1. INTRODUCTION

1.1 Zooplankton

Plankton are organisms drifting in oceans, seas, and bodies of fresh water. The word Zooplankton is derived from the Greek words- zoon meaning 'animal' and plankton meaning 'wanderer or drifter' (Thurman, 1997). Zooplankton are heterotrophic (sometimes detritivorous) plankton. Individual zooplankton are usually microscopic, but some (like jellyfish) are larger and visible to the naked eyes. Zooplankton are the important source of food for the aquatic organisms and their quality is much dependent on phytoplankton dynamic in the pond. They consume phytoplankton, their animal counterparts, which considered to be the most favorable food sources for fish and other aquatic animals.

Zooplankton are important organisms in freshwater ecosystem since they occupy central position in the food chain. They transfer energy from primary producers to higher trophic such as fish and their community structure, biomass and production influence the whole food web structure of freshwater ecosystem through trophic interactions (Mills and Forney, 1988). Fresh water zooplankton is an important biological component in aquatic ecosystems, whose main function is to act as a primary and secondary links in the food chain and they play a vital role in energy transfer of aquatic ecosystems. Zooplankton can also be used as "bio-indicators" for water pollution studies, because their occurrence, vitality and responses, change under adverse environmental conditions.

There are thought to be over 30,000 species of zooplankton. The natural zooplankton communities of freshwater bodies are composed of ciliates, rotifers, cladocerans and copepods (Thorp and Covich, 2010). Zooplankton play a vital role in the community nutrition of the fresh water system and hence assume importance in aquaculture activities. Fresh water zooplankton is dominated by four major groups of invertebrates- Ciliated protozoans, Rotifers and two subclasses of the Crustacea- the Cladocera and Copepod.

Zooplankton constitute a very important, dynamic and complex component of aquatic ecosystem. Functionally the group includes detritivores, herbivores, carnivores and omnivores, and all these groups are involved in nutrient cycling. Zooplankton are linked directly with phytoplankton, on one hand, and higher organisms and fish, on the other hand. Any impact on zooplankton is likely to influence primary as well as tertiary production. At the same time they are one of the groups most sensitive to toxic chemicals (Hanazato, 2001). So they are frequently used in eco-toxicological tests. The responses of zooplankton to toxicity tests are considered to be informative. Among the toxic chemicals, pesticides affect zooplankton at individual, population and community level (Goodrich and Reach, 1990; Dodhson et al., 1995; Hanazato, 1998, 2001).

Since zooplankton has a quick response to natural disturbances, it may be very useful for the monitoring of environmental changes. The phenology of aquatic organisms like zooplankton will also be affected by temperature increases (Edwards and Richardson, 2004). In the food web, zooplankton act not only as consumers of primary producers but also as food for invertebrate organisms (Hoxmeier and Wahl, 2004; Piasecki et al., 2004). Natural and anthropogenic disturbances (e.g. hydrology, nutrient enrichment, land-use, salinity increase) may strongly influence zooplankton species composition (Quintana et al., 1998; Gilabert, 2001; Badosa et al., 2007; Brucet et al., 2010). Zooplankton are bio-indicators of the climate change for many reasons (Richardson, 2008). Their physiological processes such as ingestion, respiration and reproductive development are highly sensitive to temperature (Mauchline, 1998). For example, Rotifers are the indicators of eutrophication (Saygi et al., 2011; Ustaoglu et al., 2012). Copepods indirectly indicate changes in the trophic status of the water body, such as eutrophication, reflecting changes in the structure of phytoplankton. They also indicate climate changes, predation, contamination with synthetic compounds and the impact of alien species (Richardson, 2008).

1.2 Physico-chemical Parameter

The physico-chemical parameters are the major components which effect the structure and dynamics of the plankton of aquatic ecosystem (Hulyal and Kaliwal, 2009). The physico-chemical properties of water quality assessment give a proper indication of the status, productivity and sustainability of a water body (Djukie et al., 1994). They immensely influence uses of water body for the distribution and richness of biota (Unanam and Akpan, 2006). Changes in the physico-chemical parameters of ecosystem have a substantial impact on the species that live within them (Sharma et al., 2016).

1.3 Malathion

Pesticides are the chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Malathion is an organophosphate compound that acts as an acetylcholinesterase inhibitor disrupts nervous impulses and chemical signaling at neurotransmitter. It is also known as Carbophos, Maldison and Mercaptothion. As it is a non-systemic, wide-spectrum insecticide, it is one of the most frequently used organophosphate pesticides. Malathion is mostly used in agriculture and in public health programs to control infestations of insects including ants, aphids, fleas, fruit flies, hornets, mites, mosquitoes, moths, spiders, wasps and weevil. It is also used as pest control for agricultural food and feed crops including strawberries, limes. Cotton, garlic, etc. It is also widely residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. In the US, it is most commonly used for various eradication programs and for public health purposes.

Malathion is produced and extensively used in Mexico in spite of the fact that it is known to cause health problems to living organisms close to the production facilities (Fuentes-Matus, 2010). It has a high mutagenic potential and high levels of residues in grains from the Mexican State of Sonora ranging from 2 to 25 ng/g (Aldana-Madrid, 2008). It also bio-accumulates up to 10-fold in primary producers and 140-fold in secondary consumers in aquatic ecosystems (Favari, 2002).

Despite the benefits of pesticide use, dispersion of a large amount of Malathion causes environmental and human health problems (Krieger, 2010). Zooplankton species are vulnerable to adverse effect of pesticide from urban and agricultural run offs (Levine, 2007). Inspite of the knowledge on the dangers of this pesticide, little quantified information is available on its toxicity to zooplankton. Malathion significantly decreased the abundance of total zooplankton, cyclopoid copepods, copepod nauplii and Ceriodaphnia. Malathion contamination of aquatic ecosystems can result in changes in the abundance and composition of zooplankton communities.

Malathion has definitely a harmful effect on phylogenetically and ontogenetically young aquatic organisms. It has harmful effects on the fishes as well. Malathion affects blood components, especially by increasing haematological components of erythrocytes and decreasing leukocytes, correspondingly increasing the haemoglobin and mean corpuscular. It also increases the plasma glucose of channel catfish, *Ictalurus punctatus* (Areechon and Plumb, 1990). It also shows harmful effect on ambhibians. A study done by the University of Pittsburgh found that Malathion was lethal to leopard frog tadpoles which killed 99% of leopard frog tadpoles. Malathion causes decrease in the survival rate, growth rate and food consumption in Indian cricket frog tadpole (Gurushankara et al., 2006). As a result of Malathion's intensive use, the amphibian population may be severely threatened (Relyea et al., 2005).

1.4 Objectives

General Objective

- To investigate the zooplankton assemblages structure in Kamalpokhari, Bhaktapur and their responses to Malathion exposure in lab.

Specific Objectives

- To investigate the diversity of zooplankton and to analyze the physico-chemical parameters of the pond.
- To assess the effect of Malathion exposure on zooplankton.

1.5 Rationale of the study

The current study will help to know the impact of Malathion on the zooplankton. It will also help to study the diversity of zooplankton of Kamalpokhari which is one of the biggest and popular ponds of Bhaktapur. It will help to analyze the physico-chemical parameters of the pond as well. This type of study has been done very rarely so its study gives knowledge about the effect of Malathion on zooplankton- its survivality and abundance. So this study will help to conduct this kind of study for the researchers as well.

2. LITERATURE REVIEW

2.1 Zooplankton

Zooplankton is derived from the Greek words- zoon meaning 'animal' and plankton meaning 'wanderer or drifter' (Thurman, 1997). Zooplankton are the most valuable indicator of tropic status than generally has been realized, since they are larger and easier to identify than phytoplankton (Ward and Wipple, 1966). Zooplankton can also be used as "bio-indicators" for water pollution studies, because their occurrence, vitality and responses, change under adverse environmental conditions (Oliver, 1996). Fresh water zooplankton is an important biological component in aquatic ecosystems, whose main function is to act as a primary and secondary links in the food chain and they play a vital role in energy transfer of aquatic ecosystems (Altaff, 2004). The natural zooplankton communities of freshwater bodies are composed of ciliates, rotifers, cladocerans and copepods (Thorp and Covich, 2010).

Natural and anthropogenic disturbances (e.g., hydrology, nutrient enrichment, land-use, salinity increase) may strongly influence zooplankton species composition (Quintana et al., 1998; Gilabert, 2001; Badosa et al., 2007; Brucet et al., 2010). Zooplankton are bioindicators of the climate change for many reasons (Richardson, 2008). Their physiological processes such as ingestion, respiration and reproductive development are highly sensitive to temperature (Mauchline, 1998). Rotifers are the indicators of eutrophication (Saygi et al., 2011; Ustaoglu et al., 2012) and Copepods indirectly indicate changes in the trophic status of the water body, such as eutrophication, reflecting changes in the structure of phytoplankton as well as indicate climate changes, predation, contamination with synthetic compounds and the impact of alien species (Richardson, 2008).

Zooplankton has occasionally been used as a tool to investigate the impact of pollution in marine as well as freshwater communities (Blanc et al., 1975; Benon et al., 1977; EPOPEM, 1979). Zooplankton species that inhabit freshwater bodies such as rivers, reservoirs and lakes are vulnerable to adverse effect of pesticide from urban and agricultural run offs (Levine, 2007). Zooplankton are one of the groups most sensitive to toxic chemicals (Hanazato, 2001).

2.2 Effects of Pesticides

Among the toxic chemicals, pesticides affect zooplankton at individual, population and community level (Goodrich and Reach, 1990, Dodhson et al., 1995 and Hanazato, 1998, 2001). The usage of pesticide in agriculture has potential hazardous impact on aquatic organisms since they are often concentrated in lakes and ponds through the agriculture runoff during rainfall (Richards and Baker, 1993). Insecticide is known to have serious impact on certain zooplankton genera, and consequently affect zooplankton community by the modification of biological interactions throughout planktonic food web (Chang et al., 2005).

Although the secondary or indirect effects of pesticides has been recognized in the past (Hulbert, 1975), most studies on the effects of pesticides on zooplankton concerns direct effects or is based on single species tests (Cairns, 1983). Experimental efforts to understand community effects primarily using single pesticides is done and focuses on a narrow range of Taxonomic groups including zooplankton (Hanazato and Yasuno, 1987, 1989, 1990 and Havens, 1995).

Surface water bodies are contaminated with many anthropogenic toxic chemicals that can affect their natural communities. It is necessary to assess the effect of these chemicals in order to conserve aquatic ecosystems. Among the anthropogenic chemicals, pesticides may cause the most serious problems because they are designed specifically to kill organisms (both the noxious target organisms and other non-target ones) and they are released into the natural environment intentionally. It has been widely documented that pesticide concentrations in the natural environment are often high enough to kill certain organisms. They affect the structure and function of natural communities (Hatakeyama et al., 1990, 1991, 1994 and Helgen et al., 1988).

The effects of pesticides may be direct, resulting in the die-off and reduction in the numbers of the affected organisms in a short period of time. Another impact commonly encountered in the use of pesticides on non-target organisms is that leading to secondary effects. These effects are visible after some period of time after exposure. The complete or partial impact of pesticides on the population of one species may lead to the imbalance of other interacting units of the ecosystem. Depending upon the severity and magnitude of these disruptions, there is always a tendency in the dynamics of the various components to return to their pre-stress state. The secondary effects could manifest themselves in a number of ways such as decreased density and intraspecific competition, emigration, recolonization, and in changes in diversity of the species in the system. Secondary effects may also result from pesticide-induced changes of the target species such as the development of resistance, necessitating higher dosages of a pesticide and thus disrupting the general homeostasis of the ecosystem (Mulla and Mian, 1981). Insecticides whether applied directly to aquatic habitats or used in agricultural crop situations may have an impact on non-target organisms in aquatic and semi-aquatic ecosystems. The nature and magnitude of the impact are influenced by several factors. The lifecycle, reproductive potential, age-susceptibility, dispersal capability, and resistance potential of living organisms as well as specificity or selectivity, rate and frequency of applications, and persistence of pesticides determine the nature and scope of the impact experienced by the affected organisms (Mulla et al., 1979).

2.3 Malathion

Malathion is a pesticide that is commonly used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. It is the most widely used organophosphate insecticide in the US (Blair et al., 2007). Dispersion of a large amount of Malathion causes environmental and human health problems despite its benefits (Krieger, 2010). The abundance of total zooplankton, cyclopoid copepods, copepod nauplii, and Ceriodaphnia decreased due the significant effect of Malathion (Smith et al, 2018).

Malathion is produced and extensively used in Mexico in spite of the fact that it is known to cause health problems to living organisms close to the production facilities (Fuentes-Matus, 2010). It has a high mutagenic potential and high levels of residues in grains from the Mexican State of Sonora ranging from 2to25ng/g (Aldana-Madrid, 2008). It bio-accumulates up to 10-fold in primary producers and 140-fold in secondary consumers in aquatic ecosystem (Favari, 2002).

3. MATERIALS AND METHODS

3.1 Materials

i. Plankton net
ii. Plastic bottles
iii. Slides
iv. Microscope
v. Dropper
vi. BOD bottles
vii. Micropipette
viii. Pipette
ix. Burette
x. Conical flask
xi. Measuring cylinder
xii. Thermometer
xiii. pH meter (HI 98107, HANNA Instrument)

3.2 Chemicals

i. Formalin
ii. Manganese sulphate
iii. Sodium thiosulphate
iv. Starch
v. Potassium iodide
vi. Conc. Sulphuric Acid
vii. Sodium sulphide
viii. Malathion
ix. Erichromeblack T
x. EDTA
xi. 0.1 N HCl
xii. Phenolphthalein
xiii. Methyl orange
xiv. 0.1 N Sodium carbonate
xv. Distilled water

3.3 Study area

Kamalpokhari is located at Kamal Binayak, Bhaktapur which is approximately 15 kilometers northeast away from Kathmandu. It is situated at the northeast end of Bhaktapur on the way to Nagarkot and at the verge of township and rural areas of Bhaktapur District. It lies in Bhaktapur municipality at ward no. 4. It covers the region between the latitude of 27°44′ N and the longitude of 85°26′ E. It is one of the famous ponds which is a religious one surrounded by picturesque natural beauty with a temple of Kamal Vinayak at the side. In Newari, it is known as ‘Lamga Pukhu’ and it is the fourth largest pond in the Bhaktapur District. The pond is made by the local people for different purposes like recreation, religious purpose, etc. It is rich in aquatic vegetation including diverse forms of plankton.

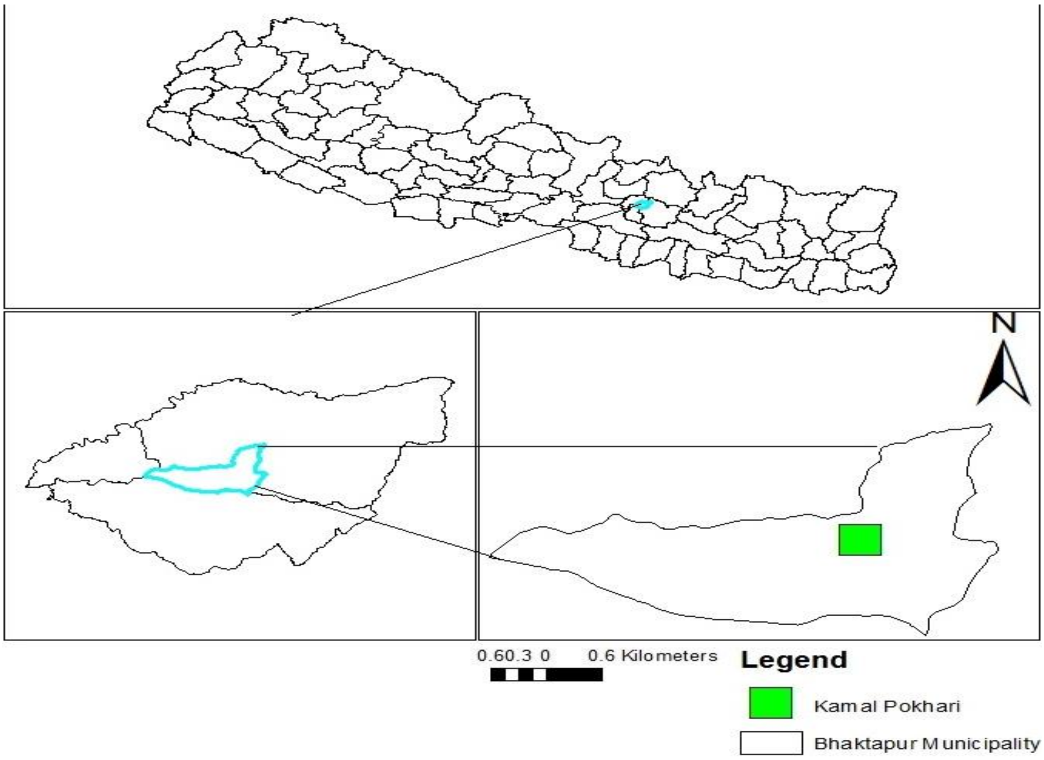

Fig 1. Map of Kamalpokhari, Bhaktapur

The sampling sites were divided on the basis of four different directions of the pond. The sampling sites were:

Site A: Eastern side

Site B: Western Side

Site C: Northern Side

Site D: Southern Side

3.4 Physico-chemical parameters

Different physical properties of pond were analyzed following standard method of Adoni (1985), Trivedy and Goel (1984), American Public Health Association. (APHA, 1998). Physical parameters of the pond like pH and temperature were measured using pH meter and thermometer respectively.

For the analysis of chemical parameters, water sample was collected 15-30 cm below the surface of water in a 1 liter plastic bottle with air-tight cap which was washed properly before collection. The sample bottle was sealed and placed in a dark environment to avoid any contamination in the sample water (Rahmanian and Nizami, 2015). The sample water was brought to the laboratory of Central Department of Zoology and the chemical parameters like dissolved oxygen, alkalinity and hardness of the pond was analyzed by titration method.

3.4.1 Hydrogen Ion Concentration (pH)

The pH was measured by using a calibrated pH meter (HI 98107, HANNA Instrument).

3.4.2 Water Temperature

Water temperature (ºC) was measured with a standard mercury thermometer by placing it in the water at a depth of 1 feet for 1 minute. The temperature was measured once at about 10 AM and the obtained value was recorded.

3.4.3 Dissolved Oxygen

Dissolved Oxygen was calculated using Winkler's method. To determine the dissolved oxygen of water, carefully filled a 300 ml Biological Oxygen Demand (BOD) stoppered bottle brim was filled carefully with sample water. Immediately 2ml of manganese sulfate was added. After the addition of Sulphate, again 2ml of alkali-iodide-azide was added in the same manner and mixing of the chemicals with the sample was done by inverting the bottle several times and left it to settle. Again 2 ml of sulphuric acid was added via a pipette held just above the surface of the sample, and the stopper was carefully tighten carefully to invert the bottle several times to dissolve the floc. After adding all these reagents, out of 300ml of water sample, 100ml of water was transformed into the conical flask and 2 ml of starch solution was added to form blue colour. Finally, sample was titrated against sodium thiosulfate (0.025N) until the sample become colourless and total consumed sodium thiosulfate was noted. The value obtained by this method was calculated by using following equation.

$$\textbf{Dissolved oxygen (DO)} = \frac{ml * N\ of\ titrant * 8 * 1000}{v2\left(\frac{v1-v}{v1}\right)}$$

Where,

V = Volume of MnSO4 and KI added.
V1= Volume of BOD bottle
V2= Volume of the part of the content titrated.

3.4.4 Water Hardness

The total hardness of water was estimated by titrating the water sample against EDTA using Eriochrome Black -T (EBT) indicator. 50 ml of sample was taken in a conical flask and 2 ml of buffer solution was added. After adding, the solution was shaken till the color change wine red and titrated with EDTA until the color of the solution changed to blue and the volume of EDTA consumed was recorded. Total hardness was calculated by using following equation.

Total hardness (mg/L) = $\frac{\textbf{\textit{ml EDTA used}} * 1000}{\textbf{\textit{ml of sample taken}}}$

3.4.5 Total Alkalinity

To determine the total alkalinity, 100 ml of water sample was taken in a conical flask and few drops of phenolphthalein indicator was added, the colour of solution did not change that indicated the absence of phenolphthalein alkalinity or carbonate and hydroxide in the water sample. Then, few drops of methyl orange indicator were added in the same solution, the colour of the solution changed into yellow. Then, the solution was titrated against 0.1 N HCl till the orange colour appeared. The total volume of HCl consumed indicates B.

The total alkalinity was calculated by the formula.

Total alkalinity (mg/L) = $\frac{\textit{(B*N) of HCl*1000*50}}{\textit{Volume of water sample}}$

Where,

B= Total volume of HCl consumed

N= Normality of HCl

3.5 Zooplankton Collection and Identification

The zooplankton sample was collected from Kamalpokhari by the help of plankton net (20cm diameter) made up of bolting silk (no.30; mesh size 25 micron). The water was collected in a 40 liters container and filtered in the plankton net. The zooplankton sample was collected in plastic bottles of 50ml and immediately preserved with 4% formalin for qualitative and quantitative analysis. The collected samples were brought in the laboratory of Central Department of Zoology, TU, Kirtipur for identification. The specimens was observed under microscope, lens 10X and 40X, photographs were taken selectively from Huawei Nova 3i. The zooplankton counting was done by drop method and identification was done up to genus level by using Identification Handbook of Freshwater Zooplankton of the Mekong River and its Tributaries by Mekong River Commission Environment Programme (Dang et al., 2015).

3.6 Culture of zooplankton

The water sample was collected from Kamalpokhari in a 1 litre bottle container for the culture of zooplankton by the help of plankton net (20cm diameter) made up of bolting silk (no. 30; mesh size 25 micron). The two groups of zooplankton i.e. Copepod and Cladoceran were cultured for two weeks. At first the water sample was examined under microscope, zooplankton like *Calanus spp, Cyclops spp* which belong to Copepod and *Daphnia spp and Moina spp* which belong to cladoceran were identified. They were counted by using drop method and were isolated by the help of dropper from the water sample. The Copepod and Cladoceran were counted and kept in two separate test tubes of 30 ml with 5 specimens each. After 24 hours their number was counted and were transferred to 500 ml beakers. Their number was counted again after next 24 hours and the temperature was maintained between 20-22℃. They were counted daily after every 24 hours and they were fed with a pinch of commercial spirulina in every 3 days interval of time which was bought from the market.

3.7 Determination of LC50

Commercial product of Malathion was purchased and the real concentration was determined at the Laboratory of CDZ, TU, Kirtipur. All concentrations of the pesticide were accordingly based on the actual concentration of the active ingredient. Because the tested cladoceran and copepod species differed in their tolerance to Malathion, we conducted a series of preliminary range finding tests. Based on these tests, four sublethal concentrations of Malathion were selected for Cladoceran and Copepod. The four Malathion concentrations were 0.01µl/L, 0.05µl/L and 0.1 µl/L. Daily prepared pesticide concentrations through serial dilution from a stock solution were used.

3.8 Malathion Exposure

The Copepod and Cladoceran species were counted from the beaker where they were cultured. 20 Copepods and 20 Cladocerans were counted and kept separately in 4 beakers of 100ml each which were filled with water. One beaker for both Copepods and Cladocerans was made control and the rest three were treated with different concentrations of Malathion. The concentrations 0.01 µl/L, 0.05 µl/L and 0.1 µl/L were treated in all the three beakers with the Copepods and Cladocerans with the help of micropipette. After 24 hours, 48 hours, 72 hours and 96 hours, the species were observed in a microscope and alive species were counted.

4. RESULTS

4.1 Physico-chemical parameters

The water temperature of the pond was found to be 20℃ and pH 8.8. The value of dissolved oxygen was obtained 5.7 mg/l. The alkalinity of the pond was 112.5 mg/l and the value of hardness was obtained to be 63 ppm (Table 1).

Table 1: Physico-chemical parameters of the pond

S,N,	Parameters	Obtained Value
1.	Temperature	20 ℃
2.	pH	8.8
3.	Dissolved Oxygen	5.7 mg/l
4.	Alkalinity	112.5 mg/l
5.	Hardness	63 ppm

4.2 Zooplankton Diversity

There were diverse groups of zooplankton found in the pond. The diversity of zooplankton were represented by different phylum such as Protozoa, Arthropoda and Rotifera including five classes- Hexanauplis, Brachiopoda, Ostracoda, Monogonata and Oligohymenophorea. Total ten genus of zooplankton were observed.

i. Cyclops spp

ii. Calanus spp

iii. Daphnia spp

iv. Moina spp

v. Hemicypris spp

vi. Keratella spp

vii. Brachionus spp

viii. Polyarthra spp

ix. Filinia spp and

x. Paramecium spp.

The zooplankton identified belong to 3 phyla- Protozoa, Arthropoda and Rotifera. Only *Paramecium spp* belongs to Protozoa phylum. *Calanus spp, Cyclop spp, Daphnia spp* and *Moina spp* belong to phylum Arthropoda whereas remaining four genus including *Keratella spp, Brachionus spp, Polyarthra spp* and *Filinia spp* belong to Rotifera phylum (Table 2).

Table 2. Classification of zooplankton

S.N.	Phylum	Sub-Phylum	Class	Sub-Class	Order	Sub-Order	Family	Genus
1.	Protozoa	Ciliophora	Oligohymenophorea		Peniculida		Parameciidae	*Paramecium*
2.	Arthropoda	Crustacea	Hexanauplis	Copepoda	Calanoida		Calanidae	*Calanus*
					Cyclopoida		Cyclopidae	*Cyclops*
			Brachiopoda		Cladocea	Cladocera	Daphniidae	*Daphnia*
								Moina
			Ostracoda		Podocopida		Cyprididae	*Hemicypris*
3.	Rotifera		Monogonata		Bdelloida		Brachionidae	*Keratella*
								Brachionus
							Synchaetidae	*Polyarthra*
							Trochosphaeridae	*Filinia*

Among the 10 genus of zooplankton, two genus belong to class Hexanauplis, two belong to Brachiopoda, one belongs to Ostracopoda, four to Monogonata and one to Oligohymenophorea. In site A which was the eastern side, only six genus were present out of the 10. Site B which was the western side, consisted of eight genus whereas site C, the northern side consisted of total nine genus. Similarly, in site D, the southern side, only seven genus were observed out of the 10. So, among the four sites of the pond, the most diverse site was site C which was the northern side and the least diverse site was site A which was the eastern side. In site C, only one genus *Paramecium spp* was not observed whereas in site A four genus of zooplankton i.e. *Hemicypris spp, Brachionus spp, Polyarthra spp* and *Paramecium spp* were not found. Among the 10 genus of zooplankton found the most abundant and dominant were *Cyclops spp*, *Calanus spp* (belong to the group Copepod), *Daphnia spp and Moina spp* (belong to the group Cladoceran) which are the two classes of Hexanauplis and Brachiopoda respectively (Table 3).

Table 3: Diversity of zooplankton of the pond

S.N.	Class	Genus	code	Site A	Site B	Site C	Site D
1.	Hexanauplis	*Cyclops spp*	Spp1	1250	650	1550	800
		Calanus spp	Spp2	2350	700	1400	650
2.	Brachiopoda	*Daphnia spp*	Spp3	3550	2550	1410	2250
		Moina spp	Spp4	1050	1350	2530	900
3.	Ostracoda	*Hemicypris spp*	Spp5	-	-	50	-
4.	Monogonata	*Keratella spp*	Spp6	215	200	115	165
		Brachionus spp	Spp7	-	80	100	-
		Polyarthra spp	Spp8	-	65	50	-
		Filinia spp	Spp9	85	215	265	165
5.	Oligohymenophorea	*Paramecium spp*	Spp10	-	-	-	100

10 different genus of zooplankton which were identified belong to four different groups of zooplankton which are Copepod, Cladoceran, Rotifer and Protozoa. Two genus i.e. *Cyclops spp* and *Calanus spp* fall under Copepod whereas *Daphnia spp* and *Moina spp* belong to Cladoceran. *Keratella spp, Brachionus spp, Polyarthra spp* and *Filinia spp* belong to Rotifer whereas Paramecium spp belongs to Protozoa (Table 4).

Table 4. Zooplankton with their respective groups

S.N.	Group	Genus
1.	Copepod	*Cyclops spp*
		Calanus spp
2.	Cladoceran	*Daphnia spp*
		Moina spp
3.	Rotifer	*Keratella spp*
		Brachionus spp
		Polyarthra spp
		Filinia spp
4.	Protozoa	*Paramecium spp*

4.3 Determination of LC50

The median lethal concentration (LC50) was determined by conducting series of preliminary tests. The different concentrations of Malathion showed different results with the survivality of Copepods and Cladocerans. The higher concentrations of Malathion increased their mortality rates and as the concentrations were decreased the mortality rate also decreased slowly (table 5). The LC50 of Malathion for Copepod and Cladoceran was determined to be 0.1µl/L.

Table 5: Concentrations of Malathion to determine LC50

S.N.	Survival rate (%) / Concentrations (µl/L)	Copepod	Cladoceran
1.	10	0	0
2.	3	5	5
3.	2	10	7
4.	1	15	15
5.	0.3	25	20
6.	0.2	40	30
7.	0.1	60	50

4.4 Effects of Malathion exposure on Copepod

Malathion affected the number of spp of Copepods (Cyclop spp and Calanus spp) and Cladocerans. The number of survival of Copepod decreased gradually with increase in the concentrations and also with the increase in the period of time. The number of survival at concentration 0.01µl/L during 24 hrs was 95%, during 48 hrs was 90%, 72 hrs was 75% and 96 hrs was 70%. The number of survival at concentration 0.05µl/L during 24 hrs was

90%, 48 hrs was 75%, 72 hrs was 65% and 96 hrs was 60%. Similarly, when concentration of 0.1µl/L was injected, the number of survival during 24 hrs, 48 hrs, 72 hrs and 96 hrs was 60%, 50%, 45% and 35% respectively (Table 6).

Table 6: Survivality of Malathion exposed group of Copepod (*Cyclop spp and Calanus spp*)

S.N.	DOSES (µl/L) \ TIME (IN HRS)	24	48	72	96
1.	0	100%	100%	95%	95%
2.	0.01	95%	90%	75%	70%
3.	0.05	90%	75%	65%	60%
4.	0.1	60%	50%	45%	35%

4.5 Effects of Malathion exposure on Cladoceran

The number of Cladoceran species also decreased with the exposure of different concentrations of Malathion. In the case of Cladocerans, the number of survival at the concentration of 0.01µl/L during 24 hrs was 90%, 48 hrs was 85%, 72 hrs was 75% and 96 hrs was 70%. When concentration of 0.05µl/L was injected, the number of alive species during 24 hrs was 80%, 48 hrs was 70%, 72 hrs was 65% and 96 hrs was 55%. At the concentration of 0.1µl/L, the number of alive species during 24 hrs, 48 hrs, 72 hrs and 96 hrs was 50%, 40%, 35% and 30% respectively (Table 7).

Table 7: Survivality of Malathion exposed group of Cladoceran (*Daphnia spp* and *Moina spp*)

S.N.	DOSES (µl/L) \ TIME (IN HRS)	24	48	72	96
1.	0	100%	100%	100%	95%
2.	0.01	90%	85%	75%	70%
2.	0.05	80%	70%	65%	55%
3.	0.1	50%	40%	35%	30%

5. Discussion

In this study, the water temperature of the pond was found to be 20°C. Water temperature ranging between 13.5°C and 32°C is reported to be suitable for the development of the planktonic organisms (Kamat, 2000, Gaikwad et al., 2008). Similar observations were made by Bhuiyan and Gupta (2007) and Park and Shin (2007). The water temperature measured in the study was nearly similar to the findings by the above authors. The water temperature plays a significant role in the diversity of zooplankton. The pH of the pond was 8.8, which indicated slightly alkaline nature. It is similar with the values of pH reported by Rajagopal et al. (2009); Santhanam and Perumal (2003). Similar observations were made earlier by Santhanam (1976); Santha Joseph (1975, 1982) and Sivakumar (1982). The alkaline nature of the pond water was may be due to low level of water and high photosynthesis of micro- macro organism resulting in high production of free carbon dioxide during the equilibrium towards alkaline side (Trivedy, 1989; Shiddamallayya and Pratima, 2008). According to Kurbatova (2005) and Tanner et al. (2005) the pH more than 8.5 indicates highly productive nature of the pond. So from the previous studies we can say that the pond is a highly productive for zooplankton.

The value of dissolved oxygen was obtained 5.7 mg/l. It is similar to the DO values reported by Santhanam and Perumal, 2003 and Rajagopal et al., 2009 which shows positive correlation with the zooplankton and similar results were also drawn by Ahmad and Krishnamurthy (1990) and Singh and Singh (1993). The result of DO in this study lies between the normal range which is 5-7 mg/L stated by FEPA (1991) and that of Ragnar (2004) which states DO should be ≥6mg/L. According to WHO (1999), the level of oxygen should be in the range of 4-6 mg/l, so the value of dissolved oxygen of the pond obtained was suitable for the growth of zooplankton. Alkalinity of the pond was 112.5 mg/l. High alkalinity value was also obtained by Rajagopal et al., 2009 and Santhanam and Perumal, 2003. It suggests that high alkalinity coincides with high planktonic yield (Singh et al., 2002; Sachidanandamurthy and Yajurvedi, 2006; Kiran et al., 2007). Higher productivity of water alkalinity should be over 100mg/l (Alikhumi, 1957).

The hardness of the water of the pond was found to be 63ppm. Similar findings were observed by Ratushnyak et al., 2006; Mathivanan et al., 2007 and Park and Shin, 2007. High range of total hardness was may be due to high loading organic substance, detergents, chlorides and other pollutants. Calcium hardness is essential for normal growth and development of many aquatic ecosystems (Meshram, 2005). So the obtained value of the physico-chemical parameters of the pond can be considered productive for the aquatic life.

Total ten different species of zooplankton were identified. Species like *Cyclop spp, Calanus spp, Daphnia spp, Moina spp, Hemicypris spp, Keratella spp, Brachionus spp, Polyarthra spp, Filinia spp and Paramecium spp*. The diversity of zooplankton were represented with different phylum such as Protozoa, Arthropoda and Rotifera which include five different classes- Hexanauplis, Brachiopoda, Ostracoda, Monogonata and Oligohymenophorea. The zooplankton species belong to the four common groups i.e. Copepod, Cladoceran, Rotofer and Protozoa. Similar zooplankton species were found by Rajagopal et al (2009); Thirupathaiah et al (2011); Khatri (1992); Dijik and Zanten (1995). The reason behind the relatively high zooplankton species density could be due to eutrophication effect. The above found species could be the index of eutrophic waters and its abundance is considered as a biological indicator for eutrophication which is stated by Nogueira (2001). *Brachionus spp* is considered to be the indicator of eutrophication (Sampaio et al., 2002) and indicators of sewage and industrial pollution (Nogueira, 2001).

Among the four sites of the pond, site C was found to be the most diverse site as nine genus of zooplankton were reported at that site and only *Paramecium spp* was absent there. Similarly, site A was the least diverse site among the four sites of the pond as only six genus were present out of the 10 species and four species like *Hemicypris spp, Brachionus spp, Polyarthra spp* and *Paramecium spp* were not observed there. Site B consisted of eight genus and two genus of zooplankton which was the second most diverse site. Similarly, in site D only seven genus were observed out of the 10. Among the 10 genus of zooplankton six genus were found in all four sites of the pond. They were *Cyclop spp, Calanus spp, Daphnia spp, Moina spp, Filinia spp* and *Keratella spp*. Cyclop spp, Calanus spp, Daphnia spp, Moina spp were found more in number than all other genus and the genus in least number was Paramecium spp and Hemicypris spp. So, the most abundant and dominant genus were *Cyclop spp, Calanus spp* (belong to the group Copepod), *Daphnia spp and Moina spp* (belong to the group Cladoceran). The most dominant group was Copepod followed by Cladoceran, Rotifer and Protozoa. Khatri (1992) also obtained a high abundance of zooplankton dominated by Copepods which was also found by Dijik and Zanten (1995); Vaidya and Yadav (2008). The result didn't match with Thirupathaiah et al., 2011 and the Rotifer was dominant in that study. The dominance of Copepod may be due to the plentiful food availability as well as due to their continuous breeding and high reproductive capacity (Kumar, 1993).

As the abundance and dominance of Copepod was highest which was followed by Cladoceran, these two groups were cultured in the laboratory and the effects of different doses of Malathion were carried out. The chemical application on zooplankton communities has been studied on artificial tanks and mesocosms (Peither et al., 1996; Hanazato, 1998). Hanazato (1991) exposed different zooplankton communities to Carbaryl and Peither et al. (1996) found that the application of lindane changed rotifer communities indirectly through effects on predators. Hernandez et al (2013) also did the similar work by exposing Malathion on Cladoceran.

After carrying out the series of preliminary range finding tests LC50 value of the pesticide was found to be 0.1 µl/L. According to this 0.01µl/L, 0.05µl/L and 0.1µl/L concentrations of Malathion was used. The median lethal concentration (LC50) for various Cladoceran and Copepod species varied from 1-16 µg /l (Tomlin, 2006; Relyea, 2009). At a sublethal level of 0.01µg/l of Malathion, the survival was affected and reproduction was reduced to about 40% (Wong, 1995). From the experiment, we could find out that different doses of Malathion affected the survivality of the two groups of species. The relative sensibilities of various aquatic invertebrates, including Cladocerans and Copepod, to Malathion toxicity vary considerably (Martinez-Tabche, 1991).

The number of survival of zooplankton of both the groups decreased gradually with increase in different doses of Malathion and with the increase in the period of time. The mortality of the Copepods increased slowly with the increase in time period whereas in the case of Cladocerans, the mortality rate increased rapidly than the Copepods. The number of alive species of Copepods in dose 0.01 µl/L during 24 hrs was 95%, during 48 hrs was 90%, 72 hrs was 75% and 96 hrs was 70%. The number of alive species in dose 0.05 µl/L during 24 hrs was 90%, 48 hrs was 75%, 72 hrs was 65% and 96 hrs was 60%. Similarly, when dose of 0.1µl/L was given, the number of alive species during 24 hrs, 48 hrs, 72 hrs and 96 hrs was 60%, 50%, 45% and 35% respectively.

Similarly, in the case of Cladocerans, when 0.01µl/L was exposed the number of alive specimen during 24 hrs was 90%, 48 hrs was 85%, 72 hrs was 75% and 96 hrs was 70%. When dose of 0.05µl/L was given, the number of alive species during 24 hrs was 80%, 48 hrs was 70%, 72 hrs was 65% and 96 hrs was 55%. When dose of 0.1µl/L was exposed, the number of alive species during 24 hrs, 48 hrs, 72 hrs and 96 hrs was 50%, 40%, 35% and 30% respectively. The number of specimen of both the groups decreased when different doses of Malathion was given with the increase in time period from 24 hrs, 48 hrs, 72 hrs and 96 hrs. While studies show that Cladoceran is quite resistant to toxicants and much sensitive to heavy metals (Garcia-Garcia, 2006), the finding of the study showed both Copepod and Cladoceran are sensitive to the effect of Malathion. Some studies show that survivorship of Cladoceran also decreased at the high concentration of Malathion but the survivorship increased at lower concentration (Hernandez, 2013). Similar result was shown by Hanazato and Yasuno (1990) and Gilbert (1988) which stated that the exposure of pesticide on Cladoceran and Copepod decreased their survivorship rate.

6. CONCLUSION

The water temperature of the pond was found to be 20℃, pH was 8.8. The value of dissolved oxygen was obtained 5.7 mg/l. The alkalinity of the pond was 112.5 mg/l which indicated the higher productivity of the pond. The total hardness of the pond was 63 ppm. So, we can say that overall the value of the physico-chemical parameters of the pond obtained was suitable and productive for aquatic life.

There were diverse groups of zooplankton found in the pond. The diversity of zooplankton were represented by different phylum such as Protozoa, Arthropoda and Rotifera. Total ten genus of zooplankton were observed which were *Cyclop spp, Calanus spp, Daphnia spp, Moina spp, Hemicypris spp, Keratella spp, Brachionus spp, Polyarthra spp, Filinia spp and Paramecium spp*. Among the four sites of the pond, the most diverse site was site C, the northern side which consisted of total nine genus. Only one genus Paramecium spp was absent. The least diverse site was site A with only six genus whereas Site B which was the western side consisted of eight genus. Similarly, in site D, the southern side, only seven genus were observed out of the 10. Among the 10 genus of zooplankton found the most abundant and dominant were *Cyclops spp, Calanus spp* (belong to the group Copepod), *Daphnia spp and Moina spp* (belong to the group Cladoceran).

Malathion affected the number of both the groups Copepods (*Cyclop spp* and *Calanus spp*) and Cladocerans (*Daphnia spp* and *Moina spp*). The number of alive specimen of zooplankton of both the groups decreased gradually with increase in the doses and also with the increase in the period of time from 24 hrs to 96 hrs. Among the two groups, the mortality of Copepods increased slowly with the increase in time period whereas the mortality of Cladocerans increased rapidly with the increase in time period. Thus, the study showed that Malathion affected the survival of zooplankton with different doses of Malathion with respect to the time period.

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APPENDIX

Appendix 1. Zooplankton of Kamalpokhari

Photo plate 1*: Cyclops spp*

Photo plate 2*: Calanus spp*

Photo plate 3*: Daphnia spp*

Photo plate 4*: Moina spp*

Photo plate 5*: Hemicypris spp*

Photo plate 6*: Filinia spp*

Photo plate 7*: Keratella spp*

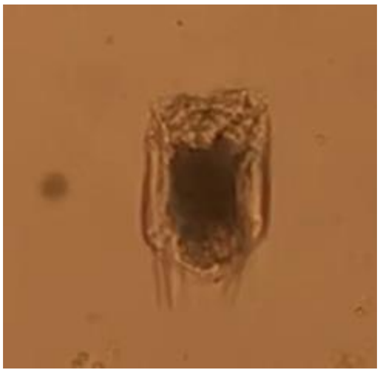

Photo plate 8*: Polyarthra spp*

Photo plate 9*: Paramecium spp*

Appendix 2. Photo plates from field work

Photo plate 10: Site A (Eastern side)

Photo plate 11: Site B (Western side)

Photo plate 12: Site C (Northern side)

Photo plate 13: Site D (Southern side)

Photo plate 14: Collecting water sample for titration

Photo plate 15: Collecting water sample for qualitative and quantitative analysis

Photo plate 16: Measuring water temperature of the pond

Photo plate 17: Measuring pH of the pond

Appendix 3. Photo plates from lab work

Photo plate 18: Titration at laboratory of CDZ

Photo plate 19: Observing zooplankton for identification

Photo plate 20: Observing zooplankton under microscope for identification

Photo plate 21: Culture of Copepod and Cladoceran

Photo plate 22: Micropipette

Photo plate 23: Injecting different doses of Malathion

Photo plate 24: Experimental Setup