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Biological Monitoring of Harnoi Stream Water Quality, Abbottabad, North Pakistan

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Abstract: Harnoi stream water quality is severely affected by urban population activities, which resides nearby the stream. The present study was conducted for the assessment of various stream water sites. The aim of the study was to check the effect of various point and nonpoint sources of pollution, on the stream water quality. The study area was divided into three main sites, upstream, polluted and downstream site. These three sites were combined together at river 'Dorr', near Havalian, which ultimately mix with river Indus at Tarbela. Main assessment biological parameters included algal, macro invertebrates, bacteriological and fungal analysis. Other physicochemical parameters included pH, temperature, electrical conductivity, Total Dissolved Solids (TDS), Dissolved Oxygen (DO), NaCl and turbidity. Higher conductivity value of 680 µS cm^{-1} was found in the month of October, which was higher in polluted site in all 8 months. The TDS higher value of 497 ppm was found in the month of October but the values were below Pak NEQS. Harnoi stream was dominated by Cyanobacteria and Oscillatoria. Macro-invertebrate at pollutant stream site included tolerant species, such as, tubifex, plectus, leech, sewage fly, dero, snails, midge fly larva, black fly larva and carp. Higher gram negative bacterial count showed fecal contamination, which is vulnerable for the stream water quality. It is concluded from the results obtained that, some immediate remediation strategies, like, wetland construction and growth of pollution control plants, are necessary to control the situation.

Key words: Biological parameters, macroinvertebrates, harnoi stream, turbidity, algal analysis

INTRODUCTION

Biological monitoring of fresh water is the use of biological responses to assess changes in the water bodies that generally change due to anthropogenic causes. It is a valuable assessment tool that is receiving increased use in water quality monitoring programmes of all types (Phillips and Rainbow, 1993; Batiuk *et al*., 1992). The biological monitoring involves a low-cost and uncomplicated method for developing a picture of a stream's health. By collecting and analyzing key indicator species of aquatic insects, one can obtain an understanding of the general condition of a stream (EPA., 2003). Biological monitoring often appears to be more appropriate in the assessment of pollution of aquatic ecosystems than traditional chemical evaluation of water quality (Thiebaut *et al*., 2006). Species diversity also declined dramatically in polluted sites e.g., *Daphnia*, which is normally prominent in clear water and vegetated site are replaced by smaller zooplankton, such as *Bosmina* of order cladocera and several rotifer species (Chow-Fraser *et al.*, 1998).

Because of the constant growth of the world population, the demand of freshwater is increasingly high, especially in the developing countries. At the beginning of 2000, it was estimated that over 1 billion people had no direct access to potable water and 40% of the world population could not afford freshwater for minimum personal hygiene (Bonanno and Lo Giudice, 2010).

Two main sources of pollution are point sources and nonpoint sources. Point sources are those that arise from a known point, such as, pipe from which a pollutant may enter into stream. Nearly every city, town and waterside settlement discharge some type of pollution to surface waters. Everyday activities, such as, laundry, flushing the toilet and using the in-sink garbage disposal add chemical and microbial pollutants to household waste water. On the other hand, nonpoint sources cannot be traced to a specific point, such as, an outfall pipe (Ball, 2001). Streams are basically polluted by municipal solid wastes, which contain high levels of heavy metals and physical and biological contaminants (Farrell and Jones, 2009). In healthy streams, all feeding groups of macro invertebrates are present but in degraded water environments,

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some feeding groups might be absent or having low relative abundance and, generally, with one or two groups dominating the community (Gamito and Furtado, 2009).

The objectives of the current research include monthly monitoring of the seasonal, biological and physicochemical variations of the Harnoi stream, where stream water quality was checked for the first time. This study has been used to check the fitness of water on "Clean water Act" that restricts fishable and swimmable water quality.

MATERIALS AND METHODS

Study area: The study area was Harnoi stream, located in the Southern part of Abbottabad (Fig. 1). Harnoi stream was characterized by all four seasons, winter from October to March, spring from April to May, summer from May to August, fall from November to December and rainfall from July to August. This stream supplies water to local community and then discharges it into river Dorr. This river water is largely used by the local farmers for irrigation, bathing and swimming. The three sites were upstream site: Karla, 10 km up from polluted area, middle polluted site: Present under Harnoi bazaar bridge and downstream site near Dhamtour, 10 km below from polluted site. Random sampling was performed and 1 L was collected in clean plastic bottle for physicochemical analysis and 100 mL in sterilized bottle for microbiological parameters.

Physicochemical analysis: The physical parameters, like, electrical conductivity, Total Dissolved Solids (TDS) and NaCl concentration, were determined, using Conductivity/TDS meter, Microprocessor HI 9835. Dissolved Oxygen (DO) and turbidity were calculated, using dissolved oxygen meter, Microprocessor Auto Cal HI 9145. Temperature and pH were calculated, using HANNA sensor checker. All the parameters were checked in the field and then in the laboratory for comparative analysis (Maqbool *et al*., 2011).

Algae analysis: Algal growths are usually visible to naked eye as vivid green or blue green growths and occasionally less than 10x hand held lens. Algal samples were scraped from stones and sediments with using clean forceps. They were also collected from water surface by hand or filters. They were then removed into sterile polythene bags. Algal and cyanobacterial samples were then examined microscopically within 24 h of collection. Anything green and stringy was classified as an alga. The results were then examined, using algal identification sheet.

Macro-invertebrates analysis: Sites were chosen that had gravelly bottoms, which were housing oodles of macro-invertebrates and to ensure consistency among sites. Samples were collected, using a kick net, which was placed downstream from the test site. Then using a foot, the ground was disturbed in approximately a two square feet area. The net was then swiped through the dusty water to collect any macro-invertebrates that were displaced into water column. The net was then emptied into a small plastic container, so that its contents could be adequately shifted through in order to count the bugs. Samples were collected into white trays, keeping each substrate separate. With the help of magnifying lens, the macro-invertebrates were categorized into taxonomic groups, according to sheet taxa, which was used for identification. Once the bugs were counted and recorded, the whole sample was returned to the water. This process was repeated six times at each of the three sites (Aazami *et al*., 2015).

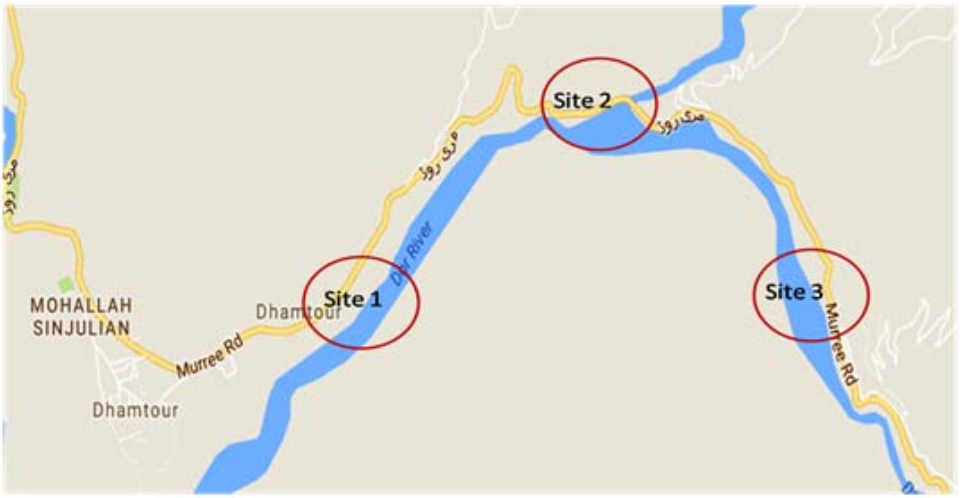

Fig. 1: Sampling site image: Site 1 in red circle is downstream, 2 is polluted and 3 is upstream site (Google map)

Bacteriological analysis: Samples were collected and necessary dilutions were made. Nutrient agar and Eosin Methylene Blue (EMB) agar plates were used for the total bacterial count and for Coliform group of gram negative organisms, respectively. Agar plates were incubated for 24 h at 35°C. EMB is a specific growth medium for Gram negative. On this medium *E. coli* gives green metallic sheen. The measurement is expressed as Colony Forming Unit (CFU) per 100 mL of water sample and aseptic conditions were maintained throughout the procedure.

Fungal analysis: Sabouraud dextrose agar (SDA) media was used for fungal analysis. Samples were collected and necessary dilutions were made and Lactophenol cotton blue solution was used for staining.

RESULTS AND DISCUSSION

Physicochemical analysis of stream water quality: Figure 2 shows monthly pH variation of three sampling sites of Harnoi stream. Water pH varies from 6.1-9, the low pH was observed at upstream site in October, which may be related to other factors, like low DO concentration, dark shadow, which enhance the fungus growth at the site.

The surface water temperature of spring and stream varied considerably during different months of the year. The value of minimum water temperature was found to be 9.3°C at upstream site in the month of December, while maximum temperature was recorded 27.6°C in May at polluted site, due to greater solar radiations, clear atmosphere and organic waste decomposition (Fig. 3). Alkaline pH was observed in the polluted sample during all sampling months. pH of water is important, because it affects the solubility and availability of nutrients and how can they be utilized by aquatic organisms. Temperature is a critical water quality parameter and it directly influences the amount of DO, which is available to aquatic organisms. Water temperature that exceeds 18°C has a deleterious effect on several fish species in the stream.

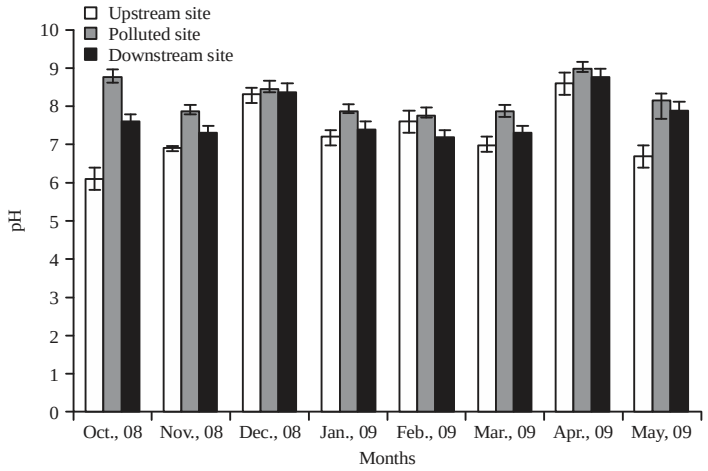

Fig. 2: Monthly pH variation of three sampling sites of Harnoi stream

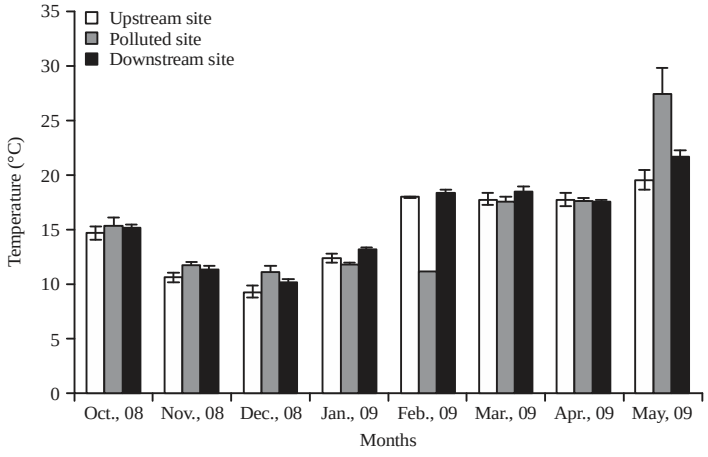

Fig. 3: Monthly temperature variation of 3 sampling sites of Harnoi stream

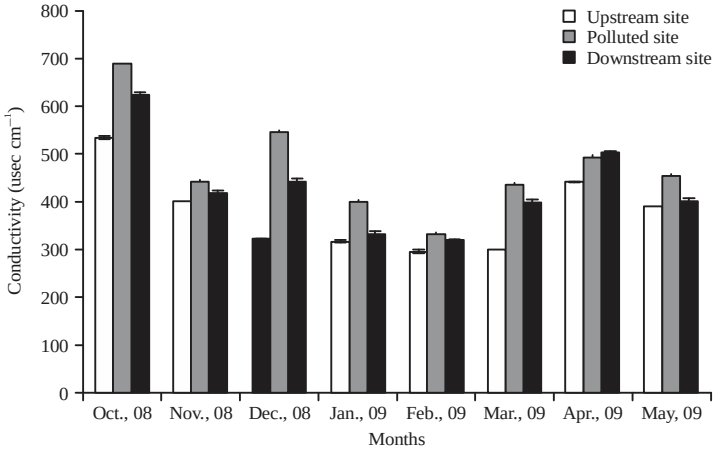

Fig. 4: Monthly conductivity variations of three sampling sites of Harnoi stream

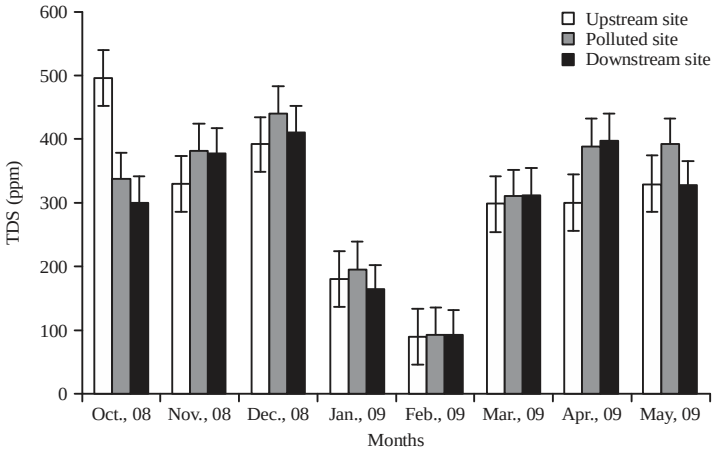

Fig. 5: Monthly TDS variations of three sampling sites of Harnoi stream

A higher conductivity value of 680 µS cm^{-1} was found in the month of October, which was higher in polluted site during 8 months of the year (Fig. 4). A higher TDS value of 497 ppm was found in the month of October, but the values were below Pak NEQS and considered in the TDS range of good water palatability (Fig. 5) (Maqbool *et al*., 2013).

Figure 6 shows that month of May has low DO at the polluted site, which was lower than other site in around all months. Lower DO values were due to more organic waste decomposition, high temperature and salinity, which releases oxygen from the stream water. The month of December also indicates low values, because this sampling was done in the cloudy weather for the last several days, so the respiring plants and other microorganisms utilized the available DO. Turbidity values were below Pak NEQS, it was the safest range of 5.8-16.5 NTU (Fig. 7). The NaCl concentration was high in the month of December and April, due to low rainfall, which accumulates the minerals (Fig. 8). As the concentration of NaCl increased in polluted sample, its ionic strength also increased. TDS and conductivity values were within the permissible limits (NWQMP., 2007). Conductivity is the ability of water to conduct an electrical current and is an indirect measure of the ion concentration. The greater the ions present, the more electricity can be conducted by water. Turbidity is a measure of the clarity of water. It is the amount of solids suspended in water and can be in the form of minerals or organic matter.

As, the month of January was cold and DO level was high during these months, the polluted samples show mostly very low level of DO, as it was high temperature and various chemical pollutants decrease the oxygen level. If water is too warm, there may not be enough oxygen in it. The ability of water to hold oxygen in solution is inversely proportional to the temperature of water.

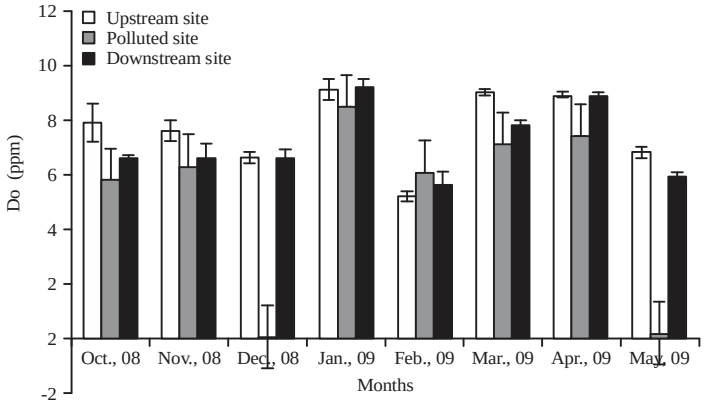

Fig. 6: Monthly dissolved oxygen variations of three sampling sites of Harnoi stream

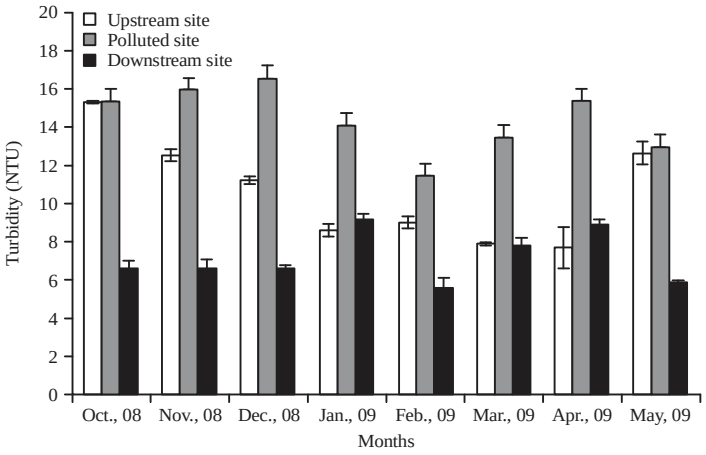

Fig. 7: Monthly turbidity variations of three sampling sites of Harnoi stream

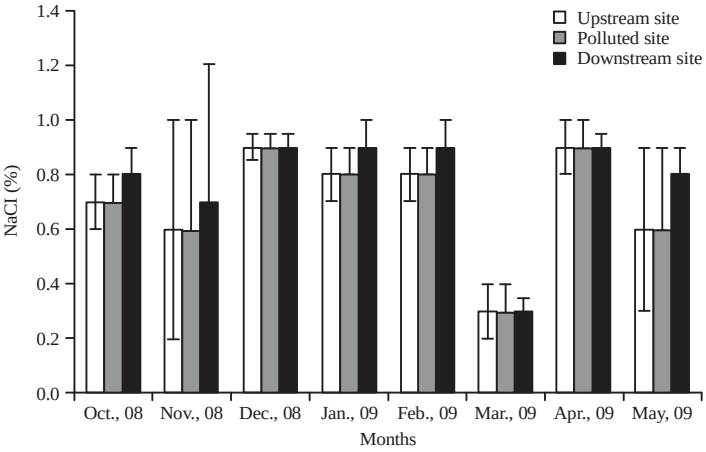

Fig. 8: Monthly NaCl variations of three sampling sites of Harnoi stream

Seasonal Algal variations of different sites: Table 1 illustrates different kinds of algae present in Harnoi stream and the month of May was ideal for them, due to high temperature, warmer water, cloudless days and lack of rainfall.

Harnoi stream was dominated by Cyanobacteria and Oscillatoria, especially, during the months of January, February and December. This is because they have wide adaptability to extreme environmental factors, like low temperature, desiccation and above

Table 1: Monthly algal variation of three sampling sites

Months	Upstream site	Polluted site	Downstream site
Oct., 8	Rhizoclonium,	Spirogyra	Volvox
	Aphanizomenon	Nitzschia	Mougeotia
	(Cyanobacteria)	Oscillatoria	Navicula
	Dichotomosiphon	Chlamydomonas	Chlamydomonas
		Arthrospira	Lepocinclis
		Stigeoclonium	
		Desmodesmus	
Nov., 8	Ulothrix	Nitzschia	Oscillatoria
	Oscillatoria	Phormidium	Euglena
	Navicula	Desmodesmus	Mougeotia
	Phacotus	Oscillatoria	Lepocinclis
		Euglena	
Dec., 8	Rhizoclonium	Phormidium	Navicula
	Aphanizomenon	Oscillatoria	Mougeotia
	Dichotomosiphon	Chlorogonium	Ulothrix
	(Yellow-green algae)	Stigeoclonium	Pandorina
Jan., 9	Navicula	Spirogyra	Spirogyra
	Ulothrix	Phacus	Oscillatoria
	Oedogonium	Lyngbya	Palmella
	Lyngbya	Palmella	Chlamydomonas
		Chlamydomona	
Feb., 9	Dichotomosiphon	Oscillatoria	Volvox
	Ulothrix	Lyngbya	Navicula
	Oscillatoria	Spirogyra	Ulothrix
		Chlorella	Lyngbya
		Desmodesmus	Desmodesmus
		Lepocinclis	
Mar., 09	Navicula	Chlorogonium	Navicula
	Ulothrix	Stigeoclonium	Ulothrix
	Oedogonium	Oscillatoria	Chlamydomonas
	Volvox	Euglena	Stigeoclonium
		Lyngbya	Euglena
		Desmodesmus	
		Phacus	
Apr., 9	Lyngbya	Lyngbya	Palmella
	Cladophora	Palmella	Chlamydomonas
	Microcoleus	Chlamydomonas	Volvox
	Dichotomosiphon	Stigeoclonium	Mougeotia
		Oscillatoria	Nitzschia
		Nitzschia	Lepocinclis
		Desmodesmus	Euglena
		Phacus	
May, 9	Navicula	Chlamydomonas	Volvox
	Ulothrix	Stigeoclonium	Mougeotia
	Oedogonium	Oscillatoria	Navicula
	Volvox	Chlorogonium	Ulothrix
		Euglena	Nitzschia
		Lepocinclis	Euglena
		Phacus	Desmodesmus
		Desmodesmus	

optimal and solar radiation. Table 1 shows that many kinds of algae were present in Harnoi stream and the month of May was ideal for them, due to higher temperature, warmer water, cloudless days and lack of rainfall, all of which contribute to the conditions which encourage algal growth and reproduction. All this led to odor and taste issues in stream water. These algae are a natural part of biodiversity, which contributes to the biological food chain. Some of these algae have the capacity to produce algal toxins, which may, at certain concentrations, cause some health concerns to those, who come into contact with water. Some of these algae may pose a risk for recreational users and were aesthetically unappealing. These algae were also involved in clogging pipes and filter lines. The greater the level of nutrients in the stream, the more food exists for algae and other vegetation to grow.

Table 2: Monthly macro invertebrate variations of three sampling sites

Months	Upstream site	Polluted site	Downstream site
Oct., 8	Mayfly nymph	Tubifex	Tubifex
	Fairy shrimp	Plectus	Dero
	Stonefly nymph	Leech	Midge larvae
	Daphnia	Midge larvae	Diving beetle
	Crayfish	Diving beetle	Beetle larvae
Nov., 8	Philodina (Rotifer)	Limnaea	Limnaea
	Stonefly nymph	Tubifex	Dero
	Mayfly nymph	Dero	Flatworm
	Caddisfly larvae	Midge larvae	Mussel
	Spider	Diving beetle	Sowbugs
	Amphipod		Beetle larvae
	Daphnia		
Dec., 8	Mayfly nymph	Limnaea	Dero
	Fairy shrimp	Tubifex	Flatworm
	Housefly larvae (Musca)	Dero	Mussel
	Dero	Midge larvae	Sowbugs
	Daphnia	Diving beetle	Scuds
Jan., 9	Mayfly nymph	Limnaea	Limnaea
	Fairy shrimp	Tubifex	Sowbugs
	Daphnia	Dero	Scuds
	Stonefly nymph	Midge larvae	Beetle larvae
	Mayfly nymph	Diving beetle	
	Small beetles		
Feb., 9	Mayfly nymph	Scud	Pelomyxa
	Fairy shrimp	Dero	Fairy shrimp
	Stonefly nymph	Limnaea	Daphnia
	Water penny	Midge larvae	Scud, Dero
	Riffle beetle	Diving beetle	Damselfly nymph
			Limnaea
			Sowbugs
Mar., 9	Damselfly nymph	Tubifex	Damselfly nymph
	Crayfish	Limnaea	Limnaea
	Mayfly nymph	Midge larvae	Sowbugs
	Fairy shrimp	Diving beetle	Scuds
	Dragonfly nymph	Scud	Beetle larvae
	Daphnia	Dero	Sowbugs
Apr., 9	Kellicottia (Rotifer)	Planaria	Planaria
	Mayfly nymph	Kellicottia	Daphnia
	Dragonfly nymph	Tubifex	Kellicottia
	Small beetles	Water scorpion	Dragonfly nymph
	Caddisfly larvae	Mosquito pupa	Clams
	Spider	Leeches	Sowbugs
	Amphipod	Blackfly larvae	Scuds
May, 9	Daphnia	Housefly larvae	Planaria
	Housefly larvae	Planaria	Plectus
	Stonefly nymph	Plectus	Sowbugs
	Crayfish	Mosquito pupa	Scuds
	Caddisfly larvae	Leeches	Beetle larvae
	Spider	Blackfly larvae	

Seasonal macro invertebrate variation of different sites: Table 2 shows that upstream and downstream had the greatest diversity and the stream with the greatest human intrusion (polluted site) had the least diversity.

The upstream site had many intolerant species, which shows that the stream is relatively free of pollutants. For example, stoneflies, mayflies, caddisflies, dobsonfly, water penny and riffle beetle are very sensitive to most pollutants and are dependent upon the stream DO level remaining above a certain point. Conversely pollutant stream site was primarily inhabited by pollution tolerant species, such as, tubifex (sludgeworm), plectus (roundworm), leech (segmented worm), sewage fly, dero, snails, midge fly larva, black fly larva and carp. The absence of different species of mussels was also representing the organic pollution at this site; similar

findings were also observed for the polluted site of Velika Morava River (Tomovic *et al*., 2012). Macro invertebrates are an important indicator of stream quality because they are relatively sensitive and can be easily impacted by pollutants or other disturbances. The goal of macro-invertebrate study was to test whether their diversity changes with the increased human development in the surrounding area of stream or not. It was demonstrated that stoneflies were no longer present in the polluted site, where they once were, because the pollutant stream dissolved oxygen content had decreased. Tubifex were more abundant in polluted site, which was the indicator of sewage pollution; their abundance was high in another study in autumn, due to the absence of rainfall and low natural filtration at polluted site (Markovic *et al.*, 2015). The productive areas for macro-invertebrates are riffles, leaf packs, soft sediments and root/bank habitat. Freshwater shrimp taxa dominated the faunal biomass of upstream and downstream, however, their role in community organization is unclear but they may cause significant reductions in sediment cover on rock substrata, reducing sedimentation and, thus, enhancing understory algal food resources through sediment removal to increase algal bio-volume.

Monthly bacterial and fungal count of different sites: Table 3 shows that few or no fungal growth was found in the cold months of December, January and February, while during the month of May, fungal diversity increased.

Water quality did not have a significant effect on composition of fungal community. Fungi do not grow at low temperature. Due to their role in energy transfer of streams, it is fundamental to further investigate the effect of pollution on structure and functioning of assemblages of aquatic fungi. Freshwater fungi involved in decay of wood and leafy material, also cause diseases of plants and animals.

In the upstream site, the highest total bacterial count of 910 and 950 CFU mL^{-1} was observed in the month of October and May, respectively. The highest gram negative load on EMB agar was 780 CFU mL^{-1}, observed in the month of October in the up-stream site, due to high temperature, which increased the biodegradable activity and rainfall, thereby, mixing the organic waste significantly into the stream. The lowest total bacterial count in the range of 100-310 CFU mL^{-1} was found in the months of December, January and February, due to low microbial activity (Table 4).

Polluted samples show total bacterial count in the range of 7.8×10^4-1.2×10^5 CFU mL^{-1} in the month of December and May, respectively. The highest bacterial load, in May and June, was due to warm weather, which increased the decomposition and the rain water, which mixed the solid waste of Harnoi area into the main stream of Harnoi. Table 5 shows that the total polluted site includes three different colored colonies obtained on EMB agar, pink colored colonies were of fecal streptococci, pink colonies with green metallic sheen were of *E. coli* and blue colonies might be of fecal coliforms. These colonies were in the range of 5.8×10^4-1.43×10^5 CFU mL^{-1}. Downstream site showed almost same bacterial count as up-stream site, which might be due to self purification of running stream water.

Downstream site showed the highest total bacterial count in the month of May, October and November, due to warm climate. On EMB agar, bacterial load was in the range of 10^2-8.1×10^2 CFU mL^{-1} (Table 6).

Table 3: Monthly fungal variations of three sampling sites

Months	Upstream site	Polluted site	Downstream site
Oct., 8	Alternaria, grayish green colony, rapidly swarming over entire plate	Mucor, white colored cottony colony, rapidly swarming over entire plate	Very low fungal growth
Nov., 8	Very low fungal growth	Rhizopus, white colored cottony colony, rapidly swarming over entire plate	Very low fungal growth
Dec., 8	Alternaria, grayish green colony, rapidly swarming over entire plate	Very low fungal growth	Very low fungal growth
Jan., 9	Very low fungal growth	Very low fungal growth	Mucor, white colored cottony colony, rapidly swarming over entire plate
Feb., 9	Very low fungal growth	Rhizopus, white colored cottony colony, rapidly swarming over entire plate	Very low fungal growth
Mar., 9	Mucor, white colored cottony colony, rapidly swarming over entire plate	Mucor, white colored cottony colony, rapidly swarming over entire plate	Fusarium, wooly, pink and white colony
Apr., 9	Mucor, white colored cottony colony, rapidly swarming over entire plate	Mucor, white colored cottony colony, rapidly swarming over entire plate	Very low fungal growth
May, 9	Alternaria, grayish green colony, rapidly swarming over entire plate	Fusarium, wooly, pink and white colony	Alternaria, grayish green colony, rapidly swarming over entire plate

Table 4: Seasonal bacterial variations of up-stream site

	Nutrient agar		EMB Agar	
Months	Colonial morphology	CFU×10^3/100 mL	Colonial morphology	CFU×10^3/100 mL
Oct., 8	Off white, circular, convex, large	50	Pink with metallic green sheen, circular, convex, large	11
	Small pin pointed off white	20	Blue, circular, convex	20
	Yellow, irregular, raised	16	Blue, circular, flat, large	16
	Orange, circular, convex, large	5	Purplish pink, circular, convex, small	22
		(91)*	Pink, large, mucoid	9
				(78)
Nov., 8	Offwhite, circular, convex, large	48	Purplish pink, circular, convex, small	33
	Small pin pointed off white	33	Blue, circular, flat, large	23
	Orange, circular, convex, large	8	Pink with metallic green sheen, circular, convex, large	5
		(89)		3
				(64)
Dec., 8	Offwhite, irregular, large	6	Purplish pink, circular, convex, large	7
	Orange, circular, convex, large	4	Blue, circular, flat, small	9
		(10)	Pink with metallic green sheen, circular, convex, large	2
				(18)
Jan., 9	Offwhite, circular, raised, small	9	Pink with metallic green sheen,	3
	Yellow, irregular, raised	4	circular, convex, large	7
		(13)	Blue, circular, convex, small	(10)
Feb., 9	Yellow, irregular, raised	12	Pink with metallic green sheen, circular, convex, large	6
	Creamy, circular, flat	3	Purplish pink, circular, convex, large	8
	Offwhite, irregular, large	9		(14)
	Yellow, irregular, raised	7		
		(31)		
Mar., 9	Offwhite, circular, convex, large	17	Pink with metallic green sheen, circular, convex, small	11
	Small pin pointed offwhite	11	Purplish pink, circular, convex, large	5
		(28)		(16)
Apr., 9	Offwhite, circular, convex, large	26	Purplish pink, circular, convex, large	33
	Small pin pointed offwhite	22	Pink with metallic green sheen, circular, convex, large	4
		(48)	Pink, large, mucoid	11
				(48)
May, 9	Yellow, irregular, raised, large	31	Light purple, circular, flat, small	36
	Opaque white, large	23	Purplish pink, circular, convex, large	21
	Creamy, circular, flat	22	Pink with metallic green sheen, circular, convex, large	9
	Orange, circular, convex, large	19	Pink, large, mucoid	8
		(95)		(74)

*Values in parentheses show total number of colonies on agar plate

Table 5: Seasonal bacterial variations of polluted site

	Nutrient agar		EMB Agar	
Months	Colonial morphology	CFU×10^5/100 mL	Colonial morphology	CFU×10^3/100 mL
Oct., 8	Off white, circular, convex, large	31	Pink with metallic green sheen, circular, convex, large	20
	Small pin pointed off white	29	Blue, circular, convex	19
	Yellow, irregular, raised	15	Blue, circular, flat, large	21
	Orange, circular, convex, large	23	Purplish pink, circular, convex, small	22
		(98)*	Pink, large, mucoid	29
			Brownish purple	18
				(129)
Nov., 8	Yellow, irregular, raised	20	Purplish pink, circular, convex, small	31
	Off white, circular, convex, large	33	Blue, circular, flat, large	23
	Small pin pointed off white	23	Pink with metallic green sheen, circular, convex, large	24
	Orange, circular, convex, large	28	Pink, large, mucoid	19
		(108)	Brownish purple	25
				(122)
Dec., 8	Offwhite, irregular, large	23	Purplish pink, circular, convex, large	24
	Orange, circular, convex, large	25	Blue, circular, flat, small	19
	Yellow, irregular, raised	32	Pink with metallic green sheen, circular, convex, large	15
	Creamy, circular, flat	23		(58)
		(103)		

Table 5: Continue

	Nutrient agar		EMB Agar	
Months	Colonial morphology	CFU×10^5/100 mL	Colonial morphology	CFU×10^3/100 mL
Jan., 9	Small pin pointed offwhite	28	Pink with metallic green sheen, circular, convex, large	19
	Offwhite, circular, raised, small	19	Blue, circular, convex, small	20
	Yellow, irregular, raised	31	Pink, large, mucoid	16
		(78)	Brownish purple	17
				(72)
Feb., 9	Yellow, irregular, raised	29	Pink with metallic green sheen, circular, convex, large	25
	Creamy, circular, flat	20	Purplish pink, circular, convex, large	17
	Offwhite, irregular, large	29	Brownish purple	13
	Yellow, irregular, raised	13	Pink, large, mucoid	20
	Small pin pointed offwhite	29	Blue, circular, flat, small	21
		(120)		(96)
Mar., 9	Offwhite, circular, convex, large	19	Pink with metallic green sheen, circular, convex, small	29
	Small pin pointed offwhite	24	Purplish pink, circular, convex, large	18
	Yellow, irregular, raised	18	Pink, large, mucoid	20
	Creamy, circular, flat	20	Brownish purple	26
		(81)	Blue, circular, flat, small	15
				(108)
Apr., 9	Offwhite, circular, convex, large	26	Purplish pink, circular, convex, large	21
	Small pin pointed offwhite	23	Pink with metallic green sheen, circular, convex, large	29
	Yellow, irregular, raised	29	Pink, large, mucoid	19
	Creamy, circular, flat	18	Brownish purple	22
		(96)	Blue, circular, flat, small	31
				(122)
May, 9	Yellow, irregular, raised, large	13	Light purple, circular, flat,	25
	Opaque white, large	23	small Purplish pink, circular, convex, large	21
	Creamy, circular, flat	30	Pink with metallic green sheen, circular, convex, large	30
	Orange, circular, convex, large	19	Pink, large, mucoid	23
		(85)	Blue, circular, flat, small	24
			Brownish purple	20
				(143)

*Values in parentheses show total number of colonies on agar plate

Table 6: Monthly bacterial variations of downstream site

	Nutrient agar		EMB Agar	
Months	Colonial morphology	CFU×10^3/100 mL	Colonial morphology	CFU×10^3/100 mL
Oct., 8	Yellow, irregular, raised	50	Blue, circular, flat, large	11
	Orange, circular, convex, large	20	Purplish pink, circular, convex, small	20
	Offwhite, circular, convex, large	16	Pink, large, mucoid	16
	Small pin pointed offwhite	5	Pink with metallic green sheen, circular, convex, large	22
		(91)*	Blue, circular, convex	9
			Blue, circular, flat, large	(78)
			Purplish pink, circular, convex, small	
			Pink, large, mucoid	
Nov., 08	Small pin pointed offwhite	48	Purplish pink, circular, convex, small	33
	Orange, circular,	33	Blue, circular, flat, large	23
	Offwhite, circular, convex, large	8	Pink with metallic green sheen, circular, convex, large	5
	convex, large	(89)		(61)
Dec., 8	Offwhite, irregular, large	6	Purplish pink, circular, convex, large	7
	Orange, circular, convex, large	4	Blue, circular, flat, small	9
		(10)	Pink with metallic green sheen, circular, convex, large	2
				(18)
Jan., 9	Offwhite, circular, raised, small	9	Pink with metallic green sheen, circular, convex, large	3
	Yellow, irregular, raised	4	Brownish purple	7
		(13)	Blue, circular, convex, small	(10)
Feb., 9	Yellow, irregular, raised	12	Pink with metallic green sheen, circular, convex, large	6
	Creamy, circular, flat	3	Brownish purple	8
	Offwhite, irregular, large	9	Purplish pink, circular, convex, large	(14)
	Yellow, irregular, raised	7		
		(31)		

Table 6: Continue

	Nutrient agar		EMB Agar	
Months	Colonial morphology	CFU×10^3/100 mL	Colonial morphology	CFU×10^3/100 mL
Mar., 9	Offwhite, circular, convex, large	17	Pink with metallic green sheen, circular, convex, small	11
	Small pin pointed offwhite	11	Purplish pink, circular, convex, large	19
		(28)		(30)
Apr., 9	Offwhite, circular, convex, large	26	Purplish pink, circular, convex, large	33
	Small pin pointed offwhite	22	Pink with metallic green sheen, circular, convex, large	4
		(48)	Pink, large, mucoid	11
			Brownish purple	(48)
May, 09	Creamy, circular, flat	31	Light purple, circular, flat, small Purplish pink,	36
	Orange, circular,	23	circular, convex, large	21
	Yellow, irregular, raised, large	22	Pink with metallic green sheen, circular, convex, large	9
	Opaque white, large	19	Pink, large, mucoid	8
	convex, large	(95)	Brownish purple	7
				(81)

*values in parentheses show total number of colonies on agar plate

CONCLUSION

Current investigation of different parameters of water quality suggested that the Harnoi stream water is highly polluted by the anthropogenic activities of nearby community including sewage disposal, organic and inorganic solid waste dumping and vehicle washing in the Harnoi stream, which results in the destruction of flora and fauna of this stream. Thus, the surface water cannot harbor the fish and does not provide suitable breeding ground for macro-invertebrates. Enactment of strict legislation for the protection of stream water from disposal of any type of contaminant should be recommended. It is concluded from the results obtained, that some immediate remediation strategies, like, wetland construction or growth of pollution control plants are necessary to control the situation. For future research prospective, it is suggested that more appropriate method of MPN should be used for total coliform count, which comprises fecal and thermo-tolerant separately. One important parameter, i.e., Biological Oxygen Demand (BOD) should also be analyzed.

REFERENCES

Aazami, J., A. Esmaili-Sari, A. Abdoli, H. Sohrabi and P.J. van den Brink, 2015. Monitoring and assessment of water health quality in the Tajan River, Iran using physicochemical, fish and macroinvertebrates indices. J. Environ. Health Sci. Eng., Vol. 13. 10.1186/s40201-015-0186-y

Ball, P., 2001. Life's Matrix: A Biography of Water. Farrar Straus and Giroux Publ., New York, USA., ISBN-13: 9780520230088, Pages: 417.

Batiuk, R.A., R.J. Orth, K.A. Moore, W.C. Dennison and J.C. Stevenson *et al*., 1992. Chesapeake Bay submerged aquatic vegetation habitat requirements and restoration targets: A technical synthesis. U.S. Environmental Protection Agency for the Chesapeake Bay Program, Annapolis, MD., USA., December 1992, pp: 1-246.

Bonanno, G. and R. Lo Giudice, 2010. Application of two quality indices as monitoring and management tools of rivers. Case study: The Imera Meridionale River, Italy. Environ. Manage., 45: 856-867.

Chow-Fraser, P., V. Lougheed, V. Le Thiec, B. Crosbie, L. Simser and J. Lord, 1998. Long-term response of the biotic community to fluctuating water levels and changes in water quality in Cootes Paradise Marsh, a degraded coastal wetland of Lake Ontario. Wetland Ecol. Manage., 6: 19-42.

EPA., 2003. Georgia Basin-Puget sound ecosystem indicators report. U.S. Environmental Protection Agency, Washington, DC., USA.

Farrell, M. and D.L. Jones, 2009. Critical evaluation of municipal solid waste composting and potential compost markets. Bioresour. Technol., 100: 4301-4310.

Gamito, S. and R. Furtado, 2009. Feeding diversity in macroinvertebrate communities: A contribution to estimate the ecological status in shallow waters. Ecol. Indicators, 9: 1009-1019.

Maqbool, F., A.H. Malik and Z.A. Bhatti, 2013. Water Quality Analysis of Salhad Stream Abbottabad, Pakistan. LAP Lambert Academic Publishing, Germany, ISBN-13: 978-3-659-33982-0, Pages: 100.

Maqbool, F., Z.A. Bhatti, A.H. Malik, A. Pervez and Q. Mahmood, 2011. Effect of landfill leachate on the stream water quality. Int. J. Environ. Res., 2: 491-500.

Markovic, V., J. Tomovic, A. Atanackovic, M. Kracun, M. Ilic, V. Nikolic and M. Paunovic, 2015. Macroinvertebrate communities along the Velika Morava River. Turk. J. Zool., 39: 210-224.

NWQMP., 2007. National Water Quality Monitoring Program fifth monitoring report (2005-06). Pakistan Council of Research in Water Resources (PCRWR), Islamabad, Pakistan, June 2007.

Phillips, D.J.H. and P.S. Rainbow, 1993. Biomonitoring of Trace Aquatic Contaminants. Elsevier Science Publications, Barking, UK., Pages: 371.

Thiebaut, G., G. Tixier, F. Guerold and S. Muller, 2006. Comparison of different biological indices for the assessment of river quality: Application to the upper river Moselle (France). Hydrobiologia, 570: 159-164.

Tomovic, J., K. Zoric, M. Kracun, V. Markovic, B. Vasiljevic, V. Simic and M. Paunovic, 2012. Freshwater mussels of the Velika Morava River. Water Resour. Manage., 2: 51-55.