

## COMBINED APPROACH TO ASSISTED PHYTOEXTRACTION: INFLUENCE OF CHELATORS, MICROBIAL CONSORTIUM AND CO- PLANTING

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### Introduction

Metal accumulation limits in plants can be pushed by exogenous stimulation, e.g. with the use of chelators or microorganisms; hence the name of the process: assisted phytoextraction. Supplementation with chelators increases metal mobility in soil, whereas inoculation with microorganisms enhances uptake e.g. if the strains secrete substances that favor metal mobilization (Vamerali et al., 2010). Using assisted phytoextraction it should be possible to remove metals from soil using plants with higher biomass production lacking trait of high metal accumulation (Bhargava et al., 2012). Our study was performed to assess the combined influence of co-planting, supplementation with chelators and microbial inoculation in assisted phytoextraction.

### Methods

Metal accumulating Indian mustard *Brassica juncea* was grown in greenhouse in pots filled with Zn, Pb, Cd contaminated soil collected from Piekary Śląskie (Poland), as a monoculture (Bj) or with accompanying plant species: alfalfa *Medicago sativa* (Bj+Ms) or maize *Zea mays* (Bj+Zm). One week before the harvest plants were treated with nitrilotriacetic acid (NTA), rhamnolipids (AGAE Technologies) or microbial consortium (EmFarma Plus<sup>TM</sup>, SCD Probiotics Polska: *Bifidobacterium bifidum*, *B. longum*, *B. animalis*, *Lactobacillus plantarum*, *L. acidophilus*, *L. caseito*, *L. delbrueckii*, *L. fermentum*, *Lactococcus lactis*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Streptococcus salivarius ssp. thermophilus*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*). A part of the plants treated with chelators was additionally supplemented 3 days before the harvest with microbial consortium. Material was harvested and assessed, among others, in terms of plant survival (plant count from consecutive series of cultures), plant biomass (fresh and dry weight), root and shoot length; chlorophyll a and b content, reactive oxygen species generation, activity of antioxidative enzymes (spectrophotometrically); and metal accumulation in aboveground tissues (with inductively coupled plasma mass spectrometry, ICP-MS).

### Results

Companion planting, chelators and microbial consortium had different effects depending on plant species. Here we present (Table 1) results for changes in bioconcentration factor.

Highest increase in BCFs was observed in a co-planting culture of Indian mustard and maize. However, in this culture we observed high oxidative stress and decrease in Indian mustard biomass production. Even

though BCFs for metals in Bj+Ms culture were often decreased, the metal yield from both plants was increased due to higher dry biomass production.

**Table 1.** Calculated BCF of four metals in plant species treated with different variants: K – control, E – microbial inoculation, R – rhamnolipids, N – NTA, R+E – rhamnolipids and microbial inoculation, N+E – NTA and microbial inoculation. BCF (bioconcentration factor) was calculated as a ratio of metal content in the aboveground plant organs to metal content in soil. Description: Bj – *B. juncea*, Ms – *M. sativa*, Zm – *Z. mays*, Bj+Ms – co-planting of Indian mustard and alfalfa, Bj+Zm – co-planting of Indian mustard and maize.

		min				max				
		control								
		Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn	
Bj	Bj K	0.78	0.41	0.005	1.21					
	Bj E	1.00	0.50	0.004	1.61					
	Bj R	0.77	0.26	0.003	1.13					
	Bj N	0.77	0.45	0.003	1.11					
	Bj R +E	0.99	0.28	0.004	1.34					
	Bj N +E	0.83	0.43	0.004	1.20					
Bj+Ms	Bj K	0.93	0.46	0.004	1.37	Ms K	0.37	0.20	0.004	0.43
	Bj E	0.99	0.37	0.003	1.26	Ms E	0.40	0.16	0.006	0.36
	Bj R	0.93	0.30	0.003	1.29	Ms R	0.54	0.21	0.004	0.51
	Bj N	0.82	0.30	0.005	1.20	Ms N	0.33	0.14	0.005	0.34
	Bj R +E	1.02	0.36	0.003	1.40	Ms R +E	0.43	0.16	0.004	0.41
	Bj N +E	0.85	0.38	0.002	1.25	Ms N +E	0.48	0.17	0.006	0.50
Bj+Zm	Bj K	0.16	0.20	0.001	1.13	Zm K	0.02	0.06	0.001	0.16
	Bj E	0.87	0.35	0.006	1.54	Zm E	0.13	0.28	0.002	0.28
	Bj R	1.04	0.46	0.006	1.43	Zm R	0.12	0.17	0.002	0.30
	Bj N	1.06	0.84	0.011	1.79	Zm N	0.13	0.37	0.002	0.44
	Bj R +E	1.66	0.76	0.017	2.60	Zm R +E	0.11	0.21	0.001	0.39
	Bj N +E	1.27	0.92	0.008	1.91	Zm N +E	0.10	0.32	0.002	0.32

## Conclusion

Co-planting may alter metal accumulation as a plant response to exogenous stimulation with NTA, rhamnolipids or microbial inoculation. Effect of exogenous supplementation is species specific.

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