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ASSESSING BIOAVAILABILITY OF METALS IN BIOFUEL FEEDSTOCKS, AND IMPLICATIONS FOR CONTAMINATED LAND USE STRATEGIES

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Abstract

The production of energy crops such as Camelina sativa on contaminated land offers the possibility of a high-value low-cost long-term remediation strategy and a potential counterbalance to land abandonment. This shift in agrarian practice offers a potentially viable source of income to primary stakeholders and brings the land back into useful production. We report on the development of methodologies for charting the traceability of potentially toxic elements in camelina cultivated on contaminated land from soil to plant material and raw oil. Translocation factors for Cd and Zn suggest camelina has the potential to act as accumulator, offering potential phytoremediation benefits. However careful consideration of the use and value of the co-products is needed to determine an accurate business case scenario.

Key words: *biofuel, contaminated land, bioavailability, potential toxic elements, camelina, translocation factors.*

INTRODUCTION

Land is a finite resource upon which the human race is entirely dependent for its well-being (Bridges and van Baren, 1997; Louwagie et al., 2011). The common perception of land and soil as an infinitely exploitable resource is ultimately unsustainable (Eswaren et al., 2001; Gobin et al., 2004). On a European scale, there are many areas where land has become degraded or contaminated by human activities such as poor farming practice, mining, industry and waste disposal. Whilst estimates of contaminated land widely vary from region to region and are localised in nature, a very large fraction (>70%) of the currently identified three million sites affected by chemical land degradation can be attributed to anthropogenic pollution with predominant contaminants being potentially toxic elements (PTE) such as Pb, Cd and Zn, and mineral oils (EEA, 2007; EEA, 2010).

Contaminated land represents a particular problem in that it is often abandoned and left as unsightly wasteland that can have a detrimental effect on health and the social-economics of the area. Concerns that PTEs may detrimentally enter the food chain if crop production is undertaken and issues relating to receptors, end-points and overall fitness often restrict the usage of such land.

Remedial treatment of these sites is often costly or unsustainable. However, in some cases the production of energy crops such as *Camelina sativa* (camelina) offers a possible high-value-low-cost long-term remediation strategy and a potential counterbalance to land abandonment (Keenleyside and Tucker, 2010). This shift in agrarian practice offers an attractive alternative that allows the primary stakeholders a potentially viable source of income and brings the land back into useful production (Hoogwijk et al., 2003; Campbell et al., 2008; Gallagher, 2008; Fargioneet et al., 2010; Cai et al., 2011); whilst allowing the demands of the Common Agricultural Policy (CAP) and key imperatives of the Renewal Energy Directive to be met (EEA 2007b; Directive 2009/28/EC).

Nevertheless, concerns are raised that camelina feedstock grown on contaminated land or irrigated with contaminated water, may have trace metals present within the plant matter and the economic viability of the crop may be compromised.

As part of EU FP7 project: Initiative Towards sustainable Kerosene for Aviation (ITAKA) a methodology for traceability of the PTEs in the value chain to assess impacts of cultivating camelina on contaminated land was developed using camelina crops grown on four metal contaminated field sites in Romania. This paper presents some of the initial findings from the case-study site at Rovinari, Gorj County, an overburden-dump site from the local lignite mines.

MATERIALS AND METHODS

The current study focussed on the development of methodologies for the traceability of PTEs in camelina cultivated on contaminated land. Such methodologies were needed as the cultivation of energy crops on contaminated land is an innovative approach, and there are few standard methods to draw upon. The methodology has been devised to consider the key three compartments: soil, plant material and raw oil.

Given the proposed end use of the camelina oil in aviation biofuel and the challenges posed in developing an effective methodology, the assessment of the methodology focussed on a small subset of key metals identified in Def-Stan 91-91 and by industrial stakeholders to be of greatest concern with respect to thermal instability and turbine rotor degradation.

In the field cultivation trials, camelina was cultivated at selected field sites in Romania (Figure 1). With all field study trials, these crops are subject to a much wider range of unregulated environmental parameters than in greenhouse trials, therefore rigorous and standardised sampling protocols should be adhered to in the analysis to minimise experimental uncertainty.

Figure 1. Locations of the four Romanian contaminated field sites

A comprehensive elemental analysis of the soil at each of the Romanian field cultivation trial sites was conducted, pre-cultivation and pre-harvest as a preliminary assessment for camelina's remediative potential. The soil characterisation methodology employed a random stratified sampling protocol using a minimum of 25 samples/ha in accordance with ISO 10382-1:2002 to allow for the inherent uncertainties in the distribution of metals in contaminated soils. Partial digestion of the bulk base compost was carried out using *aqua regia* (AnalaR quality), HCl (aq) (32.25%) and HNO_3 (aq) (69%), (in ratio 3:1 v/v)), with quantities compliant with ISO 11466:1995. Elemental concentration of 20 elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Ti, V and Zn) of digestion analytes were determined using ICP-OES analysis. Dutch Target and Intervention Values for soil remediation (DTIV) (VROM, 2000, VROM, 2009) and the Romanian Reference Values (RRV) have been employed to evaluate the degree of contamination in the soil samples analysed, for the 11 target-metals As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn, the presence of which have been highlighted to be of concern in bio-kerosene (Def Stan 91-91).

Analysis of plant material was undertaken the 11 target-metals using trace element analysis reagents and microwave-assisted digestion, with the methodology incorporating key elements of EN13804:2013; EN13805:2002 and EN14084:2003. For the determination of trace metals in oil, a number of industry standard methods (including UOP 389 and ASTM D7771-15 / ASTM D5185-09) were trialled and assessed with regard to reliability and reproducibility, and two indirect methods using EDTA extraction followed by ICP-OES analysis, and microwave-assisted digestion and subsequent ICP-OES analysis. Overall, the indirect method of acid microwave-assisted digestion was considered the most reliable technique.

Study site

The case study site is located at Rovinari, Gorj County. Rovinari (44°55' 17.9"N; 23°10' 51"E) is a mining town in Gorj County, Oltenia, Romania, located on the E79 and next to the River Jiu; it is approximately 288 km west-northwest of Bucharest and 24 km south-west of the county seat, Târgu Jiu.

Rovinari and its environs form one the largest open cast lignite mines in Romania. The extracted lignite is used to power the Complexul Energetic Rovinari thermo-electrical power plant situated close to the town.

The field study site is surrounded by the Carrier Gala lignite quarry located to east the town of Rovinari. The Sterile site is an area where the overburden material of the lignite quarry has been deposited. The soil matrix is composed of yellow-brown (2.5Y68) clays with pebble-cobble sized rock fragments and lignite fragments. The total carbon and total nitrogen range of the soil is determined to be between 2.6-8.9% and 0.10-0.35%, respectively, with a C:N ratio of 21-33. The soil displays a degree of alkalinity, pH 8.2-8.4, similar to pH values have reported for other sterile dumps within the locality (Cărăbis et al., 2011). The soil had been previously conditioned with lignite-based fertilizer and cropped with maize, with a crop yield estimated at 5.5 tonne/ha. The study site was ploughed to a depth of 300 mm, cultivated and was sown to a crop of *Camelina sativa*, cultivar GP202, in the autumn of 2012. During the growing season (May 2013) a crop survey was carried out; 12 randomly selected 1 metre squares being sampled for plant density, height and branching to assess crop viability.

RESULTS AND DISCUSSION

Soil

The geochemical characterisation of the metals within the soil for the case study site, Rovinari-Sterile showed pre-cultivation concentrations of the 11 target -metals As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn, to be 7.7 mg kg^{-1}; 0.80 mg kg^{-1};11mg kg^{-1}; 35 mg kg^{-1}; 19 mg kg^{-1}; 2%; 520 mg kg^{-1}; 43 mg kg^{-1};10 mg kg^{-1}; 30 mg kg^{-1} and 58 mg kg^{-1} respectively. As, Cr and Ni concentrations were found to be in excess of the DITV and RRV, although they do not exceed the Target / Threshold (sensitive areas) of the DITV and RRV, respectively. Comparison of the data of the current research with concentrations reported by other authors (Dodocioiu and Susinski, 2010; Bălăceanu et al.¸2011; Gămăneci and Căpăţînă, 2011) for such metals in soils within 7.5 km radius of the thermal power plant at Rovinari showed the concentrations of Cd, Cu, Mn, Ni, Pb and Zn determined for this study site to be predominantly within the range determined in the earlier studies; whilst the soil concentration of Co at the Rovinari Sterile site was found to below the lower limit. The comparative differences in the concentrations of Cd, Co, Cu, Mn, Pb and Zn, may arise in part due to the location of the study site to the northeast of the Rovinari power complex. It is reported, that the predominant wind direction is from the north and northeast (Bălăceanu et al.¸2011). This may have resulted in a lower degree of enrichment of the soil by atmospheric deposition from power plant stack emissions than experienced by areas to the south and south-west of the power complex (Lazar et al., 2008; Bălăceanu et al., 2011). Kruskal-Wallis one-way analysis of variance by ranks was used to identify any significant differences in the measure of central tendency for the determined soil metal concentrations from the pre-harvesting and pre-cultivation sampling. Significant differences ($P<0.05$) between the median values for the two samplings was observed for the metals, Al, Ba, Ca, Cd, Fe, K, Mg, Mo, S and Ti, of these Cd, Mg and S were the differences were found to be strongly significant ($P < 0.001$). The lack of any apparent significant difference between the pre-harvesting and pre-cultivation median soil concentration of As, Co, Cr, Cu, Ni, Pb V and Zn suggests that in terms of remediative potential, suggests that the uptake in to the camelina crop is likely to be insufficient for such metals to have a measurable remediative affect in a single cropping year.

Crop Survey and Production

There appeared to be delineation in crop morphology between the southern and northern half of the field. In the southern part vigorous crop growth is observed with crop heights of 30-82 cm, > 60% of plants displaying branched flower inflorescences and crop emergence commonly between 60-100%, with plant counts of 60-200/m^2. By contrast, the northern section of the study site show higher plant counts/m^2 (260-450) but plants are characteristically smaller (20-70 cm) and exhibit spindly growth with lower incidence of branched flower inflorescences (< 60%). Foliar effects similar to those seen at other study sites (details discussed elsewhere in ITAKA deliverable D5.17 suggesting underlying causative factors. Red margins and red-orange mottling, along with necrotic lesions and interveinal chlorotic areas on leaves (Figure 2) were also noted.

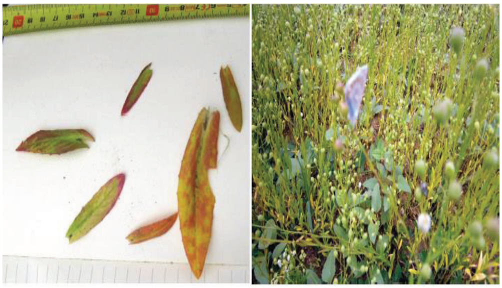

Figure 2. Stress induced variations in foliar chlorosis and discolouration at Rovinari study site

To determine the viability of contaminated land for production of camelina, comparison with camelina crops grown on uncontaminated land in similar climatic and pedological conditions is desirable. Cultivation of camelina in the agronomic trial plots have been undertaken at the didactic farm Moara Domnească (SDE Belciugatele - USAVMB), Ilfov County, to determine optimal agronomy for the camelina crop varieties including GP202 and GP204 (Dobre et al., 2014). Potential yields in excess of 1400 kg/ha were achievable but were dependent on cultivar. The best potential yields were found to occur with the camelina cultivar GP202.Consideration of the production (330 kg/ha) from the Romanian contaminated land field site, Rovinari-Sterile finds that the production was 60% of that of achieved in the agronomic trials Moara Domnească, Ilfov when no chemical fertilizer is applied. The disparities in the yield of camelina between the agronomic trials and the contaminated study sites may not arise solely from the adverse influence of contaminant levels on nutrient uptake but may also reflect differences in soil parameters such as soil OM, soil mineralogy and soil acidity.

Metal transference

Typically, other research has primarily focussed on the uptake of metals into the roots, shoots and leaves (Ebbs and Kochian, 1997; Baryla et al., 2001; Chatterjee and Chatterjee, 2000; Shanker et al., 2005; Yoon et al., 2006; Ben Ghnaya et al., 2009; John et al., 2009; Sinha et al., 2010; Pourrut et al., 2011; Tian et al., 2014). However due to need to assess whether of the uptake of the metals As, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn into the co-products, such as oil meal and silicles (seed-pods), are potentially detrimental to the business case for camelina, the concentrations of metals in the four compartments, roots, shoots, silicles and seed, were determined. The results of the analysis for the pre-harvest plant material from the 2012/2013 camelina crop cultivated at the Rovinari-Sterile site for the metals of concern to the aviation industry (Cd, Co, Cu, Fe, V and Zn) and metals of concern to the food chain (As, Cr, Ni and Pb) are presented in Figure 3.

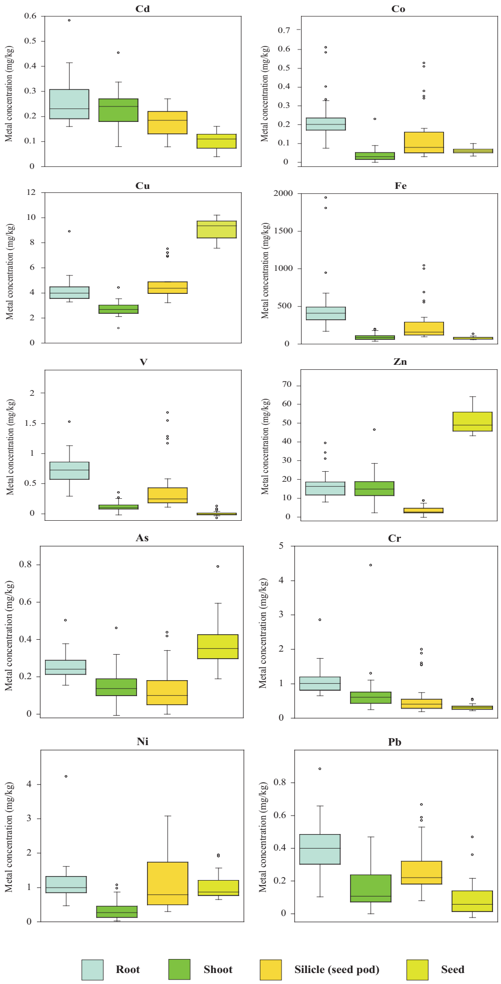

Figure 3. Concentrations of the specific target-metals in roots, shoots, silicles and seeds from *Camelina sativa* plant material harvested from the 2012-2013 cultivation at the Rovinari field site

Elemental analysis of the four plant components of roots, shoots, silicles, and seed from the Rovinari-Sterile case-study site, found that As, Cu and Zn were present in the highest concentrations within the seed. Given the well-established relationship between As and P in both soil and plant material, this is not unexpected and the management of available soil P may be key to the future successful of camelina production on As contaminated sites.

Consideration of the near parity noted between the root and shoot concentration of Cd and Zn, suggests not only a higher degree of transference of such metals from the roots into the aerial parts of the plants compared with As, Cr, Cu, Fe, Ni, Pb and V, but also possible similarities in the uptake mechanism into the plant, for example, the uptake of Cd via Zn^{2+} channels of low specificity (Clemens, 2006). Inspection of the subsequent differences in the partitioning of Cd and Zn in the silicles and seed (Figure 3) further suggest that in agreement with the findings for *Brassica napus* the storage of Cd in camelina is likely to be in the leaf and stem organelles rather than in the fruit of camelina as inferred for Zn (Baryla et al., 2001; Carrier et al., 2003; Verbruggen et al., 2009).

The phytoremediation potential in terms of the ability of *Camelina sativa* to tolerate and accumulate the metals As, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn can be estimated using bioconcentration factors (BCF) and translocation factors (TF).

Bioconcentration factors are used to assess the ability of a plant to accumulate metals from the soil and are defined as the ratio of metal concentration in the plant compartment (root, shoot, silicles, and seed) to that in the soil. A plant's ability to tolerate or accumulate through the translocation of metals in the first instance from the roots to the shoots is assessed using TFs, which are defined as the ratios of metal concentration in the shoots to the roots. To examine the extent to which metals are transported to the silicles and seed after transference from the root to the aerial parts of the plant TFs for the movement of metals from the shoot to silicles and the shoot to seed were also calculated. The BCFs for the four plant compartments analysed for the Rovinari-Sterile camelina crop along with the corresponding TFs for the metals, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn are summarized in Table 1.

Scrutiny of the BCFs determined that the camelina roots from the Rovinari-Sterile site were most efficient in taking up Cd, Cu and Zn (BCF: 0.39; 0.23; 0.26, respectively). Bioconcentration factors lower than 0.2 are expected where plants are grown on contaminated soil (McGrath and Zhao, 2003; Brunetti et al., 2010). Similarly the TF values suggest that camelina is most efficient at translocating the same three metals Cd (TF: 0.91), Cu (TF: 0.68) and Zn (TF: 1.0).

Table 1. Bioconcentration factors and translocation factors for the specific target-metals in *Camelina sativa* grown at the Rovinari site

		Elemental concentration (mg kg^{-1} dry weight)									
		As	Cd	Co	Cr	Cu	Fe	Ni	Pb	V	Zn
BCF											
Root/soil	Median	0.030	0.39	0.017	0.026	0.23	0.019	0.024	0.039	0.023	0.26
	IQR	0.0091	0.198	0.0091	0.0123	0.0365	0.00755	0.0119	0.0210	0.00964	0.0955
Shoot/soil	Median	0.018	0.38	0.0025	0.016	0.15	0.0040	0.0068	0.012	0.0037	0.24
	IQR	0.0105	0.158	0.0318	0.00821	0.0311	0.00199	0.00673	0.0173	0.00238	0.131
Silicles/soil	Median	0.012	0.28	0.0072	0.011	0.25	0.0070	0.020	0.023	0.0087	0.047
	IQR	0.0228	0.116	0.00965	0.00596	0.0745	0.00849	0.0322	0.0150	0.00877	0.0475
Seed/soil	Median	0.045	0.17	0.050	0.0077	0.53	0.0031	0.0021	0.0067	0.00018	0.81
	IQR	0.0185	0.120	0.0170	0.00233	0.148	0.00150	0.00994	0.0138	0.00126	0.236
TF											
Shoot/root	Median	0.63	0.91	0.15	0.59	0.68	0.24	0.23	0.35	0.17	1.0
	IQR	0.376	0.294	0.086	0.291	0.140	0.103	0.29	0.255	0.0957	0.417
Silicles/shoots	Median	0.57	0.77	2.6	0.81	1.7	2.4	3.5	2.1	2.4	0.18
	IQR	0.980	0.273	4.38	0.775	0.293	2.2	6.95	1.74	2.77	0.194
Seed/shoot	Median	2.3	0.45	1.8	0.53	3.3	0.90	3.2	0.38	0.058	3.6
	IQR	1.50	0.186	2.22	0.374	0.729	0.703	3.84	0.474	0.320	1.4

The transfer of metal contaminants to the shoot, silicles and seeds are found to decrease with the exception of the transfer of As, Cu and Zn from the soil to the seed. It may be anticipated that as Cu and Zn are essential plant micronutrients that the uptake and translocation in the plant would be enhanced. By contrast, the BCF for Cd is an order of magnitude higher than other non-essential metals such as As, Co, Cr, Ni, Pb and V considered. Other authors have suggested that TF values less than unity were indicative of tolerance to a given metal in the plant (Brunetti et al., 2010), and where TFs < 0.60 this may be suggestive of restricted uptake and possible exclusion mechanisms being operational in the plant (Baker and Brooks, 1989; Yoon et al., 2006). Brunetti et al. (2010) further suggested that TF values greater than one are indicative of accumulator plants, therefore it is possible that *Camelina sativa* has the potential to act as accumulator for Zn and Cd. Although it is known that other Brassica species act as hyper accumulators for Cd and Zn (Carrier et al., 2003; Verbruggen et al., 2009) further work is needed to verify the accumulator status of camelina with respect to these two metals. Consideration of TFs calculated to assess the efficiency with which metals are transferred from the shoot into the silicles and seeds, suggest that whilst the movement of Co and Ni into the shoots from the roots may be restricted, once in the aerial part of the plant such metals are readily mobilized to the silicles and seeds. Further work is needed to assess the impact of such mobility on the food chain, should the any exclusion mechanisms that are operational be negated. The degree of variability displayed by the TF data suggests that there may be genetic variation in the ability to tolerate or accumulate metals within the plant population of the camelina crop (Yang et al., 2005).

Oil

Analysis of the oil extracted from the camelina grown on the four contaminated sites indicated that concentrations of Cd, Co and V were below the limit of detection. By contrast, Cu and Fe were present in all oil samples whilst there was no consistency in the occurrence of Zn in the oil samples.

Consideration of the analysis of the oil extracted from the camelina grown on Rovinari-Sterile site indicated that concentrations of Cd, Co, Ni, V and Zn were below the limit of detection. Whilst the concentration of As (0.36 mg kg^{-1}), Cu (0.66 mg kg^{-1})and Fe (0.27 mg kg^{-1}) present in the oil samples were at least an order of magnitude greater than those discerned for Cr (0.007 mg kg^{-1}) and Pb (0.03 mg kg^{-1}).

Oil from camelina grown on a nominal non-polluted site (Moara Domnească) was also analysed for comparative purposes. Analysis of the trace element concentrations in the composite oil samples for all the Romanian study sites, focused on the six metals Cd, Co, Cu, Fe, V and Zn, previously identified as problematic within aviation fuel and in keeping with the developed methodology. Scrutiny of the oil data sets suggest that in particular, for the concentration of Cu in the oil is influenced by the cultivar of camelina grown as well as soil characteristics such as pH and external crop nutrient inputs.

CONCLUSIONS

The use of the camelina co-products (oil-meal, silicles and straw) as a valuable component to animal feeds, in particular in the poultry industry, has been fundamental to the business case scenario for camelina biofuel production. Consideration is given to whether co-products from camelina crops produced on contaminated is likely to have the same import.

Translocation factors for Cd and Zn suggest *Camelina sativa* has the potential to act as accumulator. Careful consideration of the use and value of the co-products from camelina grown on certain contaminated lands is therefore recommended: The fractional distribution of metals in the oil and seed is non-uniform, so the oil extraction process may affect a multiplicative increase in the concentration of metals in the crushed seed. Which when given the potential for direct access to the food chain from the use of meal for livestock fodder, and the use of shoot material as bedding material could be of concern. To minimize such concerns it is suggested that the use camelina straw from Cd contaminated sites is restricted to use as a soil conditioner, where appreciable cost benefits may be achieved in terms carbon sequestration and agronomic improvements in soil organic matter.

Comparison of the oil metal concentrations determined for the four contaminated sites with that of oil from the nominally unpolluted site at Moara Domnească, highlight the need for further work to determine the effect of external crop inputs, such as nitrate fertilizers, on the uptake, translocation and storage of metals in the camelina crop grown on contaminated land. This study has developed an effective methodology for the measurement of metals in the camelina value chain, and by evaluating and defining some of the specific vulnerabilities of camelina physiology, gone some way to answering the broad and rather imprecise question of ‘Can a biofuel such as camelina be grown on contaminated land?' The equally imprecise answer is 'Yes, but site specific characteristics must be duly considered'.

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