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HEAVY METAL POLLUTION PROMOTES COMPETITIVE SUCCESS OF INVASIVE SPECIES ELIMINATING SEEDBANKS OF SOFT CARBON RESOURCES IN THE GANGA RIVER CORRIDOR

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ABSTRACT

Anthropogenic perturbations affect ecosystem functional attributes and, when coupled with species invasion, exacerbate the changes including soil properties and competitive interactions among species. Here, we investigated whether invasive plant species under metal stress condition eliminates seedbanks of soft carbon resources along a 518 km stretch of the Ganga River corridor. Soil samples with natural herbaceous cover (non-infested) showed significant difference ($p<0.001$) in terms of two carbon hydrolyzing enzymes - β-D-glucosidase and FDAase, as opposed with soils rich in C residues from *Croton bonplandianus* and *Grangea maderaspatana*. Such soil functional responses were associated with high C:N ratio and concentration of phenolics (180-240 μg g^{-1}). Rising temperature and pulsed release of nutrients during summer favoured the growth of these species negating the potential effects of high concentrations of heavy metals present in soil. Understanding such indicators of ecosystem health in river corridors will allow prediction of shifts in carbon and nutrient biogeochemical cycles.

Key words : Ganga River, Heavy metal, Invasion, River corridors, Soft carbon resources.

Introduction

Anthropogenic activities constrain ecosystem functions by altering soil attributes, re-structuring vegetation composition and competitive interactions among species (Jian *et al.*, 2016 and Zhang *et al.*, 2020). Mid-reaches of the Ganga River have been dramatically modified by human activities including urban-industrial release of metal pollutants (Jaiswal and Pandey, 2019a; 2019b). River corridors act as a transition zone between the main land and the river course. Such transition zones are ecologically important because of their rich biodiversity and the role they play in land-water intersecting carbon and nutrients biogeochemical cycles (Wymore *et al.*, 2023). Also, during summer low flow, the dynamic areas of river corridors provide shelter for a variety of insect pollinators, small frugivores, grazers and seed predators being the important components of food web (Zhang *et al.*, 2023). Unfortunately, along with the pollution of the main channel, river corridors have also been contaminated by human-borne pollutants including heavy metals (Jaiswal and Pandey, 2019).

Invasion by species non-native to that region reduces seedling emergence and affects biodiversity of natural communities (Shi *et al.*, 2021). Because of their multiple environmental concern, invasive plant species and ecosystem modifications thereof have been a subject of intensive studies (Kohli *et al.*, 2006). Various chemicals involve in their allelopathic success, including those released from plant residues, root exudation and leaching from aerial parts have been well identified (Khattak *et al.*, 2024). In addition, invasive species embark fast growth, high reproductive output, huge root system, aggressiveness and tolerance to abiotic stressors. These attributes ensure competitive success of invasive plant species pressing their dominance even in polluted landscapes. The details of how such species respond in suppressing competitors when coupled with metal pollutants is still an enigma and remain a subject of debate. For example, high concentrations of heavy metals have been shown to be toxic and compromise the competitive potential of susceptible herbaceous plants (Sanjosé *et al*., 2021). But whether invasive plant species when exposed to metal stressors have a similar role eliminating seedbanks of soft carbon resources in the Ganga River corridors remains an important question. Understanding such indicators of ecosystem health of the river corridors may allow accurate prediction of shifting carbon and nutrient biogeochemical cycles under natural and anthropogenic controls; drawing mounting attention to policy makers.

Materials and Methods

The data reported here are the results of two consecutive years of studies along 518 km mid-stretch of the Ganga River corridor. The climate is tropical experiencing average rainfall over 1050 mm, major part of which is received between late June to early September when river shows a massive increase in water volume and flooding. The period between second half of the March to first half of the June represents a dry low flow season when water volume shrinks and pollutants concentrate. The Gangetic alluvium and sand-beds are nutrient rich and highly fertile.

We selected four study sites: Jajmau (Site I; situated Kanpur downstream, a metal polluted site); Sangam (Site II; situated at Prayagraj with mixed effects); Shooltankeshwar (Site III; situated upstream Varanasi city, least polluted) and Rajghat (Site IV, situated along Varanasi urban with mixed effects). We choose *Croton bonplandianus* (Family-Euphorbiaceae) and *Grangea maderaspatana* (Family- Asteraceae), occupying large patches in the river corridor (Fig. 1), as model species, because these species grow aggressively even in metal rich sand beds. Such patches have been compared with species growing in non-infested site locations.

Soil samples (0 to 10 cm depth, top soil) in triplicates were collected randomly from naturally weed-infested and non-infested site locations selected in the corridor along the mid-reaches of the river. Soil samples, collected from respective sub-sites were pooled to form composite samples. All samples were separated into two sets: with the first set, seed germination tests were performed. For this purpose, soil soaked in distilled water (85% saturation) was filled in earthen pots (2 kg soil in each pot); seedling emergence and biomass accrual recorded. The second set was processed following standard experimental protocol and used for carbon, nutrient and heavy metal analysis.

Total organic carbon (TOC) in soil was quantified following Walkley and Black (Chen *et al*., 2015); total nitrogen (TN) following Kjeldahl method and total phosphorous (TP) following the protocol of Jackson (Jackson, 1958). Microbial biomass-C (C_{mic}) was estimated following chloroform fumigation method (Jenkinson, and Powlson, 1976). Total microbial activity was estimated in terms of fluorescein diacetate hydrolytic bioassay (FDAase). Samples were incubated with fluorescein diacetate and fluorescein formed was measured following Schnurer and Rosswal (Schnurer and Rosswal, 1982). The β-D-glucosidase was assayed in terms of p-nitrophenol produced when samples incubated with p-nitrophenol- β-D-glucoside (Eivazi and Tabatabai, 1988). For metals, sample were digested in acid mixture using a microwave digestion system and analyzed in Perkin Elmer (Analyst 800, USA) atomic absorption spectrophotometer. Total phenolics in plant residues was measured using Folin-Ciocalteau reagent. The data were subjected to analysis of variance (ANOVA) to justify significant differences among chosen determinants.

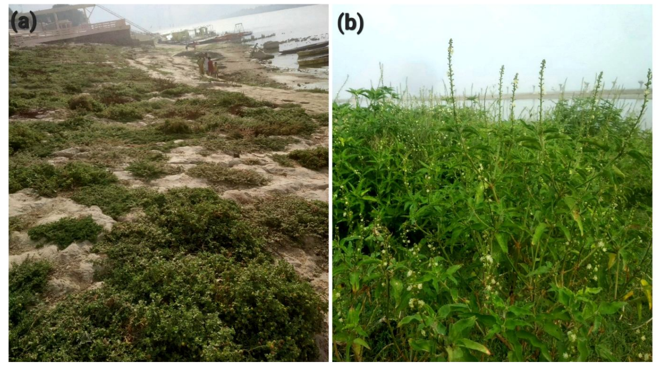

Fig. 1 : Aggressive growth of (a) *Grangea maderaspatana* (b) *Croton bonplandianus* in the Ganga River corridor between Kanpur and Varanasi.

Results and Discussion

Here, we consider soil with plant residues which impart potentially high activities of carbon (C) hydrolyzing enzymes as "soft-C" resources. Soil samples with herbaceous cover (non-infested) showed significant difference ($p<0.001$) in terms of two carbon hydrolyzing enzymes (β-D-glucosidase and FDAase) as opposed with soils rich in C residues from *C. bonplandianus* and *G. maderaspatana* (Table 1). Such soil microbial responses are probably due to high C:N ratio and concentration of phenolics (180-240 µg g^{-1}) in the latter. The enzyme β-D-glucosidase is used to address C-acquisition whereas FDAase is used as a signature of overall microbial activities (Jaiswal and Pandey, 2021).

We initiated this research to observe the effect of invasive species on seedbanks of soft carbon resources. Under field condition, the invasive species (*Croton bonplandianus* and *Grangea maderaspatana*) and

Table 1 : Soil characteristics at weed-infested (I) and non-infested (NI) locations at selected sites in the Ganga River corridor.

Parameter	Site							
	I		II		III		IV	
	I	NI	I	NI	I	NI	I	NI
Total organic carbon (%)	5.12±0.38	4.80±0.35	4.75±0.31	4.20±0.26	2.64±0.16	1.80±0.10	4.53±0.36	4.02±0.30
Total nitrogen (%)	0.65±0.04	0.75±0.05	0.60±0.03	0.72±0.05	0.32±0.03	0.24±0.05	0.62±0.04	0.71±0.05
Total phosphorous (mg g^{-1})	0.30±0.02	0.38±0.02	0.28±0.02	0.36±0.02	0.10±0.01	0.14±0.03	0.31±0.03	0.40±0.04
C:N ratio	7.87±0.53	6.40±0.44	7.91±0.47	5.83±0.30	8.25±0.65	7.50±0.60	7.53±0.66	5.63±0.31
Microbial biomass carbon(µg g^{-1})	200.50± 16.50	250.90± 21.50	520.50± 38.50	630.30± 59.50	360.20± 28.40	450.40± 36.45	470.50± 38.67	560.20± 43.50
β-D-glucosidase (µg p-NP released g^{-1} hr^{-1})	42.30±3.15	50.20±4.31	310.80± 27.90	350.20± 36.20	190.50± 13.60	225.20± 20.28	65.10±4.75	74.40±6.15
FDAase(µm fluorescein g^{-1} hr^{-1})	34.30±2.60	42.60±3.20	94.50±6.95	110.10± 10.50	88.50±7.55	104.10± 9.50	45.10±3.77	56.60±4.37
Zn (µg g^{-1})	162. 60± 11.20	170.20± 11. 50	73.30±6.10	82.20± 7.80	42.50±3.87	50.40±4.15	114.50± 9.67	122.50± 10.20
Pb (µg g^{-1})	65.30±5.33	72.10±5.77	34.50±2.40	40.40±3.65	14.50±1.05	17.70±1.32	44.30±4.60	52.60±4.15
Ni (µg g^{-1})	77.90±5.85	86.70±6.18	32.60±2.51	37.90±2.86	14.70±1.05	20.90±1.75	47.20±3.85	55.60±4.67
Cu (µg g^{-1})	74.60±5.90	82.70±7.50	34.60±2.61	41.10±3.25	13.60±0.92	18.90±1.78	54.10±3.96	62.80±5.58
Cr (µg g^{-1})	190.40± 16.20	210.10± 17.62	68.70±4.29	77.20±6.58	44.80±3.15	52.40±4.67	112.30± 9.25	155.30± 11.21
Cd (µg g^{-1})	0.39±0.02	0.46±0.03	0.26±0.02	0.30±0.03	0.18±0.01	0.22±0.02	0.30±0.03	0.34±0.03
Σ HM (µg g^{-1})	571.19	622.26	243.96	279.10	130.28	160.52	382.70	449.14

Values are mean (n=6) ±SD

metal pollution reduced the seedbanks and the effects were more pronounced where both the stressors occurred together. Because the limit of concentration of pooled heavy metals (ΣHM) that inhibited the growth of common species measured here did not appear to negate the growth of invasive species, our study indicates a new model highlighting the role of metal pollution promoting the competitive success of *Croton bonplandianus* and *Grangea maderaspatana*. In support of this, we conducted controlled studies with soil collected separately from infested and non-infested sites with variable concentration of total heavy metals. Consistent with the field results, we observed significantly ($p<0.001$) poor potential of seed germination and biomass accrual in seedlings growing in soils collected from weed infected sites and the effects were more pronounced for soils collected from metal polluted-weed infested sites.

Infestation-free soils with low concentration of ÓHM produced seedlings of multiple species (*Ageratum conyzoides*, *Amaranthus viridis*, *Convolvulus prosrtatus*, *Dichanthium annulatum*, *Eclipta alba*, *Euphorbia hirta*, *Evolvulus alsinoides*, *Heliotropium indicum*, *Oxalis corniculata*, *Solanum nigrum* and many other) with faster relative biomass accrual. Infested soils showed seedling emergence of *Amaranthus spinosus* and *Lippia nodiflora* alongwith the test weeds. We found 3 to 5 times rapid relative biomass accrual in seedlings of invasive test species even at metal polluted sites (0.09-0.15 gg^{-1} day^{-1} in invasive weeds compared to 0.015-0.05 gg^{-1} day^{-1} in common species). These observations indicate that the emerging ecosystem shifts, embarking with invasive species, will function as negative regulator of seedbank and heavy metal pollution will promote the process.

Conclusion

Evidently, capitalizing upon nutrient rich alluvium, *C. bonplandianus* and *G. maderaspatana* are able to maintain aggressive growth outcompeting the slow growing poor herbaceous species. Rising temperature and pulsed release of nutrients during summer (Singh *et al.*, 1989) will favour flushes of growth resulting higher accumulation of biomass, negating potential effects of high concentrations of heavy metals in the root environment. Furthermore, because rising CO_2-driven metabolic changes support the success of invasive weeds (Ode *et al*., 2014), the CO_2-fertilization (Rice *et al*., 2021) and high concentration of nutrients (Pandey *et al*., 2014) as expected in future, would still cause more rapid and aggressive growth potentially contributing to their invasive success. Elimination of natural cover and constrained recruitments of herbaceous plants through germination will lead to a mismatch with insect pollinators (thus, reduced pollen donation), small frugivores, grazers and seed predators.

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