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ATRIPLEX HALIMUS CLONES SELECTION TO IMPROVE MINE TAILING PHYTOTECHNOLOGIES

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Phytotechnologies are a sustainable way to clean up mining waste. Not only they can help restoring land, but they can also drive the recovery of valuable resources like secondary and critical raw materials (SRMs and CRMs). The selection of specific clones for metal extraction from soils is one of the key elements to boost metal recovery from biomass obtained in phytoremediation.

This study focused on *Atriplex halimus* (L.) to explore differences among plants collected in Sardinia Island (Italy) with the purpose of evaluating the feasibility of starting a clonal selection for metal extraction. Plant clones were obtained from six mother plants vegetating in two areas of Sardinia. The clones were then cultivated in mine tailings substrates heavily polluted by metals. After 90 and 150 days, differences among clones in terms of survival, growth, and metals accumulation were observed, both in pure and amended substrates (amendments: 20% compost, 10% sand, 10% gravel, V/V).

Phase 1) Production of *Atriplex halimus* clones plant from spontaneous mother plants

***Atriplex halimus* plants**

Plant material used to produce clones was collected from three mother plants in the C area (C1, C2, C3) and three mother plants in the NC area (NC1, NC2, NC3)

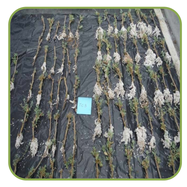
Stem cutting production

- Thirty stem cuttings were obtained from each mother plant
- Clones were named after their respective mother plant (C1, C2, C3, NC1, NC2, NC3)

Stem Cutting rooting

- Cuttings were rooted in greenhouse to obtain plant clones
- growth in controlled greenhouse was monitored for 30 days

Phase 2) Plant growth in polluted subtrates **and clones evaluation**



Rooted cuttings = young plant clones

- Clones C1, C2, C3
- Clones NC1, NC2, NC3

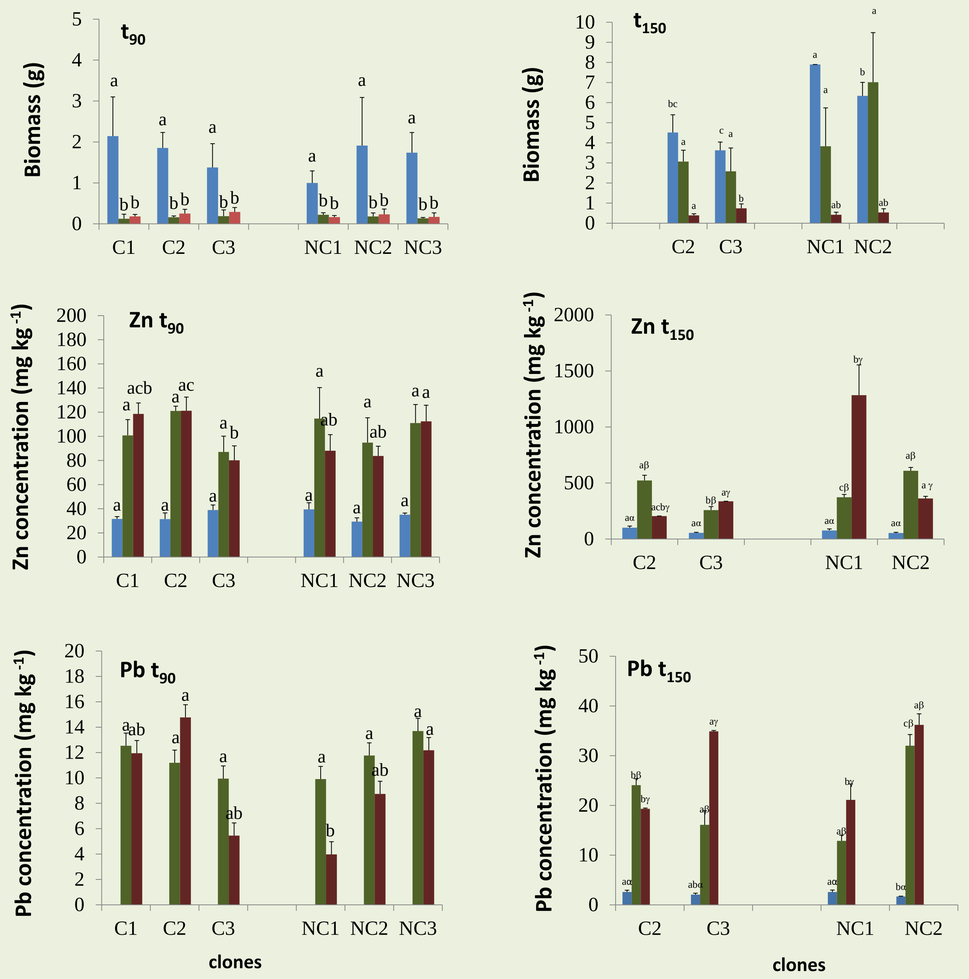
Plants growth in experimental substrates

- **3 SUBSTRATES**
- B
- CP
- CP+A

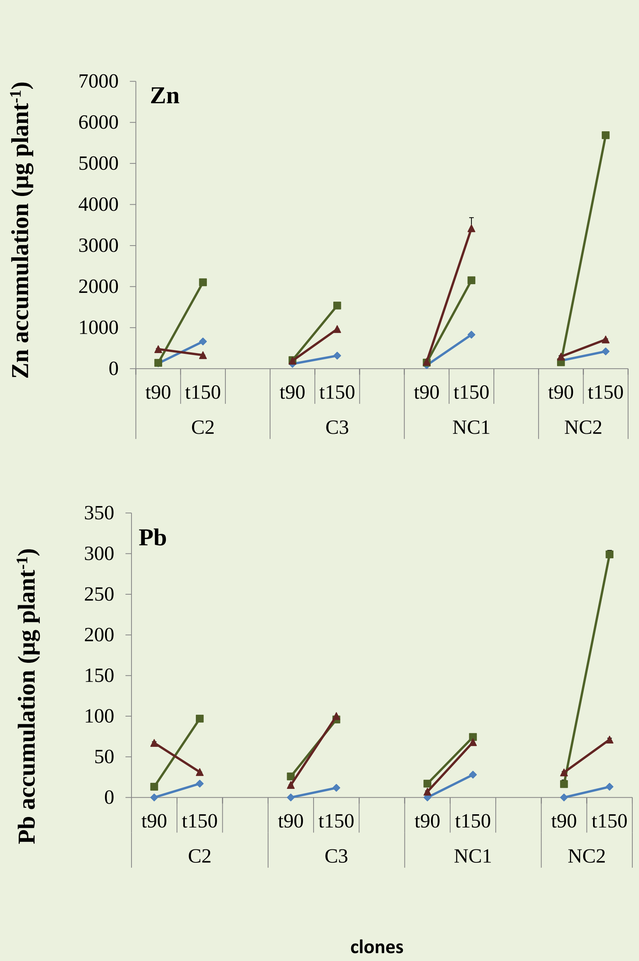
Plant evaluation and analyses

- Growth parameters, metal content
- Evaluation at 90 and 150 days after the transplant

Biomass and metal concentrations in aerial biomass



Metal accumulation in aerial biomass



Campo Pisano mine site

Element	(B)	(CP)	(CP+A)
	[mg kg^{-1}]		
	Control	Campo Pisano	Campo Pisano + Amendments
Pb	nd	3024.7	2523.4
Zn	62.3	12481.6	9187.3

*Experimental Substrates and their metal concentrations. *nd: values below the detection limits*

Element	CLONES			
	C2	C3	NC1	NC2
Pb	0.3	0.2	0.2	0.1
Zn	1.2	0.6	0.9	0.6

Translocation factors (TF) calculated at t150 on plants grown in the substrate Campo Pisano + Amendments

Biomass production, metal concentrations and metal accumulation in plants were assessed after 90 and 150 days (t90 and t150) on the 3 experimental substrates. At t90, all clones were evaluated, whereas at t150 only the plant clones C2, C3, NC1, NC2 were evaluated. In the figures above, the experimental substrates are represended with the following colors: B CP CP+A

Among the six clones examined, there was significant variability in biomass production and metal accumulation. The addition of soil amendments to the contaminated substrates improved aerial biomass production and the plants showed a marked tendency to accumulate Zn compared to Pb. The calculated translocation factor (TF) values indicated that, in general, plants had a greater propensity for root accumulation than for aerial accumulation (TF<1), although in some cases the opposite pattern was observed (TF>1). The plants effectively accumulated significant amounts of zinc and lead, with concentrations in their aerial parts reaching up to 1062 mg kg^{-1} and 36 mg kg^{-1}, respectively. Overall, metal accumulation in aerial biomass increased from t90 to t150 and varied depending on the clone and the substrate. At t150, notable differences were observed between the clones: clone NC2 showed the highest total metal content per plant, and in the presence of substrate amendments (CP+A), up to 6,000 µg of Zn and 300 µg of Pb per plant were recorded.

These findings demonstrate that *Atriplex halimus* is particularly suitable for clonal selection for phytoremediation. Clonal selection could enable the development of a cultivation system with specific production goals, enhancing both biomass production and metal accumulation in plant tissues.

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