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# Evaluation of bacteriological parameters and heavy metal contents on water of Tinau river, Butwal, Rupandehi, Nepal

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**Abstract**

The present work was undertaken to assess the surface water quality and discuss the potability of water by analyzed data of bacteriological (total Coliform and Faecalform) characters and heavy metals content (Fe, Mn, Zn, Pb, Cr and Cu) in the water of Tinau river. The study was carried out in the year 2011/2012 by selecting four spots within three different seasons: winter, summer and rainy. For bacterial enumeration, bacterial plate counts were carried out using the pour plate method with nutrient agar; while for determination of heavy metal concentration, Flame Atomic Absorption Spectro-photometry was used. The heavy metal content was within the range of WHO guideline for drinking purpose. However, on the basis of bacteriological parameters the water was not safe to consume without treatment. This study will also help to make aware those local people or adjacent farmers for proper management of waste disposal and also to minimize use of synthetic inputs.

***Key words***: Coliform and Faecalform bacteria, Drinking water, Human consumption

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## Introduction

The quality of drinking water is one of the most pressing issues in Nepal. The major sources of drinking water in Nepal are ground water and surface water. These sources of water are often contaminated by pathogens (especially Colliform bacteria) and heavy metals. The effect of heavy metal on fresh water ecosystem has become global concern. These metals are persistent and once released the environment for a prolonged period (Ghorade, 2013).

The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Lenntech, 2008). Trace quantity of many metals such as Nickel (Ni), Manganese (Mg), Lead (Pb), Chromium (Cr), Cadmium (Cd), Zinc (Zn), Copper (Cu) and Iron (Fe) are though important constituents of most waters as they are necessary for growth of biological life in water, but numerous metals have received attention as they serves as environmental contaminants and toxicological hazards to human. For instance, the occurrence of arsenic, cadmium and lead in drinking water is considered an important pathway of potential exposure of man's various deadly diseases such as cancer, kidney diseases and impaired cognitive function (Jarup *et al*., 2000). Furthermore, the usability/portability of water is being affected most especially when some metals (such as Zinc, Copper, Iron, Cadmium, etc.) are found in concentrations higher than the desirable amount as they tend to biomagnifies and pose high risk to human health especially due to the recalcitrant nature of these contaminations (Lapygina *et al*., 2002).

Microorganisms are widely distributed in nature and their abundance and diversity may be used as an indicator for the suitability of water (Okpokwasili and Akujobi, 1996). The micro-biological quality of water is measured by the analysis and enumeration of indicator micro-organisms (Briancesco, 2005). The indiscriminate release of liquid waste of organic and inorganic nature changes physico-chemical characteristics of water and causes hazard to flora and fauna including important member of food chain of man and aquatic ecosystem. So, safe drinking water should be free from coliform organisms and heavy metals with their tolerable limits (HMG/NBSM, 2002).

In Nepal, bacteriological quality of the drinking water is a neglected issue. We are aware of only a few researches done in this field. Up to best of our knowledge no researches have been conducted to identify the bacteriological quality of water from different sources supplying drinking water to most of the urban and rural areas of Nepal including Tinau river, Butwal. It is a well known fact that the quality of the water from drinking water source determines the quality of the water reaching to the consumers in significant level. The present study is aimed to investigate both heavy metals contents [such as Iron (Fe), Copper (Cu), Chromium (Cr), Lead (Pb), Cadmium (Cd), Zinc (Zn) and Fluoride (F)] and bacteriological characteristics of water in Tinau river; use for the drinking water which serves the major source of water consumed by the town bearer.

## Materials and methods

### *Collection of water samples*

The study was carried out in Tinau River (Fig. 1). Water samples were collected from four different locations; Jhomsa (27043'03.72"N to 83027'52.56" E; 497 m alt), Dobhan (270 44'42.24" N to 83027'40.44" E, 275 m alt), Chidiya Khola (27043'16.13" N to 83028´11.18″ E, 266 m alt) and Butwal Tinau (270 43'03.72" N to 83027'52.56" E, 204 m alt.) on seasonal basis. To determine the water quality, water samples were collected during winter (15$^{th}$ December, 2011), summer (12$^{th}$ April, 2012) and rainy (10$^{th}$ August, 2012) seasons. A triplicate water samples from each sampling sites were collected in standardized PET (poly-ethylene terephthalate) thermostated bottles. The PET bottles of 1.5 liter capacities and 0.5 liter capacity with stoppers were used for sample collection. The bottles were washed thoroughly with 2% nitric acid and subsequently rinsed with distilled water. Before collecting the water samples, all bottles were rinsed with sample water 2-3 times. All the sampled bottles were made watertight by air tightening it inside water. Precaution has been taken to remove any air bubble present. Each container was clearly marked with the name and date of sampling. All the samples were preserved at 4$^0$C till analysis.

### *Enumeration of total bacterial counts* (*TBC*)

Determination of bacterial load in the water samples were done in triplicates. Bacterial plate counts were carried out using the pour plate method with nutrient agar. This method was based on the serial dilution of water sample, which were then pipette into each sterile Petri-dish. About 20ml of sterilized molten Nutrient agar was cooled to 45$^o$C and poured into each Petri-dish containing 1ml of the water sample. Plates were allowed to cool and set after which were incubated in an inverted position at 37°C. After 24 hrs of incubation, the plates were counted using colony counter (Olutiola *et al*., 1991; Barrow and Feltham, 1993).

### *Estimation of total coliform count* (*TCC*)

Total coliform was estimated by using the membrane filtration technique (Olutiola *et al*., 1991). The water sample was filtered using a membrane filter thereby retaining the bacteria cells on the upper surface of the membrane filter. A 20 ml of sterile molten MacConkey agar (without salt) plate was inoculated by placing the membrane filter on it, and inoculated at 37°C for 24 hrs in an inverted position. After incubation, membrane filter was transferred to an absorbent pad saturated with methylene blue solution (0.01%) and allowed to stain for 1 minute. Subsequently, membrane filter was saturated with sterile distilled water so as to remove excess stain. Colonies are counted on membrane filter and used to calculate the number of coliforms per ml of the original sample recorded in CFU/ml unit.

### *Mineral analysis*

Zinc, iron, copper, lead, nickel and manganese were analyzed in the three matrices using a Perkin Elmer model 306 Atomic Absorption Spectrophotometer. While potassium, magne-sium, sodium and calcium were analyzed from

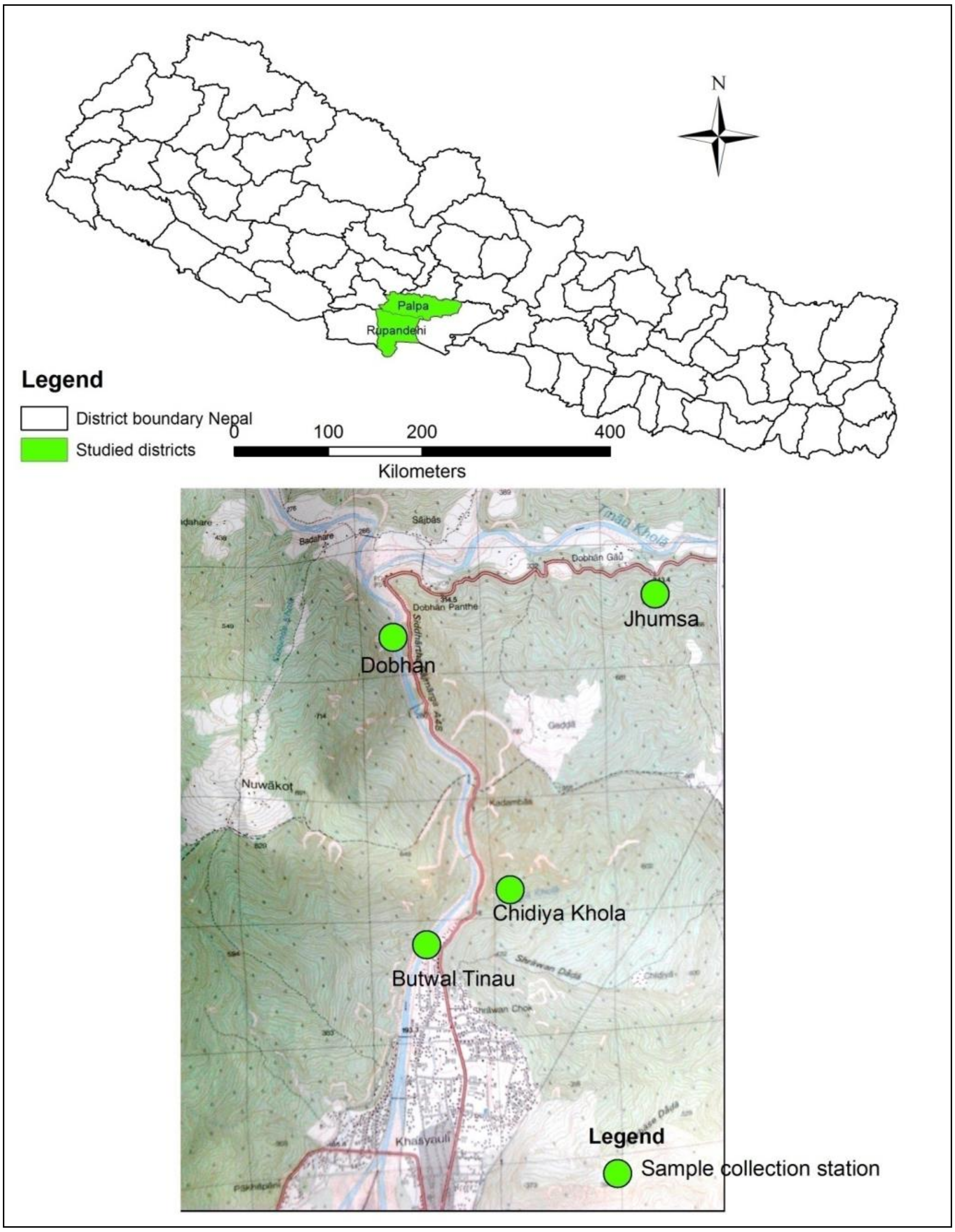


**Figure 1**. Location of study area.

solution obtained by means of Atomic Absorption Spectrophotometer (PYE Unicam Sp 9, Cambridge, UK) (AOAC, 2005) all were recorded in milligram per liter.

## Results and discussion

### ***Total Coliform* (*TC*)**

The average value of TC count in Jhomsa, Dovan, Chidiya khola and Butwal Tinau were 85.33, 113.33, 98.3 and 141 colonies/100 ml, respectively (Table 1). The highest value of TC in all sites was recorded in rainy followed by the summer season. The highest value was found in Butwal Tinau (152/100 ml) during rainy and the lowest in Jhomsa (71/100 ml) during winter season. The fairly high value of total coliform are indicative of increasing pollution of the river by organic means particularly through the discharge of sewage and domestic effluents into the river, Sullivan *et al.* (2007). The significant difference in TC count may be due to difference in pollution level as well as the variation in temperature and the discharge rate of the stream. The results of present study draw support from the finding of Radha and Seenayya (2004), who have worked on the bacteriological water quality of Gangetic river system and Husainsagar Lake respectively. The maximum TC count in Butwal Tinau may be by human and animal disposal from the human settlement on the bank of river.

**Table 1.** Total coliform in three different seasons (n=3): 2011/2012

| Water sample sources | Average colony/100 ml | | | Average |
|---|---|---|---|---|
| | Winter | Summer | Rainy | |
| Jhomsa | 71 | 90 | 95 | 85.33 |
| Dobhan | 100 | 110 | 130 | 113.33 |
| Chidiya Khola | 97 | 97 | 101 | 98.3 |
| Butwal Tinau | 141 | 130 | 152 | 141.0 |

### *Faecal Coliform* (*FC*)

Average number of FC in Jhomsa, Dovan, Chidiya Khola and Butwal Tinau were 3.40, 48.6, 18 and 74.33 colonies/100 ml, respectively (Table 2). The highest value was recorded in all sites during mid monsoon and lowest during winter. During mid monsoon, the highest number of colonies was found in Butwal Tinau (90/100ml) and the lowest in Jhomsa (6/100ml). The FC counts of the present research were somewhat lesser than Madrikhola, Bagmati and Amalabisauni reservoir in Pokhara (Shrestha *et al*., 1991). The faecal coliform found maximum in Butwal Tinau was due to discharge of excreta and waste disposal.

**Table 2.** Faecal coliform in three different season (n=3).

| Water sample sources | Average colony/100 ml | | | Average |
|---|---|---|---|---|
| | Winter | Summer | Rainy | |
| Jhomsa | 2.2 | 2 | 6 | 3.40 |
| Dobhan | 48 | 43 | 55 | 48.67 |
| Chidiya Khola | 12 | 16 | 26 | 18.0 |
| Butwal Tinau | 71 | 62 | 90 | 74.33 |

According to WHO, coliform bacteria should not be detected in drinking water supplies and according to specification of drinking water distributed through pipeline distribution system, no sample should contain faecal coliform organisms (HMG/NBSM, 2002). The analysis of bacterial parameter was made on the basis of quantitative range of FC, developed by WHO. In this classification, the FC water quality is divided into four-risk grades i.e., no risk, low risk, high risk and very high risk (ENPHO, 2001) (Table 3).

**Table 3.** Risk concepts and Faecal Coliform grading (ENPHO, 2001)

| Faecal coliform grading | Grade | Risk |
|---|---|---|
| 0 | A | No risk |
| 1-10 | B | Low risk |
| 11-100 | C | High risk |
| 101-1000 | D | Very high risk |

According to ENPHO (2001), all the water samples from the present study sites could be categorized as high-risk grade but only one sampling in each season except Jhomsa station may not be reliable to categorize these on high-risk grade.

### *Heavy metal*

Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. The released of heavy metal without proper treatment posses a significant threat to public health because of its persistence, bio-magnifications and accumulation in food chain (Rajendra *et al.*, 2003). In the present study, concentration of some of the metallic elements in the study sites were above the concentration of natural level for a stream as given by Whitton and Say (1975). Therefore, the metal elements though being in trace amount, their slight increase can also be regarded as the most useful parameter for evaluation of water pollution and its load and tread in the river. In the present study, most of the selected heavy metals were found in highest concentration in pre-monsoon and lowest in winter. But the value of Fe was lowest in the pre monsoon and the value of Cu was highest in the monsoon season. However, the Tinau river water is safe to use for drinking purpose (Table 4) according to WHO (1991) and HMG/NBSM (2002) guidelines (Table 5).

***Iron* (*Fe*):** The concentration of Iron in ppb level was varied from 0.04 (Jhomsa) to 0.26 (Butwal Tinau) (Table 4). High concentrations of iron generally cause inky flavor, bitter and astringent taste (Hassan, 2012). It can also discolour clothes, plumbing fixtures and cause scaling which encrusts pipes.

***Manganese* (*Mn*)**: The concentration of copper in ppb level was varied from <0.01 (Jhomsa, Chidiya Khola) to 0.06 (Jhomsa) (Table 4).

***Copper* (*Cu*):** The concentration of copper in ppb level was varied from <0.01 to 0.01 (Dobhan) (Table 4). High level of copper can cause harmful effect such as irritation of nose, mouth and eyes, nausea, vomiting, diarrhea, lesions in Gastro Intestinal Tract (GIT). In the study area in the months of monsoon the victims of above diseases have been recorded in the primary health centers.

***Chromium* (*Cr*)**: The concentration of chromium in ppb level was <0.05 in all station and season (Table 4). The major sources of chromium are the electroplating and metal finishing industries

**Table 4.** Heavy metal content in three different seasons (n=3)

| Heavy metals | Average value heavy metals (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Winter season 2011 | | | | Summer season 2012 | | | | Rainy season 2012 | | | |
| | a | b | c | d | a | b | c | d | a | b | c | d |
| Iron (Fe) | 0.06 | 0.13 | 0.16 | 0.26 | 0.05 | 0.08 | 0.15 | 0.23 | 0.04 | 0.09 | 0.13 | 0.16 |
| Mn | <0.01 | 0.02 | <0.01 | 0.03 | < 0.01 | 0.02 | 0.01 | 0.01 | 0.06 | 0.02 | 0.03 | 0.02 |
| Zinc (Zn) | <0.01 | 0.11 | 1.03 | 0.07 | 0.14 | 0.22 | 2 | 0.14 | 0 | 0.13 | 0.98 | 0.09 |
| Lead (Pb) | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Chromium (Cr) | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Cupper (Cu) | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.01 | <0.01 | 0.01 | <0.01 | 0.01 | <0.01 | 0.01 |

(a- Jhomsa, b- Dobhan, c- Chidiya Khola, d- Butwal Tinau)

**Table 5**. WHO, HMG/NBSM (2002) guidelines for some heavy metals in drinking water.

| Elements | WHO limit | Nepal Government/NBSM limit (mg/l) | | Remarks |
|---|---|---|---|---|
| | | Tolerable | Max. tolerable | |
| Iron (Fe) | < 0.3 | 0.3 | - | Adverse effect on domestic use, water supply structures, taste/ appearance |
| Manganese (Mn) | <0.1 | 0.1 | upto 0.5 | Adverse effect on domestic use, water supply structures, taste/ appearance |
| Zinc (Zn) | <3.0 | 3.0 | - | Toxic , imparts to water an undesirable astringent taste |
| Lead (Pb) | <0.01 | 0.01 | no relaxation | Toxic, when hardness is low |
| Chromium ($Cr_6$+) | <0.05 | 0.05 | no relaxation | May be carcinogenic |
| Copper (Cu) | <1.0 | 1.0 | upto 1.5 | Astringent taste.gastrointestinal distress, discoloration and corrosion of pipes and fittings. |

and publicly owned treatment plants relatively minor sources (other than localized contamination) are iron and steel foundries, inorganic chemical plants, tanneries, textile manufacturing, and runoff from urban and residential areas.

***Lead* (*Pb*)**: The variation of Lead metal in ppb level was <0.05 in all station and season (Table 4). The lead concentration was increased and by excess released free metal ions into the water bodies from kitchen utensils and solubility of old paintwork from building during acidic wet deposition.

***Zinc* (*Zn*)**: The concentration of Zinc in ppb level was varied from <0.01 (Jhomsa) to 2 (Chidiya Khola) (Table 4). The zinc content was higher in summer. In summer, the water volume of the river was reduced substantially, it is likely that the heavy metal concentration increases with the anthropogenic input or it may be due to the natural and anthropogenic activities, agricultural runoff, domestic activities, wastewater discharges, effluent discharges and another non-point sources opened into water bodies.

## Conclusion

Microbiologically, the water of the study sites contained high number of total coliform and feacal coliform in some extent. The result indicated that the water of Tinau river was not suitable for direct consumption as drinking purpose. Hence, proper treatment is essential before distributing water to public. Disinfection with chlorine prior to supply can easily remove the contamination of the water. Comparison among all the study sites, the pollution problem was higher in Butwal station than other sites. It showed high values of heavy metal and bacterial parameters. Among all the sites Jhomsa site was in the best condition. This study will also help to make aware those local people or adjacent farmers for proper management of waste disposal and also to minimize use of synthetic inputs. The study indicated that increase in toxic waste day by day in river produced biological magnification in food chain, which is a challenge to scientists, policy makers and administrators in the conservation of the environment.

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