EFFECTS OF AGRICULTURAL LAND USE PATTERN ON DIVERSITY AND ABUNDANCE OF AMPHIBIANS IN RAPTI MUNICIPALITY, CHITWAN, NEPAL

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A Thesis submitted in partial fulfillment of the requirements for the award of the degree of Masters of Science in Zoology with special paper Ecology and Environment

Submitted to:

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Kirtipur, Kathmandu
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DECLARATION

I hereby declare that the work presented in this thesis entitled **"EFFECTS OF AGRICULTURAL LAND USE PATTERN ON DIVERSITY AND ABUNDANCE OF AMPHIBIANS IN RAPTI MUNICIPALITY CHITWAN, NEPAL"** has been done by myself and has not been submitted elsewhere for the award of any other degree. All the sources of the information have been specifically acknowledged by references to the author(s) or institution(s).

Date: 12 April 2021

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RECOMMENDATION

This is to recommend that the thesis entitled **"EFFECTS OF AGRICULTURAL LAND USE PATTERN ON DIVERSITY AND ABUNDANCE OF AMPHIBIANS IN RAPTI MUNICIPALITY, CHITWAN, NEPAL"** has been carried out by Somika Pathak for the partial fulfillment of Master's Degree of Science in Zoology with special paper Ecology and Environment. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institution.

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CERTIFICATE OF ACCEPTANCE

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Somika Pathak

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LIST OF ABBREVATIONS

ºC	Degree Celsius
m	Meter
mm	Millimeter
ANOVA	Analysis of Variance
CCA	Canonical Correspondence Analysis
DHM	Department of Hydrology and Meteorology
GIS	Geographic Information System
GPS	Global Positioning System
PAST	Paleontological Statisticals

ABSTRACT

Amphibian diversity, abundance, and richness are abundant in the agro-ecosystem. Different agricultural practices, on the other hand, may have a direct impact on the amphibian community by altering the area's physiology and chemical makeup. The aim of this study was to determine the effect of agricultural land use patterns on amphibian diversity and abundance. To conduct the research, the study area, which is a part of Rapti Municipality's agricultural field, was separated into four blocks, two blocks each on the north and south sides of the East-West Highway. According to cropping pattern and intensification, each block was further divided into four habitats: uncultivated land, three crop rotations without vegetable cultivation, three crop rotations with vegetable cultivation, and agricultural land near the forest. A nocturnal, time-constrained visual encounter survey of amphibians was conducted along transects (100×4 m) for 30 minutes at different strata of blocks. Data was collected during June and July 2019. A total of 12 species of amphibians belonging to four families were recorded. The species diversity index was higher (H =1.79) in agricultural land near forest whereas least (H =1.589) in three crop rotations with vegetables cultivation. The total abundance of amphibians was highest (620) in three crop rotations without vegetable cultivation and lowest (234) in three crop rotations with vegetable cultivation along different strata. There was significant relation between species diversity and different agricultural land use patterns ($F = 8.039$, $df = 3,12$, $p = 0.004$) whereas abundance of amphibians was not significant ($F = 0.6408$, $df = 3,12$ $P = 0.5816$) along different agricultural land use patterns. Agricultural patterns, environmental variables, and pesticide usage, all played a role in shaping amphibian species diversity and abundance along different agricultural land use patterns.Thus, agricultural land use patterns effect on amphibians. To conserve amphibians, information should be shared among farmers, and also steps to minimize the use of pesticides and chemical fertilizers.

1. INTRODUCTION

1.1 Background

Amphibians are the diverse vertebrates among the animal kingdom. In Nepal, 56 species of amphibians (Shah and Tiwari, 2004; Khatiwada *et al.*, 2017) included in 3 different orders; Anura (Frogs and Toads), Caecilians (limbless Amphibians), and caudate (Salamander and Newts) (Shah and Tiwari, 2004) are found. Amphibians depend on both aquatic and terrestrial habitats to complete their life cycle (Daniels, 2005).

Amphibian diversity and abundance are high in different agricultural land as there is availability of water on the field with natural vegetations and earthen irrigation channel in paddy fields (Duré *et al.,* 2008). Similarly, ponds and cultivated land are the aquatic habitats that promote for amphibian population; these habitats support the amphibians for food, breeding, and metamorphosis of tadpoles (Karunakaran and Jeevanandham, 2017). The permeable sensitive skin of amphibians plays the role of an important organ for maintaining respiration, body temperature, and water level in the body, coloration, and defense mechanism (Daniels, 2005). So, small changes in aquatic and terrestrial habitats support reducing their occupants (Becker *et al.,* 2010). Moreover, amphibians are known as indicator animals because they indicate the health of their surrounding habitat (Saber *et al.*, 2017).

Paddy field agro-ecosystem supports a diverse range of vegetation and animals, so amphibians mostly prefer this habitat (Bambaradeniya *et al.,* 2004). They feed on a variety of crop pests. Therefore, they are also considered as bio-control agents. Besides that, large numbers of insects that are the vectors of zoonotic disease are also consumed by the amphibians. They provide important ecological services in an ecosystem (Khatiwada *et al.,* 2016). Moreover, their role in the community ecology is crucial as they play the role of both predators and prey. Thus, they are inseparable units of both terrestrial and aquatic ecosystems (Saber *et al.,* 2017). Amphibians are experiencing decline due to invasive animal species like (*Rana muscosa*), over-exploitation, land-use change, global change, use of pesticides, and infectious diseases (Collins and Storfer, 2003).

Agricultural intensification causes negative impacts in amphibians through decreasing their fitness by pesticides exposure (Piha, 2006). Biotic interactions and resource availability patterns of the ecosystem are changing due to land conversion and intensification (Matson *et al.,* 1997). Land use intensification is the process of attempting various activities to improve the yield and make it commercial per unit area of land use. It covers both certain land uses intensification and changes between lands use (Martin *et al.,* 2018). So, even agricultural intensification help in the higher crop yield per unit area, its result into uniformity of agricultural habitats which automatically increase soil erosion, pollution of water and air (Stoate *et al.,* 2001).

Amphibians have become major threatened taxa (Heatwole, 2013) because of their quick response towards the environmental changes making them the most sensitive animal. So, when Amphibians' larvae are exposed to the higher concentration of herbicides, they retard their development, metamorphosis, behavioral response and get affected negatively. In addition to this, defects and abnormalities were seen to the death in a short period (Cauble and Wagner, 2005). Hence, the chemicals used in the agricultural land acted as a major factor for decreasing amphibian's population. Chemicals can have an impact through their own or in combination with other products (Relyea and Mills, 2001). The immense use of pesticides and fertilizers in agricultural land results in high numbers of malformed amphibians (Ouellet *et al.,* 1997).

The number of individuals of each species of amphibians in an area mainly depends on the accessibility of food, moisture, and microhabitats (Daniels, 2005). Besides these, the distribution and abundance of amphibians are affected by the temperature and vegetation cover which play as an important environmental variables for amphibians (Contreras, 2018). Similarly, p^H, water temperature, and level of disturbance determine the diversity and abundance of amphibians in that area (Saber *et al.,* 2017).

The habitat of an organism plays an important role in the study of its diversity, distribution, and abundance (Peter, 1983). Agricultural land also acts as potential habitat for the high diversity and abundance of amphibians because it gives suitable habitat to fulfill their feeding, breeding, and settlement requirement. The change in the physiology and chemical composition of agricultural land because of pesticides and chemical fertilizers, tillage, drainage, crop rotations, intercropping has affected the diversity and abundance of amphibians as they modify the natural habitat. So, it is important to know the effect of different agricultural land use patterns on amphibians to find out the possible threats on amphibians in agricultural land and help to address these threats during the conservation of amphibians.

1.2 Rationale of the study

Agricultural lands, as well as other types of habitat, are preferred by amphibians because they provide suitable feeding and breeding grounds. Different agricultural patterns may directly influence the amphibian community by modification in the physiology and chemical composition of the area. These types of study on agricultural land use pattern and its effect on amphibian diversity, and abundance are rarely done in Nepal. The reason behind the selection of this area was maximum agricultural intensification. In this area, people cultivate different kinds of crops in a single year as there is high accessibility of irrigation facilities, agricultural equipments, seeds and pesticides. This research aimed to document the diversity index of amphibians in different agricultural land-use patterns. It also explored how different factors like pesticides use, disturbance and crop rotation influence the assemblage of amphibians. The finding gave insight on how agricultural land use pattern governs the diversity of amphibians and what factors should take into consideration to maintain the healthy population of these bio-indicators.

1.3 Objectives

1.3.1 *General objective*

The main objective of this study was to investigate the effects of agricultural land-use patterns on amphibians in Rapti Municipality, Chitwan Nepal.

1.3.2 *Specific objectives*

The specific objectives were to:

1. Quantify the diversity and abundance of amphibians in different agricultural land-use systems.
2. Access the effect of agricultural land use pattern on diversity and abundance of amphibians.

2. LITERATURE REVIEW

2.1 Diversity of amphibians

Globally, research of amphibians and reptiles is a less prioritized topic for conservationists in vertebrates than birds and mammals (Fazey *et al.,* 2005). Similar cases were also found in Nepal for amphibians and reptiles research. In Nepal, Hodgson collected herpetofauna between 1826 and 1854 which have received baseline research for herpetofauna (Günther, 1860). After that, Nepalese herpetology research lifted to another level by adding contribution to zoogeographic information of amphibians and reptiles (Swan and Leviton, 1962). Similarly, Dubois (1974), Fleming and Fleming (1974), Kramer (1977), Nanhoe and Ouboter (1987) added research on the herpetology of Nepal. After then, the diversity and distribution of amphibians and reptiles with habitat description was explored by Zug and Mitchell (1995) in Chitwan National Park. They described *Rana danieli* species as a new record from that side at that time. Likewise, Das (1998) recorded new species of Rana called *Rana chitwanensis* from the terai region of Nepal. Furthermore, by documenting the knowledge on new species or new taxa to the country, O'Shea (1996), Shrestha (2000), Schleich and Kästle (2002), Tillack and Lorenz (2003), elevated the Nepalese herpetology research. Finally, the first documentation of a total number of species of amphibians and reptiles was given and described 54 species of amphibians in Nepal (Shah and Tiwari, 2004). This documentation becomes a milestone in the research of Nepelease herpetology.

In Nepal, amphibians' research reached an advanced level through molecular and morphological comparison, from which genus *Tylototriton* was described as a new species of amphibians from Eastern Himalaya (Khatiwada *et al.,* 2015). Similarly, a new species of amphibians from Jamun Khadi belonging to Jhapa, Eastern Nepal was recorded and it represents the genus *Microhyla* of Anuran order (Khatiwada *et al.,* 2017). In addition, amphibians and reptiles were studied by some researchers reagionally in Nepal. According to them, seven species of amphibians were found in Manaslu Conservation Area (Pokhrel and Thakuri, 2016), eight species of amphibians representing three families were found in Sirdibas, Bihi and Prok Village Development Committes in Manaslu Conservation Area, Gorkha District (Shrestha and Shah, 2017), 13 species of anurans belonging to four families and eight genera of amphibians were found at Beeshazari and associateed lakes of Chitwan National Park which is also a Ramsar site (Bhattarai *et al.,* 2017), 12 species of amphibians belonging to eight genera and four families were recorded during the inventory of herpetofauna in Parsa National Park. *Microhyla nilphamariensis*, *Sphaerotheca breviceps* and *Uperodon taprobanicus* were the three species of amphibians that were new record to park (Bhattarai *et al.,* 2018). Similarly, twelve species of amphibians were found in Ghandruk, Annapurna Conservation Area, (Gautam *et al.,* 2020).

The study of the diversity of amphibians along paddy fields was carried out in Northeastern Argentina. This study identified 26 species of amphibians representing four families namely Cycloramphidae, Bufonidae, Hylidae, and Leptodactylidae. Among the selected five microhabitat type in rice fields, diversity of amphibians was higher in natural vegetation followed by rice fields per se, ditches and paths, aquatic vegetation, and gullies (Duré *et al.,* 2008). Likewise, 19 species were recorded from the study of diversity and distribution of amphibians conducted in Romania. Among them, 9 species were endemic to Romania (Cogălniceanu *et al.,* 2013).

Various climatic factors like temperature and rainfall plays a major role in the diversity, distribution, and richness of amphibian species (Pyron, 2014). However, a higher level of temperature and p^H in aquatic habitat increases the death of the embryo or increases morphological malformation in amphibians (Boyer and Grue, 1995). The presence of water resources nearby terrestrial habitats also influences the diversity and abundance of amphibians (Wells, 2007) as amphibians need water sources along with terrestrial habitats to complete their life cycle. Similarly, quantity and species of vegetation within the aquatic and nearby terrestrial habitat determine the status of amphibians within the habitat. In addition to that, hydro-period, water characteristics, the existence of predators, competitors, diseases, and human disturbances periodicity are the factors that affect the amphibian's habitat (Hamer and Mcdonnell, 2008). Conditions of the soil of habitat also affect the amphibians' occurrence. The dry condition of soil reduces the occurrence of amphibians. Besides this, linear infrastructure like roads, firelines, etc also affects the occurrence of amphibians. Amphibians mostly prefer increased distance from roads (Aryal *et al.,* 2020). Moreover, the abundance and diversity of amphibians are affected by the presence of leaf litter as it is an important component of habitat and prefers mostly.

2.2 Effect of agricultural land use pattern on amphibians

The amphibian population declined as a consequence of agricultural expansion which is one of the major factors of habitat loss of amphibians (Gallant *et al.,* 2007). Pesticides, herbicides, fungicides, and fertilizers are used as agricultural chemicals that play a crucial role in decreasing amphibian species. Chemicals alone give a serious impact and even after mixing with other cause the decline of amphibian species (Relyea and Mills, 2001). In agricultural land, higher hind-limb and digit malformations of amphibian species have been recorded (Linzey *et al.,* 2003). Different types of agricultural chemicals like pesticides, herbicides, fungicides, and fertilizers act as a set of pollutants (Mann *et al.,* 2009) which become the major sources for immunosuppression, vulnerable to infections, deformities in limb and a decrease in the population of amphibians (Linzey *et al.,* 2003). Thus, land-use modification contributes to reducing the richness of amphibians (Wanger *et al.,* 2010).

In an ecosystem, agricultural land after transformation from natural habitat can alter the figure of species composition, abundance, and diversity at the local, regional and global level. However, different taxa receive a different level of reaction after changing from their natural habitat (Robinson and Sutherland, 2002). Homogenization of land is the cause through which diversity of ecosystem decreases after a change in land use pattern (Flather *et al.,* 1998). Similarly, original characteristics of natural habitat change after the expansion and change in agricultural land use pattern as it causes the destruction, fragmentation, introduction of invasive species, human disturbance, and impact through pesticides and fertilizers spraying. These are the factors that affected the native species (like birds, carnivorous, herbivores) of that area and consequences in decreasing diversity and abundance at an alarming rate (Medan *et al.,* 2011). Globally, for the loss of biodiversity, agricultural practice play a significant role. It is due to extreme physical manipulation along the large area of land with extensive use of pesticides and fertilizers. In addition to that tillage, drainage, crop rotation, intercropping also affect the flora and fauna of agricultural land (Mclaughlin and Mineau, 1995) by changing the soil food web structure and function (Moore, 1994). Similarly, the rate of development, growth, reproduction, and survivability of amphibians is restricted by chemicals. So, immense use of pesticides causes a lethal and sub-lethal effect on the amphibian population through declining their fitness and survival (Mann *et al.,* 2009).

Various amphibian species occur throughout Nepal but the researcher has less interest in amphibians as they are cryptic species. So, most research work being carried out are focused on higher taxa. After studying the different kinds of literature from different sources shows that there is less information on the diversity, distribution, and abundance of amphibians along different habitats like agricultural land in Nepal. Similarly, there is lacking information on the effect of agricultural land use patterns on amphibians which were not been done yet. As a result, this study showed amphibian diversity, abundance, and relationship along different agricultural use patterns in Rapti Municipality, Chitwan, Nepal.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Rapti Municipality, Eastern part of Chitwan in Bagmati province of Nepal. Different types of cropping patterns are followed by farmers of Rapti Municipality within one year. From mid-March to mid-June, major crops productions are paddy and maize. Similarly, paddy from mid-June to October, from November to January, major crops are mustard, wheat, and vegetables or cash crops. Some follow maize crops within January to March also.

Climate: Chitwan has a tropical monsoon climate with high humidity throughout the year.

Temperature: March to early June is the usually hot month, with high temperatures progressively to a peak in May. During April, despite the heat of the day, the nights can be quite cold. The average minimum temperature was 2ºC in January and the average maximum temperature was 25ºC in May- June.

Rainfall: In Chitwan, the minimum rainfall was in December-January. Maximum rainfall was during July i.e. 1000 mm.

Drainage systems: Main drainage systems of the study area are Rapti River, Pampha Khola, Dhumre Khola, Lothar Khola.

Flora and fauna: Main vegetation of Chitwan is Sal (*Shorea robusta*), Bhaller (*Trewia nudiflra*) Botdhayero (*Anogeissus latifolia,),* Chir Pine (*Pinus roxburghii),* Harro (*Terminalia belerica)*, Bhorla *(Bauhinia vahlii),* and Sal Lahara *(Spatholobus parviflorus)*, riverine forest associations (*Trewia-Bombax*, *Acacia- Dalbergia*, and mixed riverine), etc. The main faunas of Chitwan are one-horned Rhinoceros, Bengal Tiger, and Gharial Crocodile, 49 species of reptiles and amphibians, 120 species of fishes, etc (CNP, 2019).

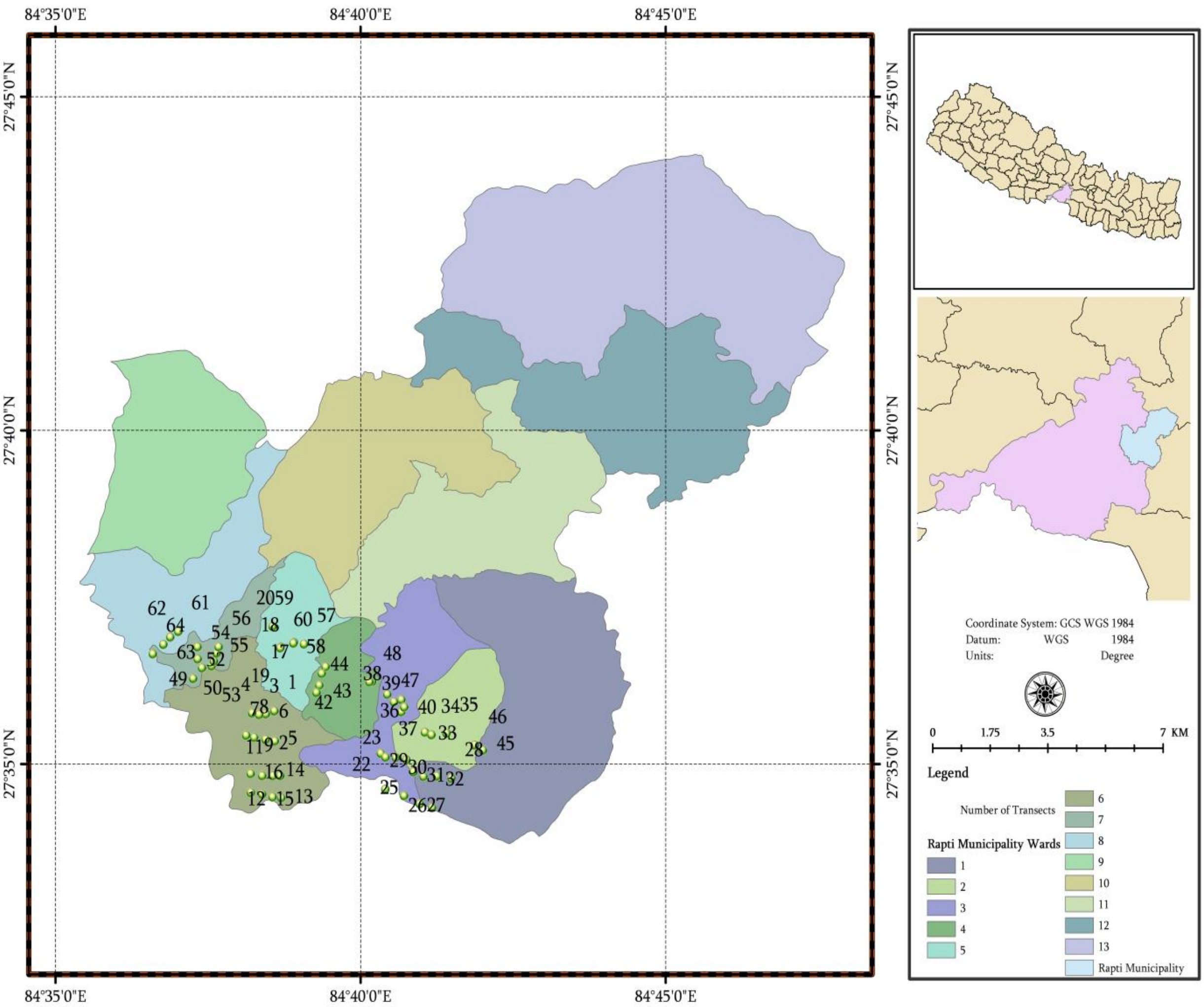

Figure 1. Map of study area showing the sampling sites

3.2 Methods

3.2.1 *Field sampling design*

A preliminary survey was conducted during June 2019 to find out the detail about the cropping patterns, use of pesticides, and chemical fertilizers of the study area. Birendranagar, Bhandara, Pratappur, Piple, Dhamaura, Mudabhar, Piyaridhap, Parsauni, Bhawanipur were the study area of Rapti Municipality ward number 1-8. Based on the field observation and questionnaire survey, the study area was divided into four blocks; two blocks north of East-West Highway and two blocks south of the same highway. Each block was further stratified into four different habitats according to the different land-use systems; i.) uncultivated land ii.) three crop rotations without vegetable cultivation iii.) three crop rotations with vegetable cultivation and iv.) agricultural land near the forest.

So, there were a total of sixteen strata within the four blocks. Then, within each stratum, four transects were laid out to carry out the amphibians collection.

Questionnaire survey

The semi-structured questionnaire survey was conducted with the farmers of transect within strata of four blocks of the study area during June 2019. The questionnaire included general information on cropping patterns, use of pesticides and chemical fertilizers, potential threats to amphibians, perception towards the population of amphibians. A total of 79 farmers were present in the questionnaire.

Focus group discussion was conducted among the pesticides and chemical fertilizers sellers of the local market to gather information on pesticides and chemical fertilizers.

I. Uncultivated land (Uncul_land): Land which is not in use for an agricultural purpose like plotting area, grassland, and land nearby river area were taken as uncultivated land. These areas were less in use and no direct use of chemicals, and having natural vegetation.

II. Agricultural land having three crop rotations without vegetables (3crp_noveg): Agricultural land in which three crop rotations without vegetable farming within a year was taken. Major crops within three crop rotations were rice, mustard/ wheat, and rice. In this area, pesticides, and fertilizers were used in a moderate range i.e. every month. Cultivated land covered with little more water than in three crop rotations with vegetable farming.

III. Agricultural land having three crop rotations with vegetables (3crp_withveg): Agricultural land in which three crop rotations with vegetable farming within a year was taken. Major crops within three rotations were rice, vegetables, and rice/vegetables. Farmers used insecticides, pesticides along with chemical fertilizers more i.e. daily and weekly during vegetable farming.

IV. Agricultural land near forest area (Near_forest): In this area, three crop rotations were without vegetable farming was taken. Major crops within three crops were rice, mustard/ wheat, rice. In this area, pesticides and chemical fertilizers were used in low to moderate range i.e. in monthly and in 3-4 months/annually. Nutrients from the forest were also runoff during the rainy season.

3.2.2 *Data collection*

Nocturnal time-constrained visual encounter survey (Campbell and Christman, 1982) was carried out during June and July 2019 in each stratum which means walking along the designated transects for documenting the specific species for the prescribed time. The survey was carried along line transects (100×4 m) for 30 minutes using torchlight from 19:00 to 23:00 hrs. Two observers walked along the line transect maintaining a distance of 2 meters. While walking along transects, every encountered amphibian species were collected in the cotton bag (20×20 cm) and then further identification and photograph process were carried out at the nearby station. After the identification and photographing process, all of these collected species were released back to avoid repetition. Global Positioning System (GPS) locations of each block and stratum were noted using Garmin Etrex 10.

Variables that affect the diversity and abundance of amphibians such as distance to the nearest water source, distance to nearest vehicle road, and distance to the nearest settlement were measured with the help of Google Earth Pro.

3.2.3. *Identification of amphibians*

Captured individuals were identified by using the field guide books "Amphibians and reptiles of Nepal" (Schleich and Kästle, 2002) and "Herpetofauna of Nepal" (Shah and Tiwari, 2004). Unidentified species were later identified by field experts through photographs (Canon EOS 200 D, 24 megapixel-55X Zoom).

3.3.4 Data analysis

The collected data were arranged, organized, and entered into MS-Excel Sheet for further analysis.

Shannon-Weiner diversity index was used to calculate the species diversity of the amphibians along different agricultural land use patterns which is calculated as:

$$H' = -\Sigma (ni/N) \log (ni/N)$$

Where,

H = Index of species diversity

Pi = the proportion of individuals in the ith species = ni N

Ni = Importance value for each species (number of individuals)

N = Total important value (Total number of individuals)

Evenness Index was calculated by the following equation;

E = H'/log S

Where, H' = Shannon- Wiener's diversity index

S = Species richness; the total number of species

Diversity, abundance, and One-way ANOVA of species along different agricultural land use patterns were calculated in PAST 3.25 (Hammer *et al.*, 2001). Similarly, results of diversity and abundance of amphibians within different agricultural land-use systems along with four blocks, and farmers' perception towards threats and population trend of amphibians were displayed as a bar graph and pie-chart in MS-Excel 2007.

Canonical corresponding analysis (CCA) was carried out in CANOCO version 4.5 (Ter Braak and Šmilauer, 2012; Team, 2020) to examine the influence of block, strata, environmental variables, and mode of pesticides and fertilizers use on amphibians distribution. The final result was presented in the form of a biplot with Monte- Carlo permutation test by using 499 permutations under the model to identify which variables have a significant effect on amphibians species distribution in the study area.

The coordinates points of the study area were imported in Arc GIS 10.7 and a map of the study area was prepared (Bajjali, 2017).

4. RESULTS

4.1 Diversity and abundance of amphibians

A total of 12 species of amphibians belonging to four families were recorded from the study area. All 12 species of amphibians belonged to order anura. Dicroglossidae was the dominant family consisting of 6 species followed by 2 species of each family Bufonidae, Microhylidae, and Rhacophoridae.

Table 1. Amphibian species recorded along with different Agricultural land use pattern

S.N	Family Name	Species Name	Species code used in the analysis
1.	Bufonidae	*Duttaphrynus melanostictus* (Schneider, 1799) *Duttaphrynus stomaticus* (Lütken, 1864)	Dut_melo Dut_sto
2.	Dicroglossidae	*Hoplobatrachus tigerinus* (Daudin, 1802) *Hoplobatrachus crassus* (Jerdon, 1854) *Euphlyctis cyanophlyctis* (Schneider, 1799) *Minervarya teraiensis* (Dubois, 1984) *Sphaerotheca maskeyi* (Schleich and Anders, 1998) *Minervarya syhadrensis* (Annandale, 1919)	Hop_tig Hop_cra Eup_cya Min_ter Sph_mas Min_syh
3.	Microhylidae	*Microhyla nyphymerensis (*Duméril and Bibron,1841) *Uperodon globulosus* (Günther, 1864)	Mic_nyp Upe_glo
4.	Rhacophoridae	*Polypedates maculatus* (Gray, 1830) *Polypedates taeniatus* (Boulenger, 1906)	Pol_mac Pol_tae

The highest species diversity (H =1.79) was observed in agricultural land near the forest and the least species diversity value (H =1.59) in three crop rotations with vegetables. Similarly, the Evenness index value was observed high (E = 0.70) in three crop rotations with vegetables and least (E = 0.54) in agricultural land near the forest.

Table 2. Diversity of amphibians along different agricultural land use patterns

Strata ⟶ Diversity ↓	Uncultivated land	Three crop rotation without vegetables	Three crop rotation with vegetables	Agricultural land near the forest
Shannon_H	1.73	1.68	1.59	1.79
Evenness_êH/S	0.63	0.59	0.70	0.54

The highest species diversity of amphibians along uncultivated land was calculated in Block D (H = 1.60) and least in Block C (H = 1.32). The highest species diversity of amphibians along with three crop rotations without vegetables was in block A i. e. H = 1.78 and least in Block C i.e. H = 1.35. Likewise, along with the three crop rotations with vegetables, species diversity was recorded higher in Block D i.e. H = 1.65, and lower in Block B i.e. H = 1.24. Block A was found to have the highest species diversity of amphibians in agricultural land near forest areas and Block C i.e. H = 1.48.

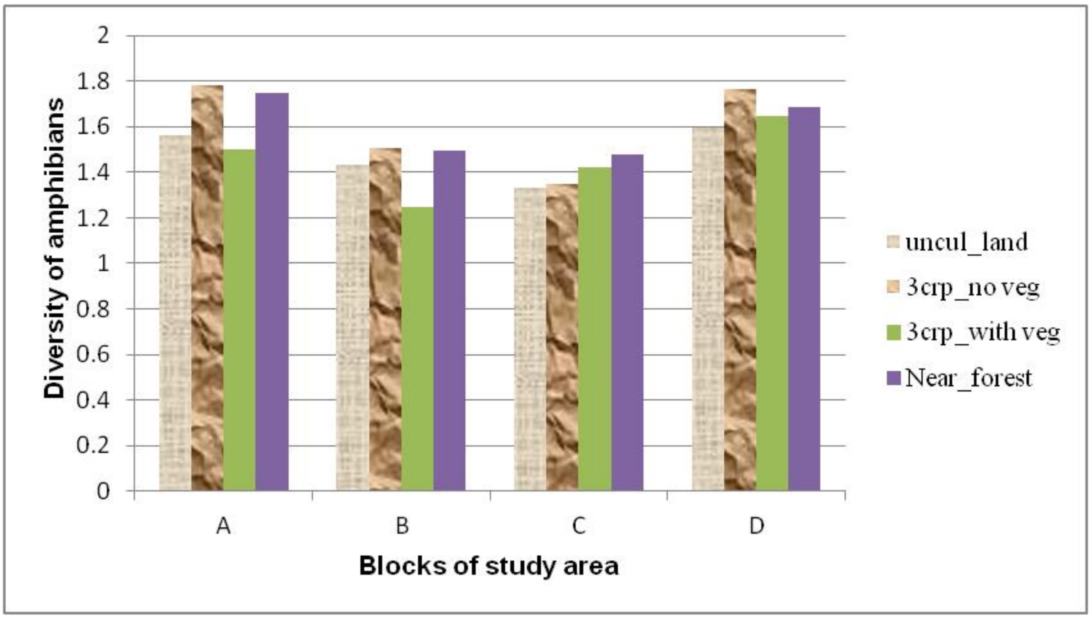

Figure 2. Diversity of amphibians in different strata of four block

The highest total abundance of amphibians along different agricultural land use patterns was found in three crop rotations without vegetable farming and the lowest in three crop rotations with vegetable farming.

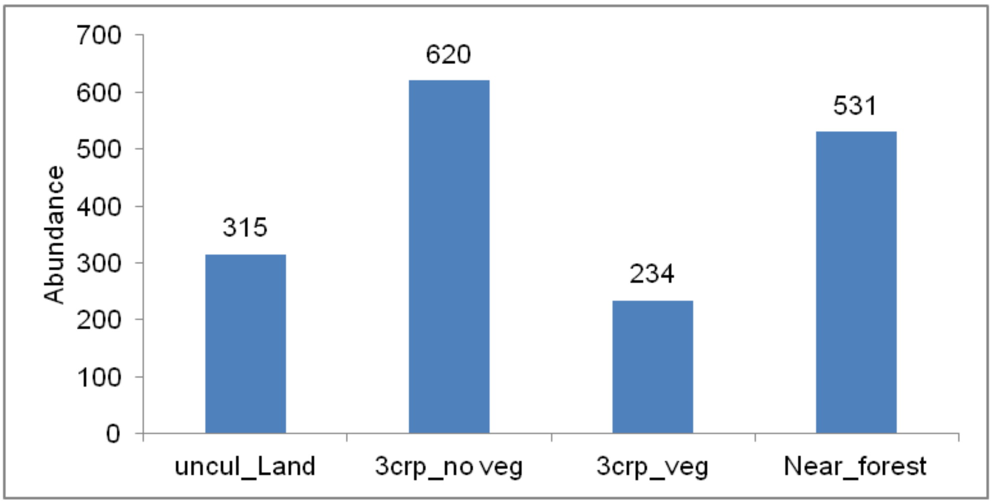

Figure 3. The abundance of amphibians along different land-use patterns

The abundance of amphibians was recorded the highest in block B among four blocks and least in block C.

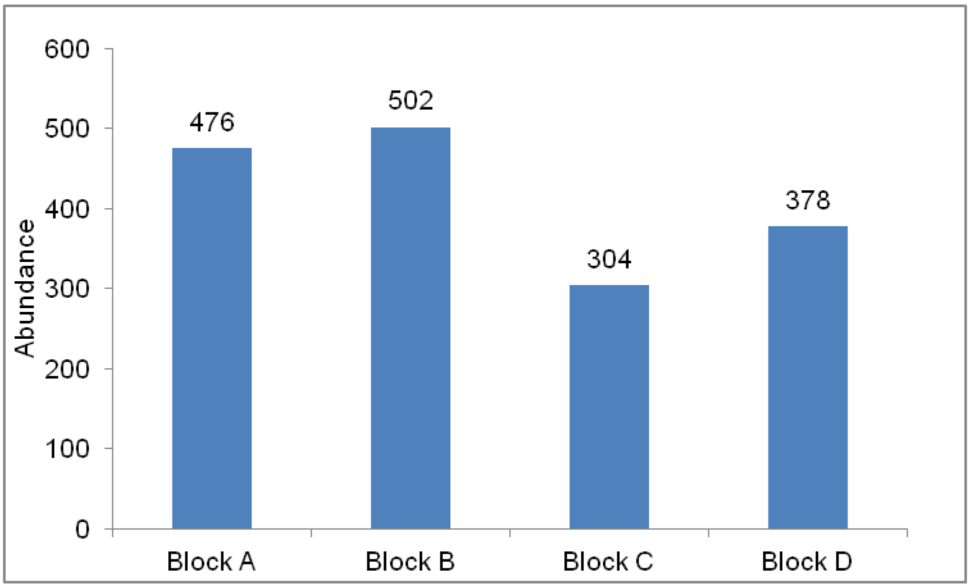

Figure 4. The abundance of amphibians along four blocks

4.2 Effects of agricultural land use pattern

The result from one-way ANOVA for diversity revealed that there was a significant difference (F = 8.039, df = 3,12, p = 0.004) in the diversity of amphibians along with different agricultural land-use patterns of blocks. There was no significant difference (F = 0.6408, df = 3,12, P = 0.5816) within the abundance of amphibians along different agricultural land use pattern of blocks.

In this study, the CCA plot shows that there is a strong influence of block D basically on amphibian species. Clusters of amphibians like *Hoplobatrachus tigerinus, Polypedates maculatus, Duttaphrynus stomaticus, Microhyla nyphymerensis, Minervarya syhadrensis, Duttaphrynus melanostictus,* and *Sphaerotheca maskeyi* were found to be strongly correlated with this block. Similarly, *Hoplobatrachus Crassus, Minervarya teraiensis,* and *Polypedates taeniatus* were weakly associated with block D. As stated above, amphibians like *Euphlyctis cyanophlyctis, Uperodon globulosus* were highly correlated with block C. Besides, *Hoplobatrachus crassus* and *Minervarya teraiensis* were strongly correlated with block B where *Polypedates taeniatus* with block A.

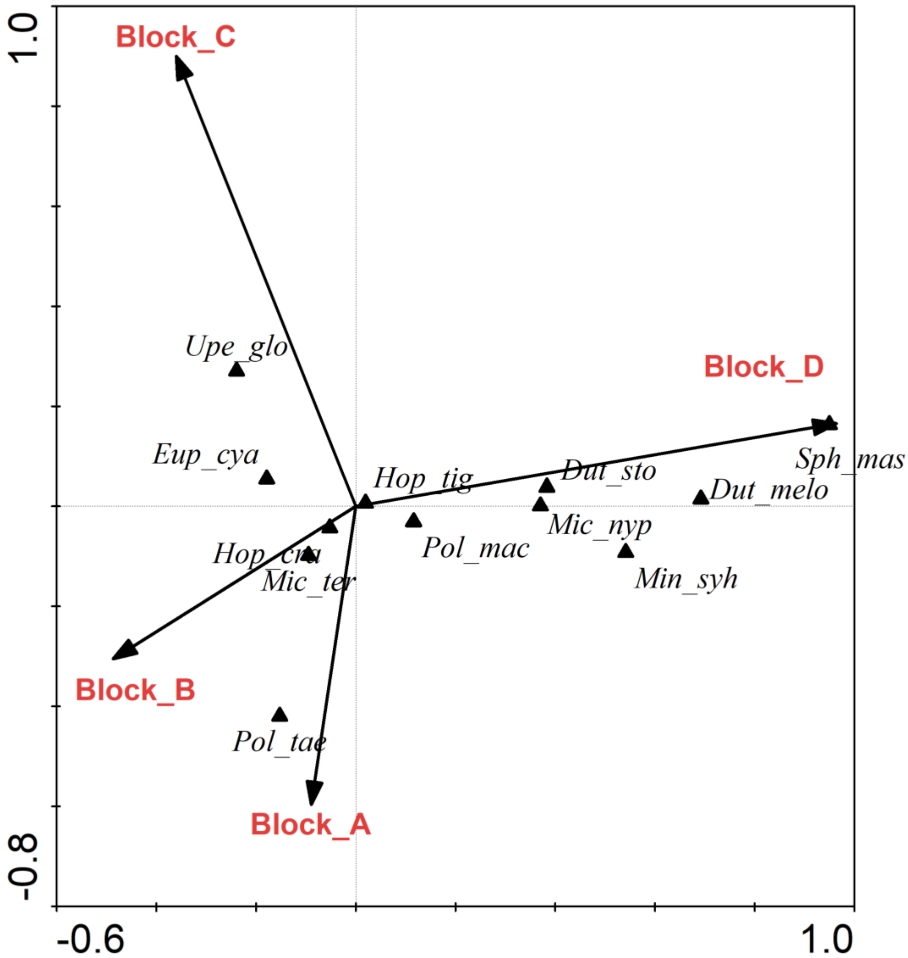

Figure 5. CCA ordination diagram (biplot) showing the effect of the different blocks on amphibians' species in Rapti Municipality, Chitwan. Monte Carlo permutation test of significance of all canonical axes: Trace = 0.148, F-ratio = 4.787, P-value = 0.0020 , with 499 permutations.

In the present study, the CCA plot shows that higher amphibian species are correlated with agricultural land near the forest. A cluster of amphibian species like *Hoplobatrachus crassus, Polypadates maculatus, Minervarya teraiensis, Polypadates taeniatus, Uperodon globulosus, and Sphaerotheca maskeyi* showed a strong correlation with agricultural land near the forest. Similarly, *Hoplobatrachus tigerinus, Duttaphrynus stomaticus,* and *Duttaphrynus melanostictus* were significantly correlated with uncultivated land. Likewise, *Euphlyctis cyanophlyctis, Microhyla nyphymerensis,* and *Minervarya syhadrensis* showed a higher correlation with 3 crop rotations without vegetables. In contrast to the above result, 3 crop rotations with vegetables showed no significant correlation with amphibian species in the study area.

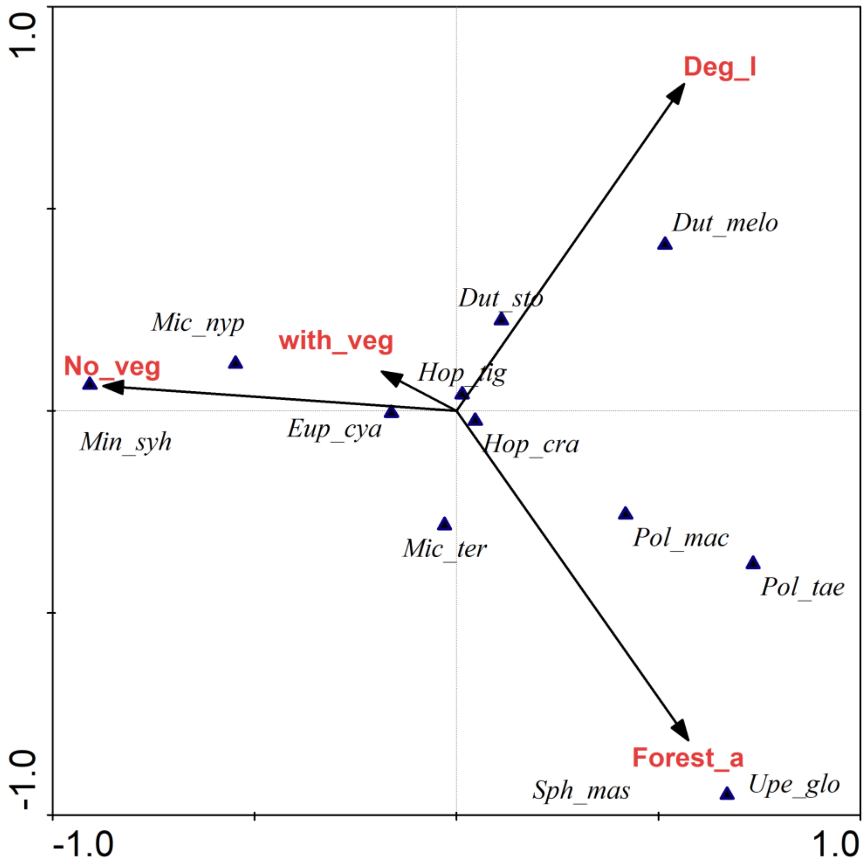

Figure 6. CCA ordination diagram (biplot) showing correlation of amphibian species with different agricultural land use patterns (strata) in Rapti Municipality, Chitwan. Monte Carlo permutation test of significance of all canonical axes: Trace = 0.093, F-ratio = 2.770, P-value = 0.0020, with 499 permutations.

In the present study, the CCA plot shows that *Polypedates maculatus, Hoplobatrachus tigerinus, Duttaphrynus stomaticus, Hoplobatrachus crassus* were strongly correlated with distance to nearest water sources. Similarly, *Minervarya syhadrensis*, *Duttaphrynus melanostictus*, *Sphaerotheca maskeyi*, and *Microhyla nyphymerensis* were slightly correlated with distance to nearest settlement and water sources. Likewise, *Euphlyctis cyanophlyctis* and *Minervarya teraiensis* show a high correlation with distance to settlement. In addition to that, *Polypedates taeniatus* is nearly correlated, and *Uperodon globulosus* is weakly correlated with road distance, but the distance to road shows no significant correlation with most amphibian species. It means that only fewer species were observed nearby roads**.**

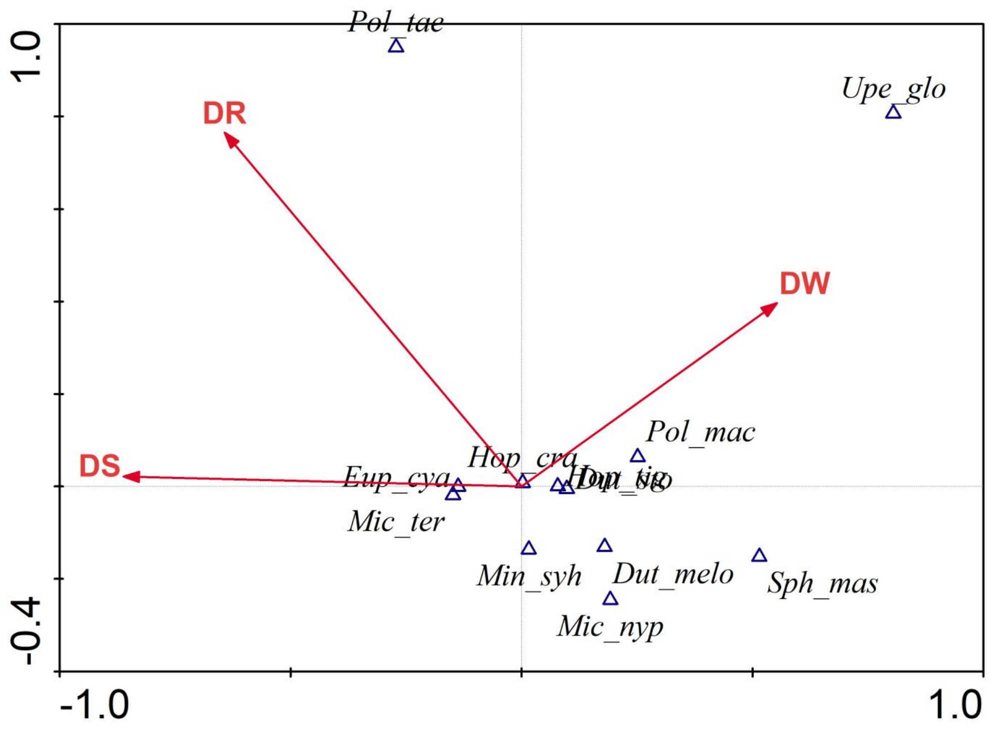

Figure 7. CCA ordination diagram (biplot) showing the effect of environmental variables on amphibians' species in Rapti Municipality, Chitwan. Monte Carlo permutation test of significance of all canonical axes: Trace = 0.058, F-ratio = 1.641, P-value = 0.0501, with 499 permutations.

In the present study, the effect of the pesticides was the greater threat to the amphibians followed by road-killed, habitat loss, pollution and disease, hunting, and temperature increasing.

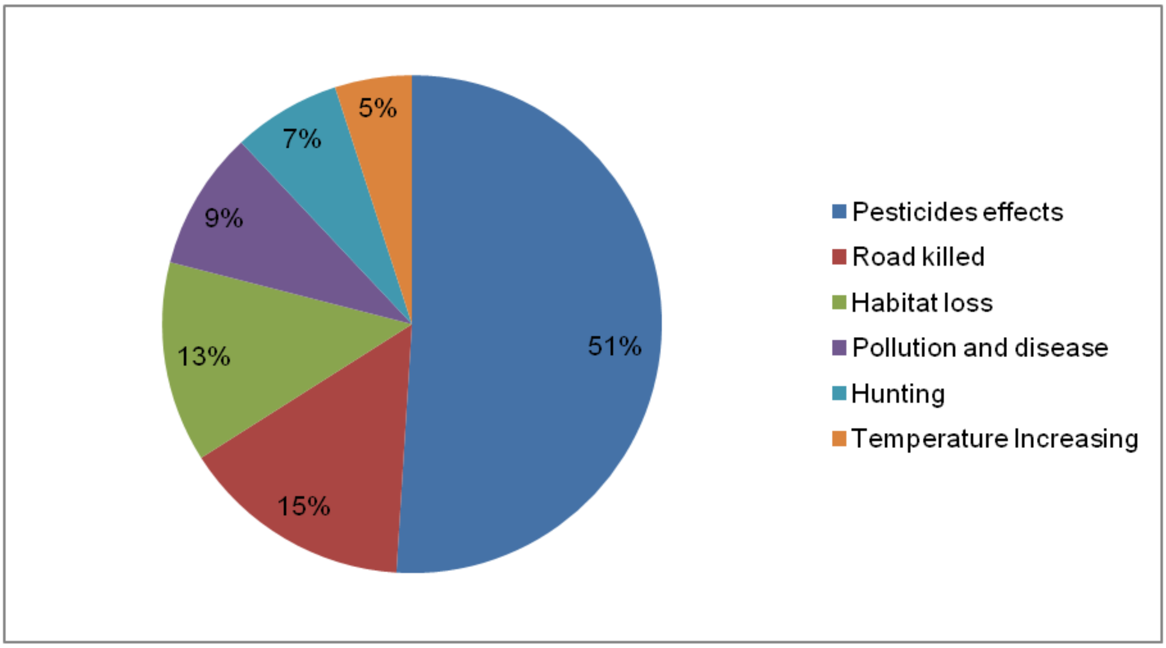

Figure 8. Perception of farmers towards potential threats to amphibians' populations

In the present study, the CCA plot shows that *Hoplobatrachus tigerinus, Hoplobatrachus crassus, Polypedates maculatus, Duttaphrynus stomaticus,* and *Sphaerotheca maskeyi* showed a strong correlation with low pesticides mode. These amphibian species are found highly in low-pesticides areas. Similarly, *Duttaphrynus melanstictus* and *Minervarya syhadrensis* were recorded as high correlation with moderate pesticides mode. In contrast to that *Euphlyctis cynophlyctis,* and *Minervarya teraiensis* were strongly correlated and *Uperodon globulosus* was weakly correlated with high pesticides mode whereas *Microhyla nyphymerensis* showed the negative correlation with high pesticides mode. These species showed its presence in both moderate and low pesticides mode areas.

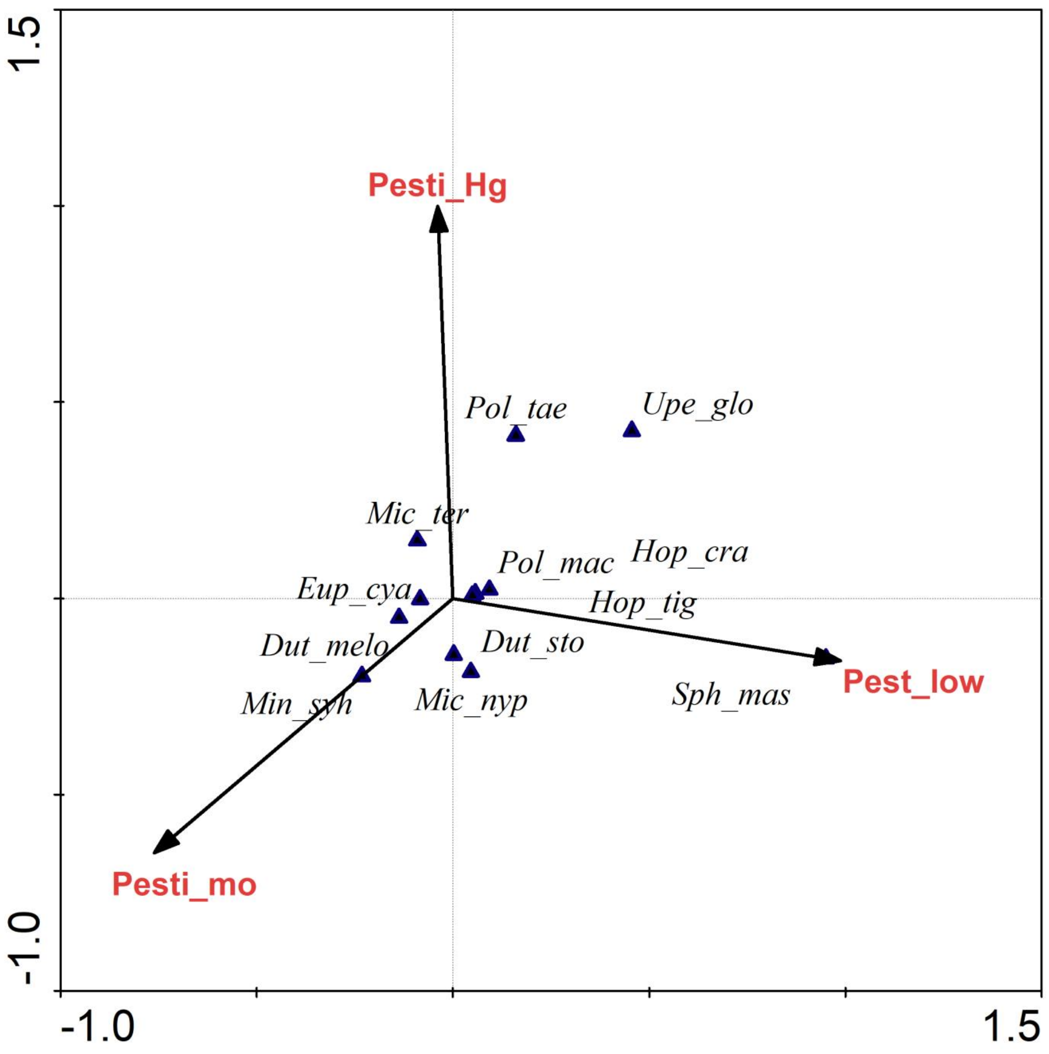

Figure 9. CCA ordination diagram (biplot) showing correlation of amphibian species with pesticides mode in Rapti Municipality, Chitwan. Monte Carlo permutation test of significance of all canonical axes: Trace = 0.055, F-ratio = 2.351, P-value = 0.0040, with 499 permutations.

The most of the respondents believed that the population of amphibians is decreasing over time and the least respondents said that the population of amphibians is increasing.

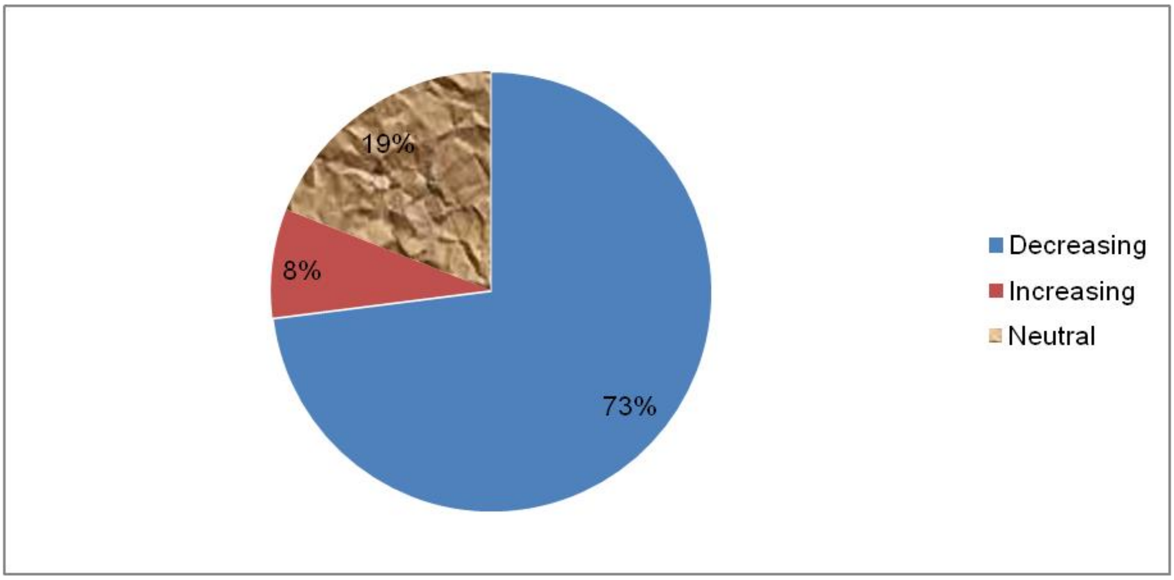

Figure 10. Perception of farmers towards population trend of amphibians in the study area

5. DISCUSSION

5.1 Diversity and abundance of amphibians

A total of twelve species of amphibians in different agricultural land of Rapti Municipality, Chitwan, Nepal was recorded. The high species diversity of amphibians were recorded from agricultural land near the forest. Agricultural land near forests serves as an edge zone with a variety of microclimates that support amphibian diversity and abundance (Lehinen *et al*., 2003). Amphibians travel to agricultural land in search of food and breeding grounds (Karunakaran and Jeevanandham, 2017). Furthermore, as compared to other agricultural land, pesticide use in this region was very low. Similar type of result was found that was amphibian species richness high in organic paddy fields where pesticide use is limited (Attademo *et al.,* 2018). The high abundance of amphibians was recorded along three crop rotations without vegetables. It was due to the availability of adequate water in the paddy field, which helps for amphibian breeding. , there were high numbers of amphibian in that area. Mathwin *et al.,* (2021) also suggested that maintaining water sources has an impact on the amphibian community and species, hence assists in amphibian conservation.

The least diversity and abundance of amphibians was observed in three crop rotation with vegetables. Pesticides, herbicides, and chemical fertilizers were widely used in these regions, resulting in poor habitat quality for amphibians. Hence, pesticides and chemical fertilizers have an adverse effect on individuals development, growth, and reproduction, leading in deformed individuals and, in extreme cases, death (Mann *et al.,* 2009).

In this study, species diversity index of ampbhibians along uncultivated land was found to be higher in Block D and lesser in Block C. This wa due to the presence of water sources and agricultural land nearer to uncultivated land in Block D. Water sources (Wells, 2007) and agricultural land (Duré *et al.,* 2008) supports a high diversity of amphibians since it provides foraging and breeding grounds for amphibians. The highest diversity of amphibians along three crop rotations without vegetables was observed in Block A and least in Block C. This was due to the sufficient amount of soil moisture and water in the paddy field. Amphibians mostly prefer water sources and found in proximity to water, moist places, and soil for the breeding purpose (Daniels, 2005). In addition, block A was far from human settlement compare to block C. Amphibians are negatively impacted by direct human activities or disturbances due to a reduction in the spatial and temporal availability of resources (Rodríguez-Prieto and Fernández-Juricic, 2005). The highest species diversity of amphibians in three crop rotations with vegetables was found in Block D. This was due to the fact that pesticides were used far less in that region than in other blocks. Relyea *et al.,* (2005) also suggested that Amphibians may be affected by pesticides in both direct and indirect ways. Abundance of amphibians was recorded high in three crop rotations without vegetables and less in three crop rotations with vegetables. This was due to the minimal use of pesticides and chemical fertilizers in three crop rotations without vegetable strata. Additionally, these strata were filled with water. These are the important variables that supports the abundance of amphibians.

Unexpectedly, relatively high diversity of amphibian was observed in uncultivated land. This was due to these lands were left undisturbed with natural vegetation and no direct use of pesticides and chemical fertilizers, it was a refuge for amphibians. Additionally, there was a near water source, which provided an excellent breeding location for the species. (Le Cœur *et al.*, 2002) also suggested that natural and semi-natural leftover regions serve as important refuges for a diversity of individuals.

5.2 Effect of agricultural land use pattern on amphibians

There was significant difference for species diversity and different agricultural land-use patterns but not for abundance. It was because abundance was directly influenced by the number of individual of species whereas diversity was the total number of species found in that area.

The diversity and abundance of amphibian species are significantly influenced by environmental variables (Contreras, 2018). The higher numbers of amphibian species were associated with water sources. Water sources are one of the most important environmental factors for tadpole breeding and metamorphosis (Daniels, 2005). Availability of water sources along with terrestrial habitat supports the amphibian population (Wells, 2007). The hydroperiod of water sources has an impact on amphibian diversity and abundance because it controls the rate at which juveniles metamorphosis into adults (Pechmann *et al.,* 1989). Diversity and abundance of ampbhibians was significantly positive with distance to road and settlement. Fewer amphibian species were found near road networks and settlement while the majority of species were found near water sources. This was due to urban characteristics such as traffic and noise pollution near roads being introduced to frog habitats. According to Aryal *et al.,* (2020), modification and loss of natural habitat and water sources for amphibians are influenced by road and it also acts as a barrier for adult amphibians to reach suitable breeding habitat. Diversity and abundance of amphibians were low in areas near roads due to more human movement and anthropogenic disturbances.

The higher numbers of amphibian species are strongly correlated with low and moderate pesticides mode. This was because amphibians are highly sensitive to chemicals like pesticides, herbicides, fungicides, and fertilizers (Mann *et al.*, 2009). Pesticide exposure can inhibit the immune system, preventing amphibians from developing a natural and effective immune response to pathogens (Carey *et al.,* 1999; Christin *et al.,* 2004). The immense use of pesticides causes infectious diseases like chytrid fungus. which have sub-lethal effects like impaired mobility, decreased food intake, and high vulnerability to predators that eventually affect the population viability of amphibians (Blaustein and Johnson, 2003). They are highly affected by chemicals in their growth, development, reproduction and survival (Mann *et al.*, 2009). In contrast to that, amphibian species like *Euphlyctis cyanophlyctis*, *Duttaphrynus melanosticus*, *Minervarya teraiensis* were observed even in a land where a high amount of pesticides are used. These species are generalists due to their high adaptive range and tolerance towards chemicals and pollution (Schleich and Kästle, 2002). Several species of frogs can develop active genetic resistance to a class of pesticides that are frequently used (Gilbert, 2015).

Pesticides are often considered of as a quick, easy, and low-cost way to control weeds and insect pests. Beneficial soil microbes, insects, plants, fish, amphibians, and birds, seem to be at risk from pesticide contamination (Aktar *et al.*, 2009). Pesticides are among the most serious threats to amphibians, since they have a negative impact on their growth, development, abnormalities, reproductive pattern and sex ratio, immunological suppression, and mortality (Mann *et al.,* 2009).

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Diversity of amphibians was rich in the study area. The high species diversity of amphibians was in agricultural land near forests due to assemblages of forest species minimal use of pesticides whereas least in three crop rotations with vegetable cultivation. Species diversity was significantly different along different agricultural land use patterns, though amphibians' abundance was not. Diversity and abundance of amphibians were supported by agricultural land that was closer to water sources and area where less use of insecticides and fertilizers. Thus, agricultural land use patterns effect on amphibians. Awareness campaigns among local farmers about the negative effects of pesticides and fertilizers should be done and they must be encouraged towards organic farming.

6.2 Recommendations

1. Dietary assessment in different agricultural land use patterns is suggested to understand the feeding ecology as feeding ecology influences diversity and abundance of amphibians.
2. Awareness campaign on negative effect of pesticides and fertilizers could be done and encouraged for organic farming.

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APPENDICES

Appendix 1. Questionnaire set

Date: ……………….. Name of farmer: ……………………………..

Age: …... sex: ………

Location: ………………….

QN.1

Crop Land Information

Kattha	**No. of Crop Rotation**	**Crops types**	**On which season**	**Fertilizers used to grow a crop**	**Disease**	**Pesticides Or herbicides used or not**	**Name of Pesticides Or herbicides /frequency**

Q. N 2 What is the Population trend of amphibians in your area?

( ) Increasing ( ) Decreasing ( ) Neutral

Q. N 3 What are the potential threats to amphibians in your area?

( ) Pesticides effect ( ) Road killed ( ) Habitat loss ( ) Pollution and disease

( ) Hunting ( ) Climate change

Appendix 2. Pesticide mode and frequency along strata and block

Block	**strata**	**Frequency**	**Mode of pesticides**
A	Uncult_ land	Zero	No use
A	3crp_without veg	Monthly	Moderate
A	3crp_ with veg	Daily/Weekly	High
A	Near_forest	Monthly	Moderate
B	Uncult_ land	Zero	No use
B	3crp_without veg	Monthly	Moderate
B	3crp_with veg	Daily/Weekly	High
B	Near_forest	Monthly	Moderate
C	Uncult_ land	Zero	No use
C	3crp_without veg	Monthly	Moderate
C	3crp_with veg	Daily/Weekly	High
C	Near_forest	Monthly	Low
D	Uncult_ land	Zero	No use
D	3crp_without veg	Monthly	Moderate
D	3crp_with veg	15days/Monthly	Moderate
D	Near forest	Monthly/Annually	Low

Appendix 3. List of Photograps

1. *Hoplobatrachus tigerinus*

2. *Polypadates maculatus*

3. *Duttaphrynus melanostictus*

4. *Duttaphrynus stomaticus*

5. *Sphaerotheca maskeyi*

6. *Microhyla nyphymerensis*

7. *Euphlyctis cyanophlyctis*

8. *Minervarya teraiensi*