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EVALUATING ECOSYSTEM RESPONSES TO METAL POLLUTION IN THE GANGA RIVER USING FLUORESCEIN DIACETATE HYDROLYTIC ASSAY

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Abstract

The Ganga River, which is one of the largest river systems of India with great ecological and social values, is under strong influence of multiple anthropogenic perturbations. Although a large number of monitoring and assessment programs has been initiated by the Government of India and other agencies, there is still a lack of studies explicitly considering ecosystem responses towards human-induced alterations in rivers. The present study was targeted to measure ecosystem responses towards metal pollution in the Ganga River. This study was conducted during summer low flow of the year 2019 at four study sites along 518 km middle segment of the Ganga River. The study shows that fluorescein diacetate hydrolytic activity (FDAase) in the bed sediment can be used as an ecosystem 'response' to carbon, nutrients and metal pollution in human-impacted rivers. The FDAase activity showed dependence on substrates (carbon and nutrients) when the heavy metal concentrations were below the toxic threshold. We found a decrease in FDAase activity at Wpdr Site despite the presence of sufficient amount of carbon. This site is characterized by high concentration of total heavy metal (ΣTHM) and total bioavailable fraction (ΣTBF) exceeding 360 $\mu g\ g^{-1}$ and 174 $\mu g\ g^{-1}$ respectively and able to induce negative response. The results of this study will help understanding the ecosystem responses towards human perturbations and planning management strategies for the Ganga River rejuvenation.

Key words: Carbon; Ecosystem response; FDAase; Ganga River; Heavy metal pollution; Nutrients

Introduction

Recent global efforts have yielded bountiful return to our basic knowledge on surface water ecology and associated responses against human perturbations. However, these have opened the ways for a number of new domains to be undertaken as a goal of future research (Jaiswal and Pandey 2019a). Knowledge of how a riverine ecosystem is responding to anthropogenic perturbations is central in designing conservation strategies and approaches to rejuvenate (Jaiswal and Pandey 2019b). During recent past, a number of studies contributed to assess the fate of carbon, nutrients and heavy metals in rivers and streams (Pandey *et al.,* 2014; Jaiswal and Pandey 2019c; Siddiqui and Pandey, 2019). These studies are important for monitoring fluxes, transport, and storage of carbon, nutrients and trace metals. Some of the studies have used similar database to establish mechanistic linkage between the determinants and river ecosystem responses (Jaiswal and Pandey 2019c). However, because river ecosystems are highly dynamic, establishing mechanistic linkages are constrained due to hydrodynamic forcing.

Authors generally use primary productivity/ chlorophyll a biomass, macro-invertebrates, fishes and diatom diversity linkages to uncover river responses to anthropogenic perturbations (Pandey *et al.,* 2014; Pandey *et al.,* 2017, Turley *et al.,* 2016; Siddiqui *et al.,* 2019). However, using these determinants are often constrained by hydrologic factors, mobility, trophic status and feeding behaviour of the test organism/variable. Recent studies suggest the use of sediment based parameters not only to uncover relatively stable determinants but also in bridging the narrative criteria with policy implementation on variable ecological scales. Riverbed sediment acts as an imprint of the ecological processes and therefore can

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serves as a test bed to acknowledge the functioning of riverine ecosystem as well as the alteration induced by human interferences. In this context, microbial functional attributes, which are extremely sensitive to changing physico-chemical characteristics of the sediment, have great relevance (Sinsabaugh *et al.,* 2009). In recent field trials, we have identified microbial extracellular enzymes as an important group of determinants to assess river responses against eutrophy (Jaiswal and Pandey, 2019b), nutrients (Pandey and Yadav, 2017), metal pollution (Jaiswal and Pandey, 2019d) and oxygen demanding substances (Jaiswal and Pandey 2019c).

During recent year, the Ganga River has experienced tremendous shifts in its water quality due to large input of carbon, nutrients and heavy metals all along its 2525km long course from various point and non-point sources (CPCB 2013; Pandey *et al.,* 2016; Yadav and Pandey, 2017; Siddiqui *et al.,* 2019a). The Ganga River basin is one of the most densely populated and urbanized river basin in the world. The basin experiences intensive agriculture covering over 73% of its geographical area. This demarcates largest non-point source of pollution input specially carbon and nutrient pollutants. In addition, the river receives large input of carbon, nutrients and metal pollutants from an intensive network of tributaries. Among the point sources, untreated and partially treated urban sewage and industrial effluents are the major driver of pollution in the main channel. The main channel receives pollutants from 36 class I and 14 class II cities. These together generate over 2723 million litres of sewage every day (MLD). Out of these however, only 44% is discharged in the river after treatment. Also, there are 138 drains that discharge over 6708 MLD of wastewater and 764 industries that release over 500 MLD of wastewater directly to the river. As a result of cumulative effect of these inputs, the river is expected to undergo unpredictable shifts in its ecological assimilation capacity and ecosystem responses.

Here we examine the metal pollution-driven shifts in ecosystem responses along a 518 km long stretch of the Ganga River. Given that, the riverbed sediment based attributes are relatively stable and microbial extracellular enzyme activities are highly sensitive even to minor alteration in environmental conditions, we selected sediment microbial activities in terms of substrate induced respiration (SIR), microbial metabolic quotient (qCO$_2$) and fluorescein diacetate hydrolytic bioassay as response determinants. Because, the complete hydrolysis of fluorescein diacetate require all the three major groups of enzymes (esterases, proteases and lipases), we consider fluorescein diacetate hydrolytic assay (FDAase) as a holistic measure of total microbial activity (Schnürer and Rosswall, 1982).

Materials and methods

Study area

The present study was conducted in the middle segment of the Ganga River during summer (April to June) low flow for two consecutive years (2018-2019). The Bhagirathi River originates in Garhwal Himalaya and joins Alaknanda River at Dev Prayag. This combined stream is known as River Ganga, the most sacred river of India. The river travels ~2525 km distance and merge in the Bay of Bengal. The Ganges basin (21°40^{1}39º and 31°27^{1}29º N latitude; 73°13^{1}00º and 89°09^{1}53º E longitude), which encompasses an area of 1,086,000 km^2, is the largest river basin of India covering 26.2% of its geographical area.With highest population density in India, this basin is considered as one of the most fertile river basins of the world. There are 29 megacities, 23 small cities and 48 townships which contribute to pollution input to the river. The climate of the region shows distinct seasonal pattern; a cold winter season (November to February), a hot and dry summer (April to June) and a humid monsoon season (July to September).March and October are transition months. The average rainfall in the basin ranges from 750 to 2500 mm and the relative humidity reaches close to saturation during rainy season. The basin experiences extensive temperature variation which may exceeds 46°C in summer and drop below 4°C in winter nights. Soil of the basin is highly fertile and ranges from mountain soil, submontane, alluvial, laterite, red, yellow to black soil.

For the purpose of this study, a total of 4 sites were selected along a 518 kilometer middle stretch of the Ganga River between Kanpur (26°302 N; 80°192 E) and Varanasi (25°192 N; 83°012 E). The sites includeWazidpur (Wpdr), Adalpura (Adpr), Assi confluence (Asdr) and Varuna confluence (Vrna) Fig. 1. These sites differ markedly with respect to the sources of pollution input. Each site was divided in to three sub-sites for detailed sampling and analysis. This middle stretch of the river is considered as the most polluted partof the Ganga River. There are three megacities; Kanpur, Allahabad and Varanasi along the study stretch that acts as major pollution source to the river. The Kanpur city with about 5 million human population have many tanneries and other industrial units that dispose effluents together with 339.3 MLD sewage (of which only 171.1 MLD is treated sewage) to the river. Allahabad with about 1.3 million urban population dispose 119 MLD untreated and 89 MLD treated sewage to the river. A large floating population during Kumbh

Mela, which is considered as one of the world's largest congregation of devotees, add large amount of human waste to the river. The holy city of Varanasi with over 1.6 million population dispose about 46 MLD untreated and 141 MLD treated sewage to the river.

Sample collection and Analysis

From each site the riverbed sediment samples (0-10 cm depth) were collected from 10-50 m reach using sediment corers. The samples were carried to the laboratory in acid-rinsed polyethylene bags in an ice-box. After air-drying at room temperature, the samples were homogenized, grinded to powder and sieved using a 2-mm mesh prior to analysis. A part of the sample was kept in desiccator to maintain the moisture level at 80% which were used for measurement of extracellular enzyme activity. For the measurement of total organic carbon (TOC), the samples were digested with $K_2Cr_2O_7$ and H_2SO_4 and measured titrimetrically following modified Walkley and Black method (Chen *et al.,* 2015). Brucin sulphanilic acid (Voghel 1971) and phenate method (Park *et al.,* 2009) was used for the measurement of NO_3^- and NH_4^+ respectively. The PO_4^{3-} was quantified using ammonium molybdate stannous chloride method (Murphy and Riley, 1962).

Table 1: Range of bioavailable fraction of heavy metals in the riverbed sediment at study sites.

Metal fraction (%)	Wpdr	Adpr	Asdr	Vrna
Cd	62-70	53-56	60-64	61-66
Cr	48-52	10-11	41-44	43-47
Cu	54-57	20-23	44-47	49-52
Ni	81-83	36-40	78-80	80-82
Pb	71-74	44-46	64-67	68-70
Zn	52-55	14-17	43-45	50-53

For the estimation of total heavy metals, the sediment samples were digested in 5:1:1 tri-acid mixture (HNO_3: HCl: $HClO_4$) in a microwave digestion/extraction system (SINEO model MDS-6G) and analyzed using atomic absorption spectrophotometer (Perkin Elmer model Analyst 800, USA). The bioavailable fraction of metals was measured following Ure (1996). For this, the samples were shaken with 0.05 mol/l EDTA (pH = 7.0) for 1 h and with 0.43 mol/l acetic acid (pH = 5) for 16 h. Then the samples were centrifuged and the supernatantswere used for the estimation of bioavailable fraction in an atomic absorption spectrophotometer (Perkin Elmer model Analyst 800, USA). The fluorescein diacetate hydrolytic activity (FDAase) was estimated spectrophotometrically following Schnurer and Rosswall (1982). The microbial metabolic quotient (qCO_2) was determined in terms of ratio of basal respiration (BR) to substrate induced respiration (SIR) (Wardle, 1993).

Results and Discussion

The concentrations of total organic carbon (TOC) and nutrients (NO_3^-, NH_4^+ and PO_4^{3-}) showed variable

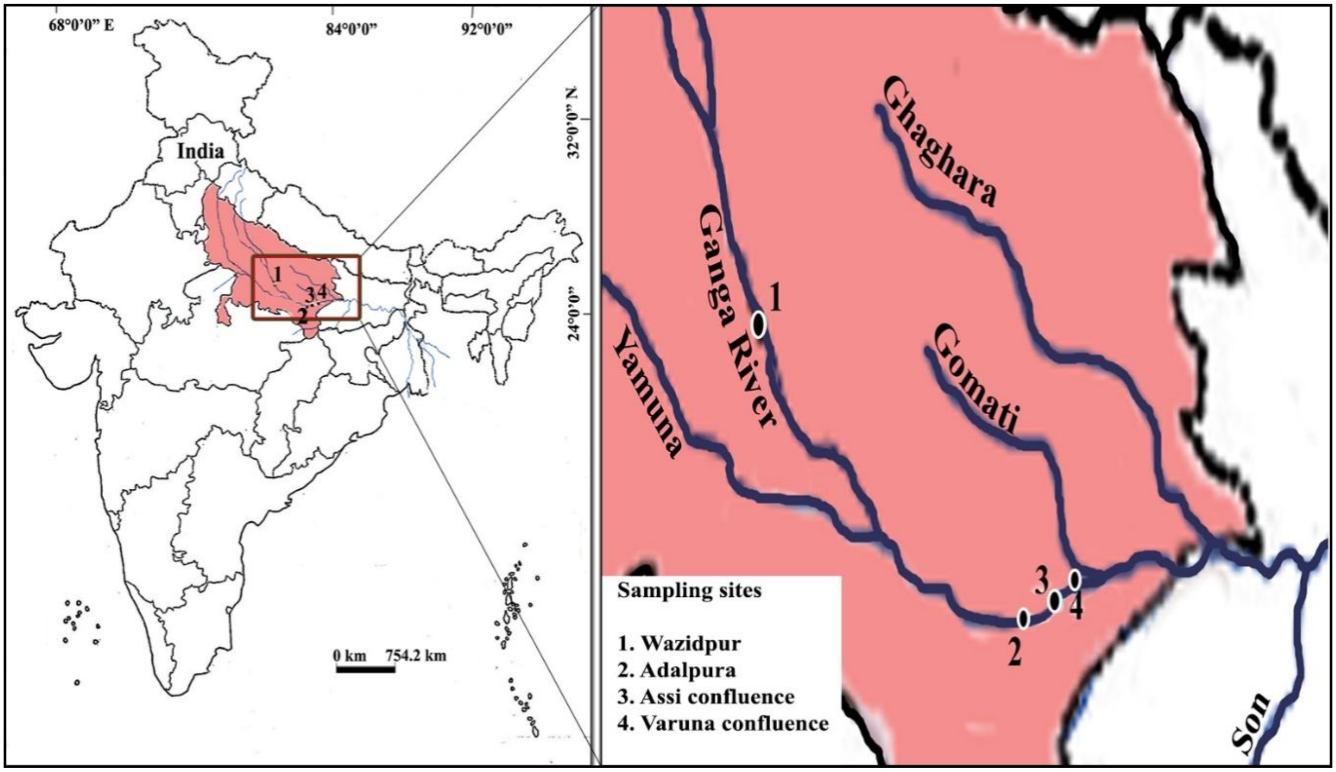

Fig. 1: Map of the study area showing geographical locations ofstudy sites.

trend on spatial scale and a slight increasing trend from April to June Fig. 2. The concentrations of TOC and PO_4^{3-} was highest at Assi confluence Site (Asdr) and the values were 7.34% and 5.73 mg g^{-1} respectively. The Assi drain, situated upstream to Assighat, was previously a rivulet now turned into a drain. It joins the Ganga River at south of the Varanasi city and adds about 66.45 MLD urban sewage into the river. These inputs are rich in organic carbon and detergent semerging from the households and because of this reason the concentration of PO_4^{3-} was very high at this site. The Wazidpur Site (Wpdr) was next in this order which is situated in Kanpur city near the Wazidpur drain of Jajmau area. The Kanpur City is well known for its leather industries and the river in Jajmau area receives about 168 MLD untreated and about 171 MLD treated sewage. The Varuna confluence Site (Vrna), situated downstream Varanasi city, comes next in order with respect to the concentration of TOC and PO_4^{3-}. Here, the concentration of TOC ranged from 3.45 to 4.21% and that of PO_4^{3-} from 3.03 to 3.46 mg g^{-1}. At this site, the Varuna tributary joins the Ganga River and adds a large amount of highly polluted water in the main channel. Additionally, the Rajghat drain situated close to this site adds over 16 MLD urban sewage. Also, the Ramnagar industrial area, situated ~11 km upstream to this site, adds ~25 MLD industrial effluents rich in metals and other chemicals. Further, the Varanasi city releases ~410 MLD sewage, of which only 101.8 MLD (<25%) is discharged after treatment (CPCB, 2013). Unlike TOC and PO_4^{3-}, the concentration of NO_3^- was highest at Vrna (3.54 mg g^{-1}) followed by Asdr (1.45 mg g^{-1}) and Wpdr (1.32 mg g^{-1}) whereas the NH_4^+ concentration was highest at Wpdr (3.97 mg g^{-1}) followed by Asdr Site (2.71 mg g^{-1}). The lowest values for NO_3^- as well as NH_4^+ were recorded at Adpr Site Fig. 2. The main reason behind this might be the prevailing hypoxic condition at these

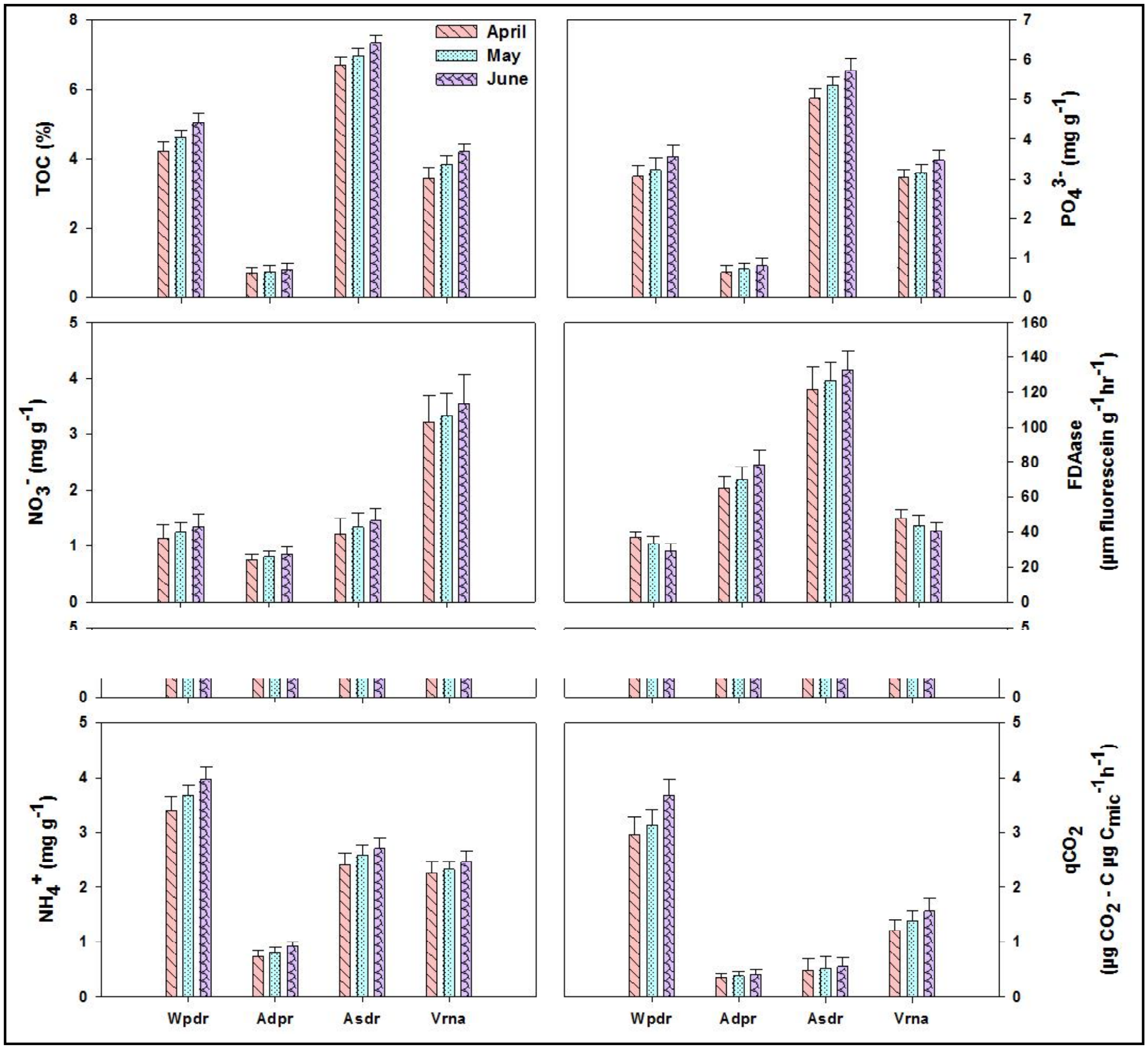

Fig. 2: Spatio-temporal trends in carbon, nutrients (NO_3^-, NH_4^+ and PO_4^{3-}), FDAase and microbial metabolic quotient (qCO_2) in riverbed sediment at study sites. Values are mean (n = 9)±1SE.

sites as reported in our earlier field trials (Jaiswal and Pandey, 2019c). The hypoxic condition causes reduction of oxidized nitrogen species leading to prevalence of NH_4^+ at these sites.

The concentration of study metals (Cd, Cr, Cu, Ni, Pb and Zn) were highest at Wpdr Sites followed by Vrna Sites and showed an increasing trend from April to June Fig. 3. The values of these metals varied between 0.12 and 0.44 µg g^{-1} for Cd, 29.68 and 220.39 µg g^{-1} for Cr, 5.97 and 97.94 µg g^{-1} for Cu, 4.03 and 99.06 µg g^{-1} for Ni, 4.23 and 86.17 µg g^{-1} for Pb, and between 33.61 and 179.84 µg g^{-1} for Zn. The main reason behind the highest concentration of metals at Wpdr Site is the presence of tanneries in areas surrounding this site. Tanneries release large amount of heavy metals (especially Cr) and other chemical rich effluents in the Ganga River.The metals are present in different forms in the riverbed sediment and show different degree of mobility, chemical interactions, bioavailability, and associated risk to the ecosystem (Xu *et al.,* 2017). For this reason, the estimation of total metal concentration alone cannot provide sufficient and reliable cue to understand transformation, transport and associated risks to aquatic flora and fauna (Singh *et al.,* 2005; Morelli and Gasparon, 2014). Because, the bioavailable fractions of metals have more direct and toxic impacts (Chapman *et al.,* 1998; Eggleton and Thomas, 2004), understanding the metal bioavailability associated ecosystem responses is important and has relevance from aquatic ecosystem restoration and management perspectives(van der Geest and Leon Paumen, 2008). The bioavailable fraction of study metals followed a trend similar to the total metal concentrations and the values were 3.82 to 28.69 times higher at most polluted site (Wpdr) compared to those at the least polluted site (Adpr) Fig. 4. The bioavailable fraction of metals ranged from 53-70% for Cd, 10-52% for Cr, 20-57% for Cu, 36-83% for Ni, 44-74% for Pb and from 14-55% for Zn (Table 1). The Wazidpurdra in near Wpdr Site adds over 54.0 MLD industrial effluent to the river in this region. Additionally, over 202 MLD of effluents and about 400 tonnes of solid wastes emerging from ~450 leather and ~150 other industries are dumped in the river in this area causing high concentration of total metal as well as their bioavailable fractions at Wpdr

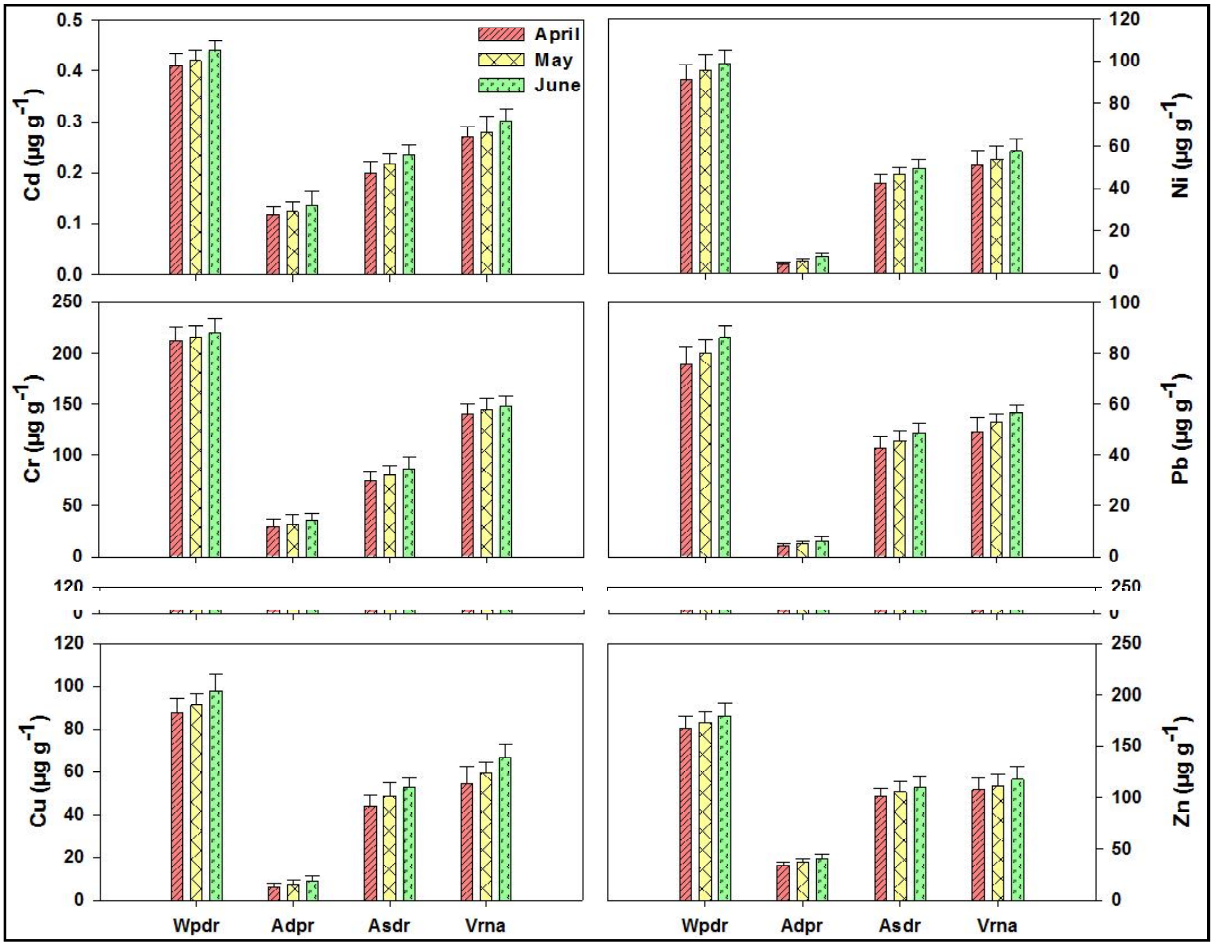

Fig. 3: Spatio-temporal trends in heavy metalsin riverbed sediment at study sites. Values are mean (n = 9)±1SE.

Site.

Understanding the shifts in ecosystem 'response' against multiple human perturbations is necessary for management of human-impacted riverine ecosystems. However, measurement of a 'response' of a riverine ecosystem is constrained by hydrological factors and the 'response variable' in question due to its dynamic nature, trophic status, sensitivity, universality and linkage with the ecosystem structure and functioning (Peterson and Stevenson, 1992; Friberg, 2014). Earlier studies have established that changes in microbial biomass/activities in riverbed sediment can be used more accurately to get cues about 'response' of human-impacted riverine ecosystems (Gibbons *et al.,* 2014). The extracellular enzyme (EE) activities are intricately linked with their surroundings and show quick and quantifiable response even to small changes occurring in their environment (Pearl *et al.,* 2003). Given that the substrates such as carbon and nutrients accelerate and the toxicants such as heavy metals inhibit the EE activities, the microbial enzymes can be used as an ecosystem 'response' towards metal pollution in large rivers. We used fluorescein diacetate hydrolase activity (FDAase) as an ecosystem 'response' towards heavy metal pollution in riverbed sediment of the Ganga Riveras it is a widely used and easy to measure EE activity across different ecosystems (Green *et al.,* 2006; Wallenius *et al.,* 2011). The FDAase involves all the three classes of enzymes (lipases, proteases and esterases) responsible for organic matter degradation and is used as a measure of total microbial activity (Schnürer and Rosswall, 1982). In our earlier recent field trial, we found that FDAase can also be used as an indicator of C-eutrophication and metal pollution (Jaiswal and Pandey, 2019b).

We found a very high FDAase activity at AsdrSite which is characterized by high concentration of TOC and nutrients, and low concentrations of metals as well as their bioavailable fraction (Figs. 2-4). Further, we found

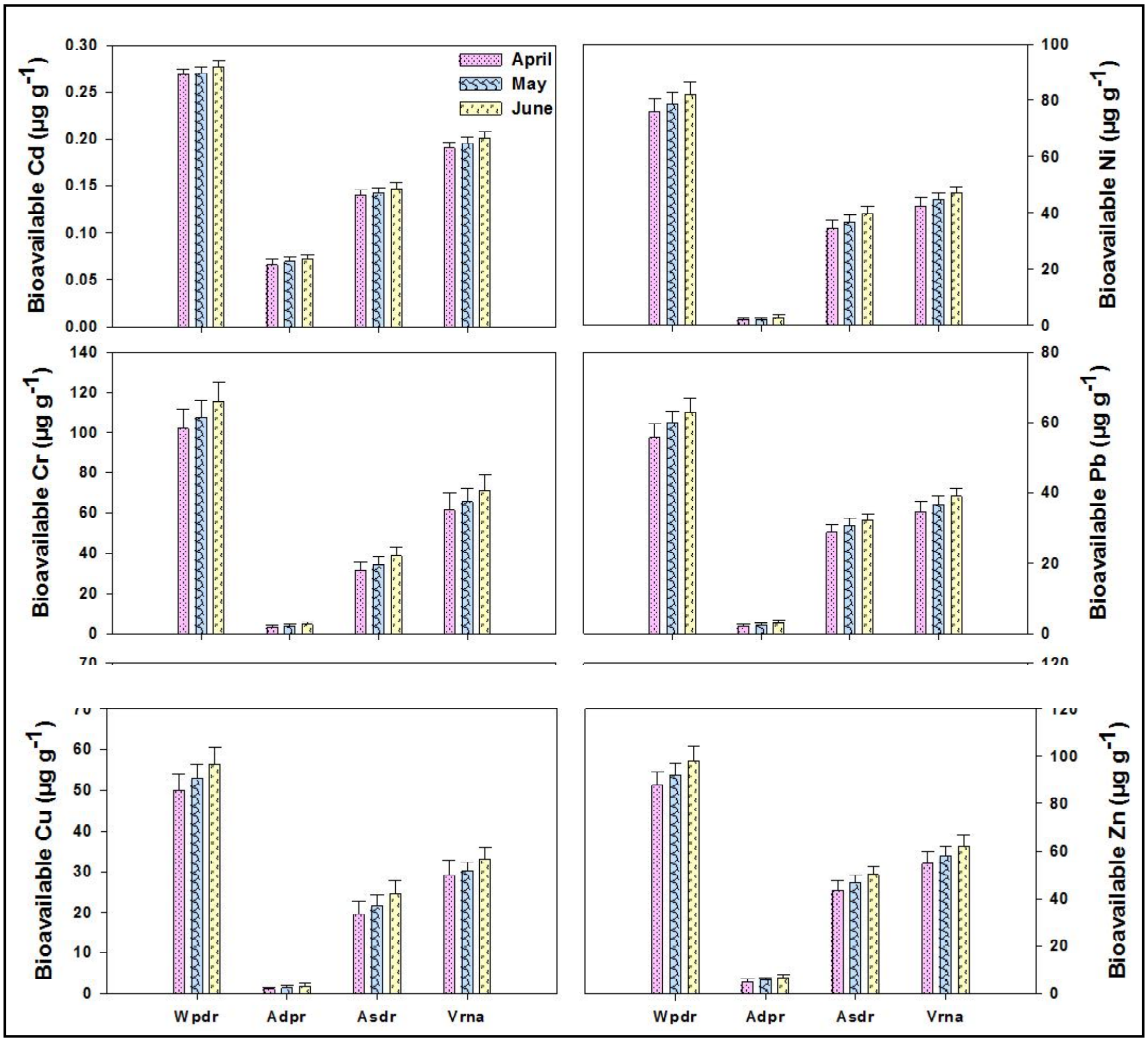

Fig. 4: Spatio-temporal trends in bioavailable fraction of heavy metalsin riverbed sediment at study sites. Values are mean (n = 9)±1SE.

low FDAase activity at Wpdr and Vrna Sites which are characterized by high concentration of total heavy metals (ΣTHM) and total bioavailable fractions (ΣTBF). The microbial metabolic quotient (qCO_2) followed a trend similar to NH_4^+ with values being highest at Wpdr Site (3.67 µg CO_2-C µg $C_{mic}^{-1}h^{-1}$) and lowest at Adpr Site (0.35 µg CO_2-C µg $C_{mic}^{-1}h^{-1}$). The qCO_2 is used as an indicator of change in microbial activities in response to environmental stressors (Wardle *et al.,* 1993). We found that despite high concentrations of carbon and nutrients at Wpdr and Vrna Sites, the qCO_2 values were high indicating stress condition at these sites (Fig. 2). The FDAase did show curvilinear negative relationships with ΣTHM and ΣTBF ($R^2 = 0.45$ to 0.64; $p<0.01$-0.02) (Fig. 5) indicating that high concentration of heavy metals is able to induce negative impacts on microbial/EE activities even in the presence of high amount of substrates. Our earlier study has also revealed that, even the high concentrations of organic carbon, which acts as a chelating agent for metals and as a substrate for EE activity, is not able to enhance the EE activity when ΣTHM exceeds 360 µg g^{-1} (Jaiswal and Pandey, 2019b). This was further evidenced by very strong positive correlations between qCO_2 and ΣTHM($R^2 = 0.98$; $p < 0.01$) and between qCO_2 and ΣTBF ($R^2 = 0.99$; $p < 0.01$) indicating stressed condition at study sites due to presence of high amount of metals and their bioavailable forms (Fig. 5). The dynamic fit models showed a

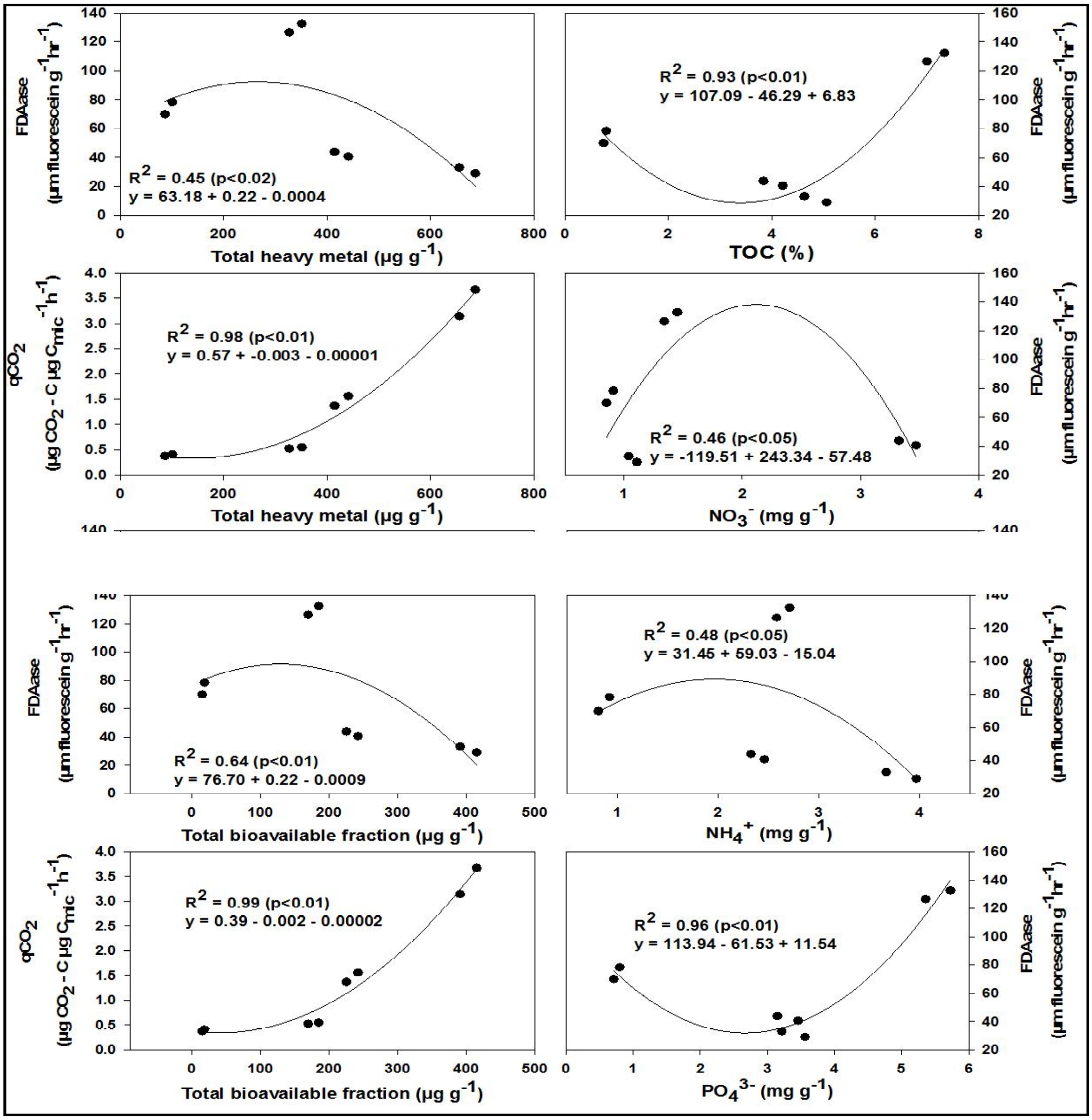

Fig. 5: Dynamic fit model showing significant correlations of FDAase, total heavy metal, microbial metabolic quotient (qCO_2), total bioavailable fraction, carbon and nutrients(NO_3^-, NH_4^+ and PO_4^{3-}) measured in riverbed sediment at study sites.

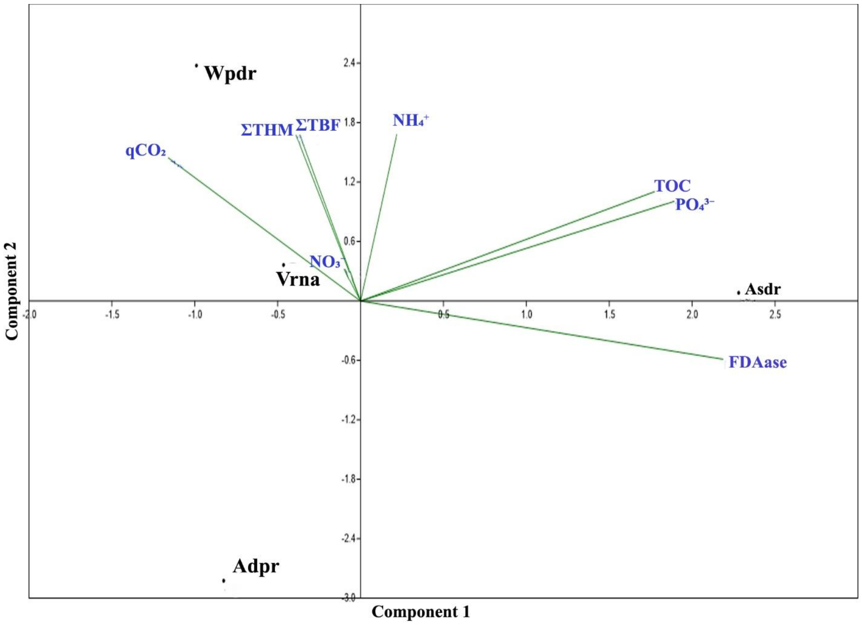

Fig. 6: Principal component analysis considering sediment quality determinants measured at four study sites of the Ganga River.

curvilinear positive relationship between FDAase and TOC ($R^2 = 0.93$; $p < 0.01$) and between FDAase and PO_4^{3-} ($R^2 = 0.96$; $p < 0.01$). These correlations first showed adecreasing trend and then an increasing trend with increasing TOC and PO_4^{3-} concentrations indicating that even with increasing concentration of substrates the FDAase activity showed a decreasing trend at sites where ΣTHM and ΣTBF were very high. The reason behind increasing the trend after a decline is that at sites where the carbon and phosphorus exceeds 6.0% and 5.0mg g^{-1}respectively and where the metal concentrations were low, the enzyme activity has dependence on substrate in a major way. Contrary to this, the FDAase showed a significant negative curvilinear correlation with NH_4^+ ($R^2 = 0.48$; $p < 0.05$) and NO_3^- ($R^2 = 0.46$; $p < 0.05$) (Fig. 5) indicating that even with increasing nutrient concentrations, the FDAase activity decreases due to metal induced toxicity. Further, the PCA results segregated FDAase opposite to ΣTHM, ΣTBF, TOC and nutrients indicating the negative response of microbial/enzyme activity towards heavy metal pollution (Fig. 6) despite the presence of high amount of substrates. The Wpdr and Vrma sites were separated with ΣTHM, ΣTBF, qCO_2 and NO_3^- indicating that these two sites are under strong control of these variables. Contrary to this, the Asdr Site was segregated with NH_4^+, TOC and PO_4^{3-} which have strong influence at this site. Further, the FDAase was placed closed to Asdr Site which is placed opposite to ΣTHM and ΣTBF showing that this site is free from metal induced toxicity and here the substrates are able to induce enzyme activity concurrent to their concentrations.

Conclusions

Assessing riverine ecosystem responses to anthropogenic perturbations using water column variables is constrained by hydrological forcing. Sediment-based determinants can be proved more appropriate in this respect. The data generated here revealed clearly that the riverbed sediment-based fluorescein diacetate hydrolytic assay (FDAase) can be an important predictor of carbon, nutrient and metal pollution. The study shows that even a sufficient availability of substrate such as carbon did not able to favour FDAase activity at sites where ΣTHM > 360 µg g^{-1} and ΣTBF > 174 µg g^{-1}. On the contrary, the enzyme activity responded almost in a synchronous manner where ΣTHM and ΣTBF concentrations were low. Our study has relevance understanding the magnitude of river responses to eutrophy and metal pollution and has relevance in rejuvenation of large rivers.

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