

Genotoxicity and cytotoxicity induced in *Zygophyllum fabago* depends on the population's redox plasticity

Antonio López-Orenes¹; Conceição Santos²; Maria Celeste Dias³; Helena Oliveira⁴; María Á. Ferrer¹; Antonio A. Calderón¹; Sónia Silva^{5*}

¹ Department of Agricultural Engineering, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena (Spain), mail: antonio.orenes@upct.es, mail: mangeles.ferrer@upct.es, mail: antonio.calderon@upct.es

² Department of Biology & LAQV/REQUIMTE, Faculty of Sciences, University of Porto, Campo Alegre, 4169-007, Porto, Portugal mail: csantos@fc.up.pt

³ Centre for Functional Ecology (CEF), Department of Life Science, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra (Portugal), mail: celeste.dias@uc.pt

⁴ Department of Biology, CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal mail: holiveira@ua.pt

⁵ Department of Chemistry, LAQV-REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal mail: soniasilva@ua.pt

* Corresponding author

E-mail: soniasilva@ua.pt

Tel: + 351 234 370 766

fax: + 351 234 372 587

Table S1: Effect of Pb exposure on seed germination (%), shoot and root biomass and size, foliar RWC and photosynthetic pigments, and Pb accumulation seedling from non-mining (NM) and mining ('Agustin' and 'Mercader') *Z. fabago* populations exposed to 0, 5 and 20 μM $\text{Pb}(\text{NO}_3)_2$ for 4 weeks. *.

	0 μM	NM 5 μM	20 μM	0 μM	'Agustin' 5 μM	20 μM	0 μM	'Mercader' 5 μM	20 μM
Germination (%)									
Day 6	71.67 \pm 8.23	23.33 \pm 5.21	25.00 \pm 6.15 bB	76.67 \pm 8.81	86.67 \pm 6.87	75.00 \pm 5.89	26.67 \pm 5.32	36.67 \pm 6.58 aB	28.33 \pm 4.11 aB
Day 12	76.67 \pm 6.86	55.00 \pm 3.29	50.00 \pm 4.88 bB	93.33 \pm 8.11	95.00 \pm 7.65	76.67 \pm 6.28	68.33 \pm 6.59 aB	80.00 \pm 7.99 aA	71.67 \pm 7.18 aA
Day 18	76.67 \pm 4.79	55.00 \pm 5.77	54.00 \pm 6.53 bB	93.33 \pm 6.98	95.00 \pm 6.56	78.33 \pm 7.54	78.33 \pm 7.85	81.67 \pm 6.59 aA	76.67 \pm 7.12 aA
Shoots									
Biomass (mg)	51.7 \pm 4.07 aB	37.5 \pm 3.28 bC	35.4 \pm 2.96 bB	79.2 \pm 10.2 aA	62.3 \pm 8.96 aB	77.1 \pm 6.84 aA	96.4 \pm 7.69 aA	119.9 \pm 6.28 aA	100.3 \pm 9.87 aA
Size (cm)	2.2 \pm 0.08 aC	1.3 \pm 0.15 bC	1.0 \pm 0.06 bC	2.9 \pm 0.13 aB	2.5 \pm 0.17 aB	2.5 \pm 0.21 aB	4.1 \pm 0.38 aA	3.6 \pm 0.31 aA	4.0 \pm 0.42 aA
RWC (%)	71.9 \pm 1.21 aB	78.0 \pm 1.75 aB	65.6 \pm 3.31 aB	76.0 \pm 1.25 bB	87.9 \pm 2.10 aA	87.2 \pm 1.78 aA	72.8 \pm 1.56 bB	85.4 \pm 2.15 aA	82.2 \pm 1.00 aA
Leaves									
Chl <i>a</i> ($\mu\text{g g}^{-1}$ FW)	123.8 \pm 2.51	105.1 \pm 0.47	96.3 \pm 3.07 bC	161.8 \pm 1.07	93.6 \pm 4.11 bB	111.2 \pm 1.04	205.4 \pm 1.72	184.7 \pm 6.34 bA	192.2 \pm 2.57 bA
Chl <i>b</i> ($\mu\text{g g}^{-1}$ FW)	79.0 \pm 1.57 aC	75.4 \pm 0.67	72.0 \pm 2.63 bC	103.3 \pm 0.55	77.3 \pm 2.75 bB	82.4 \pm 1.02 bB	116.3 \pm 0.75	109.8 \pm 3.43 bA	110.7 \pm 1.63 bA
Car ($\mu\text{g g}^{-1}$ FW)	27.8 \pm 0.83 aC	24.1 \pm 0.19 bB	24.9 \pm 0.52 bC	41.7 \pm 0.30 aA	39.6 \pm 0.84 aA	42.2 \pm 0.35 aA	38.3 \pm 0.88 aB	40.9 \pm 0.96 aA	40.0 \pm 0.46 aB
Roots									
Biomass (mg)	40.2 \pm 2.87 aB	23.9 \pm 3.90 bC	27.0 \pm 4.12 bB	98.1 \pm 11.75	88.1 \pm 5.92 aB	90.7 \pm 6.71 aA	105.6 \pm 9.65	115.9 \pm 10.89	100.9 \pm 7.92 aA
Size (cm)	4.6 \pm 0.13 aB	3.8 \pm 0.22 abC	3.3 \pm 0.12 bC	8.9 \pm 1.71 aA	7.8 \pm 0.32 aB	6.1 \pm 0.66 aB	10.4 \pm 2.56 aA	12.5 \pm 1.66 aA	11.5 \pm 1.93 aA
Pb content									
Pb shoots (ng g^{-1})	0.30 \pm 0.08 cB	1.02 \pm 0.21 bB	2.70 \pm 0.52 aB	0.48 \pm 0.11	2.24 \pm 0.62 bA	21.86 \pm 7.89	0.52 \pm 0.13 cA	2.42 \pm 0.59 bA	15.01 \pm 4.68 aA
Pb roots (ng g^{-1} DW)	0.28 \pm 0.10 cB	14.41 \pm 3.78	33.15 \pm 10.11	0.86 \pm 0.38 cA	8.89 \pm 2.56 bA	92.24 \pm 21.56	3.48 \pm 1.60 bA	11.76 \pm 4.58 bA	125.73 \pm 38.96
Transfer coefficient		0.07	0.09		0.27	0.25		0.23	0.13

*Values are given as mean \pm SE ($n=4-6$). For each phase and organ, different lowercase mean significant differences between treatments among the same population, and different uppercase letters mean significant differences between populations among the same Pb dose ($p < 0.05$).

Table S2. Percentage (%) of nuclei in each of the three identified peaks in leaves and roots of control and exposed plants: Peak I – G₀/G₁ phase of the subpopulation A; Peak II - G₀/G₁ phase of subpopulation B together with the G₂ phase of subpopulation A; Peak III - G₂ phase of the subpopulation B*.

		Pb (μM)	Nuclei (%)		
			G ₁ ^A	G ₁ ^B +G ₂ ^A	G ₂ ^B
NM	Leaves	0	44.0 ± 7.6 bB	43.8 ± 4.3 aA	12.2 ± 5.8 aA
		5	54.2 ± 2.2 aB	38.0 ± 3.4 abA	7.8 ± 2.4 aA
		20	59.2 ± 3.8 aA	34.7 ± 3.6 bA	6.0 ± 1.8 aA
	Roots	0	44.6 ± 6.9 aA	42.3 ± 3.7 aA	13.1 ± 3.9 aA
		5	50.5 ± 6.3 aA	38.1 ± 3.2 aA	11.4 ± 3.8 aA
		20	53.2 ± 9.6 aA	39.7 ± 6.2 aA	7.1 ± 3.7 aB
‘Agustin’	Leaves	0	63.5 ± 5.8 aA	26.8 ± 3.4 aB	9.7 ± 3.8 aA
		5	64.1 ± 3.6 aA	27.2 ± 2.4 aB	8.8 ± 1.7 aA
		20	62.4 ± 5.5 aA	30.2 ± 4.6 aA	7.4 ± 3.5 aA
	Roots	0	51.4 ± 3.0 bA	39.71 ± 3.5 aA	8.9 ± 3.2 aA
		5	59.4 ± 9.0 abA	34.2 ± 5.0 abA	6.5 ± 4.7 aA
		20	69.4 ± 10.4 aA	24.8 ± 8.8 bB	5.8 ± 2.9 aC
‘Mercader’	Leaves	0	59.4 ± 7.2 bA	32.3 ± 7.5 aB	8.3 ± 3.4 aA
		5	70.8 ± 6.3 aA	21.8 ± 3.6 bB	7.4 ± 2.7 aA
		20	61.3 ± 5.6 abA	30.9 ± 5.5 aA	7.8 ± 1.9 aA
	Roots	0	47.6 ± 7.1 aA	35.3 ± 5.2 aA	17.1 ± 3.4 aA
		5	55.0 ± 13.6 aA	32.7 ± 6.6 aA	12.3 ± 4.7 aA
		20	56.7 ± 7.4 aA	29.6 ± 6.5 aAB	13.7 ± 1.9 aA

*Values are given as mean ± SE (n=4-6). For each phase and organ, different lowercase mean significant differences between treatments among the same population, and different uppercase letters mean significant differences between populations among the same Pb dose (p < 0.05).

Table S3. Total antioxidant activity, AsA (ascorbate), DHA (dehydroascorbate), reduced glutathione (GSH) and soluble total non-protein thiols (NPT), total soluble phenolics (TPC), hydroxycinnamic acids (HCA), flavonoids, flavanols, lignin, H₂O₂, MDA, and carbonyl (C=O) contents in shoots from non-mining (NM) and mining ('Agustin' and 'Mercader') *Z. fabago* populations exposed to 0, 5 and 20 μ M Pb(NO₃)₂ for 4 weeks. *.

Shoots	NM			'Agustin'			'Mercader'		
	0 μ M	5 μ M	20 μ M	0 μ M	5 μ M	20 μ M	0 μ M	5 μ M	20 μ M
ABTS (μ mol GA eq. g ⁻¹ FW)	3.45 \pm 0.09 cA	4.63 \pm 0.08 bA	5.16 \pm 0.14 aA	3.50 \pm 0.06 bA	3.65 \pm 0.11 bB	4.53 \pm 0.09 aB	3.39 \pm 0.03 aA	3.30 \pm 0.18 aB	3.29 \pm 0.04 aB
DPPH (μ mol GA eq. g ⁻¹ FW)	1.97 \pm 0.12 aA	2.18 \pm 0.20 aA	2.38 \pm 0.23 aA	2.18 \pm 0.09 aA	2.01 \pm 0.07 aA	2.34 \pm 0.10 aA	1.91 \pm 0.10 aA	1.73 \pm 0.16 aA	1.72 \pm 0.09 aB
FRAP (μ mol Fe(II) g ⁻¹ FW)	10.80 \pm 0.35 bC	10.29 \pm 0.30 bB	12.98 \pm 0.24 aB	14.43 \pm 0.21 bA	13.67 \pm 0.39 bA	15.53 \pm 0.21 aA	13.60 \pm 0.31 aB	10.49 \pm 0.71 bB	9.28 \pm 0.16 bC
AsA (μ mol g ⁻¹ FW)	0.64 \pm 0.01 aB	0.58 \pm 0.01 aC	0.67 \pm 0.01 aB	0.94 \pm 0.02 aA	0.99 \pm 0.02 aA	1.00 \pm 0.05 aA	0.88 \pm 0.01 aA	0.77 \pm 0.01 bB	0.66 \pm 0.02 cB
DHA (μ mol g ⁻¹ FW)	0.32 \pm 0.03 aA	0.25 \pm 0.04 aA	0.14 \pm 0.01 bC	0.27 \pm 0.02 bA	0.34 \pm 0.02 aA	0.39 \pm 0.02 aA	0.18 \pm 0.03 bB	0.18 \pm 0.01 bB	0.26 \pm 0.01 aB
AsA/DHA	1.98 \pm 0.20 b C	2.33 \pm 0.40 b B	4.85 \pm 0.61 a A	3.53 \pm 0.36 a B	2.93 \pm 0.27 a B	2.58 \pm 0.23 a B	4.96 \pm 0.82 a A	4.32 \pm 0.29 a A	2.51 \pm 0.21 b B
GSH (nmol g ⁻¹ FW)	20.61 \pm 0.26 bA	34.26 \pm 1.29 aA	39.23 \pm 0.60 aA	22.59 \pm 0.23 cA	26.84 \pm 0.32 bB	39.02 \pm 1.41 aA	17.95 \pm 0.28 cB	25.46 \pm 0.46 bB	29.64 \pm 1.13 aB
NPT (nmol g ⁻¹ FW)	393.44 \pm 14.02 cA	571.43 \pm 15.86 bA	660.17 \pm 11.22 aA	333.82 \pm 11.09 bB	336.44 \pm 6.54 bB	436.08 \pm 24.32 aB	377.59 \pm 6.73 aA	326.50 \pm 7.25 aB	339.45 \pm 26.51 aC
TPC (μ mol gallic acid eq. g ⁻¹ FW)	14.24 \pm 0.10 c A	16.54 \pm 0.24 bA	19.94 \pm 0.18 aA	14.28 \pm 0.24 bA	16.05 \pm 0.43 bA	19.13 \pm 0.12 aB	14.33 \pm 0.18 aA	13.11 \pm 0.65 abB	12.84 \pm 0.20 bC
HCAs (μ mol caffeic acid eq. g ⁻¹ FW)	3.10 \pm 0.11 a B	2.38 \pm 0.07 bC	2.44 \pm 0.09 bB	4.43 \pm 0.05 aA	2.86 \pm 0.08 bB	2.46 \pm 0.14 bB	4.29 \pm 0.12 aA	4.28 \pm 0.22 aA	4.18 \pm 0.18 aA
Flavonoids (μ mol rutin eq. g ⁻¹ FW)	0.96 \pm 0.08 a A	1.33 \pm 0.22 aA	0.49 \pm 0.08 bA	1.12 \pm 0.04 aA	0.63 \pm 0.11 bB	0.66 \pm 0.04 bA	1.01 \pm 0.04 aA	0.48 \pm 0.04 bB	0.57 \pm 0.09 bA
Flavanols (nmol catechin eq. g ⁻¹ FW)	30.71 \pm 1.09 a B	8.30 \pm 2.97 bC	5.24 \pm 1.81 bC	43.12 \pm 1.05 aA	19.82 \pm 1.49 bB	23.77 \pm 0.80 bB	44.81 \pm 1.41 aA	38.86 \pm 3.18 aA	49.21 \pm 3.03 aA
Lignin (mg LTGA g ⁻¹ DW)	8.03 \pm 0.17 aA	7.29 \pm 0.06 aA	8.01 \pm 0.05 aA	5.02 \pm 0.15 aB	5.63 \pm 0.05 aB	6.88 \pm 0.06 aB	3.49 \pm 0.03 aC	3.66 \pm 0.18 aC	2.89 \pm 0.17 aC
MDA (nmol g ⁻¹ FW)	7.23 \pm 0.62 aAB	7.08 \pm 1.46 aA	8.78 \pm 1.51 aB	6.58 \pm 0.34 bB	8.81 \pm 0.53 aA	9.66 \pm 1.91 aA	8.80 \pm 0.48 aA	8.68 \pm 0.81 aA	9.87 \pm 0.63 aA
C=O (nmol mg protein ⁻¹)	21.05 \pm 0.43 bA	43.77 \pm 0.50 aA	38.52 \pm 1.47 aA	14.24 \pm 0.34 bC	13.85 \pm 0.88 bC	17.50 \pm 1.50 aB	17.97 \pm 0.11bB	20.65 \pm 0.37 aB	20.40 \pm 1.37 aB
H ₂ O ₂ (μ mol g ⁻¹ FW)	0.60 \pm 0.01 cA	0.82 \pm 0.01 bA	0.93 \pm 0.02 aA	0.45 \pm 0.02 cB	0.49 \pm 0.01 bB	0.62 \pm 0.04 aB	0.46 \pm 0.01 bB	0.51 \pm 0.01 abB	0.56 \pm 0.04 aB

*Values are given as mean \pm SE ($n=4-6$). For each phase and organ, different lowercase mean significant differences between treatments among the same population, and different uppercase letters mean significant differences between populations among the same Pb dose ($p < 0.05$).

Table S4. Total antioxidant activity, AsA (ascorbate), DHA (dehydroascorbate), reduced glutathione (GSH) and soluble total non-protein thiols (NPT), total soluble phenolics (TPC), hydroxycinnamic acids (HCA), lignin, H₂O₂, MDA, and carbonyl (C=O) contents in roots of nonmetalliferous (NM) and metalliferous (‘Agustin’ and ‘Mercader’) *Z. fabago* seedlings grown in the presence of increasing Pb concentrations (0, 5, and 20 µM) for 4 weeks*.

Roots	NM			‘Agustin’			‘Mercader’		
	0 µM	5 µM	20 µM	0 µM	5 µM	20 µM	0 µM	5 µM	20 µM
ABTS (µmol GA eq. g ⁻¹ FW)	0.64 ± 0.10 aB	0.46 ± 0.09 aB	0.52 ± 0.13 aB	0.91 ± 0.05 aA	0.77 ± 0.03 bA	0.94 ± 0.04 aA	1.04 ± 0.05 aA	0.76 ± 0.06 bA	0.56 ± 0.05 cB
DPPH (µmol GA eq. g ⁻¹ FW)	2.93 ± 0.28 aA	2.83 ± 0.21 aA	3.18 ± 0.34 aA	2.03 ± 0.08 aB	1.43 ± 0.13 bB	1.81 ± 0.08 aB	2.02 ± 0.11 aB	1.22 ± 0.18 bB	0.93 ± 0.07 bC
FRAP (µmol Fe(II) g ⁻¹ FW)	6.82 ± 0.17 aB	3.63 ± 0.43 bB	4.92 ± 0.47 bB	7.28 ± 0.26 aB	4.50 ± 0.22 bA	6.96 ± 0.14 aA	10.17 ± 0.53 aA	3.50 ± 0.25 bB	2.96 ± 0.18 bC
AsA (µmol g ⁻¹ FW)	0.23 ± 0.01 aA	0.21 ± 0.01 bA	0.17 ± 0.03 bB	0.15 ± 0.01 bB	0.14 ± 0.01 bB	0.24 ± 0.01 aA	0.23 ± 0.01 aA	0.14 ± 0.01 bB	0.12 ± 0.01 bC
DHA (µmol g ⁻¹ FW)	0.23 ± 0.03 bA	0.20 ± 0.01 bA	0.36 ± 0.03 aA	0.19 ± 0.01 aA	0.13 ± 0.01 bB	0.14 ± 0.02 bB	0.16 ± 0.01 aB	0.14 ± 0.01 aB	0.18 ± 0.01 aB
AsA/DHA	1.00 ± 0.16 a B	1.05 ± 0.10 a A	0.48 ± 0.16 b B	0.79 ± 0.05 b B	1.04 ± 0.17 abA	1.75 ± 0.36 aA	1.41 ± 0.82 aA	1.08 ± 0.29 bA	0.68 ± 0.21 cB
GSH (nmol g ⁻¹ FW)	30.03 ± 1.80 aA	32.86 ± 0.47 aA	33.13 ± 1.37 aA	29.16 ± 0.37 bA	34.03 ± 0.66 aA	36.38 ± 2.34 aA	28.76 ± 0.27 cA	32.64 ± 0.65 bA	37.86 ± 1.69 aA
NPT (nmol g ⁻¹ FW)	823.52 ± 50.59 aA	653.22 ± 12.95 bA	674.39 ± 42.68 bA	325.97 ± 10.60 aB	267.36 ± 17.59 aC	350.52 ± 16.06 aB	363.99 ± 25.69 aB	373.28 ± 28.04 aB	263.69 ± 8.25 bC
TPC (µmol gallic acid eq. g ⁻¹ FW)	3.00 ± 0.07 bA	2.91 ± 0.03 bA	3.60 ± 0.25 aA	2.50 ± 0.04 bB	2.43 ± 0.03 bB	3.31 ± 0.18 aA	2.94 ± 0.09 aA	2.56 ± 0.05 bB	2.17 ± 0.03 cB
HCAs (µmol caffeic acid eq. g ⁻¹ FW)	0.62 ± 0.17 aB	0.20 ± 0.16 abB	0.11 ± 0.05 bB	1.12 ± 0.21 aA	0.91 ± 0.12 aA	0.55 ± 0.05 bA	0.41 ± 0.06 aB	0.19 ± 0.07 bB	0.15 ± 0.03 bB
Lignin (mg LTGA g ⁻¹ DW)	10.28 ± 0.33 aC	11.37 ± 0.38 aA	10.47 ± 0.52 aC	11.44 ± 0.56 aB	8.06 ± 0.57 aC	12.10 ± 0.09 aA	12.84 ± 0.15 aA	10.61 ± 0.05 aB	11.48 ± 0.16 aB
MDA (nmol g ⁻¹ FW)	3.55 ± 1.15 bA	3.30 ± 1.39 bA	6.22 ± 1.00 aA	1.49 ± 0.79 aA	2.73 ± 0.91 aA	3.60 ± 0.72 aB	2.36 ± 0.73 aA	1.94 ± 0.47 aA	3.03 ± 0.26 aB
C=O (nmol mg protein ⁻¹)	65.37 ± 0.78 bA	62.70 ± 2.10 bA	97.70 ± 1.49 aA	49.74 ± 2.97 bB	56.32 ± 0.69 aB	59.76 ± 1.12 aB	40.39 ± 0.19 bC	38.32 ± 0.61 bC	52.35 ± 0.78 aC
H ₂ O ₂ (µmol g ⁻¹ FW)	1.05 ± 0.05 aA	0.84 ± 0.01 bA	0.83 ± 0.06 bA	0.41 ± 0.01 aC	0.33 ± 0.02 bC	0.40 ± 0.02 aB	0.48 ± 0.04 aB	0.47 ± 0.04 aB	0.32 ± 0.01 bC

*Values are given as mean ± SE (*n*=4-6). For each phase and organ, different lowercase mean significant differences between treatments among the same population, and different uppercase letters mean significant differences between populations among the same Pb dose (*p* < 0.05).