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AIR POLLUTION AND QUALITY OF HONEY FROM RURAL AND URBAN AREAS OF THIMPHU DISTRICT, BHUTAN

Govinda PRASAD SHARMA[1]*, Enna MOOL[1], Ramesh PRASAD SAPKOTA[1], Tulsi GURUNG[2], Shiva PRAVEEN PUPPALA[3], Rejina MASKEY BYANJU[1]

[1]Central Department of Environmental Science, IOST, Tribhuvan University, Nepal
[2]College of Natural Resources, Royal University of Bhutan, Punakha, Bhutan
[3] Economics and Insights Division, Department of Planning and Environment, NSW, Australia
*Corresponding author: govinda.76575@cdes.tu.edu.np

ABSTRACT

Honey is prepared by bees from nectar or honeydew and has great nutritional and therapeutic benefits. However, air pollution affects honey quality as bees collect nectar, pollen, water and propolis from polluted and unpolluted areas. For this study, air pollution measurement and bee hive experiment were carried out concomitantly in Olakha (urban) and Ngabiphu (rural) in the Thimphu District in 2022. It was found that the annual mean $PM_{2.5}$ was 19.43 µg/m^3 and 8.87 µg/m^3 while PM_1 recorded were 16.63 µg/m^3 and 4.05 µg/m^3 in Olakha and Ngabiphu respectively.

The honey produced by using *Apis mellifera* hive experiment from Ngabiphu and Olakha was collected, and the honey produced from *Apis cerana* sold by farmers at the Thimphu Centenary Farmers Market was purchased to compare pH, ash content, and heavy metals mainly Pb, Cd, Ni, and Cu. Twenty four samples from three places (8 each) were kept at room temperature and in the dark until laboratory analysis was carried out in Nepal Environmental and Scientific Services in Kathmandu. The data were analyzed using One-way Anova. It was found that the ash content and pH of the honey were within the international permissible limit. The Pb and Ni were not detected but the Cd content was detected only in six honey samples. There was a statistically significant difference of Cu content ($p= 0.003816$, $\alpha=0.05$) in honeys and the mean readings were 2.86 ±0.84 µg/g, 2.55±0.91 µg/g, and 4.24±1.05 µg/g in Olakha, Ngabiphu and Market honey respectively.

Keywords: *Apis mellifera, Apis cerana, heavy metals, pollutants, Thimphu.*

INTRODUCTION

Bhutan, a tiny nation has 3966 invertebrate species from which 3511 are insect species (NBC, 2017). Among insects, honeybee of six types namely *Apis cerana*, *Apis mellifera* and *Trigona iridipennis* species are managed in the traditional and modern hives while *Apis laboriosa*, *Apis dorsata* and *Apis florea* exist in wild state (Bhujel et al., 2022*)*. ICIMOD (2017) stated that beekeeping is a part-time job along with other farm activities for the Bhutanese. From the annual report of the Department of Livestock (DOL) of 2020, there were 13,812 *Apis cerana* (local bee) honey hives and 3,676 of *Apis mellifera (*improved*)* hives in Bhutan. An increasing trend in the adoption of *Apis mellifera* is taking place due to higher production of honey and easy in handling (Sherpa, 2020; NHRDC, 2020). However, the MoAF (2020) mentioned that the apiculture developmental in Bhutan are faced with multiple constraints and challenges and some of the these are lack of research and innovation, weak institutional linkages, limited financial resources, low adoption of improved technologies, inadequate processing and value addition facilities, use of pesticide/weedicides in agriculture (Gurung et al., 2012), high rate of pests and predation, high absconding rate of local bees (Verma,1990) and currently air, water and soil pollution.

Every year, there is a continuous rise of gaseous air pollutants such as CO, NO_x, SO_2, $PM_{2.5}$, PM_{10}, lead, and polyhydric aromatic compounds in Thimphu city (NEC, 2016). The major sources of air pollution are emerging from transportation, firewood burning for cooking and heating, wind-blown dust from building construction and bare agricultural fields, smoke from a forest fire, emission from industries, and transboundary air pollution (NEC, 2010, ICIMOD, 2019). The CO, hydrocarbons, and benzene emissions were high in gasoline vehicles compared to diesel vehicles in Bhutan (NEC, 2002). With the inflow of human population and rising vehicles, air pollution in Thimphu is becoming a new concern. Around 51.6 % of the total vehicles of the country are available under Thimphu region and the emission is increasing steadily (Dorji, 2019; MoIC, 2021). Increasing indoor and outdoor pollutants mainly PM_1, $PM_{2.5}$ and PM_{10} and other volatile compounds by burning firewood and vehicles' fuels may impair the health of the residents in Thimphu (Khumsaeng and Kanabkaew, 2021).

The anthropogenic influence on the environment results in a decrease of honeybee population across the globe (Balos et al, 2021). The bees are facing numerous problems from human related activities such as transportation, agriculture, industries and other undertakings (Rollin et al, 2013; Kaya et al., 2015; Christen et al., 2019). Celli, et al. (2003) revealed that the honeybees were killed by pesticides when applied inappropriately in agriculture fields or private gardens. Effects on bees will also decrease in pollination of crops thereby reducing crop productivity that will upset sustainable development goals of many nations (Patel, 2021). Many studies have revealed that honey can be used as a bioindicator of environmental pollution/quality (Turhan, 2007; Bastias et al, 2013; Mutlu et al., 2017; Cunningham et.al; 2022). Contaminated water and agricultural fertilizers are some major causes of heavy metal contamination in the plant tissues (Addis and Malede, 2014). Heavy metals such as Co, Fe, Mn, Ni, Zn, and Cu are essential elements, but become toxic at higher concentrations for growth of plants (Addis and Malede, 2014. Nevertheless, heavy metals such as Pb, Cd, Cr, and Hg have been marked with high toxicity for plants (Czipa Net al, 2015). During the honey-making

process, bees transport pollutants to the beehives following contact with polluted plant species or from drinking contaminated water (Mejías and Garrido, 2017).
The bees close to cement plant were detected more deposition of PM on their bodies than the bees 7 km away from cement plant in Italy (Pellecchia, 2018). Tong et al. (1975) had found forty-seven elements in honeys produced near highway, industrial, and mining areas. Among the elements, Al, Ba, Ca, Cu, Mg, Ni, Pd, and Silicon were emitted in higher level from traffic/near highways. Honey may contain minerals and heavy metals as a result of air pollution. Gallagher (2016) narrated that air pollution acts as a retardant, shortening the life span and affecting bees to reach plants emitting aromas from flowers. The chemical reaction breaks down the plant scents from which bees gets confused and take more time to locate nectar and pollen. Leonardo et al. (2019) examined the indirect effect of petrol exhaust pollution on olfactory learning and memory (short and long term) in honeybees. Formicki, et al. (2012) traced out the contaminations (Cd, Ni, Pb, Fe, Mg, and Zn) of multi-floral honey, propolis, bee pollen, and wax coming from apiaries located in different locations in Southern Poland.
Some areas/lands are characterized by heavy metals in soil, water, and air. Plants can absorb soil pollutants and enter the nutrient cycle. High accumulation of toxic heavy metals in plants is hazardous for the food chain and may result in damages to human and animal health (American Academy of Pediatrics, 1996; Tesfaye et al, 2016; Mantovi et al., 2003; Marcovecchio et al, 2007). The present study aimed at finding the effect of air pollution on bees and the quality of honey. The status of environment of rural or urban areas of Thimphu district will be known by observing air pollution levels and the presence of heavy metals in honeys. The honey samples were analyzed for pH because it has an important link to the growth of bacteria. The ash content of the honeys was carried out as it determines physiochemical and nutritional properties.

MATERIAL AND METHODS

Description of study the site

The detail description of the study sites for keeping bee colonies and Airvisual Pros are given in the Table 1. For honey hive experiment, A*pis mellifera* were raised in the premises of the Pelkhil School in Olakha (urban) and the Royal Thimphu College in Ngabiphu (rural). These two places are situated in Chang Geog (Geog means block- combination of Geogs make a district) under Thimphu District in Bhutan (Figure 1). Olakha is situated at the southern flank of Thimphu city (see Figure1) and comparatively polluted as compared to Ngabiphu which is like a village. The degraded blue pine forest is available close to Olakha but in Ngabiphu, the mixed-forest with different layers of plant species are found and some of them are good sources of nectar, propolis, and pollen for bee foraging. The agricultural farming is almost non-existence in Olakha, but the apple orchard and small-scale vegetable cultivation is practiced in Ngabiphu. To measure PM_{10}, $PM_{2.5}$, PM_1, CO_2, AQI, humidity and temperature, one AirVisual Pro was placed in Olakha and another in Ngabiphu for the whole year 2022. The AirVisual pros were sheltered from direct sunlight, rain, snow, ice, and high winds, and the natural airflow around the device were steady. To validate data obtained from the field, AirVisual Pros, a low-cost sensor was collocated and compared with the standard GRIMM-EDM 180 Sampler's data at Air Quality Monitor Station (AQMS) in International Centre for Integrated Mountain Development (ICIMOD), Kathmandu. The particulate matters (PM) correction equations were derived from the linear regression and applied to the AirVisual Pros Pro data to get corrected PM values

Table 1. Description of the study sites

Site	Altitude(m)	Longitude	Latitude	Setting	Road Distance (m)	Vegetation
Ngabiphu	2594	89^{0} 39'32.9"E	27^{0} 24'08"N	Rural	400 (Feeder)	Mixed forest
Olakha	2279	89^{0} 39'29.3" E	27^{0} 26'21.8" N	Urban	300 Highway)	Pine forest

Data collection

The AirVisual Pros (AirVisual Outdoor & Indoor Sensors) were set to record one reading in every ten seconds and data collection was done for the whole year, January-December, 2022. Every month, data was downloaded and prepared month-wise records for analysis. The honey from the experimental sites (Olakha and Ngabiphu) were harvested in October and kept in cool dark place for laboratory test in Nepal Environmental and Scientific Services (NESS) in Kathmandu. From each study site, eight honey samples produced from *Apis mellifera* were collected and eight samples from *Apis cerana* were purchased through farmers who were selling honey in the Centenary Farmers Market in Thimphu. The farmers from various villages of different districts in Bhutan sell honey produced from *Apis cerana*. To rear *Apis Mellifera*, the movable frames in the box were used while the *Apis cerana* are reared in wooden log–hive by the local farmers in the villages. The standard procedure was followed for rearing bees (*Apis mellifera*), harvesting, storing, and packing of honey. The utmost care of handling honeys was carried out as it could be contaminated by natural toxins, environmental contaminants (like, air, water and soil), and by human through production, processing, transport and even testing in the laboratory.

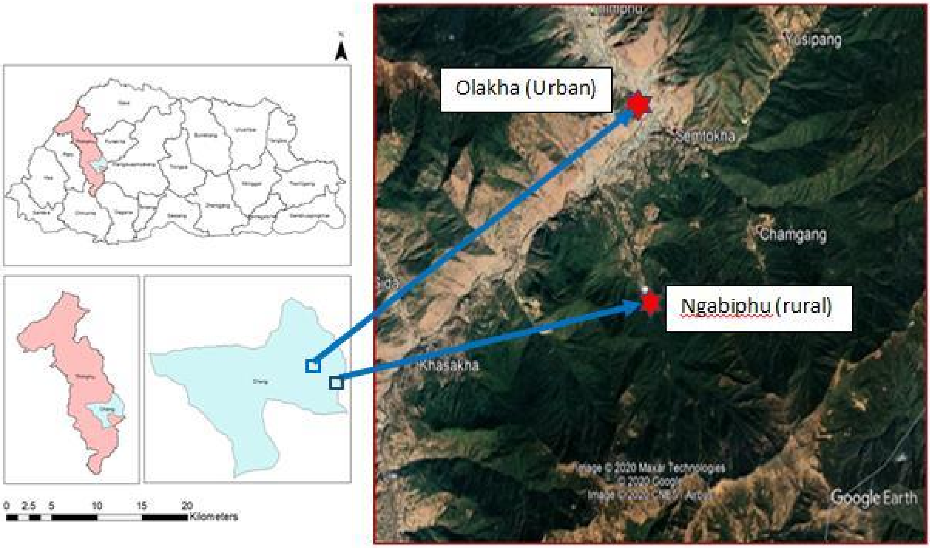

Figure 1: Location of Olakha (Urban) and Ngabiphu (Rural) in Thimphu District in Bhutan (Source: Google Earth, 2023)

Laboratory Test

Collected /sampled honeys were analyzed for heavy metals (Pb, Ni, Cd, and Cu) using the Atomic Absorption spectrophotometer in the NESS, (AAS, USEPA, 1986 - Method 3050). The ash content was tested using Gravimetric Method, 923.03 AOAC, 20th Edition 2016. pH of the honeys was tested using standard pH meter.

Statistical Analysis

The basic descriptive statistics such as mean, standard deviation, maximum and minimum were used for analysis. Use of R-studio for Anova-test and t-test were done and the statistical significance level was considered at alpha 5%. Further, graphs and tables were also used for interpreting data.

RESULTS AND DISCUSSION

$PM_{2.5}$ Comparison

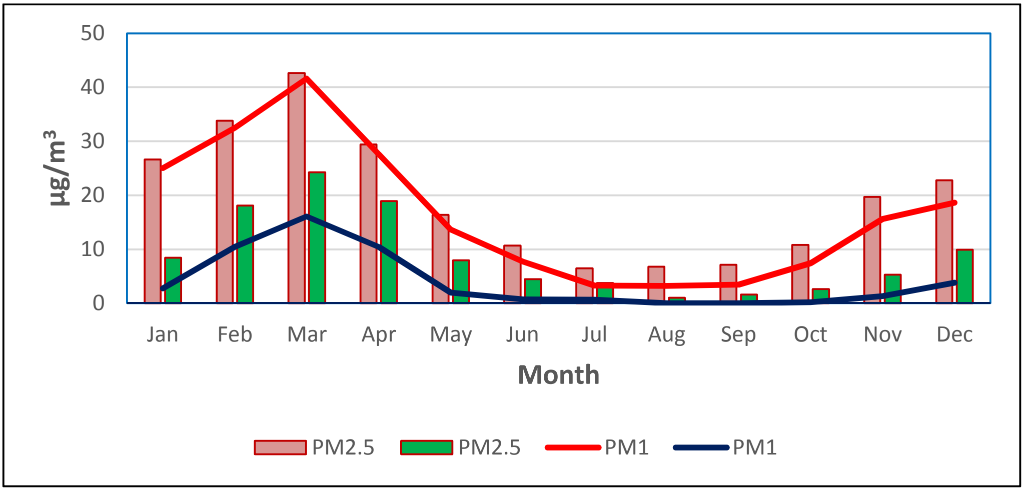

Figure 2: $PM_{2.5}$ and PM_1 concentrations in Olakha and Ngabiphu in the year 2022.

There was a statistically highly significant difference of $PM_{2.5}$ concentrations (p =0.0000 <α=0.05) between Olakha and Ngabiphu. The mean annual $PM_{2.5}$ were recorded as 19.43 µg/m^3 in Olakha and 8.87 µg/m^3 in Ngabiphu exceeding WHO 2021 permissible level of 5 µg/m^3 in both the study sites. There was a statistically high significant difference (p= 0.0001, Confidence level= 95%) of PM_1 between Olakha and Ngabiphu. The annual mean PM_1 of Olakha was 16.63 µg/m^3 and in the range of 0-139.83 µg/m^3, while in Ngabiphu, it was 4.05 µg/m^3 in the range of 0-96.50 µg/m^3. The coefficient of variation (CV= mean/SD *100%) of $PM_{2.5}$ concentration were 79.02% and 86.59% in Olakha and Ngabiphu respectively. Both PM_1 and $PM_{2.5}$ were higher in dry months (November- April) and lower in wet months (May-October) in both the study areas (Figure 2).
Figure 2 shows that the maximum reading of $PM_{2.5}$ was 42.66 µg/m^3 in Olakha and 24.25 µg/m^3 in Ngabiphu and both of these were ensuing in the month of March. The pollutants levels were very low from May to October as it is the time of monsoon rain. The bees collecting nectar, pollen, propolis and wax are not affected by $PM_{2.5}$ and PM_1 concentration during monsoon season as the pollutants concentration were very low (Figure 2). They do not get affected by pollutants even in winter months particularly in November, December, January and until mid of February every year as bees do not forage due to low temperature in both the study sites.

PM1 and PM2.5 comparison in summer and winter

As per the National Centre for Hydrology and Meteorology of Bhutan (NCHM, 2022), October to March is considered as winter and April to September as Monsoon season-(rainy season). In general, there were high concentrations of $PM_{2.5}$ and PM_1 in the winter season and very low in Monsoon in both the study sites (Figure 3). The low $PM_{2.5}$ and PM_1 in Monsoon are due to the precipitation setting down the suspended particulate matter from the atmosphere.

In winter, bees could be affected by air pollution only from mid-February to Aril (2.5 months) in Olakha. Bees start to come out from the hives for foraging only from mid of February and hence there could be some effects but not that seriously as the maximum level of $PM_{2.5}$ in 2022 reached up to 42.66 µg/m^3 and 24.25 µg/m^3 in Olakha and Ngabiphu respectively. The bees will be able to get the floral scent and forage successfully in wet season (April to September) when there will be blossom of flowers in both study areas. Dust contamination to bees may take place from mid-February to April in Olakha when the rainfall will be very less. As compared to Olakha, the bees are not affected at all by air pollution during winter and Monsoon in Ngabiphu due to low $PM_{2.5}$ and PM_1 (Figure 3).

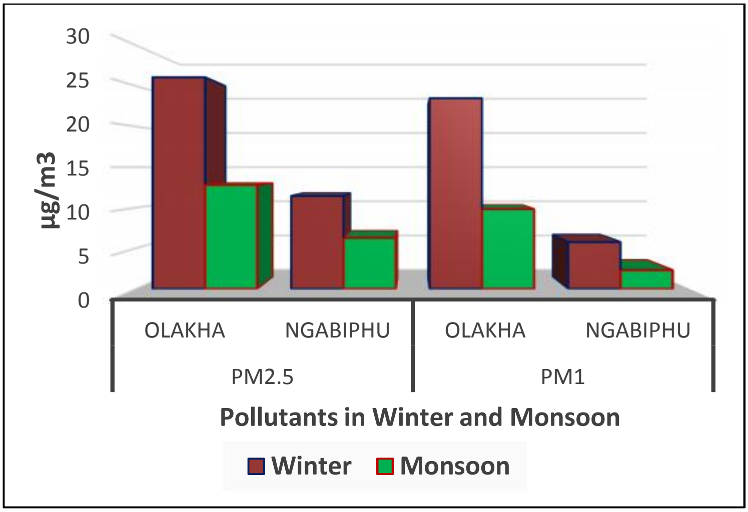

Figure 3: PM1 and PM2.5 levels in Olakha and Ngabiphu during Winter and Monsoon in 2022

$PM_{1,}$ with a diameter less than 1 µm in size can contribute to deadly diseases like heart attacks, lung cancer, dementia, emphysema, edema and other diseases and also lead to premature death (Kulshrestha, 2018). When PM_1 is inhaled, it travels into the lungs, enter the bloodstream, damage the inner walls of arteries, penetrate tissues in the cardiovascular system and spread to organs (Wang et Al., 2021). Like humans, bees could be affected with this pollutant (PM_1) but no study have been carried out as yet.

Quality of honey

It was found that there was a statistically significant difference of ($p<0.05$) pH means between honey samples collected from three places. The pH was lower in rural honey than urban honey but lying within the international permissible limit of 3.5- 6.1 set by CAC, 2001. The pH of two honey samples were 6.2 and 6.3 from Olakha (Urban) that crossed the permissible limit of 6.1. The reasons of higher values in pH could be due to exposure of honey in open air, not fully capped honey, or it could be from error in laboratory test. If the pH of honey exceeds 6.1, then the bacteria will start fermenting honey due to the presence of various types of organic acids (Terrab et al., 2004). The pH will determine the stability, shelf-life and influence the texture of honey. Lower the pH level in honey, better it is as the microorganisms could not affect honeys in the long run (Terrab et al., 2004).

Table 2. Compariosn of honey from different bee species and places

	Apis Mellifera				***Apis Cerana***	
Variables	Olakha (Urban)		Ngabiphu (Rural)		Market (Rural)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
pH	6.05 ± 0.17	5.80-6.30	5.33 ± 0.10	5.20-5.50	5.25 ± 0.05	5.20-5.30
Ash (%)	0.15 ± 0.08	0.03-0.29	0.11 ± 0.05	0.01-0.15	0.11 ± 0.06	0.01-0.19
Cu (µg/g)	2.86 ± 0.84	1.48-3.83	2.55 ± 0.91	1.32-3.80	4.24 ± 1.05	2.81-6.03
Cd (µg/g)	2 *	0.0-0.52	3*	0.0-0.32	1*	0.0-0.32
Pb (µg/g)	ND		ND		ND	
Ni (µg/g)	ND		ND		ND	

Note : ND= not detected, 1*= one honey sample Contain Cd , 2 * = two honey samples contain Cd 3*= three honey samples contain Cd, n= 8 samples from each place

Table 2 shows that the ash content was 0.15% in the honey of *Apis mellifera* from Olakha, but it was 0.11% in the honey of both *Apis mellifera* and *Apis cerana* from rural parts of Thimphu district. There was no statistically significant difference ($p=0.079 > 0.05$) in the means of ash contents in honeys and the average were within permissible limit of 0.02 - 0.6 % (CAC, 2001). It is a known fact that the ash content of honey is related to its origin, colour, and flavor. The Pb content in the honey from all three places were not detected indicating that the air is still clean in Thimphu district and in Bhutan. It is evident from figure 2 and 3 that the air pollutants were low and hence no Pb were detected in all the honey samples tested. Pb occurs in air which originates mainly from motor traffics besides other natural sources and anthropogenic activities (Bogdanaov, 2006). The Ni content in all the honey samples were also not detected. Absence of Pb and Ni in the honeys samples indicates a low level of contamination in the air and water (Przybylowski and Wilczska , 2001).

The copper content in honey samples ranged from 0.84 - 6.03 µg/g with the mean values of 2.86 µg/g, 2.55 µg/g, and 4.24 µg/g in Olakha, Ngabiphu, and Market respectively (Table 2). There was a statistically high significant difference ($p=0.00382 < 0.05$) in the means of Cu content in honeys. Figure 4 shows that in two honey samples from Market, the Cu content were 6.03 µg/g, and 5.37 µg/g exceeded the permissible limit of 5 µg/g (WHO, 2002). However, the average Cu content in the honey of *Apis cerana* of Market honey was 4.24µg/g which is still below international permissible level of 5 µg/g (WHO, 2002). The Cu might have entered in the Market honey due to improper procedure during harvest (keeping longer duration in open, excessive use of smoke, packing and storing in plastic bottles and other uncleansed glass containers), hygienic behavior of beekeepers/ honey harvesters and also could be the honey from honeydew which contains a bit more heavy metals. Honey from honeydew are containing more heavy metals than nectar honeys (Dzugan et al., 2018). It is a known fact that the Cu is required in our bodies but when it exceeds the required level, it may become toxic and impair the health of humans.

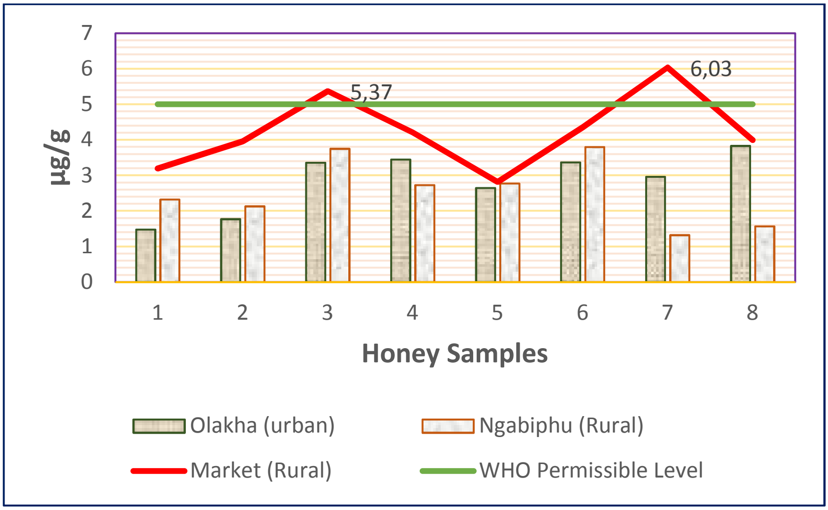

Figure 4. Cu Comparison in honey of Olakha, Ngabiphu, Market, and WHO (2002)

The Cd content in six honey samples (1 from Olakha, 2 from Market and 3 from Ngabiphu) were detected out of twenty-four samples (Table2). Only one honey sample containing Cd from the Market honey was detected and the reading was 0.32 µg/g. From Olakha, two honey samples were found containing Cd with the values of 0.54 µg/g and 0.31 µg/g. Three honey samples were tested Cd with the values of 0.19 µg/g 0.20 µg/g, and 0.31 µg/g from the honey samples of Ngabiphu. The Cd in the honey samples of Olakha were exceeding the permissible level of 0.30 µg/g. Honey produced even in the rural areas of Ngabiphu and Market from *Apis Mellifera* and *Apis cerana* were detected Cd. It is stated that the Cd originates from industries and incinerators and reach to plants through soils (Bogdanov 2006, Ruschioni,et al 2013). In some areas, soils and water contain more amount of Cd and plants take through roots and reach to nectar and bees collect them (Fakhimzadeh, 2000; Linden et al., 2003). As such, it is imperative to do further study about the sources of Cd which may be coming from polluted water, soils, smoking , improper storage or equipment-related contamination, handling of honey, hygienic behavior of beekeepers, exposure of honey in open air, floral species, and availability of honeydew. The research area need to be extended, and comparative analysis should be conducted in future studies.

CONCLUSIONS

The annual average $PM_{2.5}$ were 19.43 µg/m^3 in Olakha and 8.87 µg/m^3 in Ngabiphu confirming a good air quality. The annual mean concentration of PM_1 was 16.63 µg/m^3 and 4.05 µg/m^3 in Olakha and Ngabiphu respectively. The air pollution effect on bees could be low as the pollutant's levels were low in summer, and in winter, the bees are inactive due to low temperature. It was found that there was a significant difference ($p<0.05$) in the means of pH between honey samples. The ash contents in honeys were within permissible limit of 0.02 -0.6 %. The Ni and Pb content in all the twenty-four honey samples were not detected which is an indication of healthy air and water. The Cu concentration in two honey samples from *Apis cerana* of Market were exceeding the permissible limit. The Cd presence in four honey samples exceeded the limit of 0.3 µg/g. Further study/research needs to be done to find out the sources of Cd in honey of rural and urban areas. A nationwide study with representative samples would be recommended as this study was limited to a few samples of honey and of one year only.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The opinions and/or conclusions expressed are exclusively those of the authors.

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