the producing zones due to the field raids early mentioned, or even so the use of inaccurate information provided by the users and dealers for the Law Enforcement Officers.

It is important to observe that *Cannabis* cultivation has been recovered in Pernambuco in the last years due to a decrease in the field raid activities and an increase in precipitation levels, which favor the plant growth [34]. Furthermore, according to specialists, the price increase due to low local production causes the appearance of new plantations [35].

Outliers samples from Pernambuco and Ceará were reclassified using the LDA model. Amongst 13 samples, 4 of them (3 from Ceará and 1 from Pernambuco) presented profiles similar to the ones of Mato Grosso do Sul ($P > 0.83$) (Fig. 4b). The remaining ones could not have their origin clearly identified due to the high $\delta^{15}\text{N}$ values, as can be seen in Fig. 4b.

The Northeast of Brazil covers an area of approximately 1.5 million km^2 . It is estimated that 50% of this area is classified as semi-arid, with annual precipitation varying from 400 to 800 mm [36]. Generally, the drier sites are inland and

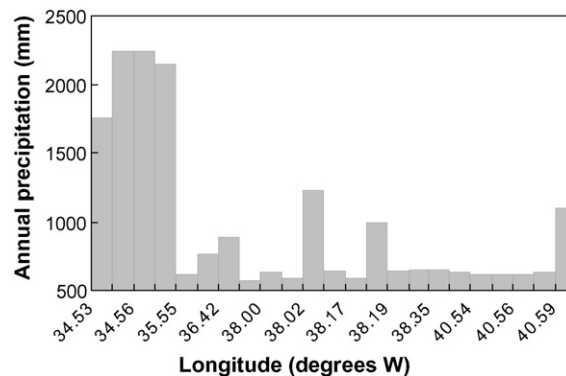


Fig. 5. Average annual precipitation for several cities in the Northeast Brazil. Coastal areas are located between $34^{\circ}5'$ W to $35^{\circ}02'$ W. Data summarized by Alcoforado-Filho et al. [37].

wetter sites on the coast. As an example of such climatic variability the annual precipitation data were plotted against longitude for various sites in the Brazilian Northeast (Fig. 5). This inland precipitation gradient forms three different phytogeographic zones. The *Sertão* is the driest inland zone, *Zona da Mata* the wettest sites on the coast, and a transition zone called *Agreste* [37]. If the N availability was also to be distinct along this gradient, it is likely that the $\delta^{15}\text{N}$ values of marijuana plants would also be distinct. Managemental practices may also add more variability to the $\delta^{15}\text{N}$ values of marijuana. For instance, the use of leguminous plants as green manure to marijuana crops would tend to lower their $\delta^{15}\text{N}$ values, since leguminous are N-fixers. The same would be true if marijuana was a fertilizer with mineral N that also has a $\delta^{15}\text{N}$ near 0‰. On the other hand, the use of animal manure would increase the $\delta^{15}\text{N}$ value of the crop.

Based on the information obtained by the Brazilian Federal Police that marijuana is widely produced in the whole Northeast, it is reasonable to conclude that the variability observed in the $\delta^{15}\text{N}$ of marijuana plants may be due to the cultivation of this drug in zones where there are enough environmental or managemental differences as the use of animal manure. However, for conclusive results regarding to the origin of these nine samples (Fig. 4b), additional parameters such as inorganic or organic profile must be studied and the use of information related to native plants, which were not found in the literature would be very useful.

As previously observed, the results of seized samples were in agreement with the climatic conditions of the seizures locations, sustaining the theory that most of them were cultivated in the same region where they were seized. In this way, since samples taken from eradicated plantation are not available, street samples seized near the producing zones can be used to establish their regional isotopic profile. If, on the one hand this approach can bring some level of uncertainty (due to the quality of sampling and lack of the knowledge of the growth conditions), on the other hand it overcomes the difficulties and risks related to the field raids. It is important to notice that despite of the reduced number of samples, no trends were observed regarding the year of apprehension.

5. Conclusions

Despite of reduced number of samples this work demonstrated the potentiality of the use of carbon and nitrogen stable isotopes associated to linear discriminant analysis in sourcing the provenance of marijuana samples seized in the different Brazilian producing regions. Although these parameters alone cannot determine the drugs origin, when used together with existing information such as the probable traffic routes, appear as a powerful tool to monitor the drug trade and even so to detect the appearance of new routes in the country, providing information regarding to producing zones and plant growth conditions. This methodology can be used to help solving one of the main problems in criminal area in Brazil, using very low samples quantity thus preserving material for further analysis that could be

necessary. Additional analytical techniques for further information can also be useful to improve the resolution among groups from regions with similar climatic conditions such as Amazon region and Mato Grosso do Sul and could be tested in order to improve the classification of samples whose geographical origin could not be clearly identified.

It can be concluded that most of the samples seized in the São Paulo analyzed in this work appears to have the same origin of those seized in Mato Grosso do Sul, and probably it is coming through the Paraguai-Mato Grosso do Sul route. In spite of the status of Maranhão as the main *Cannabis* producing State in Brazil, this work shows no evidence of existence of traffic routes between this region and the city of São Paulo. In this case, a continuous and systematic monitoring using a larger number of samples are necessary for conclusive results which could also allow us to evaluate possible changes in these illicit trade routes throughout the years.

By now, the results seem to confirm the existence of traffic routes between Paraguai/Mato Grosso do Sul and Northeast region, especially Ceará State, which lead us to believe in the low local marijuana demand, probably as a consequence of the Federal Government efforts to eradicate *Cannabis* plantations. This feature stands out if considered the fact that samples from Marijuana Polygon were not substantially traced in São Paulo. Along with these early results came an strengthen in the law enforcement efforts to monitoring all possible routes originated from Paraguay, leading to a notable increase in apprehensions, making the São Paulo State Police Department a reference agency in the country.

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