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MONSOON-DRIVEN REVERSAL OF METAL PARTITIONING REGULATES OXYGEN DYNAMICS AND MICROBIAL EFFICIENCY IN THE GANGA RIVER

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ABSTRACT

Monsoon-driven hydrological forcing plays a key role in regulating metal mobility and biogeochemical functioning in large tropical rivers. Although its influence on riverine interfaces and associated microbial responses remains poorly understood. In this study, we investigated seasonal variations in heavy metal distribution across the sediment-water interface, land-water interface and water column of the middle stretch of the Ganga River during the pre-monsoon (PRM) and post-monsoon (POM) period for 2024. We also looked at how organic carbon dynamics, oxygen availability, microbial respiration, biomass and extracellular enzyme activities changed with the seasons. During PRM, most of the heavy metals deposited at the sediment-water interface. There were lower concentrations at the land-water interface and in the water column. This demonstrates that benthic sequestration is effective under conditions of low flow. In contrast, POM conditions showed clear metal redistribution, with higher concentrations in the water column and at the land-water interface and lower concentrations at the sediment-water interface. This was due to hydrological control and lateral transport. This seasonal shift in metal partitioning coincided with substantial biogeochemical reorganization at riverine interfaces. After the monsoon, there was more total organic carbon, dissolved oxygen, microbial biomass carbon, substrate-induced respiration and extracellular enzyme activities. There were also less dissolved oxygen deficit and microbial metabolic quotient, which showed that microbes were better at using carbon. In general, the monsoon changes the pre-monsoon regime from one that is mostly about benthic metal sequestration, limited oxygen and limited microbial activity to one that is mostly about metal redistribution, increased oxygenation and increased microbial metabolism. These findings highlight the central role of monsoon dynamics in synchronizing metal transport, microbial activity and carbon cycling in heavily impacted tropical river systems such as the Ganga.

***Key words*:** Monsoon hydrology; Heavy metal partitioning; Sediment-water interface; Land–water interface; Microbial efficiency; Oxygen dynamics

Introduction

Large tropical rivers are very important in regulating the movement, change and long-term fate of pollutants and organic carbon along the land-water continuum. The Ganga River, for example, supports almost one-tenth of the world's population and is under a lot of pressure from human activities like industrial waste, urban waste water, agricultural runoff and changes in land use on a regional scale. Heavy metals that get into rivers through these pathways stay there (Yang *et al.*, 2024). Hydrological variability and biogeochemical processes that happen at the sediment-water and land-water interfaces have a big effect on how mobile they are and how they affect the environment (Lucas *et al.*, 2025).

In river systems, sediments serve as both sinks and secondary sources of heavy metals. In the low flow situation, fine particles and organic matters effectively trap metals in benthic sediments (Peng *et al.*, 2018). But this apparent immobilization is very dynamic. Seasonal hydrological forcing, especially discharge pulses caused by the monsoon can make sedimentary metal pools less stable by physically resuspending them, changing their redox state and moving them laterally across interfaces (Van *et al.*, 2005). This makes metals more available in the water column and in nearby riparian zones (Gupta and Pandey, 2025; Tang *et al.*, 2014). Although there is more awareness of these processes, most research on large tropical rivers has looked at metal contamination in either sediments or water on their own. This does not give us much information about how seasonal hydrology controls the movement of metals between different parts of the river.

Metal redistribution has major implications for microbial communities that facilitate organic matter decomposition and carbon cycling (Condron *et al*., 2010). High levels of metal pollutants can hinder extracellular enzyme activity, slow down microbial respiration (Jaiswal and Pandey, 2018). Low oxygen levels can also make aerobic carbon processing even harder. As a result, riverine interfaces that are constantly loaded with metals often show a disconnect between the availability of organic carbon and microbial activity. This changes the pathways that stabilize carbon (Li *et al*. 2025).

Monsoon hydrology adds another layer of complexity by affecting metal mobility, oxygen dynamics and microbial functioning all at once (Chaturvedi *et al.*, 2024). According to Kumar *et al*., (2013) more discharge after the monsoon can help oxygenate the water column bring in more organic matter from the catchment and encourage microbial growth and enzyme activity. At the same time, stronger hydrodynamic energy can move metals that are trapped in sediment and send them back into the water column and the land-water interface (Bouwman *et al*., 2013). How these two opposing forces-metal stress and better oxygen and substrate availability work together to control microbial efficiency and carbon processing across riverine ecosystems is still not well understood, especially in heavily polluted tropical rivers like the Ganga.

Riverine studies seldom amalgamate metal partitioning, oxygen dynamics, microbial respiration, biomass and extracellular enzyme activities into a cohesive seasonal framework (Prajapati and Pandey, 2024). We are limited to predict how monsoon-driven changes in water flow affect the biogeochemical functioning and ecological risk of large river systems because we do not have a good understanding of how these changes work together on a larger scale (Khadanga *et al.*, 2025). It is important to fill this gap because changes in the intensity and timing of the monsoon caused by climate change are likely to make metals move more easily and change the way biogeochemistry works in South Asian rivers.

In this context, the current study examines monsoon-induced influences on metal distribution across the sediment-water interface, land-water interface and water column of the middle stretch of the Ganga River. This study also examines the concomitant alterations in organic carbon pools, dissolved oxygen dynamics, microbial respiration, biomass and extracellular enzyme activities during the pre-monsoon and post-monsoon periods. We hypothesize that pre-monsoon low-flow conditions facilitate benthic sequestration of metals and inhibit microbial efficiency, whereas post-monsoon alterations in hydrology results in a shift in metal partitioning towards the water column and land-water interface. It results in increased oxygen availability and microbial carbon processing. This study shows the connections between seasonal metal redistribution and microbial metabolic responses at riverine ecosystem and offers a novel insight on the interplay of hydrology, contamination and carbon cycling in tropical river ecosystems.

Materials and Methods

Study area and sampling design

Field investigations were conducted during the summer low-flow period (15 April–14 June) and post-monsoon period (28 September- 15 October) for 2024 along a ~520 km stretch of the middle stretch of the Ganga River, extending from Kanpur upstream (26°502 N; 80°312 E) to Varanasi downstream (25°322 N; 83°042 E) (Fig. 1). The study reach was divided into eight

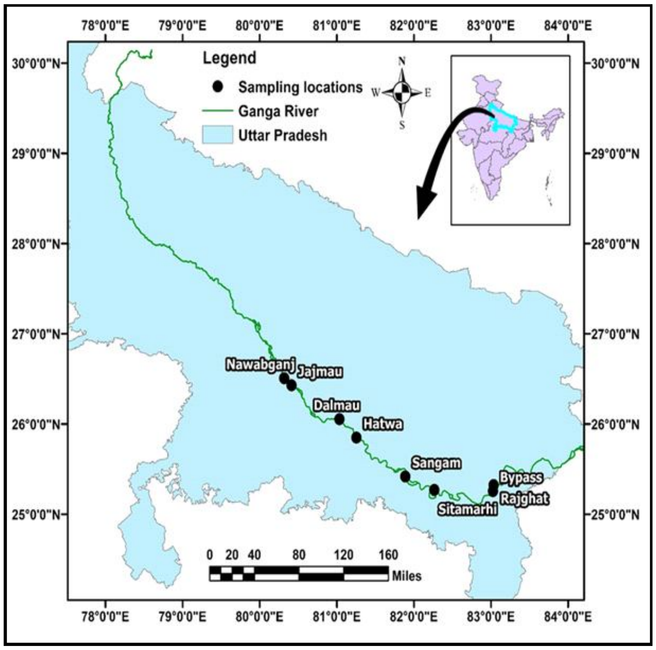

Fig. 1: Map showing all sampling stations in middle stretch of Ganga River.

monitoring stations: Nawabganj (Nwbj), Jajmau (Jjmu), Dalmau (Dlmu), Hatwa (Htwa), Sangam (Sngm), Sitamarhi (Stmh), Bypass (Byps) and Rajghat (Rjht). The region experiences a tropical monsoonal climate characterized by three distinct seasons: a hot-dry summer (March–June), a humid monsoon season (July-October), and a cool winter (November–February). More than 90% of the annual rainfall occurs during the monsoon period, with the southwest monsoon contributing over 80% of total precipitation.

Sample collection

Water and sediment samples were collected during the low-flow season (April–June) and for post-monsoon season for 2024. At each monitoring station, three sub-sites were selected along two reach scales (25 and 50 m) of the main river course. Triplicate samples of water and sediment were collected from each sub-site. Water samples were collected from near-bottom depths using a depth-integrated sampler. Dissolved oxygen (DO) was fixed immediately after collection and samples were transported to the laboratory in ice boxes maintained at 4°C until further analysis. Sediment samples were collected from the upper 0–5 cm layer using sterilized stainless-steel corers and stored in polyethylene bags. All sampling, preservation and transportation procedures followed standard protocols. For extracellular enzyme assays, fresh sediment samples were adjusted to 80% moisture content and pre-incubated in desiccators prior to analysis.

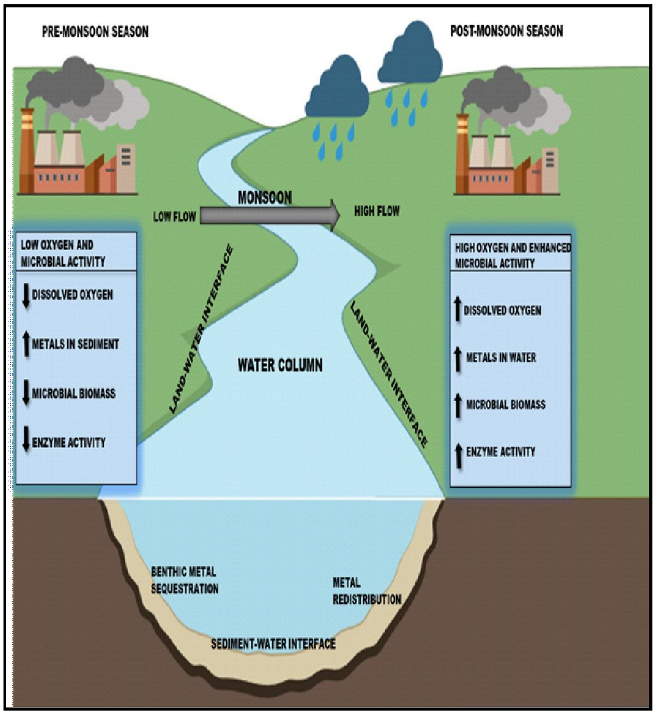

Fig. 2: Monsoon-driven hydrological forcing reverses metal partitioning from benthic sequestration during low flow to water-column redistribution during high flow, coupling oxygen dynamics with microbial activity across river interfaces.

Physicochemical and microbial analyses

The dichromate oxidation method (Nelson and Sommers, 1982) was used to find the total organic carbon (TOC) in sediment. Potassium dichromate and concentrated sulfuric acid were used to digest finely ground sediment samples. The leftover dichromate was then titrated against ferrous ammonium sulphate. The concentrations of TOC were given in mg g^{-1} dry weight. Dissolved oxygen (DOsw) was measured in situ using a calibrated portable dissolved oxygen meter (YSI Professional Plus, USA). Dissolved oxygen deficit (DOD) was calculated as the difference between temperature-corrected oxygen saturation concentration and the observed DOsw .We measured substrate-induced respiration (SIR) by adding a glucose solution (1% w/w) to moist sediment samples and letting them sit in aerobic conditions (Anderson and Domsch, 1978). We trapped evolved CO_2 in NaOH and then used HCl to titrate it after $BaCl_2$ precipitation. The microbial metabolic quotient (qCO_2) was calculated as the ratio of basal respiration to microbial biomass carbon (Cmic). Microbial biomass carbon (Cmic) was determined using the chloroform fumigation-extraction method (Vance *et al.*, 1987). Fumigated and non-fumigated sediment samples were extracted with 0.5 M K_2SO_4, and extractable organic carbon was quantified using a TOC analyzer (Shimadzu, Japan).

Extracellular enzyme activities

We measured fluorescein diacetate hydrolase (FDAase) activity by following Green *et al.*, (2006). We did this by incubating sediment samples with fluoresce in diacetatein phosphate buffer and measuring the release of fluorescein at 490 nm. The activity of β-D-glucosidase was evaluated using p-nitrophenyl-β-D-glucopyranoside as a substrate, by quantifying the released p-nitrophenol at 410 nm (Eivazi and Tabatabai, 1988). The Folin-Ciocalteu method at 700 nm (Ladd and Butler, 1972) was used to measure the amount of amino acids that were released when casein was used as a substrate for protease activity. We measured phenol oxidase activity calorimetrically by mixing 2 mL of sample suspension with 2 mL of 5 mM L-DOPA and letting it sit for an hour while tumbling. Then, we centrifuged the mixture and measured the absorbance at 460 nm.

Heavy metal analysis

Heavy metal concentrations were determined after ternary acid digestion following Allen *et al.*, (1986).

Sediment samples were digested with a tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4; 5:1:1) using a microwave digestion system (SINEO MDS-6G), diluted with distilled water, filtered (Whatman No. 42), and analyzed by atomic absorption spectrophotometer (PerkinElmer Analyst 800, USA).

Statistical analysis

We have used IBM SPSS Statistics version 26 to perform the statistical analyses. Two-way analysis of variance (ANOVA) was used to calculate spatial and temporal variations among sampling sites and across years. When substantial differences were identified. We used Tukey's HSD post-hoc test to compare means with a significance level of $p < 0.05$.

Results and Discussion

Monsoon-driven reversal of heavy metal distribution across riverine interfaces

Heavy metal concentrations displayed strong, statistically significant and interface-specific seasonal variability along the middle stretch of the Ganga River (Figs. 3 to 5; Table 1). Across all sites, monsoon transition from pre-monsoon (PRM) to post-monsoon (POM) resulted in a consistent redistribution of metals from benthic sediments to more mobile compartments. In the water column, dissolved concentrations of metal pollutants increased significantly during POM, with enrichment ranging from 35-90% for Zn, 40–100% for Ni, 40–95% for Cr, 45-110% for Cu, 50-115% for Pb and reaching up to 120% for Cd relative to PRM values (Fig. 4; $p \leq 0.005$).These increases show that metals can move a lot after the monsoon and be transported downstream when the discharge is high. At the land-water interface, there was a similar but less extreme increase in metal levels. During POM, the levels of Zn, Cr, Ni, Cd, Cu, and Pb increased by 25–40%, 25–45%, 30–50%, and 30–55%, respectively (Fig. 5; $p \leq 0.005$). This pattern reflects enhanced lateral connectivity, surface runoff and catchment-derived inputs activated during and following monsoon rainfall.

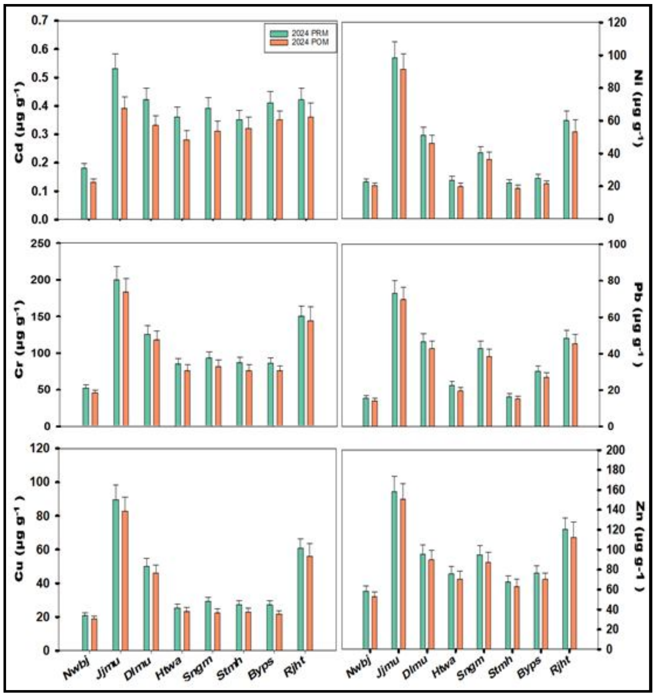

Fig. 3: Seasonal variation in metal pollutants concentrations at the sediment-water interface of the middle Ganga River during the pre-monsoon (PRM) and post-monsoon (POM) periods. Data illustrate the distribution and relative enrichment of metals within benthic sediments under contrasting hydrological conditions.

In contrast, metal concentrations at the sediment-water interface declined significantly during the post-monsoon period, with decreases of 20-35% for Zn and Cu, 20-40% for Cd and Ni and up to 45% for Pb and Cr compared to PRM levels (Fig. 3; Table 1; $p \leq 0.005$). The uniform reduction across all elements suggests hydrologically induced remobilization of sediment-bound metals driven by increased flow velocity, sediment resuspension and monsoon-related shifts in redox and boundary-layer dynamics. The strong and opposite

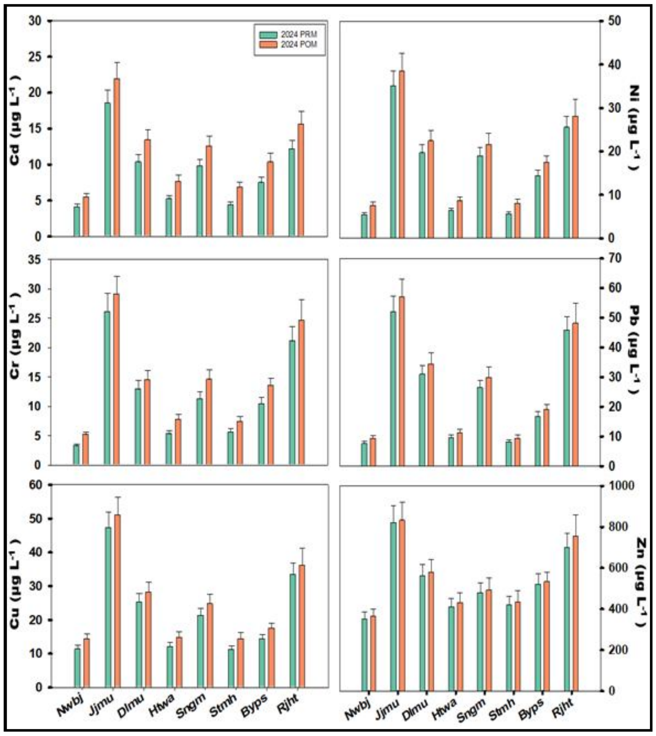

Fig. 4: Seasonal variation in dissolved metal pollutants concentrations in the water column of the middle stretch of Ganga River during the pre-monsoon (PRM) and post-monsoon (POM) periods, highlighting changes associated with monsoon-driven hydrological forcing.

seasonal trends across interfaces show that the monsoon caused a clear change in metal partitioning. This change was marked by less benthic retention and more metal accumulation in the water column and land-water interface after the monsoon season. Pandey and Singh, (2017) also reported the similar pattern of heavy metals accumulation in sediment of Ganga River during pre-monsoon season. This redistribution fundamentally alters metal exposure pathways and sets the stage for subsequent biogeochemical and microbial responses examined in the following sections.

Oxygen dynamics and organic carbon responses

Seasonal redistribution of heavy metals was accompanied by significant and statistically significant changes in the availability of oxygen and the movement of organic carbon across the middle stretch of the Ganga River (Fig. 6; Table 1). In the post-monsoon (POM) period, the amount of dissolved oxygen at the sediment-water interface (DOsw) increased steadily as compared to the pre-monsoon (PRM) season, with increases of 25% to 60% across sites ($p \leq 0.005$). During POM, the dissolved oxygen deficit (DOD) also dropped sharply, by 30–55% compared to PRM conditions ($p \leq 0.005$). At the same time, the dissolved oxygen deficit (DOD) decreased sharply during POM, going down by 30–55% as compared to PRM conditions ($p \leq 0.005$). These contrasting and statistically significant trends show effective post-monsoonal reoxygenation of the river system. It is enabled by enhanced hydrological mixing, increased flow velocity, and augmented air-water gas exchange following monsoon discharge.

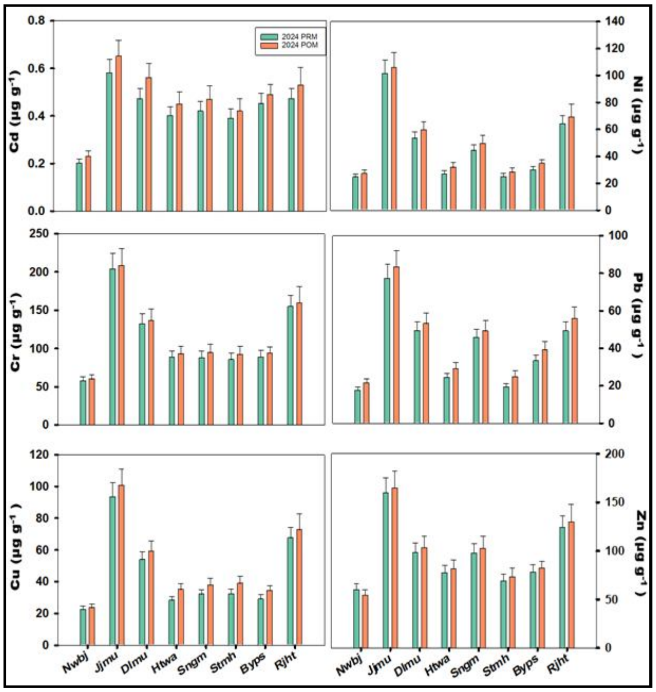

Fig. 5: Seasonal variation in metal pollutant concentrations at the land-water interface of the middle stretch of the Ganga River during the pre-monsoon (PRM) and post-monsoon (POM) periods, reflecting lateral metal exchange between terrestrial and aquatic compartments.

Table 1: Two-way analysis of variance (ANOVA) showing the effects of site, season and their interactions (site × season) on biogeochemical determinants, microbial attributes and metals pollutants in the Ganga River.

Parameter	Site	Season	Site × Season
TOC	100.76***	11.98***	0.237NS
DO	50.47***	8.86***	0.109NS
DOD	46.86***	8.27***	0.101NS
SIR	81.77***	7.94***	2.560**
qCO2	205.18***	18.52***	1.257NS
Cmic	97.74***	0.54NS	0.430NS
FDAase	52.23***	0.27NS	0.262NS
β-D-glucosidase	260.65***	0.80NS	0.213NS
Phenol oxidase	256.53***	0.71NS	0.195NS
Protease	242.30***	1.67NS	0.319NS
Cd	61.59***	42.46***	0.47NS
Cr	152.71***	6.03**	0.062NS
Cu	253.50***	10.67***	0.138NS
Ni	284.8***	13.67***	0.260NS
Pb	226.87***	7.97***	0.045NS
Zn	107.35***	1.09NS	0.004NS

At all interfaces, total organic carbon (TOC) clearly and significantly increased after the monsoon, going up by 15–30% at the sediment-water interface (Fig. 6; Table 1; $p \leq 0.005$). The sediment-water interface had the most enrichment, which was due to a lot of fresh organic matter from land being moved during monsoon runoff and catchment flushing.

The fact that TOC and dissolved oxygen both went up at the same time after the monsoon is important because it shows that the buildup of organic carbon did not cause oxygen stress when the flow was high. This is very different from what happened before the monsoon, when higher levels of TOC were linked to higher levels of DOD and less oxygen. The seasonal separation of carbon accumulation from oxygen depletion during POM underscores the preeminent role of hydrological forcing in governing oxygen dynamics. It is effective in protecting the system from organic matter-induced hypoxia despite increased carbon loading.

Microbial biomass, respiration, and metabolic efficiency

Microbial functional attributes displayed pronounced and statistically significant seasonal variations along the middle stretch of the Ganga River, which is a characterstic feature of to post-monsoonal hydrological reorganization (Fig. 6; Table 1). During the post-monsoon (POM) period, microbial biomass carbon (Cmic) increased markedly at all sampling sites at SWI, with enhancements ranging from 30-60% at the sediment-water interface relative to pre-monsoon (PRM) levels ($p \leq 0.005$). These increases indicate substantial post-monsoonal recovery and expansion of microbial biomass following seasonal reoxygenation. Substrate-induced respiration (SIR) exhibited a more pronounced seasonal variation, escalating by 35-70% at the sediment–water interface during POM in contrast to PRM ($p \leq 0.005$). This big increase in SIR shows that microbes can use more substrates and have more metabolic potential after the monsoon, when there is more oxygen and organic carbon.

In contrast, the microbial metabolic quotient (qCO_2) declined significantly during the post-monsoon period, with reductions of 25-45% at the sediment-water interface relative to PRM values (Fig. 6; Table 1; $p \leq 0.005$). Lower qCO2 during POM indicates reduced maintenance respiration and enhanced microbial carbon-use efficiency, whereas elevated qCO_2 during PRM reflects metabolically stressed microbial communities. A similar trend in qCO_2 was also reported by earlier studies (Jaiswal and Pandey, 2018).

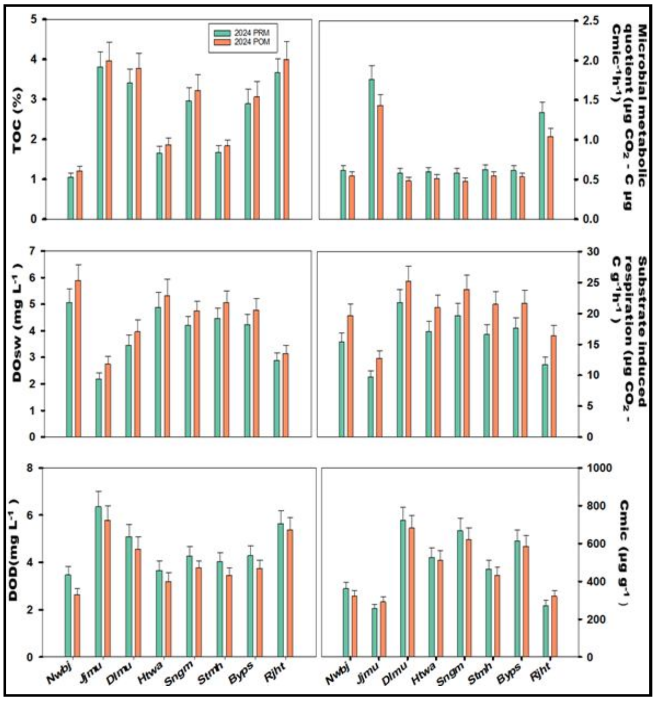

Fig. 6: Spatio-temporal variation in total organic carbon (TOC), dissolved oxygen in sediment-water interface (DOsw), dissolved oxygen deficit (DOD), microbial metabolic quotient (qCO2), substrate-induced respiration (SIR) and microbial biomass carbon (Cmic) across sampling sites during the pre-monsoon (PRM) and post-monsoon (POM) periods

These improvements in microbial metabolic efficiency occurred despite increased dissolved metal concentrations in the water column during the post-monsoon (POM) period. This pattern indicates that oxygen availability and hydrological reoxygenation exert a more substantial influence on microbial functioning than direct metal exposure under high-flow conditions (Palmer and Ruhi, 2019). In contrast, during the pre-monsoon (PRM) season the simultaneous increase in sediment-associated metal concentrations and oxygen limitation likely created synergistic metabolic constraints. This leads to less efficient microbes and a higher qCO_2.

Extracellular enzyme activity responses

During the post-monsoon (POM) period, extracellular enzyme activities indicated a clear and statistically significant increase in the middle stretch of the Ganga River (Fig. 7; Table 1). Total hydrolytic activity, indicated by fluorescein diacetate hydrolase (FDAase) exhibited an increase of approximately 30-55% at the sediment-water interface compared to pre-monsoon (PRM) levels ($p \leq 0.005$). This increase reflects a broad stimulation of microbial hydrolytic capacity following post-monsoonal reoxygenation and improved substrate availability.

Activities of carbon and nitrogen-acquiring enzymes also rose significantly during POM. β-D-glucosidase activity increased by 35-60%, while protease activity increased by 25-45% across all sites at sediment-water interface as compared to pre-monsoon (PRM) values (Fig. 7; $p \leq 0.005$). It indicates enhanced microbial capacity to depolymerize labile carbohydrate and protein substrates under post-monsoon (POM) conditions. Oxidative enzyme activity exhibited a comparable seasonal variation, with phenol oxidase activity increasing

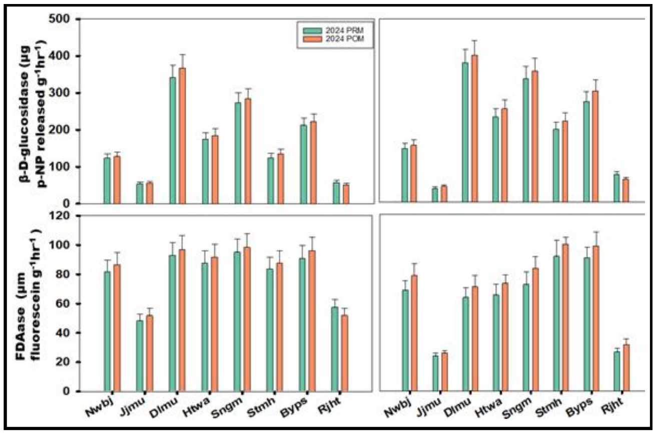

Fig. 7: Seasonal variation in extracellular enzyme activities, including fluorescein diacetate hydrolase (FDAase), β-D-glucosidase, protease and phenol oxidase across sampling sites during the pre-monsoon (PRM) and post-monsoon (POM) periods, indicating changes in microbial functional potential

by 30-80% during the post-monsoon season (POM), especially notable at polluted locations (Jajmau and Rajghat). It shows that microbes can get to chemically complex and more resistant organic matter reservoirs more easily after hydrological flushing and oxygen replenishment.

In contrast, extracellular enzyme activities were consistently suppressed during the pre-monsoon period across all enzyme classes (Fig. 7). Reduced FDAase, β-D-glucosidase, protease and phenol oxidase activities during PRM coincided with elevated sediment-associated metal concentrations and higher dissolved oxygen deficits (Jaiswal and Pandey, 2019). It is showing that enzymes are being blocked by both metal stress and a lack of oxygen. The statistically significant and coordinated rise in hydrolytic and oxidative enzymes after the monsoon shows that the microbial functional capacity is recovering across the board, mostly because of seasonal hydrological forcing and reoxygenation, not just changes in metal exposure.

Conclusion

This research illustrates that monsoon-induced hydrological forces significantly regulate heavy metal distribution, oxygen dynamics and microbial activity within the interconnected riverine interfaces of the middle stretch of the Ganga River. Pre-monsoon conditions favored strong sequestration of metals within benthic sediments, accompanied by reduced oxygen availability, elevated microbial metabolic stress and suppressed extracellular enzyme activity. In contrast, post-monsoon hydrology caused a clear change in how metals were divided, moving them from watershed and sediments to the water column and at the same time increasing the amount of oxygen and organic matter that came into the system. Even though there was more metal exposure in more bioavailable compartments after the monsoon, microbial communities had more biomass, better respiratory potential, better metabolic efficiency, and higher enzymatic activity. These responses show that hydrologically mediated oxygen recovery and substrate availability can help microbes work better even when metals are present. This can cause a seasonal change from carbon stabilization during pre-monsoon stress to more efficient carbon turnover during the post-monsoon period.

In general, our results show that the ecological effects of metal pollution in large tropical rivers cannot be understood without taking into account seasonal hydrology. Monsoon-driven interface pumping synchronizes metal mobility, oxygen dynamics and microbial metabolism, resulting in alternating phases of biogeochemical constraint and recovery. It is important to understand how these two processes work together in order to predict how metals will move, how carbon will cycle, and how ecosystems will be able to adapt in the Ganga River and other rivers that are affected by the monsoon.

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