ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN
2019 International Nuclear Atlantic Conference - INAC 2019
Santos, SP, Brazil, October 21-25, 2019

Agência Brasileira do ISBN
ISBN 978-85-99141-08-3
9 788599 141083

Characterization of the *Scaptotrigona aff. Postica* bee from Brazil using analytical techniques

Luís Guilherme M. Leal[1], Cibele B. Zamboni[1], Roberto M. do Nascimento[2], Ronaldo Z. Mendonça[2] and Simone M. Simons[2]

[1]Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN – SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil.
luguileal@hotmail.com
czamboni@ipen.br

[2]Instituto Butantan
Av. Vital Brasil, 1500
05503-900 São Paulo, SP, Brazil
roberto.nascimento@butantan.gov.br
ronaldo.mendonca@butanta.gov.br
simone.simons@butantan.gov.br

ABSTRACT

The study aimed to propose the characterization of the *Scaptotrigona aff. Postica* Bee ("tubi") from Barra do Corda (MA-Brazil). This species produces honey, propolis and pollen with several medical applications. Two analytic techniques were applied for this investigation: Instrumental Neutron Activation Analysis (INAA) and Energy Dispersive X Ray Fluorescence (EDXFR). The elements Br, Ca, Cl, Co, Cu, Fe, K, Mn, Na and S were identified in all samples. The results obtained from INAA were compared with EDXRF technique and they are in good agreement. Both analytical techniques proved to be adequate and complementary, offering a new contribution to the understanding of the relation of these bees to the vegetation that surrounds the meliponary.

1. INTRODUCTION

In recent years, the demand for natural and healthy products has grown significantly and products from Meliponicultura (breeding of stingless bees) have been consumed on larger scale in the world. Brazil is the third global produce: there are more than 8000 producers with production around 150-170 tons/year. About 75% is exported (around US$300 million/year) [1, 2]. The stingless bee keeping is still not entirely standardized, and studies are necessary to obtain knowledge about this species. This study aimed to propose the characterization of the *Scaptotigona aff. Postica* Bee ("tubi") from Barra do Corda (MA-Brazil). This species produces honey, propolis and pollen using saliva [2-5] with several medical applications [6-13]. In this investigation, Energy Dispersive X-ray Fluorescence (EDXRF) and Instrumental Neutron Activation Analysis (INAA) measurements were performed in "tubi" bee sample. The X-ray Fluorescence measurements were performed using a XRF spectrometer (X-123 SDD, Ampetek®) and the INAA measurements in the IEA-R1 nuclear reactor (both facilities of IPEN, Nuclear Research Institute in São Paulo city, Brazil).

2. METHOD

2.1 Sample Preparation

The sample were provided by beekeepers in the region located in the municipality in Barra do Cord, in the central region of the State of Maranhão, Brazil. The collection was performed with the approval of the Conselho de Gestão do Patrimônio Genético (AF18A78). The IBu staff (IBu: Instituto Butantan, Research Center in São Paulo city, Brazil) was responsible for collection and identification of the samples. For sample preparation, the bees were transferred to a conical tube (~500 mg), singly macerated and kept in the tube at room temperature until use. No chemical pretreatment is performed on the samples. Six samples were investigated by both techniques. The number of repetitions was two for INAA and three for EDXRF analysis.

2.2 Instrumental Neutron Activation Analysis (INAA)

In the Instrumental Neutron Activation Analysis (INAA) procedure, samples and standard (NIST 1573a) were irradiated simultaneously and the comparison of the intensity of the gamma rays from sample with those emitted by a standard permit a quantitative measure of the concentrations. Duplicate samples (50-100mg) were prepared. Each sample was weighed and sealed into a polyethylene capsule and irradiated in the IEA-R1 nuclear reactor at IPEN-CNEN/SP (3.5 – 4.5 MW, pool type) for minutes to hours. The measurements of the gamma induced activity of the samples and standard were carried out using an ORTEC Model, GEM-60195 detector and an ORTEC 671 amplifier coupled to a MCA ORTEC 919E connected to a PC. The concentration of each element in each biological sample was obtained by using in-house software [14].

2.3. Energy Dispersive X-Ray Fluorescence (EDXRF)

EDXRF data were obtained using an X-Ray Spectrometer (X-123 SDD, Amptek®) with Ag X-ray mini-tube with a Si Drift (25 mm^2 x 500 μm) Be window (12.5μm). Samples (the same used in the INAA measurements), prepared in triplicate (~10mg), and placed inside sample holder (SPEX-contaminant-free micro X-cell – 31 mm sample holder with 6.3 mm in the window diameter). The excitation was performed using 30 kV and 5μA for a counting time of 300 s. The analysis was performed using WinQxas software [15].

3. RESULTS AND DISCUTION

The element concentrations by EDXRF and INAA techniques are present in **Table 1** as the mean value (MV) and standard deviation (±1 SD). The elements Br, Ca, Cl, Cu, Fe, K, Mn, Na, S and V were activated using the (n,γ) nuclear reaction and P by (n,α) nuclear reaction. Potential interference from $^{23}Al(n,\gamma)^{28}Al$ and $^{27}Al(n,p)^{28}Al$ nuclear reactions in P concentration results, by $^{31}P(n,\alpha)^{28}Al$, was checked comparing the INAA results with EDXRF. The element Na was determined only by INAA and, the element Co only by EDXRF.

Table 1: The element concentrations of *Scaptotrigona aff. Postica* Bee ("tubi") samples by EDXRF and INAA techniques.

Elements	INAA	EDXRF
	MV ± 1 SD	
Br, g/kg	0.026 ± 0.005	0.040 ± 0.012
Ca, g/kg	2.99 ± 0.68	2.21 ± 0.37
Cl, g/kg	1.53 ± 0.05	1.58 ± 0.09
Co, mg/kg	nd	0.97 ± 0.14
Cu, mg/kg	49 ± 11	36.2 ± 1.2
Fe, mg/kg	555 ± 28	536 ± 32
K, g/kg	9.74 ± 0.44	9.25 ± 0.51
Mn, mg/kg	194 ± 23	229 ± 35
Na, mg/kg	5.8 ±0.7	nd
P, g/kg	12 ± 3	8.13 ± 0.48
S, g/kg	6 ± 2	3.9 ± 0.2

nd: not determined

The elements Br, Ca, Cl, Co, Cu, Fe, K, Mn, Na, P and S were identified in all samples. The Student's *t-test* was applied for results comparison between EDXRF and INAA techniques. The results for Ca, Cl, Cu, Fe, K, Mn, Fe, P and S were considered statistically equal ($p > 0.05$). Only Br was considered significantly different ($p < 0.05$).

These analytic techniques are complementary, requiring a small amount of samples (very import condition when the biological material is scare), multielemental analysis, short time of analysis and simple sample preparation. Particularly the EDXRF technique offers an efficient and fast evaluation.

4. CONCLUSION

The multielemental analyses using the INAA and EDXRF analytics techniques provide the knowledge of elemental composition of the *Scaptotrigona aff. Postica* Bee. These data can indicate the botanical origin of their resins and contribute to understand the relationship of these bees with the vegetation surrounding the meliponary.

ACKNOWLEDGMENTS

The authors thank the financial support of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 15/01750-9), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 470974/213-3) and Fundação Butantan.

REFERENCES

1. H. T. Inoue, E. A. Sousa, R. O. Orsil, S. R. C. Funari, L. M. R. C. Barreto, A. P. S. Dib, "Produção de própolis por diferentes métodos de coleta," *Archivos Latinoamericanos de Producción Animal*, **v. 15, n. 2**, pp. 65-69 (2007).
2. A. S. Pereira, F. R. M. S. Seixas, F. R. Aquino-Neto, "Própolis: 100 anos de pesquisa e suas perspectivas futuras," *Química Nova*, **v. 25**, pp. 321-326 (2002).
3. W. Kerr, "Abelhas indígenas brasileiras (meliponíneos) na polinização e na produção de mel, pólen, geoprópolis e cera," *Inf Agropec* **v. 13**, p. 15-27 (1987).
4. P. Nogueira-Neto, *A vida e criação de abelhas indígenas sem ferrão (Meliponinae)*, Tecnapis, São Paulo & Brazil (1997).
5. V. Bankova, R. Christov, M. C. Marcucci, S. Popov, "Constituents of brazilian geopropolis," *Z Naturforschung*, **v. 53**, pp. 402-406 (1998).
6. "Catalogo de abelhas moure" http://moure.cria.org.br (2016).
7. M. C. Marcucci, F. Ferreres, C. Garcia-Viguera, V. S. Bankova, S. L. De Castro, A. P. Dantas, P. H. M. Valente, N. Paulino, "Phenolic compounds from Brazilian própolis with pharmacological activities. *Journal of Ethnopharmacol*," **v. 74**, pp. 105-112 (2001).
8. T. Takemura, T. Urushisaki, M. Fukuoka, J. Hosokawa-Muto, T. Hata, Y. Okuda, K. Kuwata, "3, 4-dicaffeoylquinic acid, a major constituent of Brazilian propolis, increases TRAIL expression and extends the lifetimes of mice infected with the influenza A virus," *Evidence-Based Complementary and Alternative Medicine* **v.2012,** pp. 1-7 (2011).
9. E. M. Maia Filho, C. D. C. R. Maia, A. C. S. C. Bastos, T. M. G. Novais, "Efeito antimicrobiano in vitro de diferentes medicações endodôntica e própolis sobre Enterococcus faecalis," *Rev Gaúcha Odonto*, **v. 56, n.1**, pp. 21-25 (2009).
10. S. A. Libério, A. L. A. Pereira, M. J. A. M. Araújo, R. P. Dutra, F. R. F. Nascimento, V. Monteiro-Neto, M. N. S. Ribeiro, A. G. Gonçalves, R. N. M. Guerra, "The potential use of propolis as a cariostatic agent and its actions on mutans group streptococci," Journal *of Ethnopharmacology,* **v. 125** pp. 1-9 (2009).
11. "Efeito químico preventivo de variedades de própolis brasileira sobre a carcinogênese oral experimentalmente induzida." http://ww3.unit.br/mestrados/saudeambiente/ wpcontent/uploads/2012/04 (2016).
12. M. M. Possamai, A. C. Honorio-França, A. P. B. Reinaque, E. L. França, P. C. D. S. Souto, "Brazilian Propolis: A Natural Product That Improved the Fungicidal Activity by Blood Phagocytes," *BioMed Res Int.*, **v. 2013**, pp. 1-9 (2013).
13. G. R. Coelho, R. Z. Mendonça, K. De S. Vilar, C. A. Figueiredo, J. C. Badari, N. Taniwaki, G. Namiyama, M. I. De Oliveira, S. P. Curti, E. P. Silva, G. Negri, "Antiviral Action of Hydromethanolic Extract of Geopropolis from *Scaptotrigona aff postica* against Antiherpes Simplex Virus (HSV-1)," *Evid Based Complement Alternat Med*. **v. 2015**, pp 1-10 (2015).
14. J. A. G. Medeiros, C. B. Zamboni, G. S. Zahn, L. C. Oliveira, L. Dalaqua Jr, "Software para realização de análises hematológicas utilizando processo radioanalítico", *39º Congresso Brasileiro de Patologia Clinica/Medicina Laboratorial*, São Paulo & Brasil (2005).
15. R. Capote, E. López, E. *Mainegra. "WinQxas Manual (Quantitative X-Ray Analysis System for Widows) Version 1.4*." IAEA, Viena & Austria (2002).