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Impact of heavy metal on activity of some microbial enzymes in the riverbed sediments: Ecotoxicological implications in the Ganga River (India)

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ABSTRACT

We studied the extracellular enzyme activity (EEA) in the riverbed sediment along a 518 km gradient of the Ganga River receiving carbon and nutrient load from varied human sources. Also, we tested, together with substrate-driven stimulation, if the heavy metal accumulated in the sediment inhibits enzyme activities. Because pristine values are not available, we considered Dev Prayag, a least polluted site located 624 km upstream to main study stretch, as a reference site. There were distinct increases in enzyme activities in the sediment along the study gradient from Dev Prayag, however, between-site differences were in concordance with sediment carbon(C), nitrogen (N) and phosphorus (P). Fluorescein diacetate hydrolysis (FDAase), β-glucosidase (Glu) and protease activities showed positive correlation with C, N and P while alkaline phosphatase was found negatively correlated with P. Enzyme activities were found negatively correlated with heavy metal, although ecological risk index (E_R^i) varied with site and metal species. Dynamic fit curves showed significant positive correlation between heavy metal and microbial metabolic quotient (qCO_2) indicating a decrease in microbial activity in response to increasing heavy metal concentrations. This study forms the first report linking microbial enzyme activities to regional scale sediment heavy metal accumulation in the Ganga River, suggests that the microbial enzyme activities in the riverbed sediment were well associated with the proportion of C, N and P and appeared to be a sensitive indicator of C, N and P accumulation in the river. Heavy metal accumulated in the sediment inhibits enzyme activities, although C rich sediment showed relatively low toxicity due probably to reduced bioavailability of the metal. The study has relevance from ecotoxicological as well as from biomonitoring perspectives.

1. Introduction

Knowledge of ecosystem status is central in designing conservation planning and approaches to surface water management. In the past decades, a number of studies contributed to assessment of the fate of carbon and nutrients in river and streams. These studies were seen critical to monitor the effect of fluxes of carbon and nutrients, linking eutrophication as one of the key factors of river status. More recent studies establish sediment microbial enzyme activities as an indicator of carbon and nutrient limitation, which can be used to uncover the influence of regional scale anthropogenic stressors (Sinsabaugh et al., 2009; Hill et al., 2010; Gibbons et al., 2014; Yadav and Pandey, 2017; Pandey and Yadav, 2017). The substrate specific nature of the extracellular enzymes make them an important tool for investigations on functional profile of microbial communities as influenced by anthropogenic activities (Hill et al., 2010). Rivers receive, in addition to other inputs, a number of heavy metals of natural and anthropogenic origin, and riverbed sediments are important depositories of these metals. The wide distribution and persistent nature of these metals make them potentially toxic to aquatic biota influencing the ecological functioning. Factors such as the magnitude of external loading, source partitioning, mineralogical composition, adsorption, river flow, sediment delivery and urban-industrial discharge influence metal distribution in riverbed sediment. Depending upon their concentration and bioavailability, these may lead toxic effect on river biota including microbial activity in the sediment (Baran and Tarnawski, 2015; Dell'Anno et al., 2003). Organic carbon in sediment forms complexes and often buffer the bioavailability of heavy metals (Griscom et al., 2000). Thus, the data on microbial enzyme activities linking bioavailable concentrations along with total content of heavy metals provide important cues on the river status and eco-toxicology that research should focus on. Understanding the biological effects of heavy metals on microbial activities in the riverbed sediment is an important scientific challenge, because the microbial community in riverbed sediment plays a key role regulating nutrient cycling and breakdown of organic matter. Detrimental effects of heavy metals on sediment microbial community might have

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important consequences on benthic detrivory and biogeochemical cycles.

Heavy metals may affect sediment metabolism. The proximate measures of sediment metabolism are ectoenzymes that break organic matter and produce soluble substrates to microbial assimilation (Sinsabaugh et al., 2008). Ectoenzyme activities are linked with microbial metabolism, nature and availability of resources, and, the presence of environmental stressors. Thus, enzyme activities in the riverbed sediment may provide cues to monitor microbial activity, nature of available substrate and the influence of anthropogenic factors. Further, based on the linkages between carbon and nutrient content in water and those in the sediments, it is possible to monitor water quality status using sediment microbial properties as surrogates for sediment and water chemistry (Hill et al., 2006). Furthermore, chemical measurements alone are not sufficient to accurately predict eco-toxicological effects and assessing changes in biological components alone would not reliably predict the ecosystem status. Integrating chemical data with changes in biological responses provides an overall picture of ecosystem status (O'Connor and Paul, 2000). Because the sediment microbial activity is directly linked with carbon and nutrient limitation; and mineralization of organic matter by heterotrophs creates trophic base for detrivory (Sinsabaugh et al., 2009). Field studies in this direction may provide useful data base on river metabolism as well as on metal-associated environmental health assessment. For the Ganga River, limited information on these aspects is available. The present study aimed to investigate the interactions between heavy metals and microbial enzyme activities in the Ganga River (India). Because a major portion of nutrient and energy flow in aquatic ecosystems is through the microbial loop, linking microbial enzymatic activities with heavy metal concentrations in riverbed sediment may be a sensitive indicator of regional scale anthropogenic stressors.

2. Materials and methods

2.1. Study area

The Ganga River is the largest river system of India. The river Bhagirathi that originates in Garhwal Himalaya joins river Alaknanda at Dev Prayag and becomes the River Ganga, the most sacred river of India. The River travels approximately 2525 km from Himalaya to Bay of Bengal. The river basin (1086,000 km^2 area) covers four countries: India, Nepal, Bangladesh, and China (Fig. 1). The basin is bounded by the Himalaya in the north and the Vindhyan range in the south. In India, it covers ~26.20% of the geographical area of the country. The climate of the region is tropical monsoonal distinguishable into three seasons; winter (November to February), summer (March to June) and a humid monsoon season (July to October). The average rainfall ranges from 750 to 2500 mm and the relative humidity reaches close to saturation in rainy season. Among 10 classes of soils found in the Ganges basin, alluvial (52.44%), red (11.80%) and medium black soils (10.78%) constitute approximately 75.02% of total basin. Alluvial soil is highly fertile and constitute extensive agricultural land. Over 60% of water in the Gangatic plain comes from the Himalayan sources while ~ 40% from the peninsular region. The Ganges basin with 1949 cities and towns and population over 500 million, has highest population density (712 persons per square kilometer) in India (2011 census). The government of India is giving much attention for restoration of this holy river because of its rapidly degrading water quality.

For detail study, we selected a 518 km stretch of the middle segment of the Ganga River from Kanpur upstream (26°30′ N; 80°19′ E) to Varanasi downstream (25°19′ N; 83°01′ E). Due to a diversity of pollutant sources and population density in the watershed, the river stretch considered here, from Kanpur upstream to Varanasi downstream, provides a model zone for comparing anthropogenic influences and other environmental conditions. The overall study stretch has been divided into 9 stations with three sub-sites in each (Fig. 1). The sites include: Dev Prayag (Dvpg), Nawabganj (Nwbj), Jajmau (Jjmu), Dalmau (Dlmu), Hatwa (Htwa), Sangam (Sngm), Sitamarhi (Stmh), Bypass (Byps), and Rajghat (Rjht). Since pristine values are not available, to validate data comparison, we considered Dev Prayag, a least polluted site situated 624 km upstream to main study stretch, as a control in backdrop.

2.2. Sample collection and analysis

The sediment and water samples were collected during base flow of two consecutive years (2016 and 2017). Sampling was done for nine months (October to June) for each study year. Triplicate samples of sediment (0–10 cm depth) from each site were collected from 10 to 50 m reach using sediment corers. Samples were carried to laboratory in acid-rinsed polyethylene plastic bags in an ice-box, and then air-dried at room temperature, homogenized, grinded to powder, and sieved using a 2-mm mesh prior to analysis. Sediment samples were digested in tri-acid mixture and analyzed for total concentration of Cd, Cr, Cu, Ni, Pb and Zn in an atomic absorption spectrophotometer (Perkin Elmer model Analyst 800, USA). Bioavailable fraction of heavy metals was measured following Ure (1996). For estimation of total organic carbon (TOC), samples were digested in the presence of $K_2Cr_2O_7$ and H_2SO_4 and measured titrimetrically following modified Walkley and Black method (Chen et al., 2015). For the measurement of NO_3^- and NH_4^+; brucin sulphanilic acid (Voghel, 1971) and phenate method (Park et al., 2009) respectively was used. PO_4^{3-} was determined as orthophosphates using ammonium molybdate stannous chloride method (Murphy and Riley, 1962). Basal respiration and substrate-induced respiration (SIR; a relative measure of active microbial biomass) was determined by following Wardle et al. (1993).

Because different sediment types may have variable water content, to investigate enzyme activity, the moisture content of each sediment sample was adjusted to 80% of water holding capacity. Alkaline phosphatase activity (AP) was determined spectrophotometrically by estimating *p*-nitrophenol formed when sediment samples are incubated in buffered solution of p-nitrophenyl phosphate (PNP) (Tabatabai and Bremner, 1969). Fluorescein diacetate hydrolytic activity (FDAase) was measured in terms of fluorescein formed when sediment suspension was incubated with fluorescein diacetate (Schnürer and Rosswall, 1982). Assessment of protease was done in terms of concentration of aromatic acid released by proteolytic cleavage of casein and expressed in terms of L-tyrosine equivalents (Ladd and Buttler, 1972). β-D-glucosidase activity was measured by incubating sediment samples with buffered p-nitrophenyl-β-D-glucoside (PNG) (Eivazi and Tabatabai, 1988).

2.3. Microbial metabolic quotient

Microbial metabolic quotient (qCO_2) was determined in terms of ratios of basal respiration to SIR (Wardle, 1993). It is used as a bio-indictor of changes in microbial biomass in response to the disturbances. Odum (1985) considered this index as an alternative measure of changes in microbial biomass/activity in response to ecosystem development, disturbance or stress condition, as the microbial efficiency declines under the influence of disturbances or stress related factors.

2.4. Potential ecological risk index (PERI)

Potential Ecological Risk Index (PERI) proposed by Hakanson (1980) is used to evaluate the degree of ecological risk of heavy metals. This method broadly considers the interaction, toxic level, concentration and ecological sensitivity of the heavy metals (Singh et al., 2010; Douay et al., 2013). Along with the heavy metal level in the sediment it also considers ecological and environmental effects and evaluates pollution level using equivalent and corresponding property index grouping method (Qui, 2010). It considers three basic elements: degree