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Microtox Toxicity Assay for the Sediment Quality Assessment of Ganga River

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Abstract: The objective of this study was to determine the sediment quality assessment of Ganga River at Kanpur city where effluents from tannery industries are discharged. Sediment samples from control, upstream and downstream area were collected and analyzed for bacterial toxicity bioassay along with a reference sediment (LGC6137). The control samples collected from the point of origin of Ganga River at Deoparyag were found non toxic to the bacteria and served as negative controls for the test method. The EC_{50} of upstream sediment sample were >10,000 mg L^{-1} sediment (>1%) and categorized as non toxic according to toxicity classification. The down stream sediment samples were very toxic to the bacteria and average EC_{50} value was 4,266 mg L^{-1} (0.43 %) that falls in very toxic category. The downstream sediment and reference sediment were toxic but the later was 10-fold more toxic. It is indicated in our study that the Microtox SPT assay can differentiate between toxic and non-toxic samples over a wide range of toxicity. The present study demonstrated the efficiency of the Microtox SPT assay in the sediment quality assessment and confirms the existing pollution in Ganga River contributed by tannery industries.

Key words: Ganga river sediment, elutriate, reference sediment, microtox, solid phase test

INTRODUCTION

Toxicity measurement of wastewater, sediments and contaminated water bodies is a very important part of environmental pollution monitoring. Evaluation of biological effects using a rapid, simple, sensitive and cost effective method can indicate specific information on toxicity and ecotoxicity and allow incorporation of toxicity parameters in the regulatory framework[1]. Toxicity tests are desirable in water pollution evaluations because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota. The majority of chemicals discharged into aquatic system eventually end up in sediments that may act as a sink of pollution as well as a source of pollution[2-4]. Sediments are ecologically important components of the aquatic habitat, which play a significant role in maintaining the trophic status of any water body[5]. Sediments near urban areas commonly contain high levels of contaminants[6-8], constituting a major environmental problem faced by many anthropogenically impacted aquatic environments[9].

This study is a part of study on the sediment quality assessment of Ganga River at one of its most polluted segment. Sediment samples from upstream and downstream of Ganga River passing through Kanpur city were collected and analyzed for toxicity bioassay. Bioassays are complementary approaches for characterizing the biological effects and hazards of contaminated sediments[10-13].

At Kanpur, major contamination is received from tannery industries[14]. Waste from leather industries are of great concern to agencies responsible for environmental management and it is considered one of the ten most harmful industries responsible for pollution leading to deterioration of a wide range of organisms[15]. Many toxicity bioassays that use aquatic organisms require the toxicants to be in liquid form; therefore, contaminants must first be extracted from sediments[16-17]. In solid phase Microtox bioassay (SPT-assay) bacteria is placed directly in close vicinity of solid particles and its response reflect in totality the action of toxicants along with synergists and antagonists present in a given sample[18-22]. Microtox toxicity assay has been in use due to its toxicity screening ability, reproducibility and easy application[23-26], it has been studied that luminescent property of *Photobacterium Vibrio fischeri* in Microtox assay was the most sensitive parameter in toxicity evaluation of tannery effluent[27]. Therefore in this

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study the data obtained against Microtox for the direct toxicity of sediment as well as toxicity of sediment elutriates are described. In toxicity assessment certain pure chemicals have been used as reference substances in a specific bioassay against which toxicity index (EC_{50}/LD_{50}) has been determined e.g., zinc sulfate and phenol have been used in Microtox basic (liquid phase) toxicity assay. However, for solid phase toxicity assay no such reference material is available that accounts for the solid matrix effects as well as contaminants effect on the assay. Therefore, in the present study a certified reference sediment sample was commercially procured with known contamination and has been used for toxicity determination for the comparison and reproducibility of Microtox assay.

MATERIALS AND METHODS

The locations of sampling stations are shown in Fig. 1. Two sampling areas were selected; sampling area-1 was up stream at Bithoor village from where river Ganga enters towards Kanpur City and sampling area-2 was down stream at Jajmau, which is situated at the exit point of river Ganga from Kanpur city area. The sediment samples were collected from six stations covering around 1 km stretch at each station using grab sampler, kept in clean glass jars and transported to the laboratory in the cold box. The samples were stored in the freezer till analysis. Reference sediment sample (LGC6137) was obtained from LGC Company, UK.

The Microtox Toxicity Test System records the light output of luminescent bacteria, *Vibrio fischeri* (Microtox reagent) before and after exposure to test

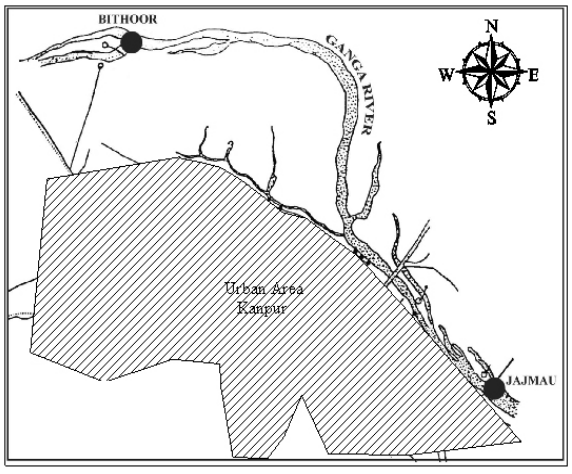

Fig. 1: Map showing location of sampling area in Ganga River at Kanpur

samples and process the raw data statistically to produce reports on the toxicity of the samples[28]. The basic test was used to assay sediment elutriate samples. Sediment elutriates were prepared by shaking sediment in water at 1:4 ratio for 24h. The supernatant was separated by centrifuging at 6,000 rpm for 60 min at 4 ºC[29].

Whole sediment was assayed using Microtox Solid Phase Test (SPT) assay. The assay protocols were as given in Microtox Manual[30]. The data were captured in computer and EC_{50} along with 95% confidence limit determined by the software provided with Analyzer.

RESULTS AND DISCUSSION

Kawn and Dutka[31] standardized the Microtox SPT-assay by using incinerator ash as a positive control and clean lake sediment as negative control and found that EC_{50} varied greatly between the two samples. Based on their observations the same authors rated sediment samples as very toxic (EC_{50}<0.5%), moderately toxic (EC_{50}>0.5-≤1%) and non toxic group (EC_{50}>1.0%). Accordingly this broad categorization has been used to rate the sediment collected from the two locations for their toxicity.

The six sediment samples, which were collected from Deoparyag, the point from where Ganga River enters the urban area and no source of anthropogenic pollution present, served as control sediment. The samples analyzed with the Microtox system were not toxic to the bacteria and the EC_{50} value was higher than the test concentration i.e., >100,000 mg L^{-1} (Table 1). Sediment samples from upstream area showed average toxicity (EC_{50}) in SPT-assay 51308 mg L^{-1} (5.13%) of fresh sediment samples. The highest EC_{50} 69870 mg L^{-1} (6.98%) was found at station-5 and the lowest EC_{50} 33490 mg L^{-1} (3.35%) was at station-1. According to the sediment toxicity rating the samples of sediments from upstream area was not toxic to Microtox, since EC_{50} of all the samples was well above 10,000 mg L^{-1} (>1%). The samples collected from downstream area tested in SPT-assay revealed average EC_{50} 4266 mg L^{-1} (0.43%) with a range from 2104-6300 mg L^{-1} (0.21-0.63%). The samples from station-3 and 4 were moderately toxic, however, the average EC_{50} value of all the sediment samples from downstream area place them under the category of very toxic samples against Microtox. The average solid phase Microtox toxicity of the reference sediment (LGC6137) was found to be 430 mg L^{-1} (0.043%) and a range of 197-654 mg L^{-1} (0.0197-0.0654%) from six replicates. The reference sediment was categorized as very toxic.

Table 1: Microtox SPT Assay for fresh sediments from control, upstream, downstream area and reference sediment (LGC6137)

Solid Phase Microtox Toxicity Assay			
Sampling Station	EC_{50} (mg sediment/L)	95% CL	Toxicity Rating
Control Area			
ST-1-6	>Highest test conc.	-	NT
Upstream Area			
ST-1	33490	27760-40410	NT
ST-2	34450	29230-40610	NT
ST-3	44840	40040-50210	NT
ST-4	56340	33540-94650	NT
ST-5	69870	43960-111100	NT
ST-6	68860	19930-237900	NT
Average	51308	32410-95813	
Downstream Area			
ST-1	3135	1029-9548	VT
ST-2	2243	1490-3374	VT
ST-3	6300	5784-6862	MT
ST-4	7091	3056-16450	MT
ST-5	2104	1174-3770	VT
ST-6	4727	3465-6448	VT
Average	4266	2666-7742	
Reference Sediment			
REP-1	197	129-300	VT
REP-2	325	181-582	VT
REP-3	346	154-772	VT
REP-4	603	239-1519	VT
REP-5	654	443-964	VT
REP-6	455	276-748	VT
Average	430	237-814	

NT =not toxic, MT = moderate toxic, T = toxic, VT =very toxic

Thus, the control samples collected from the point of origin of Ganga River at Deoparyag were found non toxic to the bacteria with EC_{50} higher than the sediment test concentration used in the assay system and served as negative controls for the test method. Certified contaminated sediment that was commercially procured exerted extreme toxic effect and served as positive control. At the study location the upstream sample (Bithoor) though exerted some toxic effects on bacteria but EC_{50} values were >1% sediment (≡10,000 mg L^{-1} sediment) and categorized as non toxic according to toxicity classification[31]. The down stream (Jajmau) sediment samples were very toxic to the bacteria except location 3 and 4 which were moderately toxic, the average EC_{50} value for the sediment from the down stream area was 0.43 %(≡ 4,266 mg L^{-1} sediment) that falls in very toxic category as per toxicity classification. It was interesting to note that both the downstream sediment and reference sediment were very toxic but there was a difference of about ten-fold in their toxicity. The toxicity of reference sediment sample was ten times higher than the down stream samples (Fig. 2).

The segment of Ganga River chosen for the present study receives effluent mostly from organized and

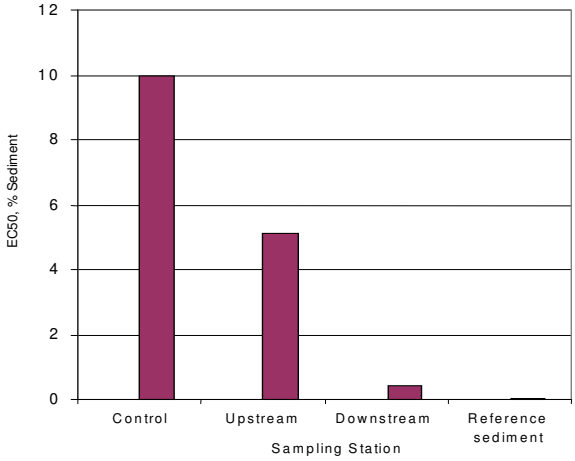

Fig. 2: MTX assay from control, upstream, downstream area and reference sediment

unorganized tanneries in Kanpur city. The tanning industry, which commonly utilizes "Chrome liquor" in the tanning process, discharges the effluent into the environment containing chrome, salts in excess of the maximum permissible limit [32]. The chrome discharges chromium however, the vegetable tanneries discharge chromium and phenolics both in high quantity. These are retained in effluent due to its toxic and recalcitrant nature during the activated sludge process, which causes environmental pollution. Recently, Chandra *et al.,*[27] examined untreated and treated tannery effluent collected from the Common Effluent Treatment Plant (CETP) and observed EC_{50} % values of untreated tannery effluent in the range of 3.12-5.09 % while with treated effluent the toxicity was greatly reduced and EC_{50} ranged between 63.49-76.07%. The CETP discharges goes to the Jajmau region the down stream area from where the samples were collected for the present studies[32]. The toxicity observed with downstream sediments was possibly due to tannery effluent contaminating sediment of the influenced area. In our study EC_{50} with sediment was lower than the EC_{50} with untreated effluent showing higher toxicity of sediment to Microtox.

In order to assess the leachability of contaminants present in bulk sediment elutriates were prepared and tested for toxicity. Sediment elutriates from downstream areas showed no toxicity to Microtox indicating that toxic principles were not leachable in aqueous medium or their concentration eluted was lower than their toxic levels (Table 2). Low elutriate: bulk sediment concentrations ratios have been reported in the literature as one of the factor showing no toxicity of elutriates from otherwise toxic sediment[33] and there

Table 2: Microtox Toxicity Assay (Basic Test) in sediment elutriates control, upstream, downstream area and reference sediment (LGC6137)

Sampling Station	Microtox Toxicity Assay (EC_{50}%)	Toxicity Rating
Control Area		
ST-1-6	>Highest elutriate conc.	NT
Upstream Area		
ST-1-6	>Highest elutriate conc.	NT
Downstream Area		
ST-1-6	>Highest elutriate conc.	NT
Reference Sediment (LGC6137)		
REP-1-6	>Highest elutriate conc.	NT

NT=not toxic

are numerous results on harbour elutriates showing absence of metal release from sediments[34-35]. This indicated that the characteristic of sediment from downstream area was of aged sediment. Slow sorption of chemicals on solid matrix over weeks, months and years leads to a chemical fraction that then resist desorption as that found with sediment from this location suggesting polluted sediment accumulation over the years. Sediment geochemical properties determine the type of metal bindings and its trend to desorb, while factors such as pH and salinity can also determine the bioavailability of chemicals bound to sediments[36]. Also organic matter affects metal speciation[37] and plays a major role in binding different contaminants and may be responsible for the negative elutriate toxicity. Viguri *et al.,*[38] also reported in their study where sediment EC_{50} ranged from 0.03% to 2.35% in Microtox SPT assay but elutriate testing (related to the toxic response of the aqueous available contaminants) was unsatisfactory in predicting bulk sediment toxicity obtained after testing on whole sediment. These results are in agreement with results obtained by other researchers[39-41]. This observation refutes that elutriates can predict the bioavailability and toxicity of the sediment.

CONCLUSION

Therefore, the present study revealed that in order to assess the quality of a given sediment direct sediment toxicity assay of whole sediment is the most suitable method and Microtox SPT proved to be an appropriate method in discriminating nontoxic and toxic samples over a wide range of toxicity.

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