ASSESSMENT OF THE PHYTOREMEDIATION POTENTIAL OF CANOLA (*BRASSICA NAPUSL.*) AND VETIVER (*VETIVERIA ZIZANIOIDES L.*) FOR TOXIC ELEMENTS

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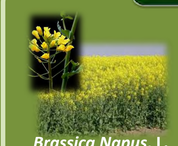
Phytoremediation permits to contain or remove contaminants in soils by using pollutant-accumulating plants that can immobilize or extract and translocate them to the harvestable parts. To better understand translocation mechanisms and to optimize phytoremediation processes, many bioavailability evaluation methods have been developed. These methods use single-step or sequential extraction procedures based on cationic exchange, acidification or complexation [1-4]; although being rapid and suitable for large applications, they not always provide reliable results in terms of under- and over-estimation problems [1,5,6]. For improving the elemental bioavailability evaluations, it could be convenient to determine, at the same time, the parameters that mainly influence availability, diffusibility and mobility of the elements in soils, such as: elemental total content, pH, Cation Exchange Capacity (CEC), or organic matter [7-9]. These soil characterizations are time-consuming, but permit to obtain information about the current and potential physical-chemical transformations in soil, by means of which strategies for increasing toxic element uptake by plants can be developed and predictive models can be applied. We report about a study carried out for assessing the phytoremediation potential of canola (*Brassica Napus*, L.) and vetiver (*Vetiveria Zizanioides*, L.) in soils contaminated by toxic elements, in the framework of a pot-experiment. The plants were grown in soils sampled in a contaminated area, using two different agricultural conditions: with and without phosphatic fertilization. With the aim to consider all the input of toxic elements, also the contribution due to irrigation waters (simulated rainwater) and the added fertilizer were considered. Main physical-chemical properties of soils were determined; the total contents of toxic elements before and after the plant growing were compared. As concerns canola and vetiver plants, the toxic element contents were determined in the different tissues apart. Moreover, considering that the total elemental content in soils is insufficient – in respect to the bioavailable fractions – to explain their translocation from soils to plants, soils were submitted to selective extraction procedures for obtaining information about the mobile (or mobilizable) fractions of toxic elements. In this way, it was possible to determine the Translocation Factor (TF) of each toxic element in the two plant species in the two different agricultural conditions and to evaluate the Bioconcentration Factors (BF), in respect not only to the elemental total contents in soils, but also to the bioavailable fractions.

CANOLA



Brassica Napus, L.

- Essential-oil crop of the *Cruciferae* family
- It is an erect annual or biennial plant, normally 700 to 1500 mm tall with branching stems, yellow flowers in spring and globular black seed in a cylindrical pod
- Fast-growing, high biomass crop plant, with adaptive capacity to variable environments
- It grows well in dry environments and can tolerate moderately saline soil conditions
- It is the second largest oil producing crop in the World, providing up to 13% of the world's oil supply
- Its oil also has a great potential in the biodiesel market (biodiesel made from canola gels at a lower temperature than biodiesel produced from other feedstocks, making canola biodiesel a more suitable fuel for colder regions)
- In addition to oil production, the leaves and stems of canola provide high quality forage because of its low fiber and high protein content
- It has displayed a significant heavy metal tolerance, showing to be able to accumulate substantial amounts of metals
- It has a tap root system and profuse root hairs that allow the plant to be well-equipped for hyperaccumulation

VETIVER

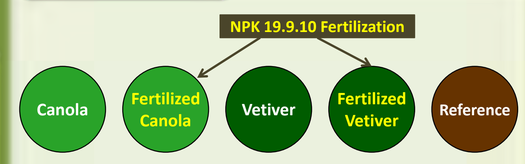
Vetiveria Zizanioides, L.

- Perennial, tropical/sub-tropical grass
- It is about four feet high (1.2 m), with stiff erect leaves and aromatic roots
- It is characterized by a strong, extensive and deep root system reaching to 4 m depth in the soil
- Vetiver plants can be expected to survive for at least 50 years
- It tolerates a wide range of climatic and edaphic conditions, including waterlogging, drought, salinity, extremes of pH, and fire
- Vetiver growth isn't affected by very high levels of heavy metals
- It has been used extensively world wide for soil erosion, sediment control and steep slope stabilization
- Vetiver leaves are used as animal fodder and for roof thatching and mulching
- Roots are the most useful part of the plant. They are used – other than for absorbing water, maintaining soil moisture, arresting soil erosion – for making sieves, blinds, hand fans, baskets, handbags, skin care substances, oil for making perfumes and aromatic ingredients in soaps and in insect repellents.
- The essential oil is obtained from the roots by distillation and is used in perfumery

EXPERIMENTAL PROCEDURE

Plant growing

Pot Experiment



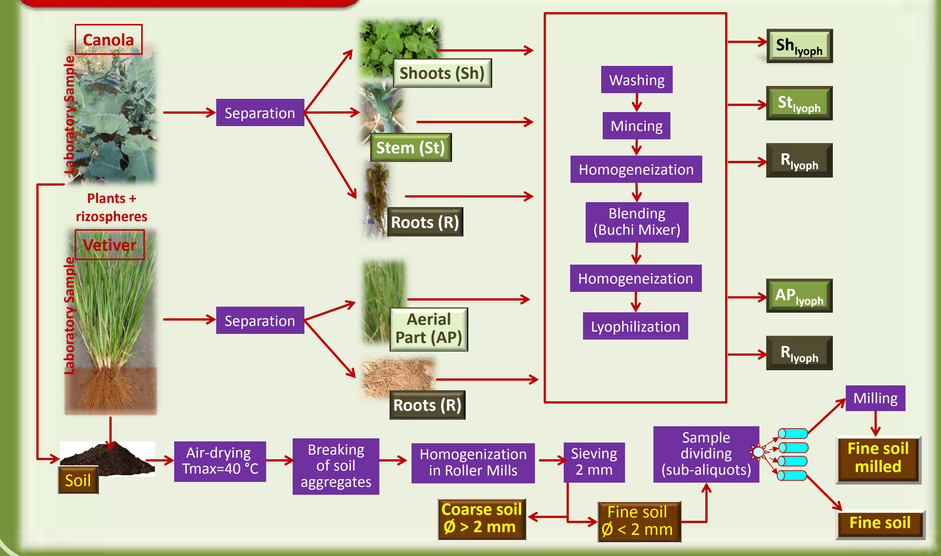
- ✓ **Plant species:** *Brassica Napus*, L. var. *Oleifera* (Canola); *Vetiveria Zizanioides*, L. (Vetiver)
- ✓ **Planting:** 15 canola seeds/pot; 15 vetiver rooted-culms/pot
- ✓ **Growing period:** 5 months
- ✓ **Irrigation:** simulated rainwater pH=5.9; 4 or 8 l/week/pot basing on growing and climatic needs

Simulated rainwater for irrigation

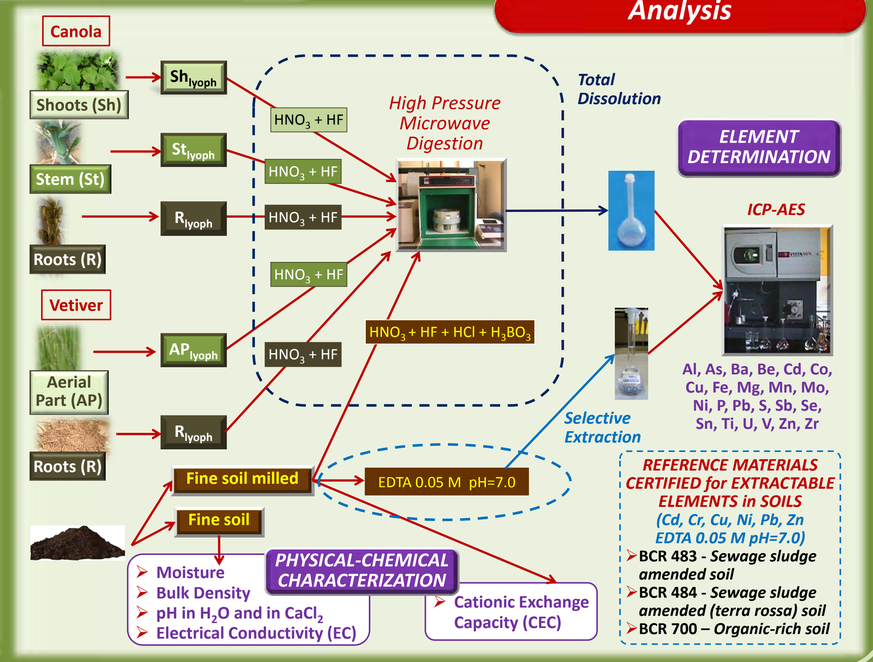
CATIONS			Used compounds		ANIONS			TOTAL	
	Conc. (eq l^{-1})10^6	Conc. (mg l^{-1})		mg l^{-1} ml l^{-1}	Conc. (mg l^{-1})	Conc. (eq l^{-1})10^6		Conc. (eq l^{-1})10^6	Conc. (mg l^{-1})
NH_4^+	10	0.18	NH_4Cl	0.53	0.35	10	Cl^-	10	0.35
Ca^{2+}	5.0	0.10	CaF_2	0.2	0.09	5.0	F^-	5	0.09
Na^+	5.0	0.11	$NaNO_3$	0.41	0.3	4.8			
Mg^{2+}	3.0	0.036	$Mg(NO_3)_2 \cdot 6H_2O$	0.22	0.18	3.0	NO_3^-	20	1.25
H^+	12	0.012	HNO_3 67%	0.78	0.77	12			
H^+	28	0.028	H_2SO_4 96%	1.36	1.3	28	SO_4^{2-}	30	1.43

Further addition of 0.5 M Na_2CO_3 *in order to reach* **pH = 5.9**

Sample pre-treatment



Analysis



RESULTS

Soil Physical-Chemical Characteristics

	Before planting	After harvesting	
		Canola	Vetiver
Coarse soil (g/kg)	8		
Classification *for Coarse soil*	Absent [< 10 g/kg]		
Moisture (%)	5.6		
Bulk Density (g/m^3)	0.92		
pH (H_2O)	8.1	7.8	7.9
Classification *for pH (H_2O)*	Moderately Alkaline [7.9 ÷ 8.4]		
pH ($CaCl_2$)	7.7		
EC (dS/m) - 25°C 2:1 soultion:soil ratio	0.40	0.35	0.20
CEC ($cmol_{(+)}$/kg)	14.4	14.2	14.1
Classification *for CEC*	Medium [10 ÷ 20 cmol(+)/kg]		

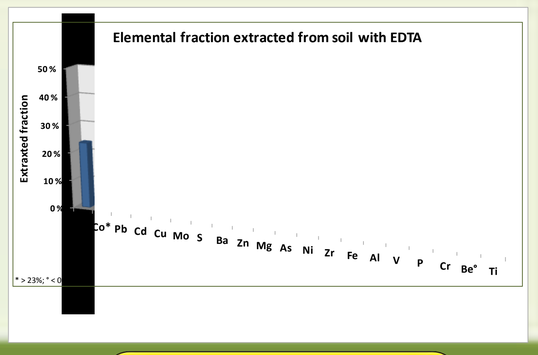
Elements in soil

Total content of elements in soils (mg/kg)

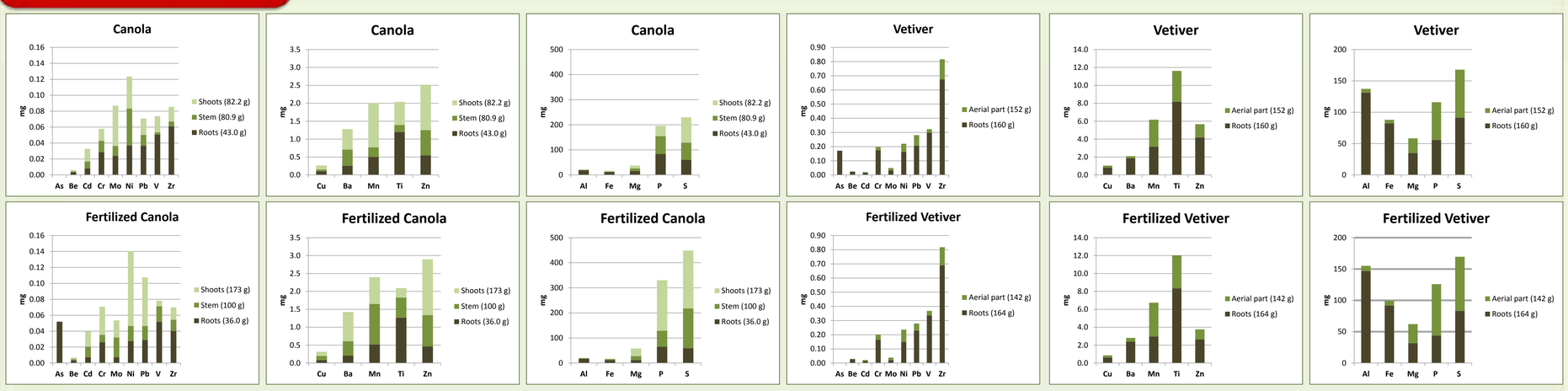
		Al	As	Ba	Be	Cd	Co	Cr	Cu	Fe	Mg	Mn	Mo	Ni	P	Pb	S	Ti	V	Zn	Zr
Before planting		4.4E+04	28	9.4E+02	8.8	1.5	< 15	75	65	3.9E+04	2.6E+03	1.12E+03	2.6	47	1.4E+03	1.0E+02	2.7E+02	4.2E+03	1.7E+02	1.1E+02	2.9E+02
After harvesting	Canola	3.9E+04	27	8.9E+02	8.1	1.2	< 15	74	55	3.7E+04	2.1E+03	1.07E+03	2.6	43	1.3E+03	1.1E+02	2.2E+02	3.9E+03	1.5E+02	99	2.6E+02
	Fertilized Canola	4.1E+04	29	9.3E+02	8.4	1.4	< 15	71	57	3.7E+04	2.2E+03	1.10E+03	2.2	43	1.4E+03	98	2.6E+02	3.9E+03	1.6E+02	1.0E+02	2.7E+02
	Vetiver	4.1E+04	31	9.2E+02	8.4	1.5	< 15	72	59	3.8E+04	2.2E+03	1.09E+03	2.1	43	1.4E+03	1.0E+02	2.7E+02	4.0E+03	1.6E+02	1.0E+02	2.7E+02
	Fertilized Vetiver	4.0E+04	30	9.4E+02	8.4	1.3	< 15	68	58	3.8E+04	2.3E+03	1.06E+03	1.7	43	1.3E+03	97	2.8E+02	3.9E+03	1.6E+02	1.0E+02	2.7E+02

Elements extracted in EDTA (mg/kg)

		Al	As	Ba	Be	Cd	Co	Cr	Cu	Fe	Mg	Mn	Mo	Ni	P	Pb	S	Ti	V	Zn	Zr
Before planting		1.1E+02	0.70	78	< 0.005	0.25	3.5	0.05	9.9	1.1E+02	1.2E+02	2.63E+02	0.37	1.1	1.7	22	24	0.32	0.26	8.8	3.6
After harvesting	Canola	76	0.69	68	< 0.005	0.17	3.2	0.05	7.6	89	99	2.51E+02	0.29	0.96	1.1	16	10	0.75	0.46	6.7	3.3
	Fertilized Canola	86	0.67	63	< 0.005	0.16	3.3	0.05	7.7	89	1.0E+02	2.59E+02	0.30	1.1	1.6	16	28	0.70	0.33	6.6	2.9
	Vetiver	93	0.79	73	< 0.005	0.18	3.3	0.05	8.5	98	1.1E+02	2.57E+02	0.32	1.1	1.1	17	13	0.86	0.43	7.4	3.0
	Fertilized Vetiver	90	0.64	70	< 0.005	0.18	3.4	0.05	8.4	96	1.1E+02	2.59E+02	0.37	1.0	1.2	18	25	0.98	0.45	7.3	3.4



Elements in vegetables



Translocation Factors (TF)

	Al	Ba	Be	Cd	Cr	Cu	Fe	Mg	Mn	Mo	Ni	P	Pb	S	Ti	V	Zn	Zr
Canola	0.037	1.05	0.182	0.777	0.267	0.388	0.110	0.340	0.784	0.700	0.611	0.351	0.243	0.730	0.184	0.118	0.951	0.105
Fertilized Canola	41	1.35		1.64	4.11	2.91	13	2.27	1.71	3.28	2.10	2.02	2.99	1.00	9.31	12	1.43	13
Vetiver	0.023	0.756		0.579	0.223	0.361	0.065	0.479	0.473	0.829	0.532	0.528	0.356	0.858	0.085	0.067	0.685	0.097
Fertilized Vetiver	12	3.81	7.53	5.37	4.79	1.65	8.68	2.27	0.734	1.88	2.07	4.65	1.63	3.31	1.57	9.01	1.34	1.20

Translocation Factor (TF) = C_{AP}/ C_R

C_{AP} = element al concentration in the plant aerial part

C_R = elemental concentration in the roots

TF < 1 → the plant accumulates the element in the roots

TF ~ 1 → the element is fairly distributed between the roots and the aerial part

TF < 1 → the plant accumulates the element in the aerial part

From soil to plants

Bioconcentration Factors (BF)

BF in respect to the elemental total contents in soil

		Al	As	Ba	Be	Cd	Cr	Cu	Fe	Mg	Mn	Mo	Ni	P	Pb	S	Ti	V	Zn	Zr
Canola	Aerial part	0.0004		0.007	0.002	0.102	0.002	0.015	0.001	0.050	0.008	0.150	0.011	0.495	0.002	3.88	0.001	0.001	0.113	0.001
	Whole plant	0.002		0.007	0.003	0.109	0.004	0.020	0.002	0.071	0.009	0.164	0.013	0.686	0.003	4.18	0.002	0.002	0.114	0.001
Vetiver	Aerial part	0.001		0.002	0.001	0.026	0.002	0.022	0.001	0.060	0.018	0.042	0.008	0.284	0.005	1.87	0.005	0.001	0.091	0.003
	Whole plant	0.010	0.020	0.007	0.009	0.044	0.008	0.051	0.007	0.073	0.018	0.061	0.015	0.268	0.009	2.01	0.009	0.006	0.170	0.009

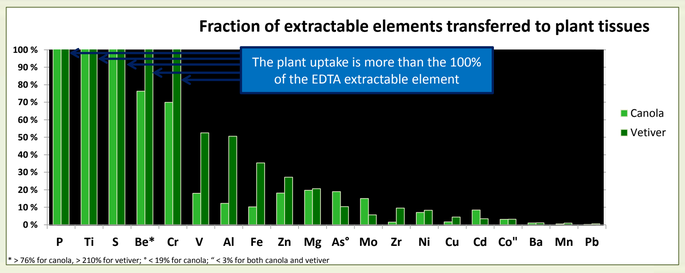
BF in respect to the element extracted in EDTA

		Al	As	Ba	Be	Cd	Cr	Cu	Fe	Mg	Mn	Mo	Ni	P	Pb	S	Ti	V	Zn	Zr
Canola	Aerial part	0.146		0.080		0.611	3.40	0.097	0.291	1.07	0.035	1.05	0.475	395	0.010	43.4	16.1	0.537	1.37	0.041
	Whole plant	0.937		0.080		0.648	5.35	0.128	0.783	1.51	0.037	1.14	0.538	547	0.016	46.8	31.1	1.37	1.39	0.115
Vetiver	Aerial part	0.340		0.020		0.155	2.90	0.143	0.363	1.28	0.075	0.294	0.336	226	0.023	21.0	70.8	0.614	1.10	0.258
	Whole plant	3.83	0.785	0.086		0.263	12.0	0.335	2.679	1.56	0.075	0.429	0.631	213	0.041	22.5	117	3.97	2.06	0.725

Bioconcentration Factor
BF = C_P/ C_S

C_P = elemental concentration In plant

C_S = element concentration in soil



Preliminary remarks

As preliminary remarks it should be pointed out that:

- ✓ for many elements, soil contamination levels are better evaluated by the extractable fraction rather than the total content
- ✓ for analytical purposes, extraction procedures should be chosen in relation to the element, its chemical state and the physical-chemical characteristics of soil
- ✓ the EDTA extractable fractions are resulted in many cases higher than 10 % (Mo, Cu, Cd) and 20 % (Pb, Co, Mn)
- ✓ after only a 5 months growth, for many elements (e.g. Al, Cd, Cu, Fe, Pb, Zn) there was a significant decrease of the EDTA extractable fraction
- ✓ in some cases (e.g. Ti and V) it was noted an increase of the EDTA extractable fraction after harvesting
- ✓ both for canola and vetiver, soil EC is decreased after harvesting (for vetiver is halved)
- ✓ for many elements vetiver shows an higher bioconcentration factor than canola, but the translocation factor is generally lower in respect to canola
- ✓ phosphatic fertilization increases the translocation factors both in canola and vetiver
- ✓ the bioconcentration factors calculated in respect to the elemental total contents are not significant, while those calculated in respect to the EDTA extractable fractions are much more relevant, especially for some elements (e.g. Cr, Ti, Zn)

Work in progress

- use of different extraction procedures (e.g. with CH_3COOH 0.43 M) to identify different bioavailable fractions of elements
- new pot-experiments
- field experiments, also to evaluate long term effects especially for perennial and mowable species (such as vetiver)

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