

Supplementary material

Title: Biochemical responses of oysters in evaluating environmental quality of tropical Indian estuarine systems

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Table SM1: Post hoc Dunnett's multiple comparison (2 sided) for indigenous bacteria and coliforms during pre-monsoon (PM), monsoon (M) and post-monsoon (pM) seasons. The Chapora estuary (#S1) was compared with the Nerul (#S2), Zuari (#S3) and the Sal (#S4) estuaries. Mean difference is significant at $p < 0.05$ level.

Dependent Variable	(I) Locations	(J) Locations	p (PM)	p (M)	p (pM)
SalW	#S2	#S1	0.97	0.96	0.99
	#S3		0.06	0.06	0.000
	#S4		0.44	0.44	0.03
SalS	#S2	#S1	0.02	0.02	0.29
	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.001
SalO	#S2	#S1	0.06	0.06	0.000
	#S3		0.000	0.000	0.000
	#S4		0.01	0.01	0.000
VibW	#S2	#S1	0.97	0.97	0.27
	#S3		0.001	0.001	0.000
	#S4		0.15	0.15	0.005
VibS	#S2	#S1	0.44	0.44	0.27
	#S3		0.000	0.000	0.000
	#S4		0.001	0.001	0.000
VibO	#S2	#S1	0.14	0.14	0.25
	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.000
AeroW	#S2	#S1	0.99	0.99	0.98
	#S3		0.01	0.01	0.001
	#S4		0.18	0.18	0.02
AeroS	#S2	#S1	0.73	0.73	0.96
	#S3		0.000	0.000	0.000
	#S4		0.02	0.02	0.000
AeroO	#S2	#S1	0.44	0.44	0.04
	#S3		0.000	0.000	0.000
	#S4		0.004	0.004	0.000
CbotS	#S2	#S1	0.62	0.62	0.04

	#S3		0.01	0.01	0.000
	#S4		0.29	0.29	0.001
	#S2	#S1	0.04	0.04	0.003
TCW	#S3		0.000	0.000	0.001
	#S4		0.000	0.000	0.000
	#S2	#S1	0.01	0.006	0.001
TCS	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.000
	#S2	#S1	0.02	0.02	0.04
TCO	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.000
	#S2	#S1	0.11	0.11	0.05
FCW	#S3		0.002	0.002	0.001
	#S4		0.000	0.000	0.000
	#S2	#S1	0.04	0.04	0.01
FCS	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.000
	#S2	#S1	0.12	0.12	0.35
FCO	#S3		0.001	0.001	0.003
	#S4		0.000	0.000	0.000

Table SM2: Post hoc Dunnett's multiple comparison (2 sided) for toxic metals (Cd, Pb and Hg) during pre-monsoon (PM), monsoon (M) and post-monsoon (pM) seasons. The Chapora estuary (#S1) was compared with the Nerul (#S2), Zuari (#S3) and the Sal (#S4) estuaries. Mean difference is significant at $p < 0.05$ level.

Dependent Variable	(I) Locations	(J) Locations	p (PM)	p (M)	p (pM)
CdS	#S2	#S1	0.78	0.75	0.89
	#S3		0.4	0.4	0.49
	#S4		0.000	0.000	0.000
CdO	#S2	#S1	0.03	0.03	0.91
	#S3		0.001	0.001	0.91
	#S4		0.000	0.000	0.000
PbS	#S2	#S1	0.23	0.23	0.28
	#S3		0.000	0.000	0.000
	#S4		0.002	0.002	0.01
PbO	#S2	#S1	0.99	0.99	0.99
	#S3		0.000	0.000	0.000
	#S4		0.4	0.4	0.75
HgS	#S2	#S1	0.13	0.13	0.12
	#S3		0.83	0.83	0.77
	#S4		0.000	0.000	0.001
HgO	#S2	#S1	0.34	0.34	0.31
	#S3		0.16	0.16	0.28
	#S4		0.000	0.000	0.000

Table SM3: Post hoc Dunnett's multiple comparison (2 sided) for antioxidant enzymes (CAT, SOD, GST and MT) level during pre-monsoon (PM), monsoon (M) and post-monsoon (pM) seasons. The Chapora estuary (#S1) was compared with the Nerul (#S2), Zuari (#S3) and the Sal (#S4) estuaries. Mean difference is significant at $p < 0.05$ level.

Dependent Variable	(I) Locations	(J) Locations	p (PM)	p (M)	p (pM)
CAT	#S2	#S1	0.99	0.99	1.0
	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.000
SOD	#S2	#S1	0.94	0.91	0.21
	#S3		0.000	0.000	0.005
	#S4		0.001	0.000	0.004
GST	#S2	#S1	0.19	0.19	0.47
	#S3		0.000	0.000	0.000
	#S4		.000	0.000	0.000
MT	#S2	#S1	0.000	0.000	0.008
	#S3		0.000	0.000	0.000
	#S4		0.000	0.000	0.000

Table SM4: Multiple regression model for predicting stress in the oysters using factor scores obtained from principal components analysis ($p < 0.05$).

Dependent variable	Predictors	Standardized Coefficients (β)	t-value	Significance (p)	R^2	Adjusted R^2
CAT	Component 1	0.31	7.92	<0.001	0.95	0.95
	Component 2	0.85	21.98	<0.0001		
	Component 3	0.36	9.3	<0.001		
	Component 4	0.01	0.23	0.82		
SOD	Component 1	0.34	10.03	<0.001	0.96	0.96
	Component 2	0.63	18.47	<0.0001		
	Component 3	0.59	17.52	<0.0001		
	Component 4	-0.32	-9.48	<0.001		
GST	Component 1	0.5	7.89	<0.001	0.87	0.86
	Component 2	0.75	11.74	<0.0001		

	Component 3	0.19	3.03	0.005		
	Component 4	0.18	2.78	0.009		
	Component 1	0.02	0.67	0.51		
MT	Component 2	0.62	17.08	<0.0001	0.96	0.95
	Component 3	0.35	9.69	<0.001		
	Component 4	-0.67	-18.42	<0.0001		

Table SM5: Spatio-temporal variations among indigenous bacteria analyzed through one-way ANOVA at different tropical estuaries along Goa coast and their significance ($p < 0.05$).

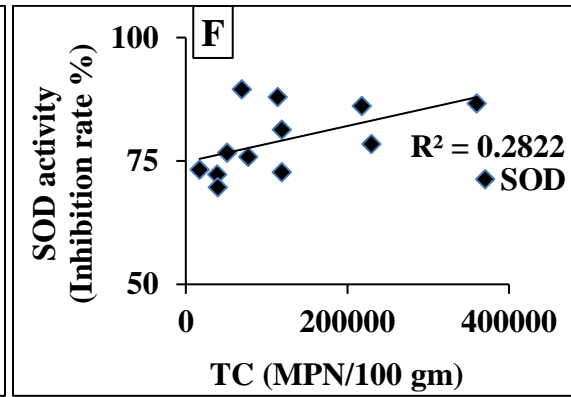
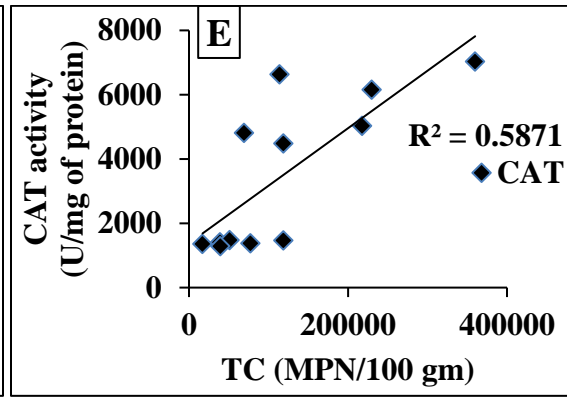
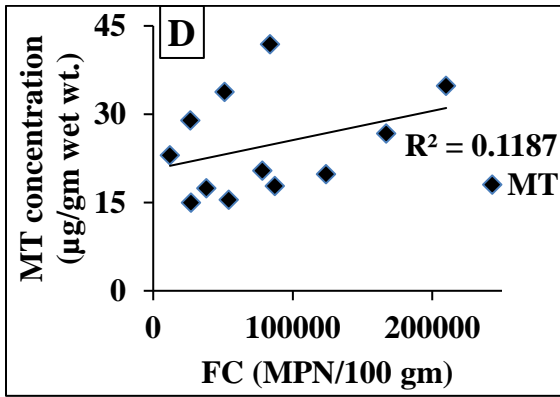
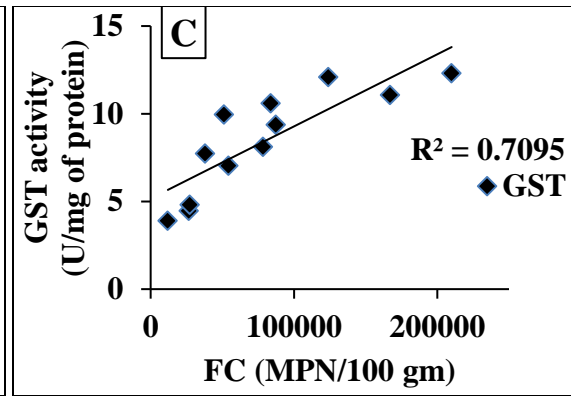
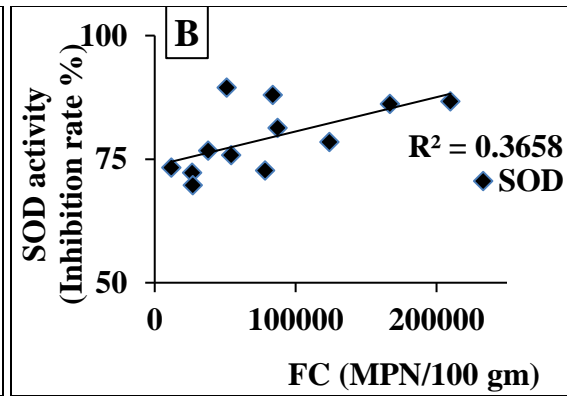
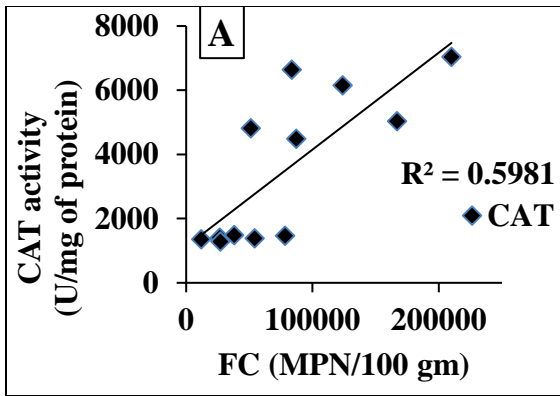
	F value	<i>p</i>
SalW	33.73	<0.001
SalS	126.13	<0.001
SalO	197.32	<0.001
VibW	42.12	<0.001
VibS	202.25	<0.001
VibO	232.7	<0.001
AeroW	25.13	<0.001
AeroS	216.36	<0.001
AeroO	67.98	<0.001
CbotS	64.84	<0.001
TCW	93.66	<0.001
TCS	74.66	<0.001
TCO	80.19	<0.001
FCW	44.4	<0.001
FCS	43.9	<0.001
FCO	21.84	<0.001

Table SM6: Temporal and spatial variations among toxic metals in oyster tissue and sediment analyzed through one-way ANOVA at different study locations and their significance ($p < 0.05$).

	F values	<i>p</i>
CdS	377.17	<0.001
CdO	88.78	<0.001
PbS	392.18	<0.001
PbO	329.79	<0.001
HgS	15.18	<0.001
HgO	30.83	<0.001

Table SM7: Variation in antioxidant enzymes (SOD, CAT, GST and MT) level across the study **locations** and their significance ($p < 0.05$), analyzed by one-way ANOVA.

	F values	<i>p</i>
CAT	150.82	<0.001
SOD	23.07	<0.001
GST	222.52	<0.001
MT	369.42	<0.001



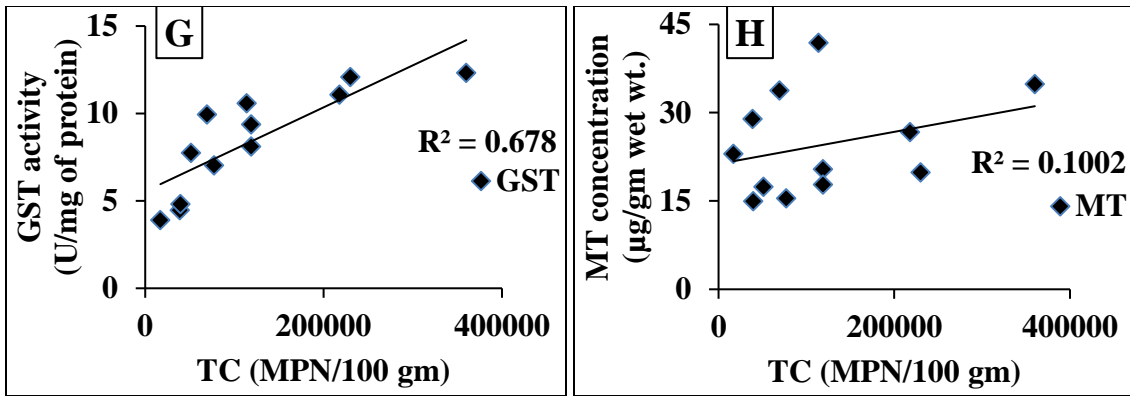
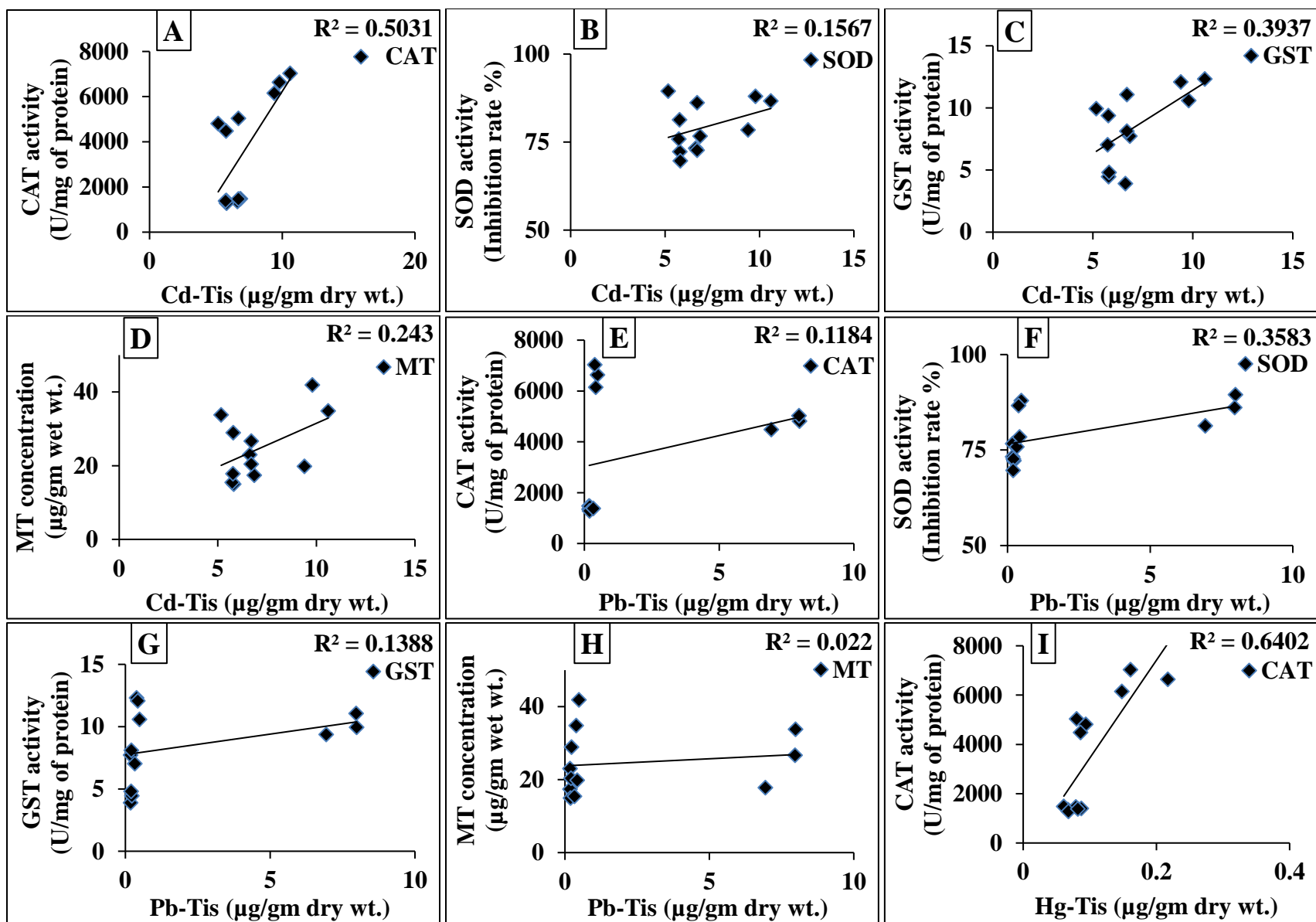


Fig. SM1- Regression of antioxidant enzymes levels (CAT, SOD, GST, and MT) on fecal (A, B, C, and D) and total coliforms (E, F, G, and H) linearly increased, suggesting stress in oyster.



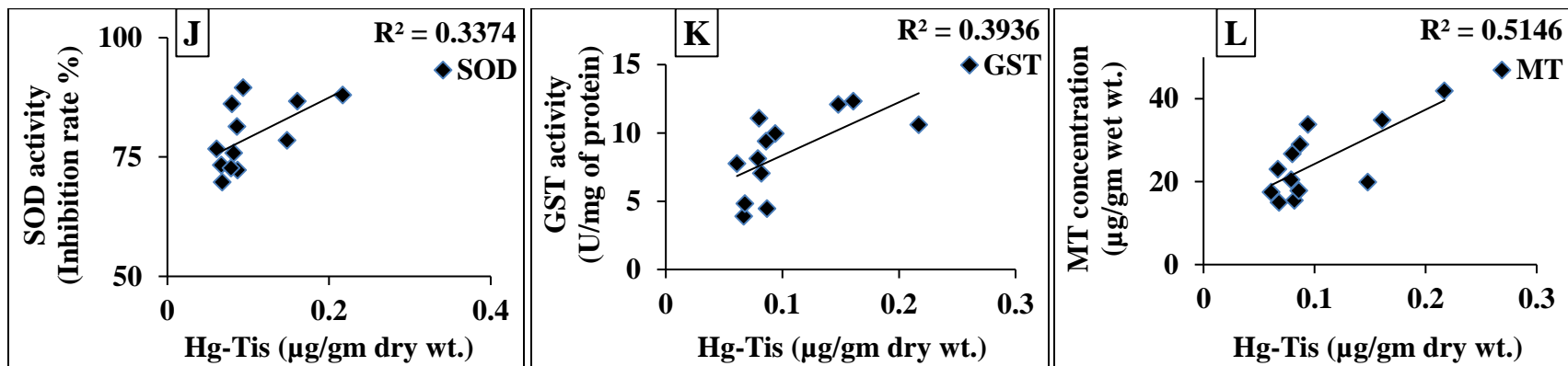


Fig. SM2- Regression of antioxidant enzymes levels (CAT, SOD, GST, and MT) on concentration of Cd (A, B, C, and D), Pb (E, F, G and H), and Hg (I, J, K and L) in the oyster tissue linearly increased, suggesting oxidative stress in the oysters.