

NORWEGIAN UNIVERSITY OF LIFE SCIENCES



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Abstract

The composition of macroinvertebrate assemblages at 6 different stations of the Bagmati River was studied by field sampling in February 2012. A total of 2583 benthic macroinvertebrates representing 10 orders and 29 families were recorded in the river. Diptera formed the major part of the benthic invertebrates with 45.1% followed by Ephemeroptera, 24.5%, Oligochaeta 15.9% and Trichoptera 5.6%. The abundance of the macroinvertebrates varied significantly between the stations. The EPT index and EPT to Chironomidae ratio showed that highly sensitive taxa were abundant in the upstream sites of the river whereas the pollution tolerant taxa were abundant at the downstream sites.

The biotic indices (BMWP, ASPT, FBI, CLI, and EPT) revealed that the ecological condition of the river was good at the upstream sites and very poor in the downstream direction. The relationship between biotic indices and dominant taxa of the sampling stations of the Bagmati River with the physico-chemical parameters was highly significant. This study shows that distribution of benthic macroinvertebrates in river reaches with differing pollution can function as biological indicators in the bioassessment of the river and can also be used in the evaluation of the water quality in other freshwater localities in Nepal.

(Key words: Macroinvertebrates, Bioindicator, Biotic Indices, Bagmati River)

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1. Introduction

Studies on the use of benthic macroinvertebrates assemblages in biomonitoring technique have been broadly described in the literatures (Beyene et al. 2009; Ogbeibu & Oribhabor 2002; Parr & Mason 2003; Pires et al. 2000; Varnosfaderany et al. 2010). Specifically, associations between macroinvertebrate community structure and environmental variables have been the subject of numerous studies (Davis et al. 2003; Gray & Delaney 2010; Iliopoulou-Georgudaki et al. 2003).

An ideal situation to assess the quality of running waters is to use the physical, chemical and biological parameters to provide the entire range of information for appropriate water management. However, such a study is more time consuming and expensive than the use of only biological parameters which is broadly accepted and gives reliable information about the quality of running water (Iliopoulou-Georgudaki et al. 2003). Invertebrate communities are good indicator of water quality (Resh 1995) since freshwater macroinvertebrate species vary in their sensitivity to organic pollution (Rosenberg & Resh 1993). Benthic macroinvertebrates are highly suitable for monitoring the ecological condition and identifying the natural and human impacts to rivers (Barbour 2008; Korte et al. 2010).

Benthic invertebrate based river quality assessments are potentially appropriate tools for water management in Asia due to steep pollution gradients, and the impacts of other stressors which are well manifested by the biota (CPCB 2006). Application of benthic macroinvertebrates in assessment of the ecological quality of rivers in Nepal started in 1985 following the Trent Biotic Index (FPAN-DIVIS 1988). However, in recent years, many studies have been carried out on this topic with the formulation of site-specific biotic scores in this region (Ofenböck et al. 2010; Pradhan 1998; Sharma 1996).

The Bagmati River is the major water source of the Kathmandu valley providing approximately 92% and 60% of wet and dry season water supply respectively (CBS & HMG 1998). Almost 20-30 years ago, the river was suitable for use as drinking water (Erlend 2002) but in recent years, the surface waters are deteriorated due to lack of sufficient water treatment facilities that have expedited the release of wastewaters and untreated waste materials from hospitals, domestics and industries into the river (MOPE 2000).

The main purpose of this study was to (1) examine the overall benthic macro-invertebrate composition along a pollution gradient of the Bagmati River, (2) determine the biological water quality based on the benthic macro-invertebrates, by applying biotic indices such as BMWP/ASPT and other metric values described by Plafkin et al. (1989), (3) relate the biotic indices to the environmental variables and (4) determine the association between dominant macro-invertebrates identified in the river and the physico-chemical variables. To address the above mentioned issues, I tested the following hypotheses:

- a) There is no significant difference in the abundance of benthic macroinvertebrates between the different Bagmati River sampling stations.
- b) There is no relationship between BMWP and ASPT scores.
- c) There is no significant association of environmental variables with the biotic indices and the dominant taxa identified in the river.

2. Methodology

Description of the study area

The Bagmati watershed is located in the central mountain region of Nepal which encompasses nearly 3719 km² within Nepal and joins the Ganges River in India (Sharma & Shakya 2006). The present study covered an approximately 42 km segment of the river (Fig. 1). Altogether six stations were selected from the river of which middle three stations (S3, S4 and S5) cover the Kathmandu valley. The Kathmandu valley is located in the midland of the lesser Himalayas and is almost round in shape measuring approximately 30 km east-west and 25 km north-south direction (Dill et al. 2001). The present study sites were selected on the basis of effluent discharge, land use patterns, other human impacts, solid waste disposal, substrate structure and bank types.

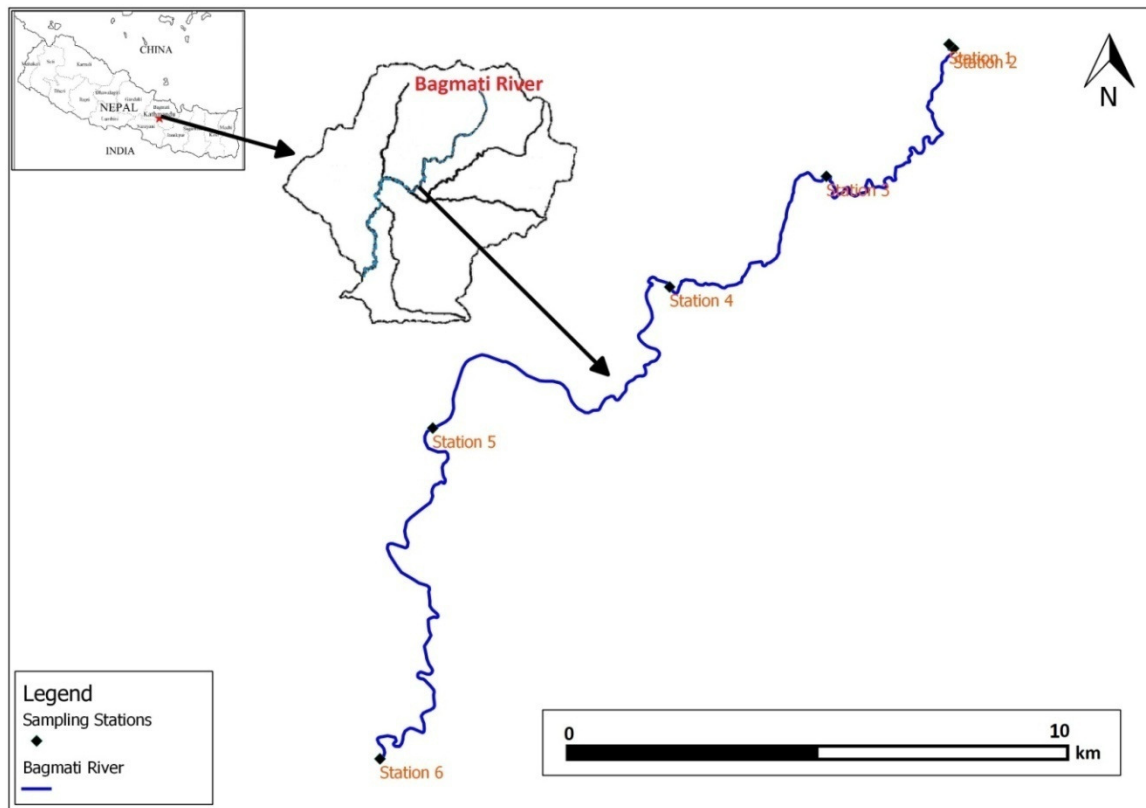


Fig. 1. Location of the Bagmati watershed and the study area in the Bagmati River, showing all six stations (stations 1 and 2 are overlapping due to small distance between them).

Station 1

The station 1 (S1) was selected as the reference site (fig. 2) and is situated just above the Sundarijal dam at $27^{\circ}46'23''$ N latitude and $85^{\circ}25'33''$ E longitude and at 1631 m a.s.l. The substratum was composed of the coarse cobbles, cobbles, boulders and coarse gravels (Annex 3). The banks were eroding with herbs, shrubs and trees on both sides. The site was not influenced by human activities (fig. 2).



Fig. 2. Station 1 (just above the Sundari jal drinking water supply dam)

Station 2

Station 2 (S2) (Fig. 2) was selected just below the Sundarijal dam, 0.32 km downstream of the reference station. The site is situated between 27°46'18" N latitude and 85°25'36" E longitude and at an elevation of 1611 m a.s.l.



Fig. 3. Station 2 (Below the sundarijal Drinking water supply dam)

The banks of the river were rocky, steep with herbs, shrubs and trees on both sides (Fig. 3). The site was not impacted except from the dam. The substratum was dominated by boulders and coarse cobbles 35% and 30% respectively.

Station 3

Station 3 (also mentioned as S3) (Fig. 4) is second downstream station from the reference site located 8.53 km downstream from the S2. This station was selected as the entry point of the pollution, is situated between $27^{\circ}44'23''$ N latitude and $85^{\circ}23'24''$ E longitude at 1338 m a.s.l. The banks of the river were eroding with some herbs and shrubs on both sides but without any trees (Fig. 4). The substratum of the river was dominated by the cobbles (32%) followed by the coarse gravel. The site lies in the vicinity of human habitation area, hence highly influenced by the human impact and agricultural activities.



Fig. 4. Station 3 (near Gokarneshor temple)

Station 4

Station 4 (also mentioned as S4) (Fig. 5) situated between $27^{\circ}42'37''$ N latitude and $85^{\circ}21'15''$ E longitude, is in the middle of the Kathmandu city. This site is 6.7 km downstream from the station 3 and is situated at 1319 m a.s.l. The left bank of the river was artificial where as the right bank was natural with some herbs and shrubs (Fig. 5). The substratum structure was dominated by the Pelal (mud and sludge, 40%) followed by the Psammopelal (sand and mud). The river at this station was heavily affected by waste materials and was loaded with sewage.



Fig. 5. Station 4 (near Guheshori temple)

Station 5

Station 5 (mentioned as S5) (Fig.6) was selected at the exit point of the river from the Kathmandu valley and is far downstream from S1. The distance between S4 and S5 is 12.03 km.



Fig. 6. Station 5 (near Chovar)

This site is situated between $27^{\circ}40'26''$ N latitude and $85^{\circ}17'35''$ E longitude, at 1283 m a.s.l. The left bank of the river was artificial at some points whereas the right bank was natural with herbs and shrubs (Fig 6). The substratum of the river at this site was dominated

by coarse gravel (35%) followed by gravel, cobbles and Psammopelal (sand and mud). The bank of the river was full of debris and dead bodies of animals.

Station 6

Station 6 (mentioned as S6) (Fig.7) is the farthest station from the reference site, approximately 42 km downstream and 14.1 km from S5. The site is situated between 27°35'46" N latitude and 85°16'57" E longitude, at 1235 m a.s.l. The substratum of the river was dominated by boulders (40%) followed by coarse cobbles, psammopelal (sand and mud) and pelal (mud and sludge). Though this site was very far from the human settlement area, the river water was highly polluted. The banks of the river were full of flood flashes and debris of dead animals, plants and other solid wastes (Fig. 7).



Fig. 7. Station 6 (near Dukuchhap)

Physico-chemical characteristics of Bagmati River

A preliminary survey was undertaken from 2nd- 4th of February 2012 to obtain the physical and biological information at different sites of the Bagmati River. Benthic macro-invertebrate sampling and the water sampling was performed during the same period (5th-15th February 2012). During the period, geographical locations (latitude, longitude and altitude) of the sites were also recorded with the help of GPS (Garmin e Trex Vista C). The substrate composition of the river was visually estimated from clay to boulders following the categories given in Moog (2007 b). The details of the substrate composition are shown in Annex 3. Water was also sampled for measuring the chemical and physical parameters of the river water.

Different methods and instruments were used to calculate the characteristics: pH meter for pH; Conductivity meter for electrical conductivity; Turbidity meter for Turbidity; UV Spectrophotometric Brucine Absorbitivity for Nitrogen-Nitrate; Spectrophotometric Pot. Persulphate digestion and followed by Ascorbic Acid redⁿ for Total Phosphorus; Argentometric for Chloride; Membrane filtration for fecal coliform bacteria. All the analyses were done following the guidelines of American Public Health Association (Clesceri et al. 1998).

Sampling

Sampling was conducted during the period 5th-15th February 2012. Semi-quantitative (time-bound) sampling was used to collect the benthic macro-invertebrates. Fast-flowing habitats (riffles) were sampled holding the net downstream and by kicking in front of the net opening whereas stream edge habitats were sampled by sweeping along stream margins and disturbing the bottom substratum (Lock et al. 2011). Macroinvertebrates were collected using a standard hand net with a square metal frame 30×30 cm, with a conical net with a mesh size of 450µm. Five samples were taken from each station, with a total sampling time of 5 minutes in each station (5×1 min.). The samples were preserved in 80% ethanol and brought to the laboratory for further examination. In the laboratory, all the biological samples were fully examined under a dissecting compound microscope. All the macroinvertebrates were identified to family level, using standard keys (Dudgeon 1999; Neseemann et al. 2007).

Bio-classification of the Bagmati River using biotic indices

The biotic integrity of the Bagmati River was evaluated by using Rapid Bio-assessment Protocol II (RBP II) (Plafkin et al. 1989). The data analysis design of the RBP II incorporates several community, population and functional parameters to make a single evaluation approach to biotic integrity. Each parameter or metric used in this protocol is different from each other as regards the measurement and the range of sensitivity to the pollution load (Plafkin et al. 1989). Bio-assessment of the different sites of the Bagmati River was undertaken on the basis of the following metrics:

Taxa richness

It is the total number of families present in the study area. It measures the health of the community by measuring the variety of the taxa.

Modified Family Biotic Index

The Family Biotic Index (Hilsenhoff 1988) was used to detect the organic pollution of the different stations of the Bagmati River and was calculated by the following formula:

$$FBI = \sum \frac{x_i t_i}{n}$$

Where,

x_i = number of individuals within a taxon

t_i =tolerance value of taxon

n =total number of individuals in the sample (100)

The tolerance given by (Bode et al. 1996; Hauer & Lamberti 1996; Hilsenhoff 1988; Plafkin et al. 1989) for the macroinvertebrates were used.

The tolerance value ranges from 0 to 10 and decreases when water quality increases.

Ratio of scrapper and filtering collector functional feeding group

Assignment of the macroinvertebrates to functional feeding group was undertaken after Merritt and Cummins (1996) and (Dudgeon 1999).

Ratio of EPT to Chironomidae abundance

In this metric, the ratio of total abundance of the Ephemeroptera, Plecoptera and Trichoptera (EPT) to Chironomidae was calculated.

% contribution of dominant taxon

This index was used to measure the abundance of a dominant group relative to the abundance of the remaining community. This index estimates the balance of the community.

EPT Index

This metric was calculated by counting the total number of families within the insect groups (Ephemeroptera, Plecoptera and Trichoptera). The index value increases with increase in water quality.

Community Loss Index

This index estimates the loss of benthic taxa between the reference site and the site of comparison. To calculate this index, it was assumed that station 1 was a reference station with which remaining stations were compared. This index measures the compositional dissimilarity between the reference and the station of comparison. The value increases as the dissimilarity increases. The value of this index ranges from 0 to infinity. The formula for this index is as follows:

$$\text{Community Loss} = \frac{d - a}{e}$$

Where,

Sample A= reference station

Sample B=station of comparison

a=number of taxa present in both samples

d = total number of taxa present in Sample A

e = total number of taxa present in Sample B

Ratio of shredder functional group and total number of individuals collected

The relative abundance of shredder to the total number of individuals was calculated to find the value of this index.

Bioassessment of the Bagmati river stations

All the metric values were converted into bioassessment score following the biological condition scoring criteria (Plafkin et al. 1989).The total score of each station was compared in terms of percentage with the reference site (% comparison to reference site). Finally the bioassessment of the Bagmati River stations was made. The details of biological scoring categories are given in Annex 1 and Annex 2.

BMWP and ASPT

The Biological Monitoring Working Party (BMWP) biotic score formulated for the characterization of rivers in the UK was applied to assess the biological condition of the Bagmati River. Scoring is based on the tolerance of different families to organic pollution.

Each family is assigned a score between 1 and 10 (where 1 is extremely tolerant and 10 extremely sensitive). The scores of the families were successively added to make a single score for a particular station. To take account of the difference in the total scores caused by the variation in the sampling and seasonality, average score per taxon (ASPT) has been common practice, giving a single score out of 10 (Armitage et al. 1983; Walley & Hawkes 1996).

$$\text{ASPT} = \text{BMWP}/N$$

Where N is the total number of families used in the calculation of BMWP score.

Statistical analysis

The Shapiro-Wilk normality test was applied (at $\alpha = 0.05$) to check the normal distribution of the data. The data were normally distributed so parametric tests were performed. Software R was used for the statistical analysis. Differences between sites based on the macroinvertebrate abundance were measured using one –way analysis of variance (ANOVA). The relationship between biological data and chemical data were determined using the Pearson Correlation (Gray & Delaney 2010). All the statistical tests were performed at 95% confidence level.

Results

Water Quality

The pH values at the stations ranged from 5.65 to 6.84 (Table 1), and S1 was more acidic than the other downstream stations.

Table 1. Spatial variation of environmental variables along the sampling site in Bagmati River. The samples were taken in February 2012.

Parameters/sites	S1	S2	S3	S4	S5	S6
Average stream Depth (cm)	19.25	21.5	24.75	31.25	39	78.5
Average stream width (m)	10	8.5	11	15.5	35	43
pH at (15.6°C)	5.65	5.72	5.90	6.18	6.74	6.84
Electrical conductivity ($\mu\text{S}/\text{cm}$)	37.2	31.8	106	128	725	758
Turbidity (NTU)	2.3	8.3	101	143	130	119
Chloride (mg/L)	2	2	7	11	67	73
Nitrogen-Nitrate (mg/L)	0.73	0.47	2.66	3.25	3.32	4.52
Total phosphorus (mg/L)	0.15	0.10	0.11	0.34	1.11	0.90
Dissolved Oxygen (DO) (mg/L)	8.0	8.6	6.8	7.0	1.82	2.8
Bio-chemical Oxygen Demand(BOD) (mg/L)	24.0	22.4	90.0	101	224	202
Fecal Coliform Bacteria (CFU/1 ml)	888	880	59600	85600	812000	361000

Electric conductivity was in the range 37.2-758 $\mu\text{s}/\text{cm}$ and the turbidity ranged from 2.3 to 143 (NTU). Electric conductivity increased from S1 to S2, and reached very high values at S5 and S6 (Table 1). Likewise, turbidity increased from S1 and S2, reached a maximum at S4 thereafter decreased downstream. Dissolved oxygen (DO) diminished to 1.82mg/l at S5 (Table 1). Similarly, biochemical oxygen demand (BOD) increased by 10 times downstream from 22.4mg/l at S2 to 224 mg/l at S5. Nutrients like total Phosphorus, Nitrogen-Nitrate and Chloride clearly increased downstream (Table 1).

Macroinvertebrate assemblages

A total of 2583 benthic macroinvertebrates representing 10 orders and 29 families (Table 2) were recorded in the Bagmati River. Dipterans had the highest abundance (Figure 6), representing 45.1% of the total count. Maximum number of Dipterans was recorded at station 4, which constituted 34.2% of the total Dipterans. Chironomidae alone were responsible for 34.6% of the total macroinvertebrate count. The second most dominant taxon was Ephemeroptera, comprising 24.5%, followed by Oligochaeta 15.9%, Trichoptera 5.6%, Pulmonata 3.5%, Coleoptera 2.4%, Plecoptera 0.7%, Megaloptera 0.5%, Odonata 0.5% and the least dominant taxon, Rhynchobdellida occupying only 0.46% of the total benthos.

Macroinvertebrate abundance varied between the sampling stations. The abundance of the invertebrates showed a diminishing trend in the downstream direction (Fig. 8). Maximum numbers of benthos were recorded from S1, S3 and S4 whereas lower numbers were recorded from S2, S5 and S6. The macroinvertebrate abundance at S1 (reference station) and S2 were significantly different ($F=17.05$; $P=0.0033$). Similarly, the difference between S1 and S5 was highly significant ($F=16.96$; $P=0.0033$). Furthermore, S6 had also significantly different distribution of macroinvertebrates compared to the S1 ($F=15.78$; $P=0.0041$). On the other hand, S1 was not significantly different with regard to macroinvertebrate abundance when compared to S3 ($F=0.12$; $P=0.7345$) and S4 ($F=1.81$; $P=0.21$).

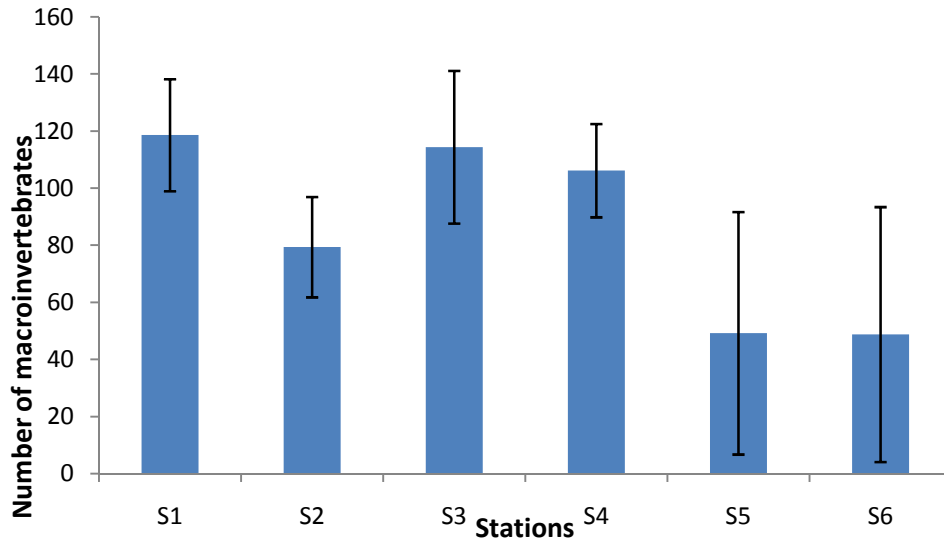


Fig. 8. Average abundance of benthic macroinvertebrates at the sampling stations S1-S6 in Bagmati River. The bars shows the mean and the whiskers are the error bars (Mean \pm SEM). The number of the samples is 5 in each station.

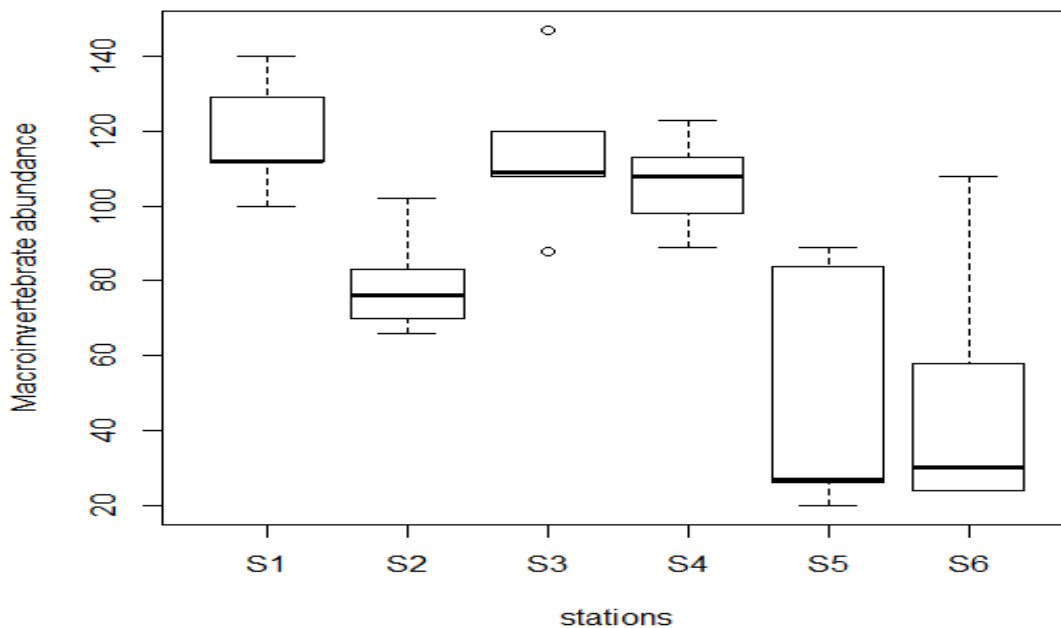


Fig. 9. Average numbers of macroinvertebrates from stations 1-6 in the Bagmati River. The vertical bar in the figure indicates the maximum and minimum values; the boxes represent the numbers between 25 and 75 percentiles. The circles indicate the outliers to the data. The horizontal thick line in the box represents the median values.

Table 2. The overall composition and distribution of macrobenthic invertebrates in the Bagmati River sampling stations (S1-S6), February, 2012.

Taxa	S1	S2	S3	S4	S5	S6
Diptera						
Tabanidae	10	10	2	1	–	1
Simuliidae	21	8	4	–	–	–
Ceratopogonidae	32	38	25	3	–	–
Blephariceridae	6	3	–	–	–	–
Chironomidae (non red)	42	28	59	96	11	9
Chironomidae (red)	–	–	241	288	51	69
Limoniidae	10	7	3	–	–	–
Tipulidae	4	7	1	–	–	–
Culicidae	–	–	–	12	18	4
Psychodidae	–	–	–	–	24	19
Ephemeroptera						
Ephemerellidae	7	8	2	–	–	–
Heptageniidae	29	18	–	–	–	–
Baetidae	273	159	72	–	–	–
Caenidae	31	17	18	–	–	–
Plecoptera						
Perlidae	13	7	–	–	–	–
Trichoptera						
Rhyacophilidae	13	14	3	–	–	–
Hydropsychidae	24	16	29	–	–	–
Philopotamidae	12	7	–	–	–	–
Glossomatidae	9	4	–	–	–	–
Limnocoetopodidae	9	7	–	–	–	–
Coleoptera						
Psephenidae	12	14	5	–	–	–
Elmidae	8	5	1	–	–	–
Hydrophilidae	10	8	6	2	–	–
Megaloptera						
Corydalidae	8	6	1	–	–	–
Odonata						
Gomphidae	6	4	3	–	–	–
Rhynchobdellida						
Glossiphoniidae	–	–	5	7	–	–
Oligochaeta						
Lumbricidae	4	2	6	4	4	15
Tubificidae	–	–	53	70	134	121
Pulmonata						
Physidae	–	–	33	48	4	6
Total	593	397	572	531	246	244

Bio-classification of Bagmati River

The bioassessment scores of the Bagmati River ranged from 9 to 39 in the different stations (mean \pm SD: 24.5 \pm 12.21). On the basis of the score results, the biological conditions of the river stations were ranked as **S1=S2 > S3= S4 = S6 > S5**. This indicated that S1 (reference site) and S2 (just below the dam) were very good in terms of biological condition and were categorized as non-impaired sites. The scores of S3, S4 and S6 fell below the reference site and categorized these sites as moderately impaired, while the S5 was classified as a severely impaired site (Table 3). The details of the biological condition categories are given in Annex 2.

Table 3. Metric value, percent comparison and bio-assessment scores for benthic macroinvertebrates.

Metric	Metric value						Percent comparison						Bio-assessment score					
	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
1. Taxa richness	23	23	21	10	7	8	100	100	91	43	30	35	6	6	6	3	0	0
2. Family Biotic Index (modified)	3.9	3.8	6.6	7.5	8.1	8.0	100	97	169	192	208	205	6	6	6	6	6	6
3. Ratio of Scrapers/Filtering Collectors	1.1	1.4	1.1	4	0.2	1.5	100	131	100	364	18	136	6	6	6	6	0	6
4. Ratio of EPT and Chironomid Abundance	10	9.1	0.4	0	0	0	100	91	4	0	0	0	6	6	0	0	0	0
5. % Contribution of Dominant Family	46	40	52	74	59	50	46	40	52	74	59	50	3	3	0	0	0	3
6. EPT Index	10	10	5	0	0	0	100	100	50	0	0	0	6	6	0	0	0	0
7. Community Loss Index	0	0	0.2	1.8	3	2.5	-	-	-	-	-	-	6	6	6	3	3	3
8. Ratio of Shredders/Total	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
Total Score													39	39	24	18	9	18
Biological condition*													NI	NI	MI	MI	SI	MI

*NI=non-impaired; MI=moderately impaired; SI=severely impaired
 S1= station 1, S2=station 2, S3=station 3, S4=station 4, S5=station 5, S6=station 6

Evaluation of water quality using the family level Biotic Index

There was a variation in water quality among the different stations (Table 4). According to the family biotic index (FBI), S1 and S2 had a very good water quality which indicated that there is possibly slight organic pollution whereas, S3 and S4 had a poor water quality indicating the possibility of substantial pollution. Furthermore, S5 and S6 had very poor water quality indicating the possibility of severe organic pollution.

Table 4. Water quality at different stations of Bagmati River classified on the basis of Family Biotic Index (FBI).

Station	Family Biotic Index	water quality	Degree of organic pollution
1	3.9	Very good	Possible slight organic pollution
2	3.8	Very good	Possible slight organic pollution
3	6.6	Poor	Very substantial pollution likely
4	7.5	Poor	Very substantial pollution likely
5	8.1	Very poor	Severe organic pollution likely
6	8.0	Very poor	Severe organic pollution likely

Classification of the water quality using BMWP score and ASPT score

The BMWP score diminished in a downstream direction (Table 5). The highest scores were at the S1 (BMWP=92) and S2 (BMWP=92) reflecting the good water quality and the good condition at the upstream sites. S3 (BMWP= 68) had very low BMWP score as compared to the upstream sites indicating the degrading quality of water. From S3, the BMWP score sharply declined in downstream stations and reached 9 at the S5 and S6 indicating the loss of EPT and dominance of Diptera and other low scoring organisms. Furthermore, ASPT index also showed the similar water quality of the sites as indicated by the BMWP index and the indices are strongly correlated ($r=0.99$, $P<0.05$).

Table 5. Score of sampling sites according to BMWP and ASPT.

Sampling Stations	BMWP Score	ASPT
S1	92	6.1
S2	92	6.1
S3	68	4.5
S4	17	2.4
S5	9	1.8
S6	9	1.8

Relationship between BMWP and ASPT

Regression between BMWP and ASPT found statistically significant association (P=0.0287) (Fig. 10). BMWP is directly proportionate to ASPT, highlighted by the positive value in the equation. The high r -squared value indicates that the points are on the trend line. Only one point is slightly deviated from the line.

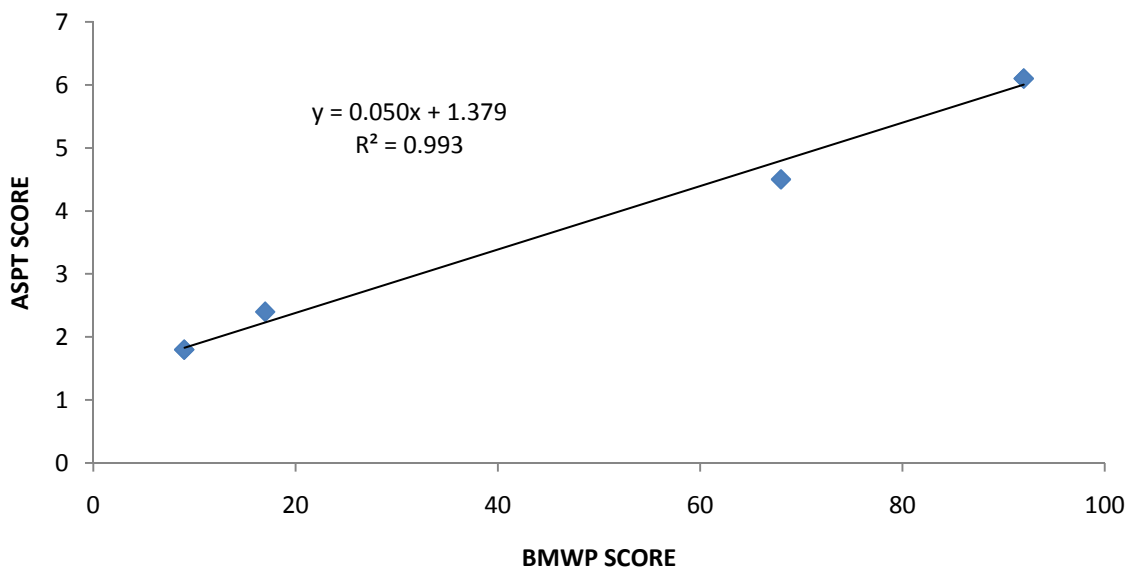


Fig.10. Relationship between BMWP score and ASPT score for six stations in Bagmati River in February 2012.

Correlation of dominant families with water physico-chemical variables

Correlation analysis revealed that Baetidae, Tubificidae and Ceratopogonidae are significantly correlated with the physico-chemical parameters (Table 6). Baetidae was strongly correlated with NO₃ (P=0.017), Turbidity (P=0.0042), BOD (P=0.049) and pH (P=0.049). Similarly, Ceratopogonidae was also significantly correlated with NO₃ (P=0.0090), Total Phosphorus (P=0.0398), Turbidity (P=0.013), DO (P=0.043), BOD (P=0.016) and pH (P=0.011). Tubificidae showed significant correlation with all the parameters: F.Coli (P= 0.029), Total Phosphorus (P=0.0099), NO₃ (P=0.0097), Chloride (P=0.012), Electrical Conductivity (P=0.010), Turbidity (P=0.0275), DO (0.0033), BOD (P=0.00095) and pH (P=0.0012).

Table 6. Pearson correlation (two-tailed) (r) of dominant families with respect to water physico-chemical variables.

Families	Physico-chemical variables								
	F.coli	TP	NO ₃	Cl	EC	T	DO	BOD	pH
Chironomidae (not red)	-0.56	-0.57	-0.07	-0.65	-0.65	0.18	0.56	- 0.43	-0.44
Chironomidae (red)	-0.18	-0.18	0.44	-0.20	-0.19	0.66	0.086	0.081	0.03
Ceratopogonidae	-0.71	-0.83*	-0.92**	-0.79	-0.79	-0.90*	0.82*	-0.89*	-0.91*
Baetidae	-0.59	-0.65	-0.88*	-0.65	-0.65	-0.94**	0.69	-0.81*	-0.81*
Hydrosychidae	-0.63	-0.77	-0.66	-0.71	-0.70	-0.64	0.66	-0.71	-0.81
Physidae	-0.26	-0.25	0.35	-0.29	-0.28	0.59	0.18	-0.01	-0.04
Tubificidae	0.85*	0.91**	0.91**	0.90*	0.91*	0.86*	-0.95**	0.99***	0.97*

TP= Total Phosphorus, F.coli=Fecal coli, NO₃= Nitrate, Cl=Chloride, EC= Electrical Conductivity, T=Turbidity, DO= Dissolved oxygen, BOD= Biological Oxygen Demand

*The level of significance is p<0.05

** The level of significance is p<0.01

*** The level of significance is p<0.001

The relationship between Biochemical Oxygen Demand (BOD) and Tubificidae had significant positive relationship ($R^2= 0.983$ $P<0.001$) (Fig.11). Conversely, the relationship between Baetidae and Turbidity was also strongly negative and highly significant ($R^2 =0.895$, $P<0.05$) (Fig. 12).

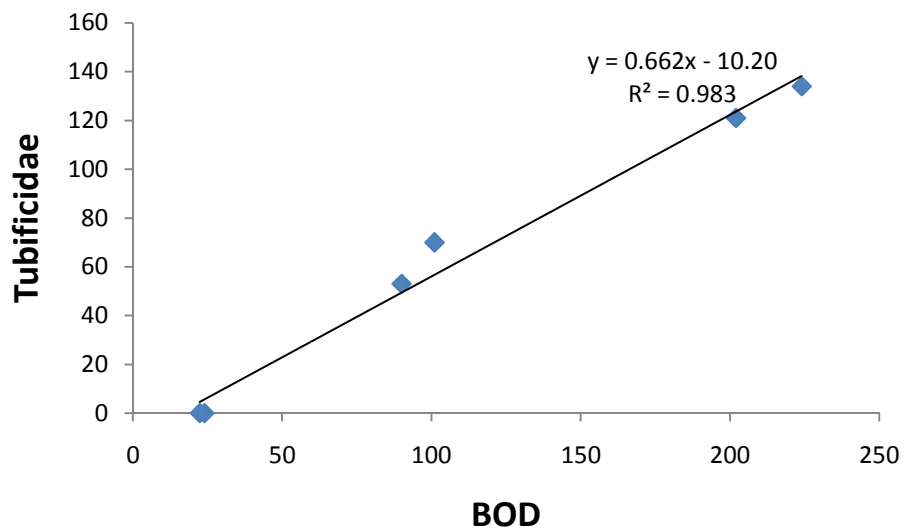


Fig. 11. Relationship between Tubificidae and BOD for six stations in Bagmati River in February 2012.

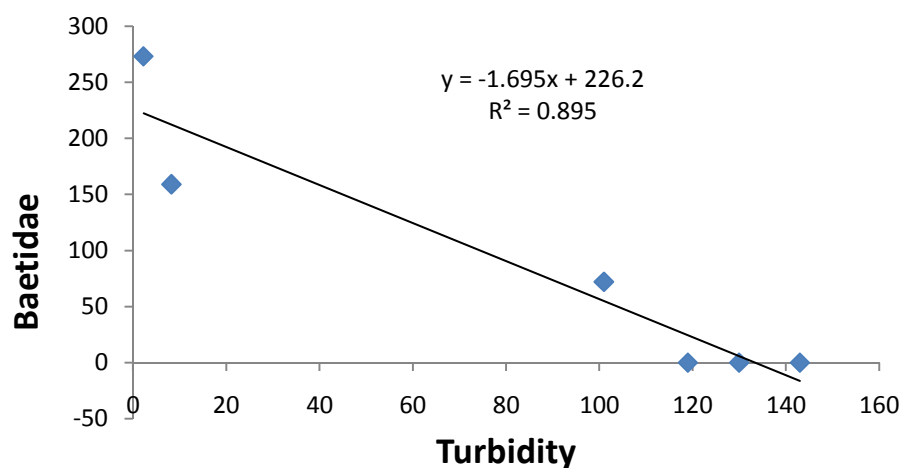


Fig. 12. Relationship between Baetidae and Turbidity for six stations in Bagmati River in February 2012.

Correlation of Biotic Indices and the Physico-chemical variables

The relationships (Pearson correlation) between the biotic indices and the physico-chemical parameters are summarized in Table 7. FBI was strongly correlated with NO₃ (P=0.0018), Turbidity (P=0.0009), DO (P=0.048), BOD (P=0.01) and pH (P=0.02). Community Loss Index (CLI) was strongly correlated with F. coli (P=0.028), TP (P=0.0046), NO₃ (P=0.035), Chloride (P=0.016), Electrical Conductivity (P=0.0163), DO (P=0.011), BOD (P=0.0042) as well as pH (P=0.0021). BMWP was also strongly correlated with TP (P=0.037), NO₃ (P=0.0086), Turbidity (P=0.011), DO (P=0.04), BOD (P=0.013) and pH (P=0.008). Similarly, ASPT was strongly correlated with TP (P=0.037), NO₃ (P=0.004), EC (P=0.049), Turbidity (P=0.0075), DO (P=0.03), BOD (P=0.009), and pH (P=0.006). EPT was strongly correlated with NO₃ (P=0.003), Turbidity (P=0.0014), BOD (P=0.02) and pH (P=0.02).

Table 7. Pearson correlation (two tailed) (r) of biotic indices FBI, BMWP, ASPT, EPT and the physico-chemical variables.

Biotic indices	Physico-chemical variables								
	F.coli	TP	NO ₃	Cl	EC	T	DO	BOD	pH
FBI	0.68	0.75	0.96**	0.74	0.75	0.97***	-0.81*	0.90*	0.87*
CLI	0.85*	0.94**	0.84*	0.89*	0.89*	0.80	-0.91*	0.94**	0.96**
BMWP	-0.72	-0.83*	-0.92**	-0.80	-0.80	-0.91*	0.82*	-0.90*	-0.92**
ASPT	-0.732	-0.83*	-0.94**	-0.81	-0.81*	-0.92**	0.84*	-0.92**	-0.93**
EPT	-0.66	-0.75	-0.94**	-0.72	-0.73	-0.96**	0.77	-0.87*	-0.87*

F.coli= Fecal Coli, TP=Total Phosphorus, NO₃= Nitrate, Cl=Chloride, EC=Electrical Conductivity, T= Turbidity, DO= Dissolved Oxygen, BOD= Biochemical Oxygen Demand, FBI= Family Biotic Index, CLI=Community Loss Index, BMWP= Biological Monitoring Working Party, ASPT= Average Score per Taxon, EPT= Ephemeroptera Plecoptera Trichoptera

*The level of significance is p<0.05

** The level of significance is p<0.01 *** The level of significance is p<0.001

Discussion

The present study revealed that most impacted sites in the Bagmati River were dominated by Diptera while the non impacted sites were dominated by Ephemeroptera, Plecoptera and Trichoptera. Chironomidae were abundant at all the stations. Chironomidae are considered probably the most diverse and abundant group of all stream macroinvertebrates (Yule 2004). Chironomidae are also considered to be capable of building up huge population quickly and tolerating abrupt changes in the habitat conditions (Solimini et al. 2003). The dominance of the Chironomidae was highest at S4 followed by S3. Both the stations were characterized by the high level of BOD, large number of F.coli bacteria, which proved Chironomidae to be a good conductor of pollution.

Ephemeroptera, Plecoptera and Trichoptera (EPT) were present only at the upstreams. Members of EPT are considered very sensitive to environmental stress, their abundance at upstream sites shows comparatively clean environment (Armitage et al. 1983), hence EPT showed to be the possible bioindicator of unpolluted ecosystem.

Among the mayflies recorded in the upstream sections of the Bagmati River, the most dominant family was Baetidae which had strong negative correlation with all the physico-chemical parameters except DO with which it was positively correlated. This relationship of Baetidae with Physico-chemical parameters showed Baetidae to be good indicator of water quality. Baetidae are known for tolerant to sedimentation and nutrient enrichment (Harrington & Born 2000). In the present study, Baetidae were abundant at S3 although this was a polluted site in agreement with Hall et al. (2006) who found Baetidae to be considerably tolerant to nutrient enrichment.

Of all the caddisflies observed, the Hydropsychidae was the most abundant family which are believed to be in the mid-range for tolerance of environmental stressors, nevertheless, they are one of the more tolerant in caddisflies group (Harrington & Born 2000). Though, the water quality of S3 was poor, Hydropsychidae was highly abundant at the station. Hall et al. (2006) also affirms that Limnephilidae and Hydropsychidae are less impinged by environmental stress than the other caddisflies.

In this study, only one family (Perlidae) of stoneflies (Plecoptera) was recorded from the first two upstream sites (S1 and S2). The two upstream sites had a good water quality according to biological indices (BMWP, ASPT and FBI) and my findings are in accordance

with (Mason 2002) who states that stoneflies require very high amount of oxygen and are very sensitive to organic pollution.

The last two downstream stations (S5 and S6) were dominated by the Oligochaeta (Tubificidae) which are known to tolerate very low oxygen and high pollutant concentrations (Zadory & Müller 1981). Strong negative correlation of Tubificidae with Dissolved Oxygen (DO) also demonstrates that this group is a good indicator of pollution.

The correlation between biotic indices and physico-chemical parameters clearly shows the sensitivity of biotic indices to the variation in the water quality. Comparatively, the biotic indices (FBI, BMWP, ASPT and CLI) were highly sensitive to water quality changes than the community structure index (EPT). Johnson et al. (2006) emphasizes that stress-specific index like BMWP are stronger than the community structure metrics. The substantial discrepancy in both BMWP and ASPT scores among the sites of the Bagmati River clearly reflected the varying ecological condition. Mason (2002) asserts that a river with good water quality has BMWP value of 100. However, none of the sites reached the values, although the index was obviously developed for the British fauna and thus may not match the communities in Nepalese rivers.

Relatively, ASPT index (Average Score Per Taxon) was more sensitive to the changes in the chemical parameters than the BMWP (Biological monitoring working party) index. This may be due to less sensitivity of ASPT to the seasonal variation, sample size, macroinvertebrate diversity and sampling effort (Armitage et al. 1983). A high ASPT value is characteristic of clean sites comprising large number of high scoring taxa (Armitage et al. 1983).

Physical modifications of river caused by urbanization may take priority over water quality in ascertaining the composition and structure of stream biota. Strange et al. (1999) asserted that alteration of the natural flow changes the composition of benthic macroinvertebrates. Field observations revealed that the sites (S3, S4 and S5) had many such modifications (eg. channelization). Consequently, there was little or no riparian vegetation. Such changes create ecological degradation (Beavan et al. 2001) and may cause spatial variation in the macroinvertebrate assemblages. Although there was no change in taxa richness between S1 and S2, the significant difference in the abundance of macroinvertebrates between the two sites may be due to change in physical habitat which influences the structure and community of the benthic communities (Downes et al. 2000).

This study shows that distribution of benthic macroinvertebrates in river reaches with differing pollution can function as biological indicators in the bioassessment of the river and can also be used in the evaluation of the water quality in other freshwater localities in Nepal.

Conclusion

A total of 2583 benthic macroinvertebrates representing 10 orders and 29 families were recorded in the Bagmati River. Dipterans had the highest abundance with 45.1% followed by Ephemeroptera 24.5%, and Oligochaeta 15.9% of the total benthos. Macroinvertebrate abundance varied between the six sampling stations. The abundance of the invertebrates diminished in the downward direction. Maximum numbers of the benthos were recorded from S1, S3 and S4 whereas small numbers of the invertebrates were recorded from S2, S5 and S6. The upstream sites were represented by Ephemeroptera, Plecoptera and Trichoptera while the downstream sites were represented by the Chironomidae and Tubificidae.

Tubificidae significantly correlated with all the physico-chemical parameters. Similarly, Baetidae and Ceratopogonidae were also significantly correlate with most of the physico-chemical parameters. Likewise, the association between biotic indices and chemical parameters remained statistically significant. Community Loss Index (CLI) was significantly correlated with the highest number of environmental variables which correlated with all the variables significantly except the Turbidity, although the correlation between them was considerably high. Family Biotic Index classified the river stations into three categories: S1 and S2 were the sites with very good water quality, S3 and S4 were poor water quality and the stations S5 and S6 were regarded very poor water quality.

This study shows that benthic macroinvertebrates can be the potential bioindicator for bioassessment of the freshwater ecosystem.

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Annex1. Flowchart of bioassessment approach given in plafkin et al, 1989.

Criteria for characterization of biological condition for Protocol II			
Metric	Biological condition scoring criteria		
	6	3	0
1.Taxa richness ^(a)	>80%	40-80%	<40%
2.Family Biotic Index (modified) ^(b)	> 85%	50-85%	<50%
3.Ratio of Scrapers/Filtering Collectors ^(a,c)	> 50 %	25-50%	<25%
4. Ratio of EPT and Chironomid Abundance ^(a)	>75%	25-75%	<25%
5. % Contribution of Dominant Family ^(d)	<30 %	30-50%	>50%
6. EPT Index ^(a)	>90 %	70-90%	<70%
7. Community Loss Index ^(e)	<0.5	0.5-4	>4
8. Ratio of Shredders/Total ^(a,c)	> 50%	25-50%	<25%
<p>(a) Score is a ratio of study site to reference site X 100</p> <p>(b) Score is a ratio of reference site to study site X 100</p> <p>(c) Determination of Functional Feeding Group is independent of taxonomic grouping</p> <p>(d) Scoring criteria evaluate actual percent contribution, not percent comparability to the reference station</p> <p>(e) Range of values obtained. A comparison to the reference station is incorporated in these indices</p>			

Annex 2. Biological condition categories and attributes (Plafkin et al 1989).

% Comparison to Reference Score ^(a)	Biological Condition Category	Attributes
>79%	Non-impaired	Comparable to the best situation to be expected within an ecoregion .Balanced trophic structure. Optimum community structure (composition and dominance) for stream size and habitat quality.
29-72%	Moderately impaired	Fewer species due to loss of most intolerant forms. Reduction in EPT index.
<21%	Severely impaired	Few species present. If high densities of organisms, then dominated by one or two taxa. Only tolerant organisms present.
<p>(a) Percentage values obtained that are intermediate to the above ranges will require subjective judgment as to the correct placement. Use of the habitat assessment and physicochemical data may be necessary to aid in the decision process.</p>		

Annex 3. Substrate composition of the sampling sites in the Bagmati River (after Moog, O. 2007 b).

Substrate / Station	S1	S2	S3	S4	S5	S6
Hygropetric sites	3	3	–	–	–	–
Megalithal >40 cm	17	35	–	–	–	40
Macrolithal >20 to 40 cm	33	30	–	–	–	10
Mesolithal >6 cm to 20 cm	20	18	32	10	15	7
Microlithal >2 cm to 6 cm	15	7	20	12	35	4
Akal >0.02 to 2 cm	2	5	15	5	20	7
Psammal >0.06mm to 2 mm	7	2	15	15	8	3
Psammopelal	3		10	16	15	11
Pelal <0.06mm	–	–	8	40	4	15
Argyllal	–	–	–	2	3	3

Hygropetric sites-thin water layer on solid substrates, Megalithal-upper sizes of large cobbles, boulders and blocks, bedrock, Macrolithal- coarse blocks,head-sized cobbles (with variable percentage of cobbles, gravel and sand), Mesolithal-fist to hand- sized cobbles (with variable percentage of gravel and sand), Microlithal-coarse gravel (size of a pigeon egg to child's fist) (with variable percentage of medium to fine gravel), Akal- fine to medium sized gravel, Psammal-sand, Psammopelal-mixture of sand with mud, Pelal-mud and sludge, Argyllal-silt, loam and clay.