MATERIALS AND METHODS

Physico - chemical parameters

For the study of physico-chemical parameters four sites were selected where water remained throughout the year. These sites were Koshi river (Site 1), Seepage stream at Kushaha area (Site 2), Seepage stream at Shripur area (Site 3) and Titrigachhi daha (Site 4). The three sites (Site 1, Site 2 and Site 3) were lotic water bodies and one (Site 4) was oxbow lake. The Site 1 and Site 4 were situated within the Koshi Tappu Wildlife Reserve. The Site 2 and Site 3 were situated at outskirts of the reserve. The study period was two years from July 2002 - June 2004. The water for physico-chemical studies were collected from four sites between 8 A.M. and 11 A.M., once in every month at regular interval. The air temperature and physico- chemical parameters of water were analysed in the field. However, the BOD test after 5 days incubation in the incubator was done in the laboratory of Post Graduate Campus, Biratnagar. Transparency, air temperature and water temperature were recorded between 12 noon and 1 P.M.

For the analysis of physico–chemical parameters Welch (1952), Michael (1984), Trivedi and Goel (1984), Adoni *et al.* (1985), Zobel *et al.* (1987) and APHA (1998) were adopted.

Reagents for water Analysis

Manganous sulphate

48 gm of $MnSO_4$ $4H_2O$ was dissolved in 100 ml of distilled water. Then the solution was filtered through an ordinary filter paper and kept in a reagent bottle.

Alkaline Iodide-Sodium azide (NaN_3) Solution

50 gm of NaOH and 13.5 gm NaI was dissolved in 75 ml of distilled water. The solution was cooled and diluted to 100 ml distilled water. Thus, Alkaline Iodide solution was prepared. Then, 1 gm of NaN_3 was dissolved in 4 ml of distilled water and added to the Alkaline-Iodide Solution.

Sodium thiosulphate ($Na_2S_2O_3$) Solution, 0.025N

6.205 gm of sodium thiosulphate was dissolved in distilled water and diluted to 1 litre distilled water. 5 ml of chloroform was added as a preservative and was kept in a brown glass bottle.

Starch Indicator

1 gm of starch powder was dissolved in 100 ml of warm distilled water and few drops of toluene were added to it as preservative.

Sodium hydroxide, 0. 05 N

10 gm of NaOH was dissolved in 250 ml of CO_2 free distilled water to prepare 1.0 N NaOH solution. This solution was kept in a polythene air tight bottle and again diluted 20 times to prepare 0.05N solution at the time of titration and standardized with HCl.

Phenolphthalein Indicator

0.5 gm of Phenolphthalein was dissolved in 50 ml of 95 % ethanol and 50 ml of distilled water was added. Then 0.05N NaOH solution was added drop wise until the solution turned to pink colour.

Hydrochloric acid, O.1N

12N concentrated HCl (Sp.gr.1.18) was diluted to 12 times with distilled water. Further, it was diluted 10 times. It was standardized against sodium carbonate solution.

Methyl Orange Indicator

O.5 gm methyl orange was dissolved in 100 ml of distilled water and kept in a reagent bottle.

EDTA solution 0.01M

3.723 gm of disodium salt of EDTA was dissolved in distilled water and volume was increased up to 1 litre. The solution was stored in polythene bottle.

Ammonia buffer solution

Solution a) 16.9 gm of ammonium chloride (NH_4cl) was dissolved in 143 ml concentrated ammonium hydroxide (NH_4OH).

Solution b) 1.179 gm of disodium EDTA and O.780 gm of $MgSO_4 .7H_20$ were dissolved in 50 ml distilled water. Then (a) and (b) solution were mixed and diluted to 250 ml with distilled water.

Eriochrome Black T Indicator

0.40 gm of Eriochrome Black T was mixed with 100 gm NaCl and grinded.

Silver nitrate 0.02N

3.400 gm of $AgNO_3$ was dissolved in distilled water and volume was increased up to 1 litre. The solution was kept in dark glass bottle.

5 % Potassium chromate (K_2CrO_4) Solution

5 gm of K_2CrO_4 was dissolved in 100 ml of distilled water.

Methods for Water Analysis

Temperature

The air and water temperature were measured by using a standard mercury thermometer graduated up to 50 °C with a precision of 0.1 °C. The water temperature was recorded by dipping the bulb in water. In each case the thermometer was shaded to prevent direct sunlight on the thermometer and the reading were recorded only after getting constant mercury in thermometer column.

Transparency

The transparency of water was measured with the help of Secchi-disc. It is a metallic plate of 20 cm diameter, having four alternating black and white quadrants on its upper surface and a graduated rope tied with a central hook of the disc. Disappearance and appearance length of the rope was recorded by descending and ascending the disc in the water. Then the transparency of the water was calculated by arithmetic mean of the disappearance and appearance readings.

$$\text{Transparency (D)} = \frac{\text{Just disappearance (X)} + \text{Just reappearance (y)}}{2} = \quad \text{cm.}$$

Hydrogen ion concentration (P^H)

It was measured by a portable P^H meter, model HI8314.

Dissolved oxygen

The dissolved oxygen of water samples were estimated by azide modification method For this 300 ml water sample was taken in a glass stopper BOD bottle avoiding agitation. 2 ml of manganous sulphate was added to the sample water and after few seconds 2 ml of alkaline iodide azide solution was added. The precipitation was allowed to settle down. After that 2 ml of conc. H_2S0_4 was added and shaken thoroughly to dissolve the precipitate. Then, 100 ml of sample was titrated against 0.025N Sodium thiosulphate solution using starch as an indicator. The calculation was made by following formula.

$$\text{Dissolved oxygen in mg/L} = \frac{(\text{ml x N}) \text{ of titrant x 8 x1000}}{V_2 (V_1 - v)/V_1}$$

where, V_1 = volume of sample bottle after placing the stopper.

V_2 = volume of the part of the contents titrated

v = volume of $MnSO_4$ and alkaline iodide azide solution.

Free Carbon dioxide (FCO_2)

Free carbon dioxide was tested by titrimetric method using phenolphthalein indicator. 100 ml sample water was taken in a conical flask and a few drops of phenolphthalein indicator were added. The sample water did not become pink and remained clear it indicated the presence of free carbon dioxide. Then the titration of sample water was done with 0.05N NaOH until appearance of pink colour. The used volume of NaOH was noted and the final calculation was made as follows:

$$\text{Free } CO_2 \text{ mg/L} = \frac{(\text{ml x N}) \text{ of NaOH x1000 x 44}}{\text{Volume of sample water}}$$

Total alkalinity

The total alkalinity was measured by titration method. 100 ml of sample water was taken in a conical flask and a few drops of phenolphthalein indicator were added in it. The sample did not turn pink. Then, 2 to 3 drops of methyl orange indicator was added to the same sample and the titration was done with O.1N HCl until the yellow colour changed to pink. The volume used was recorded and the total alkalinity was calculated as follows:

Total alkalinity as $CaCO_3$, mg/L = $\frac{(B \times N)\ HCl \times 1000 \times 50}{\text{Volume of sample}}$

Where, B = volume of acid used with phenolphthalein and methyl orange.

Total hardness

Total hardness of sample water was determined by EDTA titrimetric method. For the titration, 50 ml of sample water was taken in a conical flask and 1 ml of buffer solution was added. Then, about 100 mg of Erichrome Black T indicator was added and the solution turned to wine red colour. The solution was titrated with 0.01M EDTA solution until the colour changed to blue.

Total hardness, mg/L as ($CaCO_3$) = $\frac{\text{Used volume of EDTA} \times 1000}{\text{Volume of water sample.}}$

Chloride

The chloride of water sample was determined by Argentometric method. 50 ml of sample water was taken in a conical flask and 2 ml of K_2CrO_4 solution was added. That solution was titrated against O.O2N $AgNO_3$ until a persistent reddish brown colour appeared. The volume of titrant was noted and the final calculation was done as follows.

Chloride, mg/L = $\frac{(\text{ml} \times \text{N}) \text{ of } AgNO_3 \times 1000 \times 35.5}{\text{Volume of sample}}$

Biological oxygen demand (BOD)

Biological oxygen demand (BOD) was estimated by azide modification method (APHA, 1998), measuring the difference of oxygen concentration between the initial and incubated sample for five days at 20°C in a BOD incubator. The calculation of BOD was done as follows:

BOD mg/L = $D_1 - D_2$

Where, D_1 = Initial dissolved oxygen (mg/L)

D_2 = Dissolved oxygen after five days (mg/L)

Macro - Biota

The macro-biota were collected and observed randomly in every month from the study area.

Macrophytes

For the study of aquatic macrophytes, plant species were collected randomly from the whole aquatic bodies of study area. The aquatic plants were collected randomly every month from different types of wetlands by using hook stick, boat etc. Immediately after collection, photographs were taken then the plants were numbered and pressed to make herbarium. Some small plants were kept in 10 % formalin for preservation. Collected plants were identified and classified with the help of standard literature (Beddome,1883,1892; Sculthrope,1967; Subramanyum,1974; Babu,1977; Hara *et al.*,1978; Polunin and Stainton, 1984; Iwatsuki, 1988; Koba *et al.*, 1994; Kihara, 1995; Cook, 1996; APHA, 1998; and Press *et al.*, 2000).

The plant species were classified into seven categories as per the scheme of Cook (1996):

(A) Plants not physiologically bound to water
but tolerating longer periods of submergence .....**Helophytes**

(B) Plants physiologically bound to water
with at least part of the generative cycle
submerged in or floating on water :

(1) Plants with the Juvenile phase
submerged in or floating on water and the adult
(flowering) phase terrestrial .....**Tenagophytes**

(2) Plants rooted in the substrate with all
photosynthetic parts submerged :
(a) Leaves borne in rosette .....**Rosulate**
(b) Leaves arranged along elongated stems .....**Vittate**

(3) Plants with some photosynthetic parts in

contact with air :

(a) Plants free floating on the surface, not attached to or penetrating the substrate .....**Pleustophytes**

(b) Plants with roots penetrating the substrate:

(i) Leaves and / or stems floating on but not arising above the water surface .....**Epihydates**

(ii) Leaves and / or stems emerging the water surface .....**Hyperhydates**

Annelids and Arthropods

The annelids were collected with the help of forceps and long steel tounge. Collected specimens were kept in plastic containers in 6 % formalin.

The arthropods were collected with the help of fisher men and local people. They used different types of nets and traps. Then with the help of forceps the collected specimens were kept in 6 % formalin for further study. The collected specimens were identified with the standard literature (Pennak, 1953; Ward and Whipple, 1959; Dillon and Lawrence 1961; Needham and Needham, 1962; Mellanby 1963; Rose, 1965; Edmondson, 1966; Mirrit and Cummins, 1978; Tonapi, 1980; Klemm, 1982; Klemm, 1985; Ward, 1992; Datta Munshi and Datta Munshi, 1995; and APHA, 1998).

Molluscs

The molluscs were mainly collected by hand picking method and also with the help of nylon cloth net (40 meshes/cm^2), nylon scoop, forceps etc. The collected specimens were kept in polythene bags and plastic containers. Then the animals were preserved in 5 % formalin for further study. Dead specimens with dry shells were also collected. Identifications were made according to Preston (1915), Baker (1928a, 1928b), Goodrich (1932), Mellanby (1963) and Subba Rao (1989).

Fishes

Fishes were collected with the help of fisher men. Fisher men used different tools e.g. Rod and Line (Balchhi), different types of nets (Gill net, Cast net, Thakauli net, Scoop net etc.), traps (Chanch, Dhasa, Sola or Hoka, Ganj, Khunga or Badara Dhadiya etc). Sometimes fishes were also picked up from the crevices of rocks, shallow marshes and mud burrows. The collected fish specimens were preserved in 10 % formaldehyde solutions for further study and identification. For the identification of fishes different standard literature were consulted like Day (1878), Srivastava (1992), Jayaram (1981), Shrestha (1981), Jhingran (1991), and Shrestha (1994).

Herpetofauna

The amphibians were collected by the help of local people and fisher men. They used different materials like rubber glove, long iron tongue, different nets (Cast net, Scoop net etc.). The collected specimens were preserved in plastic containers in 10 % formalin for further study.

In case of reptiles, long iron tongue, rubber glove, scoop net, plastic container etc. were mainly used for collection. Some of the collected specimens were preserved in 10 % formalin for further study. Some reptiles were released after taking photographs. Informations about snakes were collected from local people. Reports about the presence of *Gavialis gangeticus* and *Crocodylus palustris* were also collected from local people.

The collected specimens of amphibia and reptile were identified with standard literature (Stebbins, 1966; Cochran and Goin, 1970; Shrestha, 1981a; Daniel, 1983; Conant and Collins, 1991; Das, 1991; Schleich, 1993; Murthy, 1995; Shrestha, 2001; and Schleich and Kaestle, 2002).

Aves

The population of birds was estimated in every month by direct counting method. Binocular and Zoom camera were used during bird watching. The main field books for the identification of birds were of Inskipp and Inskipp (1985), Ali and Ripley (1986), Fleming *et al.* (2000) and Shrestha (2000). The check list of observed birds was prepared.

Mammals

In case of mammals, they were recorded in their original habitat. In addition to the visual observations, the presence of some species was also confirmed by their markings and droppings. Their identification was done by following standard books of Corbet and Hill (1992), Shrestha (1997), and Majupuria and Majupuria (1998).

Statistical Analyses

Standard deviation, correlation coefficient were calculated by using Microsoft excel statistical function of computer software. The significance of correlation coefficient was tested by applying t- test. It was done manually. Two way ANOVA was used to test the significant and insignificant difference among sites and seasons and it was done by using Microsoft excel statistical function of computer software.