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Editorial

Journal of Nepal Science Olympiad has been started to provide a platform for publishing innovative, original, and high quality research articles dealing with both theoretical and applied aspects of academic endeavor broadly classified as science and technology. Nepal Science Olympiad (NeSO) defines that science and technology is for peace, prosperity and posterity. With this publication, NeSO wants to encourage works of national and international researchers and promote the discovery, innovation, advancement and dissemination of basic and transitional knowledge in science, technology and related disciplines.

The book now in your hand is the first issue of the Journal of Nepal Science Olympiad. The journal is scheduled to be published in quarterly basis. The contents of the publication is supposed to be helpful for wider public of researchers, planners, academicians, students, and all concerned individuals. The present issue consists of five research articles and one review article covering the broad science subjects: Chemistry, Zoology and Botany. The specific fields of the issue include Phytochemistry, Pharmacology, Conservation Biology, Anatomy, Ethnobiology and Biotechnology.

The views, opinions, and assumptions expressed in the articles are solely those of the authors and do not necessarily reflect the official policy, strategy or position of NeSO. Any questions regarding the contents of the article should be directed to the author(s).

We express our sincere gratitude to all those who have contributed in this issue. We pledge to timely optimize the quality and standard of our publication. The editorial board is fully liable for any shortcomings incurred in this issue. Valuable suggestions from our esteemed readers are always awaited and for which we are committed to improve our future issues.

JOURNAL

OF

NEPAL SCIENCE OLYMPIAD

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PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON THE SELECTED MEDICINAL PLANTS OF CENTRAL REGION OF NEPAL

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Abstract: Phytochemical and biological screenings of some medicinal plants collected from central region of Nepal were performed. Phytochemical screening revealed that the selected plant samples contain alkaloids, flavonoids, terpenoids, glycosides, quinones, reducing sugars, polyphenols and saponins. Among the ten selected medicinal plants, methanolic extracts of bark of *Myrica esculenta* showed high toxicity against brine shrimp nauplii having LC_{50} value 20.89μg/mL.

Introduction

A natural product is a chemical compound or substance produced by living organisms found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design (Herbert, 1981).Sources of natural product are plants, animals and microorganisms, which can be terrestrial or marine, like plants (e.g. Taxol from *Taxus brevifolia*), animals (e.g. vitamins A and D from cod liver oil) or microorganisms (e.g. *Streptomyces peucetius*) (Wikipedia, 2015).

Natural product chemistry is the study of primary and secondary metabolites synthesized by plants (KC, 2013). Primary metabolites are the compounds which are prepared from simple inorganic molecules found in nature, and without which living beings cannot grow up. Examples of primary metabolites are sugars, amino acids, nucleosides, mevalonic acids, fatty acids, proteins, acetyl-coA etc. Primary metabolites have a broad distribution in all living beings and are intimately involved in essential life process such as growth, development, reproduction etc.

Secondary metabolites like steroids, terpenoids, flavonoids, alkaloids, quinones, polyphenols etc. are biosynthesized from primary metabolites. These secondary metabolites are produced when living beings reached in relaxed stage and they are significant to the organism that produces them (Tapan, 2011).

With change in time and development of science and technology, the traditional medicine has become the precursor for today's modern medicine and finally the boon to human.(Herbert, 1981).Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential biological activity. Natural Products are providing their fair share of new clinical candidates and drugs in spite of competition from other drug discovery methods (Sarker et al., 2011).

Objectives

Despite the occurrence of many medicinal plants in central region of Nepal, not much work has been reported for biological and phytochemical screening. So, this is the first attempt for the screening of various medicinal plants. The plants were chosen randomly and the present work is focused mainly on the following aims and objectives.

- To perform extraction of different ten selected medicinal plants from Dhading, Kathmandu and Makawanpur districts.
- To conduct phytochemical screening of plant extracts.
- To perform brine shrimp bioassay of plant extracts.

Methods

Collection of plants: Ten different plants having medicinal values were collected from central region of Nepal. Bark of *Myrica esculenta* and*Cleistocalyx operculatus*and leaves of *Nyctanthes arbortristis* were collected from Dhading. Roots of *Berberis aristata, Astilbe rivularis* and *Bergenia ciliata,* leaves of *Urtica dioca*, aerial parts of *Swertia chirayita*and flowers of*Rhododendron arboreum* were collected from Makawanpur district (Simbhanjyang area). Aerial parts of *Aloe vera* were collected from Kathmandu.

The taxonomic identification of plants was confirmed by Prof. Dr. Krishna Kumar Shrestha, Central Department of Botany, TU, Kirtipur. The collected fresh plant materials were washed and air dried in shade for 10-15 days. The dried plant materials were ground into powder form and stored in clean and labelled plastic bags until used for further experiment.

Table 1. *Names of the plants, respective family and parts used*

Scientific name	Localname	Family	Parts used	Traditional Use
Myrica esculenta	Kaphal	Myricaceae	Bark	Asthma, Diarrhea, Fever, Dysentery
Berberis aristata	Chutro	Berberidaceae	Root	Jaundice, Inflammation, Wound healing
Astilbe rivularis	Thulo Okhati	Saxifragaceae	Root	Peptic ulcer, Headache, Infertility
Urtica dioica	Shisnu	Urticaceae	Leaves	Nephritis, Hematuria, Menorrhagia
Bergenia ciliate	Pakhanbed	Saxifragaceae	Rhizome	Fever, Diarrhea, Pulmonary affection
Swertia chirayita	Chiraito	Gentinaceae	Aerial parts	Anti-diabetic, fever, malaria, Skin cream
Aloe vera	Ghiukumari	Liliaceae	Aerial parts	Headache, Arthritis, Stomach ailment, Skin disease
Nyctanthes arbortristis	Parijat	Oleaceae	Leaves & Flowers	Stomachic, Expectorant, Chronic fever
Rhododendron arboretum	Laligurans	Ericaceae	Flowers	Blood dysentery, Cough, Menstrual disorder
Cleistocalyx operculatus	Kyamuno	Myrtaceae	Bark	Gastrointestinal disorder, Sinusitis

Extraction

The extraction of chemical constituents of plant material was carried out with dehydrated alcohol (methanol) by the process of cold percolation. 50 gram of powdered material was weighed out by digital balance and kept in clean and dry conical flask. The weighed powdered in the flask was dipped in methanol (150-200 mL) for three days at room temperature with shaking at intervals. Then the mixture was filtered with the help of general filter paper and funnel, repeating the same process about 8-10 times as per required for the complete extraction and the filtrate was concentrated by rotatory evaporator under reduced pressure maintaining temperature lower than the boiling point of the respective solvent used. The concentrated filtrate was air dried to obtain solid or semisolid residue. The dried extract was analysed for percentage yield. About 2 gram of the each extract was separated in vials in order to perform phytochemical screening and brine shrimp bioassay. The same procedure was repeated for all plants (Dhungana, 2011).

Phytochemical screening

The method employed for phytochemical screening was mainly based on the standard protocol (Alamzeb et al., 2013). In this method, all plant materials were completely extracted by percolation with methanol and subjected to phytochemical screening. The presence of main groups of natural constituents in different extractive solutions were analysed by using different specific reagents.

Biological screening

Biological screening involves the study of the effect of the crude plant extracts at arbitrarily fixed dose levels in a species of organism and prediction of its effect over the entire dosage range. The present research work involves the screening of each fraction of the extract for their brine-shrimp bioassay.

The brine-shrimp toxicity assay for each extract was carried out by following the standard protocol (Ciulei, et al., 1953). The process involves introducing the newly hatched brine shrimp naupalii to the crude plant extracts/ fractions. This method determines the LC_{50} values (µg/mL) for the crude extracts. Compound of LC_{50} values less than 1000 ppm are considered as potentially pharmacologically active.

Result and Discussion

Phytochemical screening: The result obtained from phytochemical screening for each plant is presented in the following table.

Table 2. *Phytochemical screening of different medicinal plants*

Plant Name	Alka.	Flavo.	Terp.	Glyco.	Quinone	Sugar	Polyph.	Sapon.
Myrica esculenta	-	+	+	+	+	+	+	-
Berberis aristata	+	-	+	+	+	-	-	+
Astilbe rivularis	-	+	+	-	+	+	+	+
Urtica dioica	-	+	-	-	+	-	-	+
Bergenia ciliata	-	-	+	-	-	+	+	-
Swertia chirayita	-	+	+	-	+	+	+	+
Aloe vera	-	+	+	+	+	+	+	+
N. arbortristis	-	-	-	-	+	-	+	+
R. arboreum	-	+	+	-	+	+	+	+
C. operculatus	-	+	+	-	+	+	+	-

Where, (+) = Present(-) = Absent

Brine Shrimp Bioassay: The LC_{50} (Lethal concentration for 50% inhibition) values (µg/mL) for different fractions were determined and those having values less than 1000 are supposed to be pharmacologically active. Results obtained during this study are given in table below.

Table 3. *LC_{50} values of different plant samples*

S.N.	Plant Name	LC_{50}(µg/mL)
1	*Myrica esculenta*	20.89
2	*Berberis aristata*	758.58
3	*Astilbe rivularis*	77.62
4	*Urtica dioica*	2187761.62
5	*Bergenia ciliate*	74.13
6	*Swertia chirayita*	5623413.25
7	*Aloe vera*	281838293.1
8	*Nyctanthes arbortristis*	2137962.09
9	*Rhododendron arboretum*	1258.93
10	*Cleistocalyx operculatus*	104.71

From the study of brine shrimp bioassay it was found that the methanolic extracts of barks of *Myrica esculenta*, barks of *Berberis aristata*, roots of *Astilbe rivularis*, rhizomes of *Bergenia ciliata* and barks of *Cleistocalyx operculatus* were biologically active having LC_{50} values (µg/mL) 20.89, 758.58, 77.62, 74.13 and 104.71 respectively. Roots of *Myrica esculenta* were found to be highly toxic against brine-shrimp nauplii among all plant materials.

Conclusion

Phytochemical screening of methanolic extract of all ten selected plants revealed the presence of different chemical constituents such as polyphenols, alkaloids, flavonoids, terpenoids, saponins, reducing sugar, glycosides and quinones. From brine shrimp bioassay, the bark of *Myrica esculenta* was found to be highly toxic against brine shrimp naupilii having the lowest LC_{50} of 20.89. Hence, *Myrica esculenta* is the most biologically active plant.

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CHALLENGES OF RHINO CONSERVATION IN CHITWAN NATIONAL PARK: A REVIEW

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Abstract: *Rhinoceros unicornis*, one horned rhino is one of the magnificient mammals of Chitwan National Park (CNP). Because of the habitat degradation, illegal hunting and poaching, the animal is under brink of extinction. These days, the invasive plant species, *Mikania micrantha* (Banmara) has also been one of the major factors for destroying the natural habitat of the rhino besides, human encroachment and other developmental activities. The local people along with the national and international stakeholders need to be concerned in protecting the animal because the animal is of international significances, as it is the source of attraction for many tourists worldwide.

Introduction

Five species of rhinoceros survive globally and three of the species are found in Asia - Greater one horned rhinoceros (*Rhinoceros unicornis*), Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and Javan rhinoceros (*Rhinoceros sondaicus*).The remaining two rhinoceros are found in Africa-White rhinoceros (*Ceratotherium simum*) and Black rhinoceros (*Diceros bicornis*).

The greater one horned-rhinoceros (*Rhinoceros unicornis*), also called as Indian rhinoceros has been listed as a vulnerable species on IUCN Red List of Threatened species (IUCN, 2008) and is listed in Appendix I of the Convention on International Trade in Endangered species of Wild Fauna and Flora (CITES)(NTNC, 2014/http://ntnc.org.np/publications). *Rhinoceros unicornis*, one horned rhino, has huge body but it can run at the speed of 55 km per hour and possess innate swimming features. The animal has a poor vision but their auditory and olfactory sense is high. The Indian rhinoceros has one horn and is found inboth male and female. They are solitary by nature. The animal prefers tropical and subtropical grasslands, Savannas and Shrub lands (http://www.worldwildlife.org/). The food of Rhinoceroses includes *Saccharum bengalensis, Narenga porphyrocoma, Imperata cylindrical, chrysopogon aciculatus, Eragrostis (*Acevedo,2005) but the most nutritious food for them is *Saccharum sponataneuem* (Dinerstein, 2003). Primarily, they are found in few protected areas of India and Nepal though they were found formerly in the Gangetic plains. There are more than 3,000 rhinos; India (Assam) only possesses about 2,544 rhinos according to the census 2014(http://www.pmi.org.in/manageindia) and in case of Nepal, according to 2015 census (CNP, Kasara), it is about 645 individuals and 605 individuals resides only in Chitwan National Park. The rhinoceros has been a huge concern throughout the world today because the overexploitation of an animal for illegal trade of it's body parts has led the animal prone to extinction.

Chitwan National Park (CNP)

Fig. 1 *Map of Chitwan National Park (CNP) (Source: nepal.de-keizer.net)*

Chitwan National Park (CNP) was formerly recognized as Royal Chitwan National Park. It lies in the inner terai region of Chitwan, Makwanpur, Nawalparasi and Parsa districts of Nepal. It is located between 83" 87 'to 84" 74' East longitudes and 27"34' to 27"68' North latitude in the southern part of Chitwan district. The altitude ranges from 110 m to 850m above the sea level. Chitwan National Park is in a tropical and subtropical bioclimatic zone. The mean annual rainfall is about 2000-2100 mm. The maximum temperature is 35°c during summer and it falls around 20°c during night time. Similarly, in winter the maximum temperature is around 25°c and falls below 10°c in night time. Initially, the area of CNP covered 544 sq km. In 1996, 750 sq kmwas separated as a buffer zone (DNPWC, 1997), 55% as an agricultural land and 45% as a community forest (DNPWC and PPP, 2000). The Buffer zone is situated between longitudes 830 50' 44" – 840 44' 58"E and latitudes 270 16' 56" – 270 42' 13"N. The Park now covers a total area of 932 sq km and is surrounded by Parsa wildlife reserve in the east and India in the southeast. Balmiki tiger sanctuary and Udaipur sanctuary lies across the border of India. The park has magnificient fauna and flora. The faunal diversity comprises 68 species of mammals, 544 species of birds, 56 species of herpeto-fauna, and 126 species of fishes (CNP, 2012). While, the floral diversity comprises 600 plant species that includes 3 gymnosperm, 13 pteridophytes, 415 dicotyledons, 137 monocots, 16 species of orchids (Subedi, 2010).CNP is rich in many endangered and vulnerable animal species. The Chitwan National Park (CNP) has the world's second largest population of one-horned rhinoceros (*Rhinoceros unicornis*) along with large population of tigers (*Panthera tigris*). Other animals found in the park are leopards (*Panthera pardus*), gaur bison (*Bos gaurus*), sloth bear (*Melursus ursinus*), wild Asian elephant (*Elephas maximus*), marsh mugger crocodile (*Crocodylus palustris*), gharial (*Gavialis gangeticus*) and the Gangetic dolphin (*Platanista gangetica*) (BES, 1998). CNP has been well-known globally because of it's unique and diversified ecosystems, thus has international significances. The eight types of ecosystem constituting seven forest types, six grassland types, five wetland and three main river system habitats has been an additional advantages (DNPWC, 2008).Moreover, it's listing as a world heritage site by United Nations Educational, Scientific and Cultural Organization (UNESCO) in 1984 has been more advantageous.

Table 1. *Vertebrate Diversity (Source: CNP, Kasara)*

Taxa	Nepal	Chitwan	Percentage
Mammals	208	68	37
Birds	869	545	63
Herpeto-fauna	143	49	34
Fishes	185	120	65

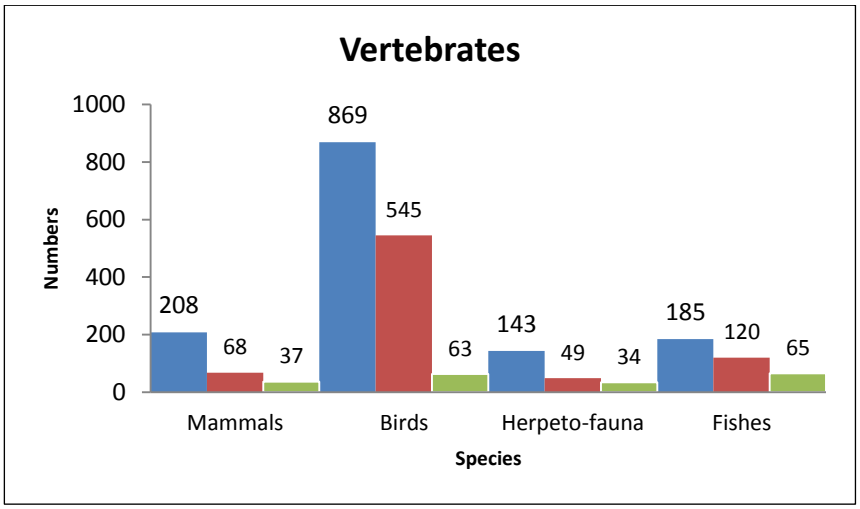

Fig. 2 *Vertebrates' diversity*

Out of 208 mammalian species, found in Nepal, 68 species of mammals are reported from CNP, Out of 869 birds species, 545 bird species are reported from CNP, Out of 143 species of herpeto-fauna, 49 species are reported from CNP and 120 species of fishes out of 185 fishes species have been reported from CNP. These data shows that Chitwan National Park is rich in vertebrates.

Rhino population trend in Nepal

More than 800 rhinos survived in Nepal until 1950s but in mid 1960s, the number of rhinos dropped below 100. The main reason for the fall in number was the agricultural expansion and poaching of an animal. The government of Nepal then established the first national park in Nepal in the year 1973 which formerly was named as Royal Chitwan National Park (RCNP) and now the name has been transformed to Chitwan National Park (CNP). After the establishment of national park, the local people was displaced from the area to extend the Park and armed forces were mobilized as a result the number of rhinos increased to 612 in 2000 which again dropped to 409 in 2005. In the year, 2008, again their number rose to 435 which subsequently increased to 534 in the year 2011 and currently according to the census 2015, the number of rhino in Nepal has reached to 645 which basically explain the progress in management of the animal's habitat along with the protection of animal. This has been determined to be the milestone in the wildlife management basically incase of magnificient animal, one horned Rhino.

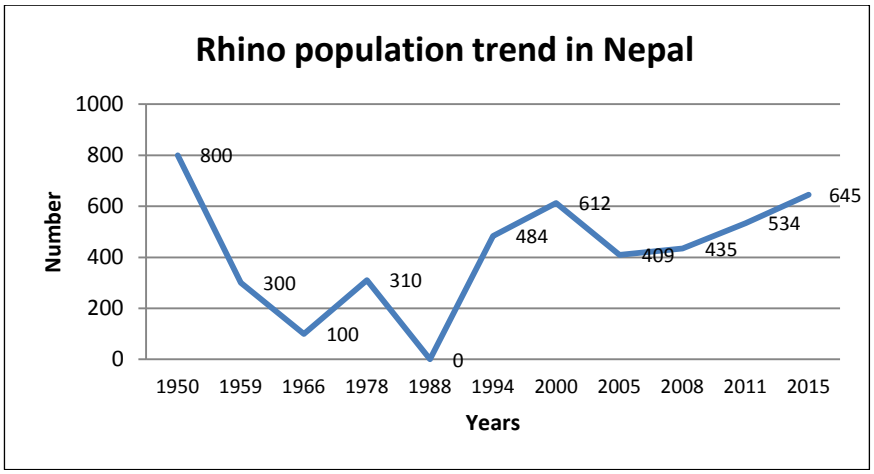

Fig. 3 *Rhino population trend in Nepal(Source: CNP, Kasara)*

Rhino Population trend in Chitwan National Park (CNP)

The recorded data on rhino population trend in CNP shows that the number of rhinos in the year 1988 was 358 which increased gradually to 446 in 1994 and 544 in the year 2000. Again because of habitat degradation and poaching pressure the number of rhino was decreased to 372 in the year 2005. This decrease in rhino population became a huge concern to the National and International level and certain policies were changed in favor of wildlife management. This showed a positive outcome because the number of rhinos gradually rose up. In the year 2008, the population of rhino was 408 which increased to 503 in the year 2011.The latest census record shows the number of rhino has reached to 605 out of 645, only in Chitwan National Park which shows a huge success in the path of Conservation of this magnificent animal, rhino.

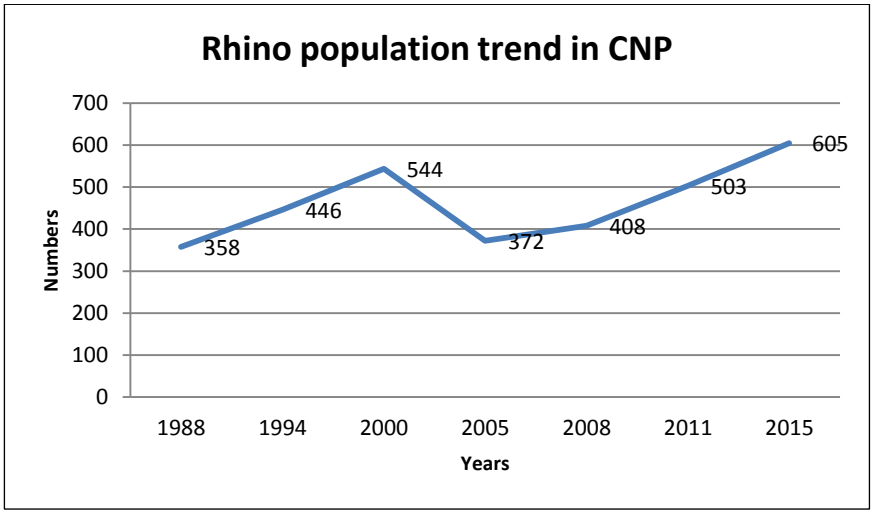

Fig. 4 *Rhino Population trend in CNP (Source: CNP, Kasara)*

Rhinoceroses are one of the major attractions of Chitwan National Park (CNP) and numerous national as well as international tourist visits to the National Park every year. Chitwan National Park (CNP) holds the second largest global population of wild greater one horned rhino in South Asia (Talukdar, 2014). During 15th century, rhinos were large in number and were found between Indo-Burmese border in the east and Pakistan in the west

(Dinerstein, 2003). In the case of Nepal, during 1960s, population of rhinos dropped below 100 and the major reason for this decline in their number was because of poaching and habitat fragmentation. After the declaration of Chitwan as a malaria free zone, population of human increased leading to habitat degradation (Laurie, 1978). Until 1950s, about 1000 rhinos were found in Chitwan valley (Dinersten, 2003). In 1954, about 72 rhinos and in 1958, about 60 were poached (Gee, 1959). By late 1960s, the population of rhino dropped to less than 100. The declaration of national park in the year 1973 was able to recover the number of rhinos to 612 because of its, implementation of the strict policies (DNPWC, 2000). Out of that, 544 were in CNP, 67 in Bardia National Park and 1 in Shuklapanta wildlife reserve. However, the population of rhino again decreased to 372 (Thapa et al. 2013) in CNP during the year (1996-2005) the period of armed conflict. The decline was about 32% from 2000. Later, 2002 was declared as the worst year because poaching has escalated. The record showed that the poaching was done for horn. The report of 2003 showed that among 19 poached rhinos, 16 rhinos were without horn and 3 without hooves. Subsequently in 2004, 9 rhinos were without horn and 2 had no hooves and in the year 2005, 12 out of 15 rhinos poached were without horns and one of them without tail (DNPWC, unpublished report). The unstable politics and insecurity was said to be responsible for the escalation of poaching (Martin & Martin, 2006). Poaching has major negative effect on rhino numbers (Rothley et al., 2004).The year 2001/2002 and 2002/2003 as compared to other years had high rate of poaching incase of rhino. The report during the war period showed that more than 37 rhinos were killed in Nepal in single year that is during the 10 years of the war, more than 141 rhinos were killed (Source: DNPWC). But, after signing Comprehensive Peace Agreement (CPA), only 23 rhinos were found killed which showed reduced poaching rate. In 2008, 7 rhinos were poached in and around CNP, 6 within the park and 1 outside; all had been found shot. In 2009, 10 rhinos were shot dead, 7 inside the park and 3 in the Buffer Zone (Martin and Martin, 2010). In 2010, 9 individuals were poached, 8 inside the park. The police were however able to arrest the poachers in February 2011 who had killed 7 of the rhinos in 2010 and they were all from the same family, according to press reports. On 3 April, 2012, again, one rhino was poached which was an adult female found shot on the western side at Sailimaili Khola, inside the park. An average of 10 rhinos were found poached a year in Nepal between 2008 to 2010, however, in 2011 and 2012, the number dropped to only 1 a year. Some effective rhino-protection strategies must have been the reason. The one reason might be the increase to 51 posts in CNP because there were only 32 posts in late 2009. Recently, the year 2014 was declared as the Zero poaching year because not a single rhino was found hunted/poached. The Park has released the data explaining that no rhinos were killed in the last 365 days beginning from February 16, 2013,only 10 rhinos died naturally (*Source: nepalnews.com, 06 Mar 2014).*The data as a whole shows that the poaching incident of rhino in the latest year seems to be negligible as compared to the past.

The major threat to rhinoceroses is due to relentless poaching especially for horn (CNP, 2012) despite the report of removal of hooves and occasionally pieces of skin have also been in record.Some analysis has been drawn out regarding the high rate of rhino poaching. The body of rhino is large and due to their large body structure, they cannot hide themselves. This let the poachers make ease in attacking and poaching them. Besides, the regular pathways that the rhino uses to travel toward and from their feeding and watering sites also makes the poachers easy to track them. These factors highlight the complex nature of rhino's survival in any environment. In Nepal, poaching of one-horned rhinos

involves local poachers, middlemen, and buyers. Mostly, it has been found that the poor and local people are used for the poaching activities.

Poaching has been one of the many reasons for the declination in the population of wild animals however it is not only the single cause. There are various other causes like drought, fragmentation of habitats and populations, slow reproductive rate, destruction and reduction of viable habitat, over-exploitation and political instability.

The number of one horned rhinos is growing throughout the world but yet the animal is prone to extinction because of illegal poaching and illegal trade.The animal has been listed currently as vulnerable by the IUCN Red List but among the Asia's three rhino species, one horned rhinos are said to be least threatened because their number is rising. The raise in the number of one-horned rhinos in Assam (India), illustrates that the species is least threatened because the announcement of 2,544 one horned rhinos has been done, the population boost of 27 percent in eight years. Similarly, in the context of Nepal also the numbers of rhino are increasing. 534 rhinos were recorded in Nepal during the year 2011, marking an increase of 99 rhinos from the last census 2008, which were 435 in numbers. Of that total 534, 503 rhinos were recorded in Chitwan National Park (an increase of 95 from 2008 data), 24 in Bardia National Park (an increase of 2 from 2008 data) and 7 in Shuklaphanta Wildlife Reserve (an increase of 2 from 2008 data). This increase in numbers of rhinos reflected the success of conservation efforts along with the protective measures adopted for this species and management of their habitat. According to the latest survey in Nepal (2015), 645 one-horned rhinos (*Rhinoceros unicornis)* were counted which was 375 in numbers about ten years ago and 534 in the year 2011.The data reflects the rise of 72 percent over the last 10 years. This achievement of Nepal has been the landmark in the history of conservation and there is no doubt that the constructive outcome on conservation of wildlife is due to the continuous and collective efforts from local community, conservationists and all national and international stakeholders. The establishment of the CNP thus is a progressive step towards the conservation of rhinos of Nepal within natural condition and gradually the conservation measure was improved by introducing the patrolling of Nepalese army, anti-poaching units (APUs) and the motives to the local people in terms of reward in case of information and to combat poaching/illegal hunting (HMGN/DNPWC, 2003). To bring this zero poaching, there have been continuous and collective efforts from local community, conservationists and all national and international stakeholders otherwise it is quite impossible. Therefore, despite the various challenges the result has become positive however, still there needs an improvement in policies in terms of wildlife conservation.

Table 2. *Mortality records of rhino from the year 1998/99-2013/14(Source: CNP, Kasara)*

Year	Natural Poaching	Artificial Poaching	Total
1998/1999	23	3	26
1999/2000	28	16	44
2000/01	12	5	17
2001/02	11	37	48
2002/03	16	32	48
2003/04	15	9	24
2004/05	11	15	26
2005/06	8	10	18
2006/07	6	14	20
2007/08	8	3	11
2008/09	6	12	18
2009/10	13	11	24
2010/11	14	2	16
2011/12	11	1	12
2012/13	9	1	10
2013/14	10	0	10

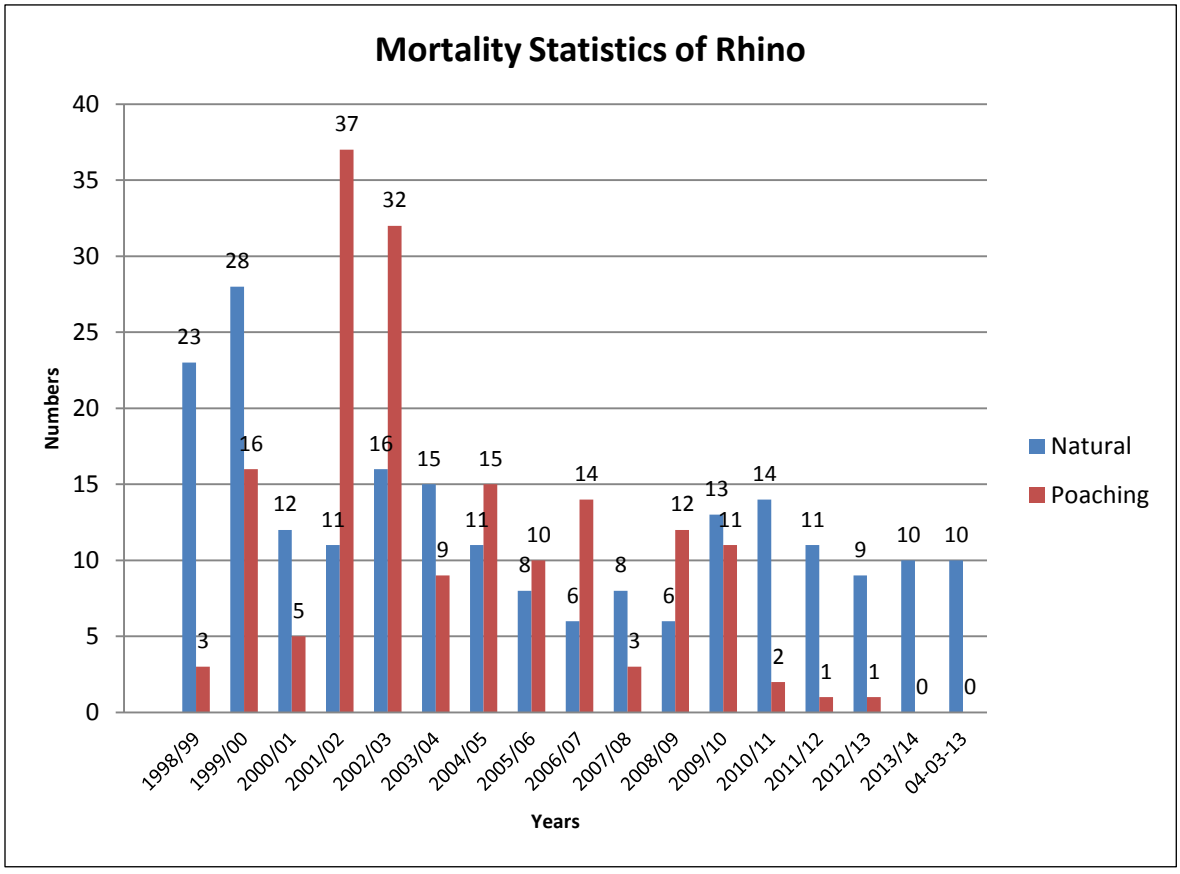

Fig. 5 *Mortality Statistics of Rhino (Source: CNP, Kasara) Threats to rhino*

Since time immemorial, rhino are being slaughtered especially for their horn though other parts of the body are used as well. The horn is about 20-60 cm long with a grey-brown hide having fold of skins. The prominent horn has made it well known globally because it is used as medicines in China, Taiwan, Hongkong and Singapore. It was the Dr. Esmond Bradley Martin who for the first time introduced the use of rhino horn and it's illicit trade in international market. According to him, traditionally, rhino's horn are used as medicines in East Asia and as dagger handles in North Yemen. Other various threats to rhino include:

Habitat loss

Destruction of their habitat since many years has been a major threat to the rhinos causing brink of extinction. Due to the rise in human population, there has been a major problem in space and food which has converted their habitat to agricultural land. The limited space and food resources have led human and rhino to compete with each other because of overlapping of the same land.

Use of chemicals in the field

Various kinds of chemicals are used in the agricultural field. These chemicals used may be harmful or injurious to wild animals. Seven types of fertilizers are used in Nepal which includes Urea, Diammonium Phosphate (DAP), Murate of Potash (MOP), Ammonium Sulphate (AS), Single Super Phosphate (SSP), Ammonium Phosphate Sulphate (APS) and NPK. This may affect the animal although at least**.**

Human –Rhinos conflict (HRC)

Human-Wildlife Conflicts has become a big concern worldwide but it has become a major initiate especially in the developing countries like Nepal where most of the people solely depend on the forest products and agriculture. Wild animals like rhinos, whenever get a chance they are found raiding the crops. Their crop raiding behavior affects the local people directly and indirectly in various ways. The problem is because the human and wild animals share same habitat which decreases the habitat of wild animal on one side and on other side they are attracted to the palatable crops grown nearby as it becomes the ease way for them to feed themselves whenever they are hungry. Sometime the entry of animals to the field might be a threatening one as they might injure or kill human being and livestock. The local people are not compensated for the losses so they are found involved in retaliatory killing of wild animals along with the adoption of various destructive measures to protect the crop against wild animals(Straede and Treves, 2006).Osborn and Parker (2003) has classified the defensive measures into two types- Active and Passive. The active methods includes frightening devices and tactics as yelling, banging metals to produce sounds, deploying fireworks (Osborn and Parker, 2003), patrolling the field (Gillingham and Lee, 2003) and also making scary dummies while the Passive methods includes biological fences (Wang et al., 2006), Construction of walls (Sekhar, 1998) digging trenches (Nyhus et al., 2000).Beyond this trapping and hunting are also done with the worst offenders (Wang et al., 2006). Hedges and Gunaryadi (2009) has explained that people are found using chilli-based repellants to reduce crop depredation. The potentiality of conflict exists in the place where human and wildlife are found together however the extent varies spatially (Heinen and Yonjon, 1994).

Hunting/ Poaching

The illicit hunting has been one of the crucial factors for decline of the greater one horned rhino. Wild animals are vulnerable to the exploitation by human and it is the means of capturing of animals illegally or hunting of endangered animals for the economic benefits. The civilization and development has made the illegal trade easier through communication, networking and technology. The biggest threats to rhinos thus has been a poaching because of the increase demand for it's horn. Rhinoceros as is highly valued and is used in traditional Chinese medicines. The horn of rhino is used in reducing fever, fear, increase sexual drive and as an aphrodisiac (Baum & Goldstein, 1993). The toenail of rhino is used in reducing fever, blood as a tonic and skin to cure skin ailments. The main threats to rhinos thus come from poaching for use in Traditional Chinese Medicine(TCM) even though rhino horn has been removed from the official TCM pharmacopeia with alternatives encouraged, and trade in rhino horn though banned under international law, demand for rhino horn still remains high.

Illegal trade

Illegal trade of wild animals constitutes illicit transport and distribution of animal body parts locally, nationally and internationally. The illegal trade of wildlife products has become a growing illicit business because at minimum it is expected to worth $ 5 billion and maximum it worths more than $ 20 billion (Wyler & Sheikh, 2008). Thus it has been estimated to be the World's third largest illegal trade (Baum & Goldstein, 1993). Wild life trade threatens around one third of mammals and aves globally (Baillie et al. 2004). The most lucrative assets include parts of tiger, ivory of an elephant, horn of rhino, and exotic bird and reptiles. Illicit trade has been growing because of ease transit, weak policies and penalties systems, corrupted officials, political corruption and ease networking system. Asia is considered to be as the place where high trade of wild animals takes place (Wyler & Sheikh, 2008). Since, 1975, CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) has been supporting biodiversity conservation however the wildlife trade has not been overall controlled. The trade of wild life threatens the flora and fauna populations along with the consistency of ecosystems. According to Leader-Williams et al. (1990), the decline of rhinos is mainly due to the increase in price of their horn in an international markets and decline of opportunities to local people living close to Protected areas (PAs).

Impact of *Mikania micrantha* on rhino's habitat

Mikania micrantha(Asteraceae) is a tropical vine with fast growing capacity which has been a major problematic invasive weed especially in Chitwan National Park (CNP).The weeds has negative impact on other native grasses and shrubs. The *Mikania* not only affect the growth of other plants but it has also created negative impacts on the wild habitat. Besides, this invasive plant has also affected the life of local community residing nearby the national park as they rely on the fodder of Park for their domesticated livestock. *Mikania* has created an extensive damage to many of the Chinese ecosystems recently so the excessive growth of these weeds in CNP has been a huge concern today in terms of wildlife conservation. Invasive alien species are now considered to be one of the serious threats to global biodiversity (Porte et. al., 2011) and Mikania has been listed among the top 100 worst invasive species and as one of the top 10 worst invaders in the world(Zhang et. al., 2004).

Conservation methods

WWF has been working on rhino conservation for over four decades. The conservation efforts are required and it can be done in following ways:

1. Translocation of rhinos from vulnerable areas reduces poaching.
2. Improvements in security- Yonzon (2002) reported that the increase in patrolling reduces poaching.
4. Adoption of anti-poaching measures
5. Penalties for poachers
6. Use of radio-tags and collars
7. Policy reforming
8. Community based conservation approaches

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ECOLOGY OF TERRACED BOG PADDY FIELD OF SANGLA KUNCHI PWAKAL VILLAGE DEVELOPMENT COMMITTEE

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Abstract: Ecology is defined as the scientific study of interactions between organisms with their environment. The physico-chemical factors like temperature, pH, dissolved oxygen, nitrate-nitrogen, phosphate phosphorus, water hardness, turbidity, and water flow and water depth govern the morphology and physiology of the biotic components of the water body of the marsh habitat. The study was carried out for thirteen months from April 2007 to April 2008 in a terraced swamp paddy field of Sangla village covering an area of 360 m^2. Total of 28 different animals were collected including aquatic, bed biota and surrounding. Twenty different animals were present throughout the study period whereas remaining eight were lacking for some month. Likewise 34 different taxa including algae and vegetation from aquatic, marsh or bog, submerged and littoral as well from the surroundings. Nineteen taxa were present throughout the months and 15 taxa were lacking for various months. Among physicochemical factors, temperature is one of the most important abiotic factors affecting the life of any ecological habitat, showing minimum average air and water temperature in January being 14^0C and 16^0C, while maximum was recorded in June i.e., 27.5^0C, 26.5^0C, respectively. The, minimum average pH was 5.6 in January, whereas maximum average recorded was 6.5 in most of the months (8 months). The mean average dissolved oxygen was 58.76 ppm during the study period. The mean average Nitrate- nitrogen ($N0_3$-N) was found to be 1.46 ppm.

Introduction

Ecology is defined as the scientific study of relationship between the organisms and their environment (Mc Naughton, Wolf, 1973 Kormondy,1965) described ecology, as the theme dealing with interactions of organisms and their environment. Whereas, Fleming, (1973) considered ecology, as the study of environment in relation to the living worlds and their interdependencies among animals and plants and the environment (among each other). Freshwater decapods occur in standing water habitats such as lakes, ponds pools, bogs, marshes, as well as wetlands i.e. in lentic environments or in the running water such as streams, rivers, rivulet, canals; each of which presents a varied sets of environmental characteristics. In Nepal there are total nine Ramsar occupying the area of 34,455 hectares, these wetlands are the most productive ecosystem on earth (Shrestha, 2009). Besides, there are more other wetlands which are being continuously converting into paddy field, real state (by refilling), fisheries pond etc. Water quality has direct influence on the growth and survival of any aqua culturable organisms as they live and grow in that medium (Govt. of India, 1992). Salinity is the most important factor for the reproduction in the coastal water (Narayanan, et al., 1988). The most obvious factor in freshwater habitat is low salinity and complex interactions of this with other environmental factors, (Vernberg and Vernberg, 1983), such as temperature, (Sastry, 1983). Temperature enhances general physiology of the organism by scheming molecular dynamics such as diffusivity, solubility, fluidity and biochemical reaction rates, (Govt. of India, 1992), as the internal tissue of the organism is typically at nearly the same temperature as the surrounding water. Hydrogen ion concentration (pH) is the next important ecological factor. It indicates the extent of acidic or basic nature of water quality, thus affecting the

metabolism and other physiological activities of the organisms of that area. In the same way, oxygen is one of the most important environmental factors for the survival of all the living beings. High turbidity may cause stratification of temperature and dissolve oxygen in water, (Govt. of India, 1992).

Objectives

To conduct a preliminary assessment of eco-biology of the crab habitat as crucial for the management as a future perspective of aquaculture.

Methods

The present study on eco-biology was carried out at Sangla Kunchi Pwakal V. D. C. (Village Development Committee). Sangla is the mid hill mountain tract, located at the bank of Baundeshwar stream. Baundeshwar stream is a perennial hill stream originating from a high mountain. The study area is a terraced bog paddy field. It carries only 30 m in length and 12 m in width. Large and small boulders and rotten woods providing the hideouts for crabs, occupy their habitat. Bushes and a few small trees on sides, grasses, weeds and reeds in the field surround it. It is situated on the north- western region of the hill where the sun sets early during both summer and winter and the location is comparatively colder. Baundeshwar stream being deep from the paddy fields, all water from the study area drains into the river. Even then there is always a direct impact of river water on the paddy fields and the vice-versa, as water from the stream is used for irrigation in the fields and seeping water from the fields drains into the river and leaching of inorganic fertilizers, pesticides, poisoning.

Ecological studies were carried out in terraced bogs and paddy field constituting habitats of the crabs. Six different sites were selected for each month and regular shifting was carried out within the same area. The area covering an expanse of 30 x 12 sq. m only was used for the study. The entire study was carried out for a period of thirteen months from April 2007 to April 2008. Since the amount of water in bogs was not sufficient for the assessment of hydrological parameters for some months in the year, at least 25 cm to 35 cm deep pits were dug at the work sites. Monthly, habitat analysis was carried out in term of hydro-geographic details such as water depth, water flow, bed texture, and bed biota, submerged and littoral vegetations. Water depths were measured by slowly inserting plastic ruler into the bottom. As soon as it touched the ground the reading was noted down. Water flow was measured keeping a small piece of paper on the surface of water, and then the distance traveled by the paper within a period of time was recorded.

Bed biota, bed texture, littoral and submerged vegetations and biota from the bogs were collected in different plastic bags and specimen bottles, without or with the preservative. A ‘D’ shaped collecting net (sieve size 0.3 mm) was used for bed biota and bed texture; and hand picking for large vegetations and biota of the habitat. For these collections, six quadrates of 50 x 50 cm square sized were randomly placed. All the material was taken in the laboratory. All aquatic and the surroundings sites were used for collections, benthic and upper region instead submerged and littoral vegetation.

To study the bed texture a little bit of the contents from the bottle were taken out, then examined under the dissecting microscope. Again, little water was added to the remaining material present at the bottom of the bottle and poured slowly to remove surface clay. Material present at the bottom of the bottle was poured in a watch glass and observed by using hand lens. The samples data were recorded. The contents of all samples were observed with naked eyes, hand lens, dissecting microscope and binocular microscope for larger biota, smaller organisms depending on sizes respectively. The specimens were identified for families or genera, for qualities and not for quantities, with the help of standard literature and records Jacobson, et al., (1997), Blindow, et al., (2000).

Month wise physico-chemical parameters of bug habitat were analyzed from 12 noon to 5.30 pm. Water and air temperatures were recorded during 12 to 1pm; dipping the tip of the mercury thermometer in water and the air in shade for two minute each. The bogs were made at least 25 to 35 cm deep so that water could be taken out easily, especially for measuring dissolves oxygen. Physico-chemical parameters, such as hydrogen ion concentration (pH), dissolved oxygen (DO), nitrate nitrogen (NO_3-N) phosphate phosphorus (PO_4-P), hardness ($CaCO_3$) and turbidity, were assessed using ENPHO (Environment and Public Health Organization, kit), water test kit. All the above-mentioned habitat analysis and physic-chemical parameters were carried out in six different sites from the crabs' habitat for 13 months. Monthly mean were calculated and recorded for all the ecological studies of crabs' habitat.

Result and discussion

All together 28 different animals were collected during thirteen months period including aquatic, bed biota and surrounding biota. All the collected taxa are listed below (Table no.1). *Paramecium, Euglena, Daphnia, Cyclops, Rotifers, Nais spp, Limpet spp, Ephemera spp, larvae, Dragon fly, larvae, Demioselle larvae, Chironomus larvae, Hydrometra spp,Coxelmis spp, Elmis spp, Renatra spp, Psepheniod spp,* Nematods, Tadpoles of different stages, *Collembola spp, Acaris spp, Opilionus spp, Scutigerella spp, Pholcus spp, Gryllotelpa spp, Gryllus spp, Onicus spp, Small centipede and Phalandidium spp.* Of 28collected taxa, Dragon fly larvae and *Demoisell larvae* (damsel fly larvae) were not found in October and November; Rotifers and Daphnia in December and January; Ephemera larvae in January and February and Euglena for three months December, January and February respectively. *Chironomus larvae* were present only in the months of December,January and February and remained absent for ten months. Different stages of tadpoles were present from the month of October 2007 until March 2008. All the remaining taxa were present throughout the experimental period, table 1. From the same habitat total of 692 crabs of *Barythelphusa lugubris* and five species of *Himalayapotamon emphysetum* were collected.

Algae and vegetation from aquatic, marsh or bog, submerged and littoral as well from the surroundings of the crab's habitat was studied. A total of 34 taxa were reported from the habitat throughout the study period, out of which nineteen taxa were present throughout the period of 13 months, (Table 2) and 15 taxa lacking for various months. *Chlorella spp, Spirogyra spp, Polygonum spp, Potamogeton spp, Rush spp, Scrophularia spp, Funcus spp, Mentha spp, Phragmites spp, Arundo spp, Arundinaria spp, Algeric spp, Aegopyron spp, Ttypha spp, Aegopodium spp,* Willow, Moss, Fungi, and *Pteridium spp* observed throughout the months. *Volvox and Diatoms* were absent for June,July, November 07 to February 08, whereas *Chlamydomonas* in November 07 to February 08respectively. *Azolla, Myriophyllum spp, Myosotis spp, Lythrum spp,* and *Heracleum spp* were lacking from December 07 to February 08. *Butomus spp* was lacking for five months as June, July, August, December and January, whereas *Menyanthes spp* was absent for five months as well, i.e., June, July, December, January and February. *Archilea spp* and *Ranunculus spp* were present in the months of April and May 07. It disappeared from June to December 07and reappeared from the month of January till April 08. *Iris and Veronica spp* were present inApril, May 07. They disappeared for eight months from June till December 07 andreappeared from January till April 08 (Table 2).

Seasonal variations of physico-chemical factors of the crabs' habitat (table 3). During the study period, the temperature of air and water was taken from 12 noon to 1 pm. The minimum average air temperature was 14^0C in January while, maximum recorded was in the month of June i.e., 27.5^0C. The air temperature started decreasing from the month of September until January and started increasing from February 08. The mean average temperature of thirteen months was 22.11^0C (Fig. no. 1). The mean average water temperature of the habitat for thirteen months was 21.71^0C with the minimum average recorded was 16^0C during the month of January. The maximum average recorded was 26.5^0C in June. The water temperature started decreasing from the month of September until January and started increasing again from February 08 (Fig. 2). The average

minimum and maximum temperature recorded were plotted to correlate with the average temperature of the habitat. In this report minimum temperature recorded was in January with the value of 2.7^0C, whereas a maximum of 27.7^0C was recorded in June (Fig. 3).

The rain started from the month of April 07 and reached its zenith in the month of September. It was 293.7 mm. In the months of November, December and February no rainfall was recorded. The minimum rainfall was recorded in January. It was 4.5mm (Fig. 4). Hydrogen ion concentration (pH) of the marsh of bog of the crab's habitat nearly remained constant throughout the study period with a slight variation in different months. The mean average pH value during the whole study period was 6.34. The minimum average pH was 5.6 recorded in the month of January while the maximum average of 6.5 was recorded in quite a few months. The pH started falling from November. An increase in the same was noticed from the month of February 08 (Fig. 5). Average DO varied during the whole study period with the mean average of 58.77 mg/lit for thirteen months. The minimum average value of was 36 mg/l recorded in June 08, whereas the maximum value was 80 mg/l recorded in January (Fig. 6). The average Nitrate in the form of nitrogen varied throughout the study period. The mean average for thirteen months was 1.46 mg/lit. The lowest average $N0_3$-N of 0.2 mg/l was recorded in March 08 and the highest average was in August with the value of 6.5 mg/l (Fig. 7). The average value of Po_4-P varied throughout the experimental period with a mean average of 0.124 mg/l. The lowest average value of $P0_4$-P recorded was 0.04 mg/l in the month of July and highest value of 0.5 mg/l was in March (Fig. 8). The average value of water hardness varied throughout the study period with a mean average of 39.92 mg/l. The minimum average value of water hardness recorded was 16 mg/l in July and August 07 while, the maximum average was 72 mg/l in January 08 and February (Fig. 9). Barring four months i.e., June, July, August and September, turbidity remained nearly constant during the remaining months of the year. The mean average value recorded for 13 months was 61.53 NTU mg/l. The minimum average turbidity recorded was 11 NTU mg/l in April 07 and December. The maximum average was 300 NTU mg/l in September. It started increasing from the month of May (Fig. 10). The water flow varied throughout the study period with a mean average of 23.8. The minimum average water flow was 2 cm/min in the months of December and January 08. The maximum average was recorded in the months of August with 60 cm/min (Fig. 11). The water depth varied from months to months with mean average value of 7.03 cm/min. The minimum average water depth was 4.2 cm in the month of February, while the maximum average of 14.2 cm was recorded in the months of September (Fig. 12). Soil texture of bed as well as of the surrounding habitat remained nearly same throughout the study period. The soil texture was sandy clay in all the samples collected.

Table 1. *List of protozoan, aquatic and terrestrial organisms of marsh paddy field of Crab*

Organisms	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr 2008**	**Total (-) in months**	**Total (+) in months**
Paramecium	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Euglena	+	+	+	+	+	+	+	+	-	-	-	+	+	03	10
Daphnia	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Cyclops	+	+	+	+	+	+	+	+	-	-	+	+	+	02	11
Rotifer	+	+	+	+	+	+	+	+	-	-	+	+	+	02	11
Nais spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Limpet	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Ephemera larvae	+	+	+	+	+	+	+	+	+	-	-	+	+	02	11
Dragonfly larvae	+	+	+	+	+	-	-	-	+	+	+	+	+	03	10
Demioselle larvae	+	+	+	+	+	-	-	-	+	+	+	+	+	03	10
Chironomus larvae	-	-	-	-	-	-	-	-	+	+	+	-	-	10	3
Hydrometra spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Coxelmis spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Elmis spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Renatra	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13

Psephenoid	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Nematoda spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Tadpole	-	-	-	-	-	-	+	+	+	+	+	+	-	07	06
Collembola spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Acaris spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Opilionus	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Scutigerella spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Pholcus spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Gryollotalpa spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Grullus spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Onicus spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Small centiped	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
Phalangidium spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13

Absent = - and Present = +

Table 2. *List of algae, aquatic and terrestrial vegetations of marsh terraced paddy field of the crab's habitat*

Algae, aquatic & terrestrial vegetation	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**Total (-) in months**	**Total (+) in months**
1. Chlorella	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
2. Spirogyra	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
3. Chlamydomonas	+	+	+	+	+	+	+	-	-	-	-	+	+	04	09
4. Volvox	+	+	-	-	+	+	+	-	-	-	-	+	+	06	07
5. Diatoms	+	+	-	-	+	+	+	-	-	-	-	+	+	06	07
6. Azolla	+	+	+	+	+	+	+	+	-	-	-	+	+	00	13
7. Ploygonum	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
8. Potamogeton	+	+	+	+	+	+	+	+	+	-	-	+	+	03	10
9. Ranunculus	+	+	-	-	-	-	-	-	-	+	+	+	+	07	06
10. Myroiphyllum	+	+	+	+	+	+	+	+	-	-	-	+	+	03	10
11. Archilea	+	+	-	-	-	-	-	-	-	+	+	+	+	07	06
12. Butomus	+	+	-	-	-	+	+	+	-	-	+	+	+	05	08
13. Menyanthes	+	+	+	-	-	+	+	+	-	-	-	+	+	05	08
14. Rush	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
15. Scrophularia	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
16. Iris spp	+	+	-	-	-	-	-	-	-	-	+	+	+	08	05
17. Funcus spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
18. Mentha spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
19. Veronica spp	+	+	-	-	-	-	-	-	-	-	+	+	+	08	05
20. Myosotis	+	+	+	+	+	+	+	+	-	-	-	+	+	03	10
21. Phragmites	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
22. Lythrum	+	+	+	+	+	+	+	+	-	-	-	+	+	03	10
23. Hydrocotyle spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
24. Arundinaria	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
25. Algeric	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
26. Aegopyron	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
27. Typha	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
28. Aegopodium	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
39. Heracleum	+	+	+	+	+	+	+	+	-	-	-	+	+	03	10
30. Willow	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
31. Moss	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
32. Fungi	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13
33. Pteridium spp	+	+	+	+	+	+	+	+	+	+	+	+	+	00	13

Absent = - and Present = +

Table 3: *Seasonal variations of physico-chemical factors of the crabs' habitat*

Months	Air temp	Water temp	pH	Dissolve O_2 ppm	Nitrate-N ppm	Phosphate-P mg/lit	Total hard mg/lit	Turbidity NTU	Water flow cm/min	Water Depth cm
April	25	19.5	6.5	56	0.9	0.07	32	11	20	6.1
May	27	24	6.45	48	1	0.06	28	61	28	7.0
June	27.5	26.5	6.5	36	0.8	0.05	24	77	35	8.3
July	26.5	26	6.5	64	0.6	0.04	16	113	50	9.1
August	26.5	26.33	6.5	64	6.5	0.2	16	115	61	11
September	23.5	22.5	6.5	64	0.6	0.05	32	300	46	14.2
October	20	20.5	6.5	56	0.4	0.3	20	18	10	7.0
November	20.5	21	6.15	56	0.4	0.075	28	20	05	5.1
December	14.5	17	6	72	6	0.05	60	11	02	5.0
January	14	16	5.6	80	0.5	0.125	72	15	02	4.6
February	19.5	18.5	6.25	56	0.7	0.05	72	13	05	4.2
March	20	22	6.5	56	0.2	0.5	60	16	12	4.7
April	23	22.5	6.5	56	0.5	0.05	20	30	34	5.1
Total	**281.5**	**282.33**	**82.45**	**764**	**19.1**	**1.62**	**480**	**800**	**310**	**91.4**
Average	**22.11**	**21.71**	**6.34**	**58.76**	**1.46**	**0.124**	**36.92**	**61.53**	**23**	**7.03**

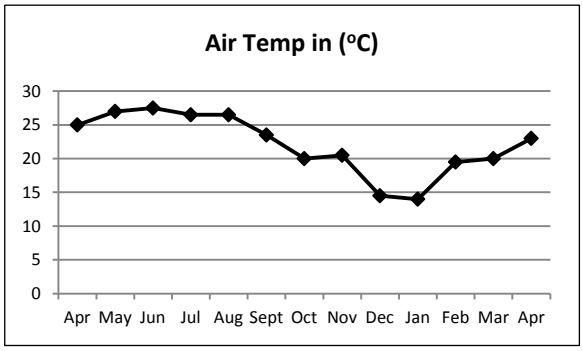

Fig. 1 *Seasonal variations of air temperature in the crab's habitat*

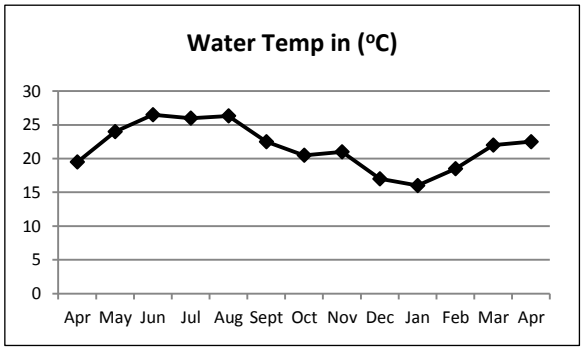

Fig. 2 *Seasonal variations of water temperature in the crab's habitat*

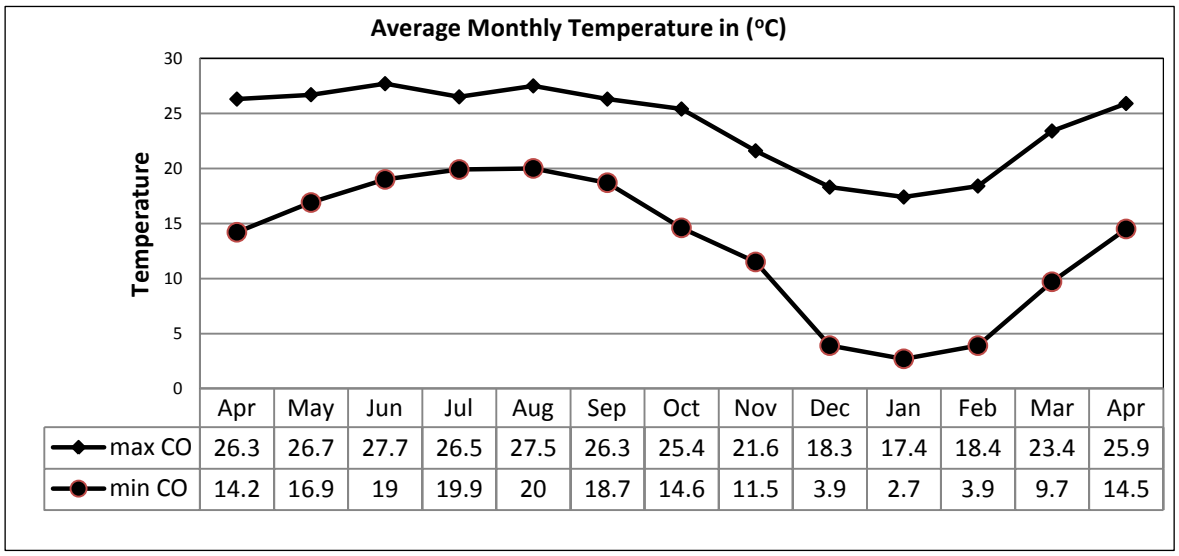

Fig. 3 *Annual report of average monthly temperature data collected from Dept. of Meteorology*

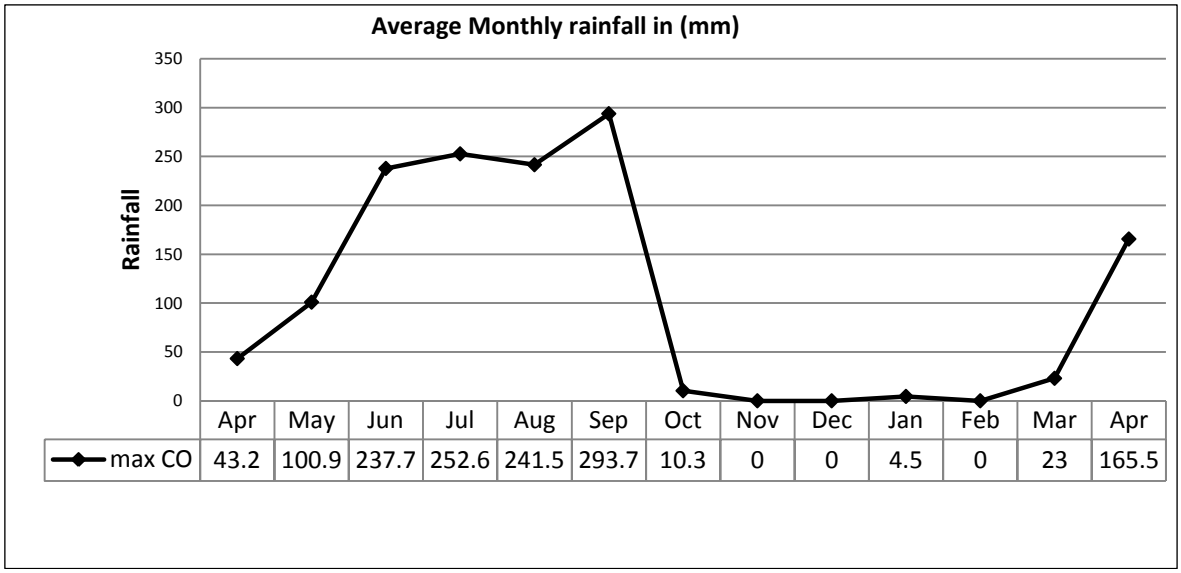

Fig. 4 *Annual report of average monthly rainfall data collected from Dept. of Meteorology*

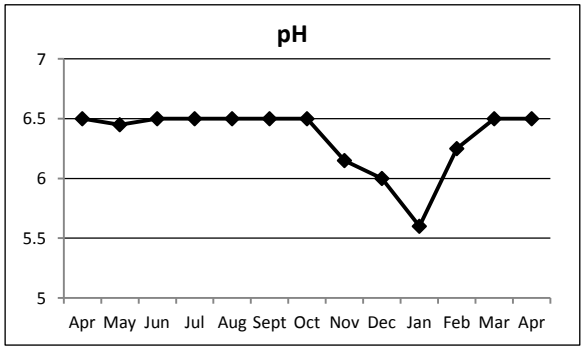

Fig. 5 *Seasonal fluctuation of pH in the crab's habitats*

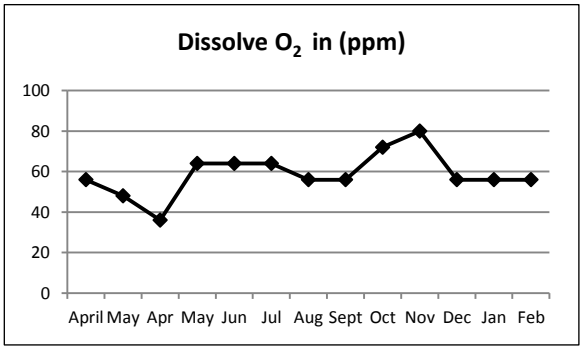

Fig. 6 *Seasonal variations of Dissolve oxygen in crabs' habitat*

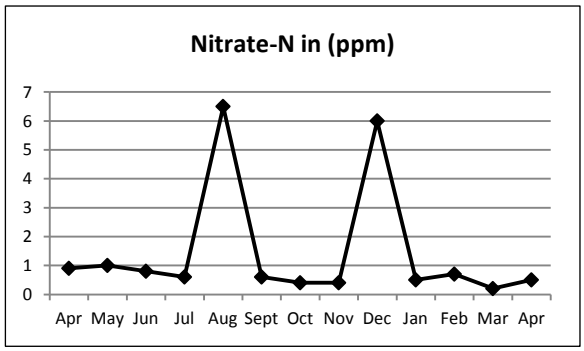

Fig. 7 *Seasonal variations of Nitrate- nitrogen in the crab's habitat*

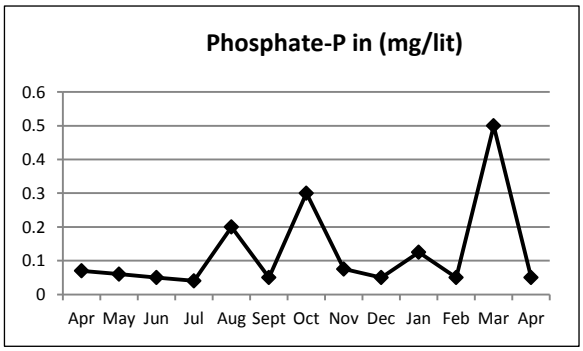

Fig. 8 *Seasonal variations of Phosphate-phosphorus in the crab's habitat*

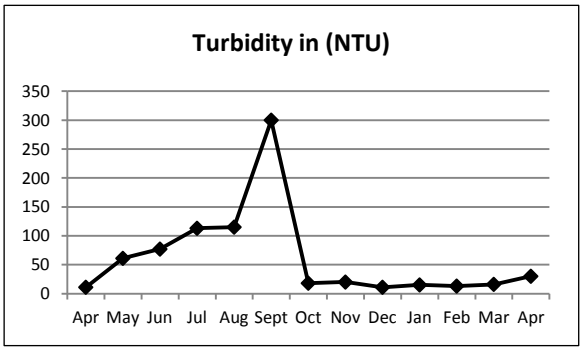

Fig. 9 *Seasonal variations of total water hardness in the crab's habitat*

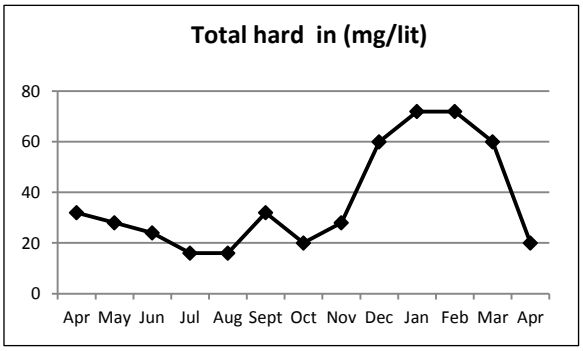

Fig. 10 *Seasonal variations of turbidity in the crab's habitat*

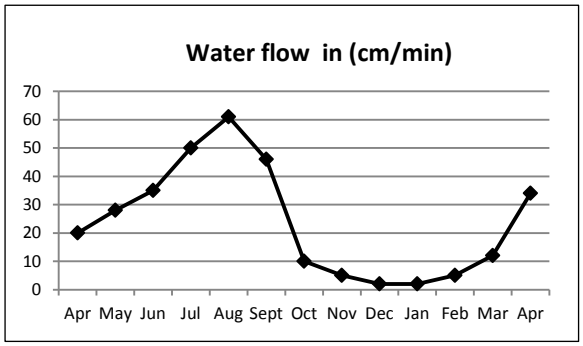

Fig. 11 *Seasonal variations of water flow in the crab's habitat*

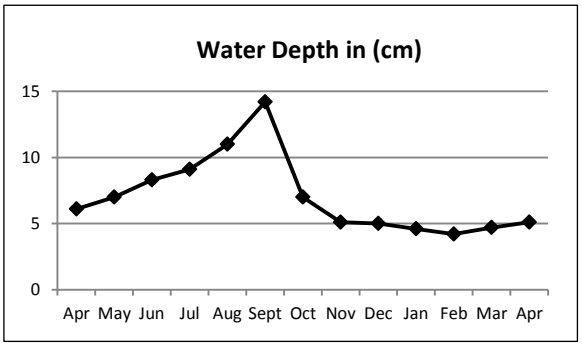

Fig. 12 *Seasonal variations of water depth in the crab's habitat*

Temperature is one of the most important factors influencing the metabolic activities of life of aquatic as well as terrestrial habitat (Miranda-Anaya, 2004; Wolcott, 1988). The maximum air and water temperatures were recorded in the month of June (27.5^0C and 26.5^0C) due to high solar light intensity. The minimum water and air temperature recorded were (14^0C and 16^0C) in winters i.e.in January due to low solar intensity. Similar pattern of temperature variation have been reported by Mahaseth, (2007) from the river Mahakali and Sultan, et al., (2003) from Pahunj reservoir. Nautiyal, (1990) recorded average water temperature as 15^0C at the foothill river of Garhwal region that fluctuated from 3^0C to 4^0C. Singh, (1990) reported water temperature of many rivers, such as Gaula river 20^0C to 29^0C, (West) Ramgagan river 25^0C to 29^0C Koshi river from 18^0C to 28^0C, and of Sarju from 14.5^0C to 26.5^0C respectively. In general, pH of the marshy terraced paddy fields recorded nearly a constant throughout the study period; except the lowest value recorded in the month of January, when the temperature was very low. The value of pH increased during the rainy season and decreased after post monsoon, especially during the winter season. Increased pH during monsoon was also reported by Singh et al., (2001) in river Brahmaputra. But Nath and Srivastava, (2001) reported increased value of pH during post monsoon and decreased value in monsoon. According to Swingle, (1976) pond water having pH values ranging from 6.5 to 9.0 is suitable for aquaculture. DO of Sangla ranged from 36 ppm to 80 ppm. Singh, (1989); Karki, (1988) and Mahaseth, (1988) reported similar observations from Godawari fish pond no. 21, closed water system of Udaipur, India and Tadi river, Nepal. In rainy season itself, another peak of DO was observed due to heavy rain and low presence of chlorophyll bearing organisms, with low photosynthetic activity (George, 1961).

According to Ruttner, (1953), each physiological activity of biota is affected by the condition of the environment i.e., temperature, light, oxygen content and other physico-chemical properties of the water. Water flow was recorded high during monsoon due to heavy rain and drainage from the upper terrace, while minimum was recorded in winters having a dry season without rain. Seeping water patches were very few which flow towards the lower terrace as well as in the stream. The

depth of the water influences the physico-chemical and biological properties of the habitat. Mahato, (1988) also reported similar type of observation in temple tank pond of Kirtipur, Kathmandu, Nepal.

The effects of turbidity reduce the abundance of zooplankton and macro invertebrate fauna of naturally and artificially turbid aquatic system. The effects of turbidity in freshwater of Alaska (USA) were studied by Lloyd and Jeffery, (1987) and it was reported that turbidity results from the scattering of light in water by organic and inorganic particles, particularly silt. Benerjee and Raychaudhary, (1961) studied the physico-chemical features of Chilka Lake and reported that during monsoon the turbidity was high due to silt and silt laden rain water, whereas low turbidity was there in winter when there was no rain. The present findings also support the observations stated earlier.

Conclusion

Among physicochemical factors, temperature is one of the most important abiotic factors affecting the life of any ecological habitat showing a marked effect on the biological and chemical properties of that area. The minimum air and water temperature being 14^{o}C and 16 ^{o}C and maximum being 27.5^{o}C and 26^{o}C along with 28 different animals, 34 algae and vegetation in which 692 crabs of the genus *Barytephusa lugusbris* and five number of *Himalalyapotamon emphysetum* were collected from same specific area. Hence it is concluded that the studied field is suitable for crabs habitat being appropriate physico-chemical factors and collected plants and animals as food.

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PREVALENCE OF AEROALLERGENS IN THE ATMOSPHERE OF KATHMANDU NEPAL AND CHITTAGONG, BANGLADESH

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The burden of aeroallergens in the atmosphere is increasing alarmingly in Nepal and Bangladesh. Nepal alone has about two million asthmatics patients. It causes approximately 5,000 deaths a year, due inhalation of biologically polluted air most of them are exposed to aeroallergens. The study of aeromycoflora was conducted to determine prevalence of aeroallergens in the atmosphere in Kathmandu, Nepal and Chittagong, Bangladesh. Gravity Slide method was used for the isolation of fungi; identified by colony morphology and microscopic method. The allergens from *Aspergillusfumigatus*, *A. flavus* and *Alternaria alternata* were extracted and identified the protein bands by Sodium Dodecyl Sulphate- Polyacrelamdide Gel Electrophoresis (SDS-PAGE) using standard proteins (Marker).In vivo allergenic immune response was evaluated by intracutaneous inoculation in laboratory animal. Total 20 different spore types and 19 genera belonging to four different classes from Kathmandu and 40 spore types from Chittagong, were identified from the atmosphere. Aspergilli/ Penicilli group was the most prevalent spora and constitute the major air spora (81.20%) and (16.32%) from Kathmandu and Chittagong respectively. The allergenic bands 30 to 67 kDa were found common in all three studied species. The most allergenic bands 20-32kDa was more prominent in *Aspergillus fumigatus* and also reported as the most allergenic to experimental animals with the highest wheal size. The fungal spores are the predominant contaminants of air, distributed uniformly during all seasons and areas. They cause a wide range of allergenic reaction to human beings and increases prevalence with the increase of concentration of spores in air.

Keyword: Allergens, aeromycoflora,SDS-PAGE, Aspergillus fumigatus, A. flavus, Alternaria alternate

Introduction

Air of every corner of the world is full of thousands of fungal spores. Some environment may exceed the concentration $10^9/m^3$ (Aimanianda et al., 2010). Human being exposed to fungal spores; aeroallergens and pathogens either in indoor or outdoor air (O'Gorman, 2008). Fungal spores are an ideal group particle of biological origin, in which to investigate allergen orthologue distribution as they are a diverse kingdom comprising over an estimated million species, are common both in the environment and as epiphytes, pathogens, gut inhabitants and endophytes of man (World Health Organization, 1996). Indoor environment provides more favourable survival conditions for the aerosolized fungi and increased culturability of fungi inside the homes (Lee et al., 2006). Nearly 10% of the people worldwide have fungal allergy (Burge, 2002). Indoor air may be the potential source of aeroallegens in outdoor air. Air-born microbes are responsible to cause disease also depend in their surviving and remaining infective for susceptible hosts (Cox, 1995).

Shrestha and Mridha (2010) reported that fungi are most prevalent in Kathmandu, Nepal and Shrestha& Sharma (1994), reported the highest number of mycoflora in the spring season followed by decrease up to autumn season and the agricultural areas comprising the highest number of genera (42 genera) while least in the airport. In Bangladesh Rajhana, (1997) reported 21 genera belonging to Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes were identified. The frequency of *Aspergillus* spp. increased in rainy season with significant prevalence during autumn but gradual decrease in winter season. The sporesof *Aspergillus*and *Penicillium* were present in the Derby air throughout the year the greatest activity and often reached maximum monthly cumulative concentrations in the autumn, although they were occasionally the dominant spores in the winter (Millington & Corden (2005). Pei-Chih et al., (2000) studied the airborne fungal concentrations reported that total fungal concentration, both indoors and outdoors of suburban homes, were significantly higher in summer, than those of urban homes and the dominant fungi contributing to such a difference were indoor *Cladosporium* spp and outdoor *Penicillium* spp.Occurrence of fungal spores in the air is markedly seasonal because of their sensitivity to weather changes (Ste˛palska et al., 2005). Fungi produce varied forms of spores which are actively or passively released (blown away, rinsed-off or shaken out); however, their further fate usually depends on the wind (McCartney, 1994). Weather conditions affect the sporulation, dispersal and deposition of spores and their elements correlate with each other (Kasprzyk, 2008). Numerous studies have shown that exposure to fungi may be associated with acute toxic effects, allergies, and asthma (Burge, 2002). There is increasing evidence that fungal growth in indoors is a risk factor for the development of childhood asthma and allergies.

More than 189 fungal species are thought to produce allergens and they are linked exposure to high levels of fungal spores with episodes of asthma, some are regarded as the life-threatening (Denning et al., 2006). It was recorded that *Alternaria* and *Aspergillus* are the most common allergens responsible for severe allergic rhinitis and asthma., as well as severe and difficult asthma, which constitutes 5-10% of asthma cases. Among the fungi, genus*Alternaria* are the most common mold causing asthma and increase the severity of the disease and mortality. They mostly cause allergy in adults prone to respiratory infections (Denning et al., 2006). Ten types of *Alternaria* allergens have been identified of which a 31-kDa glycoprotein (Alt a1) is the most damaging and frequent one (Kasprzyk et al., 2015). The antigenic extracts of eight prevalent viable airborne fungi (*A. flavus*, *A. japonicus*,*A. fumigatus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia pallescens*, *Fusarium roseum*, and *R. nigricans*) were demonstrated >60% positive reactions in the skin prick test.

Present study aimed to survey aeromycoflora and the prevalence of aeroallegens of Kathmandu (Nepal) and Chittagong (Bangladesh). The study of distribution of fungal spores in the atmosphere of Kathmandu and Chittagong as well as the study of allergens produced by *Alternaria alternata, Aspergillusfumigatus* and *A. flavus* were the specific objectives of this research.

Material and Methods

The study was carried (2007) in Kathmandu; the capital of Nepal with warm and temperate with summer monsoon, the dry season extends from about mid-October until pre-monsoon showers in late-April and mid- May. The average annual precipitation is about 1295mm

and the highest precipitation was observedin September 25. The maximum temperature (31^oC) was reported, during the hottest month July in Kathmandu valley. During late spring to early summer (April –September), the minimum temperature reported was 20^o-20.3 ^{o} C. The coldest day of 2007 was January 14, with a low temperature of 1°C. The humidity is comparatively higher from July to September with 80.2% to 90%. The annual average humidity was 69.93-82.6% and the least humid month of 2007 was April with an average daily low humidity of 55%. The most humid month was August with an average daily low humidity of 73%. The highest sustained wind speed was 7 m/s, occurring on November 9; the highest daily mean wind speed was 4 m/s (March 29). The windiest month was March, with an average wind speed of 2 m/s. The least windy month was January, with an average wind speed of 0 m/s.

Chittagong is known as Port City and honoured as business capital of Bangladesh. The climatic condition of Chittagong was warm humid during the study period with maximum temperature 38.8^0C, minimum 25^0C, relative humidity 74-93% and precipitation range from 2.0-108".

The survey of atmospheric fungi was carried in different areas of Kathmandu valley (agriculture, densely populated, river bank and airport areas). Sampling of air fungi was done by exposing Gravity Slide. A total of 576 and 72 slides were exposed in the atmosphere of Kathmandu and Chittagong respectively. Slides were exposed for three times in a day from 6-10 A.M., 2-4 P.M. and 8 - 10 P.M. separately during the months, February/March/April (spring), May/June/July (summer), August/September/October (autumn) and November/December/January (winter). In Chittagong, different areas like densely populated area, ship breaking, airport, forest and railway were selected. The exposed, slides were returned to the laboratory for the further study. The number of spores per cm^2 was calculated and reported the population of fungi in different locations and seasons.

Study of allergens from *Aspergillus flavus, A. fumigatus* and *Alternaria alternata* was done by following methods of extraction of allergens from three fungi were separately described. Extraction of allergens from *A. flavus* as described byTilak, (1989) with some modifications. Extraction of allergens from *Aspergillus fumigatus* as described by Kim & Chaparas, (1979) with a minor modification. Extraction of allergens from *Alternaria alternata* by described Vijay et al., (1979) with a minor modification.

Determination of Molecular Weight of Proteins

Molecular weight of proteins was determined by running 8% SDS-PASE as described by Maniatis et al., (1989),using the Molecular Weight Markers (BSA, Actin, Carbonic an hydrate, Viral protein purchased from Sigma Co.) for determination of approximate molecular weight of allergens produced by the studied fungi as mentioned above. The gel was stained with Coomassie brilliant blue and photograph of the stained gel was taken.

Preliminary Intracutaneous Test of Allergens in Laboratory Animal

Intracutaneous test was done as the process described by Luduena & Hoppe (1952) with some modifications, in New Zealand white rabbit.

Results

Total 11,876 fungal spores were recorded by Gravity Slides Method with an average of 20.61 spores per slide from Kathmandu. Fungi belonging to 19 genera included in four different classes were isolated from the atmosphere of Kathmandu. Among the classes, the highest number of Deuteromycetes was recorded (96.12 %), followed by Basidiomycetes (1.52%) and Ascomycetes (0.90%). Among the air spora, Aspergilli/ Penicilli group was found highest percent of about 81.20 %, which is followed by *Cladosporium* species with 8.72% and *Stemphyllium* spp. with0.03% (Table 1).

Table 1. *Total number of fungal spores collected in different locations of Kathmandu by using Gravity Slide Sampler Method.*

S.No.	Fungal types	Agriculture	Densely populated	Riverbank	Airport	Total	%
I	Zygomycetes (*Mucor,* and *Rhizopus etc.*)	27	56	44	9	136	1.46
II	Ascomycetes	52	27	22	6	107	0.90
III	Basidiomycetes	79	51	37	13	180	1.52
IV	Deuteromycetes						
1	*Alternaria* spp	96	87	69	34	286	2.41
2	Aspergilli/Penicilli	3200	3645	2629	70	9644	81.20
3	*Bispora* sp	4	3	1	0	08	0.06
4	*Botrytis* sp	11	14	3	0	28	0.23
5	*Chaetomium* sp	2	1	2	0	05	0.04
6	*Cladosporium* spp	359	412	201	64	1036	8.72
7	*Curvularia* spp	85	41	46	20	192	1.62
8	*Drechslera* spp	19	8	12	6	45	0.38
9	*Epicoccum* spp	8	3	5	2	18	0.15
10	*Fusarium* spp	19	17	14	5	55	0.46
11	*Fusariella* sp	3	2	0	0	05	0.04
12	*Fusiform* spp	3	3	2	0	08	0.06
13	*Helmithosporium* spp	5	2	3	0	10	0.08
14	*Humicola* spp	3	0	3	0	06	0.05
15	*Leptosphaeria*	4	0	2	0	06	0.05
16	*Periconia* spp	4	0	0	0	04	0.06
17	*Stemphyllium* spp	2	0	0	0	02	0.03
18	*Torula* spp	5	2	3	1	11	0.09
19	*Trichothecium* spp	5	3	3	0	11	0.09
20	Unclassified	23	25	20	5	73	0.61
	Total	**4018**	**4403**	**3220**	**235**	**11876**	**100**
Percentage		**33.83%**	**37.07%**	**27.12%**	**1.98%**		

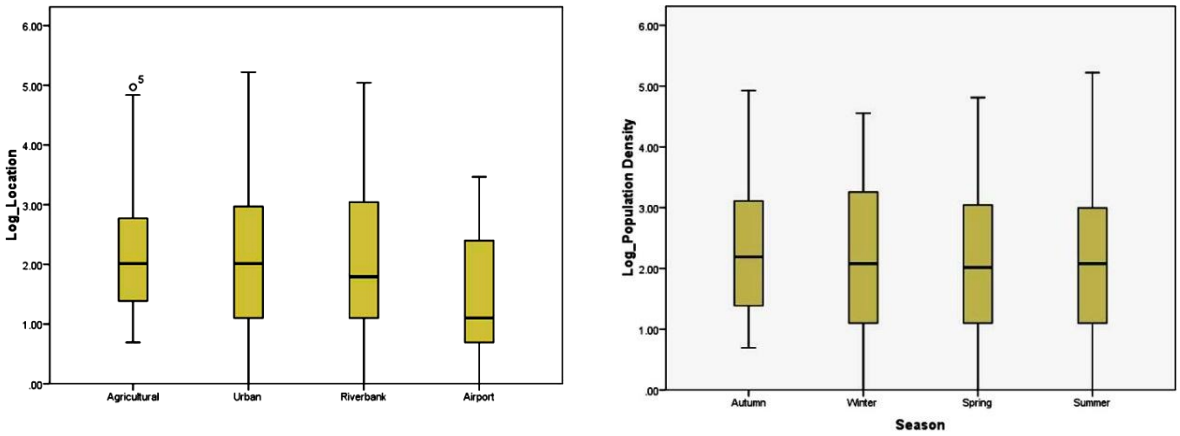

Fig. 1 Box-plots: i) *Location wise distribution of the population density of the fungi (Log_transformation of the values) and ii).Season wise distribution of the log population density in the atmosphere of Kathmandu.*

The spores of *Alternaria* spp in the air of different localities recorded the highest (126) number in agricultural area followed by urban-area (107) and lowest in the airport-area. The maximum number of colonies was found in spring season that was followed by winter and autumn and the minimum number of colonies was found in summer (Fig. 1 & 2).

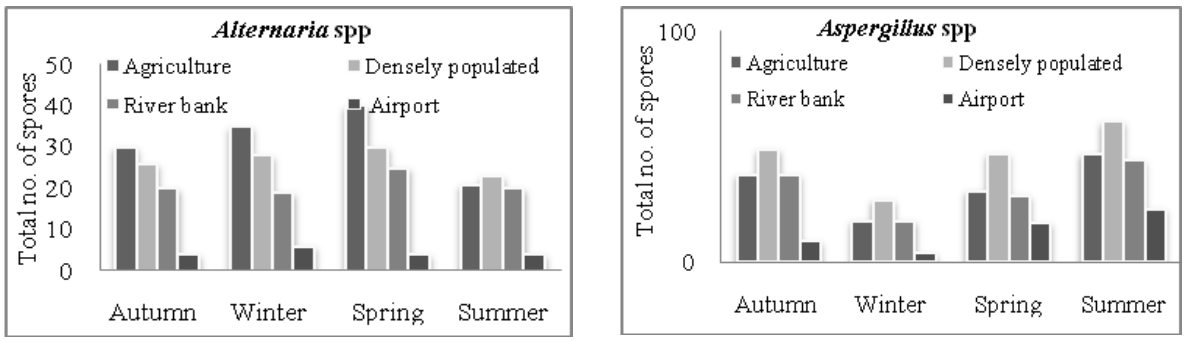

Fig. 2 *A: Distribution of Alternaria spp. B. Distribution of Aspeillus spp. in different localities of Kathmandu in various seasons.*

The prevalence of *Aspergillus* spp. from theair of Kathmandu was recorded in a range of 11.73% to 17.50%. *Aspergillus* spp. was reported the highest (185) in distribution number in densely populated area and lowest in the airport area. It was not much different during spring and winter similarly summer and autumn (Fig2B).

A total of 40 different fungal types from the atmosphere of Chittagong were identified. The spores of *Aspergilli/ Penicilli* group was reported in highest percentage (16.32%) followed by *Cladosporium* spp with 11.68% and *Tetraposporium* spp with 0.23% (Table 2, Fig. 3).

Table 2. *Total fungal spores recorded from Chittagong, Bangladesh*

S. No	Different isolates	DPA	SBA	FA	RA	AA	APA	Total	Percent
1	*Acremonium* spp	1		3	10			14	1.67
2	*Alternaria* spp		16	4	15	6	6	47	5.60
3	Aspergillus /Penicilli	42	12	35	19	9	20	137	16.32
4	*Ascospres.*	5	6	9				20	2.38
5	*Bagnisielia*		1		4			5	0.59
6	*Basidiospores*	1	6					7	0.83
7	*Bispora*	4	4	6	2	1	1	18	2.14
8	*Candida* spp	29	50		2			81	9.65
9	*Cercospora* spp	4	6	4	1			15	1.78
10	*Chytryds*			2				2	0.23
11	*Cladosporium* spp	22	55	10	11			98	11.68
12	*Colletotrichum*		4		2			6	0.71
13	*Corynesporella* spp		9			11		20	2.38
14	*Curvularia* spp		2	8	2	8	8	28	3.34
15	*Cunninghamella* spp	4						4	0.47
16	*Deightoniella*			1			2	3	0.36
17	*Dendryphiopsis*			3			1	4	0.47
18	*Drechslera* spp	6		3	4	4	1	18	2.14
19	*Daldima* spp	3	5		2			10	1.19
20	*Diplodia*				2			2	0.23
21	*Epicoccum* spp	3			1			4	0.47
22	*Fusarium* spp	11	6		3			20	2.38
23	*Haplosporella*				2			2	0.23
24	*Helminthosporium* spp			6	1			7	0.83
25	*Heterosporium*				2			2	0.23
26	*Humicola*			4	2			6	0.71
27	*Mucor/Rhizopus* spp	13	13	23		5	6	60	7.15
28	*Nigrospora* spp	3	2	8	4			17	2.02
29	*Phaeotrichoconis*		4					4	0.47
30	*Pithomyces*			3		4		7	0.83
31	*Populaspora*		2					2	0.23
32	*Sporidesmium*	2	4	3		1		10	1.19
33	*Stemphyllium* spp	3	1				1	5	0.59
34	*Tetraploa*			3		4	2	9	1.07
35	*Tetraposporium*		2					2	0.23
36	*Torula* spp	19	5	5	1			30	3.57
37	*Trichoderma* spp	6	8	4	11	6	12	47	5.60
38	Uredospores	3				11		14	1.67
39	Hyphal fragments	1	2	6	5	6	3	23	2.74
40	Pollens				1	7		8	0.95
41	Insect scale		3	2	4	2		11	1.13
42	Unidentified	1	2	4		2	1	10	1.19
Total		**186**	**230**	**159**	**113**	**87**	**64**	**839**	
Percent		**22.17**	**27.41**	**18.95**	**13.47**	**10.37**	**7.63**		

Note: *DPA- Densely populated area, SBA- Ship-breaking area, FA -Forest area (Chittagong University area), RA - Railway area, AA - Agriculture area and APA- Airport area.*

According to Friedman test, the p-value for the chi-square test is 0.853. The distribution pattern of different fungi in various locations of Chittagong is not significantly different. The difference among mean ranks of the fungi of the six areas may be due to chance or sampling fluctuation (Table 3).

Table 3. *Distribution of fungi in different locations of Chittagong by Gravity Slide Sampler Method*

S.No.	Area	N	Mean	Std. Deviation	Mean Rank	Test Statistics[a]	
1	Densely populated area	4	13.00	19.442	3.38	N	4
2	Ship-breaking area	4	6.50	4.435	3.25	Chi-Square	1.971
3	Forestry area	4	12.75	14.863	4.38	df	5
4	Railway area	4	9.25	7.500	3.75	Asymp. Sig.	0.853
5	Agriculture area	4	5.50	3.317	2.62	a. Friedman Test	
6	Airport area	4	9.00	8.756	3.62		

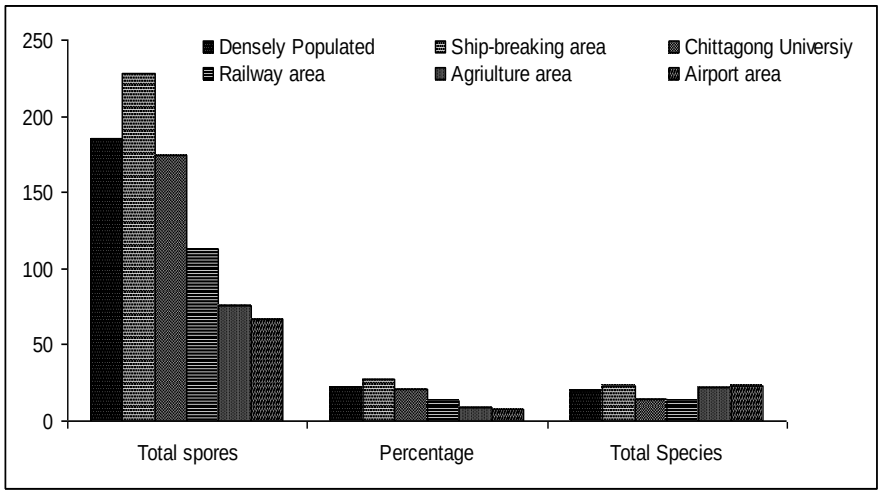

Fig. 3 *Distribution of different fungal spores in different locations of Chittagong.*

Molecular Weight of Proteins Determination by SDS-PAGE

Qualitative analysis of protein was done by determination of the molecular weight, SDS-PAGE to determine molecular weight of allergens produced by *Aspergillus fumigatus, A. flavus* and *Alternaria alternata*. The result showed that proteins extracted from mycelial mat and culture filtrate of *Aspergillus fumigatus, A. flavus* and *Alternaria alternata with common* the most common fraction of protein of about 50-67 kDa and about 20-30 kDa. The mycelial mat of *Aspergillus fumigatus* and*Alternaria alternata* with 20-30kD is regarded as the most allergic fraction. The SDS-PAGE showed that allergens from all three organisms contained the common bands of about 67 kDa. The bands of about 17 kDa molecular weight were more prominent in *Aspergillus fumigatus* and *Alternaria alternata* (Fig. 4A).

Preliminary Test of Allergens in Experimental Animal

Allergens were tested in experimental animals from extracts of *Aspergillus fumigatus, Aspergillus flavus and Alternaria alternata*. The size of wheal developed by different organisms was measured. The highest mean size of wheal was produced by *Aspergillus fumigatus* followed by *Aspergillus flavus.* Duncan multiple comparison tests, average wheal sizes (in mm) developed from each of four levels of time are not significantly different among themselves. However, each of these two levels (i.e., 80 minutes and 110 minutes) of time and, Time with level 20 minutes and time with level 50 minutes are significantly different from each other on the average wheal sizes (in mm). The highest wheal size in mm on the average of (23.67 mm) is developed from organism after 50 min by *Aspergillus fumigatus* with a standard deviation of value, 1.528 mm. It is followed by other organisms *Aspergillus flavus* with wheal size 22.33 mm. The highest wheal size in mm on the average of (19.33 mm) is developed from organism, *Aspergillus fumigatus* with a standard deviation of value, 1.528 mm after 20 minutes. It is followed by *Aspergillus flavus* with wheal size 15.33 mm (Fig. 4B).

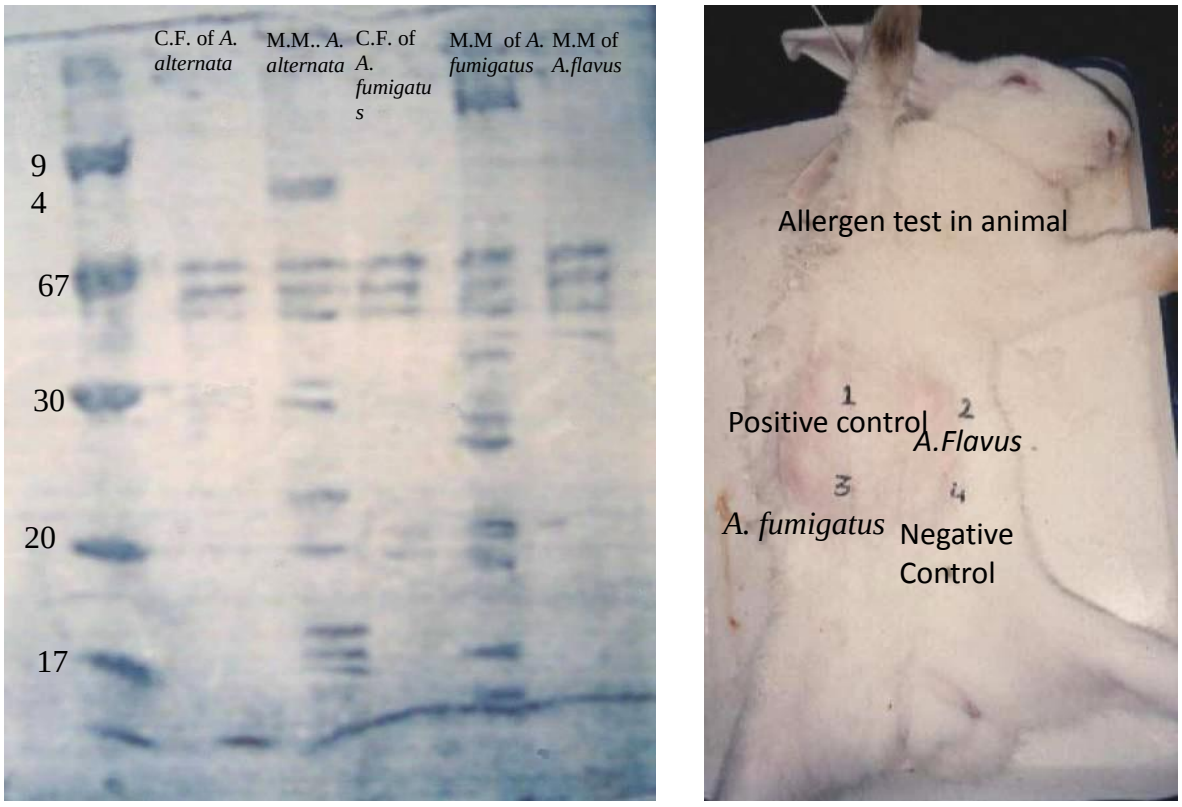

Fig. 4 *A. SDS-PAGE Lane 1. Molecualr Weight Marker, 2.Culture filtrate, 3.Mycelial mat of Alternaria alternata, 4.Culture filtrate, 5. Mycelial matofAspergillus fumigatusand6. Mycelial mat of Aspergillus flavus and B. Preliminary test of allergens in experimental animal*

Discussion

During the study *Aspergillus* spp. was found the most frequent dominant and prevalent among all isolates and recorded in all seasons, accounting 14.5% of the total air spora. However,*Aspergillus* spp. was reported 6.79% from Athens, GreecePyrri & Kapsanaki-Gotsi (2007); 47% and 12.5% from Kathmandu (Upadhayay et al., 1988 and Devkota &Sharma 1989); 27.7% in Kuwait (Khan et al., 1999);1.63% from India (Mane, 2002); 1.14% from Santa Fe city, Argentina (Basilico et al., 2007); Awad, (2005) reported 11.2–

38.9% of *Aspergillus* in Egypt and its incidence related to local micro-environments and urbanization. The highest spores (26.27%) of *Aspergillus* were recorded during autumn and the lowest (13.33 %) during winter. The fungi are abundant in distribution during favourable season because of temperature, saprophytic nature, atmospheric humidity, rain-fall and wind velocity. But particularly during summer the atmosphere is warm and humid, which is suitable for the growth and dispersal of spores of *Aspergillus*. Thus, the burden of spores played key role to cause allergic during the season by *Aspergillus.*Award, (2005) reported *Aspergillus* (11.2–38.9%) of the total spores and 38.9% of thisgenus common in the cultivated and urban areas and related to local microenvironments and urbanization.

The molecular wt. determination of*Aspergillus fumigatus*and *A. flavus* showed common bands of 30-67 kDa. *Aspergillus fumigatus*showed more prominent bands and allergenic bands of 20-30 kDa. Similarly, *Aspergillus fumigatus*produced the highest wheal size in on in vivo test. Thus *Aspergillus fumigatus* was recorded as the most allergenic as other studied fungi.

Alternaria ranked second contributing 9.97% to the total airspora. *Alternaria* spp. were recorded more frequently from agriculture areas (28.23%) and densely populated areas (36.27%), where more vegetation and plant materials were available and the lowest (6.34%) in the airport area. Atluri et al., (2002) reported 37% of *Alternaria* spp from agriculture area, Srikakulam, India.This indicating the occurrence of spores in the air correlated with the incidence of leaf spot and leaf blight disease on plants and saprophytic nature of the fungi. It was isolated throughout the year but showed more prevalent during winter with high humidity. Award, (2005) also reported *Alternaria* (7.5–59.9%) of the total fungal spora and42–59.9% of *Alternaria* spp from agriculture area and concluded that vegetation was considered its main source. Whereas, different authors reported various percentage of *Alternaria* spp from different regions, which are; 0.3% from Dublin (O'Gorman & Fuller 2008); 12.5% from Kathmandu (Devkota. & Sharma 1989); 1.9% from Santiago, Chile Henríquez et al. (2001);7.37% from Srikakulam(Atluri et al., 2002); 5.12% from Vaijapur', India(Mane 2002); 31.73% from Rohtak city, Haryana(Dahiya & Gupta 2003); 2–4.7% in Croatia (Pepeljnjak & Segvi 2003) and 8.68% from Santa Fe City, Argentina (Basilico et al., 2007). Kasprzyk and Worek (2006) also reported that the fungal spore concentrations would be higher in the countryside than at the urban site, particularly for taxa known to be plant pathogens, such as *Alternaria solani, A. tenuis* etc.

During this study the maximum concentration of *Alternaria* was recorded during spring season (28.65%), followed by winter (27.69 %), autumn (23.90%), summer (19.91%). The minimum spore concentration was found during rainy days. Spore load of *Alternaria* increased following rainy days but was found more in late spring with dry weather. The increase of spore after rainy days indicates this period favours the growth and sporulation, however the dry weather helps favours the spore liberation causing subsequent increase in conidial load in the atmosphere. Similar results were reported by Kasprzyk et al., (2004). Stepalska & Wołek (2005) found that spore concentrations are known to show a considerable daily, seasonal and annual variability. It was also reported that the conidia of *Alternaria* were prominently occur in the long duration as air spora of many European countries.Oliveira et al., (2009), Maya-Manzano et al., (2012) reported that the fluctuation of airborne fungal spores due the influence of geographic location, humidity, distance from the sea, climatic zone (latitude) and geobotanic conditions on their daily concentrations. In the present study SDS-PAGE revealed the 30-67 sharp bands which were the allergenic

band. Allergens extract of *Aspergillus fumigatus* and*Alternaria alternata* with 20-30kD is regarded as the most allergic fraction. Kasprzyk et al., (2015) also identified ten types of *Alternaria* allergens of which a 31-kDa glycoprotein (Alt a1) was found the most damaging and frequent one.

Conclusion

The distribution of aeroallergens more or less uniform throughout the study period, however, the no. and types of air spora was recorded more in certain areas like ship-breaking areas of Chittagong than in Kathmandu. The most allergenic fungus was found *Aspergillusfumigatus* among the studied fungi among the three studied fungi.

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HEALING WITH ANIMALS IN NEPAL

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ABSTRACT: Healing with animals or Zootherapy constitutes an essential part of the traditional pharmacopoeia of Nepal. Most of the zootherapeutic practices are on the verge of extinction because of rampant urbanization of cultures. Present paper studies and documents such vanishing animal healing practices amongtwo ethnic groups *Pahari* and *Danuwar* from the middle mountainous region of Nepal. Data have been collected by employing tools such as participant observation, questionnaire survey, structured interview, semi-structured interview and participatory rural appraisal. Both the groups are found to use animals in curing a number of ailments. It is interesting to note that some of the neglected animals are found to be of tremendous medicinal value. Zootherapeutic potential of such species can lead to their economic and cultural valorization. Their value in providing essential protein supplement to the people will be equally important. These animals could also substitute other endangered animals which are still in use in present day zootherapy. The study also provides base-line data for further research and valuable inputs to biodiversity conservationists.

KEY WORDS: Zootherapy, Ethnozoology, *Pahari*, *Danuwar*, Nepal, Himalaya

Introduction

Healing with animal parts or products is called Zootherapy. Zootherapy is popular among ancient cultures all across the world (Alves and Rosa, 2006; Kakati et al., 2002; Lev, 2003; Lohani, 2010a; Lohani, 2010b; Mahawar and Jaroli, 2007; Quave et al., 2010). Animal based medicines are usually obtained from animal body parts, metabolic products and other bodily secretions or non-animal materials such as nests and cocoons etc. (Costa-Neto, 2005). Not only ancient systems, modern medical system also utilizes animal based medicines. World Health Organization has reported that out of the 252 essential chemical selected, 11.1% come from plants, and 8.7% from animals (Marques, 1997).

People in Nepal have been using animals of their vicinity in a variety of ways. Of all other uses medicinal use has been found to be the most popular one. Zootherapy has been playing important role in the traditional pharmacopoeias of ancient cultures of Nepal. The ancient Nepali encyclopedia "Chandra Nighantu" describes some of the medicines derived from animal parts and products to treat a number of human ailments (Devkota, 1968). Some of the zootherapeutic practices are still prevalent among many ancient populations even though there is well developed modern medical system in the country. Need for systematic documentation of such ethnomedicinal practices has been emphasized by Lohani (2011) and the study shows that people in Nepal still have faith in zootherapeutic remedies.

Less attention has been paid to study and document the traditional knowledge regarding phenomenon of zootherapy. So this pioneer paper studies the traditional knowledge on

medicinal uses of animals in two ethnic groups *Pahari* and *Danuwar* in the middle mountainous region of Nepal before this traditional knowledge disappears forever. The study provides baseline data to carry out further investigation on animal medicines that are of high quotation frequencies. Some of these medicines could even prove to be the potential sources of future pharmaceutical agents. The paper also provides valuable information to the conservationists. It is suggested that the animals that are widely utilized in zootherapy be kept in highest priority of conservation.

Methods

Study area and study groups: Present ethnozoological studies have been conducted in two small villages of *Paharis* and *Danuwars* in the Kavrepalanchok and Lalitpur districts respectively.

Quantitative data were obtained by using techniques such as questionnaire survey and structured interview. For household level survey, a representative sample of 15% of the total households in each of the groups was obtained by simple random sampling method. Qualitative data were obtained by participant observation, semi structured interview, participatory rural appraisal (PRA) and key informant's interview. Fauna were identified using different taxonomic keys (Grimmet et al., 2003;Shah and Tiwari, 2004; Shrestha, 1981; Shrestha, 2003). Because of the strict conservation law of the country voucher specimens were not collected. Animals after identification were set free in their natural habitat.

Result

Zootherapeutic knowledge and practices: A total of 19 animal species were reported as being used in 22 traditional zootherapeutic remedies for either human or animal health purposes. A detailed description of these remedies is given in Table 1.

Faunal classification: Only three animals are Invertebrates and the rest are all Vertebrates. Only one animal is from the Phylum Arthropoda and two faunal spp. from the Phylum Mollusca. Sixteen animal spp. are from the Phylum Chordata(Class Teleostomii 4 spp., Class Aves 8 spp. and Class Mammalia 4 spp.).

Different categories of use reported: All together 22 categories of use were reported (Fig. 1). These include applications for the treatment of various forms of ailments. Reported categories in both the groups were reconstituent, neutraceutical, antirhinitic, antiasthmatic and cold remedies, anti-rheumatic, anti-arthritic, and night blindness, anti-gastritic, emollient, haemostatic, vulnerary, bed-wet controlling, stammer correcting, aphrodisiacal, digestant, anti-pyretic, dysentery control, anti-jaundice, headache curing, plastering and magico- religious applications.

Discussion

Slugs serve as reconstituent and nutraceutical agents for both the groups. These are also used in fractures and healing of bones in both.Use of eggs of Pulmonates in sprains is reported from other researchers as well (Costa-Neto 1996).

It is interesting to note that while in some cases the same animal (or a similar species) is used in the both the groups, the actual parts of the animal and the application often diverge. Examples of crab *Himalayapotamon*spp. and *A.bengalensis* can be cited to explain the above mentioned finding. In *Pahari* group roasted crab is used to stop bed-wetting whereas in *Danuwar*group cooked crab is used as reconstituents and neutraceuticals in case of tuberculosis and typhoid. Similarly tail of *Anguilla bengalensis* has magico religious value to *Paharis* while gall bladder of the same animal is used as cold remedy among *Danuwars*. Alcohol obtained by the fermentation of jackal flesh and local cereal is used as anti-arthritic applications whereas bone paste of the same animal is used to cure wounds. Similarly bile of *Histrix*sp. is used to cure headache while boiled gastro-intestinal tract along with its contents of the same animal is used as digestive disorder remedy. Same case is found in *Passer* sp. also where head has aphrodisiac value but its excreta are used in healing wound.

Bile of many animals has been found to be useful in curing a variety of ailments. In some cases it is taken orally while in others it is applied topically. Bile of *A. bengalensis, T.putitora,* and *S. plagiostomus* is used as remedies in cold, asthma and headache respectively. Use of bile and gall bladder in curing malaria, typhoid and tuberculosis has been reported by Solanki et al. (2004) from India.

Fat of a number of animals of the Classes Aves and Mammalia has been found to be used as vulnerary, emollient and anti-rheumatic agents. Similar finding has been reported from Brazil by Begossi et al. (1999), where fat of ray fish is used to cure rheumatism.

In some of the animals exoskeletal parts such as scales (*Manispentadactyla*) and feathers (*Adreola*spp.) have been used as anti-asthmatic and haemostatic applications.

Conclusion

It is found that animal-based remedies constitute an important part of traditional medicine in both the study sites. But there exists a significant difference between the two groups in actual application and preparation of animal products. There are cases where the same animal part has been used to cure entirely different ailments. It has also been found that some of the easily available but neglected animals such as crabs, slugs and eels have tremendous ethnozoological value. Mass production and proper management of such animals could help in minimizing the existing protein gap in the country and also in providing animal-based medicines to the people. Such production could also provide substitute for the highly-valued but rare and endangered animals thereby contributing to the conservation of the dwindling biodiversity of the country. Present study also provides base-line data to carry out further research in finding essential biological compounds responsible for healing in some of the most cited animals. Zootherapeutic potential of the species that are usually regarded as “useless” can lead to their economic and cultural valorization.

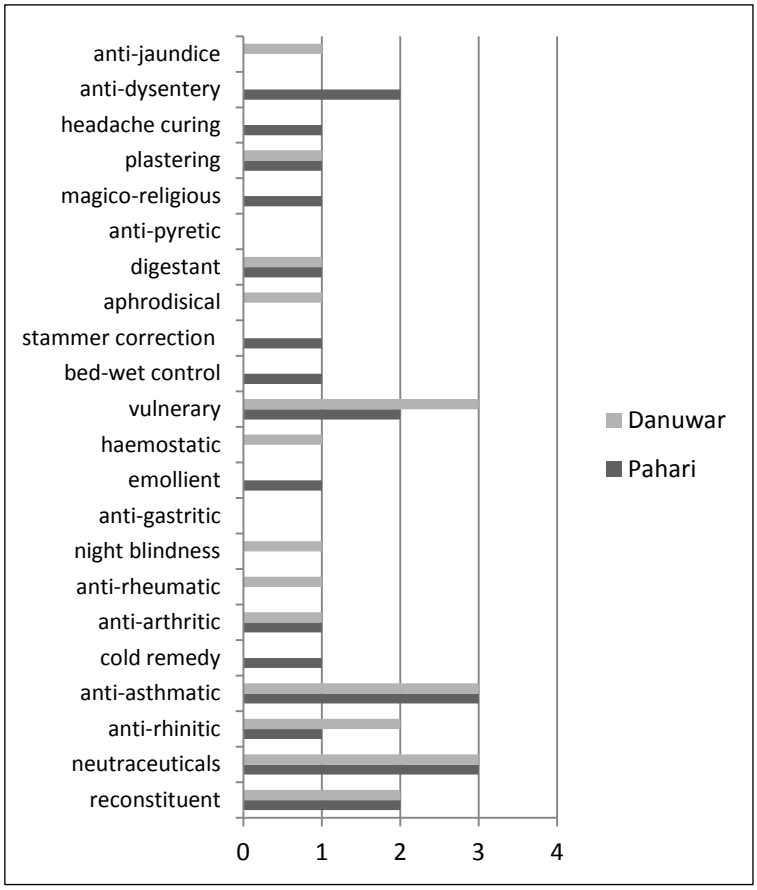

Fig. 1 *Different categories of use in Paharis and Danuwars*

Table 1 *Popular uses of zootherapeutic remedies among Paharis and Danuwarswith methods of preparation and applications. Fauna are provided with their common English and scientific name*

Phylum/Class/ Order	Scientific Name	Common English Name	Popular use *PahariDanuwar*		Preparation and Application
Arthropoda/ Crustacea/ Decapoda	*Himalayapota mon*spp.	Crab	Control bedwetting	Reconstituent/ Neutraceuticals	Roasted crab is given orally in both [P D]
Mollusca/ Gastropoda/ Stylommatophora	*Pila*spp.	Snails	Reconstituent/ Neutraceutical	*******	Cooked and eaten [P]
Mollusca/ Gastropoda/ Stylommatophora	??	Grey Slugs	Reconstituent/ Neutraceutical Plastering materials	Same as those of *Pahari*	Raw slug is swallowed whole. [P D] For plastering, raw slug is ground with stinging nettle root and applied on the fracture site.[P D]
Chordata/ Teleostomii/ Symbranchiformes	*Amphipnouscu chia*(Ham.)	Mud eel	**********	Anti-asthmatic Anti-rhinitic	Gall bladder of the fish is either used fresh or sun dried and stored for future use. In case of need, it is rubbed against a piece of stone with a little bit of water to obtain its paste. The paste is administered orally. [D]

Chordata/ Teleostomii/ Anguilliformes	*Anguilla bengalensis*(Gray &Hardw)	Fresh water eel	Magicoreligious	Anti-asthmatic Anti-rhinitic	Dried tail of the fish is soaked in water and the decanted water is given to the woman at time of her delivery. It is believed that the fish soaked water helps her to have normal and easy delivery. It is also believed that it facilitates easy expulsion of placenta. [P] Dried gall bladder is soaked in water and rubbed on the stone to obtain its smooth paste. [D]
Chordata/ Teleostomii/ Cypriniformes	*Tor putitora* (Ham.)	Carps or Mahaseer	*******	Anti-asthmatic	Bile is administered orally to cure asthma. [D]
Chordata/ Teleostomii/ Cypriniformes	*Schizothoraxplagiostomus*(Heckel)	Hill-stream trout	Digestant In curing headache	********	Gastrointestinal tract along with its contents is boiled in water and eaten to cure digestive disorder, body ache and to increase appetite. [P] Bile is eaten in curing headache.[P]
Chordata/ Aves/ Passeriformes	*Myophonuscaeruleus* (Scopoli)	Blue Whistling Thrush	In curing stammering Neutraceutical	*******	It is believed that oral intake of fresh uncooked blood corrects stammering and tuberculosis. [P]
Chordata/ Aves/ Columbiformes	*Columba*spp.	Pigeon	Anti-rhinitic	*******	Cooked meat is eaten to cure cough and cold. [P]
Chordata/ Aves/ Passeriformes	*Sturnus contra* (Linn)	Asia Pied Starling	Anti-dysentery	*******	Head is chopped off the body and thus oozing warm blood is sucked in to cure blood dysentery. Some people eat cooked meat of the bird to cure dysentery. [P]
Chordata/ Aves/ Passeriformes	*Passer* spp.	House & tree sparrow	********	Aphrodisiac (EV) Vulnerary for wounds &burns	Head part of the killed bird is inserted in a ball of kneaded flour and fed to he-buffalo. It is believed that it enhances the buffalo's sexual desire. [D] Feces of the bird are topically applied to the wound to accelerate the process of pus formation for quick healing. [D]
Chordata/ Aves/	*Athenebrama*(Temminck)	Spotted Owlet	******	In curing night	Head of the bird is cooked and eaten to

Strigiformes				blindness	cure night blindness. [D]
Chordata/ Aves/ Charadriformes	*Vanellus*spp.	Red-wattled Lapwing	*******	Digestant Anti-jaundice	Eggs of the bird are supposed to cure jaundice and gastritis. [D]
Chordata/ Aves/ Ciconiiformes	*Ardeola*spp.	Indian Pond Heron	*******	Haemostatic	Down feathers are topically applied to the fresh cut wound to stop bleeding. [D]
Chordata/ Aves/ Galliformes	*Gallus gallus* (Linn)	Domestic fowl	Vulnerary for wounds Emollient Magico-religious	Vulnerary for wounds and burns in human and ox (EV) Anti-rheumatic	Fat is topically applied to cracks and burns and to soften skin. Cocks are used in faith healing. [P] Fat is topically applied to cure burns and body is massaged with fat to cure rheumatism. It is also applied to cure wound in the neck region of oxen caused by the friction of the yolk as a result of continuous plowing for longer period.[D]
Chordata/ Mammalia/ Rodentia	*Hystrixindica*(Kerr), *H. brachyura* (Linnaeus)	Porcupine	Anti-asthmatic In cough and cold.		Gastrointestinal tract along with its contents is boiled with water and the soup is taken orally to cure asthma. Its flesh is also cooked and eaten to cure cough and cold.Items such as meat and gastrointestinal tract are also sun dried and preserved for future use as medicine. [P]
Chordata/ Mammalia/ Carnivora	*Selenarctosthibetanus*(Cuvier)	Himalayan Black Bear	Anti-asthmatic Cures dysentery	*******	Body parts like fat and liver are eaten to treat asthma and dysentery. These parts are even dried and preserved for the future use. [P]
Chordata/ Mammalia/ Pholidota	*Manispentadactyla*(Hodgson)	Scaly anteater	Anti-asthmatic	********	Scales are rubbed against the stone with a little bit of water to obtain its smooth paste. The paste is then administered orally three times a day to treat asthma of initial phase. [P]

Chordata/ Mammalia/ Carnivora	*Canisaureus*(Linnaeus)	Golden Jackal	Anti-arthritic Vulnerary for wounds	Anti-arthritic Vulnerary for wounds Neutrace-uticals	Alcoholic beverage obtained from the fermentation of the mixture of jackal meat and local grains, is used as medication. The body is massaged with it to cure gout and arthritis. Bone paste obtained by rubbing on stone is applied to wounds. Other body products such as fat and bile are also used differently to cure a number of ailments. [PD] Meat is eaten to cure above ailments among *Danuwars*.

Abbreviations used to denote ethnic groups:P: *Pahari,* ***D:*** *Danuwars,*
Types of application: E: *Ethnoveterinary remedy*

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ANIMALS IN SOLID WASTE MANAGEMENT

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ABSTRACT: With the raise of standard of people, the waste production increases. Therefore, it is the problem of today as well as of tomorrow. As the waste is everyone's business, so should be waste handling everyone's responsibility. When the waste is disposed without segregation at source, the mixed waste has little value. Further, when it is disposed haphazardly, it will lead to loss of beauty of the landscape, pollution of every type, loss of resources and hazardous growth of animal pests, vectors and disease organisms. The uncontrolled growth of pigeons and crows, sustenance of large number of street dogs and encroachment of monkey population in human territory are the some examples of haphazard disposal of mixed waste in Kathmandu valley. If the human behavior changes the other way round and every person and household segregate the solid waste and generate biodegradable and non-degradable components at the source, both types of wastes will be usable for material and energy recovery. Carefully collected source segregated biodegradable waste can be used as animal feed as has been practiced in many parts of Nepal. Further, a well-designed food chain can be established based on the biodegradable waste to generate high-level animal products. For example, the vegetable market waste can be first fed to cattle and their discharge can be further vermicomposted to produce high-level manure. Similarly, waste can be pit composted to rear house fly larva or bin composted to rear soldier fly larva so as to supply nutritious feed to poultry or any other domesticated fowl. Therefore, the using of well-designed sequence of animals can be very helpful ways of treating organic waste at the source, either at household or local community levels.

Introduction

Background

The composition of waste generated can be roughly considered as the indicator of social and economic status of the generator. Another truth is that the richer we get, the more we discard (OECD, 1999). As shown in Fig. 1, the waste generation is not dependent on population statistics rather on the GDP of the OECD countries. Therefore, solid waste is an ever-increasing problem and the challenge of the future world. Whole world is trying to address this challenge from every angle. At this point, we cannot forget that waste is everyone's business and is, therefore, everyone's responsibility.

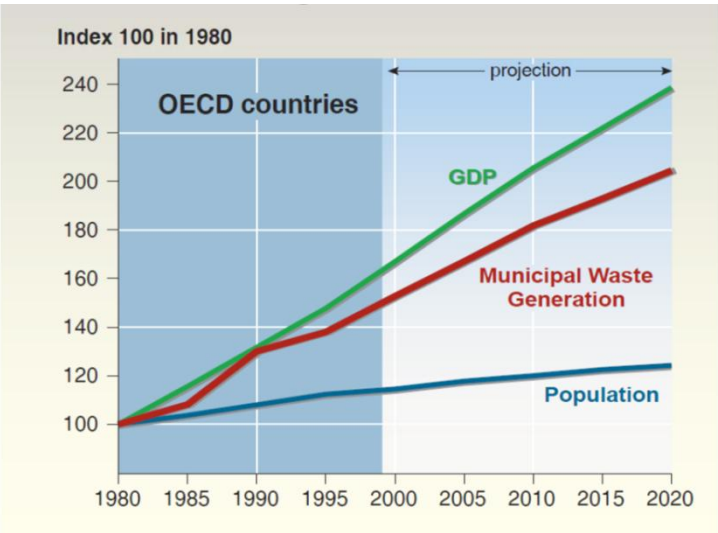

Fig. 1: *Graph showing relationship among GDP, municipal waste generation and population of OECD countries from the year 1980 to 2020. Source: The organization of Economic Cooperation and Development (OECD), 1999.*

When we carefully analyze the fundamental components of integrated solid waste management (ISWM) as shown in Fig. 2, we find that all the six components are equally important. However, if the source of waste generation do not make any effort, then all wastes are either disposed or incinerated leading to land wastage, air, water and land pollution, wastage of resources (materials and energy), growth of unwanted animals, plants and microorganism (eutrophication), etc. For this reason, modern waste management strategy put emphasis on the waste handling at the source of generation, so that reduce, reuse and recycle (3Rs) principle can be applied in various levels for material recovery and waste minimization. Non-Hazardous Waste Management Hierarchy (USEPA, 2013) places emphasis on reducing, reusing, and recycling the majority of wastes (Fig. 3). Key to the implementation of 3Rs principle is the waste segregation at the source. Just the process of segregation of discarded useless household wastes at the source of generation produces two value added products: biodegradable materials that can be used for multiple purposes and non-degradable material, which can serve as raw materials for various industries (Fig. 4). This simple step not only promotes the environmental, ecological, economical and material benefits, but also discourages the growth and multiplication of unwanted animals. Further, after this step, the animals of various types can be safely used in implementing 3Rs principle.

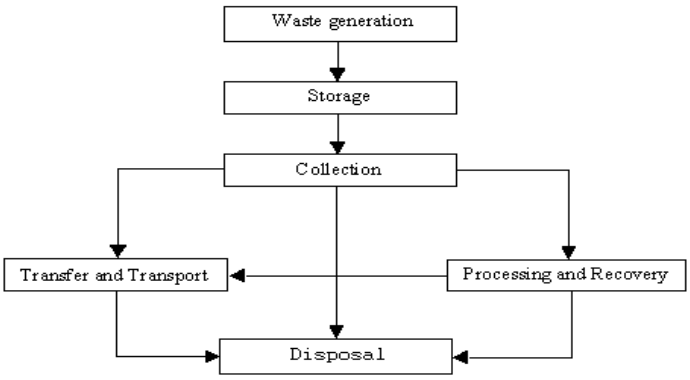

Fig. 2: *Graph showing the six elements of integrated solid waste management. Source:Tchobanoglous et. al., 1993*

Fig. 3: *Graph of a hierarchy ranking the most environmentally sound strategies for the management of non-hazardous municipal solid waste. Source: USEPA, 2013*

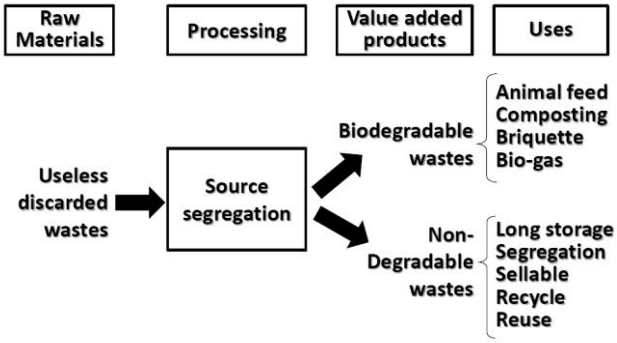

Fig. 4: *Graph showing how the simple process of segregation of solid wastes at the source produces two value added products of economic importance.*

From this consideration, we come to know that animals are playing two opposite types of roles in the waste management system. Unwanted animals can grow uncontrolled in the haphazardly disposed wastes causing ecological and environmental problem as well as threat to the human health. However, if we grow selectively chosen animals in controlled way in the wastes, they can be great boom in the waste management. This paper will discuss these two opposing roles of animals in waste management system.

Objectives

- Evaluation of uncontrolled population growth of unwanted animals in the unmanaged waste disposal system
- Exploration of the possible uses of different animals in integrated solid waste management system

Limitation

This paper has not discussed all the animal species that are associated with waste management. Further, new techniques can be developed to make use of new species of animal for the purpose. This has not been explored in the paper. Finally, neither the actual management of waste by the use of animals nor the damages caused by the animals proliferated due to the haphazard disposal of waste has been estimated in the article.

Methodology

Questionnaire survey

The status of monkey and street dog were investigated by the general survey using structured questionnaire in Kathmandu valley. For the monkey, the neighboring areas of the Pashupatinath forest, Swoyambhunath Forest, Mehpi temple and Mulstar Mall premise of Thapathali were investigated. For the survey of dog, two areas "dirty or polluted area" and "clean or unpolluted area" were chosen. The polluted areas surveyed were Bishnumati and Kalimati areas while the unpolluted areas were Kaldhara and Lainchaur.

Experimentation

Composting with various techniques: The different types of composting, like vermicomposting, pit composting, tumbler (Photo 1) and aerated bin composting were followed in a controlled environment. First, the species and population of invertebrates visible to naked eyes were enumerated in the known quantity of samples from each composting at different time interval. Second, the compost maturation and quality were investigated by growing tomato and chilly plants in the plant pot with each compost type and soil in standard proportion.

Photo 1: *Self designed tumbler for composting of kitchen wastes*

Vermicomposting with various earthworm species: Three species of earthworms, namely *Perionix*, *Lampeto* and *Esenia* were used for separate vermicomposting. The vermicompost from each species were used in different proportion of compost and soil to grow tomato plants in the plant pots. The height and number of leaves of each plant were noted down after one month period of plantation. Standard error was use to evaluate the result.

Secondary data

Several relevant secondary data were used for the article from different sources.

Result and Discussion

From Historical Perspectives

For thousands of years, human populations across the world have lived side by side with pigs that convert their unwanted kitchen scraps into pork (Colbert, 2013). Even long before that, people were keeping poultry, donkey, horse, camel, sheep, goat and other cattle in their houses. These animals were definitely feeding on food wastes and other biodegradable wastes. The dogs are supposed to be associated with human being from something like 30,000 years ago (Beam, 2009). Since that time, they are serving human beings as pet, security guard as well as waste feeders (Barbalace, 2003). Thus, the domesticated animals are serving as waste feeders from ancient time and helping naturally to manage household solid wastes.

As human started to settle in one place as communities, the waste produced by the households began to accumulate in the periphery of the settlement areas. The piles of wastes attracted many natural fauna searching for food. Because of waste piles outside of the cities and human settlement, several disease vector species of insects and other invertebrates, birds, shrews, rodents and other higher mammals proliferated unnaturally and in uncontrolled way causing several ecological imbalance, social nuisance and health hazards. One of the unfortunate hazards of this type of uncontrolled population growth of the animals in waste piles is the medieval pandemic outbreak of bubonic plague called Black Death that swept through Asia and Europe (National Geography 2008). Littering of food and other solid wastes in medieval towns - the practice of throwing wastes into the unpaved streets, roadways, and vacant land - let to the breeding of rats, with their attendant fleas carrying bubonic plague (Tchobanoglous et. al., 1993). It had eliminated one-third of European population within 5 years from A.D. 1347 to 1352 (The Middle ages 2001).

Almost in all countries, the food wastes have been used as feeds for domestic animals and to some extent to the poultry. However, this changed in 2001 when an outbreak of foot and mouth disease was linked to a farm that was illegally feedings its pigs unprocessed restaurant waste, and the English government – and later the entire EU – banned the use of leftovers in pigswill (Chynoweth, 2013). In USA, the food waste to be fed to pigs must be mandatorily heat treated (Barbalace, 2003; Westendorf and Myer, 2012). Similarly, campaign is going on in entire Europe to reuse food waste as pig feed again with necessary precautions (Colbert, 2013; Chynoweth, 2013).

Hazardous Growth of Animals in Haphazardly Disposed Solid Wastes

The best example of the hazardous growth of animals in haphazardly disposed solid waste is, as mentioned above, the bubonic plague that surged Asia and Europe in the 14^{th} century due to the uncontrolled multiplication of rat and flea in the disposed solid waste. Unmanaged landfilling of municipal solid waste (MSW) can serve as breeding ground for flies, mosquitoes, cockroaches and cause their excessive population growth (Pradyumna, 2013). The landfill site in Tansen Municipality had been closed after short period of operation in 2013 because of complain of local people. One of the reasons behind the forced closure is the intolerable population of flies. Such landfilling can also attract large avian fauna often resulting in airplane accidents. Besides aerial fauna, ground animals too grow excessively in the mixed MSW when not properly disposed. Some of the animals to be listed are shrew, rats, moles, cats, dogs, monkeys, etc.

*Street Dogs:*An estimate indicates that there are about 25,000 street dogs in Kathmandu Valley (Street Dog Care, 2012). Almost all of them survive on food waste, either intentionally given to them or found in the disposal sites (Table 1). Such a huge population of dogs removing food waste from waste stream can be considered as contribution in waste management. Further, these street dogs may have been helping communities in security. However, they are nuisance and harm to the society in many ways. While searching for food, they scatter the stored waste. When not enough, they do not hesitate to steal food from kitchen and other places. They pollute the environment by discharging their waste everywhere. The dogs have very high reproductive potential and grow rapidly every year. Uncontrolled and unsupervised growth of dogs in close association with human community is a great health hazard. Most of the street dogs are sick and infected with

various notorious diseases. Every year in Nepal, about 32,000 people are treated for dog bites and around 200 people, most of them children, die of rabies each year (KAT Center).

***Table 1**: A questionnaire survey carried out in more polluted area (Bishnumati and Kalimati) and less polluted area (Lainchaur and Kaldhara) clearly reveals that pollution of the area correlated to haphazard management of waste induce the proliferation and aggressive behavior of street dogs.*

Subject	More polluted area	Less polluted area
Waste management system	70% consider to be worse	88% consider to be satisfactory or good
Number of street dogs in the vicinity	50% claim to be 10 or more	50% claim to be less than 5
Behavior of street dogs	42% complain to be aggressive	22% complain to aggressive

Rhesus Monkey: There are around 1,000 rhesus macaque (*Macaca mulatta*), the best-known species of Old World monkeys, in Kathmandu Valley (Bhattarai, 2014). The Pashupatinath forest, Swoyambhunath Forest, Mehpi temple, Nibarahi premise, Bajrayogini temple of Sankhu and Mulstar Mall premise of Thapathali are major habitat sites of monkey in Kathmandu (Third Eye Foundation 2016). Because of distribution of the monkeys in the temple areas and because of long regular religious practice of offering fruits, cereal grains and other food items in the temples, the wild monkeys in the valley have switched themselves from the difficultly available natural foods to human given materials. Further, the openly disposed food wastes by the forests and riverbanks work as supplementary food sources for them. Without needing to do natural struggle, the monkeys in Kathmandu have become lazy, unhealthy and unnaturally attracted to human environments. Kathmandu is sustaining the monkeys' population artificially without natural predators and struggle. This has created conflict between people and monkeys.

In a questionnaire survey carried out in 2014, 63% surveyed people believed that the biggest problem monkeys pose to humans is stealing food from them, followed by biting (21.7%) and others(15.2%) which include creating havoc in the areas, dirtying areas and breaking pipelines (Fig. 5). In Mehpi, stealing food was perhaps the main and only problem while stealing and biting were the main problems in Thapathali. Other kinds of problems prevailed mainly in Swayambhunath and Pashupatinath, although stealing food was the most significant.

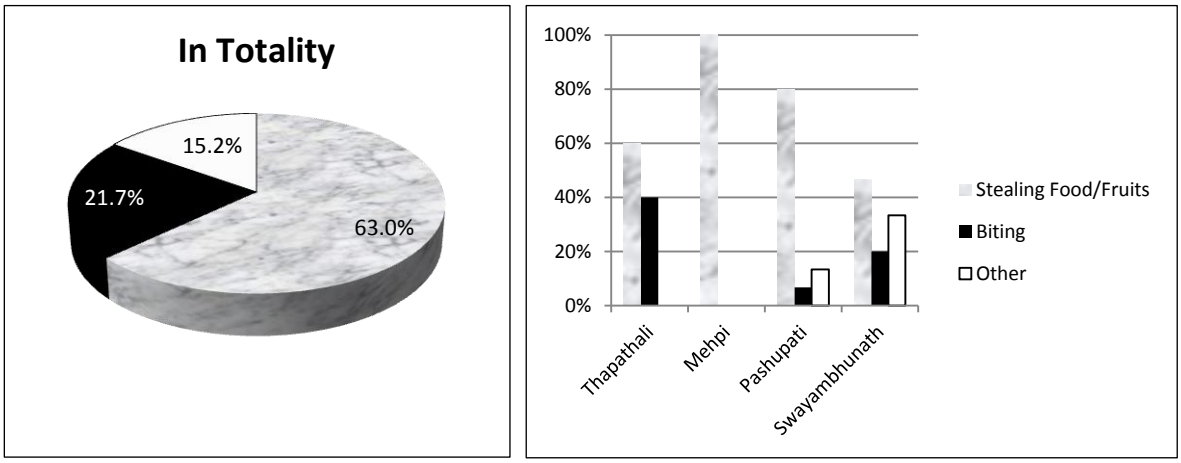

***Fig. 5:** Graph showing problems created by monkeys in totality and in different places of Kathmandu*

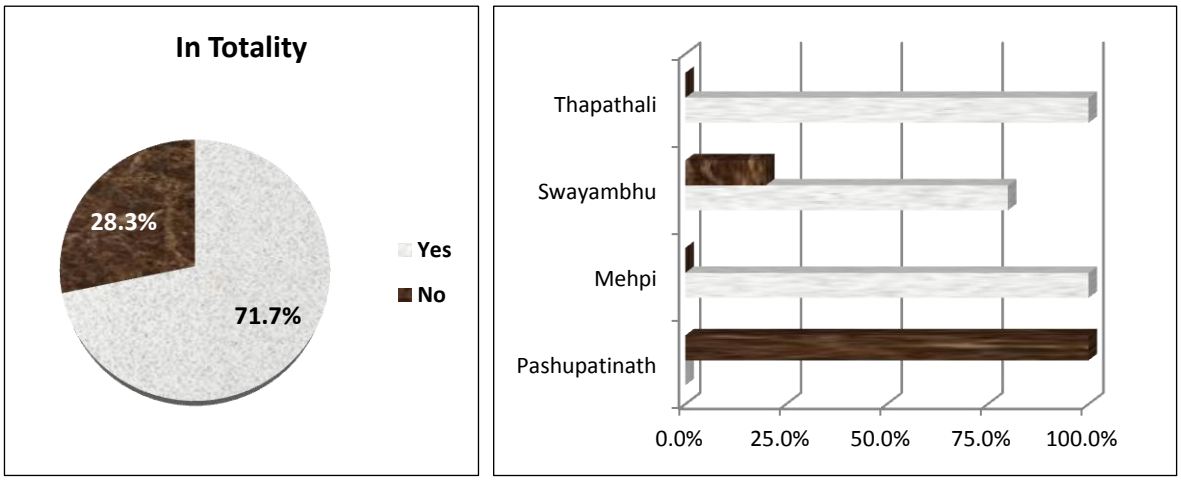

Fig. no. 6: *Graph showing people's perception that monkeys have become pest in totality and in different places of Kathmandu*

The lack of predators for these monkeys in the urbanized valley has decreased their mortality rates and increased their birth rates; so much that it is now suitable to term them as "pests" in Kathmandu (Fig. 6).

Pigeon and Crow: Another important organism that has changed from peace to pest due to haphazard disposal of food waste is the pigeon. Many people feed pigeons regularly for a religious belief of brining good fortune in their life. As mentioned above, the regular long religious practice of offering cereal grains and other food items in the temples is providing these birds lot of food. Further, the open disposal of food wastes in the streets, yards, gardens, fields, etc. is supplementing their food supply (Photo 2). In such protected human environment, the bird is increasing in exponential ratio. At the same time, it is also encouraging the predatory birds like crow in the valley (Rajbhandari, 2015a). However, their number, frequent flight in flocks, their feathers and droppings have already becoming nuisance to the people. Their droppings damage the beauty of the city, expensive buildings and historic artifacts. These pigeons are infected with various diseases, which are known harmful to human (Shutterbug, 2008). If often-reported bird flu or avian influenza A (H5N1) virus spread across the pigeon population in the most densely populated Kathmandu, the scope of the epidemics is unimaginable.

In this section, we can make a concrete conclusion that to avoid the epidemics of zoonoses like rabies, bird flu, plague, etc., to prevent the shifting of pets to pests and to conserve the natural integrity, people in Kathmandu must stop the open disposal of food waste. Further, the long religious practice of offering food items in the temple and feeding of animals and birds have to be gradually controlled. The beauty of flora and fauna is perceivable only in their controlled and balance growth.

Photo 2 : *Pigeons and crows feeding on haphazardly disposed wastes in a garden.*

Animals in the management of Solid Wastes

Domesticated Animals: As mentioned above, the domesticated animals are naturally helping us in the management of solid waste from ancient time. Especially in the rural and suburban areas, people see the resource value of biodegradable waste (kitchen and vegetable wastes) and do source segregation at the time of production. Such source segregated food wastes are either used at household level or collected by animal husbandries with or without payment. Even some of the aquaculture of catfish (indigenous Mungri, *Clarias batrachus*) is using meat, fish and food wastes as the fish food (Gurung, 2014). This type of model is found in VDCs and small municipalities of Nepal and is best exemplified by Dhankuta Municipality. Because of almost 100% source segregation and utilization of biodegradable waste at the source, the municipality collects only the non-degradable wastes for to be segregated and sold by the local waste handler (Rajbhandari, 2015b). All the five components of biodegradable municipal solid wastes, namely meat waste (MW) from butchers, fish waste (FW) from fishmongers, fruit and vegetables waste (FVW) from fruit and vegetable shops, restaurant waste (RW), and household waste (HW) have different characteristics. However, with careful collection methods, the contamination of waste can be avoided. Further, a simple heat treatment at 65^{o}C for 20 minutes can eliminate all the infectious agents from the food wastes and make them safe for animal feed (Garcia et al., 2005).

Another model of animal use in solid waste management is found in Integrated and Sustainable Solid & Liquid Resource Management (SLRM) developed by Mr. C. Srinivasan, the project director, Indian Green Service (Murali D, 2013). This model makes use of even abandoned cattle to feed on fruit and vegetable wastes to transform them into cow dong, which can be in turn utilized for biogas generation. The sludge of the biogas plant is further used for vermicomposting to generate the world's best fertilizer, the vermicompost. Furthermore, the model keeps ducks to utilize meat wastes from butchers and fish wastes from fishmongers as their feed (Srinivasan 2010).

Use of Detritivores: In an experiment, vermicomposting, pit composting, tumbler composting and aerated bin composting were carried out for several months to produce their respective compost. Then locally available tomato plants were grown in each compost mixing with soil in ratio of 1:3. As shown in Fig. 7, in 35 days, not the vegetative growth, but the production of fruits was significantly higher in the plants grown in vermicompost compare to other composts.

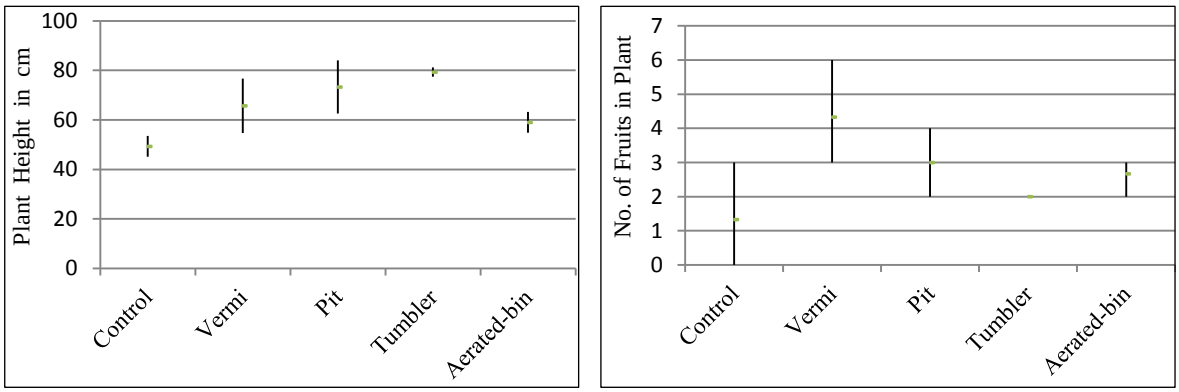

Fig. 7: *Graphs show 95% confidence limit of mean of height (left) and fruits (right) of tomato plants grown in different composts. Though the growth of the plants is better in other types of composts, the fruit yield is significantly higher in vermicompost.*

Table 2 shows the number of major invertebrate individuals present in 0.5 kg of compost from different compost plants at different stage of maturation. In the enumeration, earthworm was not counted as it was the major component of vermicomposting. As illustrated in the table, black soldier fly larvae were predominantly found in aerated bin composting, while the housefly larvae (maggot) occurred in the pit composting as well as in other types. The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), can consume a wide range of organic material in the fastest rate (Black, 2015); and has the potential to be used in waste management. In addition, the prepupae stage of this insect can be harvested and used as a valuable nutritious feed for animal livestock (Trinh et al., 2015). Same is also true with the housefly maggot. The Integrated and Sustainable Solid & Liquid Resource Management (SLRM) developed by Mr. C. Srinivasan (Murali D, 2013) also allows flies to breed on the organic wastes, because the maggots are voracious detritivores. It is not only the method of processing of organic wastes, but also the production of maggots as the very nutritious food for poultry and domestic fowl. Thus, the notorious insect can be wisely use to produce their larvae to be used in one hand in waste management and in another hand in feeding fowl the most nutritious feed.

Table 2: *Table showing the number of major invertebrates found in different compost during their maturation. The number is the counting in 0.5 kg of the compost.*

Major invertebrates	**Vermincomposting**		**Pit composting**		**Bin composting**	
	2 weeks	**4 weeks**	**2 weeks**	**4 weeks**	**2 weeks**	**4 weeks**
Black soldier fly Larvae	0	34	-	-	37	58
House fly Larvae	24	45	47	36	18	30

Efficiency of different earthworm species in vermicomposting: In another experiment, composts were produced separately from 3 popularly used earth- wormsspecies, *Esenia*, *Lampeto* &*Perionix* and used to grow local tomato plants (Fig. 8).

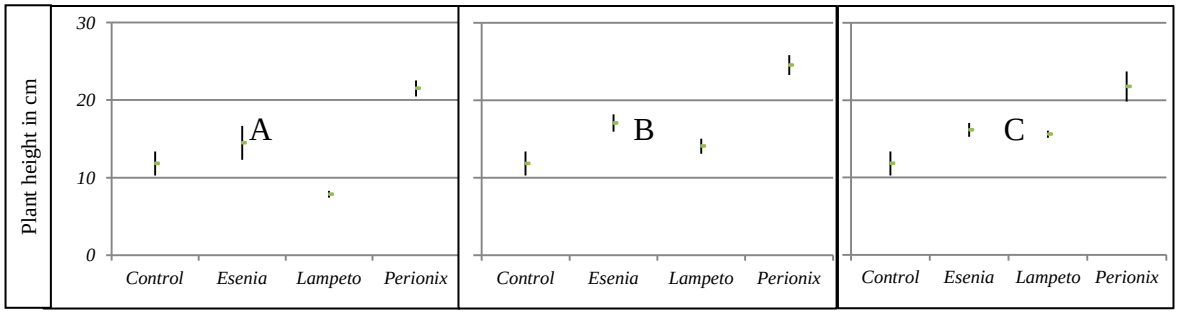

Fig. 8: *The Graphs show 95% confidence limit of mean height growth of local tomato plants within a period of two weeks in the composts of different earthworm species. Soil and compost mixing in A = 5:1, B = 3:1 and C = 1:1. The compost of Perionix showed the significantly higher growth in all but the best was in 3:1 combination. It looks that the compost of Lampeto needs to be mixed in higher proportion.*

As shown in the graph, the compost of *Perionix* showed the significantly higher plant growth of all and best growth in the soil-compost combination of 3:1. *Esenia* also showed the best plant growth in 3:1 soil-compost combination. However, the plant grew best in case of the compost of *Lampeto* in 1:1 combination. It will be too early to differentiate the composts of the three species of earthworms. One should follow not only the vegetative growth, but also the fruit production. Further, the compost produced by the mixed culture of all the three species should also be compared.

Conclusion

Animals of all kinds are heterotroph and dependent on plant or animal products. If the kitchen wastes are disposed haphazardly, unwanted animals proliferate and cause several hazards in the society. However, if the kitchen waste are segregated from other wastes at the source and used as feed for domesticated animals or for detritivores like earthworms, black soldier fly or house fly larvae in different composting plants, we can not only manage the organic wastes at the source and help in solid waste management, but also produce animal products to be used for different purposes. Well-designed use of animals in solid waste management can be great boom in green recovery of material and energy.

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HABITAT SUITABILITY ANALYSIS OF KEY MAMMALS: COMBINING CCA WITH GIS APPLICATION

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ABSTRACT: Understanding habitat requirements and potential habitats of wildlife species are inevitable for success of species conservation. Species have specific habitat preference that influences their distributions. A study was conducted to determine habitat use and potential habitat of the Himalayan Tahr (*Hemitragus jemlahicus*) and Musk deer (*Moschus chrysogaster*) in the *Sagarmatha* (Mt. Everest) National Park and Buffer Zone (SNPBZ). The habitat survey and species presence-absence data was used to establish patterns of species distribution through Canonical Correspondence Analysis (CCA). The recognized distribution patterns were used to visualize potential habitat over entire park and buffer zone in a spatially-explicit way by using Geographic Information System (GIS) application. We underline that the outcomes of this study can be useful for habitat management and species conservation.

KEY WORDS: Habitat use, Potential habitat, Himalayan Tahr, Musk deer

Introduction

Sagarmatha National Park (SNP) was established in 1976 with a mission to conserve and maintain the biological, cultural values, and scenic beauty of the park (DNPWC/SNP, 2006). The park provides habitat for many high altitude biodiversity including rare and endangered species of mammal such as, Snow leopard, Musk deer, Red panda, and Common leopards (Ale & Boesi, 2005; MFSC, 2002). These species are considered focal species for biodiversity conservation and park management (MFSC/DNPWC, 2003). The economic activities in the park are driven by tourism activities (Baroni et al., 2000; Stevens, 2003; Caravello et al., 2007). The human-centric activities can pose a serious impact on wildlife habitat specially, mammals that are seen as an indicator of ecosystem health and always been a key consideration in the park management (Jorgensen et al., 2005).

Research studies generate a better understanding of their population; threats and habitat requirements (DNPWC/SNP, 2006). The park has been a target of conservation due to its protected status- IUCN category II (Rogers and Aitchison, 1998) and their sensitivity to landscape level habitat alteration (Byers, 2005). UNESCO listed the park as a natural world heritage site in 1979 under criterion III because of its unique natural, cultural and landscape characteristics.

Habitat determination is a complex problem that requires consideration of various factors and their relationships. Assessments of habitat for a large area are not viable without a powerful tool. GIS was appropriate in addressing habitat potential (Dankas and Klein, 2002). In this context, using the habitat preference data from author's previous paper (Thakuri and Pradhan, 2010), further analysis is conducted to understand the habitat suitability and available potential habitat of Himalayan Tahr and Musk deer in the region for supporting protected area management.

Study Area

This study was conducted in the SNP and Buffer Zone (BZ) in the southern slope of Mt Everest. The SNP includes an area of 1,148 km² covering lower alpine forest areas to upper grassland and nival zone that extends from 2,845 to 8,848 m asl, the Sagarmatha (Mt. Everest)- top of the world. The BZ covers an area of 275 sq. km lying just outside the southern part of the park.

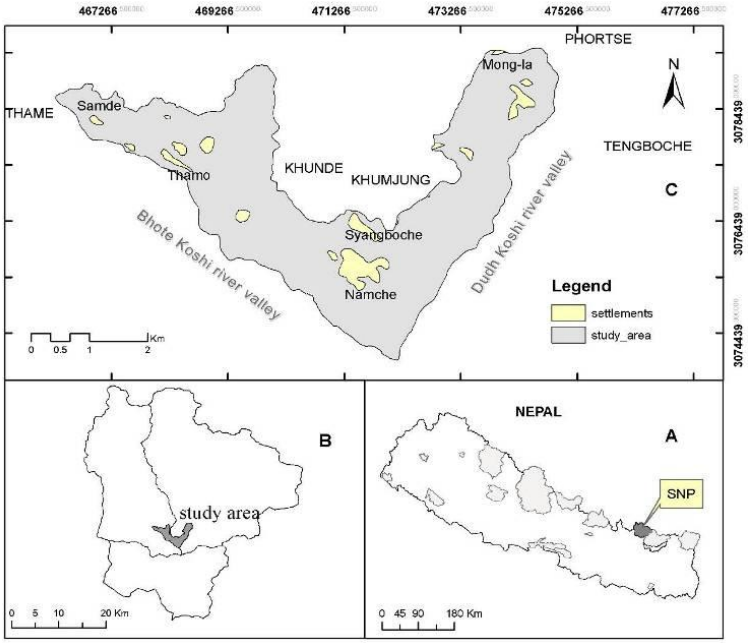


Fig. 1 *Map of study area: A) location of the Sagarmatha National Park in the map of Nepal, B) the Sagarmatha National Park and Buffer Zone, and C) enlarged view of survey site.*

A portion of the park in the southern part, along two-river valleys - Bhote Koshi and Dudh Koshi - was considered for detail sampling survey (Thakuri and Pradhan, 2010). The site extends from Samde (Thame bridge) to Mongla range (Fig.1c). The elevation extent covers from around 2,600 m to 4,000 m asl., extending from 27°47'23.61" N to 27º50'55.14" N and 86º39'28.98"E to 86º44'44"E. This region was selected due to its accessibility and considering the importance because of human activities and major concentration area of mammal species. It includes parts of Namche and Khumjung Village Development Committees with 18 small and large settlements housing 262 households mainly dependent on tourism related business. The area includes characteristics mountain topographic features, landform and vegetation pattern with humid and tropical climate regime (Joshi, 1982).

Data and Methods

Field Data Collection: This paper uses the field survey data explained and used in Thakuri and Pradhan (2010). They had collected the species presence-absence data by using direct sighting and indirect sign count methods. Habitat survey included vegetation observation, human-induced impacts, and geographical variables. For more detail, refer to Thakuri (2009), and Thakuri and Pradhan (2010).

Canonical Correspondence Analysis (CCA): The species presence-absence data used as baseline information for determining habitat preference of Himalayan Tahr and Musk deer in terms of their habitat and anthropic variables using the Canonical correspondence analysis (CCA) (ter Braak, 1987). CANOCO for windows and CANODRAW software module of CANOCO for windows package were applied for the CCA. The CCA helped to search for patterns between Himalayan Tahr and Musk deer with habitat variables.

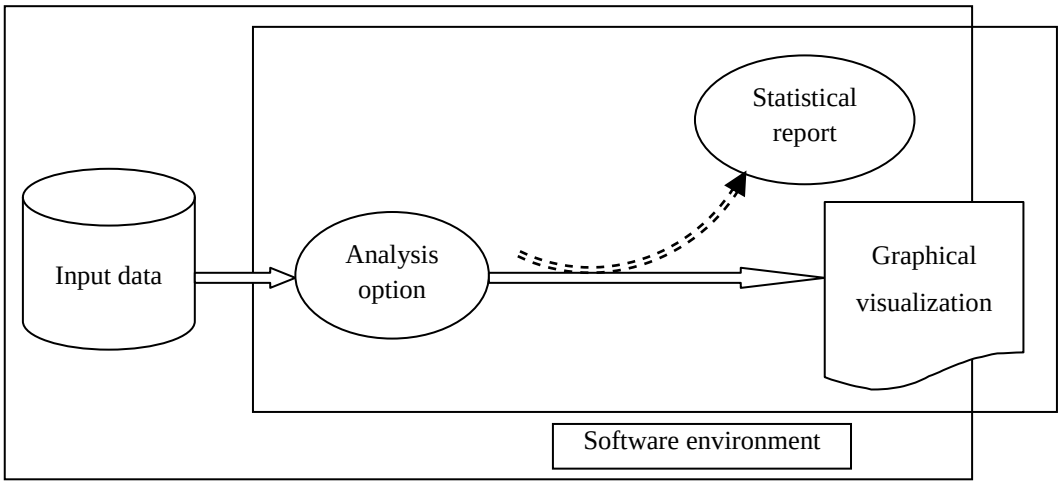


Fig. 2 *Correspondence analysis procedure*

Spatial analysis: Geographical Information Systems (GIS) were used in a study of habitat suitability analysis. By combining habitat data with GIS, potential habitat maps of Himalayan Tahr and Musk deer developed. Species presence data, satellite-based vegetation type maps, digital elevation models (DEMs) and terrain data were incorporated to produce a Himalayan Tahr and Musk deer predictive potential habitat map for SNPBZ. Four habitat variables- vegetation, aspect, altitude and slope are used as criteria for mapping potential habitat using ArcGIS software.

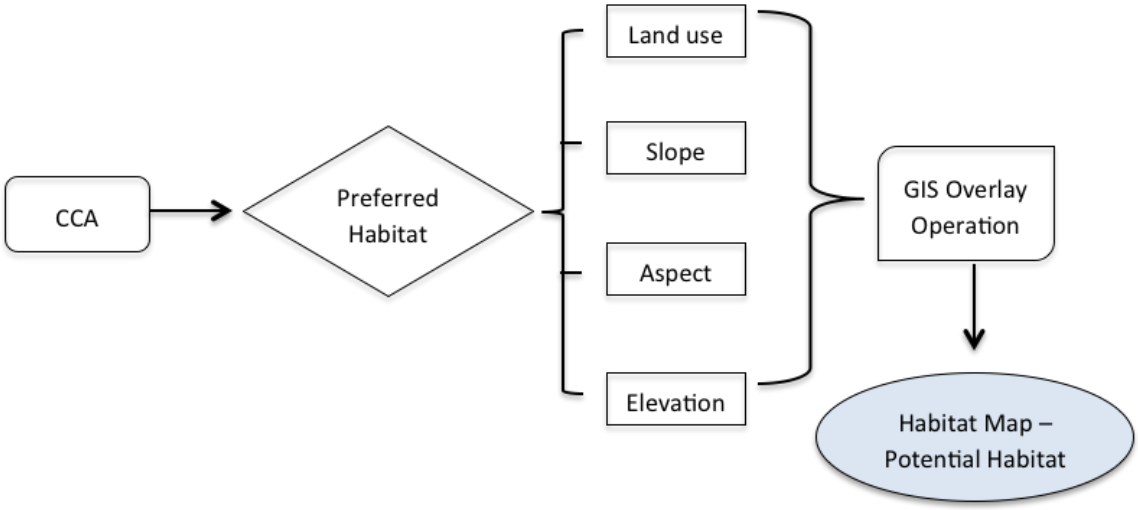


Fig. 3 *Combining CCA with GIS for deriving potential habitats*

Results and Discussion

a. Distribution and habitat utilization

The habitat utilization by Himalayan Tahr and Musk deer is quite notable. These two species are using completely different habitat in SNP. Himalayan Tahrs are observed in all vegetation types, while Musk deer mainly favor needleaved and broadleaved forest. They are distributed along southern aspects with dry and broken terrains and both high and low slopes, while Musk deer preferred northern moist rolling topography. Himalayan Tahrs are found in more disturbed area whereas Musk deer are found in relatively undisturbed area.

Himalayan Tahr (*Hemitragus jemlahicus*) and Musk deer (*Moschus chrysogaster*) have completely different habitat preference and thus, clear habitat separation exist. The distribution of Himalayan Tahr is more or less continuously distributed in all vegetation types - forestland, shrubland, and grassland such as mixed shrubland, needleaved shrubland, dwarf shrubland, broad leaved shrubland, multilayer forest and close to open herbaceous vegetation whereas Musk deer is distributed in needleaved forest and broadleaved forest ($p < 0.01$).

The detail vegetation analysis also suggests strong agreement with the result of CCA and is in line with the other studies carried out in the park and other area of country (Kattel, 1992; Aryal, 2006; Shrestha, 2006). The distributions of Himalayan Tahr and Musk deer in those vegetation types appear due to availability of their food.

These two species have high influence of geographic variables like aspect, slope and ruggedness. Himalayan Tahr is distributed along south-east (SE), south (S), west (W) and south-west (SW) aspects, broken terrains and both high (50-70°) and low (10-30°) slopes while Musk deer prefers north (N), north-west (NW), east (E) aspects, medium (30-50°) slope and rolling topography ($p < 0.05$).

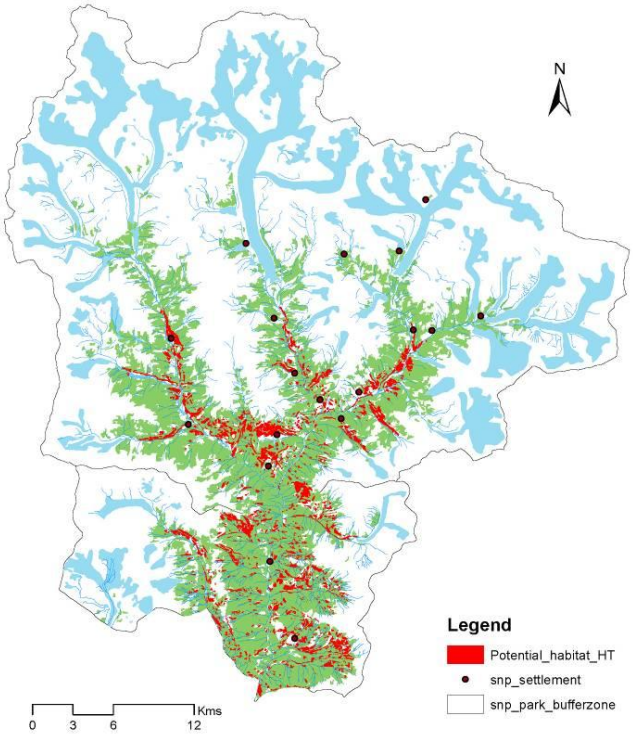
b. Potential Habitat

The potential habitat maps for Himalayan Tahr and Musk deer, showing a suitable habitat based on the considered criterion are presented below.

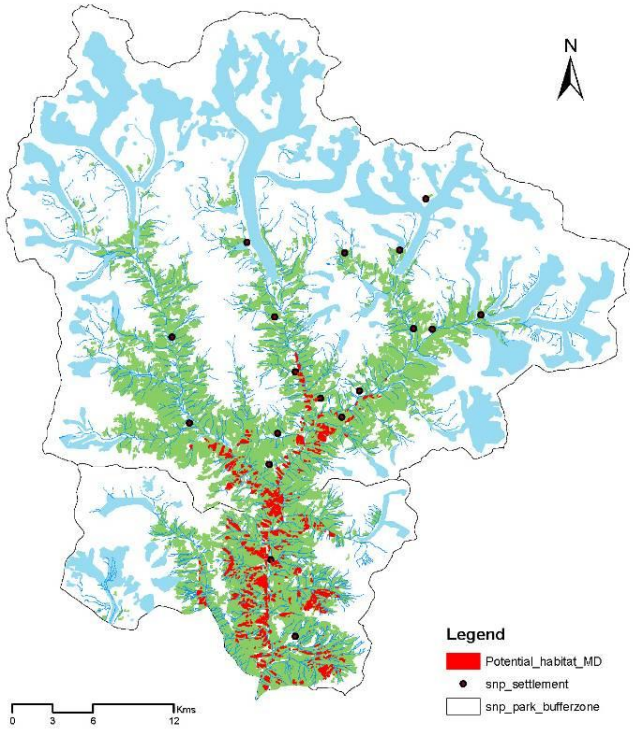
Table. *Habitat use criteria*

Criteria	Himalayan Tahr	Musk Deer
Land use	Mixed shrubland, needleaved shrubland, dwarf shrubland, broad leaved shrubland, multilayer forest and close to open herbaceous vegetation	Needleaved forest and broadleaved forest
Slope	10-30°; 50-70°	30-50°
Aspect	SE, S, W and SW	N, NW, E
Elevation	3000-5000 m	2500-4000 m

a) Himalayan Tahr



b) Musk deer



i.

Fig. 4 *Potential habitat of a) Himalayan Tahr (Hemitragus jemlahicus and b) Musk deer (Moschus chrysogaster) in Sagarmatha (Mt. Everest) National Park and Buffer Zone*

Conclusion

In this study, the habitat preferences of key mammals derived from the CCA combined with the GIS to gain their potential suitable habitat in the SNPBZ. Habitat and anthropic variables influence the distribution of mammals to a great extend separating habitat between two species. The habitat separations between Musk deer and Himalayan Tahr are quite remarkable. The mammal species have specific habitat preferences, which result into their distinct distribution in the area. The habitat preference and distribution of focal mammal species can be used to support an ecologically sustainable wildlife management. Collecting information on the habitat preference of the species is highly important for a scientifically sound park management. Habitat approach of the wildlife study is an effective way for park management purpose especially in the area with high human encroachment. The approach provides a clear overview on the management issues area to park manager. The GIS application is an effective tool for habitat suitability analysis. The potential habitat maps developed for Himalayan Tahr and Musk deer can be used as a reference for the species conservation and habitat management.

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