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Influence of the land use of the foraging area on the physico-chemical properties of the honey from the honey bee, *Apis mellifera,* in the urban and peri-urban area of Butembo, Democratic Republic of Congo

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1 ABSTRACT

From March to September 2022, a study on the influence of the land use of the foraging area on the physicochemical properties and the bacterial load of honey from the honey bee, *Apis meillifera,* in the urban and peri-urban area of the city of Butembo was carried out; in North Kivu in the Democratic Republic of Congo. The study focused on 4 beehives including 2 in urban areas (CEFADES and Mavono) and 2 in peri-urban areas (Musienene and Bunyuka). Analyses of physicochemical parameters of honey samples collected from these beehives revealed a pH value varying between 3.93 and 4.2; an electrical conductivity between 1.22 and 1.02 (x 10 $^{-3}$ mS /cm); a sugar content between 82.5 and 78 degrees Brix and a density whose value was between 1.79 and 2.02 g/cm ^{3}. The analysis of land cover revealed 3 main classes (afforestation, crop field class and bare land and built-up class) whose importance varies from one site to another. The canonical correspondence analyses revealed that the afforestation (in eucalyptus) positively influences the pH of the honey; that of crop fields influences the water content of honey while bare land influences the density of honey. On the other hand, the sugar content is not influenced by the land use in the foraging area. Ultimately, land use influences the chemical composition of honey.

2 INTRODUCTION

In Africa, agrosylvopastoral activities constitute the basis of the economy of the majority of the populations. These activities include agriculture, livestock, hunting, crafts, and the exploitation of natural resources (firewood and service wood.) and non-timber forest products, including honey (Sitou *et al.,* 2020) . Honey is one of the oldest and most appreciated foods of humanity for its taste and for its therapeutic virtues (Bouknani and Nour Elimane, 2020). Today, the beekeeper strives to obtain from his bees a quality product in sufficient quantity to meet consumers' demand (Bouet Kouanou *et al.,* 2020). From the flower to the table via the beehive; honey, formerly nectars, must meet certain qualitative standards (physico-chemical and organoleptic properties) in order for it to be identified. Consumers naturally look for a quality product whose colour, state of crystallization and firmness, smell, taste and aromas meet their expectations (Hoyet, 2005). However, the stages of honey production are complex and likely to be altered by human activities, willingly or not (Bouet Kouanou *et al.,* 2020). In Africa, as

everywhere in the world, researchers are interested in honey and other beehive products in different aspects such as the inventory of the beekeeping sector (Moujanni, 2017; Ksouri, 2019); food quality control (Si Sabeur Souad, 2019); honey production process, its composition, properties and storage (Bouet Kouanou *et al.,* 2020; Buveka Ngoma *et al.,* 2018). Currently, coming up with products that comply with standards on the national and international markets is becoming a challenge as far as honey products are concerned. It is therefore necessary to ensure not only the identity and origin of the honey but also its physicochemical and bacteriological qualities. Since honey is a food intended for commercialization, it can, be falsified or adulterated (e.g. adding sugar). Several factors can affect the physico-chemical properties of honey e.g. the quality of the honey can vary depending on the floristic composition of the vegetation in the foraging area. Its quality analysis is therefore important for the honey sector. Therefore, this study intends to determine the influence of the land use of the foraging area on the physicochemical characteristics of honey.

3 MATERIALS AND METHODS

3.1 Description of the study area: This study was carried out in Butembo city, in the east of the Democratic Republic of Congo, extending on approximately 190.34 km ^{2}. This entity spans the two territories of Lubero and Beni by the chiefdoms of Baswagha and Bashu, respectively and has 4 municipalities namely Bulengera, Kimemi, Mususa and Vulamba. The study area included 4 different sites in urban and peri-urban areas of Butembo city, as shown in figure 1 below.

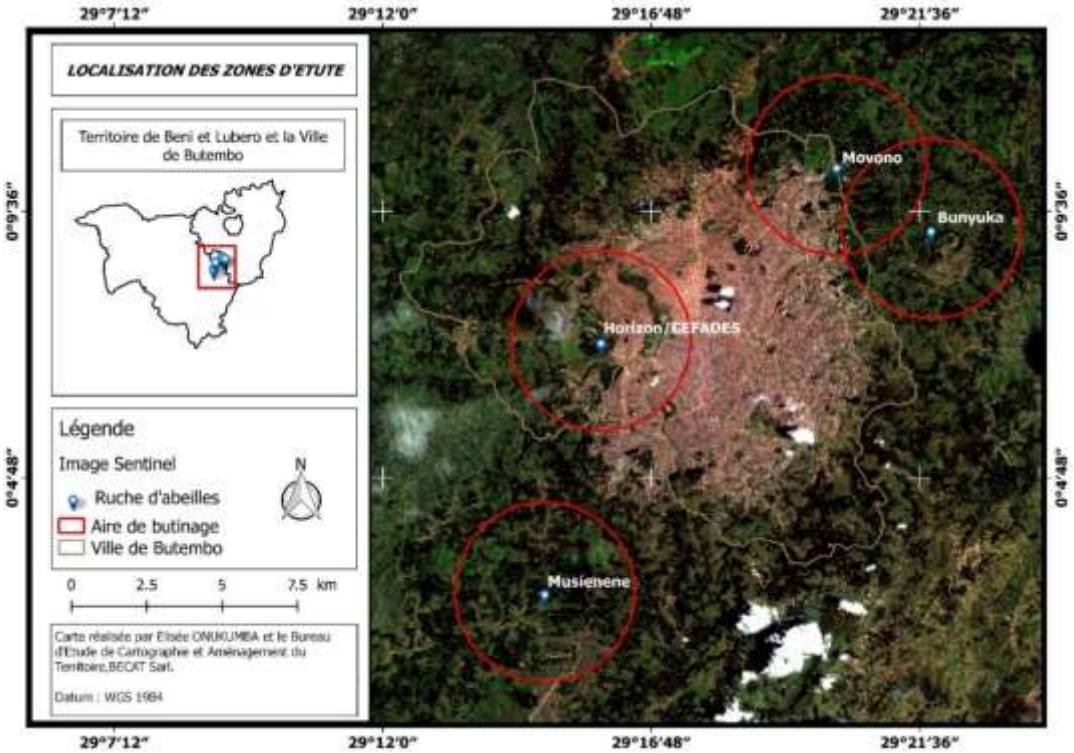

Figure 1: The different sites where honey samples were collected.

During the investigation period (from March to September 2022), data taken from the weather station of the Technical Agricultural and Veterinary Institute (ITAV/Butembo) revealed that the monthly average temperature, rainfall and humidity were respectively 25.1°C; 252.9 mm and 93.2%.

3.2 METHOD

3.2.1 Collection of honey samples: To determine the variability of the physicochemical properties of honey from beehives in urban and peri-urban areas of Butembo city; four apiaries, namely the Horizon, Mavono, Bunyuka and Musienne apiaries were selected based on their reputation and importance in the production and supply of honey in the region. In each beehive, a sample of honey was taken from the mixture of honeys from different colonies in the apiary. An average of three colonies was taken for each apiary. The honey samples were carefully collected so that it is of the most recent manufacture possible. To achieve this, the honey was taken preferentially from the super, or from the body of the hive if there was no super. The sample was taken using a clean and disinfected spoon between each sample within the apiary. The honey was kept in well-labelled sterile boxes and stored in an insulated box before delivery to the Central Research Laboratory of the Université Catholique du Graben for analyses.

3.2.2 Physico-chemical analysis of honey: In this study, it was assumed that natural honey is a Newtonian fluid. Methods to determine its physical parameters are briefly described below and are described as presented by Buveka Ngoma *et al.*, (2018) and Si Sabeur Souad (2019).

- The electrical conductivity of honey: The determination of the electrical conductivity of the honey samples was based on the measurement of the electrical resistance of an aqueous solution. A 40 g sample of honey was placed in a pot and then diluted in 10 ml of distilled water. The result is displayed on the screen of the conductimeter (immersed in the pot).
- The pH of honey: The PH-meter used is of the ST 10 Ohaus brand, previously calibrated with its thermometer. After immersing its probe in a solution of each honey sample, the pH value is read on the screen.
- The density of honey: The density of each sample was determined using the pycnometer. Then the density was calculated. The relative density of honey is the ratio of the mass of a well-known volume of the sample at 20°C by the mass of the same volume of water at the same temperature symbolized by d20.
- Free water content of honey: The determination of the water content in a food is carried out after drying the sample to a constant weight in the oven; at 160°C for 24 hours. The difference between the fresh weight and the dry weight gives this water content.

Using an analytical weighing machine, an oven and a desiccator; weigh the fresh sample (P1) in a porcelain crucible (P0). After drying in an oven at 105°C for 24 hours, the crucible containing the sample is taken to a desiccator to cool down before being weighed (P2). The sugar content of honey: The sugar content of each sample, expressed in degrees Brix, was deduced from the value of the refractive index of each sample of honey at 20°C using an Abbe refractometer (a drop of the sample is placed on the prism and the result is read in the eyepiece of the device).

3.2.3 Cartographic supports for the characterization of land use: The geomatics approach (GIS and remote sensing) adopted in this work was based on an image dated 02/04/2022 from the SANTINEL 2 satellite (L1C_T35NQA_A035396_20220402T081238). This image has the advantage of free access on the "Visioterra" platform. Its spatial resolution of 10 meters makes it possible to better identify the structural elements of the landscape of the study area (Bweya, 2022). These elements (land use) were geolocated using *Garmin GPS Maps 62Stc* within a radius of 3 km from the center of the apiary as determined by Piroux (2014). These data are ground truths which were used in the interpretation of the acquired image and

consequently in the analysis of the land use of this region. The processing and analysis of these images were carried out using ENVI 4.6.1 software and the cartographic layout in QGis 3.26. The first step in image processing consisted of a projection of the spectral bands in the UTM system (Universal Transverse Mercator)/zone 35N covering the study area, and based on the reference ellipsoid WGS 84 (World Geodesic System) as than offered by Bweya *et al.,* (2019). A composite true colour composition was made using the band of Red (band 4) Green (band 3) and Blue (band 2) (RGB). Three land use classes have been defined and mapped for each. These are the "Afforestation" class, the "Crop fields" class and the "Bare soil and built-up" class. Supervised classification is the method chosen for this study. Thanks to GPS coordinates and visual analysis of high spatial resolution Google Earth images, polygons were plotted (which will later be used as *Region of Interest*) on the images in order to outline homogeneous land use classes from field observations. The classification algorithm used is the maximum likelihood method.

3.2.4 Statistical analyses: Statistical analyses were performed with R software version 4.1.2. Different multivariate analyses were performed:

A Principal Component Analysis (PCA) was carried out using data on the honey physico-chemical properties and bacteriological load of honey according to the different sites. It consists of transforming variables (in this case, physico-chemical factors of honey) linked together into new variables uncorrelated from each other and thus makes it possible to reduce the number of variables with the aim of best describing the proximities between individuals (in this case, honeys from different apiaries)

Canonical Correspondence Analyses (CCA) were carried out using data on the honey physico-chemical properties and bacteriological load as well as that of land use. These analyses made it possible to describe the physico-chemical and bacteriological profiles of the different apiaries in relation to the land use in the foraging area. A Hierarchical Classification on Principal Components (HCPC) was used to group the physico-chemical and bacteriological profiles according to the apiaries.

4 RESULTS

4.1 Physico-chemical properties of honey produced in the urban and peri-urban areas of Butembo: Analyses of the physicochemical properties of honey harvested in the four study sites revealed that the pH of honey sampled at the CEFADES site is very acidic (3.93) followed closely by that of Mavono (3.95) then that of Musienene and Bunyuka, respectively, worth 4.17 and 4.2. Concerning the water content, it is observed that the Bunyuka site provided honey with the highest water content (15%) than the other sites, i.e. 12.8 for CEFADES and 12.6 for Mavono and Musienene. The honey sample taken at the Mavono site had the highest sugar content at 82.5 degrees Brix ; followed by CEFADES (81 degree Brix) and concomitantly by Musienene and Bunyuka (78 degree Brix each) (Table 1). In terms of electrical conductivity, the honey from Mavono had the highest value (1.22) followed respectively by honey from Musienene, CEFADES and Bunyuka with respective values of 1.18; 1.04 and 1.02. The CEFADES site produced the honey with the highest density (2.02) followed by Mavono and Musienene which had a density of 1.83 and finally Bunyuka with a density of 1.79.

Table 1: Value of physico-chemical parameters

	CEFADES	Mavono	Musienene	Bunyuka
pH	3.93	3.95	4.17	4.2
Water content (in %)	12.8	12.6	12.6	15
Sugar content Sugar (in degree Brix)	81	82.5	78	78
Electrical conductivity (in mS /cm)	1.04×10^{-3}	1.22×10^{-3}	1.18×10^{-3}	1.02×10^{-3}
Density	2.02	1.83	1.83	1.79

The Principal Component Analysis (PCA) was carried out using data on the physico-chemical composition of honey according to the different sites. The related results are shown in Figure 2 and revealed that sugar content was positively associated with the first and second principal components (Correlation coefficient r equals 0.991 and 0.136 for the first and second principal component respectively). Similarly, it is observed that the honey collected in sites 2 and 3 (Horizon/CEFADES and Mavono, respectively) had a positive contribution to the sugar content while the contribution of sites 1 and 4 (Bunyuka and Musienene, respectively) was negative for this parameter. On the other hand, the water content of honey proved to be negatively associated with the first principal component (correlation coefficient r equals -0.65) while it was positively associated with the second principal component (correlation coefficient r equals 0.76). Sites 1 and 4 (Bunyuka and Musienene, respectively) contributed positively to honey water content, while sites 2 and 3 (Horizon/CEFADES and Mavono) contributed negatively.

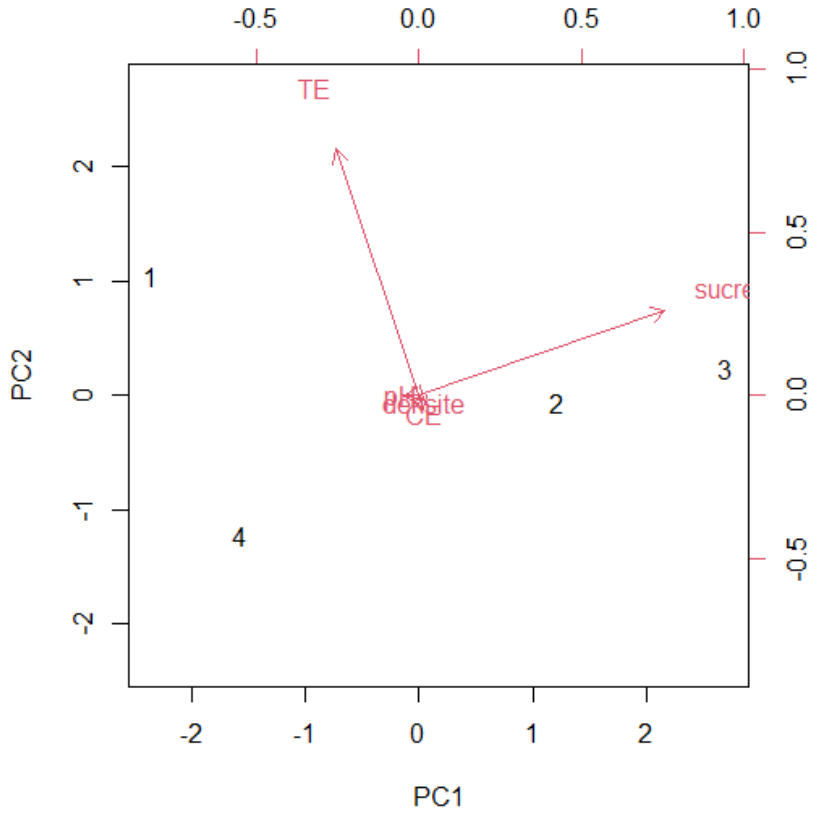

Figure 2: PCA physico-chemical quality of honey

4.2 Land use of foraging areas: The four study sites presented a fairly varied land use from one site to another, as illustrated in the figures below (figures 3, 4, 5 and 6).

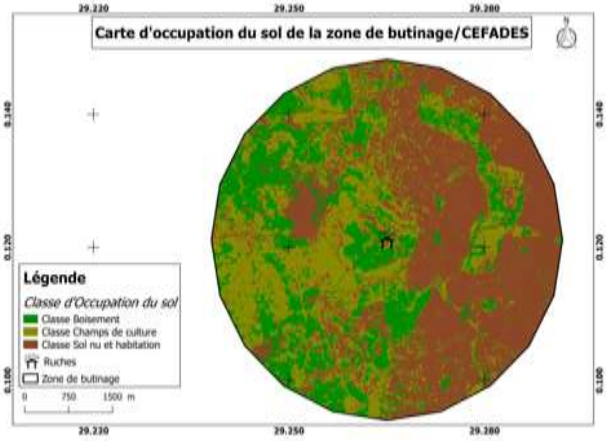

Figure 3: Land use map of the CEFADES foraging area

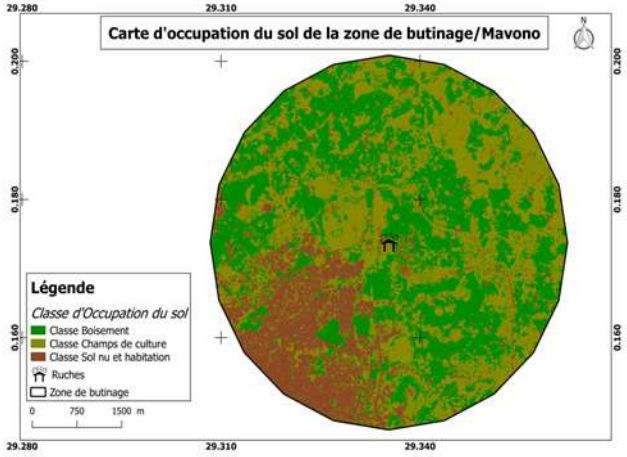

Figure 4: Land use map of the Mavono foraging area

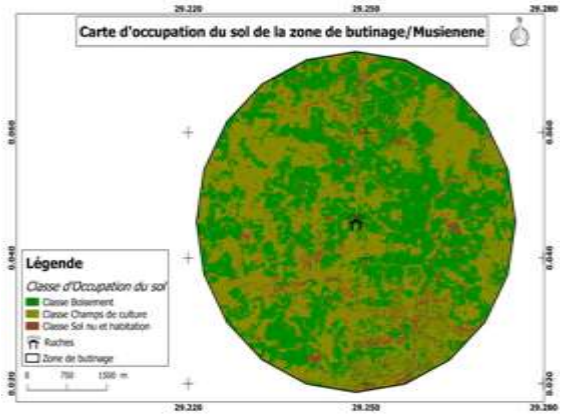

Figure 5: Land use map of the Musienene foraging area

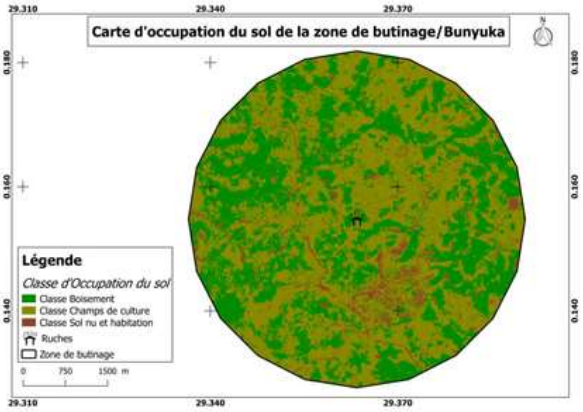

Figure 6: Land cover map of the Bunyuka foraging area

There is a difference in land use spots in different sites with a structural similarity depending on whether the site is urban (CEFADES and Mavono) or peri-urban (Musienene and Bunyuka). The class composed of bare land and built-up is the most dominant in urban areas, while afforested areas and fields predominate the foraging area in peri-urban areas. This relationship is much more highlighted by the results of the Principal Component Analysis as illustrated by Figure 7.

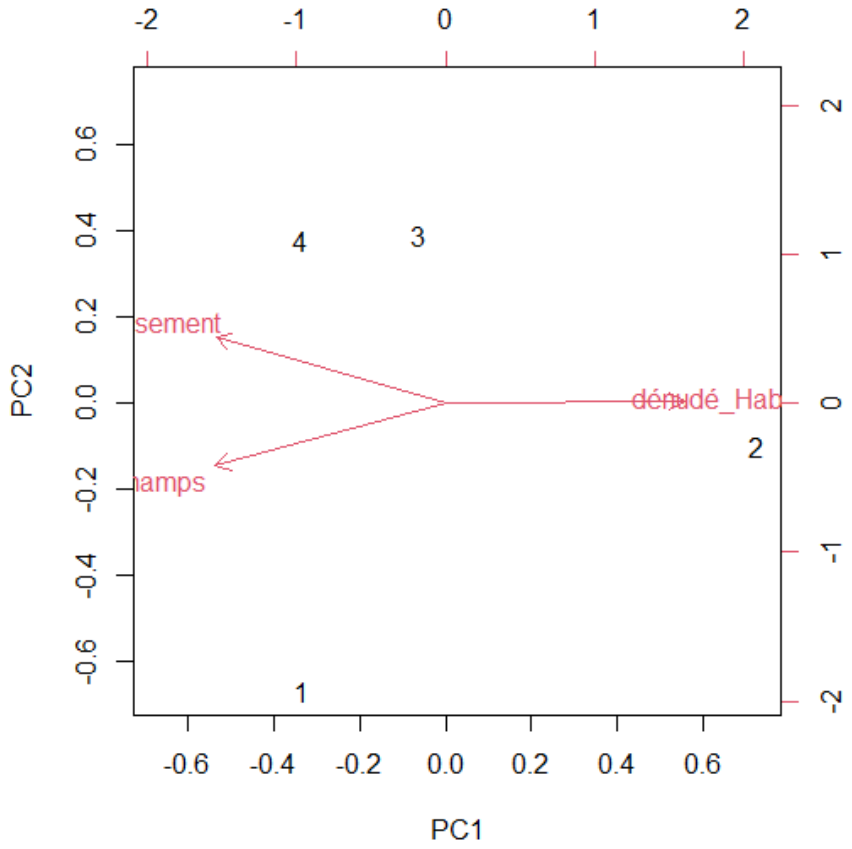

Figure 7: PCA of land use

Figure 7 shows that bare land and built-ups made a positive contribution to the first principal component, while afforestation and fields had a negative contribution. Bare land and built-ups had no contribution to the second principal component. Afforestation contributed positively to the second component while fields contributed negatively. Site 2 (Horizon/CEFADES) contributed positively to the bare land-built-ups class, while the other sites were negatively associated with it. Sites 3 and 4 (Musienene and Mavono, respectively) were positively associated with afforestation, while site 1 (Bunyuka) was more associated with the field class.

4.3 Influence of land use in the foraging area on the physicochemical properties of honey: The canonical correspondence analysis (CCA) showed that the pH and water content of honey are positively associated with the class of afforestation and fields while they are negatively associated with the class of bare lands and built-ups. This class is positively associated with honey density. Sugar content and electrical conductivity of honey were not influenced by any of the land cover classes (Figure 9).

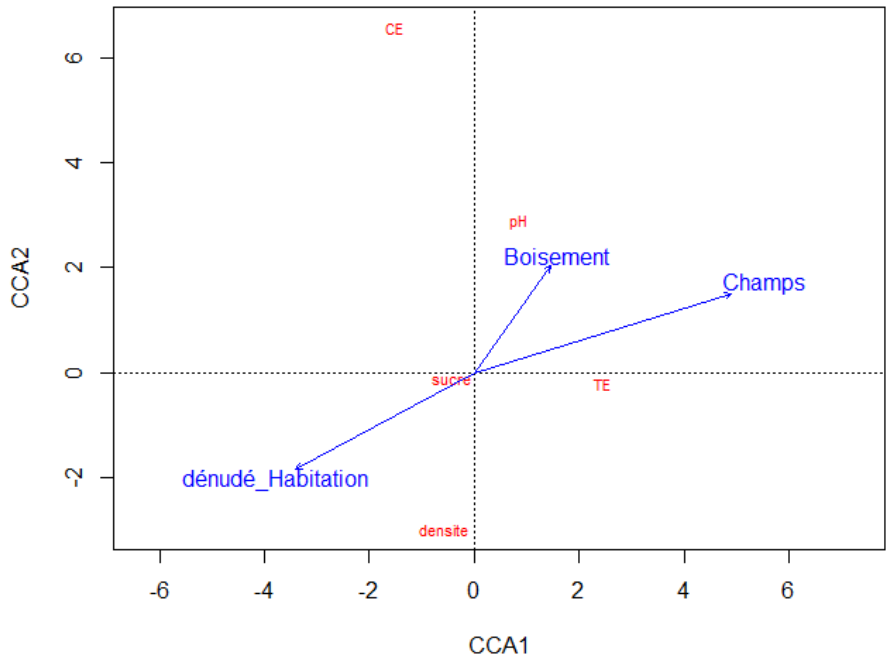

Figure 9: CCA of honey-land use

5 DISCUSSION

5.1 The physico-chemical properties of honey produced in urban and peri-urban areas of Butembo: Water content is one of the most important characteristics of honey, as it plays a crucial role in quality assessment and is used as an international standards (Codex Alimentarius) (Benkhaddra and Ghadbane, 2014). The water content of the honey samples varied between 12.6 and 15%. These results corroborate those of Si Sabeur Souad (2019) who found a water content between 13.05 and 14.35% for honey from the Beni Chograne Mascara region. All the samples of honey analysed had a water content below 15%. This is below 21%, the standard prescribed by the International Honey Commission (CIM, 1999). The pH of these samples fluctuated between 3.93 and 4.2. This range corresponds to the standard which is between 3.5 and 4.5 for nectar honeys (Si Sabeur Souad, 2019). Thus this criterion of quality and stability of honey as well as its non-falsification, proves that the honey of the urban and peri-urban region of Butembo is of good quality. These values were also found by Buveka Ngoma *et al.*, (2018), thus concluding that nectar honey is of good quality. Therefore, the samples reveal that honey from the urban and peri-urban region of Butembo is similar to nectar honey and not honeydew honey (pH 4.5 and 5.5) (Si Sabeur Souad, 2019). Conductivity represents a good criterion for determining the botanical origin of honey and is currently resorted to during routine checks of honey and which replaces the ash content (Si Sabeur Souad, 2019). Electrical conductivity is linked with the floral origin of honeys, and is below or equal to 0.8 mS/cm for nectar honeys (CIM, 1999). The values found for honey samples in the present study varied between 1.02 and 1.22 (x 10^{-3} mS /cm). These results are in accordance with the standards. They corroborate the results obtained by Si Sabeur Souad (2019) who found an electrical conductivity varying between 0.6 and 0.8. They are on the other hand lower than those reported by Buveka Ngoma *et al.* (2018) who found values ranging from 39.6 to 42.2. This low electrical conductivity of the honeys of the urban and peri-urban region of Butembo is indicative of a nectarine origin. In fact, forest honey (honeydew) has a higher electrical conductivity than nectarine honey (nectar) (Si Sabeur Souad, 2019). The honey samples in the present study

were found to have a sugar content between 82.5 and 78 degrees Brix. Bouet Kouanou *et al.,* (2020) support that the total fructose and glucose content should not be less than 60g per 100g for nectarine honey. These values make it possible to confirm that the studied honey samples were nectarine honeys, as per Rouidja (2010) specifications. The density of the honey samples varied between 2.02 and 1.79. These values are different from those reported by Bakary *et al.* (2019) at 1.06 to 1.22 g/cm ^{3} for honeys from the Worodougou Region in Côte d'Ivoire. This would be justified by the difference in the climatic conditions between the Côte d'Ivoire and the Democratic Republic of Congo. The water content of honey, which influences the density, depends on the environmental conditions, including the prevailing climatic conditions during the harvest period (Si Sabeur Souad, 2019).

5.2 The effect of land use in the foraging area on the physicochemical characteristics of honey: Three (3) land use classes identified, i.e., Afforestation class, the Cultivation field class, the bare soil and built-ups class, tended to have dissimilar importance from one site to another. The Horizon site apiary was positively associated with bare land and built-ups. This could be explained by the fact that this site is in the urban area and is the neighbourhood of residential areas, in particular the Vusenzera and Ngengere quarters of the City of Butembo. The Musienene and Mavono apiaries are positively linked to afforestation and this can simply be explained by the fact that the 2 sites are far from dwelling houses and have foraging areas dominated by trees; mainly eucalyptus whose cultivation is one of the key activities in the area. As for the Bunyuka apiary, its foraging area is associated with crop fields. In this peri-urban area, agriculture is the main activity. The canonical correspondence analyses showed that the land use class "crop fields" is positively correlated with the honey water content. The water content of honey is its free water. It is an important parameter that guarantees the conservation of honey and presides its crystallization or fermentation (Buveka Ngoma *et al.*, 2018). This positive relationship between water content and the crop fields could be explained by the presence of flowers (nectar) on seasonal food crops (beans, soybeans, peas.) found in the foraging area. Similarly, Bouet Kouanou *et al.*, (2020) found that the water content of nectar varies between 40 and 70% depending on the dominant floristic composition. Plants provide water to the bee which comes to forage and the production of nectar depends on many factors: photosynthesis and the supply of organic constituents of the nectar, the growth of the plant and the secretory process itself. Therefore, a soil water supply suitable for good vegetative and reproductive growth is a necessary condition for achieving a good beekeeping season. There was a positive relationship between the "crop field" land use class and the water content, as well as between honey density and bare soil land use class. Bare land implies an absence of vegetation and thus a scarcity of nectar, thus influencing the primary composition of honey. The portion of bare land in the foraging area had an opposite effect to that induced by the crop fields land use due to a low supply of bees in nectar. This low intake would negatively influence the water content during the transformation process in the digestive tract of the bee but also during the passage after regurgitation from one forager to another (trophallaxis) for dehydrating honey. This decrease in water content in such a honey is at the origin of its high density (Bouknani and Nour Elimane, 2020). Therefore, large portions of bare land might be the cause of the low production of honey in these areas. However, this honey is of high density (Alexandra, 2011). The pH of the honey samples was also influenced by afforestation with E*ucalyptus* tree species. This tree species has been reported to increase the soil acidity through its acidic litter fall (Vulotwa, 2021). Their flower nectar as well is reputed more acidic than other tree species of the region (Vulotwa, 2021). Hence, the predominance of the Eucalyptus tree species in an apiary might have affected the pH of honey. By increasing the honey acidity, it reduces chances of multiplication bacteria and other

germs that cannot stand an acidic medium (Balas, 2015). Bouknani and Nour Elimane (2020) specify that during trophallaxis, the enzymes contained in the gastric juice and in the salivary substances modify the starch (diastase) and facilitate the metabolism of sucrose into glycogen and fructose (invertase). This proves that the honey sugar content is linked to the biological factor intrinsic to the bee without referring to other environmental factors. This can justify why the sugar content in honey samples during the present study was not influenced by any of the studied environmental factors.

6 CONCLUSION

This study made it possible to reveal the influence of the land use and land cover of the foraging area on the physico-chemical properties of the honey. This honey is produced in areas where the foraging area is covered either with woods, crop fields or bare land whose influence on the physicochemical composition has been proven. The physicochemical properties of honey produced in the urban and peri-urban area of Butembo city reveal that it is of nectariferous origin (flower honey) and complies with international standards. However, it turns out that producing honey under woodlots is more beneficial than producing it in crop fields or on bare land. In general, land use influences the chemical composition of honey.

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