ANTIBACTERIAL ACTIVITY AND ELEMENT SPECTRUM OF NEPALI HONEY FROM DIFFERENT BEE SPECIES (HYMENOPTERA: APIDAE)

Doctoral Thesis Submitted
to the
University of Natural Resources and Applied Life Sciences Vienna
(Universität für Bodenkultur Wien), Austria

by

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October, 2004

This Ph.D. thesis is dedicated to ...

MY PARENTS, who have paved the way for me to travel

MY WIFE, who has provided me with loving support, encouragement, and comfort throughout my academic journey

Ph.D. supervisors

Univ. Doz. Dipl. Ing. Dr. Hermann Pechhacker

Prof. Dr. Jost H. Dustmann

Acknowledgements

I wish to acknowledge my deep indebtedness to the Government of Republic of Austria and Österreichischer Austauschdienst (Austrian Academic Exchange Service/ ÖAD) for providing me the scholarship.

I am extremely grateful to the person who urged me on by way of his untiring support and seemingly unlimited belief in me, to my PhD supervisor, all else pales. My supervisor, Univ. Doz. Dipl. Ing. Dr. Hermann Pechhacker has been of immeasurable help with his valuable advice, support, patience, cooperation, constant encouragement during the long process of research, writing and editing this thesis and my educational stay in Austria.

It wouldn`t have been possible to carry out research without the skilled work of members of the Austrian Agency for Health and Food Safety (Agricultural Inspection Service and Research Centre), Institut für Futtermittel, Vienna. Thank you very much Dr. Manfred Sager (element spectrum part), K. Mamdouhi and Birgit Posch (antibacterial part). In particular, I thank Univ. Doz. Dipl. Ing. Dr. Herbert Wurtzner (Head, Institut für Futtermittel) for his keen interest to the thesis`s goals.

I am also grateful to the individuals who gave of their time and care. I have benefited from the computer skills of Ernst Hüttinger and Helmut Heigl of the Austrian Agency for Health and Food Safety (Agricultural Inspection Service and Research Centre, Institut für Bienenkunde, Lunz am See), and photographic skills of Wolfgang Dukat, Vienna.

I indebted to several people for their comments and suggestions in earlier days of this work. And, I would like to thank the people for their time and effort in assisting in reviewing chapters in this thesis. Their detailed and constructive comments were helpful in ensuring the technical integrity of the analyses. I am profoundly thankful to Dr. Manfred Sager (Institut für Futtermittel, Vienna), Prof. Dr. J.H. Dustman (Tierärztliche Hochschule Hannover, Germany), Dr. R.A. Cooper (Senior lecturer, University of Wales Institute Cardiff, United Kingdom), and Prof. Dr. Josef Simuth (Head, Laboratory of Genetic Engineering, Slovak Academy of Sciences, Slovak Republic) for providing me the valuable literatures. In addition, the work and the thesis would have been impoverished without the suggestions and critical comments of these eminent experts. I am especially grateful to Prof. Dr. J.H. Dustmann for his scholarly suggestions.

I should not forget Markus Pechhacker for his unlimited cooperation of his effort and great assistance for statistical anaysis, and for reviewing chapters in this thesis. The many hours we

spent discussing in subject matter have led to many fruitful developments. Thank you very much Markus.

I believe, honestly, it's necessary to acknowledge the persons who constantly motivated me, shaped my dreams and my work. My heartfelt thanks to Dr. Surendra Raj Joshi (ICIMOD, Nepal) and Dr. Khum Narayan Paudayal (Central Department of Geology, Tribhuvan University, Nepal).

There are many that from behind the scenes have helped during my field work, and I wish to thank them. Thank you Rudra K.C., Lalitpur; Bishnu Bhusal, Suroj Pokharel, and Khem Raj Neupane, Chitwan.

There are special mentors that I must acknowledge due to their importance in my work. It is not practical to list all of those that have contributed because then I would be reciting names of many that I never met, but whose published work inspired me. Suffice it to say that to all those from Peter Molan to Stefan Bogdanov, thank you !

Many people have directly-indirectly contributed their ideas and efforts. I also want to thank the many researchers that have shared their knowledges and experiences with me in several conferences. Their testimonies have often made difficult tasks more than worth while. Thank you-all of you !

Of course, like any researcher, I am indebted always to those people who do their best to improve on my best. Thanks especially to all of the eminent scientists who have lent their work to my research. I'd list them all, but they're credited in the published works, so I'll just say thanks !

My gratitude to all staffs of Institut für Bienenkunde, Lunz am See for their patience, understanding, and friendly support throughout the thesis`s gestation. In particular, I thank Dr. Rudolf Moosbeckhofer (Head, Institut für Bienenkunde, Vienna), Dr. Karl Pfeiffer (Head, Institut für Bienenkunde, Lunz am See) for their keen interest in my research and my colleague Josef Mayr for his friendly cooperation.

I offer my thanks to all ÖAD's staffs for their unfailing cooperation throughout my educational stay in Austria.

I am especially grateful to those wonderful and warm hearted people, those that do come early to mind, I don't want to miss. Many times I have had wonderful experiences and family environment to truly feel that in some way. That means so very much to me. Let me just mention. Thank you Mrs. Maria Pechhacker, Christian & Lore Pechhacker and Michael Pechhacker.

Finally, I offer my heartfelt thanks to my parents and my wife for their patience and loving support through several years of closeting myself with this effort.

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Abstract

This work was designed to determine peroxide and non peroxide antibacterial activity against *Staphylococcus aureus* ATCC 6538 P and element spectrum of Nepali honeys from different bee species and three agro-ecozones: terai, hills and mountains.

To assess peroxide and non peroxide antibacterial activity of honeys from Nepal, a study was undertaken using 164 honey samples from *Apis cerana, A. dorsata, A. florea, A. laboriosa* and *A. mellifera* bees obtained from three agro eco-zones (terai, hill and mountain) of Nepal. The honeys were tested against *Staphylococcus aureus* ATCC 6538 P using agar (23g/L 001-7-0 DIFCO) well diffusion methods. Of the 164 honey samples assessed, the effectiveness of Nepali honeys in inhibiting *Staphylococcus aureus* ATCC 6538 P was seen across 68% (peroxide) and 10% (non-peroxide) for *A. mellifera* honey; 66.66% (peroxide) and 66.66% (non-peroxide) for *A. florea* honey; 36.66 % (peroxide) and 10 % (non-peroxide) for *Apis cerana* honey; 35.55% (peroxide) and 15.55% (non-peroxide) for *A. dorsata* honey; 33.33% (peroxide) and 33.33% (non-peroxide) for *A. laboriosa* honey. 46.34% and 13.41% samples showed peroxide and non-peroxide antibacterial activities respectively. The antibacterial activities are expressed in % phenol equivalent. Comparisons between the sensitivity to honey of different locations showed no significant differences in both activities equivalent to that of % (w/v) phenol observed among all the locations. Honey from *Apis cerana -A. mellifera* (P=0.0009) and *A. dorsata-A.mellifera* (P=0.0010) showed significant difference in peroxide activity, whereas honey produced by two native bee species *A. dorsata* and *A. cerana* did not show significant difference in peroxide activity.

For element spectrum, Li, Na, K, Ca, Mg, Sr, Fe, P, Al, B, Co, Cu, Mn, Ni, V and Zn in 41 and Cd, Cr, Mo and Pb in 32 *Apis cerana* honey samples from seven different locations were determined by means of inductively coupled plasma (ICP) and atomic absorption spectrometry (AAS) based techniques. The elemental concentrations are expressed in mg/kg and µg/kg for ICP and AAS techniques respectively. *Apis cerana* honey samples analysed from seven locations, elemental concentrations of Li 0.044 mg/kg, P 302.142 ppm, Al 34.39 mg/kg, Co 0.017 mg/kg, Ni 1.19 mg/kg , Zn 7.867 mg/kg and Cr 29.288 µg/kg, in Langtang; Na 39.423 mg/kg, Fe 8.75 mg/kg, B 5.243 mg/kg , Co 0.017 mg/kg , and Pb 122.596 µg/kg in Kathmandu; Sr 0.226 mg/kg, Cu 1.51 mg/kg, Mn 7.392 mg/kg, Cd 4.136 µg/kg , and Mo 29.347 µg/kg, in Chitwan; K 4259.50 mg/kg in Palpa; Ca 193.917 mg/kg ,and Mg 149.3 mg/kg in Arghakhachi found to be highest amongst all. Similarly, K 889.000 mg/kg and V 0.0001 mg/kg in Kathmandu, Ca 63.637 mg/kg , Mg 31.962 mg/kg , Sr 0.067 mg/kg, Mn 0.001 mg/kg, Zn 0.001

mg/kg in Kathmandu valley; Na 15.101 mg/kg in Chitwan; Fe 1.553 mg/kg, P 44.827 mg/kg, Al 0.0022 mg/kg, B 0.0025 mg/kg, Co 0.00001 mg/kg, Cu 0.0001 mg/kg, Ni 0.0001 mg/kg in Arghakhachi; Li 0.0029 mg/kg, Cd 0.312 µg/kg, Pb 7.869 µg/kg, Cr 5.839 µg/kg, and Mo 5.625 µg/kg in Jumla-Jajarkot honey found to be lowest amongst all. K, which accounts on average for 83.28 per cent, was the most abundant of the elements determined; whereas Cd, accounts on average 0.0001% was the lowest.

Difference in concentration of Ca, Fe, P, Zn, Cd, and Pb between polluted (Kathmandu, Kathmandu valley and Chitwan) and clean areas (Jumla-Jajarkot, Palpa and Langtang) were compared. Significant difference was found only in total Pb content (P=0.0001).

Similarly, Li, Na, K, Ca, Mg, Sr, Fe, P, Al, B, Co, Cu, Mn, Ni, V and Zn in total 44 and Cd, Cr, Mo and Pb in 27 representative honey samples were analysed by means of ICP and AAS respectively from *Apis cerana, A. dorsata* and *A. mellifera* collected in Chitwan. The difference in concentration of Ca, Sr, K (FES), Al, Cu, Mn in *Apis dorsata - A. mellifera* and Fe, P, K (FES), Al, B, Cu in *Apis cerana –A.mellifera* honeys were significant . Li, Na, Mg, As, Be, Cd, Co, Cr, Mo, Ni, Pb V and Zn in *Apis dorsata-A. cerana, Apis dorsata- A.mellifera* and *Apis cerana-A. mellifera* honeys did not differ significantly in individual groups. Moreover, comparison showed that the difference in concentration of Mo was significant in *Apis cerana* and *A. mellifera* honeys. The overall element contents of Chitwan honeys showed that K was the most abundant of the elements determined. Whereas, the lowest concentration noted for Cd. The highest average concentration detected in Chitwan honeys was K 1728.29 mg/kg followed by P 125.92 mg/kg, Ca 113.57 mg/kg, Mg 59.79 mg/kg, Na 19.57 mg/kg, Fe 3.88 mg/kg, Mn 2.88 mg/kg, B 2.49 mg/kg, Al 2.34 mg/kg, Zn 2.14 mg/kg, Cu 0.67 mg/kg, Sr 0.17 mg/kg and Ni 0.11 mg/kg . The overall concentrations in Chitwan honey was limited to 2.41^{-03} mg/kg, 0.013 mg/kg, 0.019 mg/kg and 0.029 mg/kg for Cd, Cr, Mo and Pb respectively. Data presented in this work are part of a study aimed to determine the overall element contents in honeys as well.The amount of the overall element present in total Chitwan honey samples found to have 2094.413 (mg/kg) . The results indicated that honeys from different bee species and produced in different locations of Nepal contain good antibacterial activity, and contain essential and toxic elements such as Pb. The distribution of the elements in Nepali honeys could be affected by the surroundings, soil, floral source, foraging range, and honey that comes into contact with metal equipments. It may be assumed that honey can be alternatively used as a environmental indicator to obtain information on pollution.

Key Words: Nepal, Different bee species, Honey, Antibacterial Activity, Element Spectrum, Environmental indicator.

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CHAPTER ONE

1.1 Introduction

Nepal : An Overview

Nepal is roughly rectangular in shape. The country's landmass stretches 885 km from east to west and has a non-uniform width of 193 km north to south. It lies within the sub-tropical to the mountainous region at 26° 22` to 30° 27 ` N latitudes and 80° 4 `to 88° 12` E longitudes, with an altitude that ranges from 90m to 8,848 m. the country is landlocked and is bordered by India in the East, West and South, and China in the North. Geographycally, Nepal represents a transitional mountain area between the fertile Gangetic Plain of India and the arid plateau of Tibet, China.

It has a total land area of 147, 181 sq.km and the population was estimated to be more than 24 million (till 2004) with an estimated growth rate at 2.27 %. 7.3 % live in the mountains (land coverage 35 %), 44.2 % in the hills (land coverage 42 %) and 48.5 % in the Terai (land coverage 23 %).

The ethnically diverse population consists of several races, tribes, languages and cultures.

The country is rich in ecological diversity. Over 80 % of the land covered by rugged hills and mountains. From the low-lying terai plains in the south, where elevation in some places is less than 100m above sea level, the landscape rises through a maze of valleys and spurs culminating in the majestic heights of the great himalayas, including the Mount Everest-the highest peak in the world.

The narrow strip of flat alluvial terrin along the southern border, known as the terai, is an extension of the Gangetic Plain and comprises about 23% of the country, including most of the fertile and forest areas. Its general slope towards the south is less than 1 per cent .

The terai: The Churia and Mahabharat Ranges punctuate the terai plains with an approximate width of 50 km. and has altitudes varying from about 60 m in the plains to about 330 m and constitutes the fertile with alluvial soil, with a good water holding capacity.Its northern edge is the Bhabar. The broad flat valleys or Duns found between successive hill ranges.The first elevation next to the Terai is the Siwaliks (also known as Churia Range).Their average altitude is 900 m (elevation difference from 120 m to 2,000 m) and is about 8 to 10 km in width. The Churia range is the youngest member of the Himalayan family.There are a number of terai-like valleys lying between the Siwaliks and the Mahabharat range, commonly called the Dun Valleys

(inner terai plains), such as Chitwan and Dang. Sal (*Shorea robusta*) is dominant toghether with Semal (*Bombox malabricum*), Asna (*Terminalia termentosa*), *Dalbergia spp.* and other species, and the Churia hill has *Pinus roxburgi*. Terai forests contain jasmin, minosa, acceacia reeds and bamboo, pipal (*Ficus religiosa*), and banyan" (*Ficus bengalensis*)etc.Tall coarse two-metre high elephant grass originally covered much of the Dun valleys but has now been largely replaced by agricultural settlements.

Middle hills: Running parallel with the Churia range is the middle mountain zone, also known as the middle hills or the mahabharat range.This zone also includes the so-called "middle hills " which extend northwards in a somewhat confused maze of ridges and valleys to the foot of the great Himalayas. The altitude ranges from 500 m in low-lying valleys and rise to a height of about 3,000 m and comprises the outer wall of the Himalayan range. The dominant trees of this zone are *Castenopsis indica* in association with *Schima wallichi*, and other species such as *Alnus nepalensis*, *Acer oblungum* and various species of oak and rhododendron, which cover the higher slopes where deforestation has not yet taken place. Orchids clothed the stems of trees and gigantic climbers smother their heads. It has great rivers such as the Karnali, Narayani and Saptakoshi flow through this area into the broad plains of the terai. This maze of valleys and spurs has been the traditional zone of human occupancy in Nepal. The rapid rise in population, and consequent problems of ecological degradation have been the most conspicuous feature of this hill region in the recent years.

Mountain: The high mountain zone, located north of the middle mountain region.This differs from the previous type in its Northward, on the long, straight and steep slopes, and narrow valleys which are sensitive to erosion.and spurs of the great Himalayas. The high mountain zone, located north of the Middle Mountain Region. This zone is rich in forests of oaks and pines up to an altitude of about 2,400 metres. Higher up, forests of various conifers, especially, Picea, Tsuga, Larix and Abies spp. tree line occur. Various rhododendron species, bamboo and maples are common at higher altitudes ranging from 3,600 to 3,900 m and associated with the coniferous zone. Composition of the forest varies considerably with coniferous predominating in the west and eracaceous in the east.

The high himal zone occupies about 23 per cent of the kingdom and is mostly snow covered. The snow line is at 5,000 m in the east and 4,000 m in the west. This zone is an area of rocky , ice-covered massifs, rolling uplands, snow-fields, valley glaciers, and sweeping meadow lands. Rhododendron, juniper scrub and other procumbent woody vegetation may extend to about 4,200 metres where it is then succeeded by a tundra-like association of short grasses, sedge mosses and alpine plants wherever there is sufficient soil. This continues upto the lower limit of

perpetual snow and ice at about 5,100 metres. It forms the northern boundary of the monsoon climate and the geo-political border between Nepal and China.This region has over 200 peaks exceeding 6,000 m . Eight of the ten highest peaks exceeding 8,000 m on earth, including the Sagarmatha (Mt. Everest), are located in this zone.

Soils in Nepal vary in characteristics from place to place and differ in the physiographic zones. The terai soils are productive with neutral to slightly high pH, ranging from low to medium in organic matter and nitrogen. In the Hills soils are low in pH, and low to medium inorganic matter, nitrogen and phosphorus. Increasing population pressure on agriculture is causing soil fertility status to decline.The main causes of soil degradation are deforestation, degradation of grasslands, poor irrigation and drainage practices, inadequate soil conservation, enroachment of steep slopes, intensive agriculture and over grazing.

Increased use of chemical fertilisers is also causing soil degradation through change in soil structure , acidification and nutrient imbalace in the soil and offsite effects from the over-use of chemicals. In the Terai, the soil is alluvial and usually fine textured, with good water-holding capacity. Bhabar, which is characterised by boulders and freely drained gravely soil thus unsuitable for agricultural purposes.The Churia has dry and immature soil. In the Siwaliks, the dominant soil texture is sandy with pebbles. These soils are poorly developed and prone to erosion, and cannot retain high-intensity precipitation. In the middle mountains, the soil type varies from medium to light textured coarse-grained sand, which is also prone to erosion. The upper region also consists of hard rocks in many places.

Nepal is continuously facing the problem of deforestation, landslide and loss of nutrient-rich soil due to the overwhelming dependence of rural people on biodiversity resources. Soil destabilization, cultivation on steep slopes and deforestation have all been leading to the loss of fertile soil that are naturally deficient in nutrients and receive only small applications of fertilizer and organic matter. According to American agricultural scientist John Melar, who has been working in agricultural planning in Nepal since the 1950s, Nepali soil was the most fertile in South Asia until just twenty five years ago. But it as now become the poorest quality in the entire region.

Climate

Nepal lies within the subtropical monsoon climatic system. Due to its varried topography different regions of the country have diverse climatic and floral conditions. The topographical orientation and its vertical extension largely affect the distribution of rainfall in Nepal. With altitude being a guiding factor in climatic classification, five different types of climates are present in Nepal.They include sub-tropical monsoon, warm and cool temperature, alpine, and

tundra climate. The terai and the Siwaliks experience subtropical climate, while the northern mountanious regions have cold, dry continental and alpine winter climate. The main source of precipitation is the summer monsoon (late june to september) of which 80 per cent falls during this period, 15 per cent during the post-monsoon (october) and pre-monsoon seasons (april to may), and the remaining 5 per cent during the winter (november to february) periods.

The climatic and physiographic conditions generate environmental problems such as soil erosion, landslides and loss of nutrient rich to soil in the uplands affects the downstream ecosystem and the farmlands, indicating a close link between the uplands and the lowlands.

Vegetation:

The vegetation of Nepal has been classified by Swan (1962) into the following seven zones:

Type	Approx.Altitudinal Range in m
1. Lower monsoon forest	152-1,066
2. Middle monsoon forest	1,066-1,981
3. Upper monsoon forest	1,981-2,590
4. Deciduous and rhododendro forest	2,590-3,200
5. Confer and rhododendron forest	3,200- 4,114
6. Wet alpine zone	4,114-5,029
7. Dry alpine zone	4,876- above

Swan and Leviton (1962)

Rice, wheat and maize are the major crops of Nepal followed by millet,oilseeds (mustard), buckwheat, barley, etc. Horticulture and animal husbandry are also important parts of rainfed agriculture in Nepal.Agriculture is mailnly based on reainfed (71% of the total cultivated land). In both rainfed and irrigated agriculture time spent by women is higher relative to that of men. In rainfed and irrigated areas women devote more time than men do. Food shortages due to seasonality contribute to malnutrition for children and women. The low consumption of fruit and fresh vegetables, which is highly dependent on local seasonal availability, contributes to nutritional disorders such as deficiencies in iron and vitamin A. Micronutrients may have a role in enhancing reproductive health of women living in the developing world. Two illustrative micronutrients, zinc and vitamin A, have received some attention in this regard. Data are also suggestive that adding zinc may negate the beneficial effect of iron and folic acid on birth

weight. Research is needed to further our understanding of nutrient-nutrient interactions (Christian, 2003). Zinc potentiate the effect of vitamin A in restoring night vision among night-blind pregnant women with low initial serum zinc concentrations (Christian et al., 2001). Iron deficiency is one of the main causes of anemia during pregnancy, although other micronutrient deficiencies may play a role. Antenatal folic acid-iron supplements modestly reduce the risk of low birth weight (Christian et al., 2003ab).

Sustainability of conventional agriculture is based upon a high input of agrochemicals, such as phosphate fertilizers. Continuous fertilization of soils or use of heavy metal contaminated sewage sludge as a fertilizer could increase the heavy metal contents exceeding natural abundances in soils, transfer and accumulation of toxic metals from sludge to soil. The subsequent uptake and accumulation of metals in the edible parts of vegetative tissue results in a direct pathway into the human food chain (Sekhar et al., 2002).

The use of pesticide in Nepal dates back to 1955 when paris green, ganunaxene and nicotine sulphates were imported from the U.S.A for malaria control. Following that in 1960, DDT was introduced in Nepal and in the same year the Department of Agriculture initiated the application of chemical pesticides for crop protection. The demand and use of pesticide for plant protection has steadily increased, consequently to fulfil the demand of the country.

An important consideration when using any agricultural chemical it effect on non-target species. Only about 4% of insects are pests of economic importance (Heading,1 983) and the problem of pesticide toxicity to beneficial insects, particularly pollinators, is worldwide (Clemson,1979; Crane, 1981; Dadant and Sons,1975; Field,1981; Johansen,1979; Mayer et al.,1983; Melksham, 1983; Mel`nichenko,1 980; Metcalfe, 1980; Rhodes et al., 1980; Ware, 1980). Poor farmers in Nepal are untrained and cannot afford to bear the high cost of the chemical pesticide. Integrated Pest Management (IPM) is expanding and is in priority for pest management. Bio-pesticides and bio-fertilizers are low-cost, environment friendly and compatible with the nature (Gautam, 1998; Tamrakar and Gautam, 1999).

Lastly, honey has been used in rural areas as a nutritious food, for medical and pharmacological purposes since ancient times. And, It has been gaining acceptance as a potential healer in modern medicine. With the continued emergence of antibiotic-resistance strains of micro-organisms in today's society, innovative and effective antimicrobial agents are urgently required. On the other hand, honey may play an important role as an environmental indicator and biomonitoring lool for environmental contamination. The country's stability and consistant development is that the utilization of the land should accomodate to the functions and activities suitable for the country's endowment, which are the topic of the next chapter.

1.2 Objectives

The specific objectives of this study are as follows:

- **to provide general information on bees and beekeeping in Nepal.**
- **to investigate peroxide and non-peroxide antibacterial activity of *Apis cerana* honey against *Staphylococcus aureus* ATCC 6538 P from different agro-ecozones of Nepal.**
- **to investigate peroxide and non-peroxide antibacterial activity of honey from different bee species (*Apis cerana, A. mellifera, A. dorsata, A. laboriosa, and A. florea*)honey against *Staphylococcus aureus* ATCC 6538 P in order to identify differences.**
- **to investigate peroxide and non-peroxide antibacterial activity of *Apis cerana, A. dorsata, and A. mellifera* honey against *Staphylococcus aureus* ATCC 6538 P from Chitwan district , Central Nepal.**
- **to determine distribution of element content of *Apis cerana* honey as a contamination indicator from different agro-ecozones of Nepal in order to identify differences.**
- **to determine distribution of element content of *Apis cerana, A. dorsata,and A. mellifera* honey as a contamination indicator from Chitwan district, central Nepal in order to identify differences.**
- **to evaluate the role or effectiveness of honey (from different bee species) as biological indicators of the presence of different trace elements in the environment, by comparing data obtained by different sampling sites.**

CHAPTER TWO

2.1 Honeybees: Species and Distribution

The hive bee species present in the Asian countries is *Apis cerana*. In Europe and Africa the native species *is A. mellifera*. America and Australia did not have any native honeybee

Figure 1a *Apis cerana*

species. The European hive bee was introduced into America in 1621 and into Australia around 1820.

The four native species of genus Apis found in Nepal are, *Apis cerana, Apis dorsata, Apis florea and Apis laboriosa. Apis mellifera* was introduced in 1993.

Ruttner (1986, 1988) classified the different Asian hive bee populations into four groups: of *A. c. japonica, A.c. cerana, , A. c. himalaya, and A c. indica* . The former three races occur in Nepal Their distribution is as follows:

The *Apis cerana* found in a very wide area comprising mainly southern and eastern Asia.In the west it extends from Afghanistan up to the Philippines in the east and in the north from Ussuria to Java in the south (Ruttner, 1985, 1986, 1987).Thus, *Apis cerana* is found not only in the tropical and sub-tropical regions of Asia, but also cooler climate such as Siberia, norther China and higher altitudes of the Asian mountains at altitude of up to 3,600 m (Koeniger, 1976; Partap, 1997). Of the *A. cerana*, three sub-species/races have so far been occur in Nepal: *Apis*

cerana cerana in the high hills, *Apis cerana himalaya in the* mid hills and *Apis cerana indica* in low lands (Verma, 1990; ICIMOD, 1994).

Apis cerana bee builds parallel combs inside a cavity.Colonies are found in forests or agricultural areas in the plains, and even in urban areas with good vegetation. Bees are larger than the dwarf bee, but are much small than the rockbee. Natural nests of the these bees occur in tree trunks, rock crevices, ant hills, underground deserted nests of white ants, or any dark enclosure, sometimes even in the open, but quite dark spaces in forests or unused rooms in buildings.

Traditional fixed comb hives made of logs, walls, and earthen pitcher frames and modern, movable frames hives are the methods of practicing beekeeping.The techniques are of are of old tradidion.The honey yield *of Apis cerana* varies between 10-20kg/colony/year, which is much less than that of *Apis mellifera* (Partap, 1997). Apiarists produce only honey and dispose their products on local market.

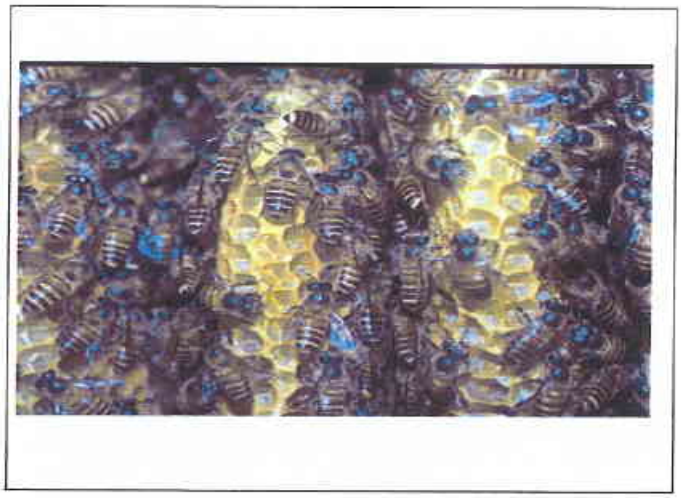


Figure 1b *Apis cerana*

The European bee *Apis mellifera* is similar to the Asian hive bee in its biology, nesting, foraging, colony defence and other behaviour features, with minor differences.

Apis dorsata (giant honeybee or rock bee) This bee has been known since ancient times. It is distributed all over the Hindu-Kush Himalaya region, in the plains as well in the hills up to a height of 2,222 metres above mean seas level (Verma, 1990). The rock bee builds a single comb nest in an open air or any terrestrial structure that offers protection from their predators and enemies. Mostly on branches of tall lofty trees such as *Ficus bengalensis* (banyan), *Ficus religosa* (Pipal), *Mangifera indica* (mango) and *Syzygium cumuni* (jamun); under a roof or rock cliff; under the water tank; bridges; in shaded places during summer; and in sunny places during

winter. As soon as the nectar supply in a particular locality depletes, they migrate to other places (Dhano, 1947;Verma, 1990;Verma, 1994; Partap, 1997). The size of a single open-air comb of rock bee, depending upon the season and stage of development of a colony measures 1.5 to 2 m from side to side and 0.6 to 1.2 m from top to bottom. The upper portions of the comb store honey and pollen and are generally 10 to 25 cm thick. Below this storage area is the brood nest (Singh, 1962). As many as 70 or more colonies may aggregate on a single tree (Joshi, 1999). Rockbee *(Apis dorsata)* is an important pollinator of several crops and is a good honey gatherer amongst different honeybee species. Its long proboscis, large flight range, large number of field workers, and its habit of collecting large quantities of pollen and nectar make it the best among the honey bees for crop pollination.

Figure 2a *Apis dorsata* **Figure 2b *Apis dorsata***

The honey is generally collected by tribals in forests, and house holders for their livelihood. Although bee products from rockbee have not been exploited to the fullest extent due to lack of proper harvesting techniques. Honeycombs are collected normally at night when bees are most docile. Since bees need daylight to navigate night harvesting results in bees losing their orientation, falling in the water and dying and the remaining bees scatter and unable to build new combs or produce any more honey. Bees are driven from their combs by smoke from torches with exposed, smouldering embers as a result many bees are burned and die. Honey is generally extracted from the combs by squeezing entire combs by hand as a result pollen is mixed with the honey, making it cloudy and less attractive. The use of crude and unhygienic methods of harvesting indiscriminately killing the bee populations and the community is losing additional benefits and income from bee products.

Apis florea F.(dwarf honeybee) is found in the plains and rarely live in places higher than 1500 m above sea level (Verma, 1990). This bee is considerably smaller than the true honey bees and

is called appropriately the dwarf or the little honey bee.This small bee also builds a single comb nests which are often suspended from the branches of bushes, hedges, trees, caves of buildinds, house chimneys, empty caves, and piles of dried sticks etc. The annual honey yeild from this species varies from one to three kg/colony and honey believed to has a medicinal value (Partap, 1997).

Figure 3a *Apis florea* **Figure 3b *Apis florea* honey combs**

There are three geographic types of *Apis florea*: one found in Sri Lanka and South India; one distributed in Iran, Oman and Pakistan and a third in Thailand (Ruttner, 1988).

Apis florea plays an important role in the pollination and can be utilized also for pollination of several agricultural and horticultural crops (Sidhu and Singh, 1961).

The dwarf bee is an important pollinator of crops in hot and dry agricultural plains. Collection of honey from the dwarf bee, *Apis florea*, had been done only for immediate consumption or for use in medicine. Honey of *A. florea* as rare and esteemed for its reputed medicinal properties (Muttoo,1956).

Population of rockbees is quite large in several forests having good bee forage. Rockbee colonies gather large quantities of pollen and nectar, and in production of honey, they may be equal to, if not better than, the hive bees. The dwarf bee works thoroughly on small patches of vegetation to gather pollen and nectar. Unfortuntely the dwarf bee population is declining from the country.

Apis laboriosa (the himalayan cliff bee): is the world's largest honeybee measuring up to 3 cm long and distributed in Nepal, Bhutan, India and China. It may also inhibit other parts of the Himalayas (Ahmed et al., 2003ab). It is found at altitudes ranging from 1200 to 3500masl. It build brood nests under overhangs on vertical cliffs at altitude between 2,500 and 3,200 and forages upto 4,100m (Underwood, 1992; Sakagami et al., 1980; Valli and Summers, 1988ab).

Like *Apis dorsata*, 70 or more colonies can be found at a single cliff site. A single comb of *A. dorsata* and *A. laboriosa* can provide up to 20 kg honey in one harvest (Joshi, 1999).

Figure 4a ***Apis laboriosa***

Figure 4b ***Apis laboriosa***

Apis dorsata/laboriosa and *Apis florea*, are eratic honey yielders and have provided honey to the people living near the forest areas (Verma, 1990).

Apart from *Apis cerana, A. dorsata, A. laboriosa* and *A. florea*, Nepal has two stingless bees of the genera *Melipona* and *Trigona*.These are quite small in size and are distributed in tropics and sub-tropics, and even in temperate regions.They build their nests in dark enclosures like cavities in branches or trunks of trees, ant hills, termite tunnels in the ground, wall crevices or any abandoned receptacle like logs, pots and tins.

Melipona spp. are distributed throughout the warmer areas of the region , below 1,000masl. Two species are known to occur in the terai areas of Nepal. In the Hindu Kush himalayan region, only a few farmers in the Dang, Rolpa, Surkhet, Dadeldhura, Doti and Dhading districts of Nepal are keeping *Melipona* in hollow logs.This species makes its nest within cavities and stores honey in special honey pots kept seperately from the brood cells. Honey yield is very low, often 1 to 2 kg per colony per year (Partap, 1997; Joshi, 1999). It is an important and efficient pollinator of crops, but ist uses and management as a crop pollinator are largely unexplored (Crane, 1990, 1992).

The nests of *Trigona*, unlike those of *Apis*, are clusters of small uniform globular cells of wax. These pots are the cells in which the young are reared. The pots are closely stacked touching each other or separated, each cell or cluster of cells being connected with others by girders or pillars of wax. Pollen and honey are stored in conspicuously large oval cells that are constructed close to the brood cell clusters or at their periphery quite apart from them. No clear separation of

honey pots is found. Because of the honey collected from this bee is rich in pollen that gets into it from pollen pots interspersed among the honey pots.

Unlike the true honey bees, these bees do not have stinging as the defence mechanism, but developed an equally effective biting behaviour, in defending their nest. When disturbed the bees attack the enemy in large numbers, usually selecting sensitive organs like eyes, nose andears as their target. Biting with the mandibles is quite irritating for several of the enemies, but can be easily protected against by man.

Despite the variety, all the Apis species have a remarkably similar life cycle. The biology of the stingless bees is also almost the same as that of the Apis. All these bees have a well developed social organization and have similar caste differentiation, division of labour, foraging, defence and reproductive behaviours.

Honey from the wild bee species is consumed locally as medicine in Ayurvedic treatments.

In recent years several factors contributed to a reduced production of honey from wild honey bees. Some of these are : large-scale deforestation, environmental degradation, uses of insecticides and consequent loss of food sources to honey bees, destruction of their nesting sites, destruction of bee colonies by improper methods of honey collection, reduction in the numbers of experienced honey collectors, leaving of this profession by traditional honey collectors for more remunerative occupations, lack of marketing facilities for the produce and exploitation of the situation by petty traders or contractors.

Pesticide formulation may offer significant advantages to agriculture and may constitute an approach to pest control that is in some ways more sound environmentally. Insecticides are essential for the control of many insect pests, but their use is often criticized adversely because of the harm it may cause to beneficial insects.

Insecticide poisoning of the immature stages of the honeybee is important to beekeepers and agriculturalists alike because replenishment of the adult population is involved.

Bee poisoning has increased in importance,with greater use of insecticides and other chemical materials on a wider range of crops. Exposure of honeybee brood to an insecticide would appear, in most cases, to be mediated by adult workers. Entry of an insecticide into adult may occur by respiratory, dermal or oral routes. For example, methyl parathion normally reduces bee visitation substantially (Sonnet, 1978). Most bee poisoning occurs when insecticides are applied to crops during the blooming period. Foragers may encounter insecticide contaminated food sources (pollen, nectar and water), alight on plant or other surfacesbearing residues, or intercept spray droplets directly (Davis,1989). Reductions in overwintering ability (Winterlin et al., 1973), honey production and pollination activity (Atkins and Kellum, 1986; Johansen,1979)

have been observed. It may lead to adverse impact on insect pollination of crops.The intensive use of insecticides is reducing the populations of honey bees.

2.1.1 Beekeeping in Nepal : Current and Emerging Scenarios

The beekeeping practice harks back to the very oldest civilization in indian sub-continent. Honey became an essential part of the life and culture of the Hindus since Vaidic period and still is a traditional household activity, integral to the cultural practices of the mountain people (Partap et al., 1997, Thapa et al., 2000). Beekeeping is traditional economic activity of Nepal and has been rooted deeply in immoral past. For the past decades it was progressing steadfastly and now gained much interest among the people.

A note-worthy feature of apiculture in Nepal through the second half of this century had been the absence of any bee disease anywhere in the country. In Nepal any serious bee disease and pest were not found until 1980, when the first outbreak of the sac brood disease caused by the Thai Sac Brood Virus (TSBV) occured along the eastern boarder areas (Kafle, 1992; Bhandari, 2001). European Foulbrood (EFB) is also causing a threat to the bees in Nepal (Saubolle and Bachmann, 1979; BDS, 1998; Thapa et al., 2000; Naomi, 2000).

Thai Sac Brood Virus was common widespread in *Apis cerana* bees whereas European Foul Brood (EFB), Nosema and Dysentery were observed in *Apis mellifera* colonies (Bhandari, 2001). The EFB disease has badly affected *Apis cerana* colonies in the Kathmandu valley (Verma, 1990).Within Nepal, the disease was prevalent in *Apis mellifera* colonies in 1994 in Jumla, the remote area of Nepal (Pechhaker , personal comunication). More than 80% of the *Apis cerana* colonies in Jumla district were collapsed due to the outbreak of the disease Thai Sac Brood Virus after introducing *A. mellifera* in Nepal (Joshi, 1999). In adition, apiculture in Nepal received a serious setback due to the appearance of the acarine and European foulbrood diseases in the indigenous bees. The wild colonies of *A. dorsata* and *A. Laboriosa* were also found to be infested with the European Foul Brood and American Foul Brood diseases, which were (also) transfered from the *A. mellifera* colonies. More than 50 per cent broods were found to be infested with European Foul Brood of one *Apis laboriosa* colony in Barabise (Pechhacker, personal comunication). *Melisococcus pluton* was detected from *A. laboriosa* colonies in Nepal (Allen et al., 1990).Today these diseases are decimating many appearies in Nepal (Manandhar, 1998).

The appearance of these diseases was attributed to the importations of the exotic bees from Europe. Infestation due to Thai sacbrood virus and *Acarapis woodi* triggered in *A. cerana* whereas *A. mellifera* colonies were found to be susceptible to *Varroa jacobsoni* and

Tropilaelaps clareae, the parasites of *A. cerana* and *A. dorsata* respectively. Although *Varroa jacobsoni* is long associated with *Apis cerana* bees but cause no serious damage as it has done in *Apis mellifera* every where in the world (Ahmad, 1984; Kafle, 1992; Bhandari, 2001). *Tropilaelaps clareae* is a serious pest of *Apis mellifera* in tropics, but not dangerous for apiculture in temperate zones (Woyke, 1985) thus becoming a problem to *Apis mellifera* colonies in Nepal. *Neocypholaelaps indica* is also occur in Nepal (Kafle, 1992; Bhandari, 2001).

Apis cerana is less susceptible than *A. mellilfera* to nosema disease not seriously affected by Varroa and is less prone to the attach of predatory wasps.

Disesease appear to constitute a major problem to beekeeping in Nepal, the simultaneously is true of pests and predators. Himalayan yellow throat martin (*Martes flavigula*), stone beech marten (*M. fonia*), rhesus monkey *(Macaca mulatta*), himalayan black bear (*Selenarctos thibetanus*) and, sloth bear (*Ursus ursinus*) are the pests of *Apis cerana* and *Apis mellifera* bees in mid and high hill areas (Thapa et al., 2000). Bee eaters (*Merops orientalis, M. leschanaulti, M. philoppinus*), Drongo *(Dicrurus acneus, D. caerulescens, D. annectas*), honey guide (*Indicator xanthonones*), Sparrows (*Passer montanus, P. domesticus*) and Magpie robin (*Copsychus saularis*) are serious predators of apiaries and feral colonies nesting. Among the insects active are wasps, *Vespa mandarina magnifica, V. affinis* and *V.auraria* and the wax moths, *Galleria mellonella* and *Achroea grisella* and sometimes *Bradymerus spp.* are locally common and also noticed to damage uncovered combs (Kafle, 1992; Thapa et al., 2000). The death head moth, *Achrontia spp.* known for desertion of the traditional hives, but other insects phorid fly, *Megaselia spp.*, different ants like *Camponotus spp.*, *Dorylus spp.*, and *Oecophyllus spp.* are serious enemies (mainly in the traditional hives) (Kafle, 1992). Pseud scorpion, Chelifer spp. is commonly seen clinging to the legs of the workers but their role in bee colonies is not clearly known (Kafle, 1992). In Nepal pests met with in and around hives in various parts of the country range through spiders, lizards, snakes and, rodents.They are legion. No data are available as to what numbers of castes of bees are eaten.

Bee diseases and pests are probably the chief impediment to Nepali beekeeping. Individual colonies which have dwindled, and some that have died out. Such an event would undoubtedly have become incorporated in Nepali beekeeping tradition.

A serious study of the pests and predators of honeybees in Nepal is long overdue.

Beekeeping with the Asian hive bee *Apis cerana* is centuries old tradition and is a part of cultural and natural heritage of the mountain communities of Nepal (Ahmad, 2003). Both *Apis*

mellifera and *A. cerana* are excellent pollinators of mountain crops (Verma and Partap, 1993; Partap and Verma, 1992, 1994; Partap and Partap, 1997, 2001).

Apis mellifera performs better when daytime temperatures are relatively high, and temperature fluctuations are low, poor tolerance of cold and requires migration to lower altitudes in winter, whereas *Apis cerana* is cold resistance and can perform well even when daytime temperatures are relatively low, on cloudy days, and at higher altitudes with marked temperature fluctuations. It thrives on mixed, diverse crops. *Apis mellifera* has larger population of bees per colony, than *A. cerana*, and needs large amounts of pollen and larger foraging grounds with monoculture-based agriculture for its survival and growth. In addition, it requires intensive management practices with standardised equipment, external inputs, and is less suitable and often unprofitable for small-scale and stationary beekeeping (Crane, 1992). To control diseases, parasites and predators, beekeeping with *A. mellifera* requires chemical treatment of colonies. In mountain areas *Apis cerana* is a better pollinator of vegetable and fruit crops during spring and early winter than *Apis mellifera*. It is more suitable for cross-pollinating entomophilous crops grown and adjusts well with the mountain characteristics, which include inaccessibility, fragility, marginality, diversity, niche and adaptation mechanisms (Ahmad, 2003). The strength of *A. cerana* over *A. mellifera* include low investment and management costs, efficiency in pollinating mountain crops and flora, resistance to the harsh mountain environments, pests, diseases and predators. Simple log hives can be used and does not need feeding, fumigating, migration in winter and is suitable for small scale and stationary beekeeping with minimum labour for maintenance.These bees are considered as mild and are easy to handle. Its sting releases half the amount of alarm pheromone as does the sting of the European bee (Morse et al., 1967). However, the mellifera beekeeping is adequately increased in Nepal in spite of the wake of the large-scale infestations of diseases, parasitic mites, and wasps into it. *Apis certana* populations are declining through out the HKH region. This decline is mainly attributed to the managed invasive arrangements, that is aggressive introduction and promotion of exotic *Apis mellifera* (Ahmad, 2003). When the *Apis cerana* population is destroyed a native and well adapted pollinator from both native and agricultural plants will be lost (Pechhacker and Juntawong, 1994). Despite the considerable advantages of *Apis cerana*, it is not to be popular among beekeepers (particularly commercial beekeepers), because of its lower honey yield (Partap and Partap, 2002).The higher honey yield of some races of *Apis mellifera* is only because of the long period of domestication and intensive selection and breeding (Pechhacker et al. 2001).The variety of geographical races/populations of *A. cerana* that exists in south and southeast Asia provides excellent opportunities for the genetic improvement of this native

species through selective breeding.Through genetic engineering techniques it may be possible to introduce desirable genes from *A. cerana* into *A. mellifera*. *Apis cerana* is sympatric in distribution and can co-exist with the two other species of Asiatic honey bees, *A. dorsata* and *A. florea*, without any adverse ecological consequences. It has shorter flight range and longer foraging hours than the European honey bee (Verma, 1989).
Since *Apis cerana* beekeeping found possible and economically viable, there is a growing demand for the European species and Ist adaptation is creating numerous problems and possibilities in Nepal. European honeybee, *Apis mellifera*, requires a higher capital investment and higher technological operation if it is to be effective (Crane, 1992). Beekeeping with *Apis mellifera* can only be more economical than *Apis cerana* when practised on a large scale (Bradbear and Roy, 1998). Therefore, all possible efforts should be made to improve the economic value of the native bee, *Apis cerana* rather than the importation of *Apis mellifera* (Pechhacker and Juntawong, 1994).

Nepal is one of the few countries in the world where substantial expansion of beekeeping is still possible, due to the diverse climate and fertile land with multi-season plants and/or crops.
In addition, it has considerable economic, social and ritual significance but still only partially exploited by beekeepers. Beekeeping is an integral component of mountain farming system and one of the economic activities which plays an active part in increasing the income of the people's community. It has very high potentials because of abundance of natural resources, easy integration in crop production, low technology requirements and indegenous knowledge & skills and a rich variety of bees. It occupies almost no agricultural land, does not involve much of human labour, and reqires little investment. It is the honey bees that do the real work of collecting pollen and nectar from flowers, the raw material, convert the latter into honey, and other materials beeswax and royal jelly constitute the products utilize both these for colony growth and reproduction.The way in which bees get nectar and pollen from different plants can be highly relevent to their pollinating effectiveness, and thus of economic importance. Beekeeping could well be a factor in improving the standard of nutrition of the population of developing countries like Nepal. Bee products such as honey, beewax, pollen, royal jelly, and propolis benefit people. Honey is a carbohydrate food, and pollen, a source of protein, could become available as a surplus hive crop. In addition, daily consumption of honey solution also found to have effect on hematological indices and blood levels of minerals and enzymes in normal individuals (Al-Waili, 2003).Therefore like sericulture, apiculture is a form of agriculture that concerns with the efficient use of natural resources to produce natural material useful to man.

For rural development against poverty it is proved as a good profitable venture by means of low cost/high yield enterprise for rural people and provide income with health food without the need for compulsory land ownership or much capital investment.
Given this fact, it is logical and rational that agriculture coupled with beekeeping are the thrust of the economic recovery to acheive economic prosperity of the people from crushing poverty.

2.2 Sources of Bee Forage

There are several species of bee flora found in Nepal. Various cultivated as well as wild floral elements are known to provide a mixed type of bee forage (Kafle, 1979, 1984; Partap, 1997) in Nepal. These bee flora have been extensively surveyed. The vegetations have been closely observed in various places, from the point of view of beekeeping (Kafle, 1984, 1992; Maskey, 1989, 1992; Partap and Verma, 1996; ICIMOD, 1996; Partap, 1997; Joshi, 1999). These surveys have found that mixed types of bee forage occur in different ecological zones of the country (Pratap, 1997). Thus, the beekeeping potential indicated for the cultivated vegetation becomes a part of the potential estimated for the natural vegetation (Suryanarayana, 2002).

2.2.1 Nepali Honey Types

Nepal has a variations in altitude, topography, mountain slopes and climatic factors from tropical to alpine, enabling a variety of plant communities to be grown.These geographical features play a dominant role in the determining the topographical, climatic and plant resources of Nepal and hence of varying degrees of success in beekeeping.
Various cultivated as well as wild floral elements are known to provide a mixed type of bee forage (Kafle, 1979, 1984) in Nepal. Except in the plains of southern terai and inner terai the plant communities in the hills and mountains change markedly within the foraging distances of the honeybees. Except from mustard and some selective cash crops such as cotton, jute, sugar cane and tobacco in the terai and innerterai, there does not exist any extensive monoculture of any crops in Nepal. Also, among wild vegetations the populations of bee forage elements are sparse and mixed well within the foraging distances of the bees.Under such situations of sparse bee forage elements, it is not possible to find pure unifloral honeys.
Bees also make honey from the sweet excretions of the insects (such as aphids). This is called honenydew honey.

The classification of honey on the basis of plant sources is not possible (Kafle, 1992). However, Nepali honey may be classified on the basis of bee species, seasonal and geographical variations.

Therefore, the honey of Nepal will be available in the following types :

- dorsata, florea, cerana and laboriosa honey.
- Spring, summer, autumn and winter honey.
- Terai, hill and mountain honey.

(Kafle, 1992)

2.3 Honey Hunting

Honey collection or honey hunting from wild nests is a very ancient art. Man and his association with honey bees had been as a hunter for thousands of years. The facets of rock portraits do give us a bulk of information on the state of honey collecting techniques known to the prehistoric man in that early stage of cultural development (Crane, 1967).An artistic impression, creative impulse and inspiration instigated by religious, cultural, social consiousness and experiences of honey hunting might have motivated the prehistoric men to create an eternal art on shelter walls and rocks. It may be austerely splendid, the ancient people typified imperial ambitions and rocks sheened with mythical aura. The artistic fascination must have forced on most people for ancient-style grandeur and seems to scattered across the world.

The bees' products were of great importance for ancient society. Medicinal use of honey, beewax and propolis was widespread.There were, therefore, good practical reasons for the interest shown by the ancients in the honeybee.

The palaeolithic paintings in rock shelters depict the rockbee honey hunting. A bulk of the Rigvaidic literature on honey and honey bees concerns with the rockbees. The Vaidic Aryans settling in the densely forested Himalayan Valleys, literally flowing with milk and honey, not only knew the social life and the life-history of honey bees, but also practised a fairly developed form of bee culture. Some of these techniques are still alive even today in entire Hindu Kush-Himalaya region (Verma, 1990) and strikingly parallels are found in examples of ancient rock art (Strickland, 1982).

Honeyhunting existed in Nepal from very ancient times. Although *Apis laboriosa* is found in Nepal, Bhutan, India, and China, traditional honey hunting activities have so far only been recorded in Nepal and informally in India; in Bhutan people only collect the wax , from nests abandoned for the winter, not the honey (Ahmad and Roy, 2000). Honey hunting from the cliffs

of *A. laboriosa* is an ancient art and still the most important part of apicultural activity in Nepal (Ahmad et al., 2003ab).

Honey hunting of the *Apis dorsata* and *A. laboriosa* is practised by Gurungs, Magars other members of the community as well (Ahmad et al., 2003ab; Strickland, 1982; Oppitz,1991). In Kaski district, honey hunting is predominantly the preserve of Gurung villages (Ahmad et al., 2003). Rajis are highly skilled traditional *Apis laboriosa* honey hunters; their technique has evolved over a long period (Ahmad et al., 2003ab). Their expertise in honey hunting being passed down from generation to generation within the family. The methods of honey hunting in the wild, cultural, religious and socio-economic factors involved in this practice have been described and reviewed (Strickland,1982; Valli and Summers, 1988ab) interestingly .

Methods of honey hunting in the wild along with socio-economic, cultural and religious aspects involved in this practice have been reviewed and described (Strickland, 1982;Valli and Summers, 1988a) interestingly. The medicinal values of honey was always appreciated. The inhabitants still enjoy honey from wild bees.

Nowadays, the social and cultural value of honey hunting is declining (Ahmad et al., 2003b). Valli and Summers (1988b) say:`this autumn, 63-year-old Manilal will hunt the honey for the last time, and this ageless quest will end, for he has no successor`.

2.4 Looking to the Future

With the favourable ecological conditions, plant biodiversity, an indigenous bees which is comparatively easily managable, make one very optimistic for the future of beekeeping in Nepal.The promotion of information and technology transfer, and promotion of beekeeping hold promise for meeting the present and future requirements of poverty alleviation and environmental protection. In a quest to stump out poverty in rural areas and create opportunity for employment beekeeping may be the occupation that answers the quest remained against poverty. Beekeeping has great importance in the agriculture based economy of Nepal by way mainly of honey production and pollination. It provide free ecosystem services in the form of cross pollination and propagation of several cultivated and wildplant species, thereby maintaining biological diversity (Verma, 1990).

In addition, there is need for a great deal of apicultural research and establishment of genetic resource centres may open new horizon for Nepali beekeeping. The economic and transport infrastructure, trained manpower and monetary resources, marketing and the expansion of products are the essential factors for the improvement of beekeeping industry in the country.

Gender equality is of importance for a country's social and economic development. The implementation of the policies targeting the women & the poor may support poverty alleviation.

Lastly, the environment in which the bees live presents many hazards. During recent years of composition of natural vegetation at all the agroclimatic belts has been extensively modified by man due to cutting trees for firewood, by lopping fodder to feed livestock and cleaning out forests for intensive farming (deforestation).The land left uncultivated around the rural settlements is also heavily grazed and no surface vegetation has reached the flowering stage (Kafle, 1992). On the other hand, the medicinal property of honey has always been appreciated and the rural communities or tribes still harnessing the potential of local honeys (from different bee species) as a traditional remedy, is discussed in the next chapter.

CHAPTER THREE

3.1 Honey as a Traditional Medicine: Fact or Fiction ?

Razors pain you;
rivers are damp;
acids stain you;
and drugs cause cramp.

(Dorothy Parker, *Not so deep as a well*, 1937)

A profound awareness, rooted in the ancient civilizations of the remote pasts, in the dawn of human cultural evolution, the art of curing was essentially magical and the benefits of local animal, plant and mineral resources in concoctions applied to wounds must have been learnt by trial and error, and accumulate wisdom disseminated by word of mouth (Cooper, 2001a). Man and his association with honey bees had been for thousands of years. Bee keeping can be traced back to about thousands of years. It's been over 6000 years, now, since people started keepingbees for honey.The medical use of honey in wound treatment is derived from diverse ancient civilizations (Jones, 2001).

All through the human history, there has been a conspicious concern for health care and the cure of the disease, though the concepts themselves took a very long time to develop into a body of knowledge. The bees' products were of great importance for ancient society. Medicinal use of honey, beewax and propolis was widespread. The art of curing by honey arose with the cultural evolution. Every human community was conscious of the burden of disease and developed its own medical system (Dunn, 1976).

The medicinal use of honey originated with ancient civilizations as a remedy for respiratory tract infections, wound infections, gastrointestinal disorders and eye problems (Forrest,1982; Cooper et al., 2000). There were, therefore, good practical reasons for the interest shown by the ancients in the honeybee.

In addition, honey is a world's oldest nourishing foods and has constituted much of the food one of the, enjoyed in every corner of human civilization for thousands of years. Some of the oldest pre-literate archaelogical records concern the medicinal use of honeys. Stone Age rock paintings in several different locations show scenes of honey hunting, and these have been dated to 6000

BC or earlier.Thus giving between eight and 10 thousand years of evidence that the human race has recognized honey as a preciuos product (Jones, 2001).

The therapeutic action of honey was mentioned in various studies in traditional (Molan, 1992) and modern medicine (Vardi, et al.1998) as being effective in the healing of various infected wounds. Its antibacterial activity is well established (Molan 1992; Cooper et al., 1999; Cooper and Molan, 1999).

Honey has been widely used in the orient as a nutritious food and for medical and pharmacological purposes (since ancient times), particularly in Buddhist medicine, in Chinese pharmacology, and Chinese medicine. In Japan, the use of honeys was adopted from around the Nara period (mid 8th century) (Watanabe, 1986). In the most ancient scripts we already find references to honey as a glorified food, an ingredient of favored drinks, a popular medice and the principal component of liniments and plasters. The congruences in the Bible, the Talmud, the Kuran, the Ved, and the sacred books of China, Persia and Egypt, all speak of honey in laudatory terms, as a food, beverage and medicine (Lau ,1976; Ali,1989; Jones, 2001).

The holy Koran as well as the Hadith, clearly refers to the efficacy of honey as a healer of diseases. It has been reported that about 181 substances are present in honey. About 2000 years ago, honey was listed in the first medical handbook for use in burns, cuts, abscesses and boils (Lau , 1976 cited in Abu T M M 1989).

Similar are some of the ancient Egyptians in 2000 BC (Gelbart, 1999), in the middle ages, a document from 1392 details would care practices including the use of honey (Naylor, 1999). The Assyrians, Greeks and Romans all used honey, in combination with other herbs and on its own, to treat wounds and diseases of the gut (Zumla and Lulat, 1989). Some systems of medicine, like the Chinese medicine, Ayurved, Tibetan medicine, Unani etc. have been well recorded and developed extensively literature over a couple of millenia (Goldwater, 1983). Descriptions of several disease conditions in the earliest works on honey are also found in the Vaidic hymns of the predecessors of Ayurved. It is in the Ayurved, a part of Atharva ved, that honey found a place of honour, as a valuable nature's gift to man. Ayurved means "the science of life" in hindu system of medicine and comes from the ancient Vaidic tradition of hindus, where it has been practiced for more than 5,000 years, based on solid foundations that are fully sustained by long experimentation and philosophical propositions. Ayurved encompasses all aspects of life and disease, such as anatomy, physiology, pathology, symptomatology and medical prescription to cure a disease. Ayurved was largely responsible for the origin and development of a well document major trend in medicine. The traditional practices have still very much alive with the written records. Concomitant with the progress in medicine are far

reaching successful developments in modern medicine, which also has an important place in Ayurved.Honey had been variously praised as a nutritious food and valuable medicine in Rigved and several later works. The acknowledged authorities on medicine in those days, Susrut and Charak (believed to have lived during the 15th and 13th centuries B.C.) Samhitas, the famous treatises of hindus, written in Sanskrit had described the medicinal uses of honey. Susrut gave the specific medicinal properties of individual honeys and emphasised the aspect of application of honey in surgery, particularly its curative properties on wounds, etc. A century or two later, Charak described in detail the properties and medicinal uses of honey, depending upon its source, method of collection, storage, etc. He recognised four types of honey. The Samhitas recorded four species of honey bees, as also their colour, nest building and characteristics of the honey produced by them (Joshi and Godbole,1970; Joshi et al., 1983).

Current day usage of honey in folk medicine are: as a traditional therapy , leg ulcers in Ghana (Ankara-Badu, 1992) earache in Nigeria (OBI, et al., 1994) and topical treatment of measles in Mali (Imperato and Traore, 1969). *Trigona* bee honey is highly prized in Ethiopia for medicinal use as a panacea for many ills such as cough, stomach disturbance, sore throat, tosillitis, stomach and intestinal ulcers, cold, disease of the mouth and mucus membrane, and wound dressing (Garedew et al., 2003). *Apis dorsata* honey used widely in Thailand to stop bleeding, prevent scaring, improve skin complexion (Wongsiri et al., 2000) and for good health in Indonesia (Lahjie and Seibert, 1990).

Reports of investigations of their clinical efficacy have been conclusively demonstrated and is now acknowledged worldwide. The antibacterial activity of honey appears to have reported first by von Ketel in 1892 (Cited in Molan, 1992a). The next report was by Sackett in 1919. More intensive study did not commence until the work of Dold et al., in 1937.

Investigations into the microbial flora of wounds began in the late 19th century. Since then improvements in techniques have facilitated the recovery, identification and enumeration of a wide variety of microbial species (Cooper, 2002 ab). The presence in honey of various amounts of inhibine as described by Dold et al., (1937) has been reported by several investigators. The rediscovery of honey in this millenium and its registration as a medical device will turn a 5000 – year-old remedy into today's unrivalled wound dressing (Postmes, 2002). Honey has proved useful in the treatment of burns, wounds, gastroenteritis, stomach and skin ulcers (McCarthy, 1995), respiratory tract infections, wound infections, gastrointestinal disorders and eye problems (Cooper et al., 2000) because of its antibacterial properties.

In the following few centuries, more detailed observations on bee behaviour, on plants that provided food to the bees and more particularly, on the characteristics of honeys produced by

different honey bees as also their medicinal properties were made. The use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of ulcers and bed sores, and other surface infections resulting from burns and wounds. Ist effectiveness in rapidly clearing up infection and promoting healing is not surprising in light of the large number of research findings in its antibacterial activity (Molan, 1992ab). Its efficacy in wound healing remains largely anecdotal, with claims that it reduces inflammation, debrides necrotic tissue, reduces oedema and promotes angiogenesis, granulation and epithelialization (Molan, 1998).The effect of honey on wound healing may in part be related to the stimulation of inflammatory cytokines from monocytic cells. Such cell types are known to play an important role in healing and tissue repair (Tonks et al., 2003).

Table1. Showing types of wound treated successfully with honey.

Type of wounds treated sucessfully with honey	**Refrences**	**Type of wounds treated sucessfully with honey**	**Type of wounds treated sucessfully with honey**
Abrasions	Blomfield, 1973; Zaiß, 1934	A fistula	Lücke, 1935
Amputations	Lücke, 1935; Zaiß, 1934	Foot ulcers in lepers	Tovey, 1991;
Abscesses	Farouk et al., 1988; Wadi et al., 1987	Infected wounds arising from trauma	Green, 1988; Hamdy et al.,1989; Effem, 1988; Ndayisaba et al.,1993; Wood et al., 1997
Bed Sores (pressure sores, decubitus ulcers)	Somerfield, 1991; Blomfield, 1973; Hutton, 1966; Hamdy et al., 1989; Effem, 1988; Weheida et al., 1991.	Large septic wounds	Braniki, 1981
Burns	Blomfield, 1973; Zaiß,1934; Farouk et al.,1988; Effem, 1988; Adesunkanmi and Oyelami, 1994; Voigtländer, 1936	Leg ulcers	Yang, 1944; Wood et al.,1997; Harris, 1994.
Burst abdominal wounds following caesarean delivery	Phuapradit and Saropala, 1992	Malignant ulcers	Effem, 1988
Cancrum	Effem, 1988	Sickle cell ulcers	Effem, 1988;
Chilblains	Yang, 1944	Surgical wounds	Lücke, 1935; Hamdy et al.,1989; Ndayisaba et al.,1993; Mclnerney, 1990; Dumronglert,1983.
Cracked nipples	Seymour and West, 1951	Tropical ulcers	Effem, 1988;
Cuts	Blomfield,1973	Wounds to the abdominal wall and perineum	Mclnerney, 1990;
Diabetis foot ulcers and other diabetic ulcers	Tovey, 1991; Farouk et al.,1988; Hamdy et al.,1989; Effem, 1988; Wood et al., 1997	Varicose ulcers	Hamdy et al., 1989; Wood et al., 1997; Bulman, 1955; Bloomfield, 1976.
Cervical ulcers	Seymour and West, 1951		
Source: Molan (2001)			

Table 2. Some infections caused by some of the species of bacteria that have been found to be sensitive to the antibacterial activity of honey.

Pathogen	Infection caused
Bacillus anthracis	Anthrax
Corynebacterium diphtheriae	Diphtheria
Escherichia coli	Diarrhoea, septicaemia, urinary infections, wound infections
Haemophilus influenzae	Ear infections, meningitis, respiratory infections, sinusitis
Klebsiella pneumoniae	Pneumonia
Listeria monocytogenes	Meningitis
Mycobacterium tuberculosis	Tuberculosis
Pasteurella multocida	Infected animal bites
Proteus species	Septicaemia, urinary infections, wound infections
Pseudomonas aeruginosa	Urinary infections, wound infections
Salmonella species	Diarrhoea
Salmonella cholerae-suis	Septicaemia
Salmonella typhi	Typhoid
Salmonella typhimurium	Wound infections
Serratia marcescens	Septicaemia, wound infections
Shigella species	Dysentery
Staphylococcus aureus	Abscesses, boils, carbuncles, impedigo, wound infections
Streptococcus faecalis	Urinary infections
Streptococcus mutans	Dental caries
Streptococcus pneumoniae	Ear infections, meningitis, pneumonia, sinusitis
Streptococcus pyogenes	Ear infections, impedigo, puerperal fever, rheumatic fever, scarlet fever, sore throat, wound infections.
Vibrio cholerae	Cholera

Molan (1992a)

Several authors are of the opinion that the sugar content of honey is exclusively responsible for its antibacterial affect (Seymour and West, 1951; White et al.,1963; Keast-Butler, 1980; Mossel, 1980; Bose, 1982; Chirife et al., 1983; Green, 1988; Somerfield, 1991;Tovey, 1991; Condon, 1993). It has a natural antibacterial activity, which is lacking in sugar paste of similar osmolarity (Postmes et al., 1996). Compared with an artificial honey solution of the same thickness and sugar concentration, natural honey kills bacteria three times more effectively (Cooper, 2002b).

Limitations to Usage

The popular literature on health and self-treatment of ailments gives the impression that honey can be taken to cure almost anything, but a rational consideration would suggest that the antimicrobial activity would be insignificant when an oral dose of honey becomes diluted after absorption from the gut into the many litres of fluid in the circulation and tissues of the body. Realistically, the potential agent, although there are some situations such as gastrointestinal infections or mastitis where the honey could remain localised and thus not become too dilute to be effectively antibacterial.

3.2 *Staphylococcus aureus*: In Brief

Taxonomy

DNA-ribosomal RNA (rRNA) hybridization and comparative oligonucleotide analysis of 16S rRNA has demonstrated that staphylococci form a coherent group at the genus level. This group occurs within the broad Bacillus-Lactobacillus-Streptococcus cluster defining Gram-positive bacteria with a low G + C content of DNA.

At least 30 species of staphylococci have been recognized by biochemical analysis and in particular by DNA hybridization. Eleven of these can be isolated from humans as commensals *S. aureus* (nares) and S epidermidis (nares, skin) are common commensals and also have the greatest pathogenic potential. *S. saprophyticus* (skin, occasionally) is also a common cause of urinary tract infection. *S. haemolyticus, S. simulans, S. cohnii, S. warneri* and *S. lugdunensis* can also cause infections in man.

Structure

Staphylococci are Gram-positive cocci about 0.5 - 1.0 μm in diameter. They grow in clusters, pairs and occasionally in short chains. The clusters arise because staphylococci divide in two planes. The configuration of the cocci helps to distinguish micrococci and staphylococci from streptococci, which usually grow in chains. Observations must be made on cultures grown in broth, because streptococci grown on solid medium may appear as clumps. Several fields should be examined before deciding whether clumps or chains are present.

Epidemiology

Because *S aureus* is a major cause of nosocomial and severe community-acquired infections (Fakih and Saravolatz, 2003), it is necessary to determine the relatedness of isolates collected during the investigation of an outbreak. Typing systems must be reproducible, discriminatory, and easy to interpret and to use. The traditional method for typing *S. aureus* is phage-typing. This method is based on a phenotypic marker with poor reproducibility. Also, it does not type many isolates (20% in a recent survey at the Center for Disease Control and Prevention), and it requires maintenance of a large number of phage stocks and propagating strains and consequently can be performed only by specialist reference laboratories.Many molecular typing methods have been applied to the epidemiological analysis of S aureus, in particular, of methicillin-resistant strains (MRSA). Plasmid analysis has been used extensively with success,

but suffers the disadvantage that plasmids can easily be lost and acquired and are thus inherently unreliable. Methods designed to recognize restriction fragment length polymorphisms (RFLP) using a variety of gene probes, including rRNA genes (ribotyping), have had limited success in the epidemiology of MRSA. In this technique the choice of restriction enzyme used to cleave the genomic DNA, as well as the probes, is crucial. Random primer PCR offers potential for discriminating between strains but a suitable primer has yet to be identified for S aureus. The method currently regarded as the most reliable is pulsed field gel electrophoresis, where genomic DNA is cut with a restriction enzyme that generates large fragments of 50-700 kb.

Clinical Manifestations

S. aureus is capable of causing various infections of the skin and other organs. *S. aureus* infections are common in people with frequent skin injury, particularly if the skin is dry. Staph skin infections are seen most commonly in pre-pubertal children and certain occupational groups such as healthcare workers. *S aureus* is notorious for causing boils, furuncles, styes, impetigo and other superficial skin infections in humans.It may also cause more serious infections, particularly in persons debilitated by chronic illness, traumatic injury, burns or immuno suppression.These infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis and meningitis, and are often associated with hospitalized patients rather than healthy individuals in the community. *S. aureus* mediates bone destruction via induction of osteoblast apoptosis (Alexander et al., 2003)

S aureus and *S epidermidis* are common causes of infections associated with indwelling devices such as joint prostheses, cardiovascular devices and artificial heart valves. About 15-40 per cent of healthy humans are carriers of *S. aureus*, that is, they have the bacteria on their skin without any active infection or disease (colonisation).The site of carriage are usually the nostrils and fexures, and can be intermittent or persistent.

Antibiotic Resistance

Multiple antibiotic resistance is increasingly common in *S. aureus* and *S. epidermidis*. Staphylococci are becoming increasingly resistant to many commonly used antibiotics including penicillins,macrolides such as erythromycin, tetracyclines and aminoglycosides.Penicillin resistance in *S. aureus* is due to production of an enzyme called beta-lactamase or penicillinase. Methicillin and flucloxacillin are lactamase-resistant penicillins so are the antibiotics of choice in most staphylococcal skin infections. These antibiotics have a broad range of action against

several bacteria and are best reserved for patients with mixed bacterial infections. Patients who are allergic to penicillin are most reliably treated with vancomycin.

Unfortunately there is now increasing methicillin resistance (MRSA). Methicillin resistance is indicative of multiple resistance. MRSA is found worldwide, predominantly in hospitals and institutions such as nursing homes. Much less commonly, MRSA is found in the general community. There are three main reservoirs (and hence sources of spread and infection) for MRSA in hospital and institutions: staff, patients and inanimate objects such as beds, linen and utensils. By far the most important reservoir is patients who may be colonised with MRSA without evidence of infection. The usual sites of colonisation with MRSA are the nostrils, skin, groin, axilla,and wounds. Penicillins with a beta-lactamase-inhibitor such as 'amoxiclav" (amoxycillin with clavulonic acid) may be used to treat *S. aureus* infections and are sometimes effective against bacteria resistant to flucloxacillin.Increasing bacterial resistance to virtually all available antibiotics causes an urgent need for new antimicrobial drugs, drug targets and therapeutic concepts (Weidenmaier et al., 2003).

There is no mechanism of resistance to either of the types of antibacterial activity in different honey types (phytochemical and hydrogen peroxide) present in *S. aureus*, in which antibiotic resistance is wide spread.

3.3 Why Test Honey From Nepal

Different types of honey from different bee species and derived from different plants available in Nepal. On the other hand, Nepal is rich in knowledge about traditional medicine. For health care rural people depend on local plants and honey based therapy, which is cheap and easily available. Many people rely on plants collected from the wild localities for food, construction materials, fuelwood, medicine and many other purposes. Socio-economically, most of the village people are very poor and the poverty compels the rural people to practice traditional medical system. Natural plant extracts or products, being indigenous resources (Tamrakar and Gautam, 1999).

Rural people through out Nepal widely use honey from different bee species with different herbs to cure several diseases like cough, fever, cold, appetite and gastritis.Honey alone also used to cure eye diseases,cuts, and burns. Raw honey is fed women after delivery of a baby.Honey from *Apis florea* alone is used to cure eye disease and snake bites (Thapa, 2002) most others certainly were used in local medicine. Some people use laboriosa honey to make traditional medicines. Honey from these bees is exported to Korea for traditional medicinal purposes (Thapa, 2001).

The herbal resources are playing important role in health care system of rural people. Local people of different ethnic groups of rural area keep sufficient knowledge about the use of local herbal resources. In the remote areas of Nepal, where modern transport rarely penetrates even today, the beehive is looked upon as a self-replenishing medicine chest (Jones, 2001; Thapa, 2002). There are more traditional practices, of people who until recently have lacked literature but are effective in handling the health problems of the respective communities. These unwritten medical cultures play a key role in rural life. Only the unwritten indigenous knowledge of the tribals and such other small communities, that are not too seriously affected by the onslaught of modern civilisation, and which knowledge has been passed by the word of mouth, from generation to generation. As repeatedly emphasised in different context, the importance of bees and ist products in our lives, in addition to being a source of food, is their immense therapeutic potential. Every culture in the world developed its own practices of treating the diseases. By all counts, ancient cultures throughout the world, nurtured till recent times, vast amounts of knowledge pertaining to uses of their hive products. Traditional culture of disease treatment still occupies a very important place in the developing world. There has been a considerable revival of interest during the past few decades. On the other hand, Staphylococci are becoming increasingly resistant to many commonly used antibiotics such as penicillins, macrolides, erythromycin, tetracyclines and aminoglycosides. New antimicrobial drugs and medication are urgently needed.

Comparison with any similar research done only a few decades ago indicates what great progress is being made in evaluating, understanding and implementing the honey as a medicine, despite the complexity of the subject.

Honey does not adversely affect human tissue, unlike other topical antimicrobial agents. Not only has it the potential to limit the growth of wound pathogens, but there is evidence that honey has the potential to promote healing. No other antimicrobial agent possesses these characteristics. Considerable contributions have been made by several researchers in this regard.

The antibacterial potency of honey types specifically selected may vary widely its clinical efficacy. Test of the potential of honey may provide us with another tool in the management of *S. aureus* infections or other microbes. Sufficiently encouraging results may lead to warrant further investigation into the role of honey (from different bee species) in wound healing and ist antibacterial activity. Wider use of honey on all wounds and microorganisms will depend on evidence from clinical research. Honey is consumed locally as medicine and as food in Nepal.The therapeutic potential of uncontaminated, pure honey is grossly underutilised. Honey

that is for consumption and available on the market is not sterilised, irradiated or assessed for antibacterial potenty and are therefore not intended for application to wounds or recommended and practiced for clinical use in Nepal. These honeys are still being used as traditional medicine by local communities but have no proven laboratory or clinical evidence the antibacterial activity. Tribes using honey as a medicine, modern clinicians remain sceptical.

In Nepal different types of honey from different bee species and derived from different plants exist. The antibacterial activity of honey, consequently its clinical efficacy, has been shown to vary with the plant source (Molan, 1992ab) it is therefore , interesting to make further invesigations such as pollen analysis of each honey types and also identify plant derived antibacterial substances along with flavonoids. Further research is clearly needed in all of these areas. This work is also an attempt to warrant further investigation into the role of honey (from different bee species) in wound healing and its antibacterial activity. Many studies support the clinical safety and efficacy of generic and specific honeys. In addition, no toxic effects have been reported in the literature. There are significant experimental data to demonstrate the antibacterial properties and histological effects of honey on the healing process. The only contraindication is known allergy to honey. It is not only antimicrobial, it is non-toxic to human tissue, and is able to accelerate wound healing without the risk of selecting novel antibiotic-resistant species.

The aim of this work is to test *S. aureus* against honey from Nepal from different bee species. Honey in Nepal is produced from many bees and floral sources, and its antimicrobial activity and clinical efficacy varies markedly with its origin and processing. There is a possibility that honey from different bee species used traditionally in Nepal species is likely to be an effective treatment for a wound infected bacteria and for other treatments. There is also the possibility that one type of honey thus be more effective than others and when applied directly to a wound, honey with an average level of antibacterial activity may be expected to be effective in preventing the growth of *S. aureus* on the wound surface and possibly deeper into the wound. The inhibitory effect of the honey is maintained on the wound surface when the honey diluted more than 10-fold by wound exudate. However, further comparative clinical trials required to provide evidence for selection of honey type or to find which type of honey gives the best therapeutic results, and what minimum level of antibacterial activity is necessary in the honey for it to be effective as a topical antiseptic treatment for infected wounds.

3.4 Literature Review

Comparison of the text with any similar work done only a few decades ago indicates what great progress is being made in studying, understanding and using honey for antimicrobial activity and wound healing, despite the complexity. Considerable contributions have been made by several workers. Advances has been fostered by the importance of problems associated with social, legal, medical and ethical issues, for instance.

The literature on apitherapy is very extensive.There are reports of the medical profession turning back to honey for treatment of ulcers, burns and surgical wounds. Recently honey was re-discovered as a topical therapy for wound infections, gastroenteritis, stomach and skin ulcers (Zumla and Lulat 1989; McCarthy, 1995). Many species of bacteria have been recovered from wounds, but *Staphylococcus aureus* is the most frequently isolated wound (Meers et al.,1981 cited in Cooper and Molan, 1999).

Staphylococcus aureus has long been recognized as an important pathogen in human disease. Staphylococci (staph) a common type of bacteria, in the genus *Staphylococcus*, are pathogens of man and other mammals. Classified as Gram-positive cocci that live on the skin and mucous membranes (eg. in nose) of humans. They may occur singly or grouped in pairs, short chains or grape-like clusters. They are usually facultative anaerobes, that is, they are capable of surviving at various levels of oxygenation, and are generally very hardy organisms. They are only able to invade via broken skin or mucous membranes, hence intact skin is an excellent human defence. Due to an increasing number of infections caused by methicillin-resistant *S. aureus* (MRSA) strains, therapy has become problematic.Therefore, prevention of staphylococcal infections has become more important. Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection. The ecological niches of *S. aureus* are the anterior nares. In healthy subjects, over time, three patterns of carriage can be distinguished: about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never *carry S. aureus*. The molecular basis of the carrier state remains to be elucidated. In patients who repeatedly puncture the skin (e.g., hemodialysis or continuous ambulatory peritoneal dialysis [CAPD] patients and intravenous drug addicts) and patients with human immunodeficiency virus (HIV) infection, increased carriage rates are found. Carriage has been identified as an important risk factor for infection in patients undergoing surgery, those on haemodialysis or CAPD, those with HIV infection and AIDS, those with intravascular devices, and those colonized with MRSA. Elimination of carriage has been found to reduce the infection rates in surgical patients and those on hemodialysis and CAPD. Elimination of carriage appears to be an

attractive preventive strategy in patients at risk. Further studies are needed to optimize this strategy and to define the groups at risk.

Despite the almost universal administration of antimicrobial agents with good anti staphylococcal activity for perioperative prophylaxis in patients undergoing clean surgery, *Staphylococcus aureus* remains the most common cause of surgical wound infection (Cooper et al., 2001). While methicillin-resistant *S. aureus* (MRSA) accounts for some wound infections, the majority are caused by methicillin-susceptible strains.

3.4.1 Definition and Explanation of Antibacterial Activity

The antibacterial activity of honey refers to the presence of inhibine which acts as an antibacterial factor (Molan, 1992a).The antibacterial activity of honey appears to have been reported first by van Ketel in 1892 (cited by Dustmann.1979).

Antibacterial activity of different honeys have been studied by many authors.

The variation of the antibacterial activity of different honey were attributed to the previously mentioned factors which influenced the antibacterial activity as osmotic properties of honey (Molan et al., 1988; Molan, 1992a, 1992b), honey pH or activity of glucose oxidase (Chirief et al., 1982), hydrogen peroxide (Roth et al., 1986; Dustman, 1987), non-peroxide substances (Effem, 1988; Bogdanov, 1984) and volatile antibacterial substances (Radwan et al., 1984).

It has been claimed, that honey contains lysozyme, a well known antibacterial agent (Mohrig and Messner, 1968). However, in an other study it was found that no lysozyme activity was present in honey (Bogdanov, 1984).

Osmotic effects

The main honey substances are sugars, which by their osmotic effect exert antibacterial action. Compared with an artificial honey solution of the same thickness and sugar concentration, natural honey kills bacteria three times more effectively (Science, Rose Cooper, Nature 19Nov., 2002).

Honey is a saturated or super saturated solution of sugars, 84% being a mixture of fructose and glucose. The water content is usually only 15-21% by weight.The strong interaction of these sugar molecules with water molecule leaves very few of the water molecules available for microorganisms. The 'free` water is what is measured as the water activity (a_w): mean values for honey have been reported from m0.562 to 0.62. Although some yeasts can live in honeys that have a high water content, causing spoilage of the honey, the aw of ripened honey is too low to support the growth of any species, no fermantation occuring if thre water content is

below 17.1%. Many species of bacteria have their growth completely inhibited if the aw is in the range 0.94-0.99. These values correspond to solutions of a typical honey (aw of 0.6 undiluted) of concentrations from 12% down to 2%(v/v). On the other hand, some species have their maximum rate of growth when the aw is 0.99, so inhibition by the osmosis (water – withdrawing) effect of dilute solutions of honey obviously depends on the species of bacteria (Molan, 1992a, 1992b).

Acidity

The inhibitory action of honey was shown by its discoverers to be affected by pH.. The investigation claimed, that the low honey pH was responsible for the antibacterial activity (Yatsunami and Echigo, 1984).

Honey is a characteristically quite acidic, its pH being between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens. The optimum pH for growth of these species normally falls between 7.2 and 7.4. The minimum pH values for growth of some common wound-infecting species is : *Escherichia coli*, 4.3; *Salmonella sp.*, 4.0; *Pseudomonas aeruginosa.*, 4.4; *Streptococcus pyogenes*, 4.5. Thus in undiluted honey the acidity is a significant antibacterial factor. But if honey is diluted, especiallly by body fluids which are well buffered , the pH will not be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria. No correlation between antibacterial activity and the pH of the honeys found (Bogdanov et al., Daghie et al., 1971; Plachy, 1944; Rachlik and Dolezal, 1961; Stomfay-Stitz and Kominos, 1960).

Hydrogen Peroxide

The inhibitory action was shown by several investigators to be affected by hydrogen peroxide. Several workers believe that differences in activity is due to hydrogen peroxide produced by honey glucose oxidase (Gauhe, 1941; White and Subers,1963, 1964ab; Dustmann,1972,1979; Burgett, 1973; Morse, 1986; Taormina et al., 2001; Schepartz and Subers, 1964), it could be due to varying levels of other antibacterial compounds coming from the nectar source (Vergè, 1951; Schuler and Vogel, 1956; Lavie and Grassè, 1963;Gonnet and Lavie, 1960; Mladenov, 1974). The presence of higher amount of hydrogen peroxide produced enzymatically by the action of glucose oxidase in honey is extremely effective in statu nascendi and responsible for antibiotic activity (Adcock, 1962; White et al., 1963; White and Subers, 1963; Dustmann, 1979; Wakhle and Desai, 1991; Molan, 1992ab). Aizzawa et al., (1974) studied a specific bio-electrochemical sensor for hydrogen peroxide. All of the glucose oxidase enzyme and the major part of the

proline and invertase in honey originated from the hypopharyngeal glands of the honey bees into the nectar to assist in the formation of honey from the nectar (Greich and Delage-Darchen, 1978; Dustmann,1967; Edelhauser and Bergner, 1984; Von der Ohe et al, 1991; Von der Ohe, 1994; Molan,1992ab).

The hydrogen peroxide and acidity produced by the reaction:

Glucose + H_2O + O_2 → Gluconic acid + H_2O_2

Glucose Oxidase **Glucose Oxidase + Glucose + H_2O ⟶ H_2O_2 + Gluconic acid** Catalase **$2H_2O_2$ ⟶ $2H_2O + O_2$**
Bogdanov and Blumer (2001d)

Gluconic acid was found to be the principal acid in honey. The acid-producing enzyme in honey is a glucose oxidase producing gluconic acid and hydrogen peroxide from glucose. The hydrogen peroxide produced in honey by the action of a glucose oxidase on glucose. The enzymatic oxidation of glucose takes place very slowly in undiluted hone and at much higher rates as honey is diluted. The addition of catalase remove the hydrogen peroxide and substantial antibacterial activity remain in many of the honey (see the reaction above).
The hydrogen peroxide produced would be of effect as a sterilising agent only during the ripening of honey. Full- strenght honey has a negligible level of hydrogen peroxide because this substance is short-lived in the presence of the transition metal ions and ascorbic acid in honey which catalyse its decomposition to oxygen and water. The enzyme has been found to be practically inactive in full-strenght honey, it giving rise to hydrogen peroxide only when the honey is diluted. This is because the acidity produced in the action of the enzyme drops the pH to a point. Thus it is important that when honey is to be used as an antimicrobial agent it is selected from honeys that have been assayed in the laboratory for antimicrobial activity.
The rates of hydrogen peroxide production by honey with respect to honey dilution were measured by Bang et al., (2003) in eight different samples of honey from six different floral sources. Concentrations of hydrogen peroxide generated were very low and cytotoxic damage by hydrogen peroxide was also found to be very low.

It is important that honey for use as an antimicrobial agent to be stored at low temperature and not exposed to light, so that none of the glucose oxidase activity is lost. Although all honey will stop the growth of bacteria because of its high sugar content, when the sugars are diluted by body fluids this antibacterial action is lost. The additional antibacterial components then become important.

When many honeys are diluted, a bee derived enzyme (glucose oxidase) present in the honey is activated and catalyses the slow generation of hydrogen peroxide which inhibits bacterial growth (White et al., 1963). The inhibition is very effective in statu nascendi of hydrogen peroxide. This activity varies markedly from honey to honey (Molan, 1992ab). In some honey, additional antimicrobial activity following dilution is due to activation of bee-derived glucose oxidase, which catalyses slow release of hydrogen peroxide from the glucose containes within honey (Cooper et al., 2002b).There is a possibility that the hydrogen peroxide produced in the honey contributes to peroxide- antimicrobial activity.

Catalase

Hydrogen peroxide was widely used at one time, but went out of favour also on the theoretical grounds that some species of bacteria possess the enzyme catalase which decomposes hydrogen peroxide. However, of the finding that the catalase activity of strains of *S. aureus* does not correlate with their sensitivity to hydrogen peroxide (Baird-Parker and Holbrook, 1971).

Some authors have considered that the non-peroxide activity is the more important one (Gonnet and Lavie, 1960; Mohrig and Messner, 1968; Radwan et al., 1984). The hydrogen peroxide amount in honey is very small and it can be produced only in after aerobic incubation of diluted honey solutions, which might mean that it is not very important for the antibacterial activity of honey (Bogdanov, 1984). Some of the non-peroxide activity is of floral origin.While honey acidity, diastase and invertase are known to have a bee origin (Bogdanov, 1996, 1997ab).

The enzyme catalase had long been thought to be present in honey (Schepartz, 1966; Schepartz and Subers, 1966). Catalase comes from the pollen and nectar of certain plants; more coming from the nectar (Dustmann, 1971). In the destruction of hydrogen peroxide associated with floral sources is due to the plants contributing catalase to the honeys (Molan, 1992ab). Honeys from some floral sources have been found to have very high levels of catalase, and these honeys accumulate low levels of hydrogen peroxide: the ones accumulating high levels of hydrogen peroxide had low levels of catalase (Dustmann, 1971, 1972).

Catalase is active with high concentrations of hydrogen peroxide but is of low activity with physiological levels (Cohen and Hochstein, 1962). Unexpectedly high levels of catalase were

found to be necessary to destroy the antibacterial activity of honey (Adcock, 1962; White et al., 1963).

Catalase is also present in plasma, at a mean level of 6.9 units/ml (6.9 mmol/litre of hydrogen peroxide removed per minute). That present in exuding plasma in a wound could be augmented by catalase released from dead leucocytes. Although this catalase would be considered to reduce the antibacterial activity of honey by removal of the hydrogen peroxide generated, it could in the process be itself generating antibacterial activity in the form of free radicals (Klebanoff, 1980).This, and the possible augmentation of the leucocytes own production of hydrogen peroxide for the killing of ingested bacteria, could account for the clinical observation that honey is a more effective bactericide in vivo than in vitro (Effem, 1988). A further consideration is that myeloperoxidase, the enzyme that generates the active free radicals from hydrogen peroxide in the leucocytes, is inactivated by excess hydrogen peroxide (Klebanoff, 1980), being denatured by levels above 2 mmol/litre (Agner, 1963).

The existence of non-peroxide antibacterial factors in honey is seen in the reports of activity persisting in honeys treated with catalase to remove the hydrogen peroxide (Adcock, 1962; Allen et al., 1991b; Bogdanov, 1984; Hodgson, 1989; Molan and Russel, 1988; Roth et al., 1986; Russel, 1983; Willix and Subers, 1964). In the first study in which catalase was added to remove the hydrogen peroxide, substantial antibacterial activity remained in many of the honey yet direct assay of the level of hydrogen peroxide present showed that the catalase had been completely effective (Adcock, 1962). It was reported that the residual activity could be removed by the addition of higher levels of catalase, greatly exceeding those required to destroy the amount of hydrogen peroxide present. It was also suggested that the catalase in this case could be having an effect on components other than hydrogen peroxide.

Although the mechanisms by which honey inhibits bacteria have not yet been completely explained, osmolarity, hydrogen peroxide generation and phytochemicals contribute to the antibacterial activity (Molan, 1992ab). In undiluted honey, the osmolarity and acidity undoubtely limit bacterial growth. Antibacterial activity of honey has been established in vitro (Molan,1992ab), but the mechanisms involved in wound healing are unexplained (Tonks et al., 2001).

Other Components

The variation in the antibacterial activity and antioxidant capacity were found from different floral sources (Popeskovic et al., 1983; Sabatier et al, 1992; Bogdanov, 1996; Hegazi et al.,

2001; Frankel et al., 1998). Some of the non-peroxide activity is of floral origin. On the other hand, honey acidity, diastase and invertase are known to have a bee origin (Bogdanov, 1996, 1997ab).

Although some have concluded that honey from certain plants has better antibacterial activity. Manuka, kanuka and, penny royal found to be provide honey additional antibacterial components activity in dilute solutions is linked to plant-derived substances, and hydrogen peroxide generation is minimal (Molan, et al., 1988). The antimicrobial potency of honey varies with its geographical, seasonal and botanical source as well as through harvesting, processing and storage conditions. Thus, only honeys of proven high potency should be used to treat infected wounds (Cooper, 2001).

Significance effect of volatile components found in Hungarian honeys against some Gram-negative pathogens (*Klebsiella pneumoniae, Escherichia coli* and *Candida albicans*) (Tòth et al., 1987). Some workers found non-peroxide activity of honey, extracteble by organic solvents, but were not able to identify the chemical nature of the substances (Roth et al., 1986; Schuler and Vogel, 1956).

There have been several studies in which dark honey from the conifer forests of the mountainous regions of central Europe have been found to have particularly high activity (Buchner, 1966; Chambonnaud, 1966,1968; Daghie et al.,1971; Dustmann,1979; Ialomiteanu and Daghie, 1973; Lindner, 1962; Plachy, 1944; Rychlik and Dolezal, 1961; Sedova and Usmanov, 1973). This honey is not from a nectar source, but from honeydew, produced by aphids sucking the sap from the leaves of the trees. Honey from sweet chestnut (*Castanea sativa*), a nectar source, has also been reported to have high activity (Buchner,1966; Dustmann, 1979), it is dark in colour and thus is considered to be partly derived from honeydew (Buchner, 1966). Another dark coloured honey, from manuka (*Leptospermum scoparium*) in New Zealand, has also been found to have a high level of activity (Molan et al., 1988; Russell et al., 1990; Allen et al., 1991ab; Price, 1991; Price, 1991). Roth et al., (1986) comented on the association of high activity with dark coloured honeys in their study of Canadian honeys. Heather (*Erica spp.*) honey, which has a fairly dark colour, has been found to have a high level of antibacterial activity in one study (Allen et al., 1991b), but a fairly low (Buchner, 1966; Rychlik and Dolezal, 1961) or low (Dustmann, 1979; Molan et al., 1988) level of activity in others. Rape (*Brassica napus*) honey has also been found to have a high level of activity in one study (Allen et al., 1991b), but a fairly low (Rychlik and Dolezal, 1961) or low (Leistner and Rödel, 1975; Nabrdalik and Skarbek, 1974; Dustmann, 1979) level of activity in others. In several studies linden (*Tilia cordata*) honey has also been found to have a fairly high level (Turner, 1983;

Nabrdalik and Skarbek, 1974; Leistner and Rödel, 1975; Rychlik and Dolezal, 1961) of activity, but a fairly low level of activity in others. Clover (*Trifolium spp.*) honey has been consistently found to have low activity (Allen et al., 1991b; Buchner, 1966; Molan et al., 1988), and cotton (*Gossypium hirsutum*) honey high activity (Sedova and Usmanov, 1973; Smith et al., 1969; White and Subers, 1963).

From New Zealand honeys, mainly manuka and viper's bugloss honey, several aromatic acidic substances with antibacterial activity have isolated (Molan,1992ab). These substances were proved to have a floral origin. The paper by Weston et al., (1999) described several methods for isolation of the antibacterially active phenolic fraction of honey derived from the native New Zealand manuka tree, *Leptospermum scoparium* (Myrtaceae). This fraction consists of phenolic derivatives of benzoic acids, cinnamic acids and flavonoids, all of which have been identified previously in honeys which do not exhibit non-peroxide residual antibacterial activity. The flavonoids had not previously been identified in manuka honey.

In addition, antibiotic factors present in honey (Gonnet and Lavie, 1960; Gonnet, 1981), carbohydrates content, enzymatic and antimicrobial activity (Gryuner and Arinkina, 1970), the occurrence of specific substances (Radwan et al., 1984), antioxidant properties of honey produced by bees fed with medical plant extracts (Rosenblat et al., 1997), effect of heat (White and Subers, 1964a), destruction of the peroxide accumulation system by light (White and Subers, 1964b; Dustmann, 1972) had been studied. The paper by Kerkvliet (1996) discussed the screening method for the determination of peroxide acumulation in honey and relation with HMF content.

3.4.2 Flavonoids

Honey contains a number of flavonoids (Sabatier et al., 1992; Barberan et al., 1993), many of which are known to have an antibacterial action.

The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants). However, their occurence is not restricted to flowers but include all parts of the plant.Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables, and certain beverages (tea, coffee, beer, wine and fruit drinks) that have diverse beneficial biochemical and antioxidant effects. The flavonoids have aroused

considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Buhler and Miranda, http://lpi.oregon.state.edu/f w00/flavonoid.html, http://www.friedli.com/herbs/phytochem/flavonoids.html).

Flavonoids present in honey could be antibacterially active and may be the markers for the botanical origin of honeys (Yao, et al., 2004; Yao et al., 2003; Tomás-Barberán et al., 2001; Weston, 1999). The antibacterially active flavonoids of honey from Nepal derived from the native plants have not identified as yet.

3.4.3 TNF-alpha

The importance of TNF-alpha in the healing process has been stressed by a number of authors (Moore et al., 1997; Schlenger et al., 1994; Groves et al., 1995). Wound healing comprises both degenerative and reparative phases of repair (Clarke, 1996). Macrophases remove damaged connective tissue and cell debris, kill pathogens; initiate new vascularization, and stimulate fibroblast proliferation, collagen synthesis and remodelling of connective tissue (Tonks et al., 2001). Macrophages, therefore play a central role in regulating wound healing (Richens, 1996).The primary response of macrophages to inflammatory stimuli involves the release of cytokines including tumour necrosis factor-alpha (TNF-alpha), the production of reactive oxygen intermediates (ROIs), hydrolytic enzymes, growth factors and vasoactive agents.TNF-alpha primes and activates phagocytes (Moore et al., 1991).In macrophages and monocytes, phagocytosis is associated with an increase in oxygen consumption and ROI generation. The reduction in ROIs seen in the presence of honey may serve to limit tissue damage by activated macrophages during the healing process (Tonks et al., 2001). The human monocytic cell line Mono Mac 6 (MM 6) constitutively or after stimulus expresses many of the properties manifested by mature peripheral blood monocytes (Zeigler-Heitbrock et al., 1994).

The investigation (Tonks et al., 2001) on effects of honey (manuka and pasture) on the production of ROI and TNF-alpha release in MM6 cells suggests that honey modulates the activation of monocytic cells in vitro without affecting viability. This modulation gives inhibitory and stimulatory effects. Spontaneous release of TNF-alpha from MM6 cells was seen with manuka and pasture honey. Both induced production of TNF-alpha from resing MM6 cells, suggesting that component (s) within these honeys may stimulated cells.This result could explain the suggested therapeutic properties of honey in promoting wound healing (Tonks et al., 2001).

Honey has been shown to have mitogenic activity on human B and T lymphocytes (Abuharfeil et al., 1999) and a protein fractionated from royal jelly stimulates U-937, a human myeloid cell

line (Watanabe et al., 1998). Proteins present in honey will be highly glycosylated because of high sugar content. Glycosylated proteins have been shown to activate a number of cell types including monocytic cells (Brownlee, 1995), it is possible that these may effect MM6 activation (Moore et al., 1997; Schlenger et al., 1994).

3.4.4 Clinical Studies of Antibacterial Activity

In india, Subrahmanyam (1998, 1999) used honey to treat burns, and in the UK, Dunford et al., (2000) successfully treated chronic infected meningococcal skin lesions with honey.

Subrahmanyam (1998) carried out a prospective, randomised clinical and histological study of superficial burn wound healing with honey and silver sulphadiazine. Early tangential excision and skin grafting of moderate burns found to be superior to honey dressing in a prospective randomised trial (Subrahmanyam, 1999).

Subrahmanyam et al., (2001a, 2001b) studied antibacterial activity of honey on bacteria isolated from wounds.

Natarajan et al., (2001) used manuka honey for a hydroxyurea-induced leg ulcer with sub clinical MRSA infection and MRSA was eradicated from the ulcer and rapid healing was successfully achieved.

Effem (1988) made clinical observations on the wound healing properties of honey.

An immunosuppressed patient who developed a hydroxyurea-induced leg ulcer with subclinical methicillin-resistance *Staphylococcus aureus* (MRSA) infection was treated with topical application of manuka honey, without cessation of hydroxyurea or cyclosporin. MRSA was eradicated from the ulcer and rapid healing was successfully achieved (Natarajan et al., 2001).

Vardi et al., (1998) studied nine infants with large, open, infected wounds that failed to heal with conventional treatment. All infants showed marked clinical improvement after 5 days of treatment with topical application of 5-10 ml of fresh unprocessed honey twice daily.

The wound had failed to heal during 3 years of treatment with conventional therapies and following four surgical procedures. After with dressings impregnated with irradiated manuka honey was initiated, the patients recurrent Staphylococcal infections ceased, and healing was achieved within 4 months (Cooper et al., 2001).

The paper by Haffejee and Moosa (1985) studied the effects of orally or intranvenously administering dilute honey for the treatment of gastrointeritis compared with a usual treatment of glucose solution (control). The study revealed that the honey treatment shortened the duration of diarrhea in patients with bacterial gastrointeritis. Patients with bacterial gastrointeritis who were treated with honey had a mean recovery time of 58.00 hours compared with 93.13 hours for the control patients (cited in McCarthy, 1995).

Recent research has revealed that various ingredients in honey and propolis have anti-cancer properties. Studies found these hive products can also kill cancer cells, which are already present.

Cooper and Molan (1999) investigated the use of honey as an antiseptic in managing *Pseudomonas* infection.

The antibacterial activity of natural honey consumption has been examined in patients suffering from head and neck cancer who developed hyposalivation following irradiation treatment (Sela et al., 2000).

3.4.5 Laboratory Studies of Antimicrobial Activity

Antibacterial activity of other bee products such as propolis, pollen, beebread (Greceanu and Enciu, 1976; Grochowski and Bilinska, 1987; Fierro and Lopez, 1997; Christov et al., 1999; Bankova et al, 2001), bee venom (Cherbuliez, 1997), and royal jelly during the treatment of childhood malignances (Kaftanoglu and Tanyeli, 1997) also were investigated.

The antibacterial activities of different types of honey (Bogdanov, 1983; Bogdanov and Blumer, 2001abc) against five species of pathogenic bacteria belonging to Gram positive and Gram negative (*Staphylococcus aureus, Streptococcus faecalis, Corynibacterium pseudotuberculosis, Pseudomonas aeruginosa and E. coli)* were studied (Hegazi et al., 2001).

$\leq$36 % honey concentration found to be complete inhibition of growth potential against *Salmonella gallinarum, Proteus vulgaris, Psedomonas aeruginosa, Salmonella pullorum, Streptococcus sp.* Dustman (1979) and 100% honey concentration found to be complete inhibition of growth potential against *Streptococcus mutans* (an agar diffusion assay-the active concentration is lower than the value given, the honey being diluted by diffusion into the agar) (Dustman, 1987).

Staphylococcus aureus is the most osmotolerant bacterium capable of causing wound infection (Chirife et al., 1983), and 29% (v/v) sugar solutions found to be prevent growth (Molan, 1992a). Similarly, 1.3% (Chistov and Mladenov, 1961ab), 1.5% (Buchner, 1966), 9% (Rychlik and Dolezal, 1961), 20% (Franco and Sartori, 1940), 50% (Adcock, 1962; Mishref et al.,1989) honey concentrations found to have complete microbicidal action against *Staphylococcus aureus*. Whereas, 0.3% (Dustman, 1979), 0.6% (Chistov and Mladenov, 1961ab), 1% (Molan and Russel, 1988), 1.56% (Rychlik and Dolezal, 1961), 50% (Jeddar et al.,1985) and 10% (Dold and Witzenhausen, 1955) honey concentrations found to have complete inhibition of growth action and 0.4 % (Willix, 1991), 1.4% (Hodgson, 1989), 17% (Dold et al., 1937), 20%

(Bogdanov, 1984) honey concentrations found to be partial inhibition of growth action against *Staphylococcus aureus.*

Eighteen strains of methicillin-resistant *Staphylococcus aureus* and seven strains of vancomycin- sensitive enterococci were isolated from infected wounds and 20 strains of vancomycin-resistant enterococci were isolated from hospital environmental surfaces. For all of the strains tested (compared with an artificial honey solution), the MIC values against manuka and pasture honey found to be below 10%(v/v), but concentrations of artificial honey at least three times higher were required to achieve equivalent inhibition *in vitro*. Comparision of the MIC values of antibiotic-sensitive strains with their respective antibiotic-resistant strains demonstrated no marked differences in their susceptibilities to honey. The study concluded that the inhibition of bacteria by honey is not exclusively due to osmolarity. For the Gram-positive cocci tested, antibiotic-sensitive and resistant strains showed similar sensitivity into honey (Cooper et al., 2002b).

Miorin et al., (2003) evaluated antibacterial activity against *Staphylococcus aureus* of honey and propolis produced by *Apis mellifera* .

Catalase added to exclude activity due to hydrogen peroxide in manuka honey to evaluate the antibacterial activity against *Staphylococcus aureus* ATCC 25923 (Allen et al., 1991b).

In vitro evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of some anti-mycotic agents (Obaseiki-Ebor and Afonya, 1984).

Bogdanov (1997b) tested non-peroxide antibacterial activity of honey with *Staphylococcus aureus* and *Micrococcus luteus*.

Cooper et al., (1999) tested the antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds.

Wound infected with the antibiotic-resistant MRSA (methicillin-resistance *Staphylococcus aureus*) were also studied (Dunford et al., 2000b).

Comparison (Molan et al., 1988), identification and quantitative levels (Weston et al., 2000b) of antibacterial components of some New Zealand honeys were studied. Activity of manuka honey found to be significantly higher than that of clover and heather/ling honey (Molan et al., 1988).

In other studies high levels of non-peroxide activity were found in some New Zealand honeys, particularly manuka honey with sufficient catalase (Molan and Russel, 1988; Allen et al., 1991ab).

The variation in antibacterial antifugal activity (against *Staphylococcus aureus, Escherichia coli* and *Trichophyton mentagrophytes*) of non-manuka honeys was assessed (agar well defusion,

measurement of minimum inhibitory concentration and modified agar well diffusion methods) using 179 unifloral honey, unpasteurized honeys from New Zealand. Of the 179 non-manuka honey samples, none showed non-peroxide or anti-yeast antimicrobial activities.Whereas, 50% of the samples tested showed antibacterial activity against *Staphylococcus aureu,* (5.0-27.9% phenol equivalent), 30% of the samples tested showed antibacterial activity against *Escherichia coli* and 35% of the samples showed antifungal activity (although the levels of activity measured were low) (Brady et al., 2004).

Tonks et al., (2001) investigated the effects of manuka, pasture and an artificial honey on macrophage function.

The sensitivity of 17 strains of *Pseudomonas aeruginosa* isolated from infected burns to two honeys, a pasture honey and a manuka honey, with median levels of activity. All strains showed similar sensitivity to honey with minimum inhibitory concentrations below 10%(v/v); both honeys maintained bactericidal activity when diluted more than 10-fold (Cooper et al., 2002a).

Cooper et al., (2000) tested twenty strains of *Burkholderia cepacia*; isolated principally from the sputum of cystic fibrosis patients, were tested for their susceptibility (agar dilution method) to eight antibiotics with a modified Kirby-Bauer Disc diffusion technique. All strains exhibited susceptibility to concentrations of honey below 6%(v/v). The result suggeted that honey may have a potential role in the clinical management of *B. cepacia* infections.

Hegazi et al., (2001) tested the antibacterial activities of different types of Egyptian bee products including honeys (citrus, cotton, sesame and clover) against five species of pathogenic bacteria (Gram positive and Gram negative) *Staphylococcus aureus, Streptococcus faecalis, Corynibacterium pseudotuberculosis, Pseudomonas aeruginosa* and *Escherichia coli* in relation to some bee products. The results revealed that the different honeys, royal jelly and bee venom were effective antibacterial agents against different pathogenic bacteria but less effective against *E. coli.*

The honey possessed significant antibacterial activity against *Escherichia coli* ATCC 25922 under in vitro and in vivo conditions (Shamala et al., 2002).

Miorin et al., (2003) tested the antibacterial activity against *Staphylococcus aureus* of honey and propolis produced by *Apis mellifera* and *Tetragonisca angustula*. The minimum inhibitory concentrations (MICs) of *Apis mellifera* honey ranged from 126·23 to 185·70 mg ml-1 and of *T. angustula* from 142.87 to 214.33 mg ml-1. For propolis, the MIC ranged from 0·36 to 3·65 mg ml-1 (*A. mellifera*) and from 0·44 to 2·01 mg ml-1 (*T. angustula*). The results showed that propolis samples had higher antibacterial activity against *S. aureus* when compared with honey.

Taormina et al., (2001) compared honeys from six floral sources for their inhibitory activity against *Escherichia coli* O157:H7, *Salmonella typhimurium, Shigella sonnei, Listeria monocytogenes, Staphylococcus aureus, and Bacillus* cereus. A disc assay revealed that development of zones of inhibition of growth depends on the type and concentration of honey, as well as the test pathogen. Growth of *B. cereus* was least affected. The inhibition of growth of *S. sonnei*, *L. monocytogenes*, and *S. aureus* in 25% solutions of honeys was reduced by treating solutions with catalase, indicating that hydrogen peroxide contributes to antimicrobial activity.

Weston et al., (2000a) studied High Performance Liquid Chromatograms of the phenolic fraction of 19 samples of New Zealand manuka honey, some with high levels of non-peroxide antibacterial activity and some with no such activity. The result indicated that phenolic components of this honey are not responsible for the presence or absence of this activity in manuka honey and the geography does not influence the phenolic composition of manuka honey.

Sela et al., (2000) examined the antibacterial activity of natural honey consumption in patients suffering from head and neck cancer who developed hyposalivation following irradiation treatment. Enumeration of total bacteria and S*treptococcus mutans* was carried out in saliva of the patients and of a normal volunteer group before and after honey consumption. Total bacteria count was not significantly different between both groups, whereas the S*treptococcus mutans* count decreased significantly in the experimental group following honey consumption.

Honey from New Zealand and Saudi Arabia at concentrations approximating 20% (v/v) inhibited the growth of *H. pylori* (28 clinical isolates) in vitro. The anti-*H. pylori* effect involved both hydrogen peroxide- and non-peroxide-mediated killing mechanisms. Broth dilution susceptibility tests were performed using solutions of honey prepared in BHI broth ranging in concentration from 5 to 35% (v/v) in 5% increments. Osmotic effects were shown to be the most important parameter for killing *H. pylori* as all carbohydrate solutions 15% (v/v) inhibited 100% of the *H. pylori* (Osato et al., 1999).

Pure cultures of *Pseudomonas sp.* (isolated from swabs from from 20 infected wounds) were assessed (on nutrient agar plates). The minimum inhibitory concentration of the manuka honey for the 20 isolates ranged from 5.5-8.7% (v/v) (mean 6.9v/v), standard deviation (1.3). The minimum inhibitory concentration of the pasture honey for the 20 isolates ranged from 5.8-9.0% v/v (mean 7.1% 8v/v), standard deviation (1.0). The study suggested that honeys with an average level of antibacterial activity could be expected to be effective in preventing the growth of *Pseudomonas* on the surface of a wound even if the honey were diluted more than ten-fold by exduation from the wound (Cooper and Molan, 1999).

Ethiopian honey from *Trigona* spp.was tested (Garedew et al., 2003) *in vitro* against four fungal (*Saccharomyces cerevisiae, Aspergillus niger, Pencillium chrysogenum*, and *Trichoderma viride*) and six bacterial *species (Bacillus subtilis, B. megaterium, B. brevis, Micrococcus luteus, Escherichia coli,* and *Pseudomonas syringae*) in different concentraions (50 %, 20 %, 10 %, 5 %, 2 %, and 1%). Fungi were found to be less sensitive than bacteria or not sensitive at all to the treatments. Only *Aspergillus niger* and *Pencillium chrysogenum* responded slightly at a higher concentration.The minimal inhibitory concentrations of catalase treated honeys from stingless bees were higher than that of the non-treated samples for most bacterial species.

Tonks et al., (2001) investigated the effects of manuka, pasture and an artificial honey on macrophage function. Reactive oxygen intermediate (ROI) production was assessed by luminol enhanced chemoluminescence and tumour necrosis factor-alpha (TNF-alpha) release was determined by immunoassay. Reactive oxygen intermediate (ROI) production found to be significantly ($P<0.001$) decreased by pasture honey and manuka honey.TNF-alpha release significantly enhanced ($P<0.001$) in unprimed MM6 cells by manuka and pasture honey but not altered in primed cells.

Tonks et al., (2003) studied the effects of each of the three honeys (manuka, pasture and jelly bush) on the release of important inflammatory cytokines from MonoMac-6 (MM6) cells. The results suggested that the effect of honey on wound healing may in part be related to the stimulation of inflammatory cytokines from monocytic cells and such cell types are known to play an important role in healing and tissue repair.

Several studies in antimicrobial activity of honey have been summarized by Molan (1992a).

Several authors (Adcock, 1962; Agostino et al., 1961; Allen et al., 1991; Bogdanov, 1984; Buchner, 1966; Cavanagh et al., 1970; Chambonnaud, 1966, 1968; Christov and Mladenov, 1961; Chwastek 1966; Daghie et al., 1973; Dold et al., 1937; Dold and Witzenhausen, 1955; Dolezal et al., 1988; Duisberg and Warnecke, 1959; Dustmann, 1979; Dustmann, 1989; Effem, 1988; Franco and Sartori, 1940; Gonnet and Lavie,1960; Greceanu and Enciu, 1976; Grochowski and Bilinska, 1987; Grochowskimand Bilinska, 1987; Hodgson, 1989; Ialomitzeanu et al., 1967; Ialomiteanu and Daghie, 1973; Ibrahim, 1981; James et al., 1972; Jeddar et al., 1985; Linder, 1962; McGarry,1961; Meier and Freitag, 1955; Mishref et al., 1989; Molan and Russel, 1988; Molan et al.,1988; Nabrdalik and Skarbek, 1974; Plachy, 1944; Popeskovic et al., 1983; Pothmann, 1950; Prica,1938; Radwan et al., 1984; Revathy and Banerji, 1980; Roth et al., 1986; Russel, 1983; Rychlik and Dolezal, 1961; Sackett , 1919; Schade et al., 1958; Sedova and Usmanov, 1973; Skrypnik and Khorol`skii, 1974; Smith et al., 1969; Stomfay-Stitz and Kominos, 1960; Tomlinson and Williams, 1985; Tysset and Durand, 1973; Tysset et al., 1980, 1987; Wadi et al., 1987; Warnecke and Duisberg, 1958, 1964; Wellford et al., 1978; White et al., 1963; White and Subers, 1963, 1964; Willix, 1991; Wooton et al., 1978) found honey to be complete microbicidal action, complete inhibition of growth and partial inhibition of growth in different concentrations (%) against *Alcaligenes sp., Alcaligenes faecalis, Bacillus sp, Bacillus alvei, Bacillus anthracis, Bacillus cereus, Bacillus larvae, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, Citrobacter freundii,*

Edwardsiella tarda, Escherichia coli, Haemophilus influenzae, Klebsiella sp. , Klebsiella pneumoniae, Listeria monocytogenes, Micrococcus luteus, Mycobacteruim tuberculosis, Neisseria sp., Pasteurella multocida, Proteus sp. , Proteus mirabilis, Proteus morganii, Proteus vulgaris, Pseudomonas spp. , Pseudomonas fluorescens, Salmonella enteritidis, Salmonella cholerae-sui, Salmonella dublin, Salmonella gallinarum, Salmonella paratyphi-A, Salmonella pullorum, Salmonella schottmulleri, Salmonella typhi, Salmonella typhimurium, Salmonella typhosa, Sarcina lutea, Sarcina orangea, Serratia marcescens, Shigella sp,. Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei , Staphylococcus sp. , Staphylococcus aureus (albus) , Staphylococcus aureus, Streptococcus pyogenes, Streptococcus salivarius, Streptomyces sp,. Vibrio cholerae biotype, *Vibrio cholerae,* and (fungi) *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Candida albicans, Candida pseudotropicalis, Candida rekaufii, Candida stellatoidea, Candida tropicalis, Candida utilis, Penicillium chrysogenum, Penicillium sp.*

Molan (1992a)

In addition, a number of other authors have also extensively reported the usage of honey concomitant with the progress in medicine are far reaching successful developments in modern science which also has an important place in apitherapy. This is supported by further findings about ist biological properties such as antibacterial (Cooper et al., 1999; Al-Jabri, 2003), antifungal (Dolezal et al., 1988; Mishref etal., 1989; Radwan et al., 1984; Revathy and Benerji, 1980; Rizvanov and Bizhev, 1962; Wellford et al., 1978), antioxidant (Frankel et al., 1998), on foot ulcers in lepers and diabetic foot ulcers (Tovey, 1991); on necrotic malignant breast ulcers (Butler,1980), epithelialization of the wound (Effem, 1988; 1993; Hejase et al., 1996; Subrahmanyam, 1994; Subrahmanyam, 1998), promotion of the formation of clear healthy granulation tissue (Armon, 1980; Braniki, 1981; Cavanagh, 1970; Dumronglert, 1983; Effem, 1988, 1993; Farouk, et al., 1988; Hutton, 1966; Subrahmanyam, 1998; Wadi et al., 1987), stimulant for the growth of new blood capillaries, fibroblasts and epithelial cells (Dunford, 2000ab), anti-inflammatory action (Subrahmanyam, 1998; Yang, 1944; Effem 1998, 1993; Hejase et al., 1996),dental health (Attar, 1982; Gedalia et al., 1997; Grobler and Basson, 1997), susceptibility of wound pathogens (Willix et al., 1992), wound treatment (Bergman et al., 1983; Tovey, 1991; Blomfield, 1973; Farouk, et al., 1988; Wood et al., 1997; Hamdy et al., 1989). Bejan et al., (1978); Kirienko et al., (1989) adviced inhalation of 50% aerosolized acacia honey and propolis to supplement conventional therapy in curing chronic and exudative bronchitis. Since the eighteenth century its benefits in treating 'tough phlegm', hoarseness, coughs, asthma and consumption have been documented (Hill, 1759).

3.4.6 Antimicrobial Activity of Other Bee Products

Honey is used to treat the hazards of radiation therapy in the mouth and throat like the honey preparation: Apicomplex (made at Medex Laboratories of Ljubljana, Slovenia) (Attar, 1982). Other bee products such as propolis, pollen and bee bread (Greceanu and Enciu, 1976; Grochowski and Bilinska, 1987) have also tested for antimicrobial activity. In addition, comprehensive research work on the volatile components of honey and ist effects was found to be very beneficial (Toth et al., 1987). Heavy hydrogen (deuterium) was discovered in honey at Cornell university (Helvey, 1953). This discovery could be helpful for further investigations on the nature of tumor therapy with honey.

Acetone and ethanol extracts of two Bulgarian propolis samples (Bur and Lov) was investigated (Prytzyka et al., 2003) by high temperature high resolution gas chromatography coupled to mass spectrometry (HT-HRGC-MS), and their activity against *Trypanosoma cruzi* was evaluated. The ethanol extracts—Et-Bur and Et-Lov—showed similar composition, with a high content of flavonoids, and strong inhibitory activity against *T. cruzi* proliferative epimastigotes, which were more susceptible than trypomastigotes. Both extracts also showed similar and significant activity against *Staphylococcus aureus* and *Candida albicans*, while being inactive against *Escherichia col.* (Russo et al., 2002; Ota et al., 2001; Bankova et al., 2001).

Russo et al., (2002) investigated the antioxidant activity of a propolis extract deprived of caffeic acid phenethyl ester (CAPE). In addition, the activity of CAPE and galangin was also examined. The experimental evidence suggested that CAPE plays an important role in the antioxidant activity of propolis.

Propolis from a cerrado area in Minas Gerais State, Brazil were tested for inhibitory activity against periodontitis-causing bacteria. All of the assayed bacterium species found to be susceptible to propolis extract (Santos et al., 2002).

Ota et al., (2001) studied the antifungal activity of propolis in sensitivity tests on 80 strains of Candida yeasts, 20 strains of *Candida albicans*, 20 strains of *Candida tropicalis*, 20 strains of *Candida krusei* and 15 strains of *Candida guilliermondii.* The yeasts showed a clear antifungal activity with the following order of sensitivity: *C. albicans* >*C. tropicalis* > *C. krusei* > *C. uilliermondii.* Patients with full dentures who used a hydroalcoholic propolis extract showed a decrease in the number of Candida.

The hepatoprotective activity of alcoholic extract of tropical Brazilian propolis found mainly due to phenolic compounds including flavonoids. The labdane-type diterpenes and some of the

prenylated phenolic compounds also possessed antibacterial activity against *Helicobacter pylori* (Bankova et al., 2001)

Three ent-kaurene diterpenoids, not previously described as constituents of propolis, were isolated from sample collected by Brazilian native bees *Melipona quadrifasciata* anthidioides. One of them, kaurenoic acid, as well as the total extract, displayed moderate antibacterial activity (Velikova et al., 2000).

Sforcin et al., (2000) observed in vitro antimicrobial activity (*Staphylococcus aureus* and *Escherichia coli*) of propolis, collected during the four seasons, on bacterial strains isolated from human infections. The growth of Gram-positive bacteria found to be inhibited by low propolis concentrations (0.4%) whereas Gram-negative bacteria were less susceptible to this substance, the minimal inhibitory concentration ranging from 4.5 to 8.0%.

Santos et al., (1999) evaluated the antibacterial activity of propolis produced in Brazil against *Actinobacillus actinomycetemcomitans, Fusobacterium spp.* and Bacteria from the *Bacteroides fragilis* group isolated from human and marmoset hosts.

Bankova et al., (1999) studied antibacterial activity of essential oils from Brazilian propolis.

Christov et al., (1999) studied antibacterial furofuran lignans from Canary Islands propolis.

Kujumgiev et al., (1999) investigated propolis samples from different geographic origins for their antibacterial (against *Staphylococcus aureus* and *Escherichia coli*), antifungal (against *Candida albicans*) and antiviral (against Avian influenza virus) activities. All samples found to be active against the fungal and Gram-positive bacterial test strains, and most showed antiviral activity.

Alcoholic extracts of propolis from four localities of Amaicha del Valle (El Paraiso, La Banda Este, La Banda Oeste and El Molino), Province of Tucumán and from Cerrillos, Province of Santiago del Estero, Argentina were prepared to test antibacterial activities against *Streptococcus piogenes*. All showed antibacterial activity. The propolis from La Banda Este found to be the most active (MIC=7.8 g/ml) (Moreno et al., 1999).

A 13-month-old female infant developed bilateral eosinophilic ulcers of the mouth. Application of the propolis ointment was associated with the rapid resolution of the ulcers within 3 weeks and they did not subsequently occur (Kiderman et al., 2001).

The effect of some UV-absorbing substances inhibiting the DNA-dependent RNA polymerases of *Escherichia coli* and *Streptomyces aureofaciens*, as well as the restriction endonuclease Eco RI isolated from the water soluble extract of propolis upon *in vitro* transcription and restriction of DNA was investigated (Simuth et al.,1986). Authors suggested that the inhibition of bacteria RNA-polymerases was probably due to the loss of their ability to bind to DNA.

The DNA strand scission due to WSSP (Water Soluble Substances of Propolis) and quercetin was investigated The single strand scission of cccDNA and the formation of oc DNA was observed. The degree of the DNA cleavage in the presence of Cu^{2+} was enhanced and the oc DNA conversion into linear DNA and fragmental DNA was observed in a study made by Simuth (1991).

Hive products also play a crucial role in cell metabolism. One of such processes is gene expression: the transcription and the translation of the genetic information (Simuth, et al., 1995). The effect of WSSP (Water Soluble Substances of Propolis) and WSH (Water Solution of Honey) on plasmid and chromosomal DNA as model biopolymers sensitive to the oxidative damage was studied by Simuth et al.,(1995). The single and double stranded scission of covalently closed circular DNA was observed after 72 hours exposition of the plasmid to the WSSP.The same effects on plasmid DNA was found after employing WSH (6% or 12% v/v). After addition of 1 mmol.$L^{-1}CuSO_4$ the total degradation of plasmid DNA was observed after 20 minutes. The results indicated that in WSH reactive oxygen free radical are produced in low amounts (the DNA damage was observed only after 3 days)which are not sufficient to overcome the protective mechanism of animal cell into oxidative stress. This state is changed by the addition of 1 mmol.L^{-1} $Cu2^{+}$ to the reaction. The total DNA degradation was observed after short time (20 min.).

3.5 Materials and Methods

All honeys produced by *Apis cerana , A. dorsata, and A. mellifera* were collected directly from the colonies at the same location and floristic region during different months from different agro-ecozones:terai, hills and mountains.

A small piece of sealed honey combs from *Apis cerana* were collected directly from traditional hives and Newton B hives in Chitwan, Daman, Dadeldhura, Jumla, Kathmandu and Langtang areas.

Apis mellifera honeys were collected from modern hives in Chitwan and Kathmandu district. *A.dorsata* honeys directly collected from the colonies in Chitwan, *Apis florea* in Mahendranagar and Dadeldhura,and *A. laboriosa* in Barabise and Bajhang.

Based on their altitudinal ranges samples were aggregated in simpler classification of terai, hills and mountains. The honey samples collected from Chitwan and Mahendranagar were grouped under terai (< 500masl); Kathmandu, Arghakhachi, Barabise, Bajhang and Dadeldhura under hills (500-2000masl); and samples from Jumla, Langtang and Daman were placed under mountains (2000-3000masl).

Overall 164 honey samples assayed had been kept in plastic bottles and stored in dark at 4°C until being used.

The laboratory testing on each relevant samples were carried out at the Institut für Futtermittel, Austrian Agency for Health and Food Safety (Agricultural Inspection Service and Research Centre), Vienna. And, the method was adopted from Molan (personal communication).

Inoculum Preparation

A culture of *Staphylococcus aureus* ATCC 6538P obtained from the Institut für Futtermittel, Vienna was added to sterile liquid nutrient agar (23g/L 001-7-0 DIFCO) at 45°C .

Plate Preparation

The template was prepared on card. A grid was drawn on the card, away from the sides , and the wells were centered on the intersections of the grid. The intersections were numbered using a white china pencil just above the intersection using a quasi-Latin square which enabled the samples to be placed on the plate.

To preapare the assay plates, 150 ml nutrient agar (23g/L 001-7-0 DIFCO) was sterilised (autoclave 15 min at 121 °C), then held at 50° C before seeding with the 1 ml of log phase culture .The agar was then, as quickly as possible, swirled to mix throughly and poured into a large square assay plate (25 X 25 cm) which had been placed on a level surface. When the agar was set the plates were placed upside-down at 4 °C for 1 hour before using.

Catalase solution

A 2 mg/ml solution of catalase from bovine liver (Sigma C-9322; 2460 units/mg) in distillated water was prepared freshly each time.Honey samples as received were mixed to homogenise (warming briefly to 37°C being necessary for this with solid samples).All samples were stored in the refrigerator , and all honey samples and solutions of honey were kept protected from light when not being handled.

Preparation of honey solutions

A 50%(v/v) solution of each honey was prepared initially to overcome the problem of the high viscosity of honey preventing it mixing properly, and being measured by pipette, in subsequent dilution procedures. This initial solution was prepared by taking a sample of honey and adding an equal volume of water. The volume of honey taken was most expediently measured by placing (approximately) 10-12 ml of honey in a stoppered 25 ml measuring cylinder and adding (by pipette) 10 ml distilled water, and warming in a waterbath at 37C for 30 minutes to aid mixing. After allowing to cool, the precise volume of honey that had been taken was determined as the difference between the volume in the measuring cylinder and the 10 ml of water that had

been added. The volume was then adjusted, by adding distilled water, to be exactly twice the volume of honey that had been taken.To prepare 25%(v/v) solutions, to 1 ml of 50%(v/v) honey solution was added 1 ml of distilled water for testing total activity, or 1 ml of catalase solution for testing non-peroxide activity.

Application of samples to the plate

Each sample was tested in quadruplicate by adding 150 µl to each of wells with the same allocated number on the assay plate. As a control to check of hydrogen peroxide activity and no detectable non-peroxide activity was included with each batch of tests: this served as a blank for the assay, as there should be no zone of inhibition around this control.

Standards

A range of phenol standards was prepared in distilled water:1,2,3,4,5,6,7,8,9,10% (w/v).
A volume of 150µl of each standard was placed in each wells on the plate to obtain a standard curve for the assay.

Using a quasi-Latin square as a template, 20 wells were cut into the agar with a flamed, cooled 13 mm cork borer and the agar plugs removed with a sterile inoculating needle after being pre-diffuse at 4°C for 1 hour.

The wells completely filled with the phenol standards and samples to be assayed and the agar plates were incubated at 37°C for 5-7 hours or until growth and zones of inhibition could be clearly seen.

***Apis mellifera* honey from Chitwan showing the zones of inhibition of growth (peroxide activity) on the agar plate.**

***Apis florea* honey from Dadeldhura showing the zones of inhibition of growth (peroxide and non-peroxide activity) on the agar plate.**

Zone measurement

The clear inhibition zones (antibacterial activity) were assessed by measuring the diameter of the zones using a projector (calliper) around each well in which no growth was seen.
Calculation of antibacterial activity

The diameter of the clear zone around each phenol standard was calculated and squared. A standard graph was plotted of % phenol against the square of the diameter of the clear zone. A best-fit straight line was plotted and the equation of this line was used to calculate (from the square of each measurement of the diameter of the clear zone) the phenol concentration (%w/v) with equivalent activity to that of each honey solution. These values were then multiplied by 4 so the activity of the undiluted honey samples could be expressed as the equivalent phenol concentration.

Statistical analysis

The statistical analysis was carried out with the SAS (Statistical Analysis System) for windows 6.12. A non– parametric test was perfomed because calculated residuals were not normally distributed. The Kruskal-Wallis test followed by the Bonferroni-Holm test was performed to analyse the antibacterial activity for significant differences.These tests compare the mean values for activity in honeys produced by each three bee species *Apis cerana* , *A. dorsata* and *A. mellifera,* and *A. cerana* honey from differnt locations equivalent to that of % phenol.

3.6 Results

Nepali honeys produced by different five bee species: *Apis cerana, Apis dorsata, Apis laboriosa, A.florea* and *A. mellifera* in different ecozones (terai, hills and mountains) were tested in a study of the peroxide and non-peroxide antibacterial action on *Staphylococcus aureus* ATCC 6538 P. Apart from *Apis mellifera,* all four are indigenous species of *Apis* found in Nepal. It was found that the activity varied when compared with that of a reference antiseptic phenol.
The peroxide and non-peroxide antibacterial activity against *Staphylococcus aureus* ATCC 6538P of honey samples produced by *Apis cerana* bee from five different locations that represent terai, hills and mountains in Nepal are represented in figure1 and table 1. Sample collection locations and antibacterial activity values are given in annexes.

The *Apis cerana* honey produced in Langtang had highest inhibitory peroxide activity against the *Staphylococcus aureus* ATCC 6538 P followed by the honeys from Chitwan, Dadeldhura, Daman and Kathmandu equivalent to that of 7.0 %, 4.8 %, 4.1 %, 2.5%, 2.3 % (w/v) phenol whereas, Jumla honey showed no peroxide and non-peroxide activities as well (fig.1, table 1). Only the Chitwan and Daman honeys showed the non-peroxide antibacterial activity 2.8 and 2.2 equivalent to that of % phenol respectively amongst all (fig.1, table 1).

Figure 1. Antibacterial activity of *Apis cerana* honeys on *Staphylococcus aureus* ATCC 6538P. Results are expressed in equivalent % phenol.

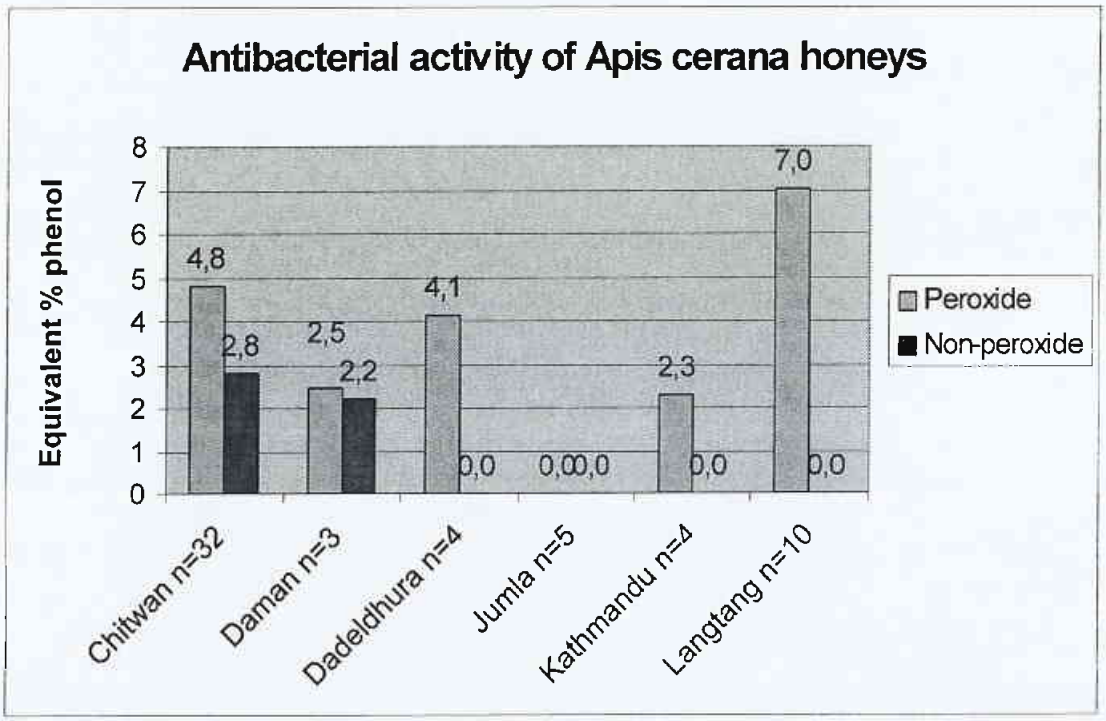


Table 1. The sensitivity of *Staphylococcus aureus* ATCC 6538P to the hydrogen peroxide and non- peroxide antibacterial activity of *Apis cerana* honeys from different locations of Nepal.

Equvalent % phenol	Chitwan n=32	Daman n=3	Dadeldhura n = 4	Jumla n=5	Kathmandu n=4	Langtang n = 10
Peroxide activity	4.8	2.5	4.1	0	2.3	7.0
Non-peroxide activity	2.8	2.2	0	0	0	0

Note: *Apis cerana* samples didnot show any significant difference among the locations

The effectiveness of *Apis cerana* honeys in inhibiting *Staphylococcus aureus* ATCC 6538 P was seen across 36.66 % and 10 % of the honey samples for peroxide and non-peroxide activities respectively from different eco-zones, except Jumla (table 4).

Comparisons between the sensitivity to honey of different locations showed no significant differences in both activities equivalent to that of % (w/v) phenol observed among all the locations.The antibacterial activity and difference in activity of honey samples produced by *Apis dorsata, A. cerana* and *Apis mellifera* in Chitwan district , central Nepal are presented in figure 2 and table 2 & 3. *Apis mellifera* honey showed the highest peroxide activity equivalent to that of 11.1 % phenol followed by *Apis dorsata* and *Apis cerana* honeys 5.0% and 4.8% respectively. Similarly, *Apis cerana* honey had non-peroxide activity equivalent to that of 2.8% phenol followed by *A. mellifera* (1.9%) and *A. dorsata* (1.7%) honey respectively (table2). Honey from *Apis cerana -A. mellifera* (P=0.0009) and *A. dorsata-A.mellifera* (P=0.0010) showed significant difference in peroxide activity, whereas honey produced by two native bee species *A. dorsata* and *A. cerana* did not show significant difference in peroxide activity (table 2).

Figure 2. Antibacterial activity of *Apis cerana, A. dorsata* and *A. mellifera* honeys from Chitwan. Results are expressed in equivalent % phenol.

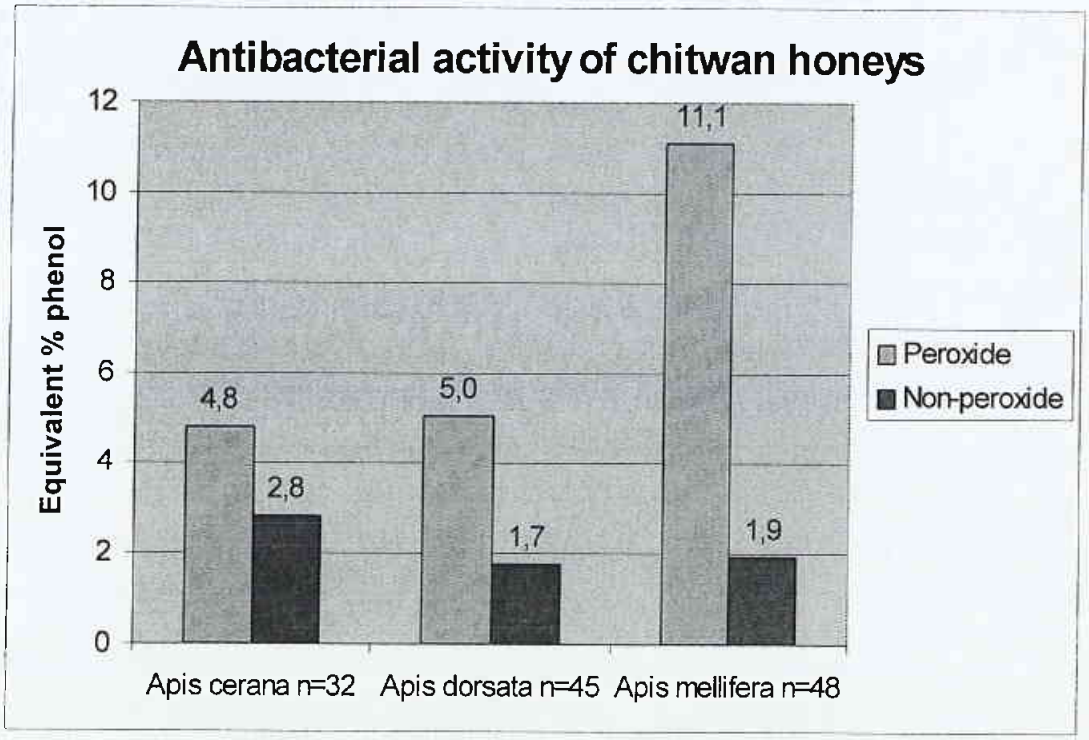


Table:2 The sensitivity of *Staphylococcus aureus* ATCC 6538P to the hydrogen peroxide and non-peroxide antibacterial activity of *Apis cerana*, *A. dorsata* and *A. mellifera* honeys from Chitwan.

Equivalent % phenol	*Apis cerana* n=32	*Apis dorsata* n=45	*Apis mellifera* n=48
Peroxide	4.8 [a]	5.0 [a]	11.1 [b]
Non-peroxide	2.8	1.7	1.9

The different letters a, b, super-scribed after the means in a row denote the statistical significance between the analytical values obtained for three honey groups.

There was no significant difference in non-peroxide antibacterial activity of honeys from all three species produced in Chitwan.

The peroxide and non-peroxide antibacterial activity of honeys produced by *Apis cerana*, *A.dorsata, A. laboriosa, A. florea* and *A. mellifera* in different eco-zones of Nepal are tabulated in table 3 and presented in fig.3.

It is apparent that *Apis mellifera* honey had highest peroxide activity equivalent to 10.7 % phenol followed by *A. florea, A. laboriosa, A. dorsata* and *A. cerana* honeys 10.4%, 6.2%, 5% and 4.5% respectively (table 3). For non-peroxide activity, mean values with *Apis florea* honey from Dadeldhura was found to be 9.3 % and with *Apis laboriosa* honey from Bajhang was 5.8 equivalent to that of % phenol (table 3). *Apis mellifera, A. dorsata* and *A. cerana* honeys also found to have non-peroxide activity as well (Fig.3, table 3).

Honey produced by *Apis florea* in Mahendranagar, *A.laboriosa* in Barabise, *A. cerana* in Arghakhachi (two samples), and *A. mellifera* (two samples) in Kathmandu from were to a level that was below the limit of detection.

Over all Nepali honey samples from different locations of Nepal had peroxide activity 6.7 % equivalent to phenol and non-peroxide activity 2 % equivalent to phenol (table 3, Fig. 3).

Figure 3. Antibcterial activity of *Apis cerana, A. dorsata, A. mellifera, A. laboriosa* and *A. florea* honeys on *Staphylococcus aureus* ATCC6538P. Results are expressed in equivalent % phenol.

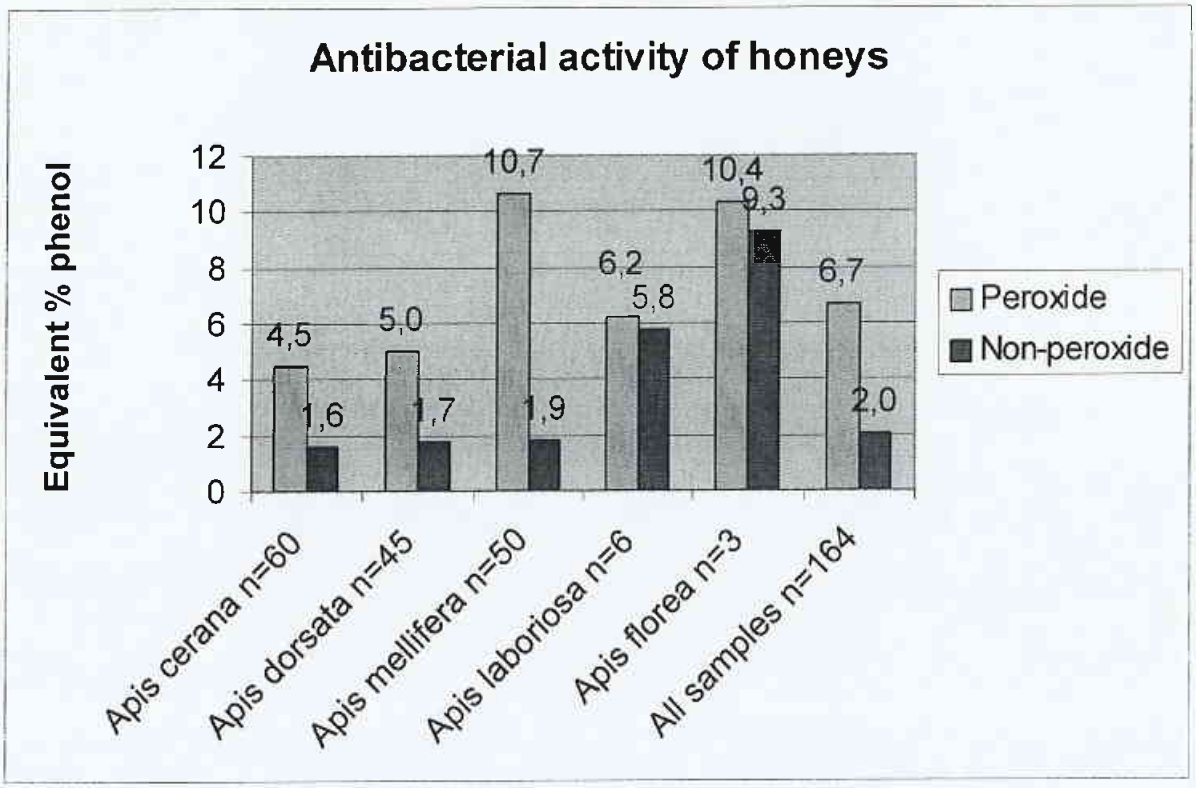


Table 3. The sensitivity of *Staphylococcus aureus* ATCC 6538P to the hydrogen peroxide and non-peroxide antibacterial activity of honeys produced by different bee species in Nepal.

Equivalent % phenol	*A.cerana* n=60	*A.dorsata* n=45	*A.mellifera* n=50	*A. laboriosa* n=6	*A. florea* n=3	Total samples n=164
Peroxide activity	4.5	5.0	10.7	6.2	10.4	6.7
Non-peroxide activity	1.6	1.7	1.9	5.8	9.3	2.0

***Note : Apis florea* honey samples collected from Mahendranagar and Dadeldhura.**
***A. laboriosa* honey samples collected from Barabise and Bajhang.**

The effectiveness in inhibiting *Staphylococcus aureus* ATCC 6538P was seen across 68 % (peroxide) and 10 % (non-peroxide) in *A. mellifera* honey, 66.66 % (both peroxide and non-peroxide) in *A. florea* honey, 36.55% (peroxide) and 10% (non-peroxide) in *A. cerana* honey,

35.55 % (peroxide) and 15.55 % (non-peroxide) in *A. dorsata* honey, 33.33 % (peroxide and non-peroxide) of the total samples samples in *A. laboriosa* honey. Of the over all 164 honey samples 46.34 % showed peroxide and 13.41 % showed non-peroxide antibacterial activities (table 4).

Table 4.Table showing percentile of the samples having peroxide and non-peroxide activity in Nepali honeys.

Bee species	Number of samples (Pe)	Number of samples (Non-pe)	Peroxide activity (%)	Non-peroxide activity (%)
Apis cerana	22/60	6/60	36.66	10
Apis dorsata	16/45	7/45	35.55	15.55
Apis mellifera	34/50	5/50	68	10
Apis laboriosa	2/6	2/6	33.33	33.33
Apis florea	2/3	2/3	66.66	66.66
All	76/164	22/164	46.34	13.41

Note: Two samples of *A. cerana* honey and two samples of *A. mellifera* honey from Arghakhachi and Kathmandu respectively are also included

3.6.1 Discussion

There have been different values reported for the sensitivity of *Staphylococcus aureus* to the antibactrerial action of honey.

This study provides conclusive evidence for the existence of antibacterial activity in honey produced by different bee species that is not due only to hydrogen peroxide. In the honeys of higher antibacterial activity, the non-peroxide activity is the important part of the total activity. There is no possibility of the inhibition of bacterial growth being due to the osmolarity of the honey.

The inhibitory concentration of honey has been reported to be for all of the eighteen strains of methicillin-resistant *Staphylococcus aureus* tested, the MIC values against manuka and pasture honey were below 10 % (v/v), but concentrations of artificial honey at least three times higher were required to achieve equivalent inhibition *in vitro*. Comparision of the MIC values of antibiotic-sensitive strains with their respective antibiotic-resistant strains demonstrated no marked differences in their susceptibilities to honey (Cooper et al., 2002b).

The antibacterial activity against *Staphylococcus aureus* of honey and propolis produced by *Apis mellifera* and *Tetragonisca angustula* was evaluated.The minimum inhibitory concentration (MICs) of *A. mellifera* honey ranged from 126·23 to 185·70 mg ml-1 and of *T. angustula* from 142·87 to 214·33 mg ml-1 (Miorin et al., 2003)

The study was undertaken to compare honeys from six floral sources for their inhibitory activity against *Escherichia coli* O157:H7, *Salmonella typhimurium, Shigella sonnei, Listeria monocytogenes, Staphylococcus aureus*, and *Bacillus cereus*. The inhibition of growth of *S. sonnei*, *L. monocytogenes*, and *S. aureus* in 25% solutions of honeys was reduced by treating solutions with catalase (Taormina et al., 2001).

In the study by Dustmann, (1979), the analysis made by very sensitive test tube dilution test after having sterilized the honey solution by sterile-filtration. The concentration of honey (%) for complete inhibition of growth was found to be 0.3 against *S. aureus*..

Previous studies have reported the concentrations of honey (%) for complete inhibition of growth was found for *S. aureus* were 0.6 (Christov and Mladenov, 1961), 1 (Molan and Russel, 1988),1.56 (Rychlik and Dolezal, 1961), 2.9 (Dold and Witzenhausen,1955; Hodgson, 1989), 50 (Jeddar et al.,1985), and 10 (Dold and Witzenhausen, 1955), 3 (Schade et al., 1958; White et al., 1963; White and Subers, 1963; 1964a), 3.1 (Dolezal et al., 1988). The concentration of honey (%) for complete microbicidal action was found to be 1.3 (Christov and Mladenov, 1961),1.5 (Buchner, 1966), 9 (Prica,1938), 20 (Franco and Sartori, 1940), 50 (Adcock, 1962; Mishref et al., 1989). 0.4 % (Willix, 1991), 1.4% (Hodgson, 1989), 17% (Dold et al., 1937), 20% (Bogdanov, 1984) honey concentrations found to be partial inhibition of growth action against *Staphylococcus aureus.*

The present study could reflect differences in antibacterial potency (% phenol equivalent) of Nepali honeys against S*taphylococcus aureu*s 6538P produced by different bee species.

Like the preliminary studies made by several workers, this study has shown that there is some variation in the levels of antibacterial activities observed for Nepali honeys.

Antibacterial activity of *Apis cerana* honeys from different eco-zones

Some differences found in the present study in the antibacterial activity of a *Apis cerana* honeys, collected from different eco-zones. *Apis cerana* honey from Langtang, which accounts on average for 7.0 equivalent to that of % phenol, was the higher peroxide activity (fig.1, table 1). It was clear that Langtang, Dadeldhura and Kathmandu honey was active without catalase added to exclude activity due to hydrogen peroxide. On the other hand, Chitwan and Daman had both peroxide and non peroxide activities as well.

An explanation for differences observed in the levels of hydrogen peroxide-induced antimicrobial activity is unknown. Several authors believe that hydrogen peroxide produced by honey glucose oxidase (Gauhe, 1941; White et al.,1963;1964; Dustmann, 1972, 1979; Burgett, 1973; Morse, 1986; Taormina et al., 2001; Schepartz and Subers, 1964) and differences in activity could be due to varying levels of other antibacterial compounds coming

from the nectar source (Vergè, 1951; Schuler and Vogel, 1956; Lavie and Grassè, 1963; Gonnet and Lavie, 1960; Mladenov, 1974) or it has been associated with floral source (Brady et al., 2004).

The findings in this study indicate that there were no significant between the *A. cerana* honey produced in different locations in their effectiveness (peroxide and non-peroxide) against *Staphylococcus aureus* ATCC 6538P, and there was no individual honey that showed a significantly greater activity equivalent to that of % phenol (w/v) to *S. aureus*.

Low levels of non-peroxide activity were found only in *A. cerana* honeys from Chitwan and Daman (fig.1, table 1) with sufficient catalase added to remove hydrogen peroxide. Manuka honey was found to have a particularly high level of this type of activity (Molan and Russel, 1988).

Apis cerana honey showing higher peroxide antibacterial activity from Langtang region (the mountain) may be due to high glucose oxidase. The glucose oxidase was found to be significantly higher in mountain honeys than those of terai and hill honeys (Joshi, 1999). Honey may also contain enzymes of plant origin from nectar or honeydew, and possibly from pollen (Crane, 1990). A major part of the antibacterial activity of honeydew honey is of bee origin (Bogdanov, 1997a).

Antibacterial activity of different honeys produced by *Apis cerana, A. dorsata, A. laboriosa, A. florea* and *A. mellifera*

Regarding the results of antibacterial activity of different honeys produced by *Apis cerana, A. dorsata, A. laboriosa, A. florea* and *A. mellifera* against *Staphylococcus aureus*, it was obvious that the *A. mellifera* honey showed the highest peroxide activity equivalent to that of % phenol followed by *A. florea, A. laboriosa, A. dorsata* and *A. cerana.* While, *A.florea* had highest non-peroxide antibacterial activity 9.3 followed by *A. laboriosa, A. mellifera, A. dorsata,* and *A. cerana* equivalent to that of % phenol (fig.3, table 3).

The manuka honey showed inhibitory activity against *Staphylococcus aureus* ATCC 25923 equivalent to that of 13% (w/v) phenol with enzyme catalase (Allen et al., 1991b).

Furthermore, different levels of antibacterial activity against *Staphylococcus aureus* were observed for a given floral source in the research presented by Allen et al., 1991b (buttercup 17.1% and 13.1%; clover 10.1% and 13.9%; kamahi 13.7% and 13.8%; kanuka 27.9% and 21.6%; rewaewa 16.9% and 20.9%, respectively). As figure 1, 2, 3 and table 1, 2, 3 revealed, the activity evaluated in this study found to be lower or roughly similar (clover honey vs florea honey) than that of different honeys from New Zealand.

The variation of the antibacterial activity of different honey were attributed to the previously mentioned factors which influenced the antibacterial activity as osmolarity and acidity of honey which undoubtely limit bacterial growth (Molan et al., 1988; Molan, 1992a), honey pH or activity of glucose oxidase (Chirief et al., 1982), hydrogen peroxide (Roth et al., 1986; Dustman, 1987), non-peroxide substances (Effem, 1988; Bogdanov, 1984) and volatile antibacterial substances (Radwan et al., 1984).

In other honeys, such as manuka, kanuka and, penny royal provided honey additional antibacterial components activity in dilute solutions is linked to plant-derived substances, and hydrogen peroxide generation is minimal (Molan, et al., 1988). From New Zealand honeys, mainly manuka and viper's bugloss honey, several aromatic acidic substances with antibacterial activity have isolated (Molan, 1992a). These substances were proved to have a floral origin. Generally, the phytochemical components make only minor contribution to the antibacterial activity of honey but, for a few honeys (eg. manuka honey), unidentified phytochemichal compounds make a major contribution (Molan, 1992ab).

According to Dunford et al., (2000a b) the additional antibacterial agents, which are plant derived chemicals, for example bioflavonoids, are present in honey.

Although the mechanisms by which honey inhibits bacteria have not yet been completely explained, osmolarity, hydrogen peroxide generation and phytochemicals contribute to the antibacterial activity (Molan, 1992ab).

It has also been claimed, that honey contains lysozyme, a well known antibacterial agent (Mohrig and Messner, 1968). However, in an other study it was found that no lysozyme activity was present in honey (Bogdanov, 1984). The antibacterial activity in honey is due to sugars, which by their osmotic effect exert antibacterial action (Molan,1992a). Honey contains a number of flavonoids (Sabatier et al., 1992), many of which are known to have an antibacterial action. In this study there is no possibility of the inhibition of bacterial growth being due to the osmolarity (and pH) of the honey. *Staphylococcus aureus* ATCC 25923 was not inhibited by the osmolarity or the acidity of the honey when pH was found to be 4.0 in study made by Molan and Russel (1988). Honeys samples assessed in this study having peroxide and non-peroxide antibacterial activity could be due to bee and plant origin.

Also, other workers found non-peroxide activity of honey, extractable by organic solvents, but were not able to identify the chemical nature of the substances (Roth et al., 1986; Schuler and Vogel, 1956).

Some workers believe that differences in activity is because of hydrogen peroxide produced by honey glucose oxidase (White et al., 1963, White and Subers 1964ab; Dustmann, 1972,

1979; Morse, 1986; Taormina et al., 2001) and other factors also play an important role for (varying levels of) antibacterial activity coming from the nectar source (Vergè, 1951; Schuler and Vogel, 1956; Lavie and Grassè, 1957, 1960; Gonnet and Lavie, 1960; Mladenov, 1974; Dustmann, 1979), other authors have considered that the non-peroxide activity is the more important one (Gonnet and Lavie,1960; Mohrig and Messner,1968; Radwan et al.,1984) and the presence of higher amount of hydrogen peroxide produced by the action of glucose oxidase in honey responsible for antibiotic activity (Adcock, 1962; White et al., 1963; White and Subers, 1963; Wakhle and Desai, 1991; Molan, 1992a) or peroxide-induced antimicrobial activity has been associated with floral source (Brady et al., 2004). All of the glucose oxidase, and the major part of the proline and invertase in honey originated from the hypopharyngeal glands of the honey bees (Greich and Delage-Darchen, 1978; Dustmann, 1967; Edelhauser and Bergner, 1984; Von der Ohe et al, 1991; Von der Ohe, 1994). Some of the non-peroxide activity is of floral origin (Bogdanov, 1996, 1997a,1997b) while honey acidity, diastase and invertase (White,1967, 1978) are known to have a bee origin.

The invertase and glucose oxidase were found to be higher in Nepali honeys from *Apis florea, Apis dorsata, A. laboriosa* and *A. cerana* than that of *A. mellifera* from the same location (Joshi, 1999). Honey bees (*Apis mellifera* L.) feed mainly on nectar and pollen (Imdorf et al., 1998). *Apis dorsata, A .laboriosa, A. florea* and *A. cerana* bees collect more honeydew and have higher electrical conductivity than *A. mellifera* bees. The honeydew honey has higher content of enzyme invertase, and glucose oxidase than that of nectar or blossom honey (Joshi, 1999). In addition, generally it was found that higher the electrical conductivity higher is the pH value (Joshi et al.,1998). Another investigation claimed, that the low honey pH was responsible for the antibacterial activity (Yatsunami and Echigo, 1984). Joshi (1999) found different pH values 3.78 for *A. florea*, 3.93 for *A. laboriosa,* 3.68 for *A. dorsata* (Chitwan), 3.2 for *A. cerana* (Chitwan), and 3.52 for *A. mellifera* (Chitwan), 3.75 for *A. cerana* (Kahmandu), 3.23 for *A. mellifera* (Kahmandu) honeys from Nepal. However, studies in which acidity was taken into account found no correlation between antibacterial activity and the pH of the honeys studied (Bogdanov et al., Daghie et al., 1971; Plachy, 1944; Rachlik and Dolezal, 1961; Stomfay-Stitz and Kominos, 1960). Only in undiluted honey the acidity is a significant antibacterial factor.

Peroxide antibacterial activity exhibited by the honeys from native bee species. *A. florea, A. laboriosa A. dorsata* and *Apis cerana* may also be due to honeydew honey.

Antibacterial activity of *Apis cerana, A. dorsata and A. mellifera* honeys from Chitwan

Comparisions between the sensitivity to honey produced by *Apis cerana, A. dorsata, and A. mellifera* in Chitwan showed significant differences in peroxide antibacterial activity. Whereas, no significant differences between the activity to local bee species *Apis cerana* and *Apis dorsata* honey was observed (fig. 2, table 2). The mean values with *Apis cerana* honey was 4.8 (table 2).

Apis mellifera bees in Nepal is being treated with oxytetracycline to control bacterial diseases such as AFB and EFB. The oxytetracycline content in two *Apis mellifera* honey samples from Kathmandu was amounted 5730 μg/kg and 932μg/kg. This could be the reason behind good antibacterial activity showed by *Apis mellifera* honey. But rest of the honey samples from *Apis mellifera*, and *A. cerana* honeys from Kathmandu & Chitwan and *A. dorsata* honey from Chitwan did not show sulfonamide and oxytetracycline residue.

There is a possibility that the hydrogen peroxide produced in the honey contributes to peroxide antibacterial activity.Variations in antibacterial agents and in the amount of hydrogen peroxide explain the wide ranging effects of honey from different plant sources (Molan, 1992ab).

Surprisingly the honey samples from Jumla, where honeydew flow is high, did not show sensitivity to the bacteria (fig 1, table1). This result has came through beyond the expectations. Dustmann (1979) suggested that the glucose oxidase in honeys derived from certain floral sources may be more susceptible to destruction by light. As well, the limited number of samples do not preclude its quality for antibacterial activity. The amount of enzymes added to nectar or honeydew depends on various factors such as age, diet and physiological stage of the bees, strength of the colony, pollen consumption, temperature etc. (Brouwers, 1982, 1983; Simpson, 1968; Persano Oddo et al., 1999). The concentration of the enzymes also depends on the period of time; how long and how intensively bees work in making honey (Von der Ohe, 1996).

The hydrogen peroxide amount in honey is very small and it can be produced only in after aerobic incubation of diluted honey solutions, which might mean that it is not very important for the antibacterial activity of honey (Bogdanov, 1984) therefore non-peroxide activity is more important (Gonnet and Lavie, 1960; Mohrig and Messner, 1968; Radwan et al., 1984). On the other hand, White et al., (1963); White and Subers (1964ab); and Dustmann, (1972, 1979) demonstrated that glucose oxidase-hydrogen peroxide system is the most important factor. The quality of glandular secretion may also vary from species to species, for instance within a same period of time *Apis dorsata* may add more glandular secretion in the honey.

Therefore in the case of Nepali honeys, the major part of anibacterial antibacterial activity (peroxide) observed in this study came from bees.

There is a highly significant correlation between the free acidity, the diastase and the invertase activity on one hand and the bacterial inhibition on the other a part of the non-peroxide antibacterial activity has a bee origin (Bogdanov, 1996).When many honeys are diluted, a bee derived enzyme (glucose oxidase) present in the honey is activated and catalyses the slow generation of hydrogen peroxide which inhibits bacterial growth (White et al,. 1963). This activity varies markedly from honey to honey (Molan, 1992a). In some honey, additional antimicrobial activity following dilution is due to activation of bee-derived glucose oxidase, which catalyses slow release of hydrogen peroxide from the glucose containes within honey (Cooper et al., 2002).

The enzyme catalase had long been thought to be present in honey (Schepartz, 1966). Catalase comes from the pollen and nectar of certain plants; more coming from the nectar (Dustmann, 1971). Schepartz and Subers (1966) have suggested that certain floral sources have different levels of peroxide activity as a result of different amounts of catalase being present from different types of plants. In the destruction of hydrogen peroxide associated with floral sources is due to the plants contributing catalase to the honeys (Molan, 1992a). Honeys from some floral sources have been found to have very high levels of catalase, and these honeys accumulate low levels of hydrogen peroxide: the ones accumulating high levels of hydrogen peroxide had low levels of catalase (Dustmann, 1971, 1972). The variation in the antibacterial activity or non-peroxide activity was found from different floral sources (Popeskovic et al., 1983; Bogdanov, 1996, 1997; Hegazi et al., 2001) and the presence of flavonoid in honey may attribute to the antibacterial action (Sabatier et al., 1992; Dunford et al., 2000a).

Apart from mustard and some selective cash crops such as cotton, jute, sugar cane and tobacco in the terai and inner terai, there does not exist any extensive monoculture of any crops in Nepal. Also, among wild vegetations the bee forage elements are sparse and mixed well within the foraging distances of the bees. Under such intensive situations it is not possible to find pure unifloral honeys (Kafle, 1979, 1984; Kafle, 1992).

Adcock (1962) found that increasing the amount of catalase added removed the residual activity (hydrogen peroxide). The amount of catalase added was sufficient to destroy the antibacterial effect of hydrogen peroxide. In other study (Allen et al., 1991) finding similar results, it could be seen that the catalase was effective in use, in that it removed all detectable activity from New Zealand honeys with very high levels of activity.

In this study, the honey, which has a non-peroxide antibacterial activity that is affected by catalase, may be from different plant sources or due to other substances exist in honey. No significant differences in non-peroxide activity were found between the bee species and locations. On the other hand, peroxide antibacterial activity showed by *A. cerana* and *A. dorsata* (which often feed on honeydew) could be due to hypopharyngeal gland secretion like *A. florea* and *A. laboriosa.* Although the concentration of hydrogen peroxide in the honey samples was not measured in this study.

The organism used for this assay, *Staphylococcus aureus*, was found by Dustmann (1979) to be the most sensitive to hydrogen peroxide. It was also selected by Dold and Witzenhausen (1955) for determining the inhibine number of honeys. The bioassay used would of course not give a correct result if other antibacterial substances were present, but would give the (total) activities of hydrogen peroxide that could be present, including other form of activity (non-peroxide) by addition of catalase.

In the present study in which non-peroxide antibacterial activity were found, may explain the different findings of some other workers. Dustmann (1979) reported the existence of non-peroxide activity but concluded that it was only a minor portion of the total activity.

Those who found that all of the activity could be attributed to bee origin may have been working with honeys (especially from native bees) which had relatively good peroxide antibacterial activity. Interestingly, not a single honey sample found to have only non-peroxide activity in this study. The data presented from the present study show the variation between different honey types, the existence of non-peroxide activity occuring in honeys with high overall activity.

It is very important to mention that the agar well diffusion technique used is one of the good techniques.The test solution becoming diluted as it difuses into the agar. The inhibine number of Dold and Witzenhausen (1955) is determined by incorporating the honey, at various standard dilutions, in the agar before the plates are poured. The highest inhibine number was assigned to inhibitory activity with the honey diluted to1/20 of its original strength. Even with the agar well diffusion technique, several honeys used by Molan and Russel (1988) was found to have quite high activity when tested at 1/30 of their original concentration and the most active prevented growth around the well at a dilution of 1/40.

As in the research presented by Allen et al., (1991), an agar well diffusion assay was used. In such assays, sensitivity is often low as samples are diluted as soon as they diffuse into the agar (Cooper, 1963). The logarithm of the concentration of an antibacterial substance is proportional to the square of the extent of the zone of inhibition in an agar diffusion assay

(Cooper, 1963). However, the technique allows the comparison of the relative activity of different honeys to be made reliably. The method has advantage, however, that it allows large numbers of samples to be analysed quickly and efficiently. It is also considered an appropriate model for the application of topical antibacterial agents into infected areas where there is little blood supply (Heggers et al., 1987). Despite this, it is plausibe that honeys previously thought to be inactive may actually have antimicrobial activity when measured using a more sensitive assay, albeit at low levels. Such low levels of activity are likely to not be useful as antibacterial agents (Brady et al., 2004).

It can be clearly seen that in general, where a honey has a high antibacterial activity, this is because it has a good non-peroxide activity in addition to the more generally recognized hydrogen peroxide in contrary to White et al., (1963); White and Subers (1964ab) and Dustmann, (1972, 1979).

Finally, the antimicrobial potency of honey varies with its geographical, seasonal and botanical source as well as through harvesting, processing and storage conditions.

Thus, only honeys of proven high potency should be used to treat infected wounds (Natarajan et al., 2001).

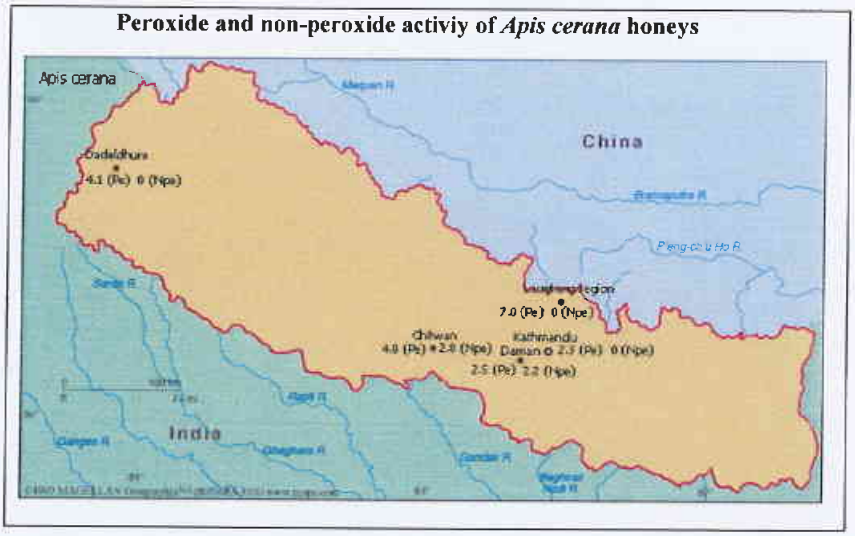
The honeys produced in Chitwan from *Apis cerana, A. dorsata* and *A. mellifera* showed peroxide and non-peroxide antibacterial activity as well. Only the honey from *Apis cerana* bees produced in Chitwan and Daman showed both peroxide and non-peroxide antibacterial activities.Whereas, *A. cerana* honey Langtang showed relatively high peroxide activity than those of Dadeldhura and Kathmandu. Honeys from *Apis cerana, A. dorsata, A. florea, A.laboriosa and A. mellifera* exhibited peroxide and non peroxide activities as well.

The levels of peroxide antibacterial activity did not differ dramatically within a floral source. It is possible that the levels of antibacterial activity observed may have been due to bee origin. Although, hydrogen peroxide was not only antibacterial substance involved in Nepali honey samples there may be phytochemical or other additional factors contributing for antibacterial activity in Nepali honey. This additional activity is presumably of floral origin, the high activity being generally associated with honeys from some floral sources (Molan et al.,1988). The presence of antibacterial factors in honey other than hydrogen peroxide is also a possibility, with research indicating that antioxidants may contribute to the antibacterial activity of a honey. More understanding of the mechanisms involved in different bee species and floral sources would be beneficial, enabling information to be generated regarding the heat and light stability of particular factors, the diffusibility of different factors, as well as any

effects of long-term storage and synergistic/ antagonistic effects with other antibacterial agents. Further investigation is required.

In this study of 164 samples, peroxide activity as found to be associated with all bee species exist in Nepal (except *A. carana* honey from Jumla). Non-peroxide activity was also found to be associated with all honey types except *A. cerana* honeys from Dadeldhura, Jumla, Kathmandu and Langtang. Peroxide activity of the total honey samples accounted for 46.34%. Whereas non-peroxide activity accounted for 13.41% of the total samples. The antibacterial activity shown by honeys from different bee species may have been due to bee origin. It was found that in the honeys with good antibacterial activity a main part of this activity was due to a factor hyrogen peroxide. However, there may be phytochemical or other additional factors presumably of floral origin contributing for (non-peroxide) antibacterial activity in Nepali honeys other than hydrogen peroxide.

Peroxide and non-peroxide activiy of *Apis cerana* honeys



CHAPTER FOUR

4.1 Introduction

Distribution of Elements: An overview and Environmental Monitoring

The past century has seen a tremendous expansion in the number of synthetic chemicals employed by humankind as materials, drugs, preservatives for foods and other products, pesticides, cleaning agents and even weapons of war.

An estimated 64,000 chemicals are currently in use commercially, with 5 billion tons being produced worldwide. Four thousand of them are used as medicinals and at least 1,200 more as household products. An estimated 700 new chemicals are synthesized each year (Philp, 1995). Add to this the numerous natural substances, both inorganic and organic, that possess toxic potential, and it is little wonder that the public expresses concern, and sometimes even panic, about the harmful effects these agents may exert on their health and on the environment. Many of these agents, perhaps 50,000 of them, have never been subjected to thorough toxicity testing (Philp, 1995).

About 500 chemicals have been evaluated for carcinogenic potentials. Some 44 have been designated as possible human carcinogens on the basis of evidence, either limited or conclusive, obtained from human studies. Of these, 37 tested positive for carcenogenicity in animals tests prior to the identification of this effect in humans (Philp, 1995).

Metals are are components of all biological system, stable environmental contaminants, therefore, they tend to accumulate in the biosphere, being preferentially stored in soils, sediments and present in foods either naturally, as a result of human activities, from contamination during manufacture/processing and storage, or may be added directly. Metalloids are distributed everywhere in nature and cover a large concentration interval between mg/kg and more to ng/kg and less, and participate in a number of important geochemical and biochemical process many of these compounds have serious effects on living organisms due to their toxicity and bioaccumulation in various environmental compartments (Djingova and Kuleff, 2000; Pacyna, 1996). While metals are neither created nor destroyed in biology, they may be significantly altered chemically through metabolism and other chemical reaction (Jacobs, 1996).

Metals that are generally toxic when their exposures are excessive. When considering their impact it is necessary to distinguish between poisonous substances that have a toxic effect on organisms even at low concentration.

There are approximately 30 elements that are practical toxicants. Some are biologically essential nutrients, others may have no known beneficial effects or functions (Jacobs, 1996). There are so-called microelements that occur in low concentrations in organisms and are essential for the life of most organisms (Markert et al., 2000). Both macro- and micro- elements are nutrients that are necessary for the growth and normal development of organisms and whose function cannot be taken on by any other element.The are therefore èssential`. For this reason, macro- and micro- elements are also called macro-or micro-nutrients (Markert et al., 2000). Minerals such as copper, chromium, Iron, magnesium, chromium, zinc, manganese, molybdenum selenium, and cobalt are the elements that are essential for human health, whose inevitably results in deficiency symptoms or loss of proper functioning, therefore needed to sustain life (Suzuki and Suzuki, 1996). The absence or deficiencies produce multiple and diverse clinical signs and symptoms but may be toxic at sufficiently high intakes (Khursid and Qureshi, 1984; Hu, 2002) and their excess intake possess an important threat to human health. Trace elements occur in the body in very small or `trace` amounts (i.e. mg or µg/kg body weight); they generally constitute less than 0.01% of the body mass (Markert et al., 2000). They are considered essential when a deficient intake produces an impairment in biological function, and when supplementation with physiological levels of that reverses the impaired function or prevents an impairment.

Toxic metals (such as lead, cadmium, mercury and arsenic) are widely found in our environment (Markert et al., 2000) in different forms that may have health implications leading to adverse effects and directly related to human health (Hu, 2002). For instance, Lead causes various health effects on virtually every system in the body , and the severity is dependent on the level of exposure and individual sensitivity (Fan, 1996).

Exposure to (these) metals can occur through a variety of routes such as dietary intake (one of the major routes of human exposure to the contaminants), breast milk and in various food products and drinking water, which are also transferred to the foetus, constitute a severe threat to the health of infants and children (Zetterström, 1999; Markert et al., 2000; Ercal et al., 2001). The intensity and extent of intake influence the actual concentration of an element in the organism (Baker, 1981). The tendency of these metals to accumulate in select tissues of the

human body; and their overall potential to be toxic even at relatively minor levels of exposure (Hu, 2002).

Ideally, intake by the organism is directly proportional to the availability of nutrients. In this case the specific element concentration in the organism reflects the concentrations in the environment. A positive relationship exists between the dose of a pollutant and the harmful effect it has on the organism, i.e. the more of substance is taken in , the greater is the effect. The receptor theory assumes that a pollutant that acts as an against or antagonist is present in a concentration sufficient to occupy all the receptors of an organism, organ or tissue. Instead of the dose-i.e. the quantity of the substance taken in per kg of body weight- the concentration of a pollutant resulting from exposure through water in the case of aquatic organisms or through the ambient air may be used as a reference value (Markert et al., 2000).

The geographic distribution and concentrations of metals are commonly found in different environmental media such as contaminated air, water, soil, plant and food are important factors contributing to the extent of human or ecological exposures (Markert et al., 2000).

Since ancient times, the trace element deposition onto soils has been the cause of many human ailments (Anonymous, 1989). Problems of the deteriorating environmental quality which has brought about when ancient nomadic tribes first settled into villages and started utilizing fire, cultivating land, and producing wastes, have become increasingly acute because of the exponential population growth and global industrialization (Chang and Page, 1996). Soil contaminants are common in industrialized countries, causing widespread contamination directly of soil and indirectly of ground water and food (Monarca, 2002).

The potential environmental impact of the trace elements depends on thier location within a catchment. If they are situated in the upper part, a large area and many different environmental compartments will be affected. Local contamination of soils, plants, sediments and water occurs almost in every case, the degree to which a whole catchment is affected by contaminated waters and glacial or alluvial sediments depends on the size of the ore deposite, the slope and the climate of a given area (Pfeifer et al., 2000). Trace elements in terrestrial and aquatic environments are characterized by being spatially variable in their concentrations. Heavy metals and organic pollutants are adsorbed by plankton at the base of the food web and biomagnified to significant levels at higher trophic levels (Bard, 1999). The total element concentration is an initial indication of the occurrence and distribution of individual trace elements in the environment.

In general the concentration of trace elements varies in different compartments of the environment according to their input quantities and dilution behaviour, including transport and

accumulation.The inputs of many trace elements to soil, animal or plant is greater than the losses leading to a net accumulation (Markert, 2000).

Interaction between elements in the form of competitive inhibition or promotion may have a decisive influence on the physiology of certain organisms (Markert et al., 2000). Data suggest that antioxidants may play an important role in abating some hazards of heavy metals. In order to prove the importance of using antioxidants in heavy metal poisoning, pertinent biochemical mechanisms for metal-induced oxidative stress should be reviewed (Ercal et al., 2001).

The nature of soil is constantly evolving in response to inputs of matter from natural and anthropogenic sources and to interactions among soil constituents. During the course of manufacturing industrial processing, and product consumption, these chemicals may be released deliberately or inadvertently into the environment. Environmental contamination of air, water and soil has become a potential threat to the safety of food.

The soil-borne trace elements are particularly important, because food chain transfer is by far the most significant route of human exposure to trace elements (Prasad, 1993). Food fiber and many products essential to sustain human life come directly or indirectly from plants which are supported by soils. Plant roots penetrate into the subsoil from which they may take up heavy metals, resulting in accumulation in the plant. Through food grown on soils, human obtain energy and essential elements (Chang and Page, 1996) and also may be exposed to harmful substances that area absorbed by plants. Both the presence of potentially toxic substances and the absence of essential elements in the soil could result in disorders (Chang and Page, 1996; van Lune and Zwart, 1997). A high input of agrochemicals, such as phosphate fertilizers may cause an inadvertent addition of heavy metals, which are contained as impurities (de Lopez Camelo et al., 1997).Under many circumstances, elements may bioaccumulate through food chain transfer and affect organisms not directly exposed to the toxic substances. If the soil is not contaminated, human exposure to trace elements through food consumption is significantly below the provisional tolerable intake published by the FAO/WHO Expert Committee on Food Additives (WHO, 1989) and is not substantially affected by the diets. (Chang and Page, 1996). Therefore, trace elements deposition in soils and bio geochemical interactions between those present in soils and the vegetative cover are controlling factors in human exposure to toxic elements in the environment (Chang and Page, 1996).

Asian people have been subjected to long-term exposure to various pesticides (Zetterström, 1999), and in addition to organic mercury and heavy metals, such as lead and cadmium in huge quantities through numerous sources, including contaminated air, water, soil and food.

Recent study (Hasspieler et al., 1997) indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities associated with these metals might be due to oxidative tissue damage. Redox-active metals, such as iron, copper and chromium, undergo redox cycling whereas redox-inactive metals, such as lead, cadmium, mercury and others deplete cells' major antioxidants, particularly thiol-containing antioxidants and enzymes. Molecular biology and DNA probe novel in vitro methods for assessment of environmental toxicity using a human cell-line and other several studies including undesirable health effects or tissue pathology at exposure or intake levels (of the nutritionally essential metals) are underway to determine the effect of antioxidant supplementation heavy metals which are components of all biological system.

Table 1. Metals of Practical Toxicological Significance

Biologically Essential Metals		
Cobalt (Co)	Copper (Cu)	Chromium (Cr)
Iron (Fe)	Magnesium (Mg)	Manganese (Mn)
Molybdenum (Mo)	Selenium (Se)	Zinc (Zn)
Metals with no established biological functions		
Al	Sb	As
Ba	Be	Bi
Cd	Ga	Ge
Au	In	Pb
Li	Hg	Ni
Pt	Ag	Sr
Te	TI	Sn
Ti	V	U

NRC (1986)

Table 2. Essential Elements For Plants and Animals

Plants and Animals **Major elements**	Plants only
Calcium (Ca)	Boron (B)
Carbon (C)	
Hydrogen (H)	
Magnesium (Mg)	
Nitrogen (N)	
Oxygen (O)	
Phosphorus (P)	
Sulfur (S)	
Micronutrients	
Chlorine (Cl)	
Copper (Cu)	
Iron (Fe)	
Manganese (Mn)	
Molybdenum (Mo)	

Jones, Jr. (1998)

4.2 Biological Markers and Biomonitoring

Biomonitoring represents a method of assessing metal pollution of aquatic and terrestrial ecosystems (Fan, 1996). To understand biochemical changes induced by factors that are independent of environmental concentrations of contaminants, such as the influence of food levels and the potential for metabolic adaptation in biomonitoring organisms is needed (Hickeya et al., 1996).

Exposure to toxic metals is often assessed by measurements in air, foods, and/or water, and sometimes also in soil and other materials, ie. environmental monitoring (Gerhardsson and Skerfvig, 1996). Attentions to environmental contaminants investigation in bee and bee products have also been devoted. In order to determine exposure to or intoxication from a metal, it is usually necessary to conduct some type of metal analysis of either a tissue and/or fluid and /or the exposed substance (Jacobs, 1996).

In past decade, new possibilities for biological monitoring of elements have been developed. The use of biomarkers for assessment of exposure to metals may be employed for biological monitoring. The use of biomarkers for assessment of the exposure to elements have been employed to give information about the risk of toxic effects in the exposed individual/groups. Biomarkers may also be used for health monitoring/surveillance. i.e., as bioindicators of effects, early health effects, and/or overt health impairment. The biomarkers take into account exposure from different sources (air, foods, water, soil, etc.) and through various routes (inhalation, gastrointestinal, and skin) and also, they may reflect exposure during different time periods. In some cases, the biomarker may reflect exposure during long periods, up to decades. In others, it only indicates the most recent uptake (Gerhardsson and Skerfvig, 1996). The determinations of the chemical pollutants in food and human body are important in environmental monitoring for the prevention, control and reduction of pollution as well as occupational health, legal decisions and epidemiological studies (Hura et al., 1998). For certain geographical areas, current dietary exposures to methyl-mercury and to cadmium are high enough to indicate a need for public health measures (Berdanier, 2002).

Numerous types of analytical methods employing a variety of determinative techniques are available to the analytical chemist for determining the analytes in biological tissues. For a long time, the possibilities of biomonitoring were restricted by the limited performance of the analytical techniques. However, the situation was dramatically improved by the introduction a few decades ago of the neutron activation and atomic absorption techniques. Recently, new possibilities have been offered by the inductively coupled plasma/mass spectrometry technique,

which allows analysis of low concentration of small samples, and determination of many elements simultaneously (Gerhardsson and Skerfvig, 1996).

Mostly, the content of the metal itself is analysed in biological samples. In most cases, blood (whole blood, plasma, or erythrocytes), urine, or hair are used as the index media for biological monitoring. For some elements, subclinical biochemical effects of the exposure have been widely used for biological monitoring of exposure. For instance, effects on heme synthesis have been widely used for assessment of lead exposure (U.S.EPA, 1986; Skerfving, 1988, 1993) with lead concentration in urine (Skerfving et al., 1993), calcified tissues (Needleman et al., 1979; U.S. EPA, 1986; Skerfving, 1988, 1993, Skerfving et al., 1993). In addition, Cadmium concentration in urine (Nordberg and Nordberg, 1988), kidney and liver (Vartsky et al., 1977; Roels et al., 1981; Ellis et al.,1984; Morgan et al., 1990; Chettle and Ellis, 1992), aluminium in lung, liver, bone, muscles, and brain (Skalsky and Carchman, 1983; Elinder and Sjögren, 1986), cobalt in blood and urine (Ichikawa et al., 1985; Stebbins et al., 1992; Alexandersson and Lidums, 1979), manganese in urine (Roels et al., 1987b), and nickel in serum,urine and feces (WHO, 1991b; Hassler et al., 1983; Sunderman, 1988). Plasma boron concentration below 25 ng/mL might be indicative of low boron status (Nielsen, 2002). The intensity and extent of intake influence the actual concentration of an element in the organism (Baker, 1981).

The potential human exposure to these chemicals with the associated toxicological impacts have been receiving increased attention. The studies on the behaviour of trace metals in the environment have concluded that many of these compounds create seroius problems in human health due to their toxicity.

Therefore biological monitoring has the advantage of measuring integrated exposure from all sources.

4.3 Elements: Human and Plant

Trace elements essential for life generally occur in the body in microgram per gram of tissue, and are usually required by humans in amounts of milligrams per day; these elements are copper, iron, manganese, and zinc (Nielsen, 2002).

Bilological role

Trace elements have at least five roles in living organisms. In close association with enzymes, some trace elements are integral parts of catalytic centers at which the reactions necessary for life occur. Working in concert with a protein, and frequently with other organic coenzymes , trace elements are involved in attracting substrate molecules and converting them to specific end

products. Some trace elements donate or accept electrons in reactions of reduction or oxidation. In addition to the generation and utilization of metabolic energy, redox reactions frequently involve the chemical transformation of molecules. One trace element, iron , is involved in binding, transporting, and releasing oxygen in the body. Some trace elements have structural roles; that is, imparting stability and three-dimensional structure to important biological molecules. Some trace elements have regulatory roles.They control important biological processes through such actions as inhibiting enzymatic reactions, facilitating the binding of molecules to receptor sites on cell membranes, altering the stucture or ionic nature of membranes to prevent or allow specific molecules to enter a cell, and inducing genes to express themselves resulting in the formation of proteins involved in the life processes (Nielsen, 2002). In addition, homeostatic regulation of mineral elements is important which describes the ability of the body to maintain the content of a specific substance within a certain range despite varying intakes. It involves the processes of absorption, storage, and excretion. The relative importance of these three processes varies among the trace elements. The amount absorbed from the gastrointestinal tract often is a primary controlling factor for trace elements needed in the cationic state such as cooper, iron, and zinc. Trace elements absorbed as negatively charged anions, such as boron and selenium, are usually absorbed freely and completely from the gastrointestinal tract. Excretion through the urine, bile, sweat, and breath is, therefore, the primary mechnism for controlling the amount of these trace elements in an organism. By being stored in inactive sites, some trace elements are prevented from causing adverse reactions when present in high quantities. For example,the storage of iron in the form of ferritin. Release of a trace element from a storage site also can be important in preventing deficiency (Nielsen, 2002).

Table 3. Approximate concentrations of trace elements in mature leaf tissue generalized for various species.

Element	Sufficient or Toxic (mg/kg)	Excessive (mg/kg)
Cadmium (Cd)	0.05-0.20	5-30
Chromium (Cr)	0.1-0.55	5-30
Lead(Pb)	5-10	30-300
Nickel (Ni)	0.5-5	10-100
Vanadium (V)	0.2-1.5	5-10
Cobalt (Co)	0.02-1	15-50
Lithium (Li)	3	5-50

Pendias and Pendias (1994)

Studies suggest that arsenic, chromium, copper, nickel and zinc are unlikely to cause problems for plants, animal, or human health, primarily because they are not found in high concentartions in MSW (Metropolitan Solid Waste) compost and/ or are not readily taken up by plants.

4.4 Why Honey From Nepal

Honey as an Environmental Marker and Biomonitoring Tool for Environmental Contamination.

Environmental pollution from natural and anthropogenic sources heavy metals may contributes to the poor health of people living in the polluted areas. The modern life style in connection with an enormous increase in the use of vehicles, the massive increase in power generation and the industrialization of agriculture have added dramatically to the number of pollutants in the environment. Each year thousands of man-made chemicals are released without knowing their effects on mankind and environment (Breulmann et al., 2000).

On the other hand, traditional foods such as honey are also an economic necessity in many communities. The concept of health in indigenous groups includes social, cultural, and spiritual dimensions. The harvesting, sharing and consumption of traditional foods are an integral component to good health among aboriginal people influencing both physical health and social well-being. Consequently, the contamination of country food raises problems which go far beyond the usual confines of public health and cannot be resolved by health advisories or food substitutions alone. The primary exposure pathway for the contaminants considered in this work is through the traditional diet. Consumers of traditional foods are exposed higher than non-consumers of traditional foods due predominantly to the bioaccumulation of natural elements in the food chain. The deterioration of human health with increasing infant mortality rate, declining life expectancy at birth and increasing prevalence of serious infectious diseases in country is thought to be due to a combination of several factors such as inadequate nutrition, poor sanitation, collapse of the health care system and pollution from agriculture and industries. Risk determination for contaminants in country food involves a consideration of the type and amounts of food consumed and the sociocultural, nutritional, economic, and spiritual benefits associated with country foods. Risk management options that minimize the extent to which nutritional and sociocultural aspects of aboriginal societies are compromised must always be considered (Oostdama et al., 1999).

The elemental composition of plants usually reflects the geochemical features of the environment where they grow (Djingova and Kuleff, 2000). Plants act as intermediate reservoirs of heavy metals from primary sources, e.g. soils, water or air, through which they move on to the organisms (Breulmann et al., 2000). Plant play an important role among the ecological components and are responsible for transmitting elements to further to man and animals . Heavy metals accumulated by plants may lead to the presence of heavy metals in bee collected pollen and nectar (and other bee products). The existance of essential or toxic elements in the honey

may reflect the concentrations in the environment and honey quality is also affected. Environmental pollution effects the chemical composition of honey and honey quality (Antonescu and Mateescu, 2001) and may be an indicative character. In addition, there is positive correlation between honeydew excreting insect population density and honeydew honey flow (Pechhacker, 1976) and correlated with an increase in the potassium and phosphorus levels (Horn, 1985) and may be other elements as well. Honey bees (*Apis mellifera* L.) feed mainly on nectar and pollen (Imdorf et al., 1998) and *Apis cerana* bees collect more honeydew honey (Joshi,1999). Honeydew honey has a higher total content of minerals (ash) than nectar or floral honeys (Crane, 1990; Bicik and Kaspar, 1986). Therefore honey bees would appear to have potential in this respect, since by foraging they effectively `sample `their surroundings for the constituents in or on the forage plants, and hence in the soil and the atmosphere of the area (Jones, 1987) and could reflect the honeydew flow in the same floristic region.

In addition, honey is widely used food and has impressive array of trace elements and minerals that are important to human nutrition and has been considered a suitable material for monitoring environmental contamination (Tong et al., 1975; Crane, 1984; Bromen-Shenk et al., 1985; Pinzauti et al., 1991; Raes et al., 1992; Leita et al., 1996; Jones, 1987; Przybyyiowski et al., 2001; Pechhacker, 2002). Honey can characterize the level of soil, plant and air pollution (Fodor and Molnar, 1993) and indicate the heavy metal contamination, in the environment. It is also increasingly used to monitor the distribution of various hazardous pollutants including radionuclides, heavy metals, radionuclides, pesticides, and organic contaminants such as polychorinated biphenyls (Leuzzi and Mincione, 1992; Drjuric et al., 1996; Rifai and Akeel, 1997; Fritzsch and Bremer, 1975; Bromenshenk, 1979; Bromenshenk et al., 1985, 1991; Wallwork-Barber et al., 1982; Celli, 1983; Anderson and Wojtas, 1986; Morse et al., 1987). In other words, the element content of bees and bee products recognized as useful indicator of the presence of specific minerals within their forage area (Crane, 1984; Free et al., 1983). Foraged pollen may also be used as a preliminary ´prospecting` technique for mineral ores (Pinsent in Warren, 1982). The trace elements content of pollen has been reported by other workers (Fawcett et al., 1971; Knight et al.,1972; Ernst and Bast-Cramer, 1980; Warren, 1982).

No work so far has been carried out to determine (trace) elements in bees from Nepal and its products and there is a growing interest in expanding beekeeping and other forms of natural resource production in peri-urban and rural areas of Nepal. The major areas of nutritional concern, specifically found to be related to poverty in Nepal include energy intake: undernutrition, insufficient intake of some micronutrients and scourge either through morbidity

or mortality and honey is popularly consumed health food. Metals essential in the human diet are mainly sodium, potassium, calcium, manganese, boron, zinc, copper, chromium, selenium,molybdenum, manganese and , cobalt (Crane, 1984) present in honey. Its trace elements may have a widespread impact on the health of individuals in communities. On the other hand, it is best understood that excess intake or sufficiently high intakes of an essential mineral also becomes toxic and can cause adverse effects on human health.

Monitoring of excess exposure (the goal of public health actions) of environmental contaminants through different sources (air,water, food etc.) and their reduction should be a prudent practice throughout the population. In indigenous communities in particular, this should be done without threating the social, cultural, spiritual, and physical well-being that is connected to collecting, sharing, and consuming traditional foods. Traditional foods have known nutritive value. Both research and assessment procedures are required to ensure that natural resource production and their utilization in rural areas also safeguards human health.

The nutritional programme design and evaluation at the community level could be implimented in Nepal. Research focus could mostly be link socioeconomic factors of poor and marginalized populations with health outcome, or to a limited extent, to link food and nutrient intake with poverty. The challenge becomes one of designing and implimenting effective beekeeping promotion coupled with nutrition programmes which will help to alleviate poverty, health and nutritional problems and environmental monitoring thereby promote better overall health and livelihood.

Therefore when it is not always clear what public health measures should be taken to reduce the exposure of populations who consume traditional foods consequently, it could be recommended that consumption of traditional food continue, with recognition that there is a need for dietary advice to the people so they can make informed choices concerning the foods they eat (Berdanier, 2002).

4.5 Literature Review

Elements in Bee Products

Honey bees (*Apis mellifera* L) are used to monitor the distribution and impact of various hazardous chemicals, including trace elements, heavy metals, radionuclides, pesticides, and organic contaminants such as polychlorinated biphenyls (Fritzsch and Bremer, 1975; Bromenshenk, 1979, Wallwork-Barber et al.,1982; Celli, 1983; Bromenshenk et al.,1985, 1991; Anderson and Wojtas, 1986; Morse et al., 1987).

The potential use of honey for mineral indicating levels of environmental contamination extensively documented.

Forte et al., (2001) determined As, Cd,Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn in honey as a candidate reference material for trace elements.

Przybyyiowski and Wilczyiika (2001) investigated contents of Zn, Cd, and Pb in Polish honeys from Pomeranian region using AAS. Results suggested that honey may be useful for assessing the presence of environmental contaminants.

Conti and Botre (2001) analysed Cd, Cr, and Pb contents of honeys, pollen., propolis and wax from Rome. The result revealed honeybees and, to a lesser extent, some of their products (pollen, propolis, wax, but not honey), can be considered as representive bioindicators of environmental pollution.

Caroli et al., (1999) reported certain elements measused by inductively coupled plasma (ICP) –based techniques. The ranges ascertained were as follows (in ng/g): As (<0.50-0.70); Cd (<0.50-0.74); Cr (1.03-3.93); Cu (144-216); Fe (191-651); Mn (223-580); Ni (17-49); Pb (3.20-186); Pt (<0.50); Sn (<4-27); V (1.22-1.94); and Zn (565-1144). The experience gained with this exploratory study revealed concentrations of elements in honey from different beehives were similar. A few exceptions were noted for the As, Cu, Fe, Ni and , Zn.

Caroli et al., (2000). Determined As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn by inductively coupled plasma (ICP)– based techniques. The concentrations (ng/g) found in *Robinia* honey were 1.28 $\pm$ 0.09 for As, 0.59 $\pm$ 0.08 for Cd, 2.36 $\pm$ 0.21 for Cr, 57.6 $\pm$ 3.2 for Cu, 209 $\pm$ 9 for Fe, 90.8 $\pm$ 3 for Mn, 18.1 $\pm$ 0.6 for Ni, 23 $\pm$ 1 for Pb, 8.10 $\pm$ 0.35 for Sn, 1.19 $\pm$ 0.37 for V, and 178 $\pm$ 4 for Zn.

Similarly elemental content found in *Eucalyptus* honey were 3.18 $\pm$ 0.21 for As, 0.70 $\pm$ 0.08 for Cd, 2.73 $\pm$ 0.22 for Cr, 141 $\pm$ 6 for Cu, 926 $\pm$ 16 for Fe, 1905 $\pm$ 81 for Mn, 7.77 $\pm$ 0.4 for Ni, 138 $\pm$ 4 for Pb, 7.97 $\pm$ 0.16 for Sn, 3.47 $\pm$ 0.15 for V, and 405 $\pm$ 9 for Zn.

Pollen spectra , mineral (ash) and sediment quantification in honeys produced in the region of Murca, Spain were analysed. All 18 Spanish honey samples were found to be low in mineral content (Breis et al., 1995).

Rodriguez-Otero et al., (1994, 1995) analysed element contents of the honeys produced in galicia, north-west Spain indicated that potassium was the most abundant of the elements determined, with an average content of 1572 mg/kg followed by Ca 102 mg/kg, Na 138mg/kg, Mg 106 mg/kg, and Fe 5.12 mg/kg. Coefficient of variation of minerals Na, Ca, S and F contents of the honeys were within the ranges of 0.34 to 0.71 and the values for P and Cl were found to be high.

Honeydew honeys showed elevated levels of K, Na, Mg, Fe, Cu and Mn (Crane, 1975). The colour and trace element composotion of honeys produced in major vegetation areas of Ghana studied by Yeboah-Gyan and Marfo (1998). Calcium, phosphorus, maganesium, potassium. Iron, manganese and copper were detected by Atomic Absorption Spectrophotometry (AAS). The results suggested that the concentration of all the elements was generally much higher in the dark samples than in the light ones, and it was attributed to differences in nectar-plant species .

Such concentrations do require the use of sensitive analytical techniques. In addition, reliable interpretation of results may be hampered by variables about which little is known as present. These variables may be: contamination of the sample during preparation, purification and storage; inherent differences between types of honey (light/dark-Petrov, 1970; nectar/honeydew- Kirkvliet, 1983;honey pH- Mitchell et al., 1954); the season and the flowers visited (Ernst and Bast- Cramer, 1980) and differences in the size of foraging areas (Hakonson and Bostick, 1976). Pechhacker (2002) investigated the influence of plant origin, soil, environment and storage on spectrum of mineral nutrients and trace elements in rape, robinia and sunflower honeys from three locations of Austria and compared with honeydew, floral and mixed honey samples from Austria and Greece. In the study plant origin proved to be most significant factor and most significant differences appeared in B, Cu and Mn. Cd, Co, Cu, Pb and Zn found to be most promising for environmental monitoring . Also he collected monofloral rape from three different location and found a significant effect of the soil.

Sixteen elements in honeys from the United States, Mexico, El Salvador and China were determined to observe possible significant differences in thier elemental compositions.The elemental contents found were: Cd 0.102-0.267 ppm, Cr 0.843-2.67 ppm, Co 0.456-0.770 ppm, Fe 5.80-183 ppm, Pb 1.79-3.19 ppm; Ni 0.304-1.25 ppm, Zn 2.00-172 ppm. Steel or galvanized containers used in processing, shipping or storage considered to be the source of these metals (Morse and Lisk, 1980) .

Distribution of elements in Turkish honeys and effect of a thermoelectric power plant on the element contents not found to have any contamination of lead, cadmium, iron, or zinc, and amounts of these elements were found well below the permitted limits (Üren et al., 1998). Multielemental determinations in samples of various types of bee honey, pollen and bee tissue carried out by Kump et al., 1996 using reflection X-ray fluorescence spectrometry (TXRF) and radioisotopic excited X-ray florescence spectrometry (XRF) to correlate with envorinment and determine the X-ray techniques` ability to compete with AAS and ICP-AES,

with regard to elemental sensitivity. The TXRF method applied for the study proved to be rather simple and fast, with 200s and also concluded to be a sufficiently accurate analytical tool, but in many cases authors found lack of necessary sensitivity for the detection of important trace elements (As, Pb, etc.).

Li et al.,(1995) made digestion-free determination of heavy metals (Pb, Cd, Cu) in honey using anodic stripping differential pulse voltammetry and potentiometric stripping analysis.

Bianchi (1992) determined the mineral (ash %) content in honey by conductometer amalysis. Samples of acacia meadow honeys from the region of Arandelovac, Yugoslavia, were analysed and minerals for Pb, Cd, Cu, Zn, Fe, Ni and Cr were found 0.5-1.5, 0.05, 0.10-0.45, 0.6-18.0,0.9-2.3, 0.1 and 0.1-0.2 mg/kg (wet weight), respectively. The high levels of zinc may have been cause by contamination from zinc-plate used in centrifuges and honey containers.

The paper by Jamoussi et al., (1995) described an efficient method for the determination of nanogram levels of heavy metals (As, Pb, Sb, Hg and Sn) in honey samples from Cap-Bon (North Tunisia). The honey samples were analysed by hydride generation with an inductively coulped argon plasma polychromator after a previous treatment in a microwave acid bomb. In the paper the optimum conditions for generating and determining the compounds and the effect of different parameters such as chemical nature, form and concentration of the reductant, acid concentration of the sample and the carrier gas flow rate are discussed. Detection limits range 0.05ng/ml for Hg to 1.1 ng/ml for Sb and precision values at 10 ng/ml found to be less than 6% of the standard deviation.

Vinas et al., (1997) concluded that the metal lead, cadmium, zinc and copper contents determined for honey samples of different origins directly by electrothermal atomic absorption spectrometry using hydrogen peroxide as a matrix agreed with those obtained by a slower conventional method.

Chemical composition was determined in 328 honey samples. Most values were found to be within the legal limits. Average lead content in 163 samples was 0.04 mg/kg; only 12 contained more than 0.1 mg/ kg . Average chromium content in 24 samples was 0.018 mg/kg (Sangiorgi, and Ferretti, 1996).

Yilmaz and Yavuz (1999) measured contents of Na, K, Ca, Mg, Cu, Fe, Mn, Zn and Co in 30 honey samples from different parts of south- eastern Anatolia, Turkey by AAS technique. The mean values for Na, K, Ca, Mg, Cu, Fe, Mn, Zn and Co were 118, 296, 51, 33, 1.8, 6.6, 1.0, 2.7 and 1.0 mg/kg respectively. Invert sugar, sucrose, hydroxymethylfurfural, diastase

activity, free acid, lactone, pH, ash, proline and moisture were also reported. Low ash contents, and some high mineral contents were found in honey samples.

Iskander (1996) determined 24 elements (As, Ba, Br, Ce, Co, Cr, Cs, Fe, Hf, Hg, K, La, Na, Ni, Rb, Sb, Sc, Se, Sm, Sr, Th, U, Zn and Zr) in honey produced on uranium mining reclaimed land in Hobson, Texas investigated by instrumental neutron activation analysis. The level of the measured elements in honey samples produced on uranium mining reclaimed land was found to be within the concentration range reported for honey commercially produced in The United States and other nations when compared to the concentration of the same elements in commercial honey and to the literature values for honey from different countries.

Sanchez and Pujola (1996) found potassium was in the higher concentrations and the electrical conductivity was positively correlated with ash content in honeys from all three areas of Spain.

Gürel et al., (1998) compared mineral content, total ash, pH value and acidity of honeys from honey bee colonies in areas with pine honeydew or with mixed nectar sources, and from colonies fed with sugar. Ash, total acids, K, Fe, Mn and Mg were found to be significantly lower in Sugar fed colonies than in mixed nectar sources and pine honeydew sources. Whereas pH values and contents of total soluble matter, Ca, Zn and Cu did not differ significantly. pH and K, Mg and Cu content were found to be significantly higher in pine honeydew honey than in mixed nectar sources and sugar fed honey.

Selenium, ash and chromium levels in some species of honey from eastern Croatia was determined (Petrovic et al., 1993, 1994). Chromium and Ash levels in 80 samples of sunflower, acacia, floral and wild floral honeys were determined by flame atomic absorpzion spectrophotometry (Petrovic et al., 1994). The mean values were 0.103µg/g, 0.152µg/g, 0.125 µg/g and 0.098 µg/g wet and 0.130µg/g, 0.188µg/g, 0.16 µg/g and 0.12 µg/g dry weight found for sunflower, acacia, floral and wild floral honeys respectivey. The average ash content in all 80 samples was 0.141%.

The average selenium levels found was 0.215µg/g d.w., 0.029 µg/g d.w., 0.046 µg/g d.w., and 0.128 µg/g d.w., for wild floral, floral, locust and sunflower respectively (Petrovic et al., 1993).

Petkov et al., (1998) determined 15 heavy metals in honey samples produced in ecological pollution zones of northern and southern Bulgaria. Average Pb contents (mg/kg) were in the north, 0.053 (range 0.02-0.08); and in the south 0.08 (0.05-0.14). the study indicated, Pb content was at or above 0.1 mg/kg in 21% of samples. Average values fro Zn 5.57, Cu 0.15, Ni 0.11, Fe 9.68, Sa 0.07, and Cd 0.015 in the north and Zn 5.82, Cu 0.18, Ni 0.25, Fe 10.67,

As 0.08 , and Cd 0.017 in the south were estimated. The levels of concentration were found to be below permitted limits .

Bicik and Kaspar (1986) determined 15 metals (dry weight) in the food of honeybee and in the developmental stages of queens. The youngest larvae contained particularly Na and K in very high concentrations. In the final larval stage some metals Al, Zn, Cu, Mn, Cd, Pb, Cr and Ni were excreted in larger proportions than others Na, K, Ca, Mg. Study suggests that honeydew honey was richer in minerals than floral honey particularly K, Mg, Ca, Zn, Al, Cu, Mn and Pb. Stored pollen was lower in almost all elements than in pollen collected in pollen traps.

Spunar and Jusio (1986) found the mean concentrations of Cu 0.25 ppm; Fe 4.72 ppm; Zn 2.68 ppm; Pb 0.01 ppm and Cd 0.001 ppm in Yugoslavian honey.

According to Gajek et al., (1987) most of the contaminated honeys found in the investigation in Poland were from China and N Korea in 1972-1983, and many were not permitted to be sold. The most frequent contamination was by Zn, Pb and Fe (81, 32 and 24% of the samples respectively) The levels of these metals in samples of Polish honey taken from areas far from sources of environmental pollution were well below the permissible limits.

Sodium, potassium, magnesium, calcium, chlorides, nitrates, phosphates, sulphates and ash were analysed in Eucalyptus, Castanea, Citrus, multifloral and honeydew honey samples from Calabrian, Italy. Castanea honeys were high in potassium, and citrus honeys were found to be lowest in element content (Leuzzi and Mincione, 1992).

Cd and Pb found to be 12 ng/g and 149 ng/g respectively in German honeys from various locations (Otto and Jekat, 1977).

120 µg/kg Pb content in Italian honey from Campobasso and Abruzzo region were reported (Cubadda et al., 1995; IZS, 1991).

Seven honey samples from polluted areas (autumn honey) showed 13.8 ppm Fe, 2.25 ppm Mn, and 2.37 ppm Pb, as compared to 8.2, 0.50, and 0.083 ppm, respectively, in spring honey (D`Ambrosio and Marchesini, 1982).

Cimino et al., (1984) analysed 24 elements in honey produced in the vicinity of Mount Etna volcano, in Sicily to study the effect of volcanic activity on honey. The samples appeared to contain, in relation to others from unpolluted areas, elevated levels of elements As, Ba, Co, K, Li, Mn, Sr and Zn. The metal enrichment was correlated to element contents of the Mount Etna lava ashes.

Cadmium, coppe r and lead contents were detected in honeys from Dolgellau, N. Wales. The mean content of Cu 505 ng/g and Pb 51 ng/g, Cd 28 ng/g was found in UK honeys from various locations (Jones, 1987).

Terrab et al., (2003) determined the mineral content and electrical conductivity of the honeys produced in Northwest Morocco. In the study the mineral content, ash content and electrical conductivirty of 98 honey samples were studied. Using ICP-AES K, Mg, Mn, Cu, Fe and Zn were quantified. Potassium was the predominant mineral (80%) of the total minerals followed by Mg and Fe (9 and 3% respectively).

The mean total ash content was 0.24%, mean soluble ash content 0.18% and mean insoluble ash content 0.06% were found in some Spanish honey samples (Sancho et. al., 1992).

Additional data on the radio-isotopic content of pollen and honey have also been reported (Kirkham and Corey, 1977; Gilbert and Lisk, 1978; Bunzl and Kracke, 1981). Radio-active elements (notably ^{3} H, ^{137} Cs) recovered from bees were detected in honey, but at lower concentrations than those present in bees.

Honey and other hive products from areas affected by the Chernobyl accident in the Ukraine were examined for concentrations of caesium isotopes. The result indicated that concentrations of caesium isotopes were relatively low in newly built combs and cappings, and also fairly low in honey, somewhat higher in pollen and in old combs, and highest in residual wax and in propolis. Contamination decreased with time (Alexenitser and Bodnarchuk, 1999).

Djuric et al., (1996) determined Radionucleotide (R) activity in honeys from the plains and mountains in Yugoslavia, by a Gel (Li) detector using standard gamma spectrometry. The results showed that the natural R activity of honey varied with soil type, and that Be-7 content varied with local vegatation. The content of R found in honey samples immediately after the Chernobyl accident in 1986 was primarily due to surface contamination, but a number of fission products detected later in some to surface indicated contamination of plants.

For some years honeys have been used as monitors of radionuclides around the Los Alamos National Laboratory in New Mexico, U.S.A.

In 1980 honey samples taken from areas potentially contaminated by Los Alamos laboratory there were no detectable levels of Hg or Pu, and only very low levels of ^{7}Be, ^{137} Cs, ^{3}H, and ^{22}Na (Wallwork-Barber et al., 1982).

Fresquenz et al., (1997) measured radionuclide levels in *Apis mellifera* colonies, and its honeys, around the Los Alamos National Laboratory, where nuclear and energy-related reasearch is carried out for 17 years (1979-95). Bees from 9 out of 11 hives and honey from 6

hives had tritium levels that were significantly higher than background levels. The highest average radioculide concentrations were 434 pCi /ml in bees from a hive near a low-level radioactive waste disposal site, and 709pCi/ml in honey from a hive near three tritium storage ponds. Rest of the all locations showed a decrease in tritium levels over the years. In all other honey samples tested from perimeter hives, radionuclide levels were not significantly different from background levels. Estimates of the committed effective dose equivalent (CEDE) for humans consuming the honey perimeter hives were < 0.04% of the permitted limit.

The mean total ash content was 0.24%, mean soluble ash content 0.18% , and mean insoluble ash content 0.06% were found in some Spanish honey samples (Sancho et. al., 1992).

Sugar, moisture ash and elements K, Br, As, Sb, Fe, Zn, Cr and Co were determined previously in Turkish honey (Temiz and Sengonca, 1981; Kurt and Yamankaradeniz, 1982; Sevimli et al., 1992).

The mean potassium represented 34.0% of the ash, and lied between those of dark and light North American honeys (1676 and 205 mg/kg respectively). Dark North American honeys contained 76 mg/kg sodium and 51 mg/kg calcium, and light contained 18 mg/kg sodium and 49 mg/kg calcium. More iron and manganese as 9.40 mg/kg and 4.09 mg/kg respectively found in dark honeys and 2.40 mg iron and 0.30 mg/kg manganese found in light north American honeys (White, 1978).

Mineral content and geographical origin of Canadian honeys suggest that some trace elements can be considered as local geographic indicators (Feller-Demalsy et al., 1989).

The Spanish honeys had high sodium and calcium contents (5.1% and 4.6% of the ash respectively, and contained more of these elements (Rodrìguez-Otero et al., 1992).

The ash or mineral content of honeys from the Japan, Philippines, Pakistan and India have also been reported (Tatsuno et al., 1968; Minh et al., 1971; Latif et al., 1956; Kalimi and Schonie, 1964; Phadke, 1968).

Additional data on ash content of *Apis cerana* honeys) from the Simla hills, India has also been reported by Mahajan (1984).

Tatsuno et al., (1968) reported 0-2.6 ppm copper and 0.2-6.3 ppm lead in 21 commercial honeys from Japan.

Kalimi and Schonie (1964) detected sodium, phosphorus, and iron in honey samples from Mahabaleshwar, India. Although the bee species was not specified.

Latif et al., (1956) conducted a survey on ash content in honey samples from Pakistan reported as in range 0.11 to 0.32%.

A comprehensive survey on ash content in *Apis cerana* honeys produced in Calcutta, Mahabaleshwar, Madras, and all India.The ash content ranged from 0.03 to 0.52, 0.014 to 0.048, 0.03 to 0.46 and 0.11 to 0.25 per cent respectively in those Indian honey samples (Verma, 1990).

The total ash content in *Apis dorsata* honeys from Philippines, Pakistan and India had average contents of 0.17, 0.26, and 0.39 %, respectively (Minh et al., 1971; Latif et al., 1956). Although these data are too old enough.

The honey samples from Europe used for the determination of elements may be produced by *Apis mellifera* bees.

The sensitibility (or trace element content) of pollen (Fawcett et al., 1971; Knight et al., 1972; Ernst and Bast-Cramer, 1980; Warren , 1982) and propolis (Macedo, 1997) to atmospheric pollutions have also been demonstrated in various international publications. Some other authors also have proposed honey bees and their products as suitable material for the assessment of chemical pollution (Tong et al., 1975; Crane, 1984; Bromen-Shenk et al., 1985; Pinzauti et al., 1991; Raes et al., 1992; Leita et al., 1996; Jones, 1987; Przybylowski and Wilczynska, 2001).

Bee-collected pollen has been suggested as a useful indicator of soil metal levels within the forage area (Free et al., 1983). In the study by Bromenshenk et al., (1985) some pollen samples were too few to identify any patterns or trends but the authors reported marked spatial variations in the concentartions of As, Cd, and Fe in bee tissues. Foraged pollen may be used as a preliminary `prospecting` technique for mineral ores (Pinsent in Warren, 1982) and feasibility studies have been undertaken by two mining companies in British Columbia (Lilley, 1983). Conti and Botre (2001) suggested that honeybees and, to a lesser extent , some of their products (pollen, propolis, wax, but not honey), can be considered as representative bioindicators of environmental pollution.

Foraging honeybees also found to be exhibited considerable differences in their life expectancy through a season and the trace element content of bees collected at a particular hive is known to alter according to diet and season (Nation and Robinson, 1971 cited in Jones, 1987).

Honeybee mortality rates have been used as an indication of gross aerial contamination, both for As (Terzic et al., 1984) and organic pesticide residues (Celli, 1984) and, also, it is likely that beeswax may be used to monitor hydrocarbon residues (Estep et al., 1977).

To date physico–chemical properties (Kerkvliet et al., 1995; Shrestha, 1998, 2000; Joshi, 1999; Joshi et. al., 2000) of Nepali honeys have been determined. But the implications of

contamination of honeys with heavy metals, has not been assessed previously. Apparently, no investigations on the element spectrum content of Nepali honeys have been attemted in terms of the concentration of contaminants in Nepali honeys. This work reports the concentrations of selected elements Li, Na, K, Ca, Mg, Sr, Fe, P, Al, B, Co, Cu, Mn, Ni, V, Zn, Cd, Cr, Mo, and Pb in *Apis cerana* honeys produced in terai, hills and mountains, *A. dorsata and A. mellifera* honeys produced in Chitwan (terai), Nepal.

Elements in the environment

Toxic metals are widely found in our environment. Humans are exposed to toxic metals from numerous sources, including contaminated air, water, soil, and food (Ercal et al., 2001) and are extensivety documented in various publications.

Human exposure to cadmium and lead in the Central Europe, Slovak Republic (Food Research Institute, 1997), and Czech Republic (National Instiute of Public Health in Prague, 1997) in Hungary (UNEP/FAO/WHO, 1992), in Poland (Gzyl, 1997) were evaluated.

The average concentrations of cadmium in foods (potatoes, apples, onions, milk, rice, and meat products) in Slovakia (Food Research Institute, 1997), cadmium and lead in vegetables (carrot, red beet, parsley, celery) in Poland and in Katowice Province (Gzyl, 1997), in cereals,vegetables, and fruits in Hungary (UNEP/FAO/WHO, 1992), lead from vegetarian and non-vegetarians diet (in adults and children) in Czech Republic (Ursìnyova et al., 1997); in selected foods of infants (118µg/kg body weight) in Austria (UNEP/FAO/WHO, 1992) were evaluated.

The contamination of cattle kidneys with cadmium (Pintèr, 1997), the full-term placenta samples in Slovakia (Reichrtovà et al., 1998a,1998b), in human milk samples from Bratislava (Ursìnyova and Hladìkova, 1997), cadmium in umbilical blood (in children), urine, creatinine (in adults and children, using AAS) in Czech Republic (Cerna et al., 1997); pregnant women and their newborns exposed to heavy metals (perinatal period problems: mainly respiratory effort) living in the Silesia district, Poland (Bursa,1996); in venous blood, in human placenta, in cord blood in Poland (Baranowska, 1995); blood cadmium (and lead) levels in mother-neonates pairs in Legnica Glogow Copper Basin (Zareba et al., 1996); blood cadmium concentration in young residents of Nowa Huta quarter (close to the steel and iron plant, city center and rural area) (Zielonka and Wodzien, 1993); in blood of people (n=60, including 28 children) living in a village 900 m from a copper smelter in Legnica (Andrzejak et al., 1993); in blood of the 51 healthy Austrian mothers, in umbilical cord blood and in human milk (using AAS) (Plockinger et al., 1993); in 60 autopsy tissue samples (liver,

kidney, thyroid) obtained from individuals (25-87 years of age) from Styria, a moderately industrialized region of Austria (Tiran et al., 1995); Pb, Cd, Hg, Zn, Cu,Ni in human placenta (by AAS) (Reichrtová et al., 1995) evaluated.

Lead concentrations in the placental tissue samples (Reichrtovà et al., 1998 a, b); in human milk samples (Ursìnyova and Hladìkova, 1997); in total blood and biological materials kidney, liver, blood (Sigmundovà et al., 1997); in children`s blood from the Slovak Republic and Germany (Hladìkova et al., 1997; Meyer et al., 2003), in in blood samples from the Czech Republic (Cerna et al., 1997;Cikrt et al., 1996), in blood, hair, teeth samples (men, women, pregnant women and children) from Hungary (Rudnai and Horvàth, 1996), in maternal blood and cord blood samples from Poland (Bursa, 1996); the relation of lead levels in blood and neuropsychic changes was evaluated in the Slovak population of 9-10 year old children (Sovcìkova et al., 1997, 1998 ab); Pb and Cd in blood, urine and food samples from China, Japan and Korea (by ICP-MS) (Ikeda et al., 2000); Pb and Cd (by ICP-MS) in blood, spot urine, boiled rice and 24-h total food duplicate samples from Bangkok,Thailand (Zhanga et al., 1999); the toxic element status of healthy Austrian women (n=51) and their new-born babies (Plockinger et al., 1993) studied.

The influence of the strongly polluted environment on the content of metal in human tissue was investigated (Baranowska, 1995); cadmium concentration in the cells (Caco-2 cells) determined by atomic absorption spectrometry (Lecoeur et al., 1998).

Mejare and Bulow (2001) discussed Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals trends in biotechnology.

Plants that accumulate the elements allow us to measure the metal input which is often below detection limit in water and air. Suitable plants for this purpose are mosses (Say et al., 1981; Goncalves et al., 1994; Herpin et al.,1994, 1996; Markert et al.,1996; Siebert et al.,1996; Burns et al., 1997).

Lichens were applied as biomonitors (Markert and Zhang, 1991; Markert and Wtorova, 1992; Vtorova and Markert, 1995), and applied to delineate the distribution of airborne chemical elements emitted by industrial plants such as metal works and foundries (Belandria et al., 1991; Kansanen and Venetvaara, 1991; Manninen et al., 1991; Perkins, 1992; Zanini et al., 1992; Gailey and Lloyd, 1993; Caniglia et al., 1994; Jovanovic et al., 1995).

In addition, the capability of lichens to accumulate different elements, inclusive of trace metals, is extensively documented (James, 1973; Nieboer et al., 1977, 1978; Puckett and Burton, 1981; Martin and Coughtrey, 1982; Brown and Beckett, 1984, 1985 ab; Lawrey, 1984; Arndt et al., 1987; Galun and Ronen, 1988; Puckett, 1988; Richardson, 1988, 1992;

Nash, 1989, 1996; Tyler, 1989) and reviewed (Brown, 1991; Brown and Brown, 1991; Garty, 1992, 1993; Jacquiot and Daillant, 1997).

Portuguese lichen (*Parmelia sulcata)* samples collected in 1993 were analysed for the elements Cd, Cr, Cu, Fe, Pb, Ni, Zn, Si, P, S, Cl, K, Ca, Ti, V, Mn, Fe, Na, Mg, Al,Co, Ga,As, Se, Br, Rb, Sr, Mo, Ag, Sb, I, Cs, Ba, La, Ce, Nd, Sm, Eu, Tb, Lu, Hf, Ta, W, Hg, Th, and U (Freitas et al., 2000). *Parmelia sulcata* samples collected fron Portugal in 1990-1992 were also used for the elements Cd, Cr, Cu, Fe, Pb, Ni, and Zn (Ruhling, 1992). In The Netherlands similar survey was made in 1982/83 and 1986/87 using also *Parmelia sulcata* (Sloof, 1993).

Falandysz et al., (2001) analysed Na, Zn, Ca, Fe, Cu, Mn, Rb, Ag, Cd, Hg, Pb, Cs, Sr, Al, Tl, In, Bi, Th, U, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, La, Lu, Ba, Si, K, P and Mg by ICP/MS and ICP/AES in 18 species of wild edible mushrooms from Poland.

The foliar concentration of 11 heavy metals were analysed in trees from a tropical rain forest in Sarawak (Breulmann, et al., 2000); concentrations of K, Fe, Zn,Br and Pb in the leaf veins, roots and stem of *Eucalyptus globulus* , *E. camaldulensis, E. lesouefii, E. saligna* (Orlic et al., 2002); Zn content in Mangroves (*Avicennia marina* Forsk.) was measused using SEM X-ray microanalysis and Atomic Absorption Spectroscopy (MacFarlane and Burchett, 1999); Cd and Zn in dry alfalfa (*Medicago sativa* L.) (Millera et al., 1995); Cd, Ni and Cr tolerance and its ability to accumulate by *Cannabis sativa* L (Citterio et al., 2003); effect of bioaccumulation of cadmium (Cd^{2+}) on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat (*Triticum aestivum* L.) seedlings (Shukla et al., 2003) were also evaluated.

Cd, Cu, Pb and Zn in the different constituents of biowaste (Veeken and Hamelers, 2002); cadmium and zinc uptake by volunteer willow species and elder rooting in polluted dredged sediment disposal sites (Vandecasteele et al., 2002); Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Zn and As in alluvial soils (Fluvisols and Humofluvisols) from the Velika (greater) Morava river valley (Jakovljevic et al., 1997); heavy metal pollution of soils and plants in Northern Bohemia, Korea (Ustyak and Petrikova, 1996; Myung and Thornton et al., 1996) evaluated. Accumulation patterns of environmentally relevant heavy metals (Pb, Cd and Zn) in selected saprophagous or phytophagous soil invertebrates, adults of the species *Porcellio scaber* (Isopoda), *Tetrodontophora bielanensis* (Collembola), *Julus scandinavius* (Diplopoda), *and Deroceras reticulatum* (Gastropoda) (Gräff et al., 1997); biochemical and physiological effects of prolonged feeding of the ant *Formica aquilonia*, in natural conditions with excess of cadmium or mercury (Migula et al., 1997); Cd toxicity in the isopod *Porcellio scaber* Abdel-

Lateif (1998); the plumbism in domestic and free living birds (Francisco, 2002); the effects of Cu, Cd, Zn and Cr on the survival and feeding behaviour of the sandy shore scavenging gastropod *Nassarius festivus* (Cheung et al., 2002); Li, Ga, Rb, Y, Zr, Ga, Mo, Ag, Sb, W, Bi in five typical municipal waste ashes in Japan (Zhang et al., 2002); Hg, Pb, Cd and Zn in the kidney of 164 roe deer, shot in three areas of Slovenia (Pokorny and Ribari-Lasnik, 2002); combined effects of heavy-metal contamination (Cu, Zn, and Hg) and starvation on common quails (*Coturnix coturnix japonica*) and its use as a model for comparison with a wild common guillemot (*Uria aalge*) population (at the Belgian coast) (Debacker et al., 2001); Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn in water, sediment and fish (muscle tissues) samples from Tuskegee Laket located in Southeastern United States (Ikem et al., 2003); Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Al, Cr, Ni, Cd,and Pb in the water, bottom deposits, zoobenthos, fish, and macrophytes of the Pond Bugach and in the soils near the pond (Gladyshev et al., 2001); Cd total Hg in flesh and hepatopancreas of two species of cephalopods (spider octopus, *Octopus saluti* and broad tail squid, *Illex coindeti*) from the South Adriatic Sea (Storelli and Marcotrigiano, 1999); Cu, Pb, Cd, Zn and As in the liver and eggs of 16 species of dead samples of waterfowl from the Doñana National Park (Hernández1 et al., 1999); Pb, Cd, and Zn in *Asterias rubens* as a bioindicator of metal contamination (Temara et al., 1998); assimilation of Cd, Cr and Zn by the zebrafish *Danio reiro* feeding on the freshwater zooplankton *Daphnia magna (*Liua et al., 2002); Fe and Pb content in lung and liver samples of female and male Canada geese (*Branta canadensis*) (Belinsky and Kuhnlein, 2000); Cd, Se and Cr in the eggs and in breast feathers of adult double-crested cormorant (*Phalacrocorax auritus*}, black-crowned night heron (*Nycticorax nycticorax*), and franklin's gull (*Larus pipixcan*) nesting at Agassiz National Wildlife Refuge in Marshall County, northwestern Minnesota (Burger et al., 1996) have also been studied.

And also studied As, Cd, Pb, Cu and Zn content (by ICP-AES and ICP-MS) in various vegetables samples from Bangladesh, (Alam et al., 2003); Intake of food energy, Fe, Zn, and Ca by rural Iranian pregnant women (Houshiar-Rada, 1998); Zn, Cu, Cd, Mn, Pb, Ni (by AAS) in milk, bread and daily diets as well as in serum and urine from the adults of 20-50 years old) with cancer risk (organochlorine pesticides residues, heavy metals) in some food from Eastern România districts in relation with their presence in human body (serum, urine) (Hura et al., 1998); Cd in human milk samples from urban industrial area of Poznan (Rydzewska and Krol, 1996); arsenic, copper, nickel, manganese, zinc and selenium concentration in foodstuffs and drinking water from West Bengal, India (Roychowdhury et al., 2003); Cd, Pb, and Hg in rice grain samples from Saudi Arabia (by atomic absorption

spectrometry after acid digestion) (Al-Saleh and Shinwari, 2001); Pb, Cd, Cu and Zn in different types of milk samples from Mumbai city, India (Tripathi et al., 1999); Pb, Cd and Zn in peanut, corn, pea, and wheat seeds (Stefanov et al., 1995); Pb, Cd, Cu and Zn in air particulates, water and food samples (pulses, green gram, leafy vegetables, amaranth, meat, root vegetables, and fruits) from different suburbs in Mumbai, India (Tripathi et al., 1997); Ag, As, Cd, Cr, Cu, Hg, Mg, Pb, Se and Zn in roots, stems, leaves and seeds of wild rice plant samples from USA (Bennett et al., 2000); lead, cadmium, molybdenum, nickel selenium, copper, zinc and manganese in products from the cereals group a part of a total diet from Madrid (Cuadrado et al., 2000); Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn in tea, cocoa-based, coffee, cereal-based, dairy products, fruit juices, malt drinks, carbonated soft drinks and wines and food drinks samples from Nigeria (Onianwa et al., 1999); Cd, Cr, Cu, Hg, Pb, Zn concentrations in chironomid larvae (Reinhold et al., 1999); As, Fe, Cu, Pb, Ni, Mn, Zn, Se, Mg ,V, Cr, Cd, Sb, and Hg in fallow land soils from anarsenic-affected area of West Bengal, India (Roychowdhury et al., 2002); copper and zinc concentrations in the edible tissues samples of three crops (maize, sugar beet and lucerne) from Italy (Mantovi et al., 2003); cadmium in major food groups together with liver and kidney samples from non-occupationally exposed populations (Sataruga et al., 2003) and elemental and radioactive analysis of commercially available seaweed (van Netten et al., 2000) also studied.

4.6 Materials and Methods

The man influences the state of metals in nature since ancient times. The metals are redistributed in ways different from natural ones and new metal compounds are obtained and released into the environment because of anthropogenic activities. Since metals and metalloids are distributed everywhere in nature and cover a large concentration interval between mg/kg and more to ng/kg and less, and participate in a number of important geochemical and biochemical process this disturbance leads to serious impact on living organisms (Djingova and Kuleff, 2000; Pacyna, 1996). This is one of the reasons that determination of metals and metalloids in environmental materials is an object of constant interest.There are approximately 30 elements that are practical toxicants (Goyer, 1986). Some are biologically essential nutrients (NRC, 1986). Other may have no known beneficial effects or functions. While metals are neither created nor destroyed in biology, they may be significantly altered chemically through metabolism and other chemical reactions (Jacobs, 1996).In tissues these metals may be of various physical and chemical forms: free uncompounded forms or compounded forms either organic or inorganic (Jacobs, 1996).

In principle, many analytical approaches yield accepted results for an individual metal.In practice, each has ist particular advantages, disadvantages, or practically for a given metal, tissue, and toxicological assessment. The choice of techniques is predicated on many factors, e.g., selectivity, sensitivity, ease of analysis, multi-element capabilities, lack of interferences, practicality, commercial availability of instrumentation, etc.

Quantitative techniques rather than qualitative techniques are most often selected for metals analyses.These techniques include, atomic spectroscopy, polarography, electrochemical, X-ray fluorescence etc. Techniques based on a form of atomic spectroscopy are the most widely employed for the analysis of metals, in general.Medicine, nutrition, the environment, and technical needs are continuing to fuel rapid and highly competitive development of recent commercial instrumentation for metal analysis. Recent developments in instrumentation has favored instruments with simultaneous, multielemental capabilities.Many analytical approaches have reached maturity and yield acceptable results for an individual metal. Each has ist particular advantages, disadvantages, or practicality for a given metal, tissue, and toxicological assessment. Every methodological approach should be validated for each metal type (Jacobs, 1996).

Extensive literatures in this field are found (Bacon et al.,1996; Becker and Dietze, 1998). Barnes (1996) emphasized on problems in the analysis of environmental samples because they limit seriously the number of determined elements and worsen the results for some elements such as B, Al,and Mg. Different aspects of sampling have also been presented (Cowgill, 1988; Ferguson et al.,1976; 1977; ISO, 1980; Knoechel and Prange, 1981; ASTM, 1982; Bond, 1982; EPA, 1982; Whitworth et al.,1998).

Analysis Technique

Atomemissionepectroscopy: Flamephotometry and Inductively Coupled Plasma

Principle and Definition

Inductively coupled plasma optical emission spectroscopy (ICP-OES or ICP-AES) is a major technique for elemental analysis. The sample to be analysed, if solid is normally first dissolved and then mixed with water before being fed into the plasma. In this work PERKIN-ELMER 3000 XL was used. ICP-AES is short for optical emission spectrometry with inductively coupled plasma. The plasma is formed by argon gas flowing through a radiofrequency field where it is kept in a state of partial ionisation, i.e. the gas consists partly of electrically charged particles. This allows it to reach very high temperatures of up to

approximately 10,000°C. Atoms in the plasma, at high temperature, emit light (photons) with characteristic wavelengths for each element which can be measured and used to determine the concentration. This light is recorded by one or more optical spectrometers and when calibrated against standards the technique provides a quantitative analysis of the original sample.

The sample being analysed is introduced into the plasma as a fine droplet aerosol. Light from the different elements is separated into different wavelengths by means of a grating and is captured by light-sensitive detectors. This permits simultaneous analysis of up to 40 elements and ICP-AES is generally compared to flame atomic absorption, i.e.detection limits are typically at the µg/l level in aqueous solutions.

ICP instruments comprise various spectrometers, nebulisers, spray chambers, ICP torch, and RF generators.

Nebuliser

When liquid is fed into an ICP plasma it must be in the form of fine droplets otherwise the liquid will not fully dissolvate and atomise. The first stage in forming droplets is the nebuliser. Most nebulisers are pneumatic, that is, they rely on the Venturi effect. The device which produces the IC plasma is commonly referred to as the ICP torch. It consists of two to four Argon flows depending on the manufacturer: Nebuliser gas (inner Argon flow), at about 1L/min, carries the analyte aerosol; Sheath gas (JY patent), for producing a laminar flow to improve low excitation energy elements eg group I & II elements; Auxiliary gas (if present), lifts the plasma above the injector tube, used when measuring organics plasma gas, at about 12-16L/min, sets the plasma conditions, eg excitation temperature the argon and analyte flow into a toroidal radio frequency (RF) field, usually at 40.68 MHz. The plasma is ignited by a Telsa spark (Slickers, 1993).

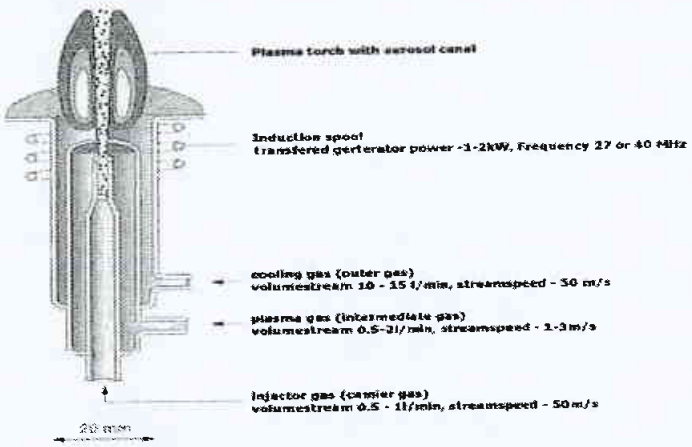


Figure 1: Burner construction of Inductive Coupled Plasmas-ICP

The nebuliser turns the analyte liquid into droplets. The largest droplets fall out into a drain in the bottom of a spray chamber and the finest droplets are carried by gas into the IC plasma (Tyler et al, http://icp-oes.com).

Vaporization, Atomization and Excitation

Aerosol vapour is transported to the plasma vapour desolvates Atomization occurs within the plasma atoms get excited to atomic and ionic states rich spectra produced because of presence of both atomic and ionic lines . Because of their different excitation energies, different emission lines will have maximum intensities at different vertical positions in the plasma.

RF Generators

ICPs generally require 1-2 kW maximum output at radio frequency (RF) to maintain the plasma. High efficiency required - especially for organics

Frequency

Fixed versus Free Running

Fixed: Crystal controlled, eg at 40 MHz

Free running: floats eg at 40 MHz ± 2 MHz

40 MHz versus 27 MHz

Because of the skin-effect in RF plasmas, higher frequency gives a thinner plasma with a wider dynamic range (less self-absorption), with lower backgrounds and fewer interferences. (Tyler et al., http://icp-oes.com).

The Spectrometer

A spectrometer consists of three main parts: an emission source which produces the spectrum, an optical system which scatters the spectrum and the device to measure the emitted lines which processes the result.Common spectrometer optical systems consists of a polychromator or a monochromator which scatters the spectrum and isolates the analytical lines of the elements to be analyzed.

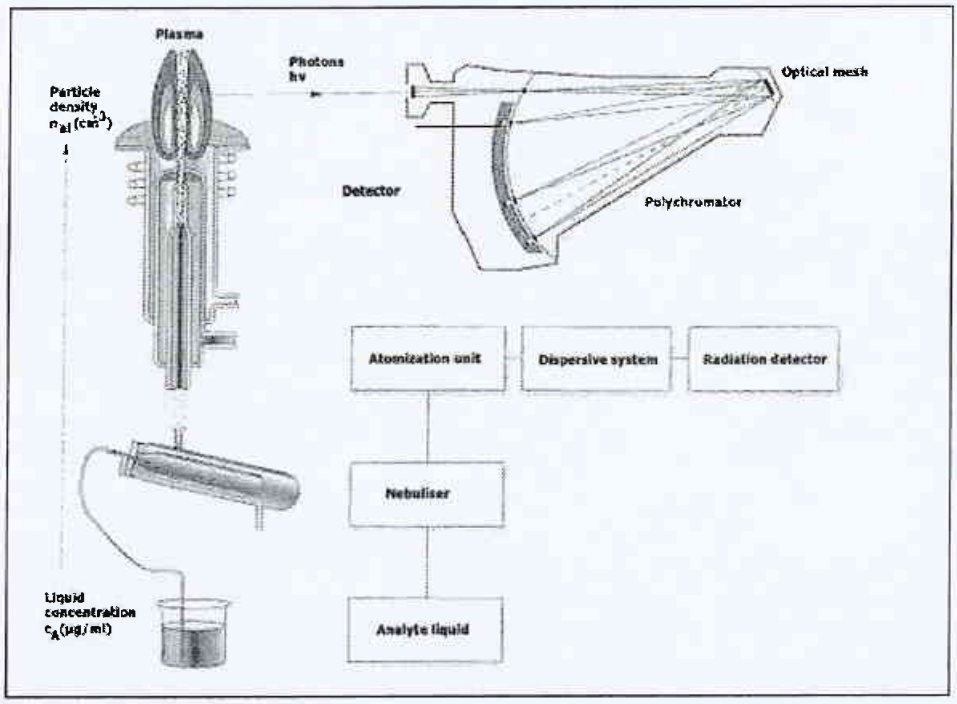


Figure 2: Diagram of a typical ICP-OES instrument with Plasma as Atomization unit (ICP-OES) (Kläntschi et al., 1996).

Figure 2 describes an ICP-OES instrument consisting a sample delivery system, an IC plasma to generate the signal, one or more optical spectrometers to measure the signal, and a computer for controlling the analysis.The most common sample delivery system consists of a peristaltic pump and capillary tube to deliver a constant flow of analyte liquid into a nebuliser. The nebuliser turns the analyte liquid into droplets. The largest droplets fall out into a drain in the bottom of a spray chamber and the finest droplets are carried by gas into the IC plasma. (Tyler et al, http://icp-oes.com).

Spray chamber

The droplets coming from the nebuliser can vary greatly in size, from less than1 μm to more than 10 μm. Since droplets going into the IC plasma should be kept below 5 μm in size, it is necessary to remove the large droplets. This is done in a spray chamber.

The liquid spray from the nebuliser enters the spray chamber. By sheer size, the larger droplets fall to the bottom of the chamber and exit through the drain. The finer droplets in the vapour are transported to the plasma.

Various types of spray chambers commonly used are: Scott , Cyclonic, Inert , Cooled , Low Volume (Tyler et al., http://icp-oes.com).

Photo Detectors and Photomultiplier Tubes

In optical emission spectrometry, photomultipliers are commonly used as detectors. They are photocell detectors. The incident photons coming from the exit slit liberate electrons from the photocathode and the electron flow is then amplified by a set of dynodes. The final anode current is proportional to the incident photon signal received by the photocathode.

The measurement dynamic range is very broad, i.e. 1015, and sensitivity is high, as the dark current is low. These detectors allow the detection of low intensities emitted by trace elements, as well as strong signals from major elements. They have very fast response times, typically 1-2 ns for a 10%-90% change in signal. The main inconvenience of photomultipliers is their cost.There are several types of photomultipliers, which differ in the nature of the entrance window, either crystal or fluoride, and in the nature of the sensitive layer on the photocathode. Some are only sensitive in the far ultraviolet while others are more sensitive in the visible. The type of photomultiplier to be used is selected according to the wavelength of the line to be detected. A fatigue lamp (a small incandescent light source) is often used with photomultipliers to keep the temperature of the tube and its associated electronics constant. The fatigue lamp is switched on when the emission source is off and switched off when the emission source is on.

Solid State Detectors

Concept: Pass charge from one capacitor to another by changing applied voltage in a coordinated fashion. The photon strikes silicon and is converted to a charge that can be transported and measured by electronic structure built on monolithic silicon chip.

Two types of optical solid state detectors

Charge Coupled Device (CCD)

Charge Injection Device (CID)

Advantages of Solid State Detectors

Wide range of elements and wavelengths.

Global analysis over the range of the chip.

Retrospective analysis for 'extra' elements.

Simultaneous' analysis.

Simultaneous background correction.

Cheap.

Disadvantages (compared with photomultiplier tubes)

Smaller signals, mainly because of the much smaller surface area of the light sensitive region.

Higher noise, chiefly counting noise.

Poorer signal to background ratios.

Worse detection limits.

Poorer spectral resolution - mathematical corrections required.

Resolution changes with wavelength in some designs.

Blooming at high intensities occurs in nearby pixels.

Slower response time.

Speed usually limited by the need to integrate to overcome counting noise.

Smaller dynamic range of intensities.

Detection Limit or level

Definition

Detection limit is commonly understood to be the smallest concentration we can measure with a particular technique. In fact it is the point at which we can make a decision whether the element or compound is present or not. To be able to measure the quantity at least three times the detection limit is needed. Three times the detection limit is often called the limit of determination.By convention and from statistics, detection limit (DL) is defined as the concentration corresponding to a signal three times the noise level of the background.

Measuring DLs

There are several ways to measure detection limits. A quick (rough) way is simply to divide the BEC (background equivalent concentration, i.e. the concentration intercept on the calibration curve) by a number, people usually use 50 or 30. The number depends on the typical noise level in the instrument. This is explained further below.

Another way is to determine the uncertainty on the BEC. A third way is called the signal-to-noise ratio (SNR) method. A favoured approach is the SBR-RSDB method.

SNR Method

The SNR method can be expressed as:

$DL=0.03 \cdot RSDB/X_A/C_o$

Where RSDB is the relative standard deviation of the background expressed as a percent and is the sensitivity (the slope of the calibration curve of intensity versus composition), where x_A is the net analyte signal (i.e. signal above background) and c_o the composition of the element in the sample. Clearly with this method, the detection limit is largely determined by the background signal: its size and its noise level, expressed as RSDB. And the sensitivity of the technique, expressed as the slope of the calibration curve.

SBR-RSDB Method

The SBR-RSDB method can be expressed in two equivalent ways:

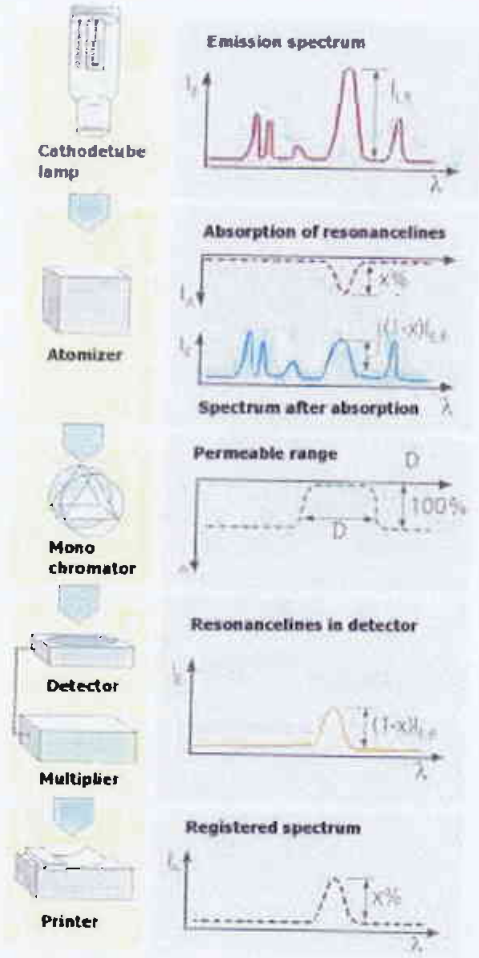
DL=0. 03 x RSDB x BEC

DL=0.03 x RSDB x C /SBR

Where c is the mass % of the element in the sample being measured, BEC is expressed as mass %, and SBR is the signal-to-background ratio. Again, the detection limit is largely determined by the background signal: its size and its noise level, exp ressed as RSDB. And the sensitivity of the technique, expressed as SBR. We can see that if RSDB is 0.7%, then DL is approximately BEC/50 and the limit of determination is 3xBEC/50. If RSDB is 1%, then DL is about BEC/30, and if RSDB is 2%, then DL is BEC/17. (Note: if the RSDB is 1%), which is often assumed, then BEC/50 corresponds to two times the noise level on the background and so the limit of determination is taken to be five times the DL, i.e. BEC/10, rather than three times in the more formal approach (Boumans, 1997).

Atomic absorption spectrophotometry (AAS)

Atomabsorptions spectroscopy



AAS is a method for elemental analysis in solution (mainly). It is very sensitive, can detect different elements and can detect elements in the range of a few ppm or less (atomization by flame) or in the range of a few ppb or less (electrothermal atomization). The AAS techniques that have commercially available instrumentation and that are suitable for these types of analyses include:Flame-AAS(FAAS),Graphite Furnace-AAS(GFAAS), and Hydride Generation-AAS (HGAAS). Although, in theory at least, AAS techniques have the ability to perform simultaneous multielemental analysis (Jacobs, 1996).The principle of AAS is based on the fact that atoms absorb radiation at the same wavelength at which they emit. The sample is atomized in the light path of a radiation source emitting the atomic spectrum of the analysed element, and the extent of absorbed radiation absorbance) in proportional to the concentration of the element (Djingova and Kuleff, 2000).

Figure 3 Measuring principle of AAS

Of these, the ones most employed are based on atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES).

AAS techniques for many of the analytes can suffer significant interferences due to the sample matrix and from the physical and chemical conditions encountered in the light path. These interferences can be due to background absorption (nanometer absorption), vaporization interferences, ionization effects, or from spectral interferences. In general, some type of interference should always be assumed, except under the most simple analyte-matrix conditions-ones that are seldom encountered in the analysis of biological samples (Dulude,1992). Most interferences can be overcome or minimized through techniques such as

matrix matching, using matrix modifiers, background correction techniques (Jackson and Mahmood,1994; Rossi, 1992).

AAS is perhaps the most popular, thoroughly tested and well established method in environmental research, and therefore regular major advances cannot be expected any more (Cresser et al., 1992).Annually appear an enormous number of contributions using AAS in environmental investigations. A number of excellent reviews discuss the state of the art and perspectives of AAS in environmental research (Welz, 1985;Broekaert and Toelg, 1987; Stoeppler, 1991;Cresser, 1994; Cresser et al., 1988;Tsalev, 1994, 1995, 1998; Pelly, 1994; Halls, 1995).

This method exploits the narrowness of atomic absorption lines to avoid the necessity to seperate a complex mixture prior to the analysis of its components. Of course, the conversion of a sample to ist atomic constituents in AAS means that it is only a method of elemental analysis, and, for various practical reasons, it is essentially suitable for analysis only of metals. Nonentheless, a very large number of elements can be analysed for at trace level, and AAS has a very wide range of applications.

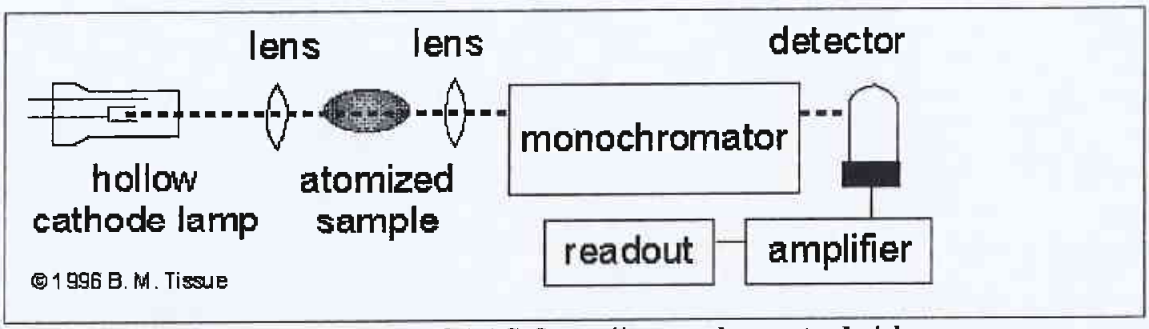


Figure 4 Schematic Diagram of AAS (http://www.chem.vt.edu/chem-ed/spec/atomic/aa.html).

Description of method

Atomic absorption spectrometry is the measurement of an absorption of optical radiation by atoms in the gaseous state. In atomic absorption, the radiation of an element-specific spectral light source is passed through the sample dissociated into atoms, and the attenuation of the radiation caused by the absorption atoms is measured.The decisive feature is that the radiation caused by the absorbing atoms is measured. The radiation of the light source is modulated at a particular freqency and the amplifier connected behind the receiver is tuned to the same modulation frequency. AAS is thus in a position to unambiguously differentiate between the

element- specific radiation of the element of enterest and unspecific radiation from the sample matrix.

Features of AAS

The method is particularly suited for single-element determinations, but also to a ceratin extent for multi-element analysis.The measuring time for aqueous solutions (without calibration, evaluation and sample preparation) is about 10 seconds(flame) and 10 minutes (graphite furnace) per sample and element. The determination limits in aqueous solutions range from µg/ml (Ti) to 0.5ng/ml(Cd, Ag) depending on the element. In most cases, they are in the range of 10ng/ml.

Hollow-cathode Lamps

Hollow-cathode lamps are a type of discharge lamp that produce narrow emission from atomic species. They get their name from the cup-shaped cathode, which is made from the element of interest. The electric discharge ionizes rare gas atoms, which are accelerated into the cathode and sputter metal atoms into the gas phase. Collisions with gas atoms or electrons excite the metal atoms to higher energy levels, which decay to lower levels by emitting light.

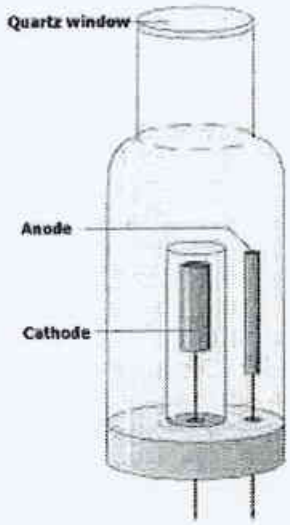
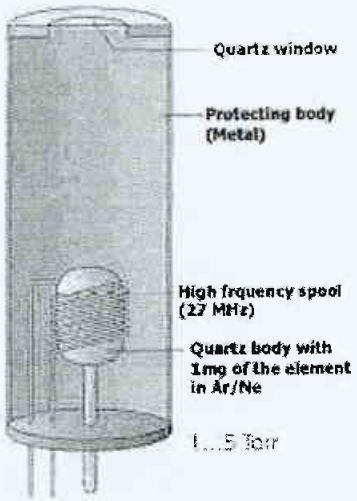


Figure: 5 Cathode tube lamp



Electrodeless discharge lamp (EDL)

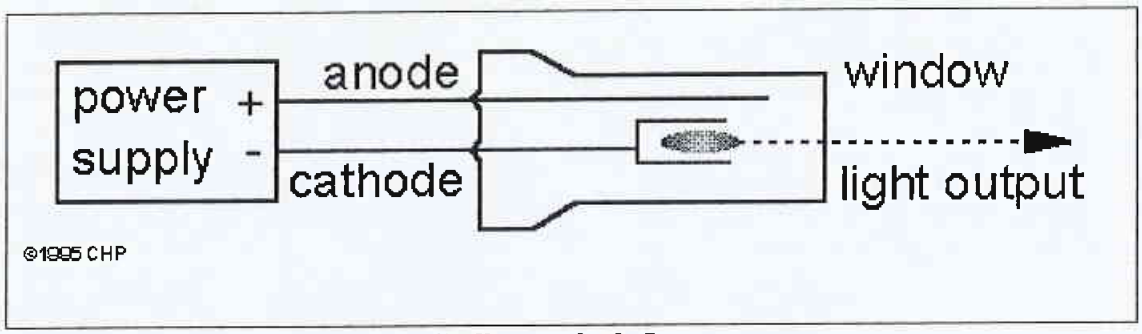


Figure 6 Schematic Diagram of a Hollow-cathode Lamp

Hollow-cathode lamps have become the most common light source for atomic absorption (AA) spectroscopy. They are also sometimes used as an excitation source for atomic fluorescence spectroscopy (AFS).

Statistical analysis

Every elemental content was corrected with the water content of the honey sample. All the results are based on dry weight. SAS (Statistical Analysis System) for Windows V8 was used. Wilcoxon test (non-parametric) was applied as a global test and for pair wise comparisons between the factors/steps after the pairwise comparisons. Bonferroni-Holm test was applied as a global significant level 0.05 was fixed. SAS software provides results in the form of p-values.The p-values say about certainity of test. If pvalue is lower than alpha the nullhypothesis is rejected. If higher than alpha then accepted. Null hypothesis were set in this way says no difference in elemental content between honey from different locations and different bee species.The mean (each) elemental concentration in honey samples compared to those of different locations in case of *Apis cerana* honey and in case of different bee species in case of Chitwan honey.

Graphical representation of results

Software for making box plots SPSS 10.0 for windows. Box plots were produced location wise and species wise for each element.

Sampling

All honey samples were collected directly from the colonies at the same location and time in Chitwan district, central Nepal. Sealed honey combs from *Apis cerana* were collected directly from traditional hives and Newton B hives in seven different locations of Nepal.

A. dorsata honeys directly collected from the colonies and *A. mellifera* honeys were collected from modern hives. Based on their altitudinal ranges samples were aggregated in simpler classification of terai, hills and mountains. The honey samples collected from Chitwan was grouped under terai (< 500masl); Kathmandu, Palpa, and Arghakhachi under hills (500-2000masl); and samples from Jumla-Jajarkot and Langtang were placed under mountains (2000-3000masl). All honey samples from different bee species assayed had been stored in plastic bottles and were preserved in a refregerator until being analysed.

Sample decomposition

Stored honey combs were carefully processed in the laboratory.

Each honey bottle was heated in a water bath to about 40°C so that the honey flowed easily enough to be mixed throughly with an acid ringed glass rod.

The honey samples dissolved completely in concentrated nitric acid after initial warming and nitrous oxides evolved vigorously. Heating upto 140°C was sufficient .Contrary to usual plant samples use of perchloric acid was not necessary.

After going to near dryness, the samples were taken up with 25 ml of water and wax were filtered . Checks revealed that the wax contained even less minerals than the honey.

Water purified by reverse osmosis had to be used exclusively. The de-ionized water prepared in-house frequently contained traces of zinc and nickel. For the zero of boron calibration, glass- distilled water must not be used, because severe and unpredictable contaminations from the borosilicate apparatus frequently occurred.

A simultaneously operating optical ICP (Perkin Elmer Optima 3000 XL) was used as a multi-element analytical tool. Mineralization of the samples does not need to be complete, but the approximate viscosity of an aqueous solution has to be reached. High residual carbon might give background noise. The sample solutions were not diluted prior to ICP determinations.

For K, the samples were very high in concentration.K was determined by flame emission on a Perkin Elmer 3030 AAS after appropriate dilution (20-100 fold).

Controls and additional parameters were performed on a Perkin Elmer 3030 Z graphite furnace. Platforms were used for Pb and Cd whereas Cr and Mo were analyzed without platforms.

Sample preparation-ICP

Flame emmission and graphite tube system wet breakdown in nitric acid in conical flask.

Stored honey combs were carefully processed in the laboratory. Each honey bottle was heated in a water bath to about 40°C so that the honey flowed easily enough to be mixed throughly

with an acid rinsed glass rod. The honey samples dissolved completely in concentrated nitric acid after initial warming and nitrous oxides evolved vigorously. Two replicas of supra pure solutions (each 4 gm) were prepared after blocking six samples one with clean nitric acid (as a control) was analysed. Breakdown by HNO3 starts with heating block at 50°C and heated up to 140°C was sufficient. Contrary to usual plant samples use of perchloric acid was not necessary. The fluid (solution) was evaporated near dryness. The samples were taken up with 25 ml of water and wax was filtered . Checks revealed that the wax contained even less minerals than the honey.Water purified by reverse osmosis had to be used exclusively. The de-ionized water prepared in-house frequently contained traces of zinc and nickel. For the zero of boron calibration, glass-distilled water must not be used, because severe and unpredictable contaminations from the borosilicate apparatus frequently occurred.
A simultaneously operating optical ICP (Perkin Elmer Optima 3000 XL) was used as a multi-element analytical tool. Mineralization of the samples does not need to be complete, but the approximate viscosity of an aqueous solution has to be reached. 5ml of the undiluted solution was used. Every 20 samples calibrated. After six samples, 2 controls (one was of pure water and other was of pure water with 1 ppm Ca and 1 ppm K) were used.The programme of the machine was already established and hence called 'water'(for water sample). For calibration multi element standards were used. High residual carbon might give background noise. The sample solutions were not diluted prior to ICP determinations.

Flameemission (FES)

For K, the samples were very high in concentration. K was determined by flame emission (FES) on a Perkin Elmer 3030 AAS in the oxidised Acetylene- Air-Flame after appropriate dilution (20-100 fold). The spectrometer was calibrated with KCL and HCl.

ICP Calibration standards

Element	Frequency nm	Unit	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Li	670.784	mg/l	2.50				
Na	589.592	mg/l	10			5.00	
Ca	393.366	mg/l	0.50	10	40	2.00	
Ca	315.887	mg/l	0.50	10	40	2.00	
Mg	280.271	µg/l	0.50		20.00		5.00
Sr	421.552	mg/l	0.52	0.04			
Fe	259.939	mg/l	0.30	0.60	2.00	10	
Fe	234.349	mg/l	0.30	0.60	2.00	10	
P	213.617	mg/l			10		5.00
K	766.490	mg/l	101		12.58		
Al	308.215	µg/l	2000	4000	10000		

B	208.889	µg/l	300	600	2500	1250	
B	249.772	µg/l	300	600	2500	1250	
B	182.528	µg/l	300	600	2500	1250	
Co	228.616	µg/l	400	800			
Cu	324.752	µg/l	100	800			
Mn	294.920	µg/l	100	200	1000		
Mn	257.610	µg/l	100	200	1000		
Ni	232.003	µg/l	1000	2000			
V	292.402	µg/l		1000		5000	
Zn	213.857	µg/l	400	800	2000		
Cd	228.802	µg/l	400	800	2000		
Cd	214.440	µg/l	400	800	2000		
Pb	220.353	µg/l	4000	8000			
Cr	267.716	µg/l	500	1000			
Cr	205.560	µg/l	500	1000			
Mo	202.031	µg/l		1000			4000

Adjustment for programme water

Sample flow rate-1.3ml/min
Flush time-20 sec
Sample flush rate-3.0 ml/min
Read delay- 50 sec
Wash rate – 1.3ml/min
Wash time 40 sec
Plasma- 151/min
Aux-0.5
Nebulizer-0.8
Source Equil. Delay- 15 sec
Power-1450 W

Detection limit or level for each element and method

Detection level was calculated out of the results for water samples using median of element content plus two times (+2) standard deviation.

Element	**Detection level**
Al [ng/g] (ICP)	183
B [ng/g] (ICP)	260
Ca [µg/g] (ICP)	0.11
Co[ng/g] (ICP)	5.9
Cu [ng/g] (ICP)	14
Fe [µg/g] (ICP)	0.3
Li [µg/g] (ICP)	0.0004
Mg [µg/g] (ICP)	0.019
Mn [µg/g] (ICP)	3.7
Ni [ng/g] (ICP)	37
P [µg/g] (ICP)	0.06
Sr [µg/g] (ICP)	0.0003
V [ng/g] (ICP)	6.8
Zn [ng/g] (ICP)	53
Na [µg/g] (ICP)	0.1
K [µg/g] (FES)	1.33
Cd [µg/g] (AAS)	0.1
Pb [µg/g] (AAS)	1
Cr [µg/g] (AAS)	1
Mo [µg/g] (AAS)	1

Graphite tube

The elements lead, cadmium, chromium and molybdenum were analysed using graphite tube (Perkin Elmer 3030 Z). The elements lead and cadmium was analysed with platform whereas chromium and molybdenum without platform. For each element different temperature time programme was used. There was slightly different programmes because characteristics of graphite tubes depend on ist age. Additional foreign substances could produce smoke during the analysis.

	Cd	Pb	Cr	Mo
Sample volume	25µl	40µl	30µl	40µl
Atomizationtime			12 sec	13 sec
Atomizationtime	3 sec	4 sec		
Gasstream			10 ml/min	10 ml/min
Integration time	2.7sec	3.0sec	10sec above the area	12 sec above the area
Delaytime			2.5sec	3 sec
Standardaddition	20/40/60µl 25 ppb Cd in 1 ml	20/40/60µl 200 ppb Pb in 1 ml	20/40/60µl 200 ppb Cr in 1 ml	20/40/60µl 200 ppb Mo in 1 ml
Ashing temperature	350°	620°	600°	700°
Atomization temperature	2300°	1.7sec	2500°	2700°
Atomization with Platform	5 sec	5sec		
Atomization without Platform	3sec	4sec		
Delay with Platform	1.0sec	1.7sec		
Delay without Platform	0.5sec	0.7sec		

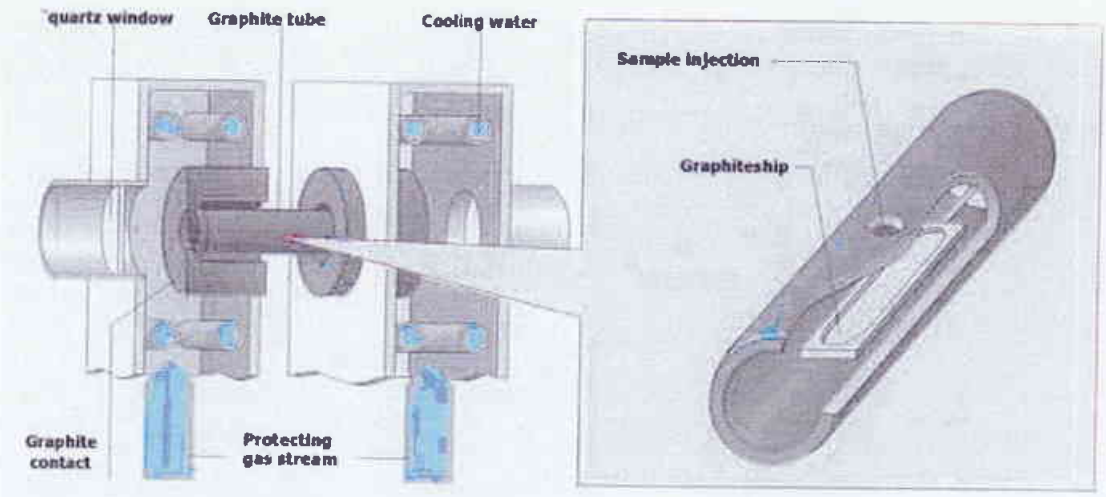


Figure 7. Construction of graphite tube oven- Graphite tube with L´vov-Plattform

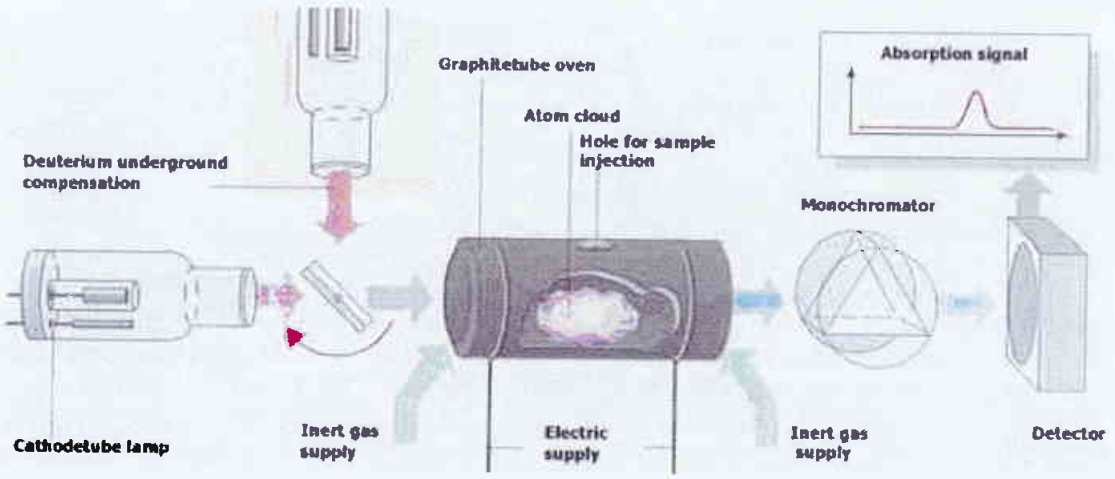


Figure 8. Construction of Atomabsorptions-Spectrometers with Graphite tube oven.

Adjustments

Drying was upto 140°C for 20 sec with Ramp 10. This temp. is adjusted over electrical current producing different wavelengths. For lead 283 mm wave length and for some low values of lead the wave length was 217.0 nm because compensation of smoke produced in a better way.Wave length for molybdenum, chromium and cadmium were 313, 357, and 228nm respectively. The breadth of the window was 0.7nm. For underground correction transvers Zeeman effect was used.

Selection of elements analyzed

In a first experiment, different amounts of a test honey samples (0, 5/ 1 /, 2/ 4 g) were dissolved in nitric acid. For sample weights below 2 grams, trace elements were in the range of the reagent blanks of p.a. HNO_3. From this, use of suprapure nitric acid is essential.

Therefore, 4g were chosen as a sample weight, which need 30 ml of suprapure nitric acid to be decomposed.

Additionally, decomposition of the test sample was done in parallel in glass Erlenmeyer flasks, and in teflon cups. Towards the end of fuming off, the samples from the teflon cups had a tendency to splash around. The glass vessels yielded some boron to the samples, but after subtraction of the blanks, the results for the teflon cups and the glasses were the same.

In order to test the regain, a cocktail of soluble trace element salts was added to the test honey before the nitric acid. Quantitative regain of added Al, As, B, Be, Cd, Co, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Sr, V and Zn was achieved. Surprisingly, even added boron and arsenic were not volatile. Regain of sulfur, given as methionine, was only about half. Barium scattered around, maybe due to interactions with the glass, which was confirmed later also.

It was not attempted to make any efforts towards the analysis of Se, Sb and Sn, which are not directly accessible by the ICP because of insufficient detection limits. Measurement of As by hydride AAS would be possible and much more sensitive than ICP-OES, but was regarded to be too laborious. The As found in some samples was in the range below 10 ng/g.

After destruction of the sugars and maybe other organics, a sample solution of K-Ca phosphate-borate in nitric acid thus remains, yielding a very smooth background spectrum in ICP-OES (except for Mo), which allows the instrument to reach its limits. In order tom control floating of the zero, every 6^{th} sample measured was purified water acidified with suprapure nitric acid.

In case of Pb, Cd, and Cr, however, the detection limit of the ICP-OES was just suitable to see contaminations, but at the normal level they were below the detection limit. Therefore, they were additionally analyzed by graphite furnace AAS. Similarly, Co, Ni and V were frequently below the detection limit of the ICP, but they were regarded not to be of the same importance in order to justify further separate single element determinations. Molybdenum in the ICP was completely interfered from a „wave“ of the background. As this is an essential element for plants acting in the nitrogen cycle, it was decided to do it separately within the graphite furnace. Also, Be was always below the detection limit. Ba scattered around due to adsorption/desorption effects of the glass vessels used, and could not be evaluated.

Fortunately, the boron from the samples was five times higher than the high blanks from the glasses, and precision among different batches was good.

Methodological investigations found in the literature

Looking at systematic investigations of methods for the analysis of trace elements in the literature, wet digestion, dilution and classical ashing in the muffle furnace gave similar results for many elements. B, and surprisingly Cu, were lost in the muffle furnace. From the diluted original honey samples, Cu was found to be volatile in graphite furnace AAS as well (Qiang et al, 1986) which is very rare. From this, wet digestion with oxidizing acids also seems the method of choice.

From soil digests containing additional phosphoric acid, boron was not volatile up to 150°C, even in presence of HF. It gets presumably stabilized as polymeric boronphosphate anion (Fodor and Molnar, 1993) This explains, why boron could be regained from honey sample digests with nitric acid, while it gets lost from most other matrices. Also, from closed vessels, BF_3 was not volatile, if the digests were allowed to cool before opening (Fodor and Molnar, 1993).

The advantage and disadvantage of ICP-AES and AAS

The advantage and disadvantage of ICP-AES among other instrumental techniques for trace analysis is well established (see table).

The multielement determination of about 15 elements is relatively easy to perform in all types of environmental matrices. However, the direct determination of some ecotoxicologically important elements such as Pb, As, Hg, Cd, Sb etc. is not possible in natural water, plant and aerosol samples (Djingova and Kuffel, 2000). After preconcentration step,applied to determine lanthanides in plants has been achieved (Markert, 1996) with excellent accuracy.

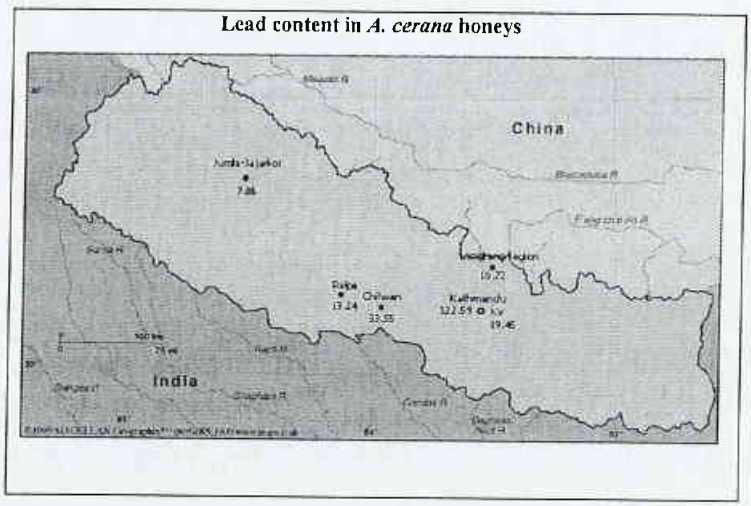
AAS

AAS is perhaps the most popular , throughly tested and well established method in environmental research, and therefore regular major advances cannot be expected any more (Cresser et al.,1992).Jackson and Mahmood (1994) and Jackson and Qiao (1992) have recently reviewed the literature with regard to AAS techniques. Hill et al., (1992, 1993) reviewed the instrumentation associated with AAS techniques. A number of reviews discuss the state of art and perspectives of AAS in environmental research (Welz, 1985; Broekaert and Toelg, 1987; Toelg, 1987; Stoeppler, 1991; Cresser et al., 1988, Cresser, 1994; Tsalev, 1994, 1995, 1998; Halls, 1995). In the period 1983-1992 more than 2000 papers were published on AAS in the environmental health practice (Tsalev, 1994). A survey on the

annual reviews in *J. Anal. At. Spectrom* on Atomic Spectroscopy Update-Environmental Analysis indicates that the articles using AAS exceed tens of times the contributions using other analytical techniques (Djingova and Kuffel, 2000). Elements such as Pb, Cd, Zn, and Cu are among the very important pollutants but they are also easily determined by AAS, a very popular method (Djingova and Kuffel, 2000).

Obviously the field in environmental trace analysis which is rapidly developing is speciation. In this respect increase in the role of electrochemical methods in determination mostly of inorganic species might be expected while for the determination of both inorganic and organometallic species the combinations between highly selective chromatographic or/and extraction techniques with sensitive methods like ICP-MS, AAS, NAA are most promising (Djingova and Kuffel, 2000).

The principle of ICP-MS is atomization of the sample in inductively coupled argon plasma and high degree ionization, after which the positively-charged ions are extracted from the plasma into the mass spectrometer where the ions are seperated according their mass to charge and detected by photomultiplier.



The advantages and the disadvantages of ICP-MS (Gray, 1985; Barnes, 1996; Jacobs, 1996; Becker and Dietze, 1998; Djingova and Kuleff, 2000); ICP-AES (Keliher et al.,1986; Sansoni, 1987; Toelg, 1987; Stoeppler, 1991;Markert, 1996; Djingova and Kuleff, 2000); AAS (Djingova and Kuleff, 2000) are:

ICP-MS		ICP-AES		AAS	
Advantage	**Limitation**	**Advantage**	**Disadvantage**	**Advantage**	**Disadvantage**
Enviable detection capabilities. More than 90% of the elements can be detected in the 0.01-1 ng/g range. About 50 elements are detectable below 10 pg/ml level. With ICP quadropole MS the precision is 0.1-2%. The dynamic range is six to seven oders of magnitude. High sample throughput.	Necessity for dissolution of samples which poses the decisive question about blank values. Matrix effects as well as problems with dissolved solids , mass resolution and spectral interferences are serious for earlier equipment and are dimished to a great extent in third generation instruments. Relatively expensive instrumentration for metal analysis.	Ability to detect up to 50 elements. Precision varies between 0.2-5%. Relatively low chemical interferences. Sensitivity lies in the ng/-µg/g range. Low detection limits for refractory elements. High sample throughput. Dynamic range about five orders of magnitude.	Spectral interferences similar to arc and spark AES. Dependence on blank values. Dependence on salt and acid content.	High sensitivity and low detection limits down to the su-nanogram range (ETAAS). Good accuracy and precision Well established methodology. High sample throughput. Relatively low cost	Single element technique. Narrow dynamic range. Works predominantl y with solutions.

4.7 Results

Elements in *Apis cerana* honey from different locations

The mean concentrations of elements found in *Apis cerana* honey samples analysed for all the sample locations are tabulated in table 1 and table2. In addition, the values of each elements in honeys produced in three representative agro-ecozones are presented in box plots 1-8. Ash content in *Apis cerana* honey samples are given in figure 1a-8a. In addition, total ash in overall *A. cerana* honeys are shown in figure 9a.

Samples analysed from seven locations, the average elemental concentrations of Li found to be highest as 0.044 mg/kg, P 302.142 mg/kg, Al 34.39 mg/kg; Co 0.017 mg/kg; Ni 1.19 mg/kg; Zn 7.867 mg/kg and Cr 29.288 mg/kg in Langtang honey; For Kathmandu honey, the concentrations estimated highest as 39.4 mg/kg, 8.75 mg/kg, 5.243 mg/kg, 0.017 mg/kg, and 122.5µg/kg for Na, Fe, B, Co, and Pb respectively. In the case of Chitwan, Sr 0.226 mg/kg, Cu 1.51 mg/kg, Mn 7.392 mg/kg, Cd 4.136 µg/kg , and Mo 29.347 µg/kg; Palpa K 4259.50 mg/kg and Arghakhachi honey Ca 193.917 mg/kg and Mg 149.3 mg/kg found to be highest amongst all (table 1). Similarly, K 889.000 mg/kg and V 0.0001 mg/kg in Kathmandu honey; Ca 63.637 mg/kg, Mg 31.962 mg/kg, Sr 0.067 mg/kg, Mn 0.001 mg/kg, and Zn 0.001 mg/kg in Kathmandu valley honey; Na 15.101 mg/kg in Chitwan honey; Fe 1.553 mg/kg, P 44.827, Al 0.0022 mg/kg, B 0.0025 mg/kg, Co 0.00001 mg/kg, Cu 0.0001 mg/kg, and Ni 0.0001 mg/kg in Arghakhachi honey; Li 0.0029 mg/kg , Cd 0.312 µg/kg , Pb 7.869 µg/kg, Cr 5.839 µg/kg, and Mo 5.625 µg/kg in Jumla-Jajarkot honey found to have lowest amongst all (table 1). The distribution (with range) of each elements is given in the annexe 1.Though these give apparent picture what concentration (and range) there is.

The total ash content of overall *Apis cerana* honey samples, commonly K, P, Ca, Na, Al, and Fe. The percentile of these elements varied from K 83.28% to Cd 0.0001 % of ash (see figure 9a).

It is instructive to compare elemental concentrations (see annexe 1) in with each bee species (for Chitwan honey) and locations. The comparison shows that (some of) the elemental concentrations in rural areas (such as Jumla-Jajarkot and Langtang) tend to be less than urban areas (Kathmandu, Kathmandu valley and Chitwan). For instance, the Pb concentration in Jumla-Jajarkot and Langtang honey (second minimum) found to be the lowest amonst all. Equivalent figures also for Cd, Cr and Mo concentrations were estimated in Jumla-Jajarkot honey. But place like Langtang surprisingly showed the second maximum (3.123 µg/kg) value of Cd in *Apis cerana* honey (see table 2).

Table 1. Showing mean (mg/kg) content of elements in *Apis cerana* honey from different locations.

Elements	Langtang	Kathmandu	Kathmandu valley	Chitwan	Palpa	Arghakhachi	Jumla-Jajarkot
	n=6	n=2	n=4	n=11	n=4	n=2	n=12
	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Li	0.044	0.007	0.003	0.007	0.004	0.003	0.0029
Na	24.378	39.423	24.510	15.101	19.507	17.878	17.789
Ca	104.132	113.959	63.637	150.923	90.761	193.917	89.037
Mg	111.807	36.449	31.962	102.711	135.658	149.395	83.399
Sr	0.099	0.169	0.067	0.226	0.120	0.181	0.084
Fe	7.297	8.758	5.085	4.981	3.723	1.553	4.121
P	302.142	68.177	72.962	213.578	73.854	44.827	183.765
K	3258.142	889.000	1074.63	1536.20	4259.50	2043.50	2353.82
Al	34.396	1.138	0.003	4.617	0.285	0.0022	0.150
B	4.578	5.243	0.003	3.850	0.676	0.0025	0.090
Co	0.017	0.017	0.000024	0.008	0.004	0.00001	0.002
Cu	1.462	0.212	0.00029	1.51	0.66	0.0001	0.647
Mn	1.362	0.937	0.001	7.392	0.658	0.0019	0.544
Ni	1.192	0.248	0.0002	0.107	0.082	0.0001	0.050
V	0.008	0.0001	1.5	0.004	3.125	2.75	0.001
Zn	7.867	0.945	0.001	2.519	0.472	0.005	0.314

Note: 41 honey samples from *A. cerana* included for ICP.

Table 2. Showing mean (µg/kg) content of elements in *Apis cerana* honey from different locations.

Elements	Langtang	Kathmandu	Kathmandu valley	Chitwan	Palpa	Jumla-Jajarkot
	n=5	n=2	n=4	n=10	n=3	n=8
Cd	3.123	0.886	0.493	4.136	0.691	0.312
Pb*	10.727	122.596	19.457	33.553	13.245	7.869
Cr	29.288	22.252	21.151	15.097	12.567	5.839
Mo	16.342	22.757	7.697	29.347	9.623	5.625

***Number of samples=4 for Pb (Langtang). 32 honey samples from *A. cerana* for AAS included.**

Box plots showing element content in *Apis cerana* honeys from different locations of Nepal. All box plots showing % of trace elements in *Apis cerana* honey from different locations.

Horizontal line in the box = median; lower boundary of the box = 25th percentile; upper boundary of the box = 75th percentile; whiskers = largest and smallest values (1.5 size of the box);

- = extreme values; o = outliers. Note: Arghakhachi honey samples are not included.

Figure1

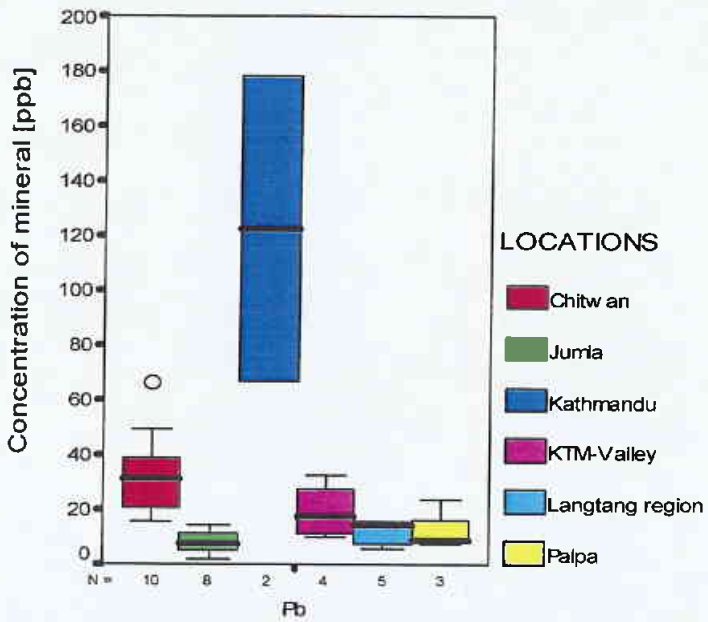


Figure 2

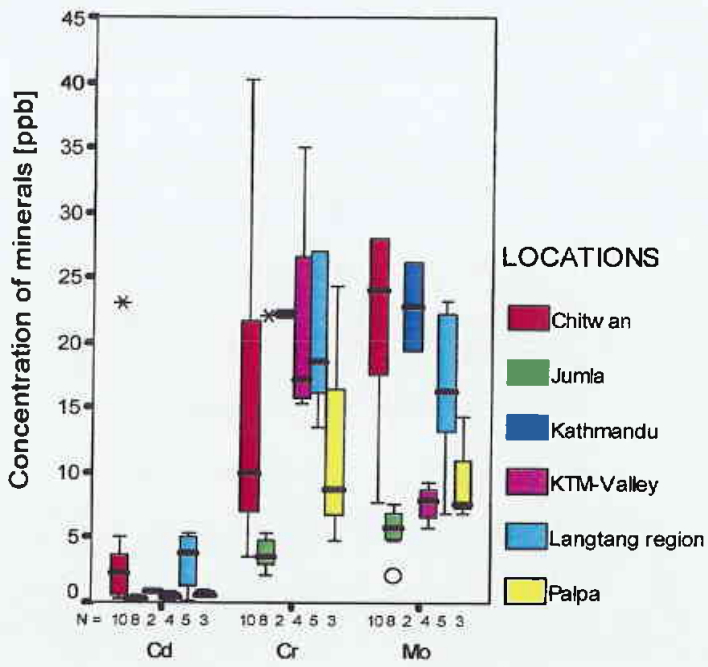


Figure 3

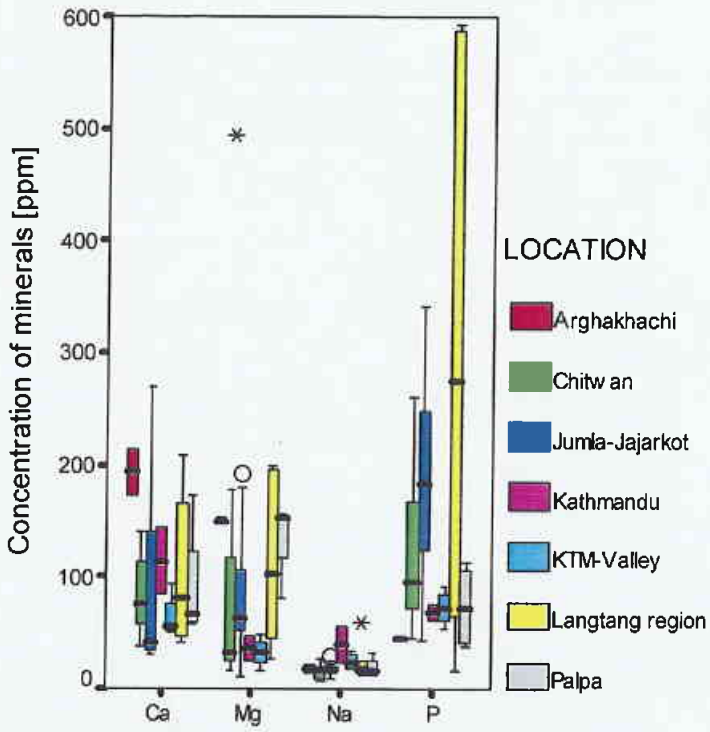


Figure 4

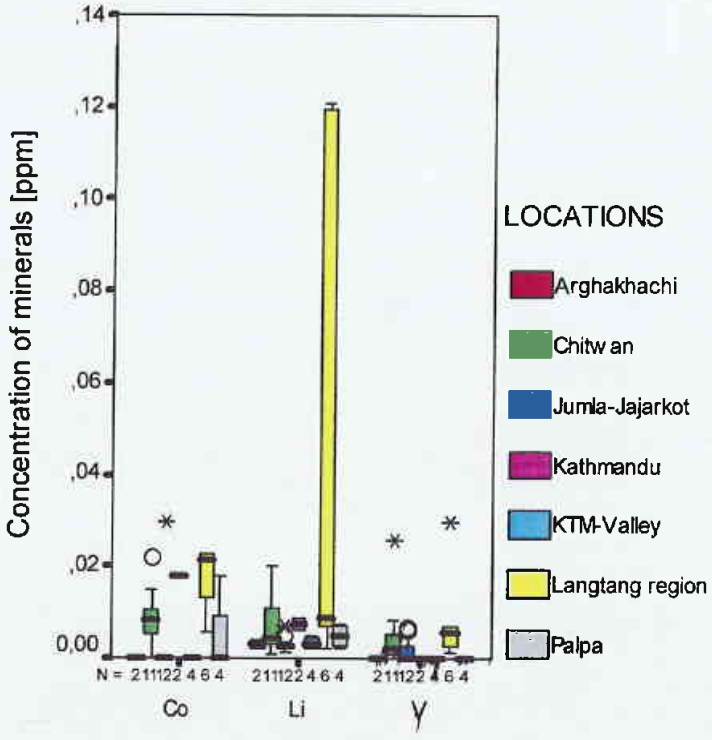
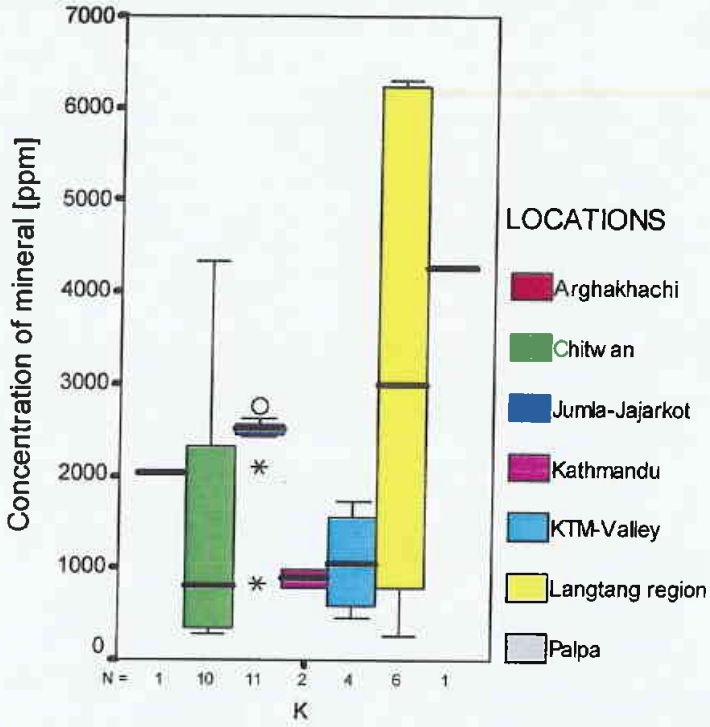


Figure 5



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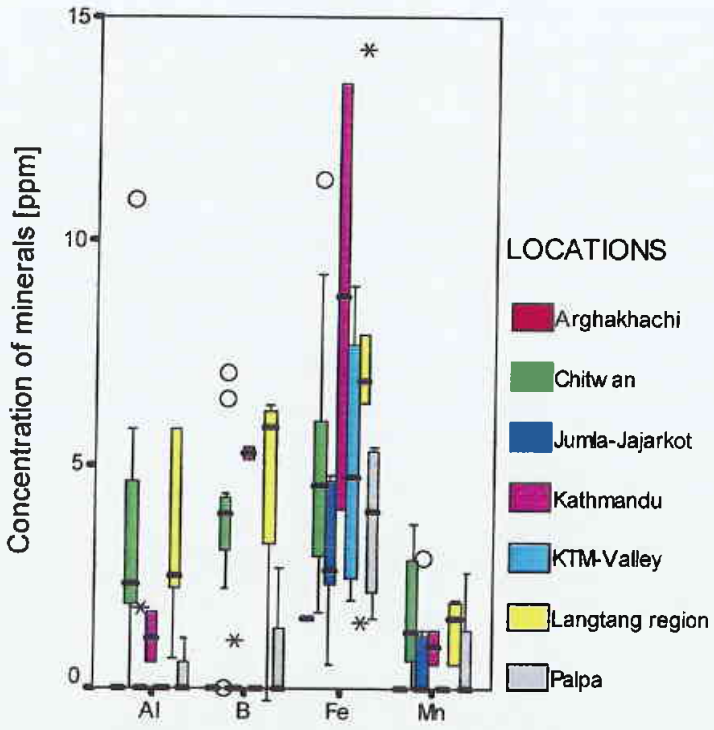


Figure 7

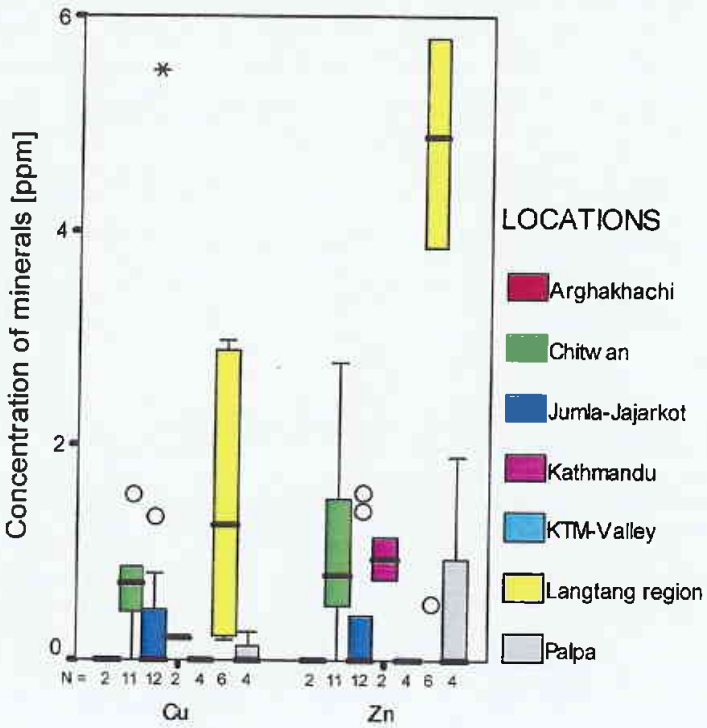


Figure 8

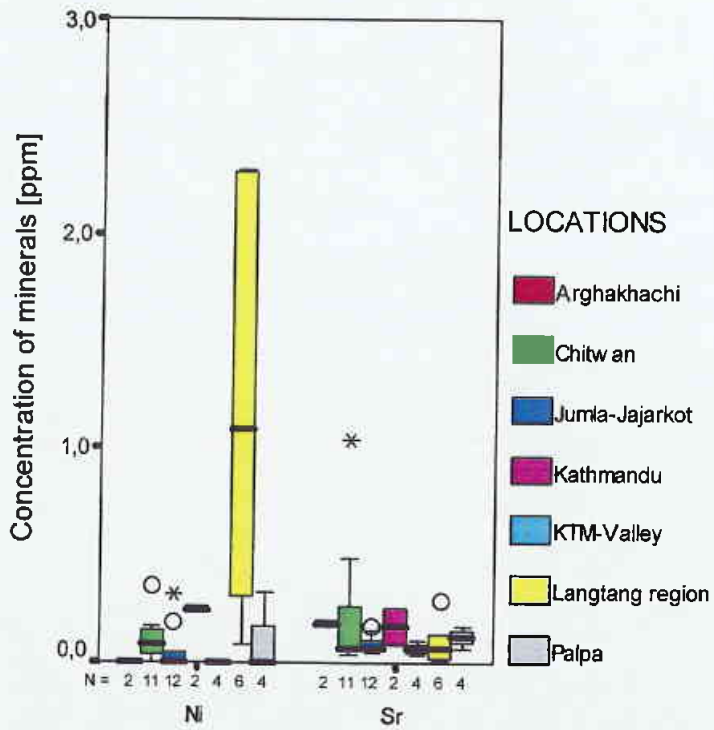


Figure 1a

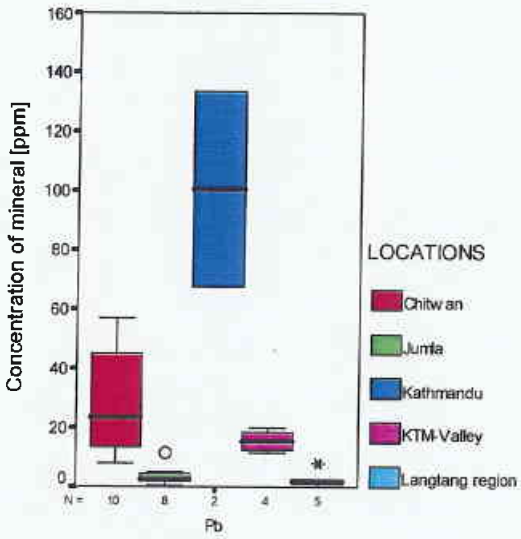


Figure 2a

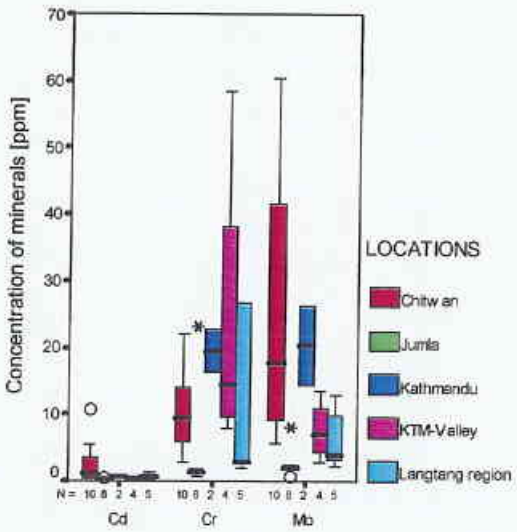


Figure 3a

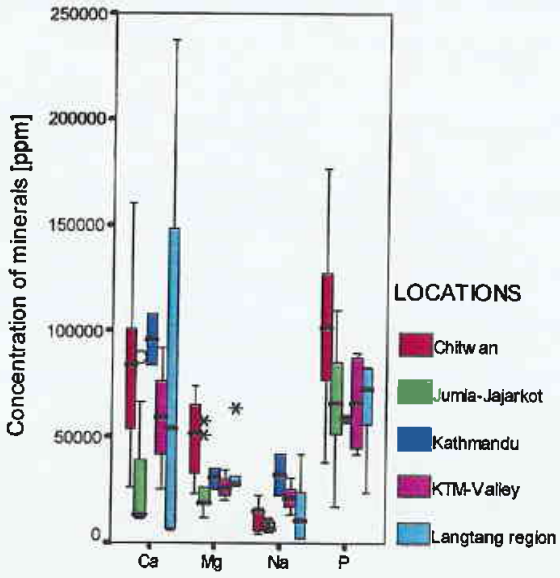


Figure 4a

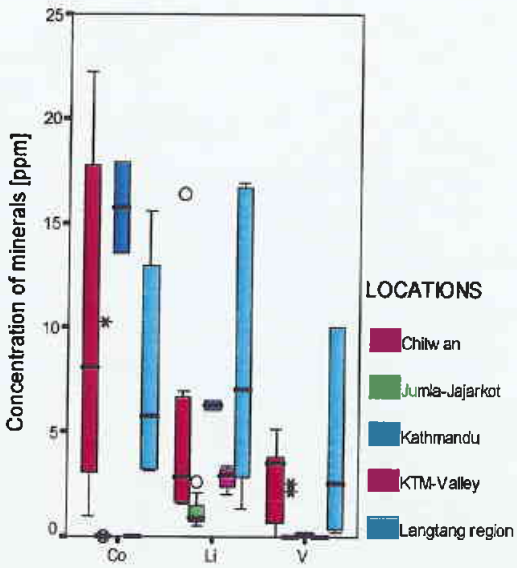


Figure 5a

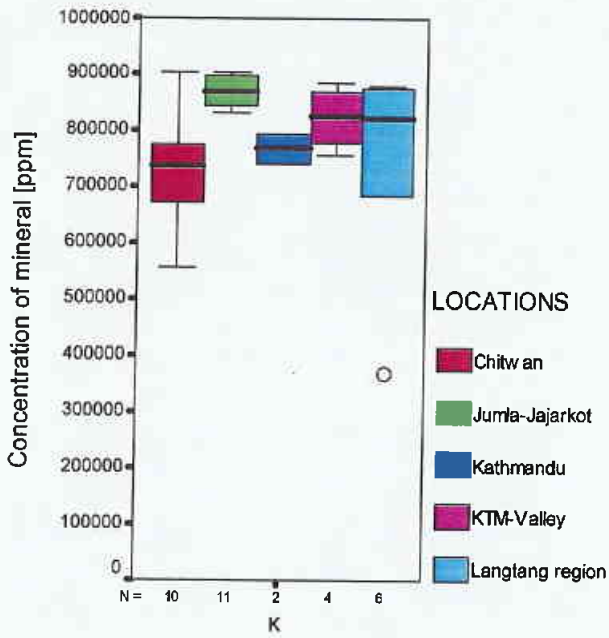


Figure 6a

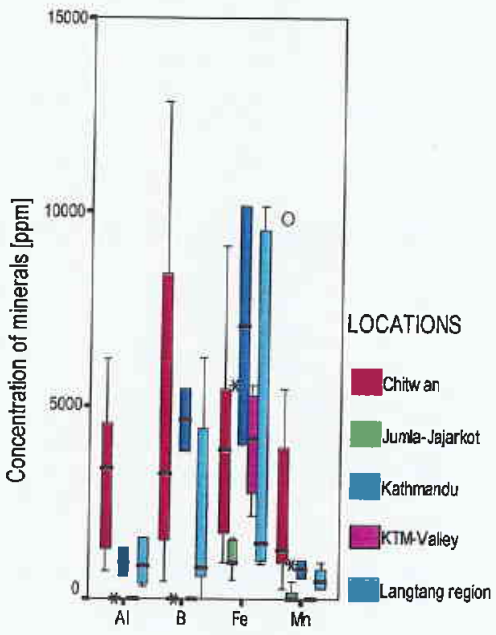


Figure 7a

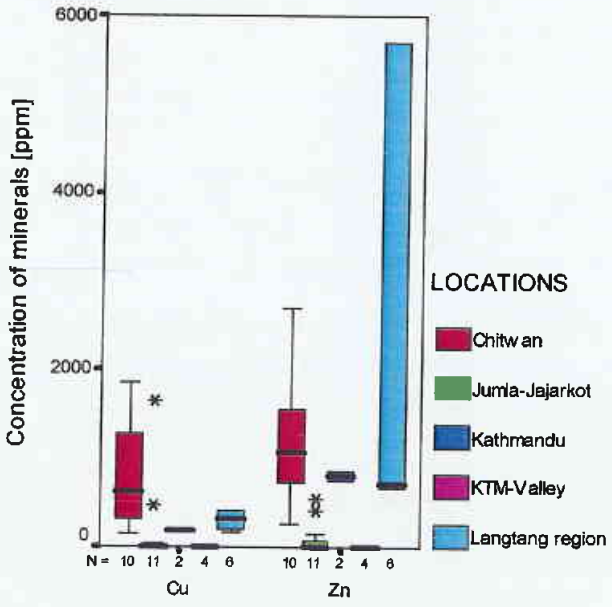
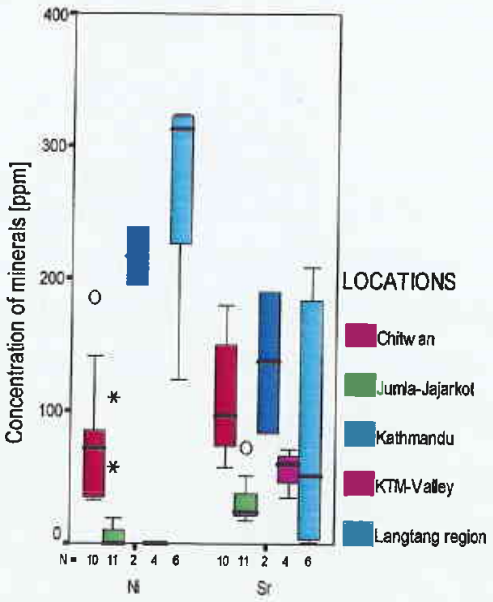
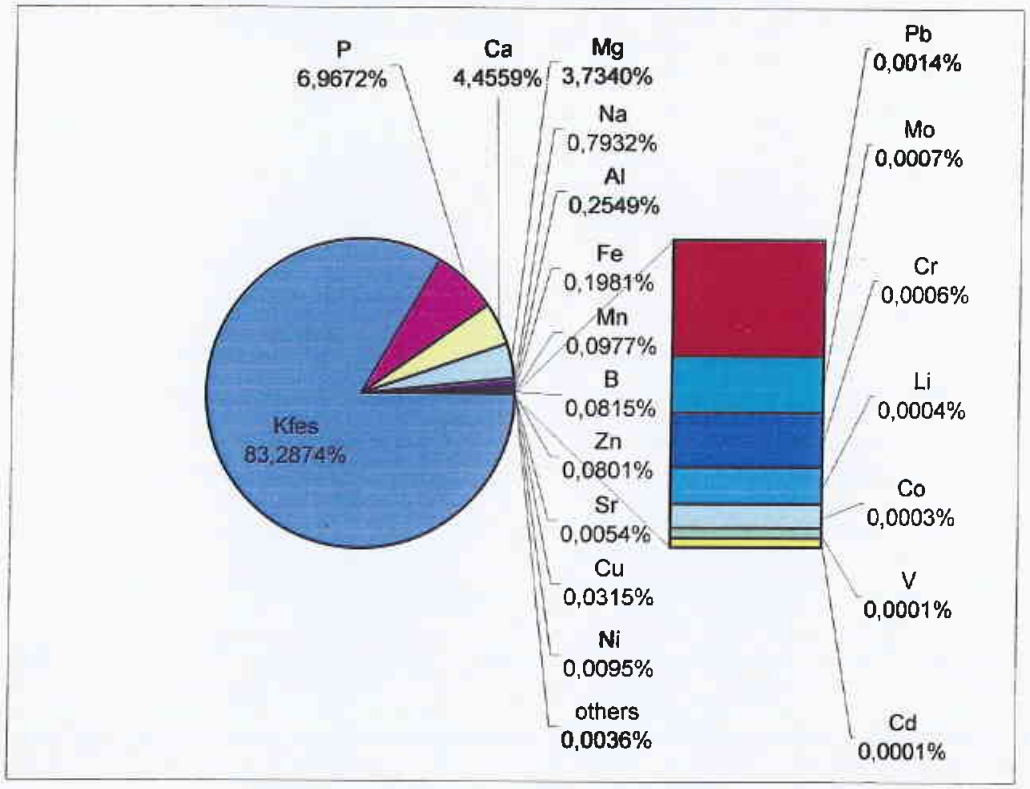


Figure 8a



Total Ash in over all *Apis cerana* honey samples (figure 9a).



Elements in *Apis cerana, A. dorsata* and *A. mellifera* honey from Chitwan.

In Tables 3 and 4 it is shown the results obtained by ICP and AAS respectively. There it is mentioned the number of analysed samples, mean values, standard deviation for each element and each bee species. Mean element contents, standard deviation with the corresponding minimum and maximum values of total *Apis cerana, A. dorsata* and *A. mellifera* honey samples are summarised in Table 5 and also given in figure 10-16. Figure 16a shows total ash content (%) in overall honey samples from Chitwan. Annexe 1 shows a range of concentrations (maximum and minimum) of each elements, with bee species, comparable with figures and tables.

Apis dorsata and *Apis mellifera* honeys showed significant difference ($P \leq 0.05$) in Ca (P= 0.0051), Sr (P= 0.006), K (FES) (P= 0.0021), Al (P= 0.0102), Cu (P = 0.001) and Mn (P = 0.0321). Whereas, rest of the elements Li, Na, Mg, Fe, P, B, Cd, Co, Cr, Mo, Ni, Pb, V and Zn were not found to be significantly different in those honeys produced by *Apis dorsata* and *Apis mellifera*. The use of Kruskal-Wallis and Bonferroni-Holm tests indicated that difference in concentration of the elements Li, Na, Mg, Cd, Co, Cr, Ni, Pb, V and Zn were not significant in *Apis cerana-A. dorsata, A. dorsata –A. mellifera, A. cerana- A. mellifera* honeys.

Comparison of the elemental concentrations showed that the difference were significant in the case of Fe (P= 0.0498), P (P= 0.0396), Al (P= 0.006), B (P= 0.044), Cu (P= 0.012), Mo (P= 0.027) in *Apis cerana* and *A. mellifera* honeys. Whereas, some elements Li, Na, Ca, Mg, Sr, Cd, Co, Cr, Mn, Ni, Pb, V and Zn were not found to be significantly different in *Apis cerana* and *Apis mellifera* honeys.

No statistically significant difference in concentration found in all elements in *Apis dorsata* and *Apis cerana* honeys.

The highest average concentration was detected K 1728.29 mg/kg followed by P 125.92 mg/kg, Ca 113.57 mg/kg, Mg 59.79 mg/kg, Na 19.57 mg/kg, Fe 3.88 mg/kg, Mn 2.88 mg/kg, B 2.49 mg/kg, Al 2.34 mg/kg, Zn 2.14 mg/kg, Cu 0.67 mg/kg, Sr 0.17 mg/kg and Ni 0.11 mg/kg (see tables and fig. 9a).

The overall concentrations of Cd, Cr, Mo and Pb in Chitwan honey is practically limited to 2.41^{-03} µg/kg, 0.013 mg/kg, 0.019 mg/kg and 0.029 mg/kg respectively. The elements Pb, Li, Mo, Cr, Co, V, and Cd were found in smaller amounts. The amount of the overall elements present in total honey samples found to have 2094.413 mg/kg.

The total ash content of overall *Apis cerana, A. dorsata and A. mellifera* honey samples from Chitwan, ranged from K 82.5% to Cd 0.0001% (see figure 16a).

Table 3. Sample statistics for element content (mg/kg) by Inductively coupled plasma (ICP).

Elements	*Apis cerana* n=11		*Apis dorsata* n=13		*Apis mellifrea* n=20	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Li	0.007	0.006	0.028	0.042	0.020	0.034
Na	15.102	6.276	31.439	31.954	14.313	6.134
Ca	150.92 ^{ab}	242.12	139.35 ^{a}	73.19	76.26 ^{b}	47.73
Mg	102.71	140.16	63.99	60.21	33.45	30.03
Sr	0.22 ^{ab}	0.30	0.30 ^{a}	0.37	0.06 ^{b}	0.02
Fe	4.98 ^{a}	3.03	5.18 ^{ab}	4.71	2.44 ^{b}	1.59
P	213.57 ^{a}	329.81	141.20 ^{ab}	121.25	67.78 ^{b}	20.59
K (FES)	1536.20 ^{ab}	1543.61	3001.09 ^{a}	1946.04	865.42 ^{b}	609.66
Al	4.61 ^{a}	5.34	2.50 ^{a}	1.72	0.98 ^{b}	1.27
B	3.85 ^{a}	1.89	2.48 ^{ab}	1.61	1.74 ^{b}	2.35
Co	8.79^{-03}	5.86^{-03}	3.693^{-03}	4.501^{-03}	6.649^{-03}	6.182^{-03}
Cu	1.51 ^{a}	2.50	0.74 ^{a}	0.56	0.21 ^{b}	0.21
Mn	7.39 ^{ab}	19.52	1.89 ^{a}	1.11	0.95 ^{b}	0.99
Ni	0.10	0.09	0.11	0.04	0.12	0.17
V	4.753^{-03}	7.512^{-03}	8.25^{-03}	10.97^{-03}	3.00^{-03}	6.39^{-03}
Zn	2.51	5.23	1.28	0.80	2.49	5.65

- **Number of sample (K) =10 for *A. cerana*; 11 for *A. dorsata* and 14 for *A. mellifera***
- **Number of sample (Cu) = 10 for *A.cerana***
- **Number of sample (Mn) =19 for *A. mellifera***

The letters a,b, super-scribed after the means in a row denote the statistical significance between the analytical values obtained for three honey groups

44 samples were included for Chitwan (ICP).

Table 4. Sample statistics for mineral content (µg/kg) by atomic absorption spectrometer (AAS)

Elements	*Apis cerana* n=10		*Apis dorsata* n=8		*Apis mellifera* n=9	
	Mean	Std. dev	Mean	Std. dev	Mean	Std. dev
Cd	4.13	6.80	1.74	1.59	1.07	0.92
Pb	33.55	15.27	31.91	26.82	22.75	10.61
Cr	15.09	11.87	11.98	11.64	13.18	9.47
Mo	29.34 ^{a}	20.06	14.19 ^{ab}	6.55	12.77 ^{b}	4.97

The different letters a,b, super-scribed after the means in a row denote the statistical significance between the analytical values obtained for three honey groups.
27 samples were included for AAS.

Table 5. Sample statistics for total elemental content of Chitwan honey (mg/kg).

Elements	Number of Samples	Mean	Std. dev.	Minimum	Maximum
Li	44	0.01	0.03	0	0.12
Na	44	19.57	19.26	5.11	117.73
Ca	44	113.57	131.68	31.05	874.59
Mg	44	59.79	82.33	0.08	493.49
Sr	44	0.17	0.26	0.02	1.14
Fe	44	3.88	3.35	0	17.87
P	44	125.92	182.19	28.94	1188.94
K (FES)	35	1728.29	1651.74	279.50	6159.50
Al	44	2.34	3.22	6.9^{-04}	18.07
B	44	2.49	2.17	0	7.41
Cd	27	2.41^{-03}	4.34^{-03}	2.73^{-04}	0.02
Co	44	6.31^{-03}	5.85^{-03}	0	0.02
Cr	27	0.013	0.01	2.87^{-04}	0.04
Cu	43	0.67	1.31	2^{-04}	8.58
Mn	43	2.88	9.94	7^{-04}	66.15
Mo	27	0.01	0.01	6.63^{-03}	0.06
Ni	44	0.11	0.12	2.5^{-05}	0.75
Pb	27	0.02	0.01	7.38^{-03}	0.08
V	44	4.99^{-03}	8.35^{-03}	0	0.03
Zn	44	2.14	4.58	1.01^{-03}	25.61

Overall (trace) element content (in mg/kg) mean: 2094.4183; Standard deviation:1898.421; Maximum: 6719.538; Minimum : 460.533; Median:1427.450.

Box plots showing element content in *Apis cerana, A. dorsata and A. mellifera* honeys from Chitwan.

Horizontal line in the box = median; lower boundary of the box = 25th percentile; upper boundary of the box = 75th percentile ; whiskers = largest and smallest values (1.5 size of the box);

- = extreme values; o = outliers.

Figure 10

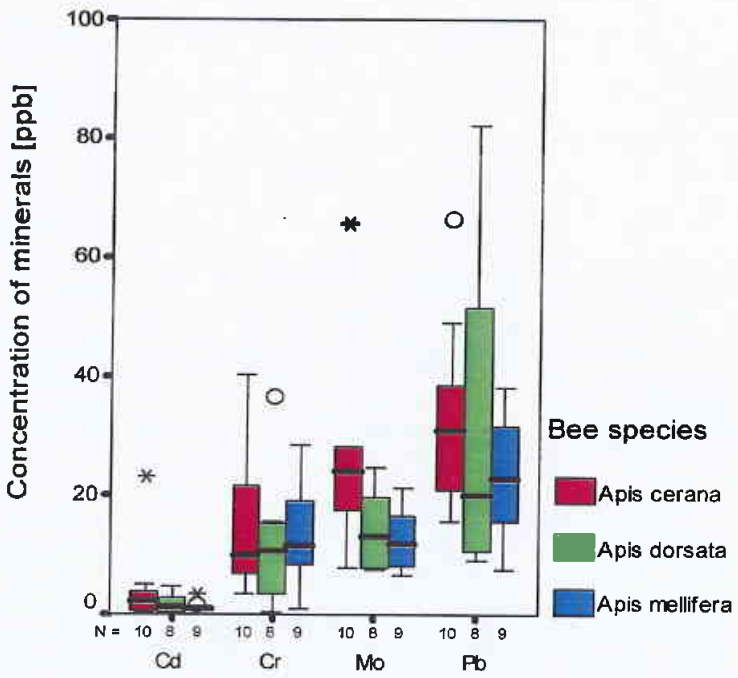


Figure 11

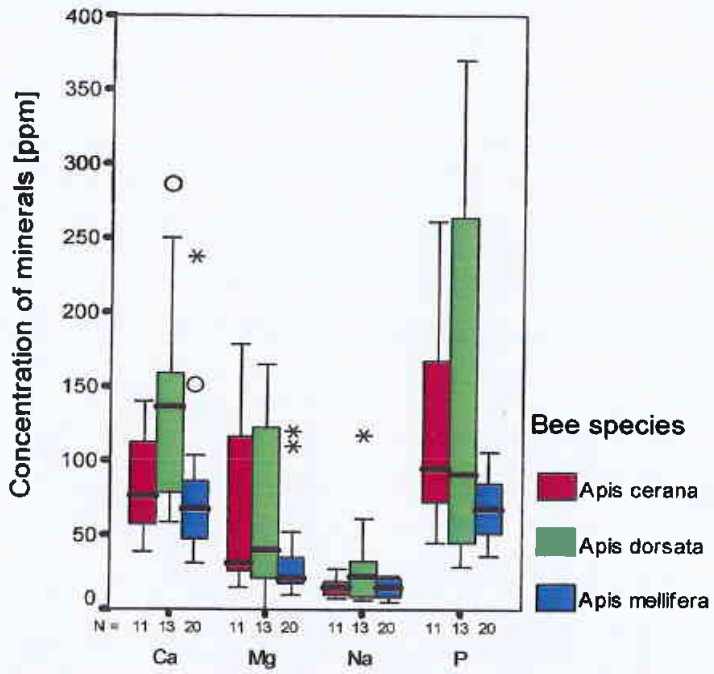


Figure 12

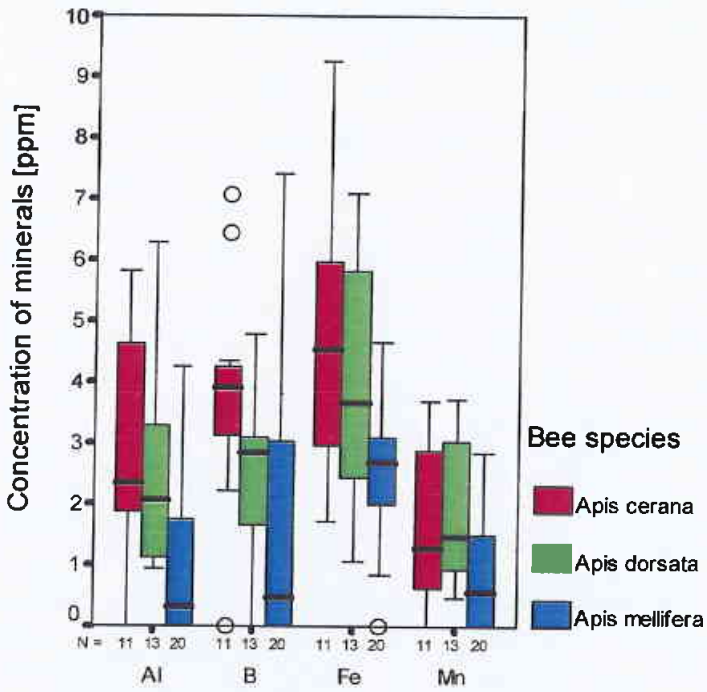


Figure 13

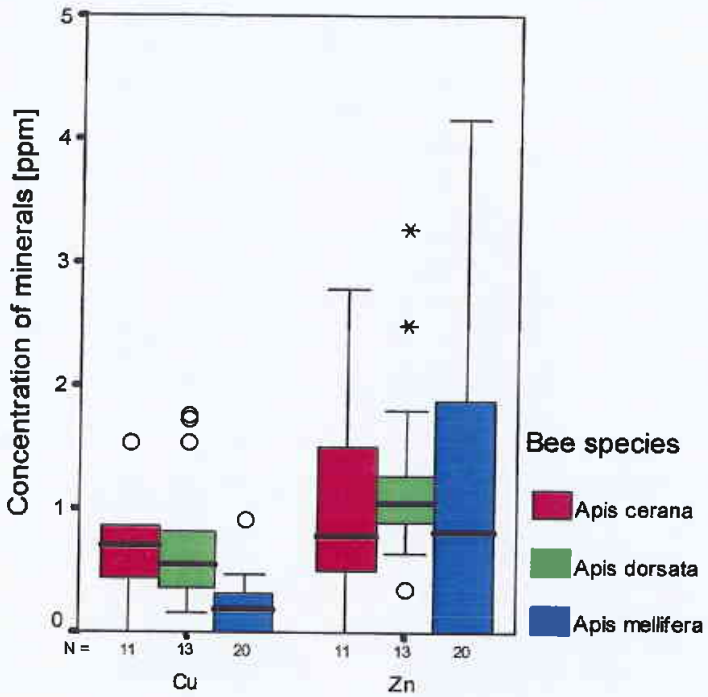


Figure 14

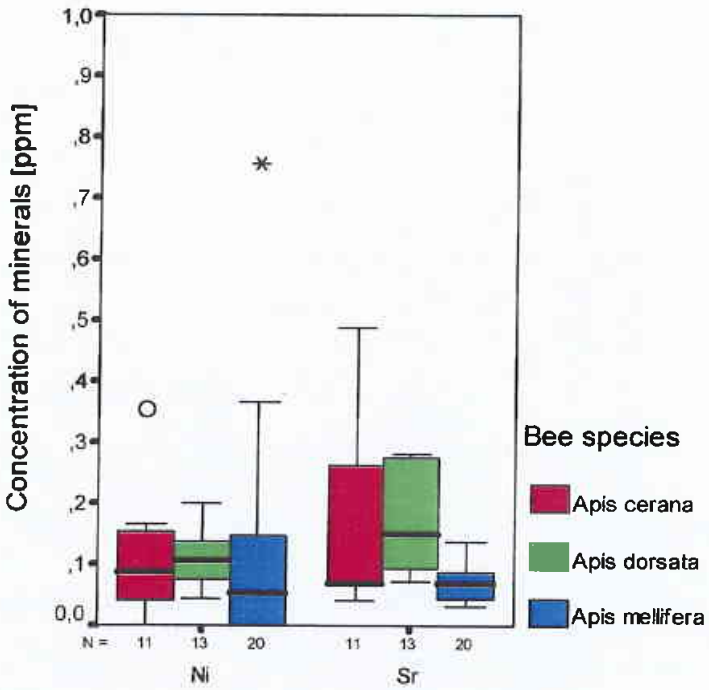


Figure 15

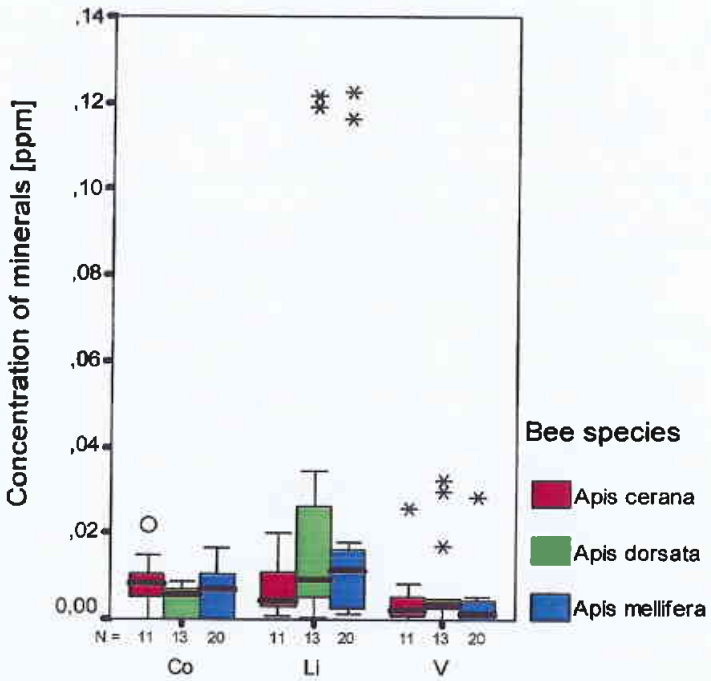


Figure 16

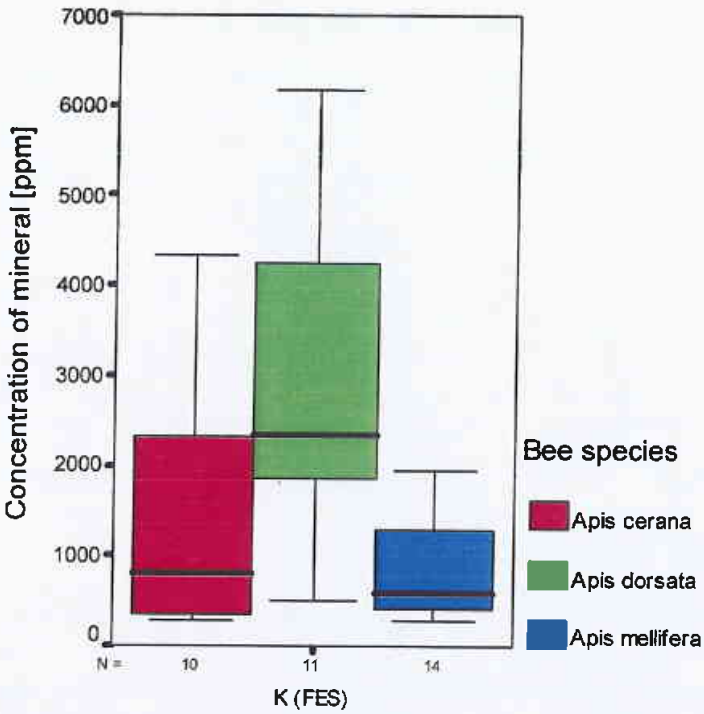
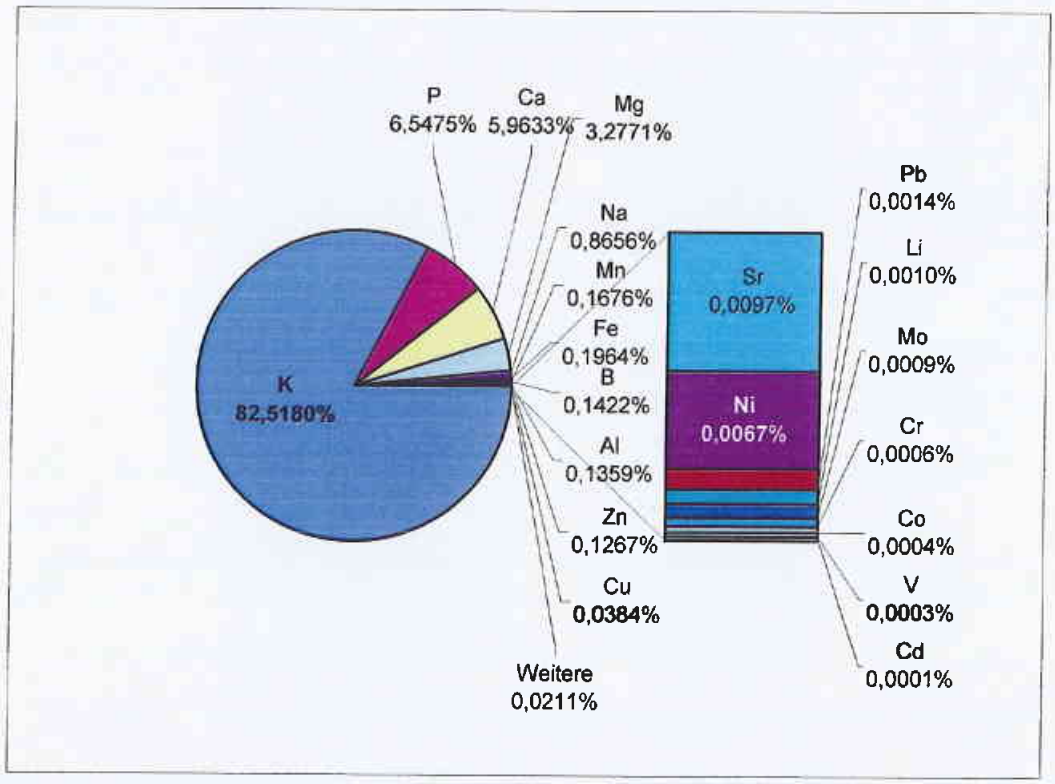


Figure 16a. Total ash in over all *Apis cerana, A.dorsata and A. mellifera* honey samples from Chitwan.



4.7.1 Discussion

Elements in *Apis cerana* honey from different locations

Heavy metals are the natural component of the geosphere and have also become the inseperable part of soil, water, air and biota (Brümmer et al., 1991). There has been growing concern about environmental contamination by heavy metals (Kabata-Pendias and Pendias, 1984; Adriano, 1986; Leith and Markert, 1990). Inputs of chemical substances constitute the greatest pollutant burden on natural ecosystem (Markert et al., 2000).

Honey is likely to be a good indicator of either the atmospheric or soil heavy metal burden, since spatial differences will be masked by inherent differences in the elemental content of honey at any one location. Analysis of honey samples has been used in previous studies to monitor a variety of environmental contaminations, including radionuclides, elements and pesticides (Djuric et al., 1996; Jones, 1987; Rifai and Akeel, 1997).

Pechhacker (2002) found soil had a significant effect on rape honey from Austria.The elements are distributed in topsoils (in relation to land use) (Bloemen et al., 1995) and elements such as cadmium, lead, and zinc transfer from industrially contaminated soil to crop plants (Dudka, et al., 1996). In addition, phosphorus is absorbed by the plant from the soil solution as primary and secondary orthophosphate ions and magnesium is absorbed by the plant as the ion Mg^{2+} it is a chemical constituent of chlorophyll. Calcium is taken up by the plant as the ion Ca^{2+}, largely from the soil solution, and perhaps, to some extent, through contact exchange with ions immobilised at the surface of the soil solids (Bakker, 1999). Plant that accumulate the (radioactive) trace elements (Ehlken and Kirchner, 2002) allow us to measure the metal input which is often below detection limit in water and air. And it is well understood fact that bees collect pollen and nectar from plants.

Concentrations of K, Fe, Zn, Br and Pb in the leaf veins, roots and stem of *Eucalyptus globulus*, *E. camaldulensis, E. lesouefii, E. saligna* (Orlic et al., 2002) were analysed. Zn content in Mangroves (*Avicennia marina* Forsk.) (MacFarlane and Burchett, 1999); Cd and Zn in dry alfalfa (*Medicago sativa* L.) (Millera et al., 1995); Cd, Ni and Cr tolerance and its ability to accumulate by *Cannabis sativa* L (Citterio et al., 2003); effect of bioaccumulation of cadmium (Cd^{2+}) on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat (*Triticum aestivum* L.) seedlings (Shukla et al., 2003) also analysed.

Bromenshenk et al., (1985) reported marked spatial variations in the concentrations of As, Cd and Fe in bee tissues, date obtained for Cu, Zn and Pb could not. Foraging honeybees also exhibit considerable differences in their life expectancy through a season and the trace

Cu, Mn, Ni, V, Zn (fig. 3(a), 4(a), 6(a), 7(a), 8(a)) in honeys from Chitwan exhibited relatively low concentration element spectrum.

The honey samples from all locations typically became enriched in K (represented in fig. 5(a)). Farmers use potassium as a fertilizer. Potassium is absorbed by the plant as the K^+ ion from the soil solution and to some extent by direct contact exchange from the surface of the soil solid. Nearly all potassium within the plant occurs in soluble form. It does not constitute part of the dry matter of the plant. Its high mobility may account for the fact that this element is involved in many physiological functions of the plant (Bakker, 1999). This could be the reason for high potassium content in honey samples from all locations.

Since the presence of elements in honey is often taken as a concise description of the environmental contamination of a place, difference in concentration of Ca, Fe, P, Zn, Cd, and Pb between polluted (Kathmandu, Kathmandu valley and Chitwan) and clean areas (Jumla-Jajarkot, Palpa and Langtang) were compared. Significant difference was found in total Pb content ($P=0.0001$) when compared urban (industrial) areas like Kathmandu, Kathmandu valley, and Chitwan with that of the rural areas like Jumla-Jajarkot, Palpa and Langtang. There were no significant differences found for Cd, Zn, Fe, Ca and P contents when compared between honey from urban and rural areas. The statistical analysis showed that the levels of Cd, Zn, Fe, Ca and P were found to be similar to levels detected in unpolluted areas. A notable but less concentrations of Pb has been found in honey samples from Jumla-Jajarkot, with an accompanying increase in Kathmandu and Chitwan honey (table 1 & 2). In other words, the lowest Cd, Pb, Cr, and Mo values were found distributed fairly even among honey samples from Jumla-Jajarkot. With respect to some elements particularly high concentrations were noted as unique to the certain areas, for example, Pb in Kathmandu and Chitwan (AAS). Values indicate that pollutant such as Pb is most likely in urban areas such as Kathmandu and Chitwan. Langtang honey showed higher amount of Zn (fig.7**(a)**) and notable Pb content (fig. 1**(a)**). There was also one sample had unexpectedly enhanced Zn concentration in Langtang honey. According to Chakrabarti (1982) in the Ganesh Himal region high grade mineralization of zinc and lead sulphides occur near the Main Central Thrust (MCT) Zone of the Himalayas in the lower region ogf Ganesh Himal range (including Langtang region) in Nepal. These honeys may have been influenced by the lithology of this area comprises a thick succession of alternating schists, quartzites and calcareous rocks which contains significant amount of lead and zinc. The lead and zinc of this area was under active exploration from 1967 till 1973. At present Nepal metal company is producing mine from the ore body.

The study by Mahajan (1984) reported 1432 ppm K, 265 ppm Ca, 118 ppm P, 109 ppm Mg, 11.9 ppm Zn, 5-3 ppm Fe, 3.71 ppm Mn in *Apis cerana* honeys from northern India. Kalimi and Schonie (1964) reported the amount of sodium in range 18.2 to 45.4, phosphorus in range 17.2 to 34.2, iron in range 169 to 389 and magnesium in range to 47 to 122 ppm. In honey samples from Mahabaleshwar, India. Na is lower in all samples, except Kathmandu honey; P is higher in all locations; Fe is lower in all locations; Mg is similar in Jumla-Jajarkot honey; higher in Langtang, Chitwan, Palpa and Arghakhachi honey but lower in Kathmandu honey that reported by Kalimi and Schonie (1964).

There are extensive reports about trace elements content in European honey from *Apis mellifera bee* more or less similar those of the other country's honey, which have been most studied, are probably related to its environmental situations. It is notable that a concentration of trace elements reported in this study Kathmandu and Chitwan resemble those of vehicular, industrial and population density, so there may be further pollutants in populated areas (see annexe 1).

Rodriguez-Otero et al., (1994) analysed element contents of the honeys produced in Galicia, north-west Spain indicates that potassium is the most abundant of the elements determined, with an average content of 1572 mg/kg followed by Ca 102 mg/kg, Na 138mg/kg, Mg 106 mg/kg, and Fe 5.12 mg/kg. The mean content of Na is much lower in all locations, but similar to K, Fe and Mg content in Chitwan honey, Ca and Fe content in Langtang honey, and Fe content in Kathmandu valley and Jumla-Jajarkot honey than that of Spanish honey reported by Rodriguez-Otero et al., (1994).

Jones (1987) reported 4.0ng/g cadmium, copper 540 ng/g and lead 66 ng/g content in honeys from Dolgellau, north Wales. The mean content of Cu 505 ng/g and Pb 51 ng/g, Cd 28 ng/g was found in UK honeys from various locations (Jones, 1987).

When compare Nepali honey samples with those reported by Mahajan (1984) it is apparent that the mean content of K, Mn and Fe found to be roughly similar to Chitwan honeys, Zn and Fe to Langtang honeys and Fe to Kathmandu and Kathmandu honeys. It seems that (see annexe 1) the city like Kathmandu or Chitwan is relatively vulnerable to pollution, perhaps because of the pollutants present in the urban conurbations may be either by vehicular, or industrial emissions. Pollutants (such as Pb and Cd) may tend to avoid hills and follow valleys, and intensify on down slopes.

As discused above, Kathmandu is the polluted capital city, with maximum Pb concentration followed by Chitwan and Kathmandu valley. There has been a striking reduction in the

concentrations of elements Cd, Pb, Cr and Mo in Jumla-Jajarkot honeys (see table). 120 μg/kg Pb content in Italian honey from Campobasso and Abruzzo region were reported (Cubadda et al.,1995; IZS Abruzzo e Molise, 1991) this value was found to be similar with that of honey from Kathmandu.

It has now generally agreed that there has been a global increase of pollutants, possibly because of more vehicular, chimney, industrial emissions. These values together reveal more apparent picture about pollutants, such as Cd, Pb, Cr and Mo, and even situation.

The traffic and industry are the major source of atmospheric pollution and it provide a major contribution to metal fallout in the urban environment. In addition, smoke emissions by industries located within the Kathmandu valley and Chitwan have become a source of air pollution. Kathmandu is especially vulnerable to air pollution due to its bowl-like topography, exploding populatic inflow, rapid urbanization, industrialization, haphazard waste disposal, and significant increase of vehicular transport in narrow streets.

The chance of pollution is greater in urban areas; the Pb concentration ranged from 67.18 – 178.00 μg/kg and 15.75 – 66. 53 μg/kg in honey samples from Kathmandu and Chitwan respectively. On the other hand, the range found in Jumla –Jajarkot honey was 1.66-14.39μg/kg (see annexe 1for details). The enrichment factor for Pb indicated the absence of (or less) atmospheric pollution in rural areas. The honey samples from polluted locations put through different environmental circumstances: Pb and Cd may be entrapped in a particulate form, as honeys (and bees) contaminated with these metals in variable ways in accordance with species and conditions of pollution. 4 hours average lead content in Kathmamndu air have been found to be within the range of 0.005 to 4.25 μg/m^3 (NESS, 1999). Concentration of Zn ranged from 0.001-0.065 mg/l; Pb <0.003-0.05 mg/l; Cu <0.001-0.03mg/l , and Cd <0.0005-0.002 mg/l in rain water collected from Kathmandu valley (Shahi and Chaulagain, 1999). The bowl like topography restricts wind movement and retains the pollutants in the atmosphere especially during the winter season when the thermal inversion period over Kathmandu, where cold air flowing down from the mountains is trapped under a layer of warmer air, which acts as a lid. As a result, the pollutants are kept sealed within the valley. Because lead is transferred less efficiently than copper between the soil-root-shoot-flower/nectar/pollen compartments (Jones, 1987), aerial deposition of lead in dust particulates may therefore have a greater influence on lead levels in honeys with deposition of some other elements (see table 1). The Pb content of honey from Kathmandu could be the determinate factor for the accumulation of chemical elements through traffic and industry in honeys in this area. The altitude also seems to be important for contaminants present in honey samples.

The elemental concentration (i.e. Pb) appeared to be least over the mountain areas (such as Jumla-Jajarkot, Langtang). These pollution free remote mountaineous areas have less amount of toxic elements. The toxic elemental values were found to reflect essentially the emission of toxic pollutants in the urban environment. There may be a correlation between air quality, soil, plants and lead (and other elements) content in honey.

Lubricating oil often contains Cd, Cu, V, and Zn (Garty, 1993) and vehicular emissions, wearing away automobile tyres (20-90 mg/tyre) and Cd containing diesel oil exhausts are among the main sources of Cd to the environment (Scheffer and Schachtschabel, 1992). Also, the phosphate fertilizers are considered as the major Cd sources (Andrews et al.,1996). Kathmandu and Kathmandu valley honey showed lower amount of copper, vanadium, zinc and cadmium in spite of heavy population of vehicles. Whereas, Chitwan honey had relatively higher cadmium amongst all (fig. 2, 4, 7). This may the research question for further work. In this study, *Apis cerana* honey samples adjudged to be influenced by the vehicular emission, industrial sources, haphazard waste disposal (in urban areas) and elements exist in soil and plants this is further magnified by leaded, substandard and adulterated gasoline. The findings were considered to provide limited but encouraging signs of a clean environmental condition in the rural areas. In adition, element content and electrical conductivity is directly correlated. Bicik and Kaspar (1986) reported additional data on the element content of floral and honeydew honey and pollen. K, Mg, Ca, Zn, Al, Cu, Mn , and Pb contents were detected higher in honeydew honey, but at lower concentrations than those present in stored pollen. *Apis cerana* honey samples analysed for this study may be came from honeydew. Therefore, the pattern of (trace) element concentration did not always coincide with air quality and the additional factors such as soil and floral sources also contributing for elemental concentrations in honeys.The relatively low concentrations of elements in honey samples also do not give the precise picture of the environmental situation and its use as an indicator of environmental contamination especially when honey samples are too few. The relative impact of soil and plant-derived and aerially derived trace elements on Nepali honey needs to be more fully assessed.

Elements in *Apis cerana* , *A. dorsata* and *A. mellifera* honey from Chitwan

The values given in table 3-5 correspond to mean and standard deviation and there is quite some chemical variation within a given honey group. Fig 10-16 show trace elements contained in the honey samples.

The elemental concentration (K) was found to be highest in *Apis dorsata* honey from Chitwan with average 3001.09 mg/kg and standard deviation 1946.04; followed by P, Ca, Mg, Mo, Cr, Mn, Al, B, Zn, Cu, Co, V (in *A. cerana* honey); Na, Fe, Sr, Li, V (in *A. dorsata* honey); and Ni (in *A. mellifera* honey) found to highest in concentration (table 3; fig. 10, 11, 12, 13, 14, 15, 16). Similarly, *Apis cerana* honey from Chitwan showed highest Pb concentration followed by Mo, Cr, Cd (fig. 10).

Amounts of elements found to be higher in honey produced by local species (*A. cerana* and *A. dorsata*), while the exotic species *Apis mellifera* honey found to be lowest in all elemental concentration (except Ni). There were minimum concentrations about all the elements (except Ni) and second maximum about the elements Li, Cr, Co and Zn (see fig. 10 and 15). Most striking is the close connection between elemental concentration and native bee species. The maximum concentration of K in *Apis dorsata* honey is at a range between 506.50- 6159.50 ppm (see annexe 1).

Differences in concentrations of the elements Ca, Sr, K, Al, Cu and Mn was found to be significant in *Apis dorsata* and *A. mellifera* honeys. Also there was a statistically significant difference found in concentrations determined in the *Apis cerana-Apis mellifera* for elements Fe, P, Al, B and Cu. Similarly difference in concentrations of elements Ca, Sr, K, Al, Cu and Mn was significant in *Apis dorsata- Apis mellifera* honeys.

No statistically significant differences were observed in concentration of all elements had been determined in *Apis cerana* and *Apis dorsata* honeys.

Consequently it appears that there were no significant differences observed in Pb,Cd,Co concentrations of honeys from *Apis dorsata* and *A. mellifera*; *A.dorsata* and *A. cerana*; and those from *A. mellifera* and *A. cerana.* Whereas, Mo content differed significantly in *Apis cerana-A. mellifera* honeys (see table 3 and 4).

Bicik and Kaspar (1986) found the honeydew honey richer in minerals than floral honey particularly K, Mg, Ca, Zn, Al, Cu, Mn and Pb. The concentration of all the elements was generally much high in dark samples than in the light ones, and it was attributed to differences in nectar-plant species (Yeboah-Gyan and Marfo, 1998). Gürel et al., (1998) found ash, total acids, K, Fe, Mn and Mg significantly lower in Sugar fed colonies than in mixed nectar sources and pine honeydew sources. K, Mg and Cu content were found to be significantly higher in pine honeydew honey than in mixed nectar sources and sugar fed honey.

Horn (1985) found German honeydew honeys were higher in potassium and phosphorus and lower in sodium when compared to floral honeys. There is positive correlation between honeydew excreting insect population density and honeydew honey flow (Pechhacker, 1976)

and correlated with an increase in the potassium and phosphorus levels (Horn, 1985). Honey bees (*Apis mellifera* L.) feed mainly on nectar and pollen (Imdorf et al., 1998). On the other hand, *Apis cerana* bees collect more honeydew honey than *Apis mellifera* bees in Chitwan and Kathmandu valley (Joshi,1999). Honeydew honey has a higher total content of minerals (ash) than nectar honeys (Crane, 1990) and has higher electrical conductivity. There is a positive correlation between electrical conductivity and element content in honey. Joshi (1999) found the significantly higher electrical conductivity in *Apis cerana* honey samples from Kathmandu, Kathmandu valley, and Chitwan.The electrical conductivity values were found to be significantly higher as 0. 79 (mS/cm) and 0.26 (ms/cm) in *A. cerana* and *A. mellifera* honeys from Kathmandu respectively (Joshi, 1999) which agreed with the results reported by Shrestha (1998). In same study Joshi (1999) reported the electrical conductivity was significantly higher in *A. dorsata* honeys collected from two different nesting sites (trees) (1.79 mS/cm and 0.49 mS/cm) than *A. cerana* and *A. mellifera* honeys from Chitwan.Whereas, pH values did not show significant differences between *Apis dorsata, A. cerana* and *A. mellifera* honeys from the same location.The limit of electrical conductivity which has been proposed for honeydew honey than (<0.7mS/cm) for nectar or blossom honey (Bogdanov et al., 1997).

Therefore, in this study, significant difference in concentration in *Apis cerana-Apis mellifera* and *A. dorsata-A. mellifera* honeys observed may also be due to honeydew honey produced by these local species. And, also, the overall concentrations of K followed by P, Ca, Mg, Na, Fe, Mn, B, Al and, Zn in honey samples found to have high.

Traffic density is a major contribution to metal fallout in the urban environment. In addition, smoke emissions by industries in the city have become a source of air pollution. Honey from the urban/industrial areas of Birmingham and Liverpool contained elevated concentrations of Ag, Cd and Pb (Jones, 1987). The combustion process in automobiles cause the release of elements such as Zn, Cd and Cu (Ormrod, 1984). Tire wear cause the release of elements such as Zn, Cd, Cu, Fe, Mn, Pb and Ni (Christensen and Guinn, 1979; Beckwith et al., 1985; Ward, 1989; Ormrod, 1984; Sadiq et al., 1989). Ward (1989) detected Cd, Cu, V and Zn in lubricating oils, Cr, Ni and V in car metal plating and Mn, Cu and Zn in brake pads. Many industrial contaminats are to some extent biodegradable but some of them, such as heavy metals, are highly persistent in the environment.The exhaust particles do not travel much beyond the road sides, but do exert an apparent influence on vegetation at roadsides, whereas gaseous pollution arising from vehicular exhausts travels much further (Garty, 2000). Many heavy metals are phytotoxic at high concentrations. Furthermore plants act as intermediate

reservoirs of heavy metals from primary sources, e.g. soils, water or air, through which they move on to the organisms (Breulmann et al., 2000). Heavy metals accumulated by plants may lead to the presence of heavy metals in bee collected pollen and nectar. Bee-collected pollen has also been suggested as a useful indicator of soil metal levels within the forage area (Free et al., 1983). The presence of Pb, Cr, Cd and Zn detected in this study suggests the samples were influenced to a great extent by vehicular/industrial emissions and floral sources as well. K was abundantly presented elements whereas Cd was the lowest in Chitwan honey. Sanchez and Pujola (1996) also found potassium in the higher concentrations in Spanish honey. Results obtained by Mahajan (1984) showed 1432 ppm K, 265 ppm Ca, 118 ppm P, 109 ppm Mg, 11.9 ppm Zn, 5-3 ppm Fe, 3.71 ppm Mn in *Apis cerana* honeys from northern india. Comparing the results with those from the northern india, it is apparent that the total potassium content is high; Ca, Zn and Mg is low; while P, Fe and Mn is roughly similar in Chitwan honeys.

The Na, Fe and Mg is much lower, but P content is higher in Chitwan honeys that reported by Kalimi and Schonie (1964).The values of overall minerals were between 460.533 ppm minimum and 6719.538 ppm maximum with an average of 2094.418 ppm.

As particle-water interactions influence the mobilization of trace metals in sediments (Hamilton-Taylor and Davison, 1995; Hong et al., 1995), bioavailability of trace metals is influenced by the extent of their sorption and (co) precipitation with the solid phase (Berg et al., 2000). The concentrations of the elements probably result from, the fact that the district is located in plain and not far below from the straightened part of the Narayani river where the low velocity of the water flow reduces the stream power so that the suspended matter settles and may be attributed to residues accumulated by foraging (native) bees by means of soil and plant.

Finally, the element concentration did not always coincide with pollution due to vehicular density, industries, solid waste disposal practice etc. Other factors such as soil and plants are also important. However, the relatively high concentrations of elements in honey samples from native bees may give the picture of environmental condition of the area and honeydew flow as well. On the other hand, low concentrations of elements in honey samples (either from native bees or from exotic bes) do not preclude its use as an indicator of environmental contamination due to very limited (or less) number of samples. The influence of soil, floral source, pollution, forage range on native and exotic bees produced honey and its quality need further research.

Honeys produced by *Apis cerana* analysed from seven locations. The average elemental concentrations of Li, P, Al, Co, Ni, Zn and Cr in Langtang honey; Na, Fe, B, Co, and Pb in Kathmandu honey; Sr, Cu, Mn, Cd, and Mo in Chitwan honey; K in Palpa honey; Ca and Mg Arghakhachi honey found to be highest amongst all. On the other hand, K and V in Kathmandu honey, Ca, Mg, Mn, and Zn in Kathmandu valley honey; Na in Chitwan honey; Fe, P, Al, B, Co, Cu, and Ni in Arghakhachi honey; Li, Cd, Pb, Cr, and Mo in Jumla-Jajarkot honey found to have lowest amongst all. The total ash content of overall *Apis cerana* honey samples, commonly K, P, Ca, Na, Al, and Fe. The percentile of these elements ranged from K 83.28% to Cd 0.0001 % of ash. Significant difference was found only in case of Pb content (P=0.0001) when compared elemental concentrations (Ca, Fe, P, Zn, Cd, and Pb) in honeys from urban (industrial) areas with that of the rural areas. In the case of honeys produced in Chitwan, *Apis dorsata* and *Apis mellifera* honeys showed significant difference only for Ca , Sr, K (FES), Al, Cu and Mn. Li, Na, Mg, Cd, Co, Cr, Ni, Pb, V and Zn were not significantly differed in *Apis cerana-A. dorsata, A. dorsata –A. mellifera, A.cerana- A.mellifera* honeys. difference were significant for Fe, P, Al , B, Cu, Mo in *Apis cerana* and *A. mellifera* honeys.
No statistically significant difference in concentration found in case of all elements in *Apis dorsata* and *Apis cerana* honeys. The total ash content of overall *Apis cerana, A. dorsata and A. mellifera* honey samples, ranged from K 82.5% to Cd 0.0001%
Elements occur naturally in the environment in different forms that may have health implications leading to adverse effects and directly related to human health. In past decade, new possibilities for biological monitoring of elements have been developed. Honey is widely used food and possesses a wide array of nutrients in trace amounts and may also be useful for identifying environmental pollution.

EPILOGUE

Why is it necessary to understand and study antibiotic activity of honey and element content in honey from different bee species? How do we account for them? These are some of the questions dealt with in this work.

Introduction- this is a chapter one, that shortly introduces Nepal and its agro-ecozones, climate, vegetation, agriculture etc.

Chapter two outlines the distribution of honeybees, beekeeping in Nepal, sources of bee forage, Nepali honey types, honey hunting, future of beekeeping etc.

Chapter three introduces honey as traditional healer and also investigates the use of honey as modern medicine for various diseases. This chapter forms a major component of the thesis and also gives a breif account on test organism *Staphylococcus aureus*. There is a focus on the *Staphylococcus aureus* as test organism, to evaluate peroxide and non-peroxide antibacterial activity of different honey types. This allows fresh insight into many possibilities. The worldwide literature on antibiotic activity of honey has been extensively reviewed and presented. It deals primarily with explanation of the antibacterial activity of honey, applicable anywhere in the world. The universal nature of the information, explanation and investigations (by other workers) on antibacterial activity/components of honey makes thesis almost equally relevant.

Based on their altitudinal ranges total 164 honey samples were aggregated in simpler classification of terai, hills and mountains. The honey samples collected from Chitwan (*Apis cerana, A. dorsata, and A. mellifera*) and Mahendranagar (*Apis florea*) were grouped under terai (< 500masl); Kathmandu (*Apis cerana* and *A. mellifera*), Arghakhachi (*Apis cerana*) Barabise (*A. laboriosa*), Bajhang (*A. laboriosa*) and Dadeldhura (*Apis florea*) under hills (500-2000masl); and samples from Jumla, Langtang and Daman were placed under mountains (2000-3000masl). A culture of *Staphylococcus aureus* ATCC 6538P obtained from the Institut für Futtermittel, Vienna was added to sterile liquid nutrient agar (23g/L 001-7-0 DIFCO) at 45°C (Inoculum preparation), plate preparation, catalase solution (bovine liver Sigma C-9322;2460 units/mg), preparation of honey solutions, Application of samples to the plate, phenol standards (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 % (w/v), zone measurement, calculation of antibacterial activity and the statistical analysis (SAS for windows 6.12) were the major components of methodology of antibacterial part.

Detail methodology has been given.The data have been analysed, statistically using SAS, graphed and tabulated. The results and findings have been written up and discussed in light of previous findings.

Discussion of each results bring together the honey from different bee species, agro-ecozones, geographical origin of honey, peroxide and non-peroxide antibacterial activity to give overall picture.

In this study, peroxide activity (hydrogen peroxide) could be the main component (associated with different bee species) of Nepali honey. On the other hand, non-peroxide activity was also in different honey types except *A. cerana* honeys from Dadeldhura, Jumla, Kathmandu and Langtang. In addition, there may be other additional factors came from floral sources responsible for (non-peroxide) antibacterial activity in Nepali honeys. One major issue to consider in the medical usage of honey (only with peroxide activity) is its interaction with blood and/or plasma. Hydrogen peroxide-in honey can be rapidly degraded by plasma catalase. Therefore, honeys with high levels of antibacterial activity (non-peroxide) are promising.

Chapter four also forms a major component of the thesis. This work provides an entry to an understanding of the role of honey (from different bee species) as an environmental indicator. Considerable attention is given to shorten (or exclude) topics of quite general interest, such as properties of each elements and much else.

The world wide literature on (trace) element related matters has been extensively reviewed. The honey samples collected from Chitwan (*Apis cerana, A. dorsata, and A. mellifera*) was grouped under terai (< 500masl); Kathmandu (*Apis cerana*), Palpa (*Apis cerana*), and Arghakhachi(*Apis cerana*) under hills (500-2000masl); and samples from Jumla-Jajarkot (*Apis cerana*) and Langtang (*Apis cerana*) were placed under mountains (2000-3000masl). A large spectrum of elements (20) was determined. Inductively coupled plasma optical emission spectroscopy (ICP-OES or ICP-AES) is a major technique for elemental analysis. K was determined by flame emission (FES) on a Perkin Elmer 3030 AAS. A simultaneously operating optical ICP (Perkin Elmer Optima 3000 XL) was used as a multi-element analytical tool and the elements lead, cadmium, chromium and molybdenum were analysed using graphite tube (Perkin Elmer 3030 Z). Every elemental content was corrected with the water content of the honey sample. All the results are based on dry weight. SAS (Statistical Analysis System) for Windows V8 was used. Sample decomposition, sample preparation-ICP, ICP calibration standards were the major steps of the method used for element spectrum.

The detail and comparative discription of methodology and instruments (ICP and AAS) has been given.

The data have been analysed, statistically using SAS, graphed and tabulated. The results and findings have been written up and discussed in light of previous and recent findings.

Elemental analysis of Nepali honey samples from different bee species has shown that honey may be an environmental indicator of heavy metal contamination. On the other hand, Honey is widely used food and contains a wide array of nutrients in trace amounts. Discussion of each results bring together the honey, bee species, agro-ecozones, environmental pollution, to give overall picture.

Epilogue is a short part, that sums up the rest of the thesis. The apparent logic of this work is contrived, imposed on a subject of enormous complexity. In fact, items in antibiotic part may depend on those in (trace) element part. This may be the topic of further investigation, because the nature is single entity, any individual aspect of which affects all the rest. Indeed, it is one of the aims to investigate and to appreciate the complexity of the interrelationships. In addition, annexes, photographs, references, and summary are the important components of the thesis.

Today, there is often a decrease in the availability of wild plant resources, indigenous bee species related to increased human activities and the efffects of competition with other forms of land use. And, also, the wide use of insecticides, diseases and parasitic mites are reducing the populations of honey bees which lead to loss of both native and agricultural plants.

Nepal is beset with poverty, malnutrition, unemployment, diseases, high mortality and scarcity of natural resources. Bees are the efficient pollinators, the abundance of bees, and of bee forage, give a good potential for various crops. Honeybees produce natural materials useful to man and increase agricultural production. Bee products such as honey, beebread, pollen, beewax, royal jelly, and propolis benefit people. Honey, beebread and pollen contain a wide array of nutrition and therefore could be one of the important source of nutrition. Beekeeping could be as one of the important activities in improving the standard of nutrition of the population of Nepal. Therefore for rural development against poverty it could be a good profitable venture by means of low cost/high yield enterprise for rural people and provide income with health food without the need for compulsory land ownership or much capital investment and enhance the use and conservation of local biodiversity resources.

Household and community level beekeeping practices essential for good nutrition and income generation for poverty alleviation. The extent of the potential conflicts, however, depends on future productivities. Increasing food production on the better agricultural lands,

while growing trees or perennial grasses for energy on marginal lands, would generally be environmentally preferable to increasing agricultural output by bringing marginal lands into food production coupled with beekeeping. Because growing beekeeping practices require no considerable land but floral resources. There is no hiding from the fact that programs for promoting beekeeping must reflect environmental and security concerns. The question is not whether this issue will affect national investment, but when and how.

On the other hand, application of insecticides and deforestation may lead to adverse impact on honey bees and insect pollination of native and agricultural plants .

The awareness should be raised about nutritional foods (such as honey) found in the local biodiversity resources. The knowledge /capability of the local people should be build to integrate beekeeping practices and marketing facilities for the produce and exploitation with local biodiversity management to stump out poverty. Support local people's, especially women's organizations, with an emphasis on their empowerment. By promoting the production, the country could address several needs at once: it could reduce poverty, create jobs in a depressed economy, and exploit idle capacity in the agro-industry. Strengthen linkages and co- ordination with relevant Institutions/NGOs involved in integrated biodiversity research and apiculture.

Not only would such attempts help protect the environment in a completely self-sufficient way, but biomass would produce useful energy alternatives with important applications in household, agriculture, and industry. In addition, biomass energy is significantly less expensive because it requires less equipment and is far simpler to use. With good management, bee products (honey , beebread and pollen) for energy and nutrition can be produced sustainably and in large quantities. The country would benefit from the implementation of the proper systems.

Social, cultural and gender (equality) factors are also important, particularly in rural areas outside the monetary economy. The policies should be implemented particularly targeting the women and the poor to assist them to enable produce and market honey, negotiate realistic prices and be able to raise tradable volumes of honey and other hive products. Among indigenous people food preparation usually has ritual significance.Thus, the beliefs and customs of our planet`s inhabitants should not be ignored when any kind of new development project is initiated for a country's social and economic development. New ideas and innovative steps to work with women in beekeeping activities would promote economic prosperity of the people from crushing poverty. This should be through the promotion of organic quality products and market organisation for household poverty reduction.

There is need for a great deal of apicultural research in Nepal. Much scientific works have yet to be done. Advantages of *Apis cerana* beekeeping makes one very optimistic for the future of beekeeping in Nepal. The honey yield of *Apis cerana* could be improved genetically by intensive selection and breeding. In addition, database building, popularizing the production of hive products toghether with honey, value addition of bee products, systematic tapping of organic –forest honeys and breeding superior stocks and establishment of genetic resource centres may open new horizon for Nepali beekeeping. The economic and transport infrastructure, trained manpower and monetary resources, marketing and the expansion of products are the essential factors for the improvement of beekeeping industry in the country. For the efficiency of economical integration, the marketing and the expansion of products should be accomplished.There are constraints to the developments of beekeeping e.g. lack of policy and legislation, training and information, quality of honey and other hive products, limited market and limited access to production credits.

However, the future of beekeeping and apicultural research is not strategically critical not only now but for the coming decades. Work (in apicultural science) under way in several industrialized countries is applicable to developing countries. While poorly designed environmental regulatory programs will dampen productivity and economic growth, environmental policies can also be designed to promote productivity and poverty reduction. Progams forcing attention on environmental issues can also focus on inefficient and unhealthy production practices, stimulate thinking about new approaches, and encourage investment in more productive equipment. In addition, market incentives must be created to overcome barriers that exist, especially in the financial community, because apicultural practice is gaining popularity and on the other hand, may be viewed as competitive with existing other farm practices. With good management, better quality honey and other bee products for different purposes can be produced sustainably and in large quantities in many parts of the country. By far the largest potential source of bee products is plantation. The establishement of plantations on deforested and otherwise degraded lands offers major developmental and environmental benefits, as well as large and secure supplies of energy as well.

In short, during the past years satisfactory progress has been made in apiculture.This development will have a profound impact on socio-economic condition of the country. It is important therefore to consider the role of apiculture in planning the future economic growth. Challenging but rationally conceived environmental regulations can stimulate investment that can enhace productivity, competitiveness, and job creation, while ensuring that national

environmental and security goals are met efficiently. Of course, there are limits to the beekeeping that can be kept where land area are high (altitude) or water is scarce or both. Honey has been used as a medicine since ancient times in Nepal and many rural areas are still using it as a folk medicine.The indigenous use of honey as a medicine for primary health care represents a heritage of socio-cultural and economic significances in developing countries like Nepal, where more than two thirds of the population are illiterate, live in villages and subsist upon local natural resources. The use of honey in rural Nepal is spiritual, cultural, and traditional.The herbal resources are also playing important role in health care system.The way of applying indigenous knowledge depends on ethnicity and methodology where the goal is somehow similar. Socio-economically, most of the rural communuties are very poor and the poverty compels the rural people to practice traditional medical system. Different ethnic groups of rural Nepal keep sufficient knowledge about the use of plant products, honey or together for treatment of various diseases.

Interest in antimicrobial and wound healing potential of honey subject has lately been greatly stimulated by the several workers and believed to have important effects both on safety and stability. It is gaining acceptance by the modern science for the treatment of various diseases. An extensive survey of literatures shows a great progress in evaluation, understanding and implementation of the honey as a medicine. On the other hand, new antimicrobial drugs and medication are urgently needed for increasingly resistant Staphylococci. No toxic effects of Honey have been reported in human tissue, unlike other topical antimicrobial agents and also it has the potential to limit the growth of wound pathogens, and to promote healing

Wider use of honey on all wounds and microorganisms will depend on evidence from clinical research. The results reported here are sufficiently encouraging to warrant further investigation into the role of honey (from different bee species) in wound healing and its antibacterial activity which could be the another tool in the management of *Staphylococcus aureus* infections or other bacterial species.

Honey consumed locally as medicine and as food in Nepal is not sterilised, irradiated or assessed for antibacterial potency and is therefore not intended for application to wounds or recommended and practiced for clinical use in Nepal.These have no proven laboratory or clinical evidence of the antibacterial activity. Different types of honey from different bees and plants species to be used must be specifically selected as antibacterial potency can vary widely its clinical efficacy. It is widely available in most communities and although the mechanism of action of several of its properties remains obscure and needs further investigation, the time has now come for conventional medicine to lift the blinds off this

'traditional remedy' and give it its due recognition. Commercial or wild honeys intended for use on wounds should be standardised antibacterial activity or that has potent antibacterial effects and should be sterilised (by gamma irradiation).

In order to elucidate the antibacterial/healing properties of honey from Nepal, randomised clinical trials and laboratory studies on cellular effects, on different species of bacterias are urgently needed. In addition, it is useful to make further invesigations such as pollen analysis (for floral origin) of each honey types and also identify plant derived antibacterial substances (such as flavonoids). We, therefore, ought to understand the antimicrobial and wound healing action of honey; and, in particular, to seek a means of estimating how much antimicrobial component (in honey) is necessary in various circumstances, as a basis for possible legislation. If the regulatory and clinical requirements have been satisfied and, practitioners and patients will be able to enjoy the benefits of a valuable and effective addition to the range of wound treatments. Anyone contemplating the use of honey in wounds is advised to use sterilised honey with standardised antibacterial activity under medical supervision and to obtain local research ethics committee approval.

The potential impacts of environmental contaminants on land use and agricultural production have received increased attention. Soil, local flora, and air act as sink for many pollutants. As explained earlier, the organic or inorganic contamination may stem from an accidental (overflow) or deliberate spill, or from a medium to long-term exposure and accumulation either through agricultural land application or atmospheric deposit. Honeybee has forage in a variety of environments and hence they effectively sample their surroundings for the constituents in or on the forage plants, the soil and the atmosphere of the area.

The way in which bees forage and their exposure to surrounding environment that have a good impact on their element status. On the other hand, advances in (trace) element content in honey has also been fostered by the importance of implications associated with soil, plant, forage area, and chemical contaminants. Accumulation of contaminants by plants represents an entry point of hazardous chemicals into the food web, initiating a biomagnification process and honey bees collect food from plants.

Honey take advantages of the presence of wide array of nutrients and medicinal properties. Ingestion of some of elements in small amounts can satisfy human micronutrient requirements, but in large amounts may cause toxicity. The effect of higher dose of elements should be addressed in the perspective of the toxicity evaluation of contaminated honeys. Honeybee and its product may be vulnerable to contaminant accumulation. The occurence of elements as chemical contaminats in honey bees and its products may attributed to

atmospheric input and have been suggested as a useful indicator of soil metal levels within the forage area. Although the relatively low concentrations of trace elements in (few) honey samples do not preclude its use as an indicator of environmental contamination and also too few to identify any patterns or trends.

Bringing many benefits to society, industrial ,vehicular expansion and agricultural practices have led to undeniable adverse effects on public health, environmental quality and ecosystem integrity and also good honey quality.

The present work was planned to determine whether it could be possible to detect significant differences in the values of trace element concentration among honeys from *Apis cerana*, *A. dorsata* and *A. mellifera* bees produced in Chitwan district, central Nepal. Additionally, data presented in this work are part of a study aimed to determine the overall element contents in those honeys. The measurements of concentrations of different trace elements revealed that the distribution of the elements depends on the surroundings, forage area (polluted and unpolluted), floral source, foraging range and forage preference of different bee species, atmospheric input and honey that comes into contact with metal equipments. From the present study it would appear that analyses of honeys may be a useful indicator of environmental contamination and mineral prospecting.

The availability of natural resources and bee population is declining through anthropogenic activities. Many chemicals associated with the contamination of foods and comodities either natural products, large molecules, or both. It is important that the levels of chemicals in foodstuffs be monitored to provide information on safety of food and on the adherence to the approved and safe use of chemicals. Exposure estimation is of particular relevance in the case of those at increased potential risk such as children, the elderly or those who because of their individual dietary habits may consume higher than average amounts of a particular food chemical.

Since the bioavailability of elements in the organism (bees) and bee products is well known and may lead to a toxic response as evidenced by studies, it is possible to resolve that the presence of (trace) elements in honey may be associated with a hazardous effect. However, this aspect is still an important challenge to workers, especially if one wishes to predict the risk to potential biological receptors, including humans, at a specific contaminated site. To understand why the use of honey as a environmental indicator has become a key element in the analysis of contaminated sites, it is necessary to examine the presence of (trace) elements in honey, their bioavailability and pathways for organism exposure. The mobility of contaminants and therefore their potential availability in honey may characterize the

environmental situation and the data could be used for ecotoxicological (environmental hazard) assessment through biological, toxicological and ecological analyses to quantify any injury, damage or disturbance caused by a source of contamination and contaminants itself. These data are necessary for through and accurate ecological risk analysis and for environmental site management for beekeeping practice efficiently. Maintaining the clean environment, beekeeping is essential to reduce poverty and sustain and protect biodiversity and ecological integrity in terrestrial ecosystem. Central to achieving this goal is the need for a great improved understanding of the potential effects of organic and inorganic contaminants. Because the release of chemicals to the environment represents a potential threat to human and ecological health.

Chemical contaminants can exert their effects directly or indirectly through toxicity to bees with associated economic and human health consequences. However, the complexity of the interactions between the contaminants, soil, plant, bee and its product dramatically complicates the interpretation of these chemical evaluations. Consequently, the elemental concentrations cannot systematically predict the toxicity of a specific element. Moreover, because of the complexity of interactions occuring in the field the effects of environmental factors would be confounding to an interpretation of results.

Furthermore, the presence of elements in honey also associated with its origin (eg.blossom honey, honeydew honey) and electrical conductivity. In addition, presence of higher amount of (trace) elements may provide information on availability of honeydew honey or floral honey and may reflect the apparent picture of honeydew flow at a particular area.

Nevertheless, many aspects could be explained satisfactorily, including the role of bee species, the interrelatedness of the various aspects of bee species and our atmospheric environment, the effect of contamination on honey, the future development and implications of the honey as a environmental marker, honey in clinical practices in coming decades, and the effect of handling ranging from a few days to a few months. In addition, honey from different bee species promises to continue to improve into the twenty-first century, especially wound healing.

It is indeed paradoxical that as concern for long-term beekeeping practices is growing, little is known about scientific aspects of native honeybees of Nepal. The issues involved simply have not received the attention they deserve, in spite of the great scope for scientific research in Nepal. Although some attempts have been made according to the bee species (and bee products) and those studies have produced relevant findings.

Research I have initiated on novel approaches to the identification (analysis) of antibiotic activity and element spectrum of honey from different bee species is not beyond the scope of the present socio-economic and environmental situation and problem of the country. Environmental concerns will play major roles in shaping the course of development in the coming decades. I am sure this research and development continues. Recent efforts, for example, have resulted in a new aspect that can reflect the ideas and future possibilities in this area. Thus, having looked at the system as a whole and subjected each element to scientific, technical, social, economic, and aesthetic scrutiny, I see a bright future for the apicultural and allied research in the country -if not immediately, then in the near future. Finally, this work of what is known about honey as an antibiotic agent and as an environmental indicator demonstrates what a fascinating subject it is. The potential human exposure to the elements with the associated toxicological impacts are most important because all may be harmful if excesive amounts are consumed. A reduction of exposures to toxic metals should be a prudent practice. Great care will need to be taken to ensure than it is done in ecologically sound ways-paying close attention to considerations of sustainability, biological diversity, other social and environmental issues, and, of course, good honey quality.

Lastly, my work combines theory, experiment, practical application and possibilities, it involves scientific, traditional and practical viewpoints, it links ideas of practices thousands of years ago to a concern for the future, it draws traditional knowledges together in scientific endeavour. In view of this, I hope that researchers will feel encouraged to persue the further study on different native bee species of Nepal and its products.

Annexe 1

Mean, standard deviation, minimum and maximum values of elements in *Apis cerana* honey from different locations.

Kathmandu (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	2	0.0073850	0.0018738	0.0060600	0.0087100
na	2	39.4230000	23.7715158	22.6140000	56.2320000
ca	2	113.9595000	43.0210837	83.5390000	144.3800000
mg	2	36.4490000	15.3343177	25.6060000	47.2920000
sr	2	0.1691000	0.1206890	0.0837600	0.2544400
fe	2	8.7585000	6.7493342	3.9860000	13.5310000
p	2	68.1770000	11.0633927	60.3540000	76.0000000
k	2	889.0000000	140.0071427	790.0000000	988.0000000
al	2	1.1388800	0.8100050	0.5661200	1.7116400
b	2	5.2438136	0.1987767	5.1032572	5.3843699
co	2	0.0179570	0.000257175	0.0177751	0.0181388
cu	2	0.2123870	0.0176540	0.1999037	0.2248702
mn	2	0.9371532	0.5449091	0.5518442	1.3224621
ni	2	0.2480448	0.0177861	0.2354681	0.2606215
v	2	0.000169100	0.000120632	0.000083800	0.000254400
zn	2	0.9450269	0.2798783	0.7471230	1.1429307

Kathmandu valley (mg/kg)

The SAS System
The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	4	0.0033750	0.0013769	0.0020000	0.0050000
na	4	24.5100000	7.4603943	18.3350000	34.2100000
ca	4	63.6375000	20.5425727	50.5500000	94.3000000
mg	4	31.9625000	13.4641112	15.5500000	48.5000000
sr	4	0.0671250	0.0265750	0.0355000	0.1005000
fe	4	5.0850000	3.2150531	1.9700000	8.9700000
p	4	72.9625000	16.0729781	54.0500000	92.4500000
k	4	1074.63	588.9938278	453.5000000	1733.50
al	4	0.0037925	0.0016383	0.0026100	0.0061100
b	4	0.0037913	0.0010873	0.0021950	0.0045100
co	4	0.000024375	0.000017090	0.000015500	0.000050000
cu	4	0.000296250	0.000148268	0.000195500	0.000514000
mn	4	0.0019150	0.000361409	0.0014250	0.0022400
ni	4	0.000264875	0.000260978	0.000087500	0.000653000
v	4	1.5E-6	2.0412415E-6	0	4.5E-6
zn	4	0.0010413	0.000317106	0.000575000	0.0012850

Arghakhachi (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	2	0.0030000	0.0014142	0.0020000	0.0040000
na	2	17.8780000	5.3061293	14.1260000	21.6300000
ca	2	193.9175000	29.2282588	173.2500000	214.5850000
mg	2	149.3950000	4.1082904	146.4900000	152.3000000
sr	2	0.1815000	0.0240416	0.1645000	0.1985000
fe	2	1.5535000	0.0190919	1.5400000	1.5670000
p	2	44.8275000	0.7389266	44.3050000	45.3500000
k	1	2043.50	.	2043.50	2043.50
al	2	0.0022050	0.0016758	0.0010200	0.0033900
b	2	0.0025048	0.0011169	0.0017150	0.0032945
co	2	0.000010000	2.1213203E-6	8.5E-6	0.000011500
cu	2	0.000151500	0.000031820	0.000129000	0.000174000
mn	2	0.0019448	0.0010207	0.0012230	0.0026665
ni	2	0.000107250	4.5961941E-6	0.000104000	0.000110500
v	2	2.75E-6	3.1819805E-6	5E-7	5E-6
zn	2	0.000583500	0.000341533	0.000342000	0.000825000

Palpa (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	4	0.0047975	0.0026212	0.0020000	0.0075000
na	4	19.5075000	8.7992853	12.3040000	31.8100000
ca	4	90.7617500	55.3907928	57.5770000	173.2500000
mg	4	135.6587500	36.8158115	80.4850000	156.1000000
sr	4	0.1205450	0.0410498	0.0671800	0.1645000
fe	4	3.7230000	1.8832815	1.5670000	5.4050000
p	4	73.8542500	37.7150571	37.8620000	112.4050000
k	1	4259.50	.	4259.50	4259.50
al	4	0.2853375	0.5677484	0.0010200	1.1369600
b	4	0.6768904	1.3452579	0.0032945	2.6947769
co	4	0.0044974	0.0089702	8.5E-6	0.0179527
cu	4	0.0661410	0.1307839	0.000174000	0.2623158
mn	4	0.6582825	1.3111439	0.0026665	2.6249983
ni	4	0.0825632	0.1647951	0.000104000	0.3297559
v	4	3.125E-6	5.2658491E-6	0	0.000011000
zn	4	0.4722701	0.9414726	0.000342000	1.8844784

Langtang (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	6	0.0444967	0.0586254	0.0020100	0.1207000
na	6	24.3783333	17.4834224	13.4220000	59.0790000
ca	6	104.1323333	68.7152994	40.7870000	208.4400000
mg	6	111.8078333	81.7417249	27.0150000	198.7780000
sr	6	0.0993150	0.1049949	0.0128500	0.2931100
fe	6	7.2973333	4.0998461	1.4800000	14.2610000
p	6	302.1428333	276.8805829	16.6810000	593.4420000
k	6	3258.92	2882.44	259.0000000	6291.50
al	6	34.3969283	77.5249073	0.6951800	192.6069500
b	6	4.5783182	2.5267341	0	6.3496503
co	6	0.0179146	0.0070889	0.0055353	0.0229408
cu	6	1.4625848	1.3887834	0.1836462	2.9753418
mn	6	1.3623487	0.6714925	0.5319106	1.9724974
ni	6	1.1920700	1.0597530	0.0863064	2.2933643
v	6	0.0086625	0.0106477	0.0012197	0.0299508
zn	6	7.8671989	9.6931484	0.5160254	27.2936106

Chitwan (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	11	0.0071464	0.0062827	0.000760000	0.0202600
na	11	15.1018182	6.2769851	7.8130000	27.6810000
ca	11	150.9230000	242.1270182	38.3710000	874.5880000
mg	11	102.7114545	140.1650209	15.2740000	493.4950000
sr	11	0.2263027	0.3027592	0.0417800	1.0363200
fe	11	4.9810909	3.0378629	1.7040000	11.3610000
p	11	213.5780909	329.8186910	44.7290000	1188.94
k	10	1536.20	1543.61	280.0000000	4322.00
al	11	4.6171118	5.3407571	0.0050200	18.0765600
b	11	3.8503030	1.8978852	0.0026000	7.0485716
co	11	0.0087971	0.0058656	0.000011000	0.0217725
cu	11	1.51958	2.507	0.24393	8.5829136
mn	11	7.3921872	19.5266668	0.0022740	66.1593750
ni	11	0.1074578	0.0994682	0.000061500	0.3528631
v	11	0.004753	0.0075122	0	0.0256891
zn	11	2.5194720	5.2351768	0.0013100	18.1411779

Jumla-Jajarkot (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	12	0.0029008	0.0015974	0.0013500	0.0070000
na	12	17.7895000	5.1073988	8.6670000	27.8750000
ca	12	89.0375000	90.5709823	29.8720000	269.1000000
mg	12	83.3993333	55.3545535	11.2640000	191.7700000
sr	12	0.0848533	0.0394931	0.0502700	0.1720000
fe	12	4.1212500	4.6831035	0.5230000	18.4100000
p	11	183.7652727	102.9040852	43.5500000	341.3750000
k	11	2353.82	531.3642476	827.0000000	2767.00
al	12	0.1508025	0.5141674	0.000970000	1.7835000
b	12	0.0909557	0.3056436	0.0012900	1.0615000
co	12	0.0024981	0.0085837	0	0.0297550
cu	12	0.6476601	1.5860830	0.000103000	5.5005000
mn	12	0.5443331	0.9207651	0.000615500	2.9230000
ni	12	0.0508500	0.1008052	0.000049300	0.3190000
v	12	0.0014622	0.0026564	2E-6	0.0063750
zn	12	0.3142227	0.5633551	0.000480100	1.5540000

Kathmandu (µg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	2	0.8860536	0.1560326	0.7757219	0.9963853
pb	2	122.5962089	78.3631970	67.1850609	178.0073569
cr	2	22.2528199	0.4041421	21.9670483	22.5385916
mo	2	22.7573529	4.7573728	19.3933824	26.1213235

Kathmandu valley (µg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	4	0.4937500	0.2713354	0.2100000	0.8150000
pb	4	19.4575000	10.3055111	10.2300000	32.7500000
cr	4	21.1512500	9.2864241	15.3000000	34.9550000
mo	4	7.6975000	1.5140536	5.7000000	9.3500000

Palpa (µg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	3	0.6913863	0.2637989	0.4900000	0.9900000
pb	3	13.2453195	8.9147127	7.3900000	23.5050000
cr	3	12.5676629	10.3069075	4.7900000	24.2579886
mo	3	9.6236588	4.0567754	6.9350000	14.2900000

Langtang (µg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	5	3.1239504	2.2832327	0.1742269	5.2878432
pb	4	10.7271816	4.8678095	5.6566435	15.5792827
cr	5	29.2888200	23.9139395	13.5318279	71.0938914
mo	5	16.3424295	6.7054626	6.9264277	23.1585959

Chitwan (µg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	10	4.1362166	6.8055221	0.3010370	23.0202369
pb	10	33.5537019	15.2722527	15.7538979	66.5334205
cr	10	15.0976715	11.8743476	3.5623279	40.1567507
mo	10	29.3473961	20.0625320	7.7445584	65.9476646

Jumla-jajarkot (µg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	8	0.3122238	0.1467104	0.0891539	0.5074629
pb	8	7.8690165	4.2516774	1.6614593	14.3956866
cr	8	5.8395261	6.6421787	2.1284288	22.1065913
mo	8	5.6253642	1.7160452	2.1167829	7.6424703

Mean, standard deviation, minimum and maximum values of elements in *Apis cerana, A. dorsata* and *A. mellifera* honey from Chitwan.

Apis cerana (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	11	0.0071455	0.0062902	0.000800000	0.0203000
na	11	15.1020909	6.2769358	7.8134000	27.6814000
ca	11	150.9245455	242.1271983	38.3700000	874.5900000
mg	11	102.7109091	140.1644347	15.2700000	493.4900000
sr	11	0.2262909	0.3027568	0.0418000	1.0363000
ba	11	0.9445818	1.2895565	-0.0548000	4.5694000
fe	11	4.9811273	3.0379753	1.7041000	11.3615000
p	11	213.5781818	329.8178609	44.7300000	1188.94
kfes	10	1536.20	1543.61	280.0000000	4322.00
al	11	4.617	5.34	0.00502	18.076
b	11	3.85030	1.89788	0.00260	7.04857
cd	11	-12.4440636	25.6957525	-75.9644000	13.5134000
co	11	0.0087971273	0.0058656431	0.0000110	0.02217725000
cr	11	24.9667364	31.2105163	-15.9865000	87.1026000
cu	10	1.51958	2.50784	0.2439300000	8.58291
mn	11	7.39219	19.52667	002700000	66.15938
mo	4	-16.7882500	28.2083778	-58.2042000	2.1866000
ni	11	0.1074577727	0.0994681818	0.0000615	0.3528631000
pb	11	15.3065636	16.6430386	-8.0453000	44.5469000
v	11	0.0047530364	0.0075122193	0	0.025891000
zn	11	2.51947	5.23518	0.0013100000	18.4118

A. dorsata (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	13	0.0280231	0.0420776	0.000300000	0.1214000
na	13	31.4392154	31.9543988	5.6551000	117.7357000
ca	13	139.3523077	73.1948475	59.3300000	286.5900000
mg	13	63.9984615	60.2188484	0.0800000	164.5000000

sr	13	0.3035077	0.3710852	0.0717000	1.1499000
ba	13	0.3919077	0.4186296	-0.2395000	0.9733000
fe	13	5.1831385	4.7128133	1.0500000	17.8727000
p	13	141.2084615	121.2510338	28.9400000	370.4600000
kfes	11	3001.09	1946.04	506.5000000	6159.50
al	13	2.50907	1.72688	0.93000000	6.27682
b	13	2.48910	1.61345	0	4.77134
cd	11	-10.3677182	22.3652089	-56.3923000	9.3530000
co	13	0.0036937923	0.0045010651	0	0.008601
cr	11	18.8426273	22.9025779	-8.4075000	72.6204000
cu	13	0.7406715385	0.5632584298	0.1586000000	1.75750
mn	13	1.89865	1.11859	0.471	3.70941
mo	11	-161.3725818	251.1099532	-815.5265000	28.8663000
ni	13	0.114277308	0.0494885140	0.0430000	0.1987311000
pb	11	23.2494364	37.0882889	-11.2455000	127.4644000
v	13	0.0082504077	0.0109760249	0.0000081	0.0325679000
zn	13	1.28106	0.8040605506	0.3480000000	3.27169

A. mellifera (mg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
li	20	0.0201350	0.0345227	0.0014000	0.1227000
na	20	14.3137700	6.1342135	5.1120000	23.0150000
ca	20	76.2685000	47.7308431	31.0500000	237.9500000
mg	20	33.4515000	30.0302853	10.0300000	120.3900000
sr	20	0.0695550	0.0274097	0.0297000	0.1366000
fe	20	2.4484700	1.5982115	-2.8087000	4.6500000
p	20	67.7885000	20.5985971	36.2000000	105.9500000
kfes	14	865.4285714	609.6611561	279.5000000	1950.50
al	20	0.9826120000	1.27522	0.00069	4.25287
b	20	1.74648	2.35199	0.00153	7.41636
cd	10	-10.5827200	23.6127219	-54.4640000	7.9665000
co	20	0.006649985	0.0061821547	0	0.0167619
cr	10	6.8776800	12.1192800	-11.3972000	26.1816000
cu	20	0.2154355	0.2189648199	0.0002	0.906
mn	19	0.9590210526	0.9997082457	0.0007	2.84200
mo	10	-116.2654600	234.3986418	-774.9111000	29.5138000
ni	20	0.1203164400	0.1768985473	0.0000255	0.7550000000
pb	10	17.5834400	31.5101170	-21.7500000	91.1731000
v	20	0.003006035	0.0063955415	0	0.0282286
zn	20	2.49286	5.65647	0.001010	25.61116

A. cerana (μg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	10	4.1362	6.8055	0.3010	23.0202
pb	10	33.5537	15.2722	15.75390	66.5334
cr	10	15.0976	11.8743	3.56230	40.1568
mo	10	29.3474	20.06253	7.7446	65.9477

A.dorsata (μg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	8	1.7490	1.59164	0.2730	4.751
pb	8	31.9117	26.8238	8.9813	82.0677
cr	8	11.9804	11.6445	0.2873	36.4973
mo	8	14.19656	6.555	7.4852	24.6324

A. mellirefa (μg/kg)

The SAS System

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
cd	9	1.0799	0.9240	0.480	3.4399
pb	9	22.752	10.6124	7.3888	38.0498
cr	9	13.1827	9.4737	0.9422	28.4013
mo	9	12.7748	4.979	6.6321	21.3731

Fig. 1

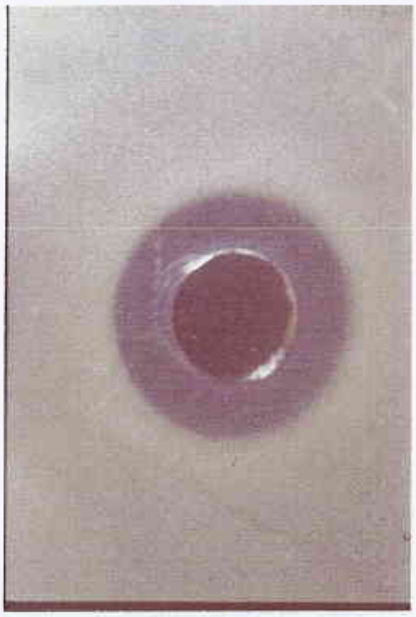


Fig. 2

Fig. 3

Fig. 4

Fig.1 *Apis laboriosa* honey from Bajahang showing the zone of inhibition of growth (peroxide activity) on the agar plate.

Fig.2 *Apis dorsata* honey from Chitwan showing the zone of inhibition of growth (peroxide activity) on the agar plate.

Fig.3 *Apis mellifera* honey from Chitwan showing the zones of inhibition of growth (peroxide and non-peroxixe activity) on the agar plate.

Fig.4 *Apis cerana* honey from Chitwan showing the zones of inhibition of growth (peroxide activity and non-peroxixe activity) on the agar plate.

REFERENCES

Abdel-Latif, H M; Donker, MH & Van, S NM (1998) 'Interaction Between Temperature and Cadmium Toxicity in the Isopod Porcellio Scaber`. Functional Ecology 12(4): 521-527 (7).

Abuharfeil, N; Al-Oran, R & Aboshehada, M (1999) ' The Effect of Bee Honey on the Proliferative Activity of Human B-and T-lymphocytes and the Activity of Phagocytes`. Food and Agricultural immunology 11: 169-177.

Adcock, D (1962) `The Effect of Catalase on the Inhibine and Peroxide Values of Various Honeys`. Journal of Apicultural Research 1: 38-40.

Adesunkanmi, K & Oyelami, O A (1994) 'The Pattern and Outcome of Burn Injuries at Wesley Guide Hospital, Iiesha, Nigeria: A Review of 156 cases`. Journal of Tropical Medicine and Hygiene 97(2):108-112.

Agner, K (1963) 'Studies on Myeloperoxidase Activity: Spectrophotometry of The MPO-H2O2 Compound`. Acta Chemica Scandinavica 17 (Suppl.1): 332-338.

Agostino, B; Rosa, AD C LA & Zanelli, C (1961) 'Attività Antibatterica di Mieli Siciliani`.Quaderni della Nutrizione 21(1/2): 30-44.

Ahmad, F (2003) 'Managed Invasive Arrangements and Plight of Asian Hive Bee, *Apis cerana* in the Himalayas`. In Proceedings of the XXXVIIIth APIMONDIA International Apicultural Congress, 216-217. Ljubljana, Slovenia.

Ahmad, F & Roy, P (2000) 'Indigenous Honeybee Project`, Bhutan Travel Report. ICIMOD, Kathmandu, Nepal.

Ahmad, F; Joshi, SR & Gurung, MB (2003a) The Himalayan Cliff Bee *Apis laboriosa* and the Honey Hunters of Kaski: Indigenous Honeybees of the Himalayas (Vol 1). ICIMOD, Kathmandu , Nepal.

Ahmad, F; Gurung, M & Joshi SR (2003b). 'Natural and Social Habitat of Himalayan Cliff *Bee Apis laboriosa* in Western Nepal`. In Proceedings of The XXXVIIIth APIMONDIA International Apicultural Congress, 214-215. Ljubljana, Slovenia.

Ahmad, R (1984) 'Some Honeybee Flora for Forest Plantations, Erodible Lands, Landscape and Agricultural Farms`. Progressive farming 4:19-23.

Aizzawa, M; Karube, I & Suzuki, S (1974) 'A Specific Bio-electrochemical Sensor for Hydrogen peroxide`. Anal.Chim. Acta 69: 431-437.

Alam,M G M; Snow, E T & Tanak, A (2003) 'Arsenic and Heavy Metal Contamination of Vegetables Grown in Samta village, Bangladesh`. The Science of the Total Environment 308(1-3): 83-96.

Alexande, E H F, Rivera, A; Marriott, I.; Anguita, J; Bost, KL & Hudson, MC (2003) In Microbiology. BMC 3:5

Alexandersson, R & Lidums, V (1979) Arb.och Hälsa 8: 1-23. Cited in Gerhardsson and Skerfvig, (1996).

Alexenitser, M L & Bodnarchuk, L I (1999) 'Beekeeping Products as Bioindicator of Radioactive Contamination in a Certain Zone`. Apiacta 34 (3): 93-96.

Ali Abu, TMM (1989) 'The Pharmacological Characterization and the Scientific Basis of the Hidden Miracles of Honey`. Saudi Medical Journal 10(3):177-179.

Al-Jabri, A A (2003) 'In vitro Antibacterial Activity of Omani and African honey`. British Journal of Biomedical Science 60(1): 1-4.

Allen, KL; Molan, PC & Reid, GM (1991a) 'Variabilität der Antibakteriellen Tätigkeit des Honigs`. Apiacta 24: 114-121.

Allen, KL; Molan, PC & Reid, GM (1991b). 'A Survey of the Antibacterial Activity of Some New Zealand Honeys`. Journal of Pharmacy and Pharmocology 43 (12):817-822.

Allen, M F (1995) `Bees and Beekeeping in Nepal` Bee World 72 (4):165-169.

Allen, M F; Ball, BV& Underwood, BA (1990) 'An Isolation of *Melissococcus pluton* From *Apis laboriosa*`. Journal of Invertibrate Pathology 55:439-440.

Al-Saleh, I & Shinwari N (2001) 'Report on the Levels of Cadmium, Lead, and Mercury in Imported Rice Grain Samples`. Biological Trace Element Research 83 (1): 91-96 (6).

Al-Waili, N S (2003) 'Effects of Daily Consumption of Honey Solution on Hematological Indices and Blood Levels of Minerals and Enzymes in Normal Individuals`. Journal of Medicinal Food 6 (2): 35-140(6).

Anderson, JF & Wojtas, MA (1986) 'Honeybees (Hymenoptera: Apidae) Contaminated With Pesticides and Polychlorinated Biphenyls`. J Econ. Entomol. 79:1200-1205.

Andrews, P;Town, RM; Hedley, MJ & Loganathan, P (1996) 'Measurement of Plant-available Cadmium in New Zealand Soils`. Austral J. Soil Res. 34 (3): 441-452.

Andrzejak, R; Antonowicz, J; Tomczyk, J; Lepetow, T& Smolik, R (1993) 'Lead and Cadmium Concentrations in Blood of People Living Near a Copper Smelter in Legnica, Poland`. Science of Total Environment (Suppl.1): 233-236.

Ankra-Badu, GA (1992) 'Sickle Cell Leg Ulcers in Ghana`. East Africa Medical Journal 69 (7):366-369.

Anonymous (1989) The Atlas of Endemic Diseases and their Environments in the People's Republic of China. Science Press, Beijing, People's Republic of China; 194 p.

Antonescu C & Mateescu C (2001) 'Environmental Pollution and Its Effects on Honey Quality`. Roum.Biotechnol.Lett., 6:371-379.

Armon, PJ (1980) 'The Use of Honey in the Treatment of Infected Wounds`. Tropical Doctor 10:91.

Arndt, U; Nobel, W & Schweizer, B (1987) Bioindikatoren. Ulmer, Stuttgart.

ASTM D3370 (1982) Standard Practices for Sampling Water. American Society for Testing Materials. Philadelphia, PA.

Atkins, EL & Kellum, D (1986) 'Comparative Morphogenic and Toxicity Studies on the Effect of Pesticides on Honeybee Brood`. Journal of Apicultural Research 25:242-255.

Atwal, AS (1970) Insect Pollinators of Crops. Punjab Agric. Univ. Press, Ludhiana, India.

Attar, Z (1982) 'Honey-An Effective Dental Remedy`. Indian Bee Journal 44(2): 48-50.

Bacon, J; Crain, J & Williams, J (1996) 'Atomic Spectrometry Update-Atomic Mass Spectrometry`. J.Anal.At.Spetrom.11: 355-393.

Baird-Parker, AC & Holbrook, R (1971) 'The Inhibition and Destruction of cocci`. In Inhibition and Destruction of the Microbia Cell (Hugo, WR, ed). Academic Press, London, UK; 369-397 pp.

Baker, A J M (1981) 'Accumulators and Excluders-Strategies in the Response of Plants to Heavy Metals`. Plant Nutr.3: 643-654.

Bakker, H (1999) Sugar Cane Cultivation and Management. Kluwer Academic/Plenum Publishers, 233 Spring Street, New York, NY 10013.

Bang, LM, Buntting, C & Molan, P (2003) 'The Effect of Dilution on the Rate of Hydrogen peroxide Production in Honey and Ist Implications for Wound Healing`.The Journal of Alternative & Complementary Medicine 9(2): 267-275 (7).

Bankova, V; Christov, R; Popov, S; Marcucci, M C; Tsvetkova, I & Kujumgiev, A (1999) 'Antibacterial Activity of Essential Oils from Brazilian Propolis`. Fitoterapia 70(2): 190-193.

Bankova, AH; Tezuka, Y; Adnyana, I K; Ishii, E; Midorikawa, K; Matsushige, K & Kadota, S (2001) ´ Hepatoprotective and anti-*Helicobacter pylori* Activities of Constituents From Brazilian propolis`. Phytomedicine 8(1):16-23(8).

Baranowska, I (1995) 'Lead and Cadmium in Human Placentas and Maternal and Neonatal Blood (in heavily polluted area) Measured by Graphite Furnace Atomic Absorption Spectrometry`. Occup.Environ. Med. 52: 229-232.

Barberan, T FA ; Ferreres, F; Garcia-Viguera, C & Tomas-Lorente, F (1993) 'Flavonoids in Honey of Different Geographical Origin` Z.Lebensm.Untersuch.Forsch. 196:38-44.

Bard, SM (1999) 'Global Transport of Anthropogenic Contaminants and the Consequences for the Arctic MarineEcosystem`. Marine Pollution Bulletin38 (5): 356-379.

Barnes, R (1996) 'Analytical Plasma Source Mass Spectrometry in Biomedical Research`. Fresenius Z.Anal. Chem.355: 433-441.

Beck, BF (1935) Bee Venom referred in Verma, LR (1990).

Becker, JS & Dietze, H-J (1998) 'Inorganic Trace Analysis by Mass Spectrometry`. Spectrochim. Acta B. Cited in Djingova R & Kuleff, I (2000).

Beckwith, PR; Ellis, JB & Revitt, DM (1985) Particles Size Distribution of Cu, Pb and Zn Across a Road Surface. In Heavy Metals in the Environment (Vol.I) (Lekkas,TD, ed). CEP Consultants, Edinburgh; 174-176 pp.

Bee Development Section (BDS) (1998) Beekeeping : An introduction. HMG, Nepal; 25 p (in Nepali).

Bejan,V; Oita, N; Caraman, C & Humita, I (1978) 'Influence of Honey Aerosols on Chronic and Exudative Bronchitis`. In Proceedings of the Third International Symposium on Apitherapy, 523-524. Protoroz, Yugoslavia.

Belandria, G; Asta, J & Garrec, JP (1991) 'Diminutions of Fluorine Contents in Lichens due to a Regression of Pollution in an Alpine valley (Maurienne, Savoie, France) From 1975 to 1985`. Rev. Ecol. Alp. De Biologie alpine, Grenoble 1:45-58.

Belinsky, D L & Kuhnlein, H V (2000) 'Macronutrient, Mineral, and Fatty Acid Composition of Canada Goose (*Branta canadensis*): An Important Traditional Food Resource of the Eastern James Bay Cree of Quebec`. Journal of Food Composition and Analysis 13 (2):101-115(15).

Bennett , J P; Chiriboga, E; Coleman, J & Waller, D M (2000) 'Heavy Metals in Wild Rice From Northern Wisconsin`. The Science of the Total Environment 246 (2- 3):261-269.

Berdanier, CD (2002) Food Constituents. In Handbook of Nutrition and Food (Berdanier,C D, ed). CRC Press LLC, Boca Raton, FL 33431; 3-95 pp.

Bergman, A; Yanai, J; Weiss, J; Bell, D & David, MP(1983) 'Acceleration of Wound Healing by Topical Application of Honey: An Animal Model`. American Journal of Surgery 145: 374-376.

Bhandari, N (2001) Status of Different Honeybee Species in Hill and Terai Areas of Nepal. Thesis (Master of Science in Entomology). Department of Entomology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Bianchi, EM (1992) 'Determining the Mineral (ash %) Content in Honey by Conductometric Analysis`. Apiacta 27(1): 19-24.

Bicik, V & Kaspar, F (1986) 'Metals in the Food of the Honeybee and in the Developmental Stages of Queens`. Apicultural Abstract 190, 41(1): 40.

Bloemen, M-L; Markert, B & Lieth, H (1995) 'The Distribution of Cd, Cu, Pb and Zn in Topsoils of Osnabrück in Relation to Land Use`. The Science of The Total Environment 166 (1-3 , 21):137-148.

Blomfield, E (1976) 'Old remedies`. Journal of the Royal College of General Practitioners 26:576.

Blomfield, R (1973) 'Honey for Decubitus Ulcers`. Journal of the American Medical Association 224(6): 905.

Bogdanov, S (1983) 'Bericht Über Apitherapiekonferenz in Israel`. Schweiz bienen zeitung 106: 342-344.

Bogdanov, S (1984) 'Honey and Medicine - Summary of Research Characterization of Antibacterial Substances in Honey`. Lebensmittel-Wissenschaft und Technologie 17:74-76.

Bogdanov, S (1996) 'Non-peroxide Antibacterial Activity of Honey`. In Proceedings of an International Conference on Bee Products: Properties, Applications, and Apitherapy, Israel; 39-47 pp.

Bogdanov, S (1997a) 'Nature and Origin of the Antibacterial Substances in Honey`. Lebensmittel-Wissenschaft und Technologie 30(7):748-753.

Bogdanov, S (1997b) Non-peroxide Antibacterial Activity of Honey. Bee products, Properties, Applications and Apitherapy, Symposium Tel Aviv 39-47.

Bogdanov, S; Martin, P & Lullman, C (1997c) 'Harmonized Methods of the European Honey

Commission`. Apidologie, extra issue.

Bogdanov, S & Blumer, P (2001a) 'Le Proprietà Antibatteriche Naturali del Miele`. L'Ape 84:14-21.

Bogdanov, S & Blumer, P (2001b) 'Natürliche Antibiotische Eigenschaften des Honigs`. Schweiz bienen zeitung 124:18-21.

Bogdanov, S& Blumer, P (2001c) 'Propriètès Antibiotiques Naturells du Miel`. RSA 98:107-114.

Bogdanov, S & Blumer, P (2001d) 'Natürliche Antibiotische Eigenschaften des Honigs`. AGRAR Forschung 8 (10):438-442.

Bond, R (ed.) (1982) Surveillance Field Procedures Review for the Great Lakes: International Surveillance Plan. International Joint Commission, Windsor, Ontario.

Bose, B (1982) 'Honey or Sugar in Treatment of Infected Wounds ?` Lancet I: 963.

Boumans, P W J M (1997) Glow Discharge Optical Emission Spectrometry (Payling, R; Jones, DG & Bengtson, A, eds). John Wiley & Sons, Chichester; 440-451 pp.

Bradbear, N & Roy, P (1998) Honeybees, People and the Himalayas. Evaluation Report on ICIMOD Beekeeping Project in Nepal, Commissioned by Austroproject Pvt.Ltd., Austria.

Brady, N; Molan, P & Bang, L (2004) 'A Survey of Non-manuka NewZealand Honeys For Antibacterial and Antifungal Activities`. Journal of Apicultural Research 43 (2):47-52.

Braniki, FJ (1981) 'Surgery in Western Kenya`. Annals of the Royal College of Surgeons of England 63: 348-352.

Breis, B F; Castillo, C; Gilabert, M E; Egea, C & Sanchez, P C (1995) 'Pollen Spectra , Mineral (ash) and Sediment Quantification in Honeys Produced in the Region of Murca, Spain`. Apicultural Abstracts 46(4): 434.

Breulmann, G; Ogino, K & Markert, B (2000) Foliar Heavy Metals in Trees From a Tropical Rain Forest in Sarawak.In Trace elements-their Distribution and Effects in the Environment (Markert, B & Friese, K, eds). Elsevier Science B V 363-373 pp.

Broekaert, J & Toelg, G (1987) 'Recent Developments in Atomic Spectrometry Methods for Elemental Trace Determinations`. Fresenius Z.Anal.Chem.326: 495-509.

Bromenshenk, JJ (1979) Monitoring Environmental Materials and Specimen Banking Using Terrestrial Insects with Particular Reference to Inorganic Substances and Pesticides. In

Monitoring Environmental Materials and Soecimen Banking (Leupke, NP, ed) Martinus Nijhoff, The Hague.

Bromenshenk, JJ; Carlson, SR; Simpson, JC & Thomas, JM (1985) 'Pollution Monitoring of Puget Sound with Honeybees`. Science 227: 632-634.

Bromenshenk, JJ; Gudatis, JL; Carlson, SR; Thomas, JM; Simpson, MA (1991) 'Population Dynamics of Honeybee Nucleus Colonies Exposed to Industrial Pollutants `. Apidologie 22: 359-369.

Brouwers, EVM (1982) 'Activation of the Hypopharyngeal Gland Activity in the Honeybee`. J.Api.Res. 22 (3): 137-141.

Brouwers, EVM (1983) 'Activation of the Hypopharyngeal Glands of Honeybees in Winter`. J.Api.Res. 21 (4): 193-198.

Brown, DH & Beckett, RP(1984) 'Uptake and Effect of Cations on Lichen Metabolism'. Lichenologist 16:173-188.

Brown, DH & Beckett, RP (1985a) Minerals and Lichens: Aquisition, Localisation and Effect.In Surface Physiology of Lichens (Brown, DH & Legaz, ME, eds). Editorial de la Universidad Complutense de Madrid, Madrid;127-149 pp.

Brown, DH & Beckett, RP (1985b) The Role of the Cell wall in the Intracellular Uptake of Cations by Lichens.In Lichen Physiology and Cell biology (Brown, DH, ed). Plenum Press, New York; 247-258 pp.

Brown, D H (1991) 'Lichen Mineral Studies Currently Clarified or Confused?`Symbiosis 11:207-223.

Brown, DH & Brown, RM (1991) 'Mineral Cycling and Lichens: The Physiological Basis`. Lichenologist 23: 293-307.

Brownle, M(1995) 'Advanced Protein Glycosolation Diabetes and Aging`. Annu Rev Med 46:223-234.

Buchner, R (1966) 'Vergleichende Untersuchungen Über die Antibakterielle Wirkung von Blüten und Honigtauhonigen, Südwestdeutsch`. Imker 18: 240-241.

Buhler, DR & Miranda, C, Antioxidant Activities of Flavonoids, http://lpi.oregon.state.edu/f_w00/flavonoid.html, Assessed on 14 July, 2004.

Bulman, MW (1955) 'Honey as a Surgical Dressing`. Middlesex Hospital Journal 55:188-189.

Bunzl, K & Kracke, W (1981) Health Physics 41:554. Cited in Jones (1987).

Burger, J; Burger, J;Gochfeld, M.& Gochfeld, M (1996) 'Heavy Metal and Selenium Levels in Birds at Agassiz National Wildlife Refuge, Minnesota: Food Chain Differences`. Environmental Monitoring and Assessment 43(3): 267-282 (16).

Burgett, DM (1973) The Occurrence of Glucose oxidase in the Honeys of Social Hymenoptera. PhD. Thesis, Cornell University, Ithaca, New York.

Burns, I; Friese, K; Markert, B & Krauss, G-J (1997). '1. The use of *Fontinalis antipyretica* L. exHedw. as a Bioindicator for Heavy Metals, 2. Heavy Metal Accumulation and Physiological Reaction of *Fontinalis antipyretica* L. ex Hedw.: In active biomonitoring in the river Elbe` . Sci. Total Environ. 204: 161-176.

Bursa, J (1996) Lead and Cadmium Concentrations in whole Maternal Blood and in Cord Blood of Their Newborns With Different Birth Weight (<1500 g;>1500 g.). In Abstracts of International Conference Environmental Pollution and Child Health: Critical Needs and Issues for Central and Eastern Europe, Sosnowiec, Poland.

Butler, K J (1980) 'Honey for Necrotic Malignant Breast Ulcers`. Lancet ii (october 11):809.

Caniglia, G; Calliari, I; Celin, L & Tollardo, A M (1994) 'Metal Determination by EDXRF in Lichens`. A contribution to Pollutants Monitoring. Biol.Trace. Res. 43-45: 213-221.

Caroli, S; Forte, G; Iamiceli, L & Galoppi, B (1999) 'Determination of Essential and Potential Toxic Trace Elements in Honey by Inductively Coupled Plasma-Based Techniques`.Talanta 50 (2): 327-336

Caroli, S; Forte, G; Alessandrelli, M; Cresti, R; Spagnoli, M; D`llio, S; Pauwels, J &, Kramer, N (2000) 'A Pilot Study for the Production of a Certified Reference Material for Trace Elements in Honey`. Microchemical Journal 67 (1-3): 227-233.

Cavanagh, D;Beazley, J & Ostapowicz, F (1970) `Radical Operation for Carcinoma of the Vulva.A new Approach to Wound Healing `. Journal of obstetrics and Gynaecology of the British Commonwealth 77 (11):1037-1040.

Celli, G (1983) ' L' apa Come Insetto Test della Salute di un Territorio`. Apic Mod 75: 133-140 (in Italian).

Celli, G (1984) L`apicoltore Moderno 75:133 (in Italian).

Cerna, M; Spevackova, V; Cejchanova, M; Benes, B; Rosser, P; Bavorova, H; Ocadlikova, D; Smid, J& Kubinova, R (1997) 'Population-Based Biomonitoring in the Czech Republic-the System and Selected Results`. Sci.Total Environ. 204: 263-270.

Chahal, B S & Gatoria, GS (1983) 'Import of Exotic Bees (*Apis mellifera* Linn.) and Spread of Bee Diseases`. Indian Bee J. 45: 11-13.

Chakrabarti, CK (1982) 'Zinc-Lead Mineralization in Ganesh Himal Region of Central Nepal`. Journal of Nepal Geological Society 1 (1): 38-45.

Chambonnaud, JP (1966) 'Etude du Puvoir Anti-bactèrien des Miels par une Technique de Diffusion en Gèlose`. Bulletin apicole 9 (1): 83-98.

Chambonnaud, JP (1968) `Contribution a la recherche des antibiotiques dans le miel` . Bulletin apicole 11(2):133-200.

Chang, A C & Page, AL (1996) Assessment of Ecological and Health Effects of Soil-borne Trace Elements and Health Effects of Metals. In Toxicology of Metals (Chang, LW; Magos , L & Tsuguyoshi, S, eds) Lewis Publishers, CRC Press, Boca Raton, FL; 29-38 pp.

Cherbuliez, T (1997) 'Bee Venom in Treatment of Chronic Diseases`. Bee products, Properties, Applications and Apitherapy symposium Tel Aviv 213-220.

Chettle, DR & Ellis, KJ (1992) Am. J. Ind. Med., 22:117-124(8). Cited in Gerhardsson and Skerfvig (1996).

Cheung, S G; Tai, K K; Leung, C K & Siu, Y M (2002) 'Effects of Heavy Metals on the Survival and Feeding Behaviour of the Sandy Shore Scavenging Gastropod *Nassarius festivus* (Powys)`. Marine Pollution Bulletin 45 (1-12): 107-113.

Chirife, J; Scarmato, G & Herszage, L (1982) 'Scientific Basis for Use of Granulated Sugar in Treatment of Infected Wounds`.The Lancet i: 560-561.

Chirife, J; Herszage, L; Joseph, A & Kohn, ES (1983) 'In vitro Study of Bacterial Growth Inhibition in Concentrated Sugar Solutions: Microbiological Basis for the Use of Sugar in Treating Infected Wounds`. Antimicrobial Agents and Chemotherapy 23(5): 766-773.

Christensen, ER & Guinn, VP (1979) 'Zinc From Automobiletires in Urban Runoff' J. Environ. Eng. Divis. 105:165-168

Christian, P; Khatry, SK; Katz, J; Kimbrough-Pradhan, E; LeClerq, SC; Shrestha, SR; Adhikari, RK; Sommer, A & West, KP Jr.(2003a) ' Effects of Alternative Maternal Micronutrient Supplements on Low Birth Weight in Rural Nepal: Double Blind Randomised Community Trial 1.` BMJ 15; 326 (7389): 571.

Christian, P; West, KP Jr; Khatry, SK, LeClerq, SC; Kimbrough-Pradhan, E; Katz , J & Shrestha SR (2001) 'Maternal Night Blindness Increases Risk of Mortality in the First 6 Months of Life Among Infants in Nepal`. J Nutr. 131 (5):1510-2.

Christian, P (2003) 'Micronutrients and Reproductive Health Issues: An International Perspective.` J nutr. 133 (6): 1969-1973.

Christian, P; Shrestha, J; LeClerq, SC; Khatry, S K; Jiang, T; Wagner, T; Katz, J & West, KP Jr. (2003b) 'Supplementation with Micronutrients in Addition to Iron and Folic Acid Does not Further Improve the Hematologic Status of Pregnant Women in Rural Nepal`. The American Society for Nutritional Sciences J. Nutr. 133: 3492-3498.

Christov, G & Mladenov, S (1961) 'Honey in Surgical Practice: The Antibacterial Properties of Honey`. Khirurgiya (Moscow) 14 (10): 937-946 (original in Bulgarian).

Christov, RV; Bankova, I; Kujumgiev,T A & Tejer, AD (1999) 'Antibacterial Furofuran Lignans From Canary Islands Propolis`. Fitoterapia 70 (1): 89-92.

Chwastek, M (1966) 'Jakosc Miodòwpszczelich Handlowych na Podstawie Oznaczania ich Skladnikòw Niecukrowcowych. CzescII. Zwartosc Inhibiny w Miodach Krajowych`. Rocziniki Panstwowego Zakladu Higieny 17 (1): 41-48.

Cikrt, M; Smerhovsky, Z; Blaha, K; Nerudova, J; Sediva, V; Fornuskova, H; Knotkova, J; Roth, Z; Kodl, M & Fitzgerald, E (1996) `Biological Monitoring of Lead Exposure Among Children in the Czech Republic`. In Abstracts of International Conference Environmental Pollution and Child Health: Critical Needs and Issues for Central and Eastern Europe. Sosnowiec, Poland.

Cimino, G; Zilno, M & Panuccio, MR (1984) 'Heavy Metal Pollution Part X: Impact of Volcanic Activity on Etnean honey`. Environ. Technol.Lett. 5 (10): 453-456.

Citterio, S; Santagostino, A; Fumagalli, P; Prato, N; Ranalli, P& Sgorbati, S. (2003) 'Heavy Metal Tolerance and Accumulation of Cd, Cr and Ni by *Cannabis sativa* L `. Plant and Soil 256 (2): 243-252(10).

Clarke, RAF (1996) Wound Repair. In the Molecular and Cellular Biology of Wound Repair (2nd ed, Clarke, RAF, ed). Plenum Press, New York and London; 3-50 pp.

Clemson, AA (1979) 'Insecticides Can Kill Bees`. Agric.Gaz.N.S.W. 4-5.

Cohen, G; Hochstein, P (1962) 'Glutathione peroxidase: The Major Pathway for the Detoxification of Hydrogen peroxide in Erythrocytes`. 141st Meeting.Division of Biological Chemistry. American Chemical Society, P58c (abstract no.137).

Condon, RE (1993) 'Curious Interaction of Bugs and Bees`. Surgery 113 (2): 234-235.

Connie, K& Asheville, NC(1994) 'Anti-Tumor Activities of Hive Products`. American Bee Journal 134(6):420.

Conti, ME & Botre, F (2001) 'Honeybees and their Products as Potential Bioindicators of Heavy Mtals Contamination`. Environmental Monitoring and Assessment 69(3): 267-282 (16).

Cooper, K.E.(1963) The Theory of Antibiotic Inhibition Zones. Analytical Microbiology.Ed F.Kavanagh.New York, San Fransisco and London: Academic Press.

Cooper, RA & Molan, PC (1999) 'The Use of Honey as an Antiseptic in Managing *Pseudomonas* infection`. Journal of Wound Care 8: 161-164.

Cooper, RA; Halas, E & Molan, PC (2002a) 'The Efficacy of Honey in Inhibiting Strains of *Pseudomonas aeruginosa* from Infected Burns`. J Burn Care Rehabil. 23: 366-370.

Cooper, RA; Molan, PC & Harding , KG (2002b) 'The Sensitivity to Honey of Gram –positive cocci of Clinical Significance Isolated from Wounds`. Journal of Applied Microbiology 93: 857-863.

Cooper, RA; Molan, PC; Krishnamoorthy, L & Harding, KG (2001)'Manuka Honey to Heal a Recalcitrant Surgical Wound`. Eur. J. Clin. Microbiol. Infect. 20:758-759.

Cooper, R (2001) How does Honey Heal Wounds. In Honey and Healing. (Munn, P & Jones, R , eds first printing). International Bee Research Association, Cardiff,UK; 27-34 pp.

Cooper, RA; Wigley, P & Burton, NF (2000) 'Susceptibility of Multiresistant Strains of *Burkholderia cepacia* to honey`.The Society for Applied Microbiology, Letters in Applied Microbiology 31: 20-24.

Cooper, RA; Molan, P & Harding, KG (1999) 'Antibacterial Activity of Honey Against Strains of *Staphylococcus aureus* from Infected Wounds`. Journal of the Royal Society of Medicine 92: 283-285.

Cowgill, U (1988) Sampling Waters: The impact of Sample Variability on Planning and Confidence Levels. In Principles of Environmental Sampling (Keith, L, ed), American Chemical Society, Washington, DC; 171-203 pp.

Crane, E (1967) 'An Excursion into Prehistoric`. Bee World 48(1):36-37.

Crane, E (1975) Honey: A comprehensive Survey. William Heinemann in Co-peration with International Bee Research Association (IBRA), London, UK.

Crane, E (1980) A book of Honey. International Bee Research Association.Oxford University Press, Oxford.

Crane, E (1981) Editorial: Endangered Honey Sources. Bee World 62 (1): 3-4.

Crane, E (1983) The a rchaeology of Beekeeping. Gerald Duckworth & Co., London, UK.

Crane, E (1984) 'Bees, Honey and Pollen as Indicators of Metals in the Environment`. Bee World 65: 47-49.

Crane,E (1986) 'Rock Paintings Related to Honey Hunting`.Bee World 67 (1): 23-25.

Crane, E (1990) Bees and Beekeeping: Science, Practice and World Resources. Oxford, Heinemann Newnes, London, UK.

Crane, E (1992) Report to Beekeeping Training and Extension Support Project on a Visit to Nepal, November, 1992. British Executive Service Overseas/BETRESP; Kathmandu, Nepal; 24 pp.

Crane, E (1999) The world history of Beekeeping and Honey Hunting. London: Duckworth

Crane, E & Walker, P (1985) 'Important Honeydew Sources and their Honeys`. Bee World 66 (3):105-112.

Crane, E & Graham, AJ (1985).'Bee Hives of the Ancient World`. Bee World 66(1): 23-41.

Cresser, M; Armstrong, J; Dean, J; Watkins, P & Cave, M (1992). 'Atomic Spectrometry Update-Environmental Analysis`. J.Anal. Atom. Spectron.7: 1-52.

Cresser, M (1994) Flame Spectrometry in Environmental Chemical Analysis: A practical Guide. Royal Society of Chemistry, Cambridge, UK; 108 p.

Cresser, M; Ebdon, L & Dean, J (1988). 'Atomic Spectrometry Update-Environmental Analysis`. J.Anal. Atom. Spectron.3: 1-44.

Cuadrado, C; Kumpulainen, J; Carbajala, A & Moreirasa, O (2000) 'Cereals Contribution to the Total Dietary Intake of Heavy Metals in Madrid, Spain`. Journal of Food Composition and Analysis 13 (4): 495-503.

Cubadda, R; La Notte, E; Esti, M; Marconi, E; Panfili, G; Mancini, MP & Trivisono, MC (1995) Studio dell' Ambiente Utilizaando 1'ape Come Bioindicatore', in Rilevamento di Contaminanti e Valutazione della Qualità dell' Ambiente Attraverso Indicatori Biologici, Università degli Studi del Molise, Dipartimento S.T.A.A.M., Campobasso; 114 p.

D'Ambriso, M& Marchesini, A (1982) 'Heavy Metal Contamination of Honey`. Atti Soc.Ital.Sci.Nat.123 (2-3): 342-348 (in Italian).

Dadant and Sons (eds) (1975) The hive and the honey bee . Dadant & Sons, Hamilton, IL.

Daghie,V; Cirnu, I; Cioca,V (1971) Contribution to the Study of the Bacteriostatic Action of Honey Produced by Physokermes sp. in the Area of Coniferous Trees. In Proceedings of the XXIIIrd International Apicultural Congress of Apimondia, 593-594. Moscow.

Daghie,V; Cirnu, I; Cioca,V (1973) 'Contributii Privind Actinuea Bacrericida si Bacretiostatica a Mierii de Lecaniide (*Physokermes sp.*) din Zona Coniferelor`. Apicultura 26 (2):13-16.

Davis, AR (1989) 'The Study of Insecticide Poisoning of Honeybee Brood`.Bee World 70(4): 163-174.

de Lopez Camelo, LG; de Miguez, SR; Marban, L (1997) 'Heavy Metals Input With Phosphate Fertilizers Used in Argentina`. The Science of the Total Environment 204 (3): 245-250 (6).

Debacker, V; Rutten, A; Jauniaux, T; Daemers, C & Bouquegneau, J M (2001) 'Combined Effects of Experimental Heavy-Metal Contamination (Cu, Zn, and CH3Hg) and Starvation on Quail's Body Condition: Parallelism with a Wild Common Guillemot (*Uria aalge*) Population Found Stranded at the Belgian Coast`. Biological Trace Element Research 82 (1-3): 87-107 (21).

Dhano, KB (1947) 'Bees`. Indian Bee Journal 1:104.

Djingova, R & Kuleff, I (2000) Instrumental Techniques for Trace Analysis. In Trace Elements-their Distribution and Effects in the Environment (Markert, B & Friese, K, eds).Elsevier Science BV 137-185 pp.

Djingova, R; Ivanova, J & Kuleff, I (1998). 'Comparative Evaluation of the Possibilities of INAA, EDXRF, ICP-AES and AAS in the Analysis of Plants`. J. Rdioanal. Nucl. Chem 237: 25-34.

Djuric, G; Popovic, D; Todorovic, D; Slivka, J & Mihaljev, Z (1996) 'Natural Fallout Redionuclides in Different Types of Honey`. Journal of Environmental Biology 17 (4): 339-343.

Dold, H; Du, DH & Dziao, ST (1937) 'Nachweis Antibakrerieller, Hitze-und Lichtempfindlicher Hemmungsstoffe Inhibine im Naturhonig Blätenhonig`. Zeitschrift für Hygiene und Infektionskranheiten 120:155-167.

Dold, H & Witzenhausen, R (1955) 'Ein verfahren zur Beurteilung der Örtlichen Inhibitorischen (keimvermehrungshemmenden) Wirkung von Honigsorten Verschiedener Herkunft`. Z.für Hygiene 141: 333-337.

Dolezal, M; Dolezal, M & Medrela-Kuder, E (1988) 'Research on Inhibine Effect Herb-honey`. Acta Biologica Cracoviensia Series Botanica 30: 9-16.

Dudka, S; Piotrowska, M & Terelak, H (1996) ' Transfer of Cadmium, Lead, and Zinc From Industrially Contaminated Soil to Crop Plants: A Fieldstudy`. Environmental Pollution 94 (2):181-188.

Duisberg, H & Warnecke, B (1959) 'Erhitzungs und Lichteinfluss auf Fermente und Inhibine des Honigs`. Zeitschrift für Lebensmittelluntersuchung und Forschung 111:111-119.

Dulude, G (1992) In Advances in Atomic Spectrometry (Vol.1) (Sneddon, J, ed). JAI Press, Greenwich, CT; 126 p.

Dumronglert, E (1983) 'A Follow-up Study of Chronic Wound Healing Dressing with Pure Natural Honey`. Journal of the National Research Council of Thailand 15 (2): 39-66.

Dunford, C; Cooper, RA & Molan, PC (2000a) 'Using Honey as a Dressing for Infected Skin Lesions`. Nursing Times 96: 7-9.

Dunford, C; Cooper, R; Molan, P & White, R (2000b) 'The use of Honey in Wound Management`. Nursing Standard 15(11): 63-68.

Dunn, FL (1976) Traditional Asian Medicine and Cosmopolitan Medicine as Adaptive Systems. In Asian Medical Systems (Leslie, C ed). Univ.of California Press, Berkeley, California; 135p.

Dustmann, JH (1967) 'Messung von Wasserstoffperoxid und Enzymatiktivität in Mitteleuropäischen Honigen`. Zeitschrift für Bienenforschung 9: 66-73.

Dustman, J H (1971) 'Über die Katalaseaktivität in Bienenhonig aus der Tracht der Heidekrautgewächse (Ericaceae)`. Zeitschrift für Lebensmittel-Untersuchung und Forschung 145: 292–295.

Dustmann, JH (1972) 'Über den Einfluss des Lichtes auf den Peroxid-wert (inhibin) des Honigs`. Z. Lebensm.Unters.Forsch 148: 263-268.

Dustmann, JH (1979) 'Antibacterial Effect of Honey`. Apiacta. 14: 7-11.

Dustmann, J (1987) 'Effect of Honey on the Cariogenic Bacterium *Streptococcus mutans*`. In Proceedings of the XXXIst International Apicultural Congress of Apimondia, 459-461. Warsaw, Poland.

Edelhauser, M & Bergner, KG (1984) 'Proteine des Bienenhonigs VIII. Honigsaccharase, Isolierung, Chromatographisches Verhalten und Eigenschaften`. Zeitschrift für lebensmittelluntersuchung und forschung 1978:189-194.

Effem, S (1988) 'Clinical Observations on the Wound Healing Properties of Honey`. British Journal of Surgery 75: 679-681.

Effem, S (1993) 'Recent Advances in the Management of Fournier's Gangrene:preliminary Observations`. Surgery 113 (2): 200-204.

Ehlken, S & Kirchner, G (2002) 'Environmental Processes Affecting Plant Root Uptake of Radioactive Trace Elements and Variability of Transfer Factor Data: A review`. Journal of Environmental Radioactivity 58 (2-3):97-112.

Ellis, KJ; Yuen, K; Yasumura, S & Cohn, SH (1984) Environ. Res. 33: 216-266. Cited in Gerhardsson and Skerfvig (1996).

Elinder, C-G & Sjögren, B (1986) In Handbook on the Toxicology of Metals (Vol.II), (Friberg, L, Nordberg,GF & Vouk,VB, eds). Elsevier, New York,1.

EPA-600/4-82-029 (1982) Handbook for Sampling and Sample Preservation of Water and Waste water. US Department of Commerce, Springfield, II.

Ercal N; Gurer-Orhan H & Aykin-Burns, N (2001) 'Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Metal-induced Oxidative Damage`. Current Topics in Medicinal Chemistry, 1(6): 529-539 (12).

Ernst, WHO & Bast-Cramer, WB (1980) 'Plant and Soil` 57:491. Cited in Jones (1987).

Estep, CB; Menon, GN; Williams, HE & Cole, AC (1977) Bulletin Environmental Contamination Toxicology 17: 168). Cited in Jones, (1987).

Fakih, MG & Saravolatz, L (2003) 'Treatment of *Staphylococcus aureus* Pneumonia`. Current Treatment Options in Infectious Diseases 5:137-142.

Falandysz, J; Szymczyk, K; Ichihashi, H; Bielawski, L; Gucia, M; Frankowska, A & Yamasaki S-I (2001) 'ICP/MS and ICP/AES Elemental Analysis (38 elements) of Edible Wild Mushrooms Growing in Poland`. Food Additives and Contaminants 18 (6): 503-513 (11).

Fan, A M (1996) An introduction to Monitoring and Environmental and Human Risk Assessment of Metals. In Toxicology of Metals (Chang, LW; Magos, L; Tsuguyoshi, S, eds). Lewis Publishers, CRC Press, Boca Raton, FL; 5-7 pp.

Farouk, A;Hassan, T; Kashif, H; Khalid, SA; Mutawali, I & Wadi, M (1988) 'Studies on Sudanese Bee Honey: Laboratory and Clinical Evaluation`. International Journal of Crude Drug Research 26 (3):161-168.

Fawcett, P; Green, D & Shaw, G (1971) Radiochemical Radioanalytical Letters 8: 37.Cited in Jones (1987).

Feller- Demalsy, MJ; Vincent, B & Beaulieu, F (1989) 'Teneur en Mineraux et Origine Geographique des Miels du Canada`. Apidology 20: 77-91.

Ferguson, R; Price, V & Baucom, E (1976) Field Manual for Stream Sediment Reconnaissance. Savannah River Laboratory, Aiken, SC; DPST-76-358.

Ferguson, R; Price, V & Baucom, E (1977) Field Manual for Stream Sediment Reconnaissance. Savannah River Laboratory, Aiken, SC; DPST-76-416.

Field, J (1981) 'Penncap-M Strikes New Hampshire Colonies`. Am.Bee J. 115: 351-353.

Fierro,MW & Lopez, GJ (1997) Clinical Evaluation of a New Hypoallergic Formula of Propolis in Dressings, Bee products. Properties, Applications and Apitherapy. Symposium Tel Aviv 101-105.

Fodor, P & Molnar, E (1993)'Honey as an Environmental Indicator: Effect of Sample Preparation on Trace Element Determination by ICP-AES`.Mikrochim Acta 112:113-118.

Food Research Institute (1997) Partial Monitoring System of Foreign Substances in Foods and in Feeds. Report From 1996. Bratislava (in Slovak).

Forrest, RD (1982) 'Early History of Wound Treatment`. J.Soc.Med. 75:198-205.

Forte, G; D`llio, S & Caroli, S (2001)' Honey as a Candidate Reference Material for Trace Elements`. Journal of AOAC International http//www.aoac.org/pubs/JOURNAL/2001/Nov_Dec/Honey_as_a_Candidate.htm.Accessed on July 22, 2003.

Franco, M & Sartori, L(1940) 'Sull 'azione Antibatterice del Miele` Annali d 'Igiene 50:216-227 (abstracted in The Lancet i: 1184 (1940)

Frankel, S; Robinson, GE & Berenbaum, MR (1998)'Antioxidant Capacity and Correlated Characteristics of 14 Unifloral Honeys`. Journal of Apicultural Research 37(1):27-31.

Free, JB; Williams, ICH; Pinset, KJKH; Townshed, A; Basi, MS; & Graham, CL (1983) Environmental International 9:9. Cited in Jones (1987).

Fresquez, PR; Armstrong; DR & Pratt, LH (1997).'Radionuclides in Bees and Honey Within and Around The Los Alamos National Laboratory`. Journal of Environmental Science and Health, Part A Environmental Science and Engineering and Toxic and Hazardous Substances 32(5):1309-1323.

Fritzsch, W & Bremer, R (1975) Bienengesundheitsdienst. Jena. Cited in Bromenshenk et al., (1991).

Gailey, FAY; Lloyd, OL (1993) 'Spatial and Temporal Patterns of Airborne Metal Pollution : The Value of Low Technology Sampling to an Environmental Epidemiology Study`. Sci. Total Environ. 133: 201-219.

Gajek, O; Nabrzyski, M & Gajewska, R (1987) 'Metallic Impurities in Imported Canned Fruit and Vegetables and in Honey`. Apicultural Abstarct (1991) 42 (2):172.

Galun, M; Ronen, R (1988). Interaction of Lichens and Pollutants. In Handbook of Lichenology (Vol. 3) (Galun, M ed). CRC Press, Boca Raton, FL; 55-72 pp.

Garedew, A; Schmolz, E & Lamprecht, I (2003) ' The Antimicrobial Activity of Honey of The Stingless Bee Trigona Spp`. Journal of Apicultural Science 47(1): 37-48.

Garty, J (1992) Lichens and Heavy Metals in the Environment. In Impact of Heavy Metals on the Environment (Vol.2) (Vernet, J-P, ed), Elsevier, Amsterdam; 55-131pp.

Garty, J (1993) Lichens as Biomonitors for Heavy Metal Pollution. In Plants as Biomonitors-Indicators for Heavy Metals in the Terrestrial Environment (Weinheim, VCH, ed). Germany; 687-711pp.

Garty, J (2000) Environment and Elemental Content of Lichens. In Trace Elements-Their Distribution and Effects in the Environment (Markert, B & Friese, K, eds).Elsevier Science BV; 245-276 pp.

Gauhe, A (1991) 'Ueber eine Glucose-oxydierendes Enzyme in der Phyrynxdrüse der Honigbiene`. Z.Vergl.Physiol 28:211-253.

Gautam, I (1998) Ovipositional Preference and Effectiveness of Some Plant Products Against Pulse Beetles *Callosobruchus chinensis* (L) and *Callosobruchus maculatus* (F)`. MSc Thesis, The Institute of Science and Technology, Central Department of Zoology, Tribhuvan University, Nepal.

Gedalia, I; Grobler, SR; Grizim, I; Steinberg, D; Shapira, L; Lewinstein, I & Sela, M (1997) Honey Contact With Teeth in situ, Bee products.Properties, Applications, and Apithrerapy symposium Tel Aviv 73-76.

Gelbart, M (1999) (cited in Dunford, C; Cooper, R; Molan, P & White, R (2000) 'The Use of Honey in Wound Management. Nursing standard 15 (11): 63-68).

Gerhardson, L & Skerfving, S (1996) Concepts on Biological Markers and Biomonitoring for Metal Toxicity. In Toxicology of Metals (Chang, LW; Magos , L & Tsuguyoshi, S, eds). Lewis Publishers, CRC Press, Boca Raton, FL; 81-107 pp.

Gilbert, MD & Lisk, DJ (1978) 'Honey as an Environmental Indicator of Redionuclide Contamination`. Bulletin Environmental Contamination &Toxicology 19: 32-34.

Gladyshev, MI; Gribovskaya, IV; Ivanova, EA; Moskvichova, AV; Muchkina, EY & Chuprov, SM (2001) 'Metal Concentrations in the Ecosystem and Around Recreational and Fish-Breeding Pond Bugach`. Water Resources 28 (3):288-296 (9) .

Goldwater, C (1983) Traditional Medicine in Latin America. In Traditional Medicine and Health care Coverage (Bannerman, RH; Burton, J & Wen-Chieh, C, eds).WHO, Geneva; 37-49 pp.

Goncalves, EPR; Soares, HMVM; Boaventura, RAR; Machado, AASC & Esteves da Silva, JCG (1994) `Seasonal Variations of Heavy metals in Sediment and Aquatic Mosses From The Cavado River basin (Portugal)`. Sci.Total Environ.142: 143-156.

Gonnet, M (1981) 'Facteurs Antibiotiques Naturels Prèsents dans le Miel.Rev`.France d ' Apicult 27-30.

Gonnet, M & Lavie, P (1960) 'Influence du Chauffage sur le Facteur Antibiotique Prèsent dans les Miels`. Annales die l' abeille (Paris) 3 (4): 349-364.

Goyer, RA (1986) Toxic Effects of Metals. In Casarett and Doull`s Toxicology (3rd ed.) (Klaassen, CD; Amdur, MO; & Doull, J, eds). Macmillan, New York; 582 p.

Gräff, S; Berkus, M; Alberti, G & Köhler, HR (1997) 'Metal Accumulation Strategies in Saprophagous and Phytophagous Soil Invertebrates: A Quantitative Comparison`. Biometals 10(1): 45-53 (9).

Gray, A (1985) In The Potential of ICP-Source Mass Spectrometry (Sansoni, B, ed). Instrumentelle Multielementanalyse,VCH Publishers, Weinheim; 227-237 pp.

Greceanu, A & Enciu,V (1976) 'Observations on The Antibiotic Effects of Propolis, Pollen and Honey`. II International Symposium on Apitherapy, 177-179. Apimondia Publishing House, Bucharest, Romania.

Green, AE (1988) 'Wound Healing Properties of Honey`. British Journal of Surgery 75 (12): 1278.

Greich, A & Delage-Darchen, B (1978) 'Nouvelles Donnes sur Lèquipment Enzymatique des Glands Salivaires de L'ouvriere d' *Apis mellifera*`. Annales des Sciences Naturelles, Zoologie et Biologie Animale 20: 401-422.

Grobler, SR & Basson, NJ (1997) 'The Effect of Honey on Human Tooth Enamel and Oral Bacteria, Bee products`. Properties, Applications, and Apitherapy 65-71.

Grochowski, J & Bilinska, M (1987) 'Biological Activity of Pollen, Beebread and Honey Relative to Selected Bacterial Strains`. In Proceedings of the XXXIst International Apicultural Congress of Apimnondia, 462. Warsaw, Poland.

Groves, RW; Allen, MH; Ross, EL; Barker, JNWN & MacDonald, DM(1995) 'TNF-alpha is Proinflammatory in Normal Human Skin and Modulates Cutaneous Adhesion Molecule Expression`. Br.J Dermatol.132: 345-352.

Gryuner, VS & Arinkina, AI (1970) 'Carbohydrates Content, Enzymatic and Antimicrobial Activity of Honey`.Pishchevaya Tekhnologiya 28-31.

Gürel, F; Karkacier, M & Özdemir, F (1998) 'Identification of Sugar Honey, Multifloral Honey and Honeydewe Honey Based on Mineral Content, Total ash, pH value and Acidity'. Apiacta 33 (3): 42-45.

Gzyl, J (1997) Assessment of Polish Population Exposure to Lead and Cadmium With Special Emphasis to the Katowice Province on the Basis of Metal Concentrations in Environmental Compartments. Cent. Haddad, I.K., Environ.Res., 26, 217-240. Cited in Gerhardsson and Skerfvig (1996).

Haffejee, IE & Moosa, A (1985) 'Honey in the Treatment of Infantile Gastroenteritis`. In British Medical Journal 290:1866-1867.

Hakonson, TE & Bostick, KV (1976) Journal Environmental Quality 5:307). Cited in Jones (1987).

Halls, D (1995) 'Analytical Minimalism Applied to the Determination of Trace Elements by Atomic Spectrometry`. J.Anal.Atom. Spectrom. 10: 169-175.

Hamdy, MH; El-Banby, MA; Khakifa, KI; Gad, EM & Hassanein , EM (1989) The Antimicrobial Effect of Honey in The Management of Septic Wounds. In International Bee Research Association Fourth International Conference on Apiculture in Tropical Climates, 1988, 61-67 . International Bee Research Association; London, UK.

Hamilton-Taylor, J & Davison, W (1995) Redox –driven Cycling of Trace Elements in Lakes. In Physics and Chemistry of lakes(Lerman, A; Imboden, DM & Gat, JR, eds). Springer, Berlin; 217-263 pp.

Harris, S (1994) 'Honey for the Treatment of Superficial Wounds: A Case Report and Review`. Primary Intention 2 (4):18-23.

Hasspieler, B M; Haffner, G D & Adeli, K (1997) 'Molecular Biology and DNA Probe Novel in vitro Methods for Assessment of Environmental Toxicity Using a Human Cell-line: Application to Assessment of Environmental Health Clinical Biochemistry`. Abstract of poster presentation 30(4):380-381.

Heading, ME (1983) 'The toxicity of Pesticides to Honeybees`. Am.Bee J.123(9): 646-647.

Hegazi, AG; Moharram, NZ; Allah, FA; Nour, MS & Khair, AM (2001) 'Antibacterial Activity of Different Egyptian Honeys in Relation to Some Bee Products`. Mellifera 1-2: 45.

Heggers, JP; Velanovich, V; Robson, MC; Zoellner, SM; Schileru, R; Boertman, J & Xing-Tao, N (1987) ' Control of Burn Wound Sepsis: A Comrarison of in vitro Topical Antimicrobial Assays`. Journal of Trauma 27: 176-179.

Hejase, MJ; Bihrle, R & Coogan, CL (1996) 'Enital Fournier's Gangrene: Experience with 38 Patients`.Urology 47 (5): 734-739.

Hernández1, L M, Gómara, B; Fernández, M; Jiménez, B; González, M J; Baos, R; Hiraldo, F; Ferrer, M; Benito, S; Devesa, V; Muñoz, O & Montoro, R (1999) 'Accumulation of Heavy metals and as in Wetland Birds in The Area Around Doñana National Park Affected by The Aznalcollar Toxic Spill`. The Science of The Total Environment 242 (1-3): 293-308.

Herpin, U; Berlekamp, J; Markert, B; Wolterbeek, B;Grodzinska, K; Siewers, U; Lieth, H & Weckert, V(1996) 'The Distribution of Heavy metals in a Transect of the Three States -The Netherlands, Germany and Poland, Determined with the aid of Moss Monitoring`. Science of Total Environment 187: 185-198.

Herpin, U; Markert, B; Siewers, U & Lieth, H (1994) 'Monitoring der Schwermetallbelastung in der Bundesrepublik Deutschland mit Hilfe von Moosanalysen`. Forschungsbericht 10802087: 94-125.

Hickeya, C W; Ropera, D S & Bucklanda, S J (1996) `Metal Concentrations of Resident and Transplanted Freshwater Mussels *Hyridella menziesi* (Unionacea: Hyriidae) and Sediments in The Waikato River, NewZealand`. Environmental Pollution 93 (3):366.

Hill, J (1989) The Virtues of Honey. pp.15-28. Lancashire:Elwood Books.(First published in 1759) .Cited in Cooper et al., (2000).

Hill, SJ; Dawson, JB; Price, WJ; Shuttler, IL & Tyson, JF (1992) J. Anal.At Spectrom. 1992.7: 215-277. Cited in Jacobs, 1996.

Hill, SJ; Dawson, JB; Price, WJ; Shuttler, IL & Tyson, JF (1993) J. Anal.At Spectrom. 8:197-237. Cited in Jacobs (1996).

Hladìkova, V; Ursìnyovà, M M; Sovcìkova, E & Cvikovà, V (1997) Children`s Blood Lead Levels Relation to the Selected Environmental Risk Factors.In Preceedings of the International Symposium on Environmental Epidemiology in Central and Eastern Europe: Critical issues for Improving Health. Smolenice, Slovak Republic; 64-66 pp.

Hodgson, MJ (1989) Investigation of the Antibacterial Action Spectrum of Some Honeys.M Sc thesis; University of Waikato, New Zealand; 83 pp.

Hong, J; Calmano,W& Förstner, U (1995) Interstitial Waters. In Trace Elements in Natural Waters (Salbu, B & Steinnes, E, eds). CRC Press, Boca Raton, FL; 117-150 pp.

Horn, H (1985) 'Die Waldtrachtkrankheit der Honigbiene. I. Der Einfluß des Mineralstoffgehaltes in Honigtauhonigen `. Apidologie 16 (2):139-156.

Houshiar-Rada, A; Omidvara, N; Mahmoodi, M; Kolahdooza, F & Amini, M (1998)

http://www.who.int/nut/malnutrition_worldwide.htm, Accessed on 17 Dec, 2003.

http://www.chem.vt.edu/chem-ed/spec/atomic/aa.html

httP://www.fao.org/es/ESNnutrition/nep-e.stm (Accessed on 17 Dec, 2003).

http://www.un.org.np/res_cor/un_reform/ccd/cca1999/nutrition.htm (Accessed on 17 Dec, 2003).

http://www.friedli.com/herbs/phytochem/flavonoids.html, Assessed on 14 July, 2004.

Hu, H (2002) In Life Suport: The Environment and Human Health (McCally, M, ed). MIT Press .

Hura, C; Leanca, M; Palamarua, I & Rusua, L (1998) 'Human Exposure at Chemical Pollutants Risk for the Public Health`. Toxicology Letters 95 (Supplement 1): 141.

Hutton, DJ (1966) 'Treatment of Pressure Sores`. Nursing Times 62 (46):1533-1534.

Ialomitzeanu, M & Daghie,V (1973). 'Investigations of the Antibiotict Qualities of Honey`. In Proceedings of the XXIVth International Apicultural Congress of Apimondia, 438-440. Buenos Aires.

Ialomitzeanu, M; Daghie, V & Mihaescu, NF (1967) 'Contribution to The Study of The Bacteriostatic and Bactericidal Action of Honeys`. In Proceedings of the XXIst International Apicultural Congress of Apimondia, 209-213. Budapest, Hungary.

Ibrahim, AS(1981) 'Antibacterial Action of Honey`. In Proceedings of the First International Conference on Islamic Medicine (Volume1), 363-365.Kuwait.

Ichikawa, Y; Kusaka, Y & Goto, S (1985) Int. Arch.Occup. Environ.Health 55: 269-276. Cited in Gerhardson & Skerfving (1996).

ICIMOD (1994) Exploration of Genetic Diversity in the Himalayan Honeybee *Apis cerana* F`. Final report, US Agency for International Development.

ICIMOD (1996) Promotion and Development of Beekeeping through Preservation of Indigenous *Apis cerana*. Annual Project Progress Report Submitted to the Federal Chancellory of Austria.

Ikeda, M; Zhang, Z.-W; Shimbo, S; Watanabe,T; Nakatsuka, H; SeokMoon,C; Matsuda-Inoguchid, N & Higashikawa, K (2000) 'Urban Population Exposure to Lead and Cadmium in East and South-East Asia`. The Science of The Total Environment 249(1-3):373-384.

Ikem, A; Egiebor, NO & Nyavor, K (2003) `Trace Elements in Water, Fish and Sediment from Tuskegee Lake, Southeastern USA`. Water, Air, and Soil Pollution 149(1-4): 51-75(25).

Imdorf, A; Rickli, M; Kilchenmann, V; Bogdanov, S & Wille, H (1998) 'Nitrogen and Mineral Constituents of Honeybee Worker Brood During Pollen Shortage`. Apidologie 29:315-325.

Impereto, PJ & Traore (1969) 'Traditional Beliefs About Measles and its Treatment Among The Banbara of Mali`.Tropical and Geographical Medicine 21: 62-67.

Iskander, FY (1996) 'Assessment of Trace Elements in Honey Produced on Uranium Mining Reclaimed Land`. The Science of Total Environment 192:119-122.

ISO (1980) International Standard Organization (Part 1). Guidance on the Design of Sampling Programs (Part 2).Water Quality, Sampling, Guidance on Sampling Techniques, ISO-5667/1.

Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise (IZS) (1991) Studio analitico die mieli abruzzesi, Teramo 82 p.

Jackson, KW & Mahmood, TM (1994) Anal. Chem. 66: 252-279. Cited in Jacobs (1996).

Jackson, KW & Qioa, H (1992) Anal. Chem. 64:50-66. Cited in Jacobs (1996).

Jacobs, R M (1996) Techniques Employed for The Asssessment of Metals in Bilogical Systems. In Toxicology of Metals (Louis, LW; Magos, L & Suzuki, T, eds). Lewis Publishers, CRC Press, Boca Raton, FL; 69-79 pp .

Jacquiot, L & Daillant, O (1997) 'Bio-accumulation des Mètaux lourds et d'autres Elèments Traces par les Lichen, Revue Bibliographique`. Bull.de l'Observ. Mycol.12: 2-31.

Jaishy, SN; Mandal, SN; Manandhar, R; Karki, TB & Maskey, KH (1999) Production and Utilization of Compost by the Farmers in Four Selected Districts of Nepal.Nepal Journal of Science and Technology 1:57-62.

Jakovljevic, M D; Kostic, N M; Stevanovic, D; Blagojevic, S; Wilson, M J& Martinovic, LJ (1997) 'Factors Influencing the Distribution of Heavy metals in The Alluvial Soils of the Velika Morava River valley, Serbia`. The Science of The Total Environment 206 (2-3):187-193.

James, OBO'L; Segroe, W & Ventura, AK (1972) 'Some Antibacterial Properties of Jamaican honey`.W. Indian med. J. 21:7-17.

James, PW (1973) The Effect of Air pollutants other than Hydrogen Fluoride and Sulphur dioxide on Lichens. In Air pollution and Lichens (Ferry, BW; Baddeley, MS & Hawksworth, DL, eds). Athlone press, London,UK; 143-175 pp.

Jamoussi, B; Zafaouf, M & Hassine, B B(1995). 'Hydride Generation/Condensation System with an Inductively Coupled Argon Plasma Polychromator for Simultaneous Determination of Arsenic, Antimony, Selenium, Lead, Mercury and Tin in Honey`. Intern.J.Environm. Anal.Chem. 61: 249-256.

Jeddar, A; Kharsany, A; Ramsaroop,UG; Bhamjee, A; Haffejee, IE & Moosa, A (1985) 'The Antibacterial Action of Honey: An in vitro Study`. South African Medical Journal 67: 257-258.

Johansen, CA (1979) 'Bee poisoning survey`. Am.Bee J.119 (4): 315-318.

Johansson, K; Bergbäck, B & Tyler G (2001)' Impact of Atmospheric Long Range Transport of Lead, Mercury and Cadmium on the Swedish Forest Environment`.Water, Air and Soil Pollution: Focus 1(3/4): 279-297(19).

Jones, KC (1987) 'Honey as an Indicator of Heavy metal Contamination`. Water, Air and Soil Pollution 33:179-189.

Jones, JB Jr (1998) Plant Nutrition Manual. CRC Press, LLC Corporate Blvd., N.W., Boca Raton, Florida 33431, USA; 9 p.

Jones, R (2001) Honey and Healing through The Ages. In Honey and Healing. (Munn, P & Jones, R first printing, eds). International Bee Research Association, Cardiff,UK; 1-4 pp.

Joshi, CG & Godbole, NN (1970) 'The Composition and Medical Properties of Honey, Madhu, as Described in Ayurveda`. Indian Bee J. 32: 77 - 78.

Joshi, M; Divan,VV & Suryanarayana, MC (1983) Bees and Honey in Ancient India. Proc. Int. Conf. Apic. trop. Climates, 1980. Indian Agric. Res. Inst., New Delhi, India; 2: 143 - 149.

Joshi, SR (1999) Physico-chemical and Melissopalynological Characteristics of Nepalese Honey. PhD Thesis, University of Agricultural Sciences,Vienna, Austria.

Joshi, SR; Pechhacker, H& Willam, A (2000) 'Physico-chemical Characteristics of *Apis dorsata*, *A. cerana* and *A. mellifera* Honey from Chitwan District, Central Nepal`. Apidologie 31:367-375.

Joshi, SR; Pechhacker, H; Sheurer, S & Shrestha, KK (1998) ' Honeydew Honey in Nepal`. Paper presented at the 4th AAA Conference, Kathmandu, Nepal.

Jovanic, S; Carrot, F; Deschamps, C; Deschamps, N & Vukotic, P (1995) 'A Study of The Air Pollution in The Surroundings of an Aluminium Smelter, Using Epiphytic and Lithophytic Lichens`. J.Trace Microprobe Tech.13: 463-471.

Kafle,GP (1979) Elementary Beekeeping. Kathmandu: Rupayan Press, Kathmandu, Nepal (in Nepali).

Kafle,GP (1984) 'A General Survey of Bee Flora in and Around Kathmandu Valley`. Nepalese Journal of Agriculture 15:89-99.

Kafle, GP (1990) Beekeeping in Integrated Mountain Development and Scientific Perspectives (Verma, LR, ed). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Kafle, GP (1992) Salient Features of Beekeeping in Nepal.In Honeybees in Mountain Agriculture (Verma, LR, ed). Oxford & IBH Publ.Co.Pvt.Ltd., New Delhi, India;155-162 pp.

Kaftanoglu, O & Tanyali, A (1997) The Use of Royal jelly During Treatment of Childhood Malignancies, Bee products. Properties, Applications, and Apitherapy 179-183.

Kalimi, MY & Schonie, K (1964) 'Mahabaleshwar Honey 1. Proximate Mineral Analysis and Paper Chromatographic Detection of Amino Acids and Sugars`. J. Nutr. Diet 1:261-264.

Kansanen, PH & Venetvaara, J (1991) `Comparison of Biological Collectors of Airborne Heavy metals near Ferrochrome and Steel Works`. Water Air Soil Pollution 60:337-359.

Keast-Butler(1980) 'Honey for Necrotic Malignant Breast Ulcers`. Lancet ii (October 11):809.

Kelner-Pillaut, S (1969) 'Abeilles Fossiles Ancetres des Apides Sociaux`. Proceedings of the Sixth Congress of the International Union for Study of Social Insects, 85-93.

Kerkvliet, J (1996) ' Screening Method for the Determination of Peroxide Acumulation in Honey and Relation with HMF Content`. J Apic Res 35: 110-117.

Kerkvliet, JD (1983) Maandschr Bijent 85, 261 (in German). Cited in Jones (1987).

Kerkvliet, JD; Shrestha, M; Tuladhar, K & Manandhar, H (1995) 'Microscopic Detection of Adulteration of Honey with Cane Sugar and Cane Sugar Products`. Apidologie 26:131-139.

Khursid, SR & Qureshi, I H (1984) `The Role of Inorganic Elements in Human Body`. The Nucleus 21:3-23 .

Kiderman, A; Torten, R; Furst, AL & Reinus, K (2001) 'Bi-lateral Eosinophilic Ulcers in an Infant Treated with Propolis`. Journal of Dermatological Treatment 12(1): 29-31(3).

Kirienko, ND; Tcherkasova, AI; Zaharteta, LI; Gladtchun, VP; Klevanik, VA & Kiiko, LA (1989) 'Complex Treatment of Chronic Bronchitis with Apicultural Products`. In Proceedings of the XXXII International Apicultural Congress of Apimondia, 282-284. Rio de Jeneiro, Brazil.

Kirkham, MB & Gorey, J (1977) Journal Nuclear Agriculture Biology 6:71 .Cited in Jones, (1987).

Kläntschi, N; Lienemann, P; Richner, P& Vonmont, H (1996) Elementanalytik. Spektrum Verlag.

Klebanoff, SJ (1980) Myeloperoxidase-mediated Cytotoxic Systems. In the Reticulo endothelial system. A comprehensive Treatise (Volume 2).Biochemistry and Metabolism (Sbarra, AJ & Strauss, RR, eds). Plenum Press, New York, USA; 270-308 pp.

Knight, AH; Crooke,WM & Shepherd, H (1972) Journal Science Food Agriculture 23: 263. Cited in Jones (1987).

Knoechel, A & Prange, A (1981) 'Analytik von Elementspuren in Meerwasser. Part I. Tracestudien zur Entwicklung von Spurenanalytischen Verfahren fuer die Multielementbestimmung von Schwermetallen in Waessrigen Loesungen mit Hilfe von Varianten der Energiedispersiven Roentgenfluorescenzanalyse`. Fresenius Z. Anal. Chem. 306: 252-258.

Koeniger, N (1976) The Asiatic Honeybee *Apis cerana*. Proc.Int. Apic.Congr. in Trop.Clim., 47-49. London,UK.

Kujumgiev, A; Tsvetkova, I; Serkedjieva, Yu; Bankova, V; Christov, R & Popov, S (1999) 'Antibacterial, Antifungal and Antiviral Activity of Propolis of Different Geographic Origin`. Journal of Ethnopharmacology 64 (3): 235-240.

Kump, P; Necemer, M & Snajder, J (1996) 'Determination of Trace elements in Bee Honey, Pollen and Tissue by Total Reflection and Radioisotopic X-ray Fluorescence Spectrometry`. Spectrochimica Acta Part B 51: 499-507.

Kurt, A & Yamankaradeniz, R (1982) 'Investigation of Honeys Consumed in Erzurum and ist surroundings in Turkey`. Food 7 (3): 115-120 (in Turkish).

Lahjie, AM & Seibert, B (1990) Honey Gathering by People in The Interior of East Kalimantan`. Bee World 71 (4): 153-157.

Latif, A; Qayyum, A & Haq, M (1956) 'Research on Composition of Pakistan Honey`. Pak. J. Sci. Res. 8: 163-166.

Lau J (1976) (cited in Abu Ali, T M M (1989) 'The Pharmacological Characterization and The Scientific Basis of The Hidden Miracles of Honey`. Saudi Medical Journal 10 (3): 177-179).

Lavie, P& Grassè, PP (1957) Etude des Substances Antibiotiques Prèsentes Chez *Apis mellifera* at Chez Quelques Insectes Sociaux`. Comptes Rendus Acad.Sc/L' Apiculteur 244/101:2653-2655/17-19.

Lavaie, P & Grassé, PP (1963) Sur l' Identification des Substances Antibacrèriennes Prèsentes dans le Miel`.Comptes Rendus Academie des Sciences, Paris 256:1858-1860.

Lawrey, JD (1984) Biology of Lichenized Fungi.Praeger, New York.

Lecoeu, S; Blais, A; Kolf-Clauw, M; Huneau, J F; Milhaud, G. & Tomé, D (1998) 'Uptake and Transport of Cadmium by CACO-2 Cells`. Toxicology Letters 95 (Supplement1): 123.

Leistner, L & Rödel, W (1975) The Significance of Water Activity for Micro-organisms in Meats. In Water relations of foods (Duckworth, RB ed). Academic Press, London,UK; 309-323 pp.

Leita, L; Muhlbachova, G; Cesco S; Barbattini R & Mondini, C (1996) 'Investigation on The Use of Honeybees and Honeybee Products to Assess Heavy Metals Contamination`. Environ.Monit. Assess. 43: 1-9.

Leuzzi, U & Mincione, B (1992) 'Research on Honey: Determination of Mineral Components by Ion Chromatogryphy of Some Calabrian Honeys (Italy)`. Apicultural Abstracts (1993) 44 (4): 361.

Li, Y; Wahdat, F & Neeb, R (1995) Digestion-free Determination of Heavy metals (Pb,Cd,Cu) in Honey Using Anodic Stripping Differential Pulse Voltammetry and Potentiometric Stripping Analysis. Fresenius J.Anal. Chem. 351:678-682.

Lilley, W (1983) American Bee Journal 123:635. Cited in Jones(1987).

Linder, KE (1962) Ein Beitrag zur Frage der Antimikrobiellen Wirkung der Naturhonige, Zentralblatt für Bakteriologie, Parasitenkunde, Infektionkrankheiten und Hygiene 115 (7): 720-736.

Liua, X-J; Ni, I-H & Wanga, W-X (2002) Trophic Transfer of Heavy metals from Freshwater zooplankton *Daphnia magna* to Zebrafish (*Danio reiro*). Water Research.36 (18): 4563-4569.

Lücke, H (1935) 'Wundbehandlung mit Honig und Lebertran`. Deutsche Medizinische Wochenschrift 61(41):1638-1640.

Macedo, FAN (1997) 'Heavy metals in Propolis`. Practical and Simple Pprocedures to Reduce The Lead Level in The Brazilian Propolis, Bee products. Properties, Applications, and Apitherapy symposium Tel Aviv 231-238.

MacFarlane, G R. & Burchett, M D (1999) 'Zinc Distribution and Excretion in The Leaves of The Grey Mangrove, *Avicennia marina* (Forsk.) Vierh`. Environmental and Experimental Botany.41(2): 167-175.

Madeddu, B & Rivoldini, A (1996) 'Analysis of Plant Tissues by ICP-OES and ICP-MS using an Improved Microwave Oven Acid Digestion`. Atom. Spectrosc. 17: 148-154.

Mahajan, U (1984) Physicochemical Analysis of Honey of Shimla Hills (Himachal Pradesh). M.Phil Thesis. Himachal Pradesh University, Shimla , India.

Malyshev, SI (1968) Genesis of the Hymenoptera. London, Methuen.

Manandhar, HS (1998) 'Study on Incidence of Parasites and Diseases on Honeybees (*Apis cerana* and *A. mellifera*) in Nepal`. In Proceeding of Fourth Asian Apicultural Association International Conference , 58-59. Kathmandu, Nepal.

Manninen, S; Huttunen, S & Torvela, H (1991) 'Needle and Lichen Sulphur Analyses on two Industrial Gradients`. Water Air Soil Pollut. 59:153-163.

Mantovi, P; Bonazzi, G; Maestri, E & Marmiroli, N (2003) 'Acumulation of Copper and Zinc from Liquid Manure in Agricultural Soils and Crop Plants`. Plant and Soil 250 (2): 249-257 (9).

Markert, B; Herpin, U; Siewers, U; Berlekamp, J & Lieth, H (1996) 'The German Heavy metal Survey by Means of Mosses`. Sci. Total Environ. 182:159-168.

Markert, B (1996) Instrumental Element and Multi- Element Analysis of Plant Samples. Wiley, Chichester; 295 p.

Markert, B & Wtorova, W (1992) 'Inorganic Chemical Investigations in the Forest Biosphere Reserve Near Kalinin, USSR.III.Comparison of the Multielement Budget with a Forest Ecosystem in Germany-aspects of Rejection, Indication and Accumulation of Chemical Elements`. Vegetatio 98: 43-58.

Markert, B; Zhang, d.L (1991) 'Inorganic Chemical Investigations in the Forest Biosphere Reserve Near Kalinin,USSR.II.The distribution of Lanthanide Elements in the Vegetation Cover`.Vegetatio 97: 57-62.

Markert, B; Kayser, G; Korhammer, S & Oehlmann, J (2000) Distribution and Effects of Trace Substances in Soils, Plants and Animals. In Trace Elements their Distribution and Effects in The Environment. Trace Metals in the Environment (Vol.4, Markert, B & Friese, K, eds). Elsevier Science BV; 3-31 pp.

Martin, MH & Coughtrey, PJ (1982) Biological Monitoring of Heavy Metal Pollution. Applied Science Publishers, London.

Maskey, M (1989) 'Inter-relationship Between Bees and Flowers in Kathmandu Valley`. Paper Presented at the Fourth International Conference on Apiculture in Tropical Climates, Cairo, Egypt.

Maskey, M (1992) Mountain Women and Beekeeping in Nepal. In Honeybees in Mountain Agriculture(Verma, LR, ed) Oxford and IBH Publishing Co.Pvt.Ltd, New Delhi; 119-132 pp.

Maurizio, A (1965) Hönigtau, Hönigtau Hönig. In Wald Hönigtau (Kloft, W; Maurizio, A & Kaeser,W, eds). Munich: Ehrewirth Verlag; 159-185 pp.

Maurizio, A (1975) Microscopy of Honey. In Honey: A Comprehensive Survey (Crane, E, ed). William Heinenmann in Co-operation with IBRA, London, UK; 240-257 pp.

Mayer, DF & Johansen, CA (1983) 'Occurence of Honeybee (Hymenoptera: Apidae) Poisoning in Eastern Washington`. Envir.Ent.12: 317-320.

McCarthy, J (1995) 'The Antibacterial Effects of Honey: Medical Fact or Fiction?` American Bee Journal 135(5): 341-342.

McGarry, JP(1961) 'The Effect of Aging on The Inhibitory Substance in Various Honeys for Bacteria `. Bee World 42 (2): 226-229.

McInerney, RJF (1990) 'Honey- A Remedy Rediscovered`. Journal of the Royal Society of Medicine 83: 127.

Meers, PD, Ayliffe, GA & Emmerson, AM (1981) 'The Use of Honey as an Antiseptic in Managing *Pseudomonas* Infection`. Journal of Wound Care 8: 161-164.

Meier, KE & Freitag, G (1955) 'Über die Antibiotischen Eigenschaften von Sacchariden und Bienenhonig`. Zeitschrift für Hygiene und Infektionskrankenkheiten 141:326-332.

Mejare, M & Bulow, L (2001) 'Metal-binding Proteins and Peptides in Bioremediation and Phytoremediation of Heavy metals`. Trends in Biotechnology 19 (2): 67-73 (7).

Mel'nichenko, AN (1980) 'Intra-farm Preservation of Insect Enemies and Pollinators and The Biological Protection of Plants`. Vest. sel'.-khoz.Nauki6:70-78 (in Russian).

Metcalfe, RL(1980) 'Changing Role of Insecticides in Crop Protection`. A Rev.Ent.25:219-256.

Meyer, I; Hoelscher, B; Frye, C; Becker K; Wichmann, H-E & Heinrich, J (2003) 'Temporal Changes in Blood Lead Levels of Children in East Germany`. International Journal of Hygiene and Environmental Health 206 (3):181-192.

Migula, P; Glowacka, E; Nuorteva, S-L; Nuorteva, P & Tulisalo, E (1997) ' Time-related Effects of Intoxication with Cadmium and Mercury in The Red wood Ant `. Ecotoxicology 6 (6): 307-320 (14).

Millera, R W; Al-Khazrajia, M L; Sisson, DR & Gardiner, DT (1995) 'Alfalfa Growth and Absorption of Cadmium and Zinc from Soils Amended with Sewage Sludge`. Agriculture, Ecosystems & Environment 53(2):179-184.

Minh, H B G; Mendozoa,V & Taigo, F M (1971) 'The Chemical Composition of Honey Produced by *Apis dorsata*`. J. Apic. Res. 10:91-97.

Ministry of health (MOH) Department of Health Services2002. Annual Report 2000/2001.

Miorin, PL; Levy Junior, NC; Custodio, AR; Bretz, WA & Marcucci, MC (2003) 'Antibacterial Activity of Honey and Propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*`. Journal of Applied Microbiology 95 (5): 913-920(8).

Mishref, A; Magda, SA & Ghazi, IM (1989) 'The Effect of Feeding Medicinal Plant Extracts to Honeybee Colonies on The Antimicrobial Activity of the Honey Produced `. In Proceedings of The Fourth International Conference on Apiculture in Tropical Climates, 80-86. Cairo. International Bee Research Association, London, UK; 80-86 pp.

Mitchell, TJ; Donald, EM & Kelso, JRM (1954) Analyst 97: 435. Cited in Jones (1987).

Mladenov, S (1974) 'Present Problems of Apitherapy`. Hive products: Food, Health and Beauty. In Proc. Int. Symp. Apitherapy, 135-136. Madrid.

Mohrig, W & Messner, R (1968) 'Lysozym als Antibacterielles Agens im Honig und Bienengift`. Acta Biologica Medica Germanica 21:85-95.

Molan, PC (1992a) `The Antibacterial Activity of Honey .1.The Nature of The Antibacterial Activity`. Bee World 73: 5-28.

Molan, PC (1992b) 'The Antibacterial Activity of Honey.2.Variation in The Potency of The Antibacterial Activity`. Bee World 73: 59-76.

Molan, PC (1998) 'A Brief Review of Honey as a Clinical Dressing`. Primary Intention 6 (4): 148-158.

Molan PC (1999) 'The Role of Honey in The Management of Wounds`. J Wound Care 8 (8): 415:418.

Molan, PC & Russel, KM (1988) 'Non-peroxide Antibacterial Activity in Some New Zealand honeys`. Journal of Apicultural Research 27 (1): 62-67.

Molan, PC; Smith, IM & Reid, GM (1988) 'A comparison of the Antibacterial Activities of Some New Zealand Honeys`. J. Apic.Res. 27 (4): 252-256.

Molan, PC (2001) Why Honey is Effective as a Medicine 1. Ist Use in Modern Medicine. In Honey and Healing (Munn, P & Jones, R, eds). International Bee Research Association , Cardiff,UK; 5-13 pp.

Monarca, S; Feretti, D; Zerbini, I; Alberti, A; Zani, C; Resola, S; Gelatti, U & Nardi, G (2002) 'Soil Contamination Detected Using Bacterial and Plant Mutagenicity Tests and Chemical Analyses`. Environmental Research 88 (1): 64-69.

Moore, FD Jr.; Socher, SH & Davis, C (1991) 'Tumor Necrosis Factor and Endotoxin can Cause Neutrophil Activationj through Separate Pathways`. Arch . Surg. 126(1): 70-73.

Moore, K; Thomas, A & Harding, KG (1997) 'Iodine Released from The Wound Dressing Iodosorb Modulates The Secretion of Cytokines by Human Macrophages Responding to Lipopolysaccharide`. Int J Biochem Cell Biol 29: 163-171.

Moreno, M I N; Isla, M I N; Cudmani, G; Vattuone, M A & Sampietro, A R (1999) 'Screening of Antibacterial Activity of Amaicha del Valle (Tucumán, Argentina) Propolis`. Journal of Ethnopharmacology 68 (1-3): 97-102.

Morgan, WD; Ryde, SJS; Jones, SJ; Wyatt, RM; Hainsworth, IR, Cobbold, SS; Evans, CJ & Braithwait, RA (1990) Biol.Trace Elem. Res., 26-27, 407-414. Cited in Gerhardsson and Skerfvig (1996).

Morse, RA & Lisk, DJ(1980) 'Elemental Analysis of Honeys from Several Nations`. American Bee Journal 120: 522-523 .

Morse, RA; Shearer, DA; Bock, R & Benton, AW (1987) 'Observations on Alarm Substances in The Genus Apis`. J. Apic. Res. 6: 113 - 118.

Morse, BA (1986) 'The antibiotic Properties of Honey`. Pan-Pacific Entomologist 62:370-371.

Mossel, D AAA (1980) 'Honey for Necrotic Breast Ulcers`. Lancet ii (November 15): 1091.

Muttoo, RN (1954) 'Beekeeping in Ancient India`. Indian Bee J. 16: 102 - 106.

Muttoo, RN (1956) 'Facts About Beekeeping in India`. Bee World 55: 125 – 133.

Myung, CJ & Thornton, I (1996) 'Heavy metal Contamination of Soils and Plants in the Vicinity of a Lead-Zinc Mine, Korea`. Applied Geochemistry11(1): 53-59.

Nabrdalik, M; Skarbek, R (1974) 'Inhibitory Properties of Bees' Honey-Inhibinowe Wlasciwosci Miodu Pszczelego`. Medycyna Weterynaryjna 30 (11): 669-670 (original in Polish).

Naomi, MS (2000) Battling with Bee Brood Disease in *Apis cerana* in W. Nepal. Programme and Abstracts. Seventh IBRA Conference on Tropical Bees:Management and Diversity.

Nash, TH (1989) Metal Tolerance in Lichens. In Heavy Metal Tolerance in Plants: Evolutionary Aspects (Shaw, Aj ed). CRC Press, Boca Raton, FL; 119-131 pp.

Nash , TH (1996) Nutrients, Elemental Accumulation and Mineral Cycling. In Lichen Biology (Nash, TH) .Cambridge University Press, Cambridge, MA; 136-153 pp.

Natarajan, S; Williamson, D; Grey, J; Harding, KG & Cooper, RA (2001) 'Healing of an MRSA-Colonized, Hydroxyure-induced Leg Ulcer with Honey`. Journal of Dermatological Treatment 12:33-36.

National Institute of Public Health in Prague (1997) Health Effect and Risks due to Contamination of Drinking Water. System of Monitoring of Health Status of Inhabitants in Relation to The Environment, Prague (in Czech).

Nation, JL & Robinson , FA (1971) Journal of Apicultural Research 10: 35. Cited in Jones (1987).

Naylor, IL (1999) 'Ulcer Care in the Middle Ages`. Journal of Wound Care 8 (4): 208-212.

Ndayisaba, G; Bazir, L; Haboniman, E & Mutegany, D (1993) 'Clinical and Bacteriological Results in Wounds Treated with Honey`. Journal of Orthopaedic Surgery 7 (2): 202-204.

Needleman, HL; Gunnoe, C ; Leviton, A ; Reed, R ; Peresie, H ; Mather, C & Barrett, PN (1979) Engl.J.Med. 300: 689-695. Cited in Gerhardsson and Skerfvig (1996).

NESS (1999) Ambient Air Quality Monitoring of Kathmandu Valley (A Report Submitted to ADB TA 2847-NEP Project, Ministry of Population and Environment, HMG, Nepal). Nepal Environmental and Scientific Services Private Limited (NESS), Kathmandu, Nepal.

Nieboer, E ; Puckett, KJ; Richardson, DHS;Tomassini, FD ;Grace, B (1977) 'Ecological and Physicochemical Aspects of the Accumulation of Heavy Metals and Sulphur in Lichens`. In Proceedings of International Conference on Heavy Metals in the Environment, Vol. 2, 331-352. Ontario, Canada.

Nielsen, FH (2002) Trace Mineral Deficiencies. In Handbook of Nutrition and Food (Berdanier, CD, ed). CRC Press, LLC, Boca Raton, FL 33431;1463 –1487 pp.

Niraula, BP (1998) 'Health` . In A Compendium on Environment Statistics, Nepal. Kathmandu, Nepal: NPC/CBS.

Nordberg, GF & Nordberg, M (1988) In Biological Monitoring of Toxic Metals (Clarkson, TW; Friberg, L; Nordberg, GF & Sager, PR, eds). Plenum Press, New York; 151 p.

NRC (1986) Subcommittee on the Ten Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences. National Research Council (NRC), Recommended Dietary Allowances , 10th ed., National Academy Press,Washington, D.C.; 174 p.

Obaseiki-Ebor, E & Afonya, T (1984) In vitro Evaluation of the Anticandidiasis activity of Honey Distillate (HY-1) Compared with that of Some Anti-mycotic Agents. J.Pharmacy and Pharmacol. 36:283-284.

OBI, CL; Ugoji, EO; Edun, SA; Lawal, SF & Anyiwo, CE (1994) The Antibacterial Effect of Honey on Diarrhoea Causing Bacterial Agents Isolated in Lagos, Nigeria`. African Journal of Medical Sciences 23: 257-260.

Omrod, DP (1984) Impact of Trace Element Pollution on Plants. In Air Pollution and Plant Life (Treshow, M ed).Wiley, New York; 291-319 pp.

Onianwa, P C; Adetola, I G; Iwegbue, C M A; Ojo, M F & Tella, O O (1999) 'Trace Heavy Metals Composition of Some Nigerian Beverages and Food Drinks`. Food Chemistry 66 (3): 275-279.

Oostdam, JV; Gilmana , A; Dewailly, E; Usher, P; Wheatley, B; Kuhnlein, H; Neve, S; Walker, J; Tracy, B; Feeley, M; Jerome, V& Kwavnick, B (1999) 'Human Health Implications of Environmental Contaminants in Arctic Canada: A review`. The Science of The Total Environment 230(1-3): 1-82.

Oppitz, M (1991) 'Beekeeping and The Hunt for Wild Honey Among the Magar of Nepal `. Bee World72 (4): 165-169.

Orlic, R; Siegelea, D; Menon, D; Markichc, S J; Cohena, DD; Jeffree, R A; McPhail, DC; Sarbutt, A & Stelcer, E (2002) 'Heavy metal Pathways and Archives in Biological tissue`. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 190 (1-4): 439-444.

Osato, MS; Reddy, SG; Graham, DY(1999) 'Osmotic Effect of Honey on Growth and Viability of *Helicobacter pylor*`. Digestive Diseases and Sciences 44 (3): 462-464.

Ota, C; Unterkircher, C; Fantinato, V & Shimizu, MT (2001) 'Antifungal Activity of Propolis on Different Species of Candida`. Mycoses44 (9-10): 375-378.

Otto, K & Jekat, F (1977) Ernahrungsumschau 2 4: 4.

Pacyna, M J (1996) Monitoring and Assessment of Metal Contaminnts in the Air.InToxicology of Metals.(Chang, LW; Magos, L & Suzuki,T, eds). CRC Press, Boca Raton, FL; 9-28 pp.

Pandey,GP (1997) Rainfed Agriculture in Asia, Report of an APO Study Meeting, 1996,

Kathmandu, Nepal, Asian Productivity Organization, 1997.

Partap, U; Joshi, SR; Verma, LR & Pachhacker, H (1997) 'Straw Hive: An Eco-Friendly Movable Frame Hive`. Ecoviews (ECOS Bulletin) 4:12-15

Partap, U (1997) Bee Flora of Hindu Kush-Himalayas: Inventory and Management.International Centre for Integrated Mountain Development (ICIMOD), Kathmandu, Nepal.

Partap, U & Partap,T (2001) Declining Crop Production and Worried Himalayan Farmers: Solution Lies in Promoting Honeybees for Pollination, Issues in Mountain Development Series 2001/2002, ICIMOD,.Kathmandu, Nepal.

Partap, U & Partap,T (1997) Managed Crop Pollination: The Missing Dimension of Mountain Agricultural Productivity, Mountain Farming Systems Discussion Paper Series No.MFS97/1, ICIMOD, Kathmandu, Nepal.

Partap, U & Partap, T (2002) Warning Signals From the Apple Valleys of The Hindu Kush-Himalayas: Productivity Concerns and Pollination Problems. ICIMOD, Kathmandu, Nepal.

Partap, U & Verma, LR (1992) 'Floral Biology and Foraging Behaviour of *Apis cerana* on Lettuce Crop and ist impact on Seed Production`. Progressive Horticulture 24: 42-47.

Partap,U & Verma, LR (1996) 'Impact of Food Resource Availability on Growth and Development of *Apis cerana* Colonies in The Kathmandu Valley of Nepal`. Ecoprint 3: 21-29.

Partap,U & Verma, LR (1994) 'Pollination of Radish by *Apis cerana*`. Journal of Apicultural Research 33: 237-241.

Pechhacker, H & Juntawong, N (1994) *'Apis mellifera* vs *Apis cerana* in The North of Thailand`. Beekeeping and Development 30: 6-7.

Pechhacker, H (1976) ' Vorhersage der Honigtautracht von Physokremes Hemicryphus Dalm (Homoptera, Coccidae) auf der Fichte (*Picea excelsa*)`.7: 209-236.

Pechhacker, H; Joshi SR & Chatt, A (2001) 'Traditional Beekeeping in Rural Areas: A Comparison of *Apis cerana* Beekeeping in Nepal and *Apis mellifera* Beekeeping in, Morocco`. Bee World 82 (2): 99-104.

Pechhacker, M (2002) Untersuchung Über die Einflussfaktoren auf das Mineralstoffspektrum des Honigs. Diplomarbeit. Universiät für Bodenkultur Wien.

Pelly, I Z (1994) Determination of Trace Elements by Atomic Absorption Spectrometry.In Determination of Trace Elements(Alfaassi, ZB ed). Balaban Publishers VCH Weinheim, Germany; 145-190 pp.

Pendias, A & Pendias, H (1994) Trace Elements in Soils and Plants. CRC Press, Boca Raton, FL.In Plant Nutrition Manual (2nd ed. Jones, JB Jr, ed). CRC Press, LLC Corporate Blvd., N.W., Boca Raton, Florida 33431, USA; 13 p.

Perkins, DF (1992) 'Relationship Between Fluoride Contents and Loss of Lichens Near an Aluminium Works`. Water Air Soil Pollut. 64: 503-510.

Petkov, R; Getchev, I & Kuntcheva, S (1998) 'Studies on The Content of Heavy Metals in Bee Honey in Ecological Pollution Zones`. Apiacta 33 (4): 107-111.

Petrov,V (1970) Journal of Apicultral Research 9: 95. Cited in Jones (1987).

Petrovic, Z.T; Mandic, M; Grgic; J, Grgic, Z & Besic, J (1993) 'Selenium Levels in Some Species of Honey in Eastern Croatia`. Deutsche Lebensmittel-Rundschau 89.

Petrovic, Z T; Mandic, ML, Grgic, J & Grgic, Z (1994) 'Ash and Chromium Levels of Some Types of Honey`. Zeitschrift für Lebensmittel Untersuchung und-Forschung 198 (1): 36-39.

Pfeifer, H-R; Derron, M-H; Rey, D; Schlegel, C; Atteia, O; Piazza, DR; Dubois J-P & Mandia, Y (2000) Natural Trace Element Input to The Soil –Sediment-Water-Plant System: Examples of Background and Contaminated Situations in Switzerland, Eastern France and Northern Italy. In Trace Elements Their Distribution and Effects in the Environment:Trace Metals in the Environment (Vol.4, Markert, B & Friese, K, eds). Elsevier Science BV 33-86 pp.

Philp, RB (1995) Environmental Hazards & Human Health. CRC Press Inc.Lewis Publishers, Boca Raton, FL.

Phuapradit, W & Saropala, N (1992) 'Topical Application of Honey in Treatment of Abdominal Wound Disruption`. Australian and New Zealand Journal of Obstetrics and Gynaecology 32 (4): 381-4.

Pinset in Warren, M.V.,1982 Geoscience Canada 9: 58. Cited in Jones(1987).

Pintèr, A (ed) (1997) Hungarian Environmental Health Action Programme. Prospektus Nyomda, Veszprèm.

Pinzauti, M; Frediani, D; Biondi, C; Belli, R; Panizzi, L; Cosimi, C & Zummo, V (1991) 'Impiego delle api nel Rilevamento dell' Inquinamento Ambientale`. Analysis 8: 354-407.

Plachy, E (1944) Studie Über die Bakterizide Wirkung des Naturhonigs (Bluten-und Blatthonig) aus Verschiedenen Höhenlagen Sowie Einige Untersuchungen über die Eigenschaft der Antibakteriellen Hemmungstoffe(Inhibine) im Naturhonig. Zentralblatt für Bakteriologie 100:401-419.

Plockinger, B; Dadak, C; Meisinger, V (1993) ´ Lead, Mercury and Cadmium in Newborn Infants and Their Mothers`. Z.Geburtshife Perinatol.197: 104-107.

Pokorny, B & Ribari-Lasnik, C (2002) ´Seasonal Variability of Mercury and Heavy Metals in Roe deer (*Capreolus capreolus*) Kidney`. Environmental Pollution 117 (1): 35-46.

Popeskovic, D;Daki, M; Bunci, S; Ruzic,P (1983) ´A Further Investigation on The Antimicrobial Properties of Honey`. Proc.29th int. Congr. Apiculture, 415-417. Budapest.

Postmes, T J; Bosch, MMC; Dutrieux, R; van Baare, J & Hoekstra, MJ (1996) Speeding up The Healing of Burns With Honey (An Experimental Study With Histological Assessment of Wound Biopsies).Bee Products: Plenum Press, New York; 57-63 pp.

Postmes, T (2002) ´After 5000 years an Old Remedy Becomes a Registered Prescription`. Proc.The First German Congress For Bee Products and Apitherypy,Pasau, Germany; 60p.

Pothmann, F-J (1950) ´Der Einfluß von Naturhonig auf das Wachstum der Tb.-Bakterien`. Zeitschrift für Hygiene und Infektionskrankheiten 130: 468-484.

Prasad, A (1993) (ed) Progress in Clinical and Biological Research. Wiley-Liss, NewYork.

Prica, M (1938) ´Über die Bactericide Wirkung des Naturhonigs`. Zeitschrift für Hygiene und Infektionskrankheiten 120: 437-443.

Price, SB (1991) Isolation of Antibacterial Components from Manuka Honey. MSc Thesis. Hamilton, NewZealand. University of Waikato.

Prytzyka, E; Andreia, P; Dantas, K S; Pereira, A S; Bankova, V S; De Castro, S L & Netoa, F R A (2003) ´Flavonoids and Trypanocidal Activity of Bulgarian Propolis`. Journal of Ethnopharmacology. 88 (2-3): 189-193.

Przybylowski, P & Wilczynska, A (2001)´Honey as an Environmental Marker`.Food Chemistry 74 (3): 289-291.

Puckett, KJ & Burton, MAS (1981) The Effect of Trace Elements on Lower Plants. In Effect of Heavy Metal Pollution on Plants (Lepp, NW, ed). Applied Science, London, UK; 213-238 pp.

Puckett, KJ (1988) Bryophytes and Lichens as Monitors of Metal Deposition. In Lichens, Bryophytes and Air Quality (Nash, TH & Wirth, V, eds). Bibliotheca Lichenologica 30. Cramer, Berlin; 231-267 pp.

Qiang, X; Zhu, R & Fres, Z (1986) Anal. Chem., 325534-538.

Radwan, SSEl-E AA & Sarhan, MM (1984) 'Experimental Evidence for The Occurence in Honey of Specific Substances Active Against Micro-Organisms`. Zentralblatt Mikrobiologie 139: 249-255.

Raes, H; Cornelis, R & Rzeznik, U (1992) 'Distribution, Accumulation and Depuration of Administered Lead in Adult Honeybees`. Science of Total Environment 113:269-279.

Reichrtovà, E; Ursìnyovà, M; Palkovicovà, L & Wsolovà, L (1998a) 'Contents and Localization of Heavy Metals in Human Placentae`. Fresenius J. Anal.Chem.361: 362-364.

Reichrtovà, E; Ursìnyovà, M; Palkovicovà, L & Wsolovà, L (1998b) 'Sites of Lead and Nickel Accumulation in The Placental Tissue`.Human Environm. Toxicol.17: 176-181.

Reichrtová1, E; Gajdoová1, J; Ursínyová1, M; Hladíková1,V; Prachar1, V; Weningerová1, M & Holíková, J (1995) 'Human Plaenta Quality in Ecologically Different Regions I. Inorganic and Organic Pollutants in Placentae`. Toxicology Letters 78 (1): 69.

Reinhold, JO; Hendriks, AJ; Slager, LK & Ohm, M (1999) 'Transfer of Microcontaminants From Sediment to Chironomids, and The Risk For The Pond Bat *Myotis dasycneme* (Chiroptera) Preying on Them`. Aquatic Ecology 33 (4): 363-376 (14).

Revathy,V & Banerji, SA(1980) 'A Preliminary Study of Antibacterial Properties of Indian Honey`. Indian Journal of Biochemistry and Biophysics 17 (supplement no.242): 62.

Rhodes, HA; Wilson,WT; Sonnett, PE; Stoner, A (1980) 'Honey Bees Die Fom Pollen Contaminated Fourteen Months Earlier With Penncap-M`. Am.Bee J.120 (8): 577-580,589.

Richardson, DHS (1988) 'Understanding The Pollution Sensitivity of Lichens`. Bot.J. Linnean Soc. 96: 31-43.

Richardson, DHS (1992) Pollution Monitoring With Lichens. Richmond, Slough, UK.

Richens, DWH (1996) Macrophage Involvement in Wound repair, Remodelling and Fibrosis. In The Molecular and Cellular Biology of Wound Repair (2nd ed., Clarke, RAF). Plenum Press, New York and London; 95-141 pp.

Rifai, J & Akeel, N (1997) 'Determination of Pesticide Residues in Imported and Locally Produced Honey in Jordan`. Journal of Apicultural Research 36(3/4):155-161.

Rizvanov, K & Bizhev, B (1962) 'Investigation of the Antibacterial and Antifungal Properties of Honey`. Nauchni Trudove 11: 433-443.

Rodriguez-Otero, J L; Paseiro, P; Simal, J & Cepaeda, A (1994) 'Mineral Content of The Honeys Produced in Galicia (North-West Spain)`. Food Chemistry 49:169-171.

Rodriguez-Otero´, J L; Paseiro, P; Simal, J; Terradillos, L & Cepaeda, A (1992) ´Determination of Na, K, Ca, Mg, Cu, Fe, Mn and Total Cationic Milliequivalents in Spanish Commercial Honeys`. Journal of Apicultural Research 31: 65-69.

Rodriguez-Otero, JL; Paseiro, P; Simal, J; Terradillos, L & Cepeda, A (1995) ´Silicon, Phosphorus, Sulphur, Chlorine and Ash Contents of Spanish Commercial Honeys`. Zeitschrift für Lebensmittel Untersuchung und-Forschung 200: 233-234.

Roels, HA; Lauwerys, RR; Buchet, JP; Bernard, A; Chettle, DR; Haevey, TC & Al-Haddad, IK (1981) Environ. Res. 26: 217-240. Cited in Gerhardsson and Skerfvig(1996).

Roels, HA; Lauwerys, R; Genet, P; Sarhan, MJ; de Fays, M; Hanotiau, I & Buchet, J-P (1987) Am.J.Ind.Med. 11: 297-305. Cited in Gerhardsson and Skerfvig (1996).

Rossi, G (1992) In Applications of Zeeman Graphite Furnace Atomic Absorption Spectrometry in the Chemical Laboratory and in Toxicology(Minola, C & Carroll, S, eds). Pergamon Press, Oxford; 3 p.

Roth, LA; Kwan, S & Sporns, P (1986) ´Use of a Disc-assay System to Detect Oxytetracycline Residues in Honey`. Journal of Food Protection 49 (6): 436-441.

Roychowdhury, T; Uchino, T; Tokunaga, H & Ando, M (2002) ´Arsenic and Other Heavy Metals in Soils From Anarsenic-Affected Area of West Bengal, India`. Chemosphere 49 (6): 605-618.

Roychowdhury, T; Uchino, T; Tokunaga, H & Ando, M (2003) ´Survey of Arsenic and other Heavy metals in Food Composites and Drinking Water and Estimation of Dietary Intake by The Villagers From Anarsenic-Affected Area of West Bengal, India`.The Science of The Total Environment 308 (1-3): 15-35.

Rudnai, P & Horvàth, A (1996) Biological Monitoring of Environmental Lead Exposure in Hungary. From Science to Action: In The Lead Hazard in Hungary- a Fact Report(Füzesi, Z; Levy, BS & Levenstein, CH, eds). Ferling Press, Pècs; 232-238 pp.

Russel, KM (1983) The Antibacterial Properties of Honey.M Sc thesis; University of Waikato; New Zealand; 147 p.

Russell, KM; Molan, PC; Wilkins, AL; Holland, PT(1990) ´Identification of Some Antibacterial Constituents of New Zealand Manuka Honey`. Journal of Agricultural and Food Chemistry 38: 10-13.

Russell, WE (1961) Soil Conditios and Plant Growth. Longmans, Green & Co.Ltd., London, UK.

Russo, A; Longo, R & Vanella, A (2002) 'Antioxidant Activity of Propolis: Role of Caffeic Acid Phenethyl Ester and Galangin`. Fitoterapia 73(Supplement 1): 21-29.

Ruttner, F (1985) Characterization and Variability of *Apis cerana* F. Proc.XXXth Inter.Apicultural Congr.Nagoya, Japan; 130-133 pp.

Ruttner, F (1986) Geographical Variability and Classification. In Bee Genetics and Breeding (Rinderer, TE ,ed). Academic Press, Orlando, FA, U.S.A.

Ruttner, F (1987) Biogeographic and Taxonomy of Honeybee. Berlin; Springer Verlag.

Ruttner, F (1988) Biogeography and Taxonomy of Honeybees. Springer-Verlag, Berlin.

Rychlik, M & Dolezal, M (1961) 'Wlasciwosci Inhibinowe Niektòrych Miodòw Polskichò`. Pszczelnicze Zeszyty Naukowe 5 (2): 53-64.

Rydzewska, A & Krol, I (1996) 'Contents of Zinc, Copper and Cadmium in Milk of Women Living in Poznan`. Ginekol.Pol. 67: 125-128 (in Polish).

Sabatier, S; Amiot, MJ; Tachini, M & Aubert, S (1992) 'Identificationof Flavonoids in Sunflower Honey`. J. Food Sci. 57: 773-774.

Sackett, WG (1919) Honeys as a Carrier of Intestinal Diseases. Bulletin of the Colorado State University Agricultural Experimental Station No.252 ; 18p.

Sadiq, M; Alam, I; El-Mubarek, A & Al-Mohdar,HM (1989) 'Preliminary Evaluation of Metal Pollution From Wear of Auto Tires`. Bull.Environ.Contam.Toxicol.42: 743-748.

Sakagami, SF; Matsumura, T & Ito, K (1980) *Apis laboriosa* Smith in Nepal. M.Sc. Thesis, Cornell University, Ithaca, Newyork.

Sanchez, D & Pujolà, M (1996) 'Mieles Andaluzas, Extremenas y Gallegas: Composiciòn Mineral`. Vida Apicola 78: 56-60.

Sancho, MT; Muniategui, S; Sanchez, P; Huidobro, J F & Simal-Lozano, J (1992) 'Study of Ash in Honey`. Bee Science 2 (3): 147-153.

Sangiorgi, E & Ferretti, E (1996) 'Controlli del Laboratorio Chimico per La Tutela del Consumatore: Analisi Effetuate e Risultati Ottenti Sul Miele Nel 1993`. Selezione Veterinaria 11: 755-761.

Santos, F A; Bastos, E M A F; Uzeda, M; Carvalho, M A R; Farias, L M & Moreira, E S A (1999) 'Antibacterial Activity of Propolis Produced in Brazil Against Actinobacillus *Actinomycetemcomitans*, *Fusobacterium* spp. and Bacteria From the *Bacteroides fragilis* group Isolated From Human and Marmoset Hosts`. Anaerobe 5 (3): 479-481.

Santos, F A; Bastos, E M A; Uzeda, M; Carvalho, MA R; Farias, L M; Moreira, E S A & Braga, F C (2002) 'Antibacterial Activity of Brazilian Propolis and Fractions Against Oral Anaerobic Bacteria`. Journal of Ethnopharmacology 80 (1):1-7.

Sapunar, J & Jusio, M (1986) 'Copper, Iron and Zinc Levels in Honey`. Apicultural Abstracts (1991) 42 (2): 172.

Sataruga, S; Baker, JR; Urbenjapola, S; Haswell-Elkins, M; Paul, EB; Reilly, PEB; Williams,DJ & Moore, MR (2003) 'A Global Perspective on Cadmium Pollution and Toxicity in Non-Occupationally Exposed Population`.Toxicology Letters 137 (1-2): 65-83.

Saubolle, BR & Bachmann, A (1979) Beekeeping: An Introduction to Modern Beekeeping in Nepal. Sahayogi Prakashan; Kathmandu, Nepal..

Say, PJ; Harding, JPC & Whittonm, BA (1981) 'Aquatic Mosses as Moniors of The Heavy Metals Contamination of the River Etherow, Great Britain`. Environ. Pollut.2: 295-307.

Schade, JE; Marsh,GL& Eckert, JE (1958) 'Diastase Activity and Hydroxy-Methylfurfural in Honey and Their Usefulness in Detecting Heat Alteration`. Food Research 23: 446-463.

Scheffer, F & Schachtschabel, P (1992) Lehrbuch der Bodenkunde. 13 Auflage, Enke-Verlag, Stuttgart; 491 p.

Schepartz, AI & Subers, MH (1964) 'The Glucose-Oxidase of Honey.1.Purification and Some General Properties of The Enzyme`. Biochimica et Biophysica Acta 85: 228-237.

Schepartz, AI & Subers, MH(1966) 'Catalase in Honey`. Journal of Apicultural Research 5 (1): 37-43.

Schepartz, AI(1966) 'Honey Catalase :Occurence and Some Kinetic Properties`. Journal of Apicultural Research 5 (3): 167-176.

Schlenger, K; Hockel, M; Schwab, R; Frischmann-Berger, R & Vaupel, P (1994) 'How to Improve the Uterotomy Healing. Effects of Fibrin and Tumour Necrosis Factor-Alpha in The Rat Uterotomy Model`. J Surg. Res. 56: 235-241.

Schuler, R & Vogel, R (1956) 'Wirkstoffe des Bienenhonigs`. Arzneimittel-Forsch.6:661-668.

Sedova, NN & Usmanov, MF (1973) 'Antimicrobial Properties of Some Types of Honey From Uzbekistan`.Voprosy Pitaniya 32 (2): 84-85 (Original in Russian).

Sekhar, KC; Supriya, KR; Kamala, CT; Chary, NS; Rao, TN & Anjaneyulu, Y (2002) 'Speciation, Accumulation of Heavy Metals in Vegetation Grown on Sludge Amended Soils and

their Transfer to Human Food Chain-A Case Study`. Toxicological and Environmental Chemistry 82 (1): 33- 43 (11).

Sela, M; Maroz, D & Gedalia, I (2000) *'Streptococcus mutans* in Saliva of Normal Subjects and Neck and Head Irradiated Cancer Subjects After Consumption of Honey`. Journal of Oral Rehabilitation 27 (3): 269-270 (2).

Sevimli, H ; Bayülgen, N & Varinlioglu, A (1992) 'Determination of Trace Elements in Honey by INAA (instrumental neutron activation analysis) in Turkey`. Journal of Radioanalytical and Neuclear Chemistry 165 (5): 319-325 (in Turkish).

Seymour, FI & West, KS(1951) 'Honey- Its Role in Medicine`. Medical Times 79:104-107.

Sforcin, J M; Fernandes, A. Jr.; Lopesa , C A M; Bankova, V & Funari, S R C (2000) 'Seasonal Effect on Brazilian Propolis Antibacterial Activity`. Journal of Ethnopharmacology 73 (1-2): 243-249.

Shahi, KB & Chaulagain, MR (1999) Monitoring of Four Trace metal Levels in The Rain water of Kathmandu valley. Nepal Journal of Science and Technology 1: 123-126.

Shamala, TR; Shri Jyoti, YR & Saibaba, P (2002) Antibacterial Effect of Honey on The in vitro and in vivo Growth of *Escherichia coli*`. World Journal of Microbiology and Biotechnlogy18 (9): 863.

Shrestha, M (1998) An overview of Physical and Chemical Properties of Nepalese Honey. Paper Presented in 4th AAA conference, Kathmandu, Nepal,

Shrestha, J.(1995) an Enumeration of Fishes of Nepal. Kathmandu:DNPWC/BPP, Nepal.

Shrestha, M (2000) Physical and Chemical properties of Nepalese honey. In Asian Bees and Beekeeping: Progress of Research and Development. Oxford and IBH, New Delhi;137-139 pp.

Shrestha, NC & Shrestha, KK (1998) 'Thai Sac Brood Virus Disease of *Apis cerana* Bees in Three Locations of Nepal`. Programme and Abstracts.Fourth Asian Apicultural Association Conference, Kathmandu, Nepal.

Shrestha, TB & Joshi, RM (1996) Rare, Endemic and Endangered plants of Nepal. WWF Nepal Program, Nepal; 235 p.

Shukla, UC; Singh, J; Joshi, P.C & Kakkar, P (2003) 'Effect of Bioaccumulation of Cadmium on Biomass Productivity, Essential Trace Elements, Chlorophyll Biosynthesis, and Macromolecules of Wheat Seedlings`. Biological Trace Element Research 92 (3): 257-274 (18).

Sidhu, AS & Singh, S (1961) 'Studies on Agents of Cross Pollination in Cotton`. Indian Cotton Grow.Rev.15: 341-353.

Siebert, A; Bruns, I; Krauss, G-J ;Miersch, J & Markert, B (1996) 'The Use of *Fontinalis antipyretica* L.ex Hedw. As a Bioindicator For Heavy Metals.1.Fundamental Investigations into Heavy Metal Accumulation in *Fontinalis antipyretica* L. ex Hedw`. Sci. Total Environ. 177: 137-144.

Sigmundovà, V; Skripenovà, A; Borosovà, B & Benasovà, A (1997) 'Content of Toxic Metals in Biological Materials in Inhabitants From Selected Regions of the Slovak Republic`.In Proceeding of Conference Health and the Environment.Milovy,Czech Republic; 242-247 pp (in Slovak).

Simuth, J (1991) Poskodenie DNA Latkami Rozpustenymi vo vode z Propolisu a Flavonoidu Kvercetinu v Pritomnosti Cu^{2+}. Bulletin PV (Bratislava) Roc. 30 (10): 335-341.

Simuth, J; Albert, S; Klaudiny, J; Visvaderova, J & Hanes, J (1995) Alternative Molecular Biological Models for Evaluation of Hive Products.The XXXIV International Apicultural Congress (APIMONDIA), Lausanne, Switzerland.

Simuth, J; Trnovsky, J & Jelokova, J (1986) Inhibition of Bacterial DNA-Depandent RNA Polymerase and Restriction Endonuclease by UV-Absorbing Components from Propolis.Pharmazie 41: 131-132.

Singh, S (1962) Beekeeping and Entomology in India. New Delhi. Indian Council Agric.Res.

Sivakoti, GP & Bista , S (1998) Major Constraints in The Performance of *Apis mellifera and A. cerana* Under Khumaltar Conditions. Asian Bees and Beekeeping, Progress of Research and Development. Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, India.

Skalsky, HLl & Charchman, RA (1983) J.Am. Coll. Toxicol.,2, 405-423. Cited in Gerhardsson and Skerfvig (1996).

Skerfving, S & Arb, O H (1993) 125-138. Cited in Gerhardsson and Skerfvig (1996).

Skerfving, S (1988) In Biological Monitoring of Toxic Metals (Clarkson, TW; Friberg, L; Nordberg, GF & Sager, PR, eds). Plenum Press, New York; 169.

Skerfving, S; Nilsson, U; Schütz, A & Gerhardsson, L (1993). Scand. J.Work Environ. Health, 19 (Suppl.1): 59-64. Cited in Gerhardsson and Skerfvig (1996).

Skrypnik EI & Khorol'skii, LN (1974) 'Persistence of Tuberculosis in Honeys`. Pchelovodstvo 94(5): 41(Original in Russian).

Slickers, K (1993) Automatic Atomic-Emission-Spectroscopy (2^{nd} ed.). Brühlsche Universitätsdruckerei, Giessen; 113p in http://icp-oes.com, assessed on 3 April, 2004.

Sloof, JE (1993) Environmental Lichenology Biomonitoring Trace Element Air Pollution. PhD. Thesis, Delft University of Technology, The Netherlands.

Smith, MR; McCaughey, WF & Kemmerer, AR (1969) 'Biological Effects of Honey`. Journal of Apicultutral Research 8 (2): 99-110.

Sommerfield, SD (1991) 'Honey and Healing`. Journal of the Royal Society of Medicine 84 (3): 179.

Sonnet, P (1978) 'Controlled-Released Pesticides`. Bee World 59 (1):112-114.

Sovcìkovà, E; Wsòlovà, L; Ursìnyovà, M; Hladìkova, V & Cvikovà, V (1998a) 'Rural-Urban Neuropsychic and Blood Lead Differences in Slovak Children`. Epidemiology 9 (Suppl.): 139.

Sovcìkovà, E; Wsòlovà, L & Ursìnyovà, M (1998b) Cognitive Changes in Lead Exposed Children. In Abstract book of the 12 th Conference of the European Health Psychology Society, Prevention and Intervention. Vienna, Austria; 89 p.

Sovcìkovà, E; Ursìnyovà, M; Wsòlovà, L; Rao, V & Lustik, M (1997) 'Effects on The Mental and Motor Abilities of Children Exposed to Low Levels of Lead in Bratislava`. Toxic Substance Mech.16: 221-236.

Stebbins, AI; Horstman, SW; Daniell, WE & Atallah, R (1992) Am.Ind.Hyg. Assoc.J.,53, 186-192, (cited in Gerhardsson and Skerfvig, 1996).

Stefanov, K; Seizova, K; Yanishlieva, N; Marinova, E & Popov, S (1995) Accumulation of Lead, Zinc and Cadmium in Plantseeds Growing in Metalliferous Habitats in Bulgaria .

Stoeppler, M (1991) Analytical Chemistry of Metals and Metal Compounds, In Metals and Their Compounds in the Environment (Merian, E, ed). VCH Publishers, Weinheim;106-206 pp.

Stomfay-Stitz, J, Kominos, SD (1960) 'Ueber Bakteriostatische Wirkung des Honigs`. Zeitschrift für Lebensmittelundtersuchung und Forschung 113: 304-309.

Storelli, M M & Marcotrigiano, G O (1999) 'Cadmium and Total Mercury in Some Cephalopods From the South Adriatic Sea (Italy)`. Food Additives and Contaminants, 16 (6): 261-265 (5).

Strickland, SS (1982) 'Honey Hunting by The Gurungs of Nepal`. Bee World 63:153-161.

Subrahmanyam, M (1994) 'Honey Impregnated Gauze Versus Amniotic Membrane in The Treatment of Burns`. Burns 20 (4): 331-333.

Subrahmanyam, M (1998) ' A Prospective Randomised Clinical and Histological Study of Superficial Burn Wound Healing with Honey and Silver Sulfadiazine`. Burns 24 (2):157-161.

Subrahmanyam, M (1999) 'Early Tangential Excision and Skin Grafting of Moderate Burns is Superior to Honey Dressing: A Prospective Randomised Trial` Burns 25 (8): 729-731

Subrahmanyam, M & Hemmady, A & Pawar, S G (2001a) 'Antibacterial Activity of Honey on Bacteria Isolated from Wounds`. Annals of Burns and Fire Disasters 14 (1): 22-24.

Subrahmanyam, M; Hemmady, A & Pawar, S G (2001b) 'Antibacterial Activity of Honey on Bacteria Isolated from Wounds`. Acta Chirurgiae Plasticae 43 (3): 22-24

Sunderman, FW Jr. (1988) In Biological Monitoring of Toxic Metals (Eds. Clarkson, TW; Friberg, L; Nordberg, GF & Sager, PR). Rochester Ser. Environ. Toxicity, Plenum Press, New York; 265 p.

Suryanarayana, MC (2002) Bees of India.http://www.mandasofpune5.homestead.com/BeesIndia.html (Assessed on 28 Dec, 2003).

Suzuki, K T & Suzuki, T (1996) An Introduction to Clinical Aspects of Metal Toxicology In Toxicology of Metals (Chang, LW; Magos, L & Tsuguyoshi, S, eds). Lewis Publishers, CRC Press, Boca Raton, FL; 333-335p.

Swan, L & Leviton, AE (1962) Herpetology of Nepal. Proc. Cal. Acad: 103-142.

Tamrakar, AS & Gautam I (1999) 'Effectiveness of Some Plant Products Against The Pulse Beetle *Callosobruchus maculatus* (Fab.)`. Nepal Journal of Science and Technology 1: 99-102.

Taormina, PJ; Niemira, BA & Beuchat, LR (2001) 'Inhibitory Activity of Honey Against Food Borne Pathogens as Influenced by The Presence of Hydrogen peroxide and Level of Antioxidant Power`. International Journal of Food Microbiology 69 (3): 217-225.

Tatsuno, T; Shirotori, T; Iwaida, M & Kawashiro, I (1968) 'Determination of Harmful Metals in Foods: Lead and Copper Contents in Honey`. Journal of Hygiene Chemistry Society, Japan 14: 327-329.

Temara, A; Skeic, M; Gillana,D; Warnaua, M; Jangouxa, M & Duboisa, P (1998) 'Validation of The Asteroid *Asterias rubens* (Echinodermata) as a Bioindicator of Spatial and Temporal Trends of Pb, Cd, and Zn Contamination in The Field`. Marine Environmental Research 45 (4-5): 341-356.

Temiz, I & Sengonca, M (1981) Investigation of Honeys Produced in Izmir and ist Surroundings in Turkey. Ege University Faculty of Agriculture, Izmir, Turkey.Report no.405 (in Turkish).

Terrab, A; gonzalez, AG; Diez, MJ; Heredia, FJ (2003)'Mineral Content and Electriacal Conductivity of the Honeys Produced in Northwest Morocco Thier Contribution to

Characterization of Unifloral Honeys`. Journal of The Science of Food and Agriculture 83 (7): 637-643 (7).

Terzic, LJ; Terzic, V; Krunic, M & Brajkovic, M (1984) Acta Veterinaria 34,57. Cited in Jones, 1987.

Thapa, R (2002) ´Apitherapy In Rural Areas of Nepal`. In The Proceedings of The First German Congress for Bee products and Apitherapy, 52.Passau, Germany.

Thapa, R (2001) ´The Himalyan Giant Honeybee and Ist Role in Ecotourism Development in Nepal`. Bee World 82 (3): 139-141.

Thapa, R; Siriwati, S; Manandhar, D; Sivakoti, GP & Bista, S (2000) ´Current Status of Predators and Disease of Honeybees in Nepal`. In Programme and Abstracts.Seventh IBRA Conference on Tropical Bees:Management and Diversity.

Tiran, B; Karpf, E &Tiran, A (1995) ´Age Dependency of Selenium and Cadmium Content in Human Liver, Kidney, and Thyroid`. Arch.Environ. health 50: 242-246.

Toelg, G (1987) ´Extreme Trace Analysis of The Elements- The State of The Art Today and Tomorrow`. Analyst 112: 365-376.

Tomás-Barberán, FA; Martos, I; Ferreres, F; Radovic, BS & Anklam, E (2001) HPLC Flavonoid Profiles as Markers for The Botanical Origin of European Unifloral Honeys. Journal of the Science of Food and Agriculture 81(5): 485-496 (12).

Tomlinson, JT & Williams, SC (1985) ´Antibiotic Properties of Honey Produced by The Domestic Honeybee *Apis mellifera (*Hymenoptera: Apidae)`. Pan-Pacific Entomologist 61(4): 346-347.

Tong, SSC; Morse, RA; Bache, C A & Lisk, D J (1975) ´Elemental Analysis of Honey as an Indicator of Pollution`. Archives Environmental Health 30: 329-332.

Tonks, AJ; Cooper, RA; Jones, KP; Blair, S; Parton, J & Tonks A (2003) ´Honey Stimulates Inflammatory Cytokine Production From Monocytes`. Cytokine 21: 242-247.

Tonks, A; Cooper, RA; Price, AJ; Molan, PC & Jones, KP (2001) ´Stimulation of TNF-alpha Release in Monocytes by Honey (Short communication)`. Cytokine 14 (4): 240-242.

Toth, G; Lemberkovics, È & Kutasi-Szabò, J (1987) ´The Volatile Components of Some Hungarian Honeys and Their Antimicrobial Effects`. American Bee Journal 127 (7) : 496-497.

Tovey, FI (1991´)Honey and Healing`. Journal of the Royal Society of Medicine 84 (7):447.

Tripathi, R M; Raghunath, R & Krishnamoorthy, T M (1997) Dietary Intake of Heavy Metals in Bombay city, India`. The Science of The Total Environment 208 (3):149-159.

Tripathi, R M, Raghunath, R, Sastry, VN & Krishnamoorthy, T M (1999) Daily Intake of Heavy Metals by Infants through Milkand Milk Products`. The Science of The Total Environment 227(2-3): 229-235.

Tsalev, D (1994) ´Electrothermal Atomic Absorption Spectrometry in Occupational and Environmental Health Practice-A Decade of Progress and Establishment`. J. Anal. Atom. Spectrom. 9:405-414.

Tsalev, D (1995) Atomic Absorption Spectrometry in occupational and Environmental Health Practice, III, Progress in Analytical Methodology . CRC Press, Boca Raton, FL; 349 p.

Tsalev, D (1998) Electrothermal Atomic Absorption Spectrometry. Ed.), In Encyclopedia of Environmental Analysis and Remediation (Vol.3). New York; 1583-1605pp.

Turner, FJ (1983) Hydrogen peroxide and other Oxidant Disinfectants.In Disinfection, Sterilization and Preservation (3rd ed, Block, SS, ed).Lea and Febiger, Philadelphia,USA; 240-250 pp.

Tyler, G; Horiba, J-Y; France & Payling, R Surface Analytical, Australia -in http://icp-oes.com, (Assessed on 3 April, 2004).

Tyler, G (1989) ´Uptake, Retention and Toxicity of Heavy Metals in Lichens`. Water Air Soil Pollut. 47: 321-333.

Tysset, C; Rousseau, M & Durand, C (1980) ´Microbism and Wholesomeness of Commercial Honey`. Apiacta 15 (2): 51-60.

U.S.EPA (1986) Air Quality Criteria For Lead. EPA-600/8-83/028Af (Vol I-IV). Environmental Protection Agency, Environmental Criteria and Assessment Office, Research Triangle Park, NC.

Underwood, BA (1992) Impact of Human Activities on The Himalayan Honeybee, *Apis laboriosa*. In Honeybees in Mountain Agriculture(Verma, LR, ed). Oxford and IBH Publishing Co.Pvt.Ltd, New Delhi; 91-112 pp.

UNEP/FAO/WHO (1992) Assessment of Dietary Intake of Chemical Contaminants,WHO/HPP/FOS/92.6.

Üren, A; Serifoglu, A & Sarikahya, Y (1998) ´Distribution of Elements in Honeys and Effect of a Thermoelectric Power Plant on The Element Contents`. Food Chemistry 61:185-190.

Ursìnyovà, M & Hladìkova, V (1997) ´The Intake of Selected Toxic Elements From Milk in Infants`. Fresenius Environ.Bull. 6: 627-632.

Ursìnyovà, M;Hladìkova, V & Sokolovic, P (1997) Intake of Toxic Elements From Vegetarian and Non-vegetarian Diet'. In Proceeding of Conference Foreign Substances in Food. Tatranskà Strba, Slovak Republic; 145-147 pp (in Slovak).

Ustyak, S & Petrikova, V(1996) 'Heavy Metal Pollution of Soils and Crops in Northern Bohemia` Applied Geochemistry 11(1): 77-80(4).

Valli, E & Summers, D (1988a) 'Honey Hunters of Nepal`. National Geographic 174: 660-671.

Valli, E & Summers, D (1988b) Honey Hunters of Nepal. Harry N Abrams, INC. Publishers, Newyork.

Van De Casteele, B; Bruno, V & Filip, M & Tack, G (2002) 'Cadmium and Zinc Uptake by Volunteer Willow Species and Elder Rooting in Polluted Dredged Sediment Disposal Sites`. The Science of The Total Environment 299 (1-3): 191-205.

van Netten, C; Hoption Cann, SA; Morley, DR; van Netten, JP (2000) 'Elemental and Radioactive Analysis of Commercially Available Seaweed`. The Science of the Total Environment 255 (1): 169-175(7).

Vardi, A; Barzilay, Z; Linder, N; Cohen, HA; Paret G & Barzilai, A (1998) 'Local Application of Honey for Treatment of Neonatal Postoperative Wound Infection`. Acta Paediatrica 87 (4): 429-432(4).

Vartsky, D; Ellis, KJ;Chen, NS & Cohn, SH & Cohn, SH (1977) Phys. Med. Biol. ,22, 1085-1096, (cited in Gerhardsson and Skerfvig, 1996).

Veeken, A & Hamelers, B (2002) 'Sources of Cd, Cu, Pb and Zn in Biowaste`. The Science of The Total Environment 300 (1-3): 87-98.

Velikova, M; Bankova, V; Tsvetkova, I; Kujumgiev, A & Marcucci, MC (2000) 'Antibacterial Ent-kaurene From Brazilian Propolis of Native Stingless Bees`. Fitoterapia 71(6): 693-696 (4).

Vergè, J (1951) 'L`activè Antibactèrienne de la Propolis du Miel et de la Gelèe Royale`. Apiculteur 95(6: Section scientifique):13-20.

Verma, LR (1989) 'Some Practical Aspects of Beekeeping With *Apis cerana* in Asia`. Proc. Int. Conf. Apic. Trop. Climates, 1988, Cairo, Egypt 4 : 438 - 441.

Verma, LR & Partap, U (1993) The Asian Hivebee, *Apis cerana*, as a Pollinator in Vegetable Seed Production.Kathmandu, ICIMOD.

Verma, LR (1990) Beekeeping in Integrated Mountain Development: Econonic and Scientific Perspectives.ICIMOD Senior Fellowship Series, No 4.Oxford & IBH Publishing Co. PVT.LTD., New Delhi, India; 139-168 pp.

Verma, LR (1994) Honeybees in Mountain Agriculture,Oxford & IBH Publ. Co. Pvt. Ltd.,New Delhi, India.

Vinas, P; Lòpez-Garcìa, L M; Hernàndez-Còrdoba, M (1997) 'Direct Determination of Lead, Cadmium, Zinc and Copprt in Honey by Eelectrothermal Atomic Absorption Spectrometry Using Hydrogen peroxide as a Matrix Modifier`. Journal of Agricultural and Food Chemistry 45 (10): 3952-3956.

Von der Ohe,W (1994) 'Chemical Conversion and Pollen Reduction`. Grana 33: 292-294.

Von der Ohe,W; Dustmann, JH & Von der Ohe, K (1991) 'Proline als Kriterium der Reif des Honigs`. Deutches Libensmittel-Rundschau 87: 383-386.

Von der Ohe,W & Von der Ohe, K (1996) 'Characterizing Honeydew Honeys Guided by Specific Sugars`. Apidologie 27: 270-272.

Voigtländer, H(1936) 'Umschau und Ausschau aus Anderen Bienenzeifungen`. Rheinische Bienenzeitung 88:305-308.

Vtorova, VN & Markert, B (1995) 'Multi-element Aanalysis of Plants of The Forest Ecosystems in Eastern Europe`. Biol.Bull. 4: 447-454.

Wadi, M; Al-Amin, H; Farouq, A; Ksshef, H & Khaled, SA (1987) 'Sudanese Bee Honey in The Treatment of Suppurated Wounds`. Arab Medico 3:16-18.

Wakhle, DM & Desai, DB (1991) 'Estimation of Antibacterial Activities of Some Indian Honeys`. Indian Bee J ournal 53 (1/4): 80-90.

Wallwork-Barber, MK; Ferenbaugh, RW; Gladney, ES (1982) 'The Use of Honeybees as Monitors of Environmental Pollution`. Am Bee J 122: 770-772.

Ward , NI (1989) Multilement Contamination of British Motorway Environments. In Heavy Metals in The Environment (Vol.I,.Vernet, J-P, ed). CEP Consultants, Edinburgh; 174-176.

Ware, G.W.(1980) 'Effect of Pesticides on Non-target Organisms`. Residue Rev. 76:173-201.

Warnecke, B; Duisberg, H (1964) 'Die Erhaltung der Honig-Inhibine durch Ausschaltung des UV-Lichtes`. Z. Lebensm.Unters.Forsch.124: 265-270.

Warren, MV (1982) Geoscience Canada 9:58. Cited in Jones (1987).

Watanabe,T (1986) 'Constituents of Honey and Their Medical Efficacy as Seen From The Standpoint of Oriental Medicine`. In Proceedings of 30 th International Apicultural Congress (Apimondia), 481-486. Nagoya, Japan.

Watanabe, K; Shinmoto, H; Kobori, M; Tsushida, T; Shinohara, K; Kanada, J & Yonekura, M (1998) 'Stimulation of Cell Growth in The U-937 Human Myeloid Cell Line by Honey Royal jelly Protein`. Cytotechnology 26: 23-27.

Weheida, SM; Nagubib, HH; El-Banna, HM & Marzouk, S (1991) 'Comparing The Effects of Two Dressing Techniques on Healing of Low Grade Pressure Ulcers`. Journal of the Medical Research Institute, Alexandria University 12 (2): 259-278.

Weidenmaier, C; Kristian, SA & Peschel, A (2003) 'Bacterial Resistance to Antimicrobial Host Defenses - An Emerging Target for Novel Antiinfective Strategies`. Current Drug Targets 4 (8): 643-649 (7).

Wellford,TET; Eadie,T; Llewellyn, GC (1978) 'Evaluating The Inhibitory Action of Honey on Fungal Growth, Sporulation, and Aflatoxin Production`. Zeitschrift für Lebensmitteluntersuchung und Forschung 166 (5): 280-283.

Welz, B (1985) Atomic Absorption Spectrometry. VCH Publishers, Weinheim; 506 p.

Weston, RJ (2000a) 'The Contribution of Catalase and other Natural Products to The Antibacterial Activity of Honey: A Review`. Food Chemistry 71(2): 235-239 (5).

Weston, RJ; Brocklebank, LK & Lu, Y (2000b) 'Identification and Quantitative Levels of Antibacterial Components of Some New Zealand Honeys`. Food Chemistry 70 (4): 427-435(9).

Weston, R J; Mitchell, K R & Allen, K L (1999) 'Antibacterial Phenolic Components of New Zealand Manuka Honey`. Food Chemistry 64 (3): 295-301.

White, JW; Subers,MH & Schepartz, AL (1963) 'The Identification of Inhibine, the Antibacterial Factor in Honey, as Hydrogen peroxide and ist Origin in a Honey Glucose-Oxidase System`. Biochim.Biophys. Acta 73: 57-70.

White, JW & Subers, MH (1963) 'Studies on honey inhibine: A Chemical Assay`. Journal of Apicultural Research 3(1): 45-50.

White, JW & Subers, MH (1964a) 'Studies on Honey Inhibine: Effect of Heat`. Journal of Apicultural Research 3: 454-450.

White, JW & Subers, MH (1964b) 'Studies on Honey Inhibine: Destruction of the Peroxide Accumulation System by Light`. Journal of Food Science 29(6): 819-828.

White, JW; Subers, MH & Schepartz, A (1962) 'The Identification of Inhibine`. Am.Bee J.102: 430-431.

White, JW Jr (1978) Honey. In Advances in Food Research (Vol.24, Chichester, CO; Mrak, EM & Stewart,GF eds). Academic Press, New York, USA; 287-374 pp.

White, JW Jr (1967) 'Measuring Honey Quality-Rational Approach`. Am.Bee J.107: 374-375.

White, JW Jr (1978) 'Honey`. Food Res. 24: 287-374.

Whitworth, D; Achterberg, E ; Nimmo, M & Worsfold, P (1998) 'Validation and in situ Application of an Automated Dissolved Nickel Monitor for Estuarine Studies`. Anal. Chim. Acta 371: 235-246.

WHO (1989) Lead-Environmental Aspects. Environmental Health Criteria 85.WHO, Geneva.

WHO (1991) Nickel, IPCS Int. Prog. Chemical Safety, Environmental Health Criteria 108, World Health Organization,Geneva; 1-383.

Willix, DJ; Molan, PC & Hartfoot, CG (1992) A Comparison of The Sensitivity of Wound-infecting Species of Bacteria to The Antibacterial Activity of Manuka Honey and Other Honey`. Journal of Applied Bacteriology 73: 388-394.

Willix, DJ (1991) A Comparative Study of The Antibacterial Action Spectrum of Manuka Honey and Other Honey. M ScThesis, University of Waikato, New Zealand; 112 p.

Winterlin,W; Walker, G & Luce, A (1973) 'Carbaryl Residues in Bees, Honey, and Bee bread Following Exposure to Carabaryl via the Food Supply`. Archives of Environmental Contamination and Toxicology 1: 362-374.

Wongsiri, S; Chanchao, C; Deowanish, S; Aemprapa, S; Chaiyawong,T; Petersen, S & Leepitakrat, S (2000) 'Honey bee Diversity and Beekeeping in Thailand`. Bee World 81 (1): 20-29.

Wood, B; Rademaker, M & Molan, PC (1997) 'Manuka Honey, a Low Cost Leg Ulcer Dressing`. New Zealand Medical Journal 110:107.

Wooton, M; Edwards, RA; Rowse, A (1978) 'Antibacterial Properties of Some Australian Honeys`. Food Technology in Australia 30: 175-176.

Woyke, J (1985) '*Tropilaelaps clareae* a Serious Pest of *Apis mellifera* in Tropics, But Not Dangerous for Apiculture in Temperate Zones`. Am. Bee J. 125: 497 - 499.

Yang, KL (1944) 'The use of Honey in The Treatment of Chilblains, Non specific Ulcers, and Small Wounds`. Chinese Medical Journal 62:55-60.

Yao, L; Jiang, Y; Singanusong, R; Arcy, B D; Datta, N; Caffin, N & Raymont, K (2004) Flavonoids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus Honeys and Their Potential for Floral Authentication. Food Research International 37 (2): 166-174 (9).

Yao, L; Datta, N; Tomas-Barberan, FA; Ferreres, F; Martos, I & Singanusong, R (2003) Flavonoids, Penolic acids and Abscisic acid in Australian and New Zealand Leptospermum Honeys. Food Chemistry 81(2): 159-168 (10).

Yatsunami, K & Echigo, T (1984) 'Antibacterial Action of Honey and Royal jelly`. Honeybee Science 5:125-130.

Yeboah-Gyan; K & Marfo, EK (1998) 'The Color and Minearl Composition of Honeys Produced in Major Vegetation Areas of Ghana`. Journal of Apicultural Research.37 (2): 79-84 (6).

Yilmaz, H & Yavuz, Ö (1999) 'Content of Some Trace Metals in Honey From South Eastern Anatolia`. Food Chemistry 65: 475-476.

Zaiß(1934) 'Der Honig in Äußerlicher Anwendung`. Münchener Medizinesche Wochenschrift 49: 1891-1893.

Zanini, E; Bonifacio, E & Cuttica, GC (1992) 'Heavy Metals in Soils Near a Steel-Making Industry: A Case Study in a Complex Valley Situation in Italy`. J.Environ.Sci.Health 27: 2019-2036.

Zareba, A; Strugala-Stawik, H; Rudkowski, Z ; Dembicka, D & Pastuszek, B (1996) Blood Levels of Lead and Cadmium in Pregnant Women , Active and Passive Smokers and their Neonates. In Abstracts of International Conference Environmental Pollution and Child Health: Critical Needs and Issues for Central and Eastern Europe. Sosnowiec, Poland.

Zetterström, R (1999) 'Child Health and Environmental Pollution in The Aral Sea Region in Kazakhstan`. Acta Paediatrica 88(429): 49-54 (6).

Zhang, F-S, Yamasaki, S & Kimura, K (2002) 'Waste Ashes For Use in Agricultural Production: Contents of Minor and Trace Metals`. The Science of The Total Environment 286 (1-3):111-118.

Zielonka, E; Wodzien, M (1993) 'Blood Cadmium and Lead Levels Among Children in Cracow`. Folia. Med. Cracov 34: 85-96.

Zumla, A & Lulat, A (1989) 'Honey- a Remedy Rediscovered`. Journal of the Royal Society of Medicine 82: 384-385.

Summary

Nepali honey from different bee species and three agro-ecozones: terai, hills and mountains were tested to determine peroxide and non peroxide antibacterial activity against *Staphylococcus aureus* ATCC 6538 P and also the element spectrum. Agar (23g/L 001-7-0 DIFCO) well diffusion method was used to assess antibacterial activity. Comparisons between the effectiveness of honey from different locations showed no significant differences in both activities equivalent to that of % (w/v) phenol observed among all the locations. Honey from *Apis cerana -A. mellifera* (P=0.0009) and *A. dorsata-A.mellifera* (P=0.0010) showed significant difference in peroxide activity, whereas honey produced by two native bee species *A. dorsata* and *A. cerana* did not show significant difference in peroxide activity.

Of the 20 elements, Li, Na,Ca, Mg, Sr, Fe, P, K, Al, B, Co, Cu, Mn, Ni, V, Zn were determined by means of ICP and Cd, Pb, Cr, and Mo were determined by means of AAS for element spectrum. Significant difference was found only in total Pb content (P=0.0001) when compared between polluted and clean areas. The difference in concentration of some elements in *Apis dorsata - A. mellifera* and *Apis cerana –A.mellifera* honeys were significant.

The results showed that honey from different bee species have both peroxide and non peroxide antibacterial activities which may be of bee and botanical origin. On the other hand, honey from Nepal consists of essential and toxic elements as well. The presence of notable amount of Pb in honey from urban areas may attributed to atmospheric input. The elements determined in Nepali honeys may be ascribed to environmental influences. From the present study it would appear that honeys produced by different bee species in Nepal have an effective antibacterial activity and are rich in elements. The distribution of the elements depends on the surroundings, soil, floral source, foraging range, eventually on contamination of honey by contact with metal equipments. It may be assumed that honey can be used as alternative of environmental indicator.

Zusammenfassung

Honige von drei verschiedenen Bienenarten und drei verschiedenen Regionen Nepals- Terai, Mittelgebirge und Hochgebirge- wurden auf ihre antibiotische Wirkung gegen Staphylococcus aureus ATCC 6538P und auf das Elementspektrum untersucht. Die Agar Bohrloch Diffusions- Methode (23g/L 001-7-0 DIFCO) wurde zur Bestimmung der antibiotischen Wirkung eingesetzt.

Die Honige der verschiedenen Regionen zeigten im Vergleich zu äquivalent (w/v) phenol signifikante Unterschiede sowohl in der Peroxyd- als auch in der Nicht- Peroxyd- Aktivität. Honige von *Apis cerana*: *Apis mellifera* (P= 0,0009) und von *A. dorsata*: *A. mellifera* (P=0,001) nicht aber die Honige der beiden autochthonen Arten A. cerana und A. dorsata Unterschiden in Peroxydaktivitäa signifikant voneinander.

Die Honige wurden auf das Spektrum von 20 Elementen untersucht: Li, Na,Ca, Mg, Sr, Fe, P, K, Al, B, Co, Cu, Mn, Ni, V, Zn mit ICP und Cd, Pb, Cr, and Mo mit AAS. Im Bleigehalt ergaben sich signifikante Unterschiede zwischen umweltbelasteten und nicht belasteten Gebieten (P= 0,0001). Bei einzelnen Elementen gab es auch signifikante Unterschiede zwischen den Honigen von *Apis dorsata* und *A. mellifera* beziehungsweise zwischen *A. cerana* und *A. mellifera*.

Die Verteilung der Elemente in den untersuchten Honigen hängt von der Umwelt, dem Boden, der Tracht, dem Sammelbereich der Bienen und möglicherweise von den metallischen Geräten des Imkers ab. Generell sind die untersuchten Honige reich an auch essentiellen Elementen. Die Honige von den urbanen Regionen enthielten aber mehr Blei. Dieser beträchtliche Bleigehalt wird von den Schadstoffen in der Luft stammen. Honig kann daher als alternativer Umweltindikator angesehen werden.