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Supplementary Material

Physiological response of *Posidonia oceanica* to heavy metal pollution along the Tyrrhenian coast

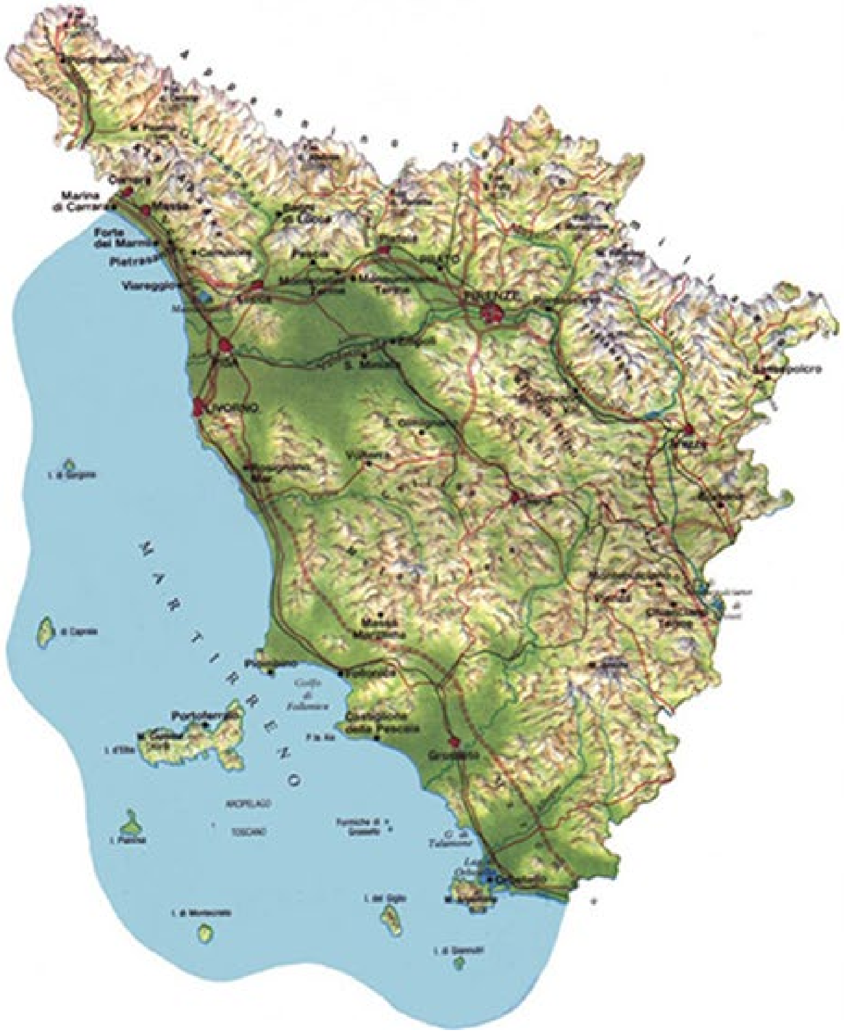
Laura Bertini[A], *Francesca Focaracci*[A], *Silvia Proietti*[A], *Patrizia Papetti*[B] *and Carla Caruso*[A,C]

[A]Department of Ecological and Biological Sciences, University of Tuscia, Largo dell'Università, 01100 Viterbo, Italy.

[B]Department of Business and Law, University of Cassino, Via S. Angelo-Campus, Folcara, 03043 Cassino, Italy.

[C]Corresponding author. Email: caruso@unitus.it

A



B

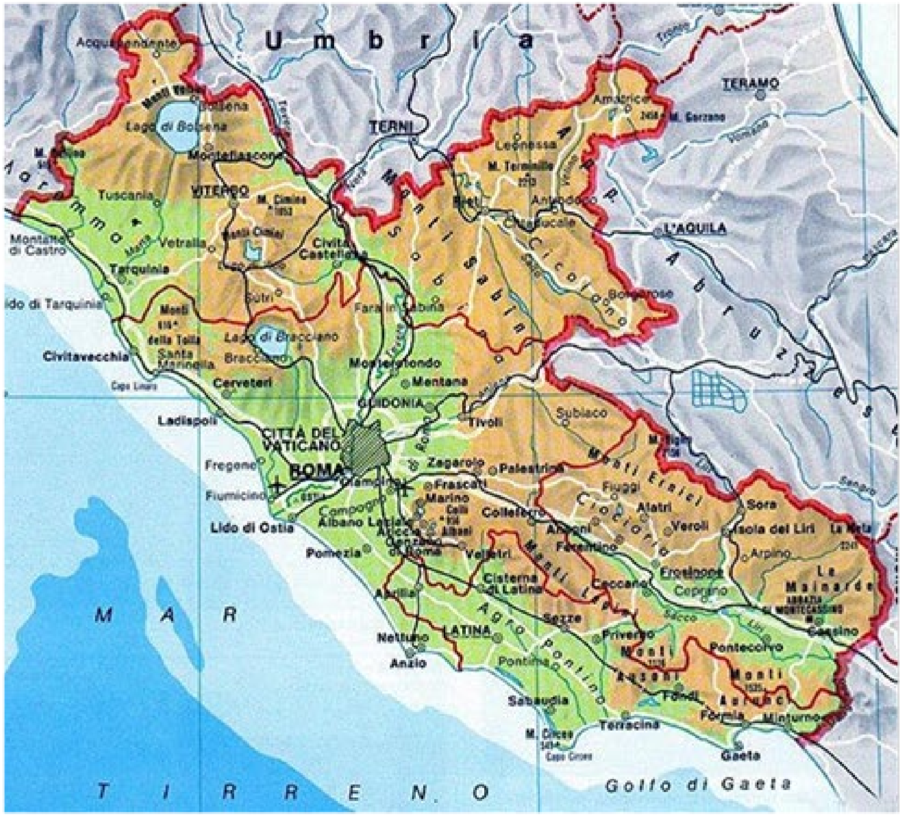


Fig. S1. Physical map of Tuscany (A) and Latium (B) regions.

Table S1. List of target and reference genes used in qPCR analysis

Primers efficiency (E) was calculated by generating standard curves for each oligonucleotide pair with at least five dilution points. The quantification cycle (Cq) value was plotted versus the logarithm of each dilution factor and the slope of the curve was used to calculate E according to the following equation $E = 10^{(-1/slope)}$. The amplification program was the following: 95°C for 3 s; 44 cycles at 95°C for 1 s and primers annealing temperature for 30 s. To detect the presence of non-specific amplicons, melting curves of all PCR products were performed (70–95°C with an increase of 0.5°C every 5s)

Gene name	Description	Gene ID	Primer sequences	Amplicon lenght (bp)	E (%)	R^2	Annealing T (°C)
CMT	CMT-type DNA-methyltransferase mRNA	JF787621.1	Primer For: 5'-TGTAATCAATAGCAAAGTCTTCATGTC-3' Primer Rev: 5'-ATCTCCGCCAAGTCCTTGTA-3'	84	101.7	0.99	60
GST	Glutatione S-transferase	Pooc_B_rp3_C10_F	Primer For: 5'-TACATGAGCAGCGACGAGTT-3' Primer Rev: 5'-CTTTGCATCTCCAGAACCTTG-3'	169	101	0.99	60
CAT	Catalase	Pooc_PC013E04	Primer For: 5'- AACTTCAAGCAGCCAGGAGA-3' Primer Rev: 5'-ATCACATGCTGGGTTTCACA-3'	202	101	0.99	60
MT-2b	partial mt2b gene for type II metallothionein	AJ249602.1	Primer For: 5'-GAAGCTGTGGCTGTGGATCT-3' Primer Rev: 5'-AGCTGCTTCCACATTTGCAT-3'	189	100	0.99	60
SOD	Superoxide dismutase	Pooc_PC034C12	Primer For: 5'-CAATGGCTGCATATCGACTG-3' Primer Rev: 5'- TGCCGGACTTTATCTTCTGG-3'	157	101.5	0.99	60
POX	Peroxidase	Pooc_Contig114	Primer For: 5'-CCCTTGTTGTCGAGGATGTT-3' Primer Rev: 5'-TTGGGCTTGTTGCTCTTCTT-3'	238	110	0.99	57
18S	Ribosomal RNA 18s	AY491942.1	Primer For: 5'-ACATAGTAAGGATTGACAGATTG-3' Primer Rev: 5'-CTGAGGTCTCGTTCGTT-3'	119	104.3	0.96	60
L23	60s Ribosomal protein L23	GO347779	Primer For: 5'-TGGTCCAACTTGTTCCTTCC-3' Primer Rev: 5'-AAGATACAGGCTGCCAAGG-3'	168	103	0.98	60

Table S2. Heavy metals concentration values (mg kg^{-1} dry weight) in *P. oceanica* leaves

	Pb	Cr	Cu	Ni	Cd
Chiarone	0.80 ± 0.08	5.00 ± 0.03	3.07 ± 0.23	3.85 ± 0.07	0.42 ± 0.014
Murelle	2.34 ± 0.22	4.52 ± 0.26	1.56 ± 0.10	3.80 ± 0.13	0.33 ± 0.003
Giannutri	14.44 ± 0.38	11.23 ± 0.26	6.48 ± 0.04	7.16 ± 0.18	0.28 ± 0.004
Tor Paterno	3.10 ± 0.10	4.73 ± 0.02	8.45 ± 0.18	8.31 ± 0.04	0.36 ± 0.003

Enzyme activity assay values

	SOD IC_{50} (□g)	CAT U mg^{-1}	GST U mg^{-1}	APX U mg^{-1}	POD U mg^{-1}
Chiarone	5.98 ± 0.30	2.22 ± 0.09	0.02 ± 0.0007	2.57 ± 0.07	13.59 ± 0.37
Murelle	3.15 ± 0.61	10.16 ± 0.23	0.03 ± 0.0015	3.48 ± 0.04	0.19 ± 0.01
Giannutri	5.16 ± 0.15	2.1 ± 0.06	0.02 ± 0.0016	1.53 ± 0.01	6.42 ± 0.03
Tor Paterno	5.84 ± 0.48	2.12 ± 0.02	0.015 ± 0.0006	1.88 ± 0.03	9.26 ± 0.06

TBARS concentration values

	TBARS nmol mL^{-1}
Chiarone	6.62 ± 0.0003
Murelle	3.73 ± 0.0014
Giannutri	5.28 ± 0.0014
Tor Paterno	5.20 ± 0.0046

Gene expression values

	CAT	**GST**	**POX**	**SOD**	**Mt-2b**	**CMT**
Chiarone	0.16 ± 0.02	0.56 ± 0.10	0.50 ± 0.16	1.31 ± 0.16	0.34 ± 0.05	0.75 ± 0.11
Murelle	15.37 ± 1.20	12.95 ± 0.24	27.65 ± 0.19	2.88 ± 0.08	26.68 ± 0.06	3.76 ± 0.37
Giannutri	0.59 ± 0.05	0.20 ± 0.03	0.22 ± 0.03	0.54 ± 0.11	0.15 ± 0.03	0.92 ± 0.04
Tor Paterno	2.79 ± 0.18	1.61 ± 0.27	0.51 ± 0.07	1.39 ± 0.21	2.30 ± 0.02	0.68 ± 0.03

Table S3. Trace element concentrations (mg kg^{-1} dry weight) in *P. oceanica* leaves sampled from different seas according to literature

Site	*Year*	*Cd*	*Cu*	*Cr*	*Ni*	*Pb*	*Reference*
Calafuria (Livorno), Tuscany Coast, Tyrrhenian Sea	2009	2.92 ± 0.07	17.50 ± 6.5	0.15 ± 0.11	15.10 ± 3.7	2.50 ± 1.35	De Biasi et al. 2009
Livorno, Tuscany Coast, Tyrrhenian Sea	2005	3.39 ± 0.12	-	0.27 ± 0.07	28.90 ± 0.65	1.40 ± 0.25	Lafabrie et al. 2007
Porto Torres (Sassari), Sardinia	2004	2.10 ± 0.10	-	0.20 ± 0.06	27.47 ± 1.10	1.80 ± 0.00	Lafabrie et al. 2007
Ustica Island, North Sicily	1997-2004	6.25 ± 1.71	24.76 ± 13.2	0.33 ± 0.12	-	1.63 ± 0.7	Conti et al. 2015
Favignana Island, Sicily	1997-2004	2.67 ± 0.36	10.95 ± 7.64	0.60 ± 0.21	-	0.94 ± 0.33	Conti et al. 2015
Linosa Island, South Sicily	1997-2004	2.37 ± 1.45	11.72 ± 4.72	0.11 ± 0.04	-	1.28 ± 0.91	Conti et al. 2015
Piombino (Livorno), Tuscany Coast, Tyrrhenian Sea	2003-2008	2.82 ± 0.05	25.87 ± 3.02	-	35.1 ± 3.7	1.97 ± 0.61	Richir et al. 2015
Porto Ercole (Grosseto), Tuscany Coast, Tyrrhenian Sea	2003-2008	2.83 ± 0.24	17.27 ± 0.76	-	6.10 ± 0.3	14.50 ± 1.90	Richir et al. 2015
Santa Marinella (Rome), Latium Coast, Tyrrhenian Sea	2003-2008	2.15 ± 0.38	11.10 ± 0.46	-	22.30 ± 1.0	0.63 ± 0.06	Richir et al. 2015
Chiarone (-10 m), Latium Coast, Tyrrhenian Sea	2012	0.25 ± 0.20	2.11 ± 0.77	2.58 ± 0.49	3.49 ± 0.40	0.46 ± 0.40	Bravo et al. 2016
Chiarone (-20 m), Latium Coast, Tyrrhenian Sea	2012	0.38 ± 0.02	2.79 ± 0.07	2.2 ± 0.45	5.21 ± 0.07	0.52 ± 0.04	Bravo et al. 2016
Murelle (-10 m), Latium Coast, Tyrrhenian Sea	2012	0.22 ± 0.02	1.01 ± 0.03	2.56 ± 0.03	1.44 ± 0.02	0.15 ± 0.01	Bravo et al. 2016
Murelle (-20 m), Latium Coast, Tyrrhenian Sea	2012	0.36 ± 0.03	1.54 ± 0.03	3.36 ± 0.04	1.86 ± 0.06	0.49 ± 0.02	Bravo et al. 2016
Chiarone, Latium Coast, Tyrrhenian Sea	2014	0.42 ± 0.014	3.07 ± 0.23	5.00 ± 0.03	3.85 ± 0.07	0.79 ± 0.08	This work
Murelle, Latium Coast, Tyrrhenian Sea	2014	0.33 ± 0.003	1.56 ± 0.10	4.52 ± 0.26	3.84 ± 0.13	2.35 ± 0.27	This work
Giannutri Island, Tuscany, Tyrrhenian Sea	2014	0.28 ± 0.004	6.49 ± 0.04	11.23 ± 0.26	7.17 ± 0.18	14.44 ± 0.38	This work
Tor Paterno, Latium Coast, Tyrrhenian Sea	2014	0.36 ± 0.003	8.46 ± 0.18	4.73 ± 0.02	8.32 ± 0.04	3.10 ± 0.11	This work

Table S4. Pearson correlation coefficient between HM and enzyme activity

	Pearson correlation coefficient	*P*-value
Pb vs POD	–0.186463116	0.620398
Pb vs SOD	0.026372452	0.96884
Pb vs CAT	–0.310025278	0.79477
Pb vs APX	–0.668001748	0.437432
Pb vs GST	–0.115726345	0.198252
Cr vs POD	–0.054616449	0.772271
Cr vs SOD	0.114638491	0.487955
Cr vs CAT	–0.386159484	0.423663
Cr vs APX	–0.668971465	0.086443
Cr vs GST	–0.150131811	**0.029677**
Cu vs POD	0.335351678	0.479127
Cu vs SOD	0.617393045	0.937163
Cu vs CAT	–0.714552538	0.781536
Cu vs APX	–0.880824165	0.206946
Cu vs GST	–0.809995895	0.053352
Ni vs POD	0.145024338	0.629847
Ni vs SOD	0.453303596	0.598522
Ni vs CAT	–0.582006754	0.513413
Ni vs APX	–0.837064318	0.052707
Ni vs GST	–0.679297918	**0.015447**
Cd vs POD	0.660530034	0.087917
Cd vs SOD	0.433962081	**0.005494**
Cd vs CAT	–0.169144435	0.153844
Cd vs APX	0.320683604	**0.018089**
Cd vs GST	–0.262577144	**0.001311**

Weak correlation

Moderate correlation

Strong correlation

P-values ≤ 0.05 are shown in bold.

Pearson correlation coefficient between HM and TBARS.

	Pearson correlation coefficient	*P*-value
Pb vs TBARS	–0.059	0.991407
Cr vs TBARS	0.1	0.539003
Cu vs TBARS	0.22	0.857662
Ni vs TBARS	0.03	0.677675
Cd vs TBARS	0.38	**0.003712**

Pearson correlation coefficient between HM and gene expression.

	Pearson correlation coefficient	*P*-value
Pb vs CAT	–0.329404968	0.929105
Pb vs GST	–0.353101536	0.769764
Pb vs POX	–0.310315243	0.797219
Pb vs SOD	–0.637279422	0.330046
Pb vs Mt-2b	–0.331133284	0.773246
Pb vs CMT	–0.244147294	0.332078
Cr vs CAT	–0.433348832	0.697296
Cr vs GST	–0.44281231	0.498483
Cr vs POX	–0.388534426	0.910405
Cr vs SOD	–0.709022819	0.05328
Cr vs Mt-2b	–0.419876999	0.889311
Cr vs CMT	–0.319673315	**0.050577**
Cu vs CAT	–0.706780354	0.968424
Cu vs GST	–0.661007368	0.771164
Cu vs POX	–0.706780354	0.758979
Cu vs SOD	–0.668994952	0.118965
Cu vs Mt-2b	–0.665528688	0.731597
Cu vs CMT	–0.711646314	0.120591
Ni vs CAT	–0.458728442	0.795186
Ni vs GST	–0.528586494	0.583496
Ni vs POX	–0.573434424	0.847682
Ni vs SOD	–0.585976585	**0.026992**
Ni vs Mt-2b	–0.530739951	0.823444
Ni vs CMT	–0.572186187	**0.026028**
Cd vs CAT	–0.191278397	0.310281

Cd vs GST	–0.149861582	0.336939
Cd vs POX	–0.172197641	0.387288
Cd vs SOD	0.161985025	0.093948
Cd vs Mt-2b	–0.168991479	0.356438
Cd vs CMT	–0.22750262	0.211945