

Supplementary Materials

Site-specific response of sediment microbial community to supplementation of polyhydroxyalkanoates as biostimulating agents

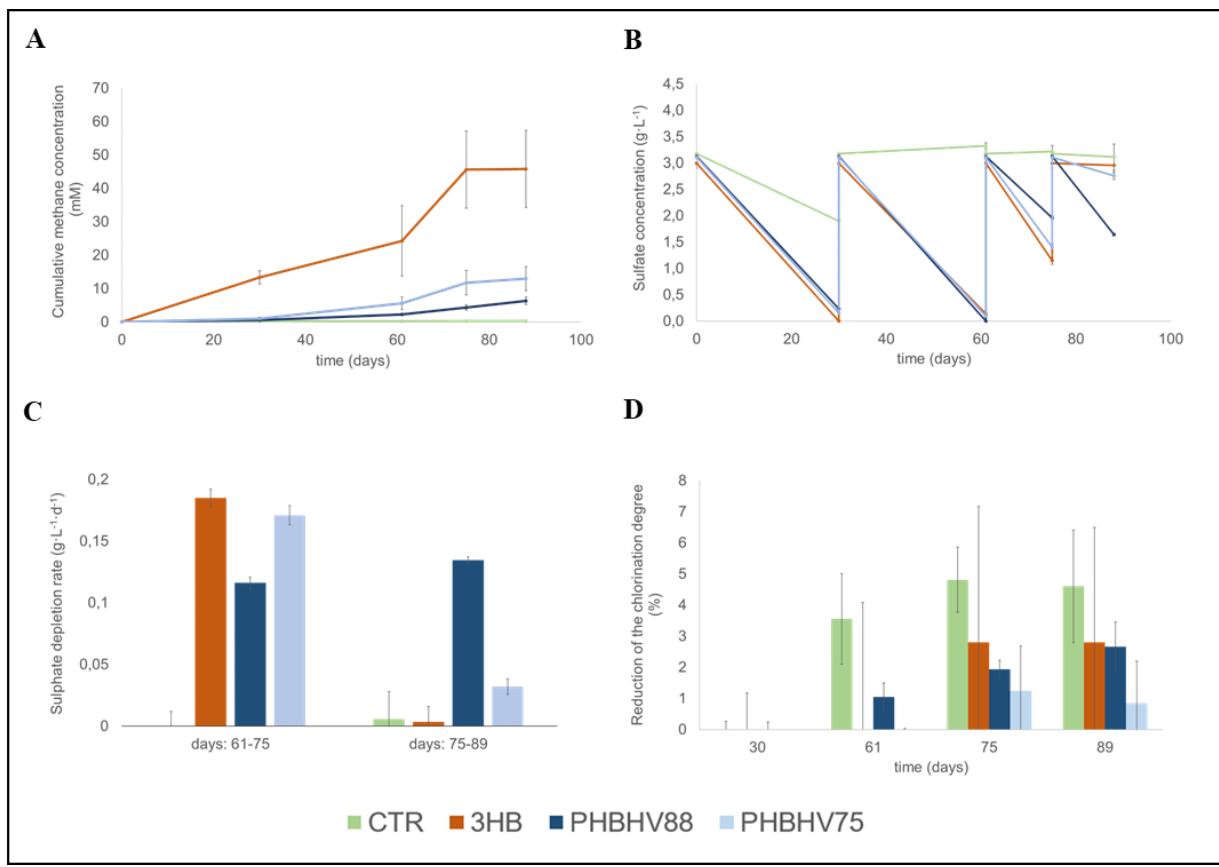
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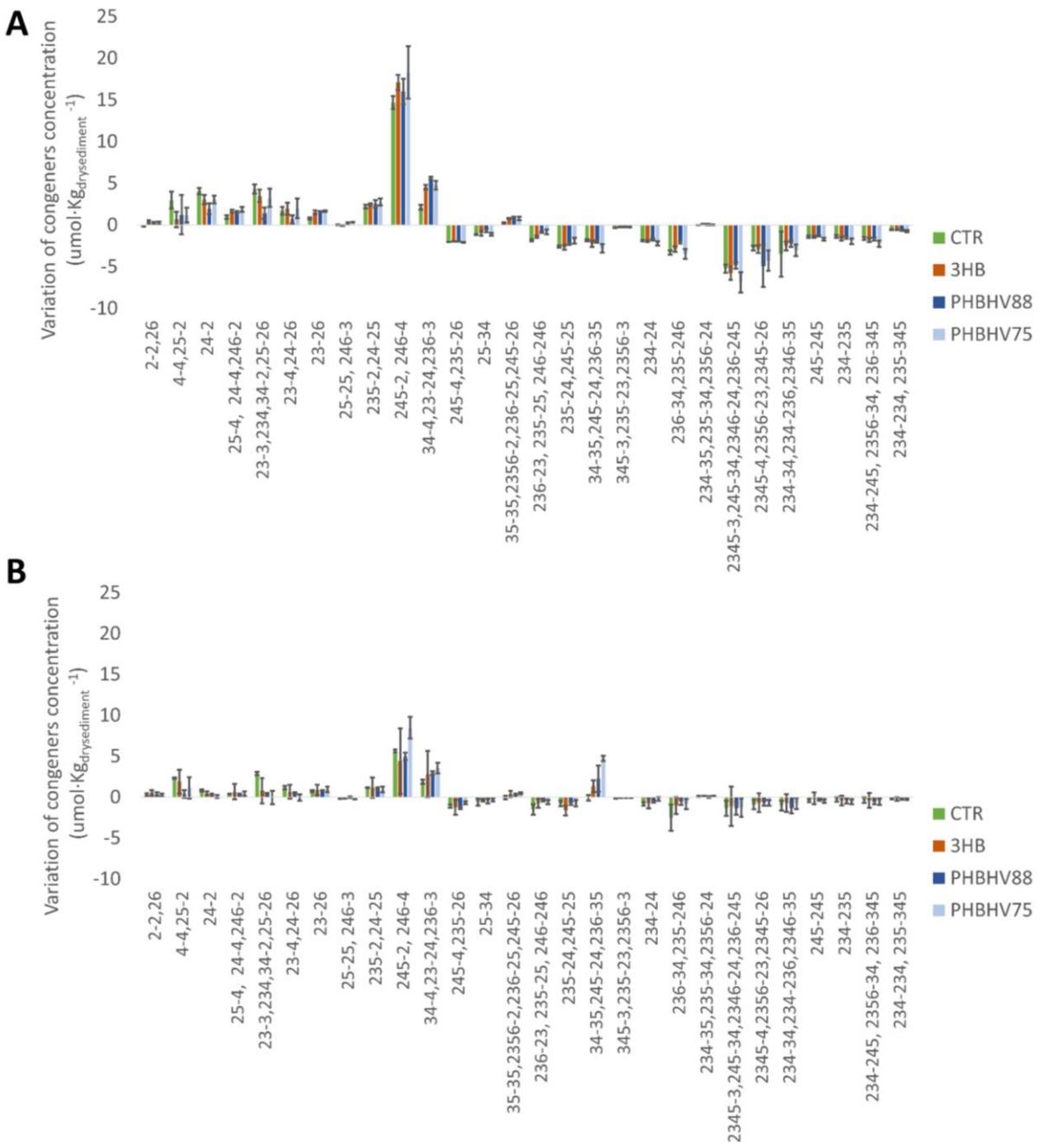
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Supplementary Figures



Supplementary Figure S1. Assessment of anaerobic metabolisms in microcosms assembled using sediments from Mar Piccolo, Taranto. **(A)** Cumulative methane concentration (mM) measured in the microcosms' headspace gas. **(B)** Profile in time of the sulfate concentrations in the aqueous phase. Sulfates were replenished on days 30, 61 and 75. **(C)** Sulfate depletion rates measured in microcosms between 61-75 days and 75-89 days of incubation. **(D)** Percentage of reduction of the chlorination degree of the PCB mixture during incubation. For all panels, values were measured in unamended microcosms (CTR, green) and in those amended with monomer (3HB, orange) and PHAs (PHBV75 and PHBV88, in light and dark blue, respectively) and are expressed as mean value \pm standard deviation. Color legend for the four panels is displayed at the bottom.



Supplementary Figure S2. Variations of the concentration of PCBs congeners in the studied sediments.
(A) Variation of the concentration of PCBs congeners for the microcosms containing sediment from Pialassa della Baiona, Ravenna **(B)** Variation of the concentration of PCBs congeners for the microcosms containing sediment from Mar Piccolo, Taranto. For all panels, values were measured in unamended microcosms (CTR, green) and in those amended with monomer (3HB, orange) and PHAs (PHBV75 and PHBV88, in light and dark blue, respectively) and are expressed as difference between the initial (0 days) and final (89 days) concentration of the congeners or mixture of congeners. Color legend for the four panels is displayed at the right.

Supplementary Tables

Supplementary Table S1. Summary of the main chemical-physical parameters reported in the literature for the studied sediments.

Reference	pH	TOC (%)	TN (%)	Sand (%) ^e	Mud (Clay and Silt) (%) ^f	Permeability (k) m·s ⁻¹
Pialassa della Baiona (Ravenna)						
Fabbri et al., 2006		10-25 ^c			44.3 ^a	
Guerra et al., 2009		4-16 ^c			16-81	
Ponti et al., 2009				12-90		
Ponti et al., 2011		3.5-2.5 ^c		12-90		
Guerra, 2012		2.81 ^{a, d}			41 ^a	
Guerra et al., 2013		0.76-3.45 ^d	0.1-0.48			
Guerra et al., 2014		5.4 ^c			33	
Borghesi et al., 2016		5.2 ^c				
Sfriso et al., 2020	7.4	1.2 ^d				
Guerra et al., 2022		2.3 ^d				
Mar Piccolo (Taranto)						
Quero et al., 2015		20.1 ^{a, c}		29 ^a	70 ^a	
Vitone et al., 2016				23 ^a	76 ^a	10 ^{-12-10⁻⁸}
Di Leo et al., 2016	7.87	4.06 ^{,d}		10	89.9	
Mali et al., 2017	7.26	2.57 ^b		19.5	77	
Todaro et al., 2019		16.7 ^{a, c}				
Sollecito et al., 2019		9-18 ^c				
Todaro et al., 2020		21.3 ^{a, c}		8.5 ^a	91.5 ^a (< 75 µm)	
Mali et al., 2020		2.5 ^d	0.22	19.5	77	
Sollecito et al., 2021				9 ^a	90 ^a (< 75 µm)	10 ⁻¹⁰⁻¹
Dominik et al., 2023		2.3 ^d	0.2	10	90	

^a value presented as average of the data reported in the article

^b method used to determine TOC is not specified

^c TOC determined via EPA 160.4 as loss of ignition (LOI)

^d TOC determined with elemental analyzer

^e particles diameter 2 mm – 63 µm

^f particles diameter < 63 µm

Supplementary Table S2. Summary of the concentrations of heavy metals and polycyclic aromatic hydrocarbons (PAH) reported in the literature for the studied sediments, expressed as mg·Kg_{dry sediment}⁻¹.

Reference	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	V	Zn	PAH
Pialassa della Baiona (Ravenna)													
Ponti et al., 2011							0.1-41						
Guerra, 2012	46300			160	47	24700	46				270		14 ^a
Guerra et al., 2014				84	38	21580	4	428	43			123	
Borghesi et al., 2016	13257	7	0.4		47	22943	5	546	51	21	31	141	
Pignotti et al., 2018					42		2		71		104	167	
Mar Piccolo (Taranto)													
Quero et al., 2015	24660 ^a	14 ^a	0.4 ^a	57 ^a	93 ^a	28029 ^a	3.9 ^a	350 ^a	41 ^a	84 ^a		261 ^a	1.8 ^a
Bellucci et al., 2016	26636 ^a	15 ^a	0.5 ^a	61 ^a	100 ^a	30156 ^a	4.2 ^a	366 ^a	44 ^a	89 ^a		281 ^a	1.8 ^a
Mali et al., 2017	31938	13	0.4	56	48	28823	2.5		50	63	56	168	1.1
Todaro et al., 2019		12	0.4		40		3.8		39	63	52	94	
Mali et al., 2020	31901	10	0.3	56	34	28791	0.9		47	47	56	2	
Todaro et al., 2020		44 ^a	1 ^a	75 ^a	124 ^a		14 ^a		51 ^a	247 ^a	95 ^a	440 ^a	5.1 ^a
Cotecchia et al., 2021													3.9 ^a

^a value presented as average of the data reported in the article.

Supplementary Table S3. Metabolic activities (methane production, sulfate depletion rate, and percentage of reduction of the chlorination degree of the PCB mixture) measured in microcosms assembled using sediments from Pialassa della Baiona, Ravenna (PB) (Botti et al., 2023) and Mar Piccolo, Taranto (MP) (this study).

	Methane production ^a (mM, mean ± standard deviation)		Sulfate depletion rate ^b (g·L ⁻¹ ·d ⁻¹ , mean ± standard deviation)		Reduction of the chlorination degree ^a (%, mean ± standard deviation)	
	PB	MP	PB	MP	PB	MP
CTR	0.3 ± 0.1	0.3 ± 0.2	0.00 ± 0.00	0.01 ± 0.02	14.4 ± 1.7	4.6 ± 1.8
3HB	53.1 ± 7.9	31.0 ± 16.4	0.11 ± 0.12	0.10 ± 0.10	14.0 ± 2.0	3.3 ± 2.9
PHBV88	14.0 ± 6.1	6.4 ± 1.9	0.12 ± 0.06	0.13 ± 0.01	12.4 ± 1.1	2.7 ± 0.8
PHBV75	16.4 ± 3.9	13.0 ± 6.3	0.11 ± 0.05	0.10 ± 0.08	15.4 ± 2.3	1.0 ± 1.2

^a measured at the end of the experiment (89 days)

^b monitored during the third month of the experiment

Supplementary References

- Bellucci, L.G., Cassin, D., Giuliani, S., Botter, M., Zonta, R., 2016. Sediment pollution and dynamic in the Mar Piccolo of Taranto (southern Italy): insights from bottom sediment traps and surficial sediments. *Environ. Sci. Pollut. Res.* 23, 12554–12565. <https://doi.org/10.1007/s11356-016-6738-6>
- Borghesi, F., Migani, F., Dinelli, E., 2016. Geochemical characterization of surface sediments from the northern Adriatic wetlands around the Po River delta. Part II: aqua regia results. *J. Geochemical Explor.* 169, 13–29. <https://doi.org/10.1016/j.gexplo.2016.06.012>
- Cotecchia, F., Vitone, C., Sollecito, F., Mali, M., Miccoli, D., Petti, R., Milella, D., Ruggieri, G., Bottiglieri, O., Santaloia, F., De Bellis, P., Cafaro, F., Notarnicola, M., Todaro, F., Adamo, F., Di Nisio, A., Lanzolla, A.M.L., Spadavecchia, M., Moretti, M., Agrosì, G., De Giosa, F., Fago, P., Lacalamita, M., Lisco, S., Manzari, P., Mesto, E., Romano, G., Scardino, G., Schingaro, E., Siniscalchi, A., Tempesta, G., Valenzano, E., Mastronuzzi, G., Cardellicchio, N., Di Leo, A., Spada, L., Giandomenico, S., Calò, M., Uricchio, V.F., Mascolo, G., Bagnuolo, G., Ciannarella, R., Tursi, A., Cipriano, G., Cotugno, P., Sion, L., Carlucci, R., Capasso, G., De Chiara, G., Pisciotta, G., Velardo, R., Corbelli, V., 2021. A geo-chemo-mechanical study of a highly polluted marine system (Taranto, Italy) for the enhancement of the conceptual site model. *Sci. Rep.* 11, 1–26. <https://doi.org/10.1038/s41598-021-82879-w>
- Di Leo, A., Annicchiarico, C., Cardellicchio, N., Cibic, T., Comici, C., Giandomenico, S., Spada, L., 2016. Mobilization of trace metals and PCBs from contaminated marine sediments of the Mar Piccolo in Taranto during simulated resuspension experiment. *Environ. Sci. Pollut. Res.* 23, 12777–12790. <https://doi.org/10.1007/s11356-015-5472-9>
- Dominik, J., Leoni, S., Cassin, D., Guarneri, I., Bellucci, L.G., Zonta, R., 2023. Eutrophication history and organic carbon burial rate recorded in sediment cores from the Mar Piccolo of Taranto (Italy). *Environ. Sci. Pollut. Res.* 56713–56730. <https://doi.org/10.1007/s11356-023-26317-6>
- Fabbri, D., Baravelli, V., Giannotti, K., Donnini, F., Fabbri, E., 2006. Bioaccumulation of cyclopenta[cd]pyrene and benzo[ghi]fluoranthene by mussels transplanted in a coastal lagoon. *Chemosphere* 64, 1083–1092. <https://doi.org/10.1016/j.chemosphere.2005.11.071>
- Guerra, R., 2012. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and trace metals in sediments from a coastal lagoon (Northern Adriatic, Italy). *Water. Air. Soil Pollut.* 223, 85–98. <https://doi.org/10.1007/s11270-011-0841-6>
- Guerra, R., Pasteris, A., Lee, S. hyung, Park, N. jin, Ok, G., 2014. Spatial patterns of metals, PCDD/Fs, PCBs, PBDEs and chemical status of sediments from a coastal lagoon (Pialassa Baiona, NW Adriatic, Italy). *Mar. Pollut. Bull.* 89, 407–416. <https://doi.org/10.1016/j.marpolbul.2014.10.024>
- Guerra, R., Pasteris, A., Ponti, M., 2009. Impacts of maintenance channel dredging in a northern Adriatic coastal lagoon. I: Effects on sediment properties, contamination and toxicity. *Estuar. Coast. Shelf Sci.* 85, 134–142. <https://doi.org/10.1016/j.ecss.2009.05.021>
- Guerra, R., Pistocchi, R., Vanucci, S., 2013. Dynamics and sources of organic carbon in suspended particulate matter and sediments in Pialassa Baiona lagoon (NW Adriatic Sea, Italy). *Estuar. Coast. Shelf Sci.* 135, 24–32. <https://doi.org/10.1016/j.ecss.2013.06.022>
- Guerra, R., Simoncelli, S., Pasteris, A., 2022. Carbon accumulation and storage in a temperate coastal lagoon under the influence of recent climate change (Northwestern Adriatic Sea). *Reg. Stud. Mar. Sci.* 53, 102439. <https://doi.org/10.1016/j.rsma.2022.102439>

- Mali, M., Dell'Anna, M.M., Mastrorilli, P., Chiaia, G., Romanazzi, G., Damiani, L., 2020. Multivariate analyses for investigating highly polluted marine ecosystem: The case study of Mar Piccolo (Taranto, South Italy). *Aquat. Ecosyst. Heal. Manag.* 23, 436–444. <https://doi.org/10.1080/14634988.2020.1807224>
- Mali, M., Dell'Anna, M.M., Notarnicola, M., Damiani, L., Mastrorilli, P., 2017. Combining chemometric tools for assessing hazard sources and factors acting simultaneously in contaminated areas. Case study: “Mar Piccolo” Taranto (South Italy). *Chemosphere* 184, 784–794. <https://doi.org/10.1016/j.chemosphere.2017.06.028>
- Pignotti, E., Guerra, R., Covelli, S., Fabbri, E., Dinelli, E., 2018. Sediment quality assessment in a coastal lagoon (Ravenna, NE Italy) based on SEM-AVS and sequential extraction procedure. *Sci. Total Environ.* 635, 216–227. <https://doi.org/10.1016/j.scitotenv.2018.04.093>
- Ponti, M., Casselli, C., Abbiati, M., 2011. Anthropogenic disturbance and spatial heterogeneity of macrobenthic invertebrate assemblages in coastal lagoons: The study case of Pialassa Baiona (northern Adriatic Sea). *Helgol. Mar. Res.* 65, 25–42. <https://doi.org/10.1007/s10152-010-0197-0>
- Ponti, M., Pasteris, A., Guerra, R., Abbiati, M., 2009. Impacts of maintenance channel dredging in a northern Adriatic coastal lagoon. II: Effects on macrobenthic assemblages in channels and ponds. *Estuar. Coast. Shelf Sci.* 85, 143–150. <https://doi.org/10.1016/j.ecss.2009.06.027>
- Quero, G.M., Cassin, D., Botter, M., Perini, L., Luna, G.M., 2015. Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Front. Microbiol.* 6, 1–15. <https://doi.org/10.3389/fmicb.2015.01053>
- Sfriso, A., Buosi, A., Tomio, Y., Juhmani, A.S., Chiesa, S., Greco, M., Gazzola, C., Mistri, M., Munari, C., Sfriso, A.A., 2020. Sediment carbon variations in the venice lagoon and other transitional water systems of the northern adriatic sea. *Water (Switzerland)* 12, 1–13. <https://doi.org/10.3390/w12123430>
- Sollecito, F., Cotecchia, F., Mali, M., Miccoli, D., Vitone, C., 2019. Geo-chemo-mechanical characterization of a polluted marine basin. *E3S Web Conf.* 92. <https://doi.org/10.1051/e3sconf/20199218001>
- Todaro, F., de Gisi, S., Labianca, C., Notarnicola, M., 2019. Combined assessment of chemical and ecotoxicological data for the management of contaminated marine sediments. *Environ. Eng. Manag. J.* 18, 2287–2296.
- Todaro, F., De Gisi, S., Notarnicola, M., 2020. Contaminated marine sediment stabilization/solidification treatment with cement/lime: leaching behaviour investigation. *Environ. Sci. Pollut. Res.* 27, 21407–21415. <https://doi.org/10.1007/s11356-020-08562-1>
- Vitone, C., Federico, A., Puzrin, A.M., Ploetze, M., Carrassi, E., Todaro, F., 2016. On the geotechnical characterisation of the polluted submarine sediments from Taranto. *Environ. Sci. Pollut. Res.* 23, 12535–12553. <https://doi.org/10.1007/s11356-016-6317-x>