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D5.7 [Methodology for traceability of the heavy metals in the value chain to assess impacts of cultivating camelina on contaminated land]

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Executive summary

Land is a finite resource upon which Mankind is entirely dependent for its well-being. The common perception of land and soil as an infinitely exploitable resource is ultimately unsustainable. 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: It is estimated that up to 40% of all EU agricultural land is seriously degraded [eg. Bot et al, 2000]. Estimates of contaminated land widely vary from region to region and are more localised in nature, though 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 lead (Pb), cadmium (Cd) and zinc (Zn), and mineral oils.

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 social-economics of the area. Concerns that PTEs may detrimentally enter the food chain if food 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 offers a possible high-value-low-cost long term remediation strategy. 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. Nevertheless, concerns are raised that camelina feedstock grown on contaminated land or irrigated with contaminated water from run-off, may have trace metals present within the plant matter and the economic viability of the crop may be compromised.

This report describes the development of a methodology for the traceability of heavy metals in camelina cultivated on contaminated land. A robust methodology needed to be developed as there is no single protocol to determine the traceability of heavy metals in a biofuel crop, such as camelina, grown on contaminated land. To deliver the task objectives, established international standards are combined with methodologies that have been trialled and developed to meet the unique character of camelina co-products. The assessment of the methodology incorporated two distinct yet complimentary approaches to validate and corroborate its applicability: i) controlled cultivation trials in which camelina was grown in soil ‘spiked’ with known metal contaminant concentrations; and ii) field study cultivation trials in which camelina was grown on four contaminated field study sites in Romania. Whilst the former approach evaluates the susceptibility of camelina to the uptake of certain metals in a controlled and well defined manor (ie. an evaluation of the plants physiological response to metals), the latter represents an initial assessment of the feasibility of using contaminated land for camelina biofuel production, though it should be emphasized that not all contaminated land is the same and so the extrapolation of the findings to other sites should be done with some caution.

Due to the challenges posed in developing an effective methodology that addressed the various interdisciplinary components of the research, the assessment of the methodology focussed in the first instance on the six metals, cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), vanadium (V) and zinc (Zn), identified in Def-Stan 91-91 and by industrial stakeholders to be of greatest concern with respect to thermal instability and turbine rotor degradation.

The controlled cultivation trials indicate that camelina has a reasonable degree of tolerance to the presence of these six metals in the soil for all but the highest concentrations. Elemental analysis of the plant matter in the controlled cultivation trials have shown that the camelina seed is naturally high in the essential micro-nutrients Fe and Zn, conversely the concentration of V was found to be below the experimental limits of detection. The trace metals Cd and Co show the greatest potential for uptake into the seed with measured concentrations reaching several hundred times greater than their corresponding natural baseline levels. Hence the uptake of Cd may prove to be problematic in certain production scenarios with camelina grown on certain soils, since the primary co-product (to the camelina oil destined for the biofuels market) is crushed camelina seed husks

destined for the animal feed market. The data indicate that Cd concentrations in the seed can potentially reach levels that could render the animal feed as unfit for consumption (given that the concentrations of Cd in food destined for human consumption typically ranges from 0.2 to 2 mg kg^{-1}, and assuming that concentrations of this order would be similarly acceptable to the animal feed market).

For the four contaminated field study sites in Romania, Câmpina, Prahova County (industrial waste land) Copşa Mică, Sibiu County (non-ferrous smelter impacted) and Rovinari, Gorj County (fly-ash and lignite overburden sterile dumps) a two phase assessment of trace metal contaminants was made throughout the agronomy chain from soil, plant matter, harvested seeds, and raw oil. In the first phase, pre-planting geochemical analysis of soils was undertaken to assess the individual soils for metal concentrations, soil acidity and total carbon and nitrogen at all four sites. Qualitative assessment of the growing crop was used to evaluate crop vigour and vitality, and a post-harvest assessment of the crop was carried out in terms of yield and the transference of the key metals Cd, Co, Cu, Fe, V and Zn into the seed and oil. In the second phase of the assessment, contaminant heavy metals were traced through all components of the system for the experimental site (Sterile dump) at Rovinari, Gorj county, Romania. The rationale for the selection of this site was based not only on the higher levels of production (330 kg/ha), that suggested that this was the most economically viable site. but also because the soil of the Rovinari Sterile exhibited overall the lowest concentrations of metal contaminants and therefore the robustness of the methodology could be validated for the field cultivations and identify if Camelina sativa hyperaccumulates specific metals at low levels that could be potential limit production of camelina on contaminated sites..

The geochemical characterisation of the metals within the soil at the four Romanian field sites highlighted the presence of high levels of As, Cd, Ni, Pb and Zn that may be of concern with regard to transference into the crop and potentially the seed. Subsequent 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, whilst Cu and Fe were present in all oil samples, and there was no consistency in the occurrence of Zn. However, 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, which does have implications for the usage of the camelina co-products. Given the well established relationship between the uptake of As and the uptake of P in plant material, the presence of As is not unexpected and the management of available soil P would be a necessary consideration for camelina production on As contaminated sites. Whereas for Cd and Zn, the shoot to root concentration ratio suggest that *Camelina sativa* has the potential to act as accumulator for such metals. Hence a careful consideration of the use and value of the co-products from camelina grown on certain contaminated lands is recommended. This non-uniform partitioning of metals between the oil and seed also has knock on effects when camelina is pressed and processed since the majority of these metals are retained in the seed. Hence the metal concentrations in the crushed seed effectively increase when the oil is extracted and its use in meal for livestock fodder could be of concern. Similarly, the use of shoot material as bedding material could be of concern for metals such as Cd that are primarily stored in the shoot material. To minimize such concerns it is suggested that the use camelina straw from Cd contaminated sites is retained on the source site and used 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 Domneasca, found that as with the four experimental sites oil concentrations Cd, Co and V were established to be below the limit of detection, and concentrations of Cu, Fe, and Zn were below 1 mg kg^{-1}. However, the concentration of Cu in the Moara Domneasca oil was shown to be the highest of all the five oils analysed. Similarly, of Zn in the Moara Domneasca oil was second highest, highlighting 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.

We must conclude that the relation between the metal contaminants present in soil and those that ultimately become resident in the various components of a mature camelina plant is undoubtedly complex, depending upon a large number of parameters of which some are independent, some are interdependent, and some are site specific. 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 camelina be grown on contaminated land?’ The equally imprecise answer is ‘Yes, but site specific characteristics must be duly considered’. Camelina can be grown on certain contaminated land sites but does show signs of stress and yields are reduced. The oil could potentially be used as a biofuel feedstock though some pre-processing may be necessary. Containment metals within the co-products are of more direct concern. Remediation of soils and the mineralisation of biologically available metal contaminants is a long term endeavour that could not be assessed within the scope of this relatively short-term study, Nevertheless, this study (and the work done by Biotehgen reported elsewhere in ITAKA) has shown that with appropriate management with regard to contaminant transfer, camelina can be grown on certain contaminated land sites.

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Abbreviations

Al	Aluminium
As	Arsenic
Ba	Barium
BF	Bioconcentration factor
C	Carbon
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
EDTA	Ethylene diamine tetraacetic acid
EU RED	European union renewable energy directive
FAO	United nations food and agriculture organisation
Fe	Iron
FLA	Farm land abandonment
GLASOD	Global assessment of soil degradation
H-ISD	Human induced soil degradation
ICP-OES	Inductively coupled plasma – optical emission spectroscopy
ISRIC	International soil reference and information centre
K	Potassium
Mha	Million hectares
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
PTE	Potentially Toxic Elements
RED	Renewable energy directive
S	Sulphur
SOC	Soil organic carbon
TC	Total Carbon
TF	Translocation Factor
Ti	Titanium
TIC	Total inorganic carbon
TN	Total nitrogen
TOC	Total organic carbon
UAA	Utilised agriculture area
UNEP	United nations environmental program
V	Vanadium
Zn	Zinc

Definitions

Accumulator:	Plants that gather and store micronutrients, macronutrients, or minerals.
Aerial part of plants:	Parts of the plant that grow above the soil–air interface. Which for purpose of this task include ***shoots:*** plant material including stems, limbs and leaves; ***silicles:*** short broad silique or seed capsule; ***seed:*** an embryonic plant enclosed in a protective outer silicle and is the source of oil.
Bioavailability:	The degree to which a substance is available for uptake into a living system.
Bioconcentration:	The accumulation or increase in concentration of a chemical in an organism.
Bioconcentration Factor:	The ratio of metal concentration in the plant compartment to that in the soil and used to assess the ability of a plant to accumulate metals from the soil.
Contaminated Land:	For the purposes of this task contaminated land is defined as land that has been chemically degraded by potentially toxic elements (PTE) such as lead (Pb), cadmium (Cd) and zinc (Zn), due to the land becoming degraded or contaminated as a result of human activities such as poor farming practice, mining, industry and waste disposal.
Cultivar:	A plant or grouping of plants selected for desirable characteristics that can be maintained by propagation and has been achieved through selective breeding.
Edaphic:	Pertaining to the nature of soil. Edaphic qualities may characterize the soil itself, including drainage, texture, or chemical properties such as pH.
Endosperm:	The tissue within the seeds of most flowering plants that surrounds and nourishes the developing embryo. It is tissue where the oil is stored.
Excluder:	Plant species that limit the translocation of heavy metals and maintain low levels of contaminants in their aerial tissues over an extensive range of soil concentrations.
Genotypic:	For the purpose of this task genotype is defined as a group of plants with the same genetic constitution.
Hyperaccumulator:	Hyperaccumulators of Co, Cu, Cr, Pb and Ni arc here defined as plants containing over 1000 mg kg $^{-1}$ of any of these elements in the plant dry matter; for Mn and Zn, the criterion is 10,000 mg kg $^{-1}$ (1%).
Macronutrients:	Substance or element that is required by the plant in large amounts for healthy growth and developments.
Metal transference:	The movement of metal salts from the soil to plant roots and subsequent movement to the aerial parts of the plants.
MIcronutrients:	Substance or element that is required by the plant in trace amounts for healthy growth and developments.
Phenotype:	The physical expression of a genotype.

Phytoaccumulation:	Accumulation of micronutrients, macronutrients, or minerals by plants.
Phytoremediation:	Describes the treatment of environmental problems, such as the metal contamination of soils, through the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere.
Roots:	Parts of the plant that grow below the soil–air interface and are the organ of a higher plant that anchors the rest of the plant in the soil, and absorbs water, micronutrients, macronutrients, or mineral salt from the soil.
Siliqua:	Narrow elongated seed capsule peculiar to the family Brassicaea.
Testa:	Hard protective outer layer (seed coat) of the seeds of flowering plants.
Translocation Factor:	Defined as the ratios of metal concentration in the shoots to the roots and is indicative of a plant's ability to tolerate or accumulate through the translocation of metals in the first instance from the roots to the shoots.

1 Context and background

Policy

Under certain cultivation scenarios biofuels may well compete with other land uses such as food production. Furthermore, this competition will only intensify as the demand for biofuels increases and the on-going challenge for the production of biofuels is finding sufficient suitable land on which to grow feedstocks. One option that has generated significant interest and is supported by a number of international organisations including the UN-coordinated Global Bioenergy Partnership is the cultivation of biofuel feedstocks on contaminated and degraded land. Although it should be recognised that degraded lands are also the target of several other initiatives such as the Bonn Challenge to restore 150 million hectares of lost forests by 2020. However, one of the problems with this scheme lies in the wide variation in the definition of 'degraded land' across the EU. This is not entirely surprising given the range of climates, cultures, ecosystems and land uses across the EU, and in a practical sense it may be the case that a single definition of degraded land is neither attainable nor desirable. For example, land that is determined as degraded in a traditionally arable farming area may actually be highly valuable to pastoralists, whilst other areas may have significant cultural and social significance. In consequence, creating a policy framework that can realistically take into account all of the issues and yet remain applicable and practical on the ground is a challenging task. Nevertheless, it is a goal worth pursuing as it could create an incentive for 'low risk, high opportunity' production on degraded land.

Whilst it is readily accepted that estimating indirect land use changes (iLUC) for a biofuel crop such as camelina is at best controversial, with determination of both positive and negative effects difficult at a local level (Gawel & Ludwig, 2011; Van Stappen *et al*., 2011). Initial assessment of the externalities of growing *Camelina sativa* on chemically degraded land maybe considered favourable. The use of chemically degraded land would allow camelina biofuel production to align with a preventative policy for biofuel production (Di Lucia *et al*., 2012). Additionally, camelina biofuel grown on contaminated land has the potential to impact positively on food security by using land deemed unsuitable for food production as well as the possible strengthening of the domestic climate mitigation policy through lessening the dependency of the EU on global energy fostering and allied carbon leakage (Gawel & Ludwig, 2011). Although long-term change in cropping are not anticipated for the production of camelina due to agronomic concerns, care is needed when selecting sites to ensure that biodiversity and water security are not compromised. The prospect of finding viable alternative strategies for chemically degraded land and minimize iLUC is one of the key drivers for the ITAKA project.

The European Commission's Directorate General for Energy has expressed a direct interest in developing such a policy framework that could be incorporated into the Renewable Energy Directive and incentivize biofuel feedstock production on degraded land. Within this policy it has been determined that a carbon credit bonus allowance of 29 g CO2,eq/MJ shall be applicable to the life cycle analysis for all biofuels whose feedstocks were cultivated on degraded or contaminated land. However, this bonus can only be applied to eligible feedstocks, which in turn requires the European Commission to agree upon a finalised definition of degraded and contaminated land. This definition had yet to be realised at the commencement of the ITAKA project in 2012.

The question of whether biofuel feedstocks can be economically cultivated on degraded and contaminated land is not addressed within the EU framework. Similarly, the question of trace metal uptake into biofuel feedstock co-products such as animal feed is considered outside the scope of the policy. The work undertaken in ITAKA task 5.1.7 takes a tentative yet pragmatic consideration of these important factors that will help decision makers understand and perhaps realise the good intention behind this policy lever to incentivize biofuel-feedstock production activities in degraded and contaminated land. Such a shift in agrarian practice may offer an attractive alternative that allows primary stakeholders a potentially viable source of income and simultaneously brings the land back into useful production.

Land and soil

Although often interchanged land and soil are different entities that are intimately linked through feedback mechanisms. Soil influences the land cover and consequently land use, and in turn, land use impacts on soil. Where unsustainable land management practices lead to soil degradation and erosion, detrimental positive feedback mechanisms are likely to be established, resulting in sustained loss of production and ecosystem services. Such losses have notable consequences at a local, regional and global scale (Jones *et al*., 2012).

1.1.1. Soil and its importance

Soil is the thin surface layer situated at the boundary between the earth's surface and the bedrock (Bridges & van Baren, 1997; EEA 2010a). Soil is a complex bio-geochemical system composed of minerals, organic matter, water and air, the proportions of which reflect soil-forming factors and processes, such as geological parent material, climate and flora and soil fauna, active at a given site (EEA 2010a). Within Europe, soil resources show diversity and spatial variation from the poorly developed soils of the Mediterranean to the organic-rich soils of Northern Europe (EEA, 2010a).

The most obvious function of soil to humanity is in the provision of biomass, food and raw materials. However, soil crucially acts to regulate the environment, filtering, transforming and storing substances such as water, nutrients and carbon (Bridges & van Baren, 1997; EEA 2010a; Louwagie *et al*., 2011). Consequently, soil has a critical role to play in a wide range of eco-processes, for example, water management and through the storage and capture of carbon, climate change mitigation (EEA 2010a; Louwagie *et al*., 2011).

Policy makers and stakeholders are becoming increasingly aware of the importance of soils and concomitantly the vulnerability of soils to mismanagement and degradation. Comparison of estimates of formation rates of soils (0.06 -0.2 mm yr-1; Wakatsuki & Rasyidin, 1992; Montgomery, 2007; EEA, 2010a) with contemporary global erosion rates for cropland (0.6 mm yr-1; Montgomery, 2007) suggest that an order of magnitude difference is likely to exist between erosion and production rates for agricultural soils. Therefore, at a human time-scale, it can be argued that soil is a non-renewable resource and as such must be carefully managed (Eswaren *et al*., 2001; Gobin *et al*., 2004).

Assessment of human-induced soil degradation

1.1.2. Historic perspective

Acknowledgement that ultimately the vitality of key environmental resources such as soil is integral to sustaining the economic prosperity of a country (Dregne, 1986; Brundtland *et al*., 1987) accentuated the lack of information for policy-makers, on a global scale, of the extent of soil degradation. The United Nations Environment Programme's (UNEP) and International Soil Reference and Information Centre's (ISRIC) Global Assessment of Soil Degradation (GLASOD) project was the first to attempt to overcome this knowledge gap and underpins subsequent land degradation assessments such as ASSOD, FAO World Soil Resources Reports, PESERA and SOVEUR (Bot *et al*., 2000;van Lynden, 2000) with the intention of strengthening the awareness of policy and decision makers to the need for sustainable soil management (Oldeman *et al*., 1991a), the resultant map presented to a first approximation the extent of human-induced soil degradation (H-ISD) on a global scale.

GLASOD related soil-degradation to land productivity. Based on experts' estimates and at a scale of 1: 10 million GLASOD recognized two main categories of H-ISD; the first considered processes that recognized the trans-boundary nature of soil degradation, leading to off-site effects, such as water and wind erosion, whilst the second category considered on-site effects and focused on processes such as compaction, sealing, nutrient loss, salinization and pollution (Oldeman *et al*., 1991a). Degradation was classified as light (some reduction in agricultural suitability); moderate (greatly reduced agricultural production); strong (biotic functions impaired such that it is considered to be non-reclaimable at farm level); or extreme (biotic functions destroyed, non-reclaimable). Severity of degradation was seen as the product of the degree of degradation and the spatial extent. As a result, the global assessment identifies the dominant type OF degradation for a region although other vectors of degradation are likely to be operational.

GLASOD determined that soil degradation affected some 15% (1964 Mha) of the total global land surface; with the area affected varied from 5% in North America to 23% in Europe (Oldeman *et al*., 1991b). Water and wind erosion were found to be the major forces, 84% of total global H-ISD being attributed to these two processes, with chemical and physical degradation accounting for 12% and 4% respectively. Compaction was seen to be the main driver of physical degradation, whilst nutrient loss and salinization as the main contributors to chemical degradation. Only in Europe was pollution found to play a significant role in H-ISD (Oldeman *et al*., 1991b).

Of the five causative factors (deforestation; overgrazing; agricultural mismanagement, over exploitation and bio(industrial) activities) GLASOD assessed, the predominant drivers (>60%) contributing to H-ISD were found to be those relating to agricultural activities, such as over-grazing and agricultural mismanagement (Oldeman *et al*., 1991b). Consideration of the extent of soil degradation finds that >60% of the soils affected are moderately to extremely degraded, of which some 25% are in need of remediative intervention, and represent a substantial loss in productivity (Oldeman *et al*., 1991b).

Comparison of the European data with the global assessment highlights both similarities and contrasts. The data shows that within Europe some 23% (219 Mha) of the land surface was affected by H-ISD compared with 15% globally. As with H-ISD affected areas worldwide, water and wind erosion remained the most dominant form of land degradation. By contrast, in Europe chemical and physical degradation play a more a significant role accounting for 28% of soil degradation compared with the global assessment of 16% (Oldeman *et al*., 1991b). Examination of the data finds that, whilst the area affected by chemical degradation in Europe (12%) is consistent with that of area affected worldwide, the main drivers vary considerably. Globally, nutrient losses and salinization are the greatest contributors to chemical soil degradation but in Europe these two degradation types account for only 27% of chemical degradation. By far the greatest source (72%) of chemical soil degradation in Europe is attributed to pollution an order of magnitude greater than the global assessment (Oldeman *et al*., 1991b). The percentage of land area affected by physical degradation in Europe is some four times greater (16%) than that observed globally. However, similar to global observations, the predominant contributors (>90%) to physical degradation soil in Europe were found to be those of compaction, sealing and crusting (Oldeman *et al*., 1991b).

Within Europe agri-centred factors remain the key causative factors, contributing some 52% of all soil degradation reported, with deforestation and bio(industrial)activities playing an enhanced role (38% and 9.5% respectively) compared with their contributions on a global scale (29% and 1.2%, respectively; Oldeman *et al*., 1991b). Scrutiny of the extent of soil degradation in Europe suggests that >70% of the affected area displays at least a moderate degree of soil degradation, with ~9% of this area in of need restoration, whilst 2% was considered beyond remediation (Oldeman *et al*., 1991b).

Table 1: Comparison of Global and European land area proportioned by degradation category

Global	**Million Ha**	**Total %**		**Europe**	**Million Ha**	**Total %**
Total Land Area	13093	100		Total Land Area	952	100
Estimated Area Degraded	1964	15		Estimated Area Degraded	219	23
		Fractional %				***Fractional %***
Water and Wind Erosion	1650	84		Water and Wind Erosion	158	72
Chemical: Nutrient loss, Salinization	236	12		Chemical: Nutrient loss, Salinization, Pollution	26	12
Physical Compaction	79	4		Physical Compaction	35	16

Subsequent reports such as theta World Soil Resources Report (Bot *et al*., 2000) and SOVEUR Soil Degradation in Central and Eastern Europe report (van Lynden, 2000) have built on the concept of GLASOD whilst looking at H-ISD on regional and country-wide scale.

FAO World Soil Resources Report (Bot *et al*., 2000) evaluated the land potential for 160 countries, in terms of soil constraints, such as erosion hazard, hydromorphology and shallowness, drylands and steeplands as well as land degradation. Bot *et al*. (2000) suggested that globally 65% of all land is subject to some degree of degradation, with 47% of it exhibiting at least moderate severity, within Europe the degree of degradation is found to be higher with approximately 90% of land exhibiting some degree of degradation. On a country level, the data suggest that 100% of land in Romania is categorized as severely degraded (the sum of Severe and Very Severe classes) with the principal causes and type being identified as deforestation and agriculture and water erosion, respectively. By contrast, 38% of land in Spain is evaluated as severely degraded, with chemical and physical deterioration being the dominant type of degradation, and the principal causative factors being those industrial as well as agricultural activities. Further assessment of the FAO World Soil Resources Report showed that 22% of severely degraded land in Europe can be attributed to agricultural activities. In Romania agricultural activities account for 46% of the total area of degraded land whilst in Spain it represents 6% of the total land degradation.

The findings of the UN’s Food and Agriculture Organization (FAO) and the Netherlands Government SOVEUR Soil Degradation in Central and Eastern Europe report (van Lynden, 2000), with respect to land degradation in Romania, suggest that 35% of the total land area is non-degraded, with 47% of land being assessed as severely degraded, the key degradation types are found to be pesticide pollution (19% ; 4.5 M ha); water erosion of topsoil (18% ; 4.3 Mha) and fertility decline (14% ; 3.4 Mha). Wind erosion was found to be important on a local scale for example between Romania and Bulgaria along the Danube Valley. Similarly, evidence suggested that pollution by heavy metals was also important on a local rather than nation scale.

1.1.3. Current European status

The European Environment – state and outlook 2010 Report, known as the SOER 2010 provided a pan-European evaluation on the current state of soil in Europe (EEA, 2010a). SOER 2010 identified a number of key processes that threaten soil integrity, including biodiversity decline, compaction, contamination, erosion, landslides, organic matter (OM) decline, salinisation and sealing. The findings of the recent 2010 SOER assessment (EEA, 2010a) show that whilst the effects of such processes are variable across Europe the nature of the threats remain the same and in many cases soil degradation processes are accelerating rather than declining (Jones *et al*., 2012b).

Current estimates suggest that a significant proportion of European soils are subject to soil degradation processes. For example it is estimated that 36-50% of soils in Europe are at least moderately susceptible to compaction from heavy agricultural machinery (Crescimanno *et al*., 2004; van Camp *et al*., 2004), with some 45% of soils in Europe displaying low soil organic content (SOC) (Rusco *et al*, 2001) and furthermore water erosion is widespread affecting approximately 130 Mha in the EU-27 countries (Jones *et al*., 2012a). Within the EU-27 countries, central, eastern and southern areas of Europe such as the Iberian Peninsula and the Black Sea region of Romania are thought to be particularly vulnerable to desertification, SOC decline and water and wind erosion. Current trends in land-use changes, for example, the increase conversion of pasture land to arable cropping, suggest that areas of central, eastern and southern Europe are likely to be at further risk of increased land degradation.

Estimates of land degradation due to chemicals tend to be at a local scale, and are hindered through lack of EU regulation, comprehensive inventories and difficulties in data validation (Rodriguez Lado *et al*., 2008; Jones *et al*., 2012b). At present, approximately three million potentially polluted sites are thought to exist, of which 250,000 may need immediate remediation (EEA, 2007), with the predominant contaminant source being heavy metals and mineral oils. However, due to improvements in data collection the number of identified sites is expected to increase by as much as 50% by 2025 (EEA, 2007; EEA, 2010a). Information on diffuse contamination, such as from the over application of agrochemicals, is even more limited, models produced by Bouraoui *et al*. (2009) suggest about 15% of the EU land surface is subject to soil nitrogen surpluses.

Accurate assessment of the economic cost of soil degradation on a Europe-wide basis is difficult, and current estimates of the cost of the combined impact of soil contamination, erosion, OM decline, landslides and salinization suggest an annual cost of up to EUR 38 billion for the EU-25 (EEA, 2010a; Jones *et al*., 2012b).

1.1.4. Changes in land use

The way land is utilized and managed may result in both beneficial (e.g. carbon sequestration) and detrimental (e.g. land degradation) outcomes. The European Environment – state and outlook 2010 Report on land use (EEA 2010b), SOER report monitored land-cover and land-use changes in 36 European countries, for the period 2000-2006. The report estimated that pan-Europe approximately 1.3% of the total area land had experienced a change in cover. Whilst the annual rate of change appears to have slowed since the 1990s, the rate of change on a country basis varied significantly. The greatest changes to land cover were seen in Cyprus, the Czech Republic, Finland, Ireland, Portugal, Sweden and Spain. One of the strongest continuing trends is that of land-use specialization (e.g. urbanization, agricultural intensification and abandonment), whilst the formation of artificial surfaces exceeds the rate at which new agricultural land is being formed. During the period 2000-2006, it was estimated that some 0.29 Mha of agricultural land was lost to other uses including land abandonment. The main drivers for such changes are those of settlement and infrastructure; food production and shift toward bio-energy production. Projections for 2020 anticipate that within the EU-27 bio-energy policies and targets will influential on EU land-use patterns. In particular, whilst the total arable cropping area is expected to decrease, the area of oilseed production is projected to rise as EU bio-fuel targets are met.

1.1.5. Marginalization and abandonment

Although the concept that marginal agriculture is agriculture at the margin of economic viability, where the income generated by present agricultural cropping cannot cover input costs or is likely to cease to cover such costs in the next few years (CEC, 1980), is commonly used to define marginal agriculture. Within Europe what constitutes marginal agriculture and consequently marginal land is not precisely defined and reflects differences in how such land is assessed in relation to productive land. For example, land considered marginal in one yield region (e.g. UK) maybe considered productive land in another (e.g. Spain; Brouwer *et al*., 2007).

Marginalization of land is a dynamic and multidimensional process occurring over time (Pinto-Correia & Sørensen, 1995). Spatially operational at different geographic levels (regional, local, farm and within holding), it is driven by the complex interaction of economic, environmental, political and social factors. Although land abandonment is commonly associated with land marginalization, it is not the only outcome, other responses to marginalization include changes to cropping regimes, restructuring of holdings and land-use changes to non-agricultural uses (e.g. forestry, urbanization). As marginalization occurs progressively over a period time, change is not necessarily permanent and can be reversed (Brouwer *et al*., 2007).

In considering areas within which agricultural land is marginal, a joint project undertaken by the Institute for European Environment Policy in the UK and the Agricultural Economics Research Institute (LEI-DLO) in the Netherlands (Baldock *et al*., 1996) recognized five regions of agricultural productivity within the EU-12. Of these, two types of regions (i) extensive farming (e.g. Spain, areas of southern France and parts of the UK, Ireland, Italy) and (ii) small-scale farming (e.g. Italy, Portugal, Spain) were found to be most susceptible for marginalization and together representing at least 30% of the utilised agriculture area (UAA).

Although Keenleyside and Tucker (2010) define farmland abandonment (FLA) as the complete withdrawal of agricultural management such that natural succession processes are able to progress, FLA is a complex and gradual process often difficult to define and can be temporary, transient or permanent. The extent of FLA varies and a number of forms are recognised, the key categories being actual, hidden or semi-abandoned and transitional. In actual abandonment, vegetation may change through natural succession to form new ecosystems such as forests and ‘steppe’ grasslands. Hidden or semi-abandonment occurs where a low level of management is maintained such that CAP cross-compliance requirements are met or so the land can be used for tourism. By contrast, transitional abandonment result from the restructuring and land reforms, for example as experienced in the central and eastern EU-12 countries since the 1990s (Keenleyside & Tucker, 2010).

Whilst land abandonment has occurred throughout the EU-27 at different periods of time and is closely associated with areas of marginal land, since the 1950s its significance has grown. It is estimated that over the period 1961-2003, 30 Mha of the EU-27 UAA was abandoned, primarily in mountainous regions and in areas of low agricultural productivity (Pointereau *et al*., 2008). However, an accurate assessment of FLA on a pan-European scale is difficult and difficulties still remain at a small scale (Keenleyside & Tucker, 2010). Cases studies for France, Poland and Spain suggest that from the end the 1980s to the late 1990s that annual of UAA loses of 0.2%; 0.7% and 0.8%, respectively were experienced in these countries (Pointereau *et al*., 2008). Since 1989, as a result of the political changes in central and eastern European countries, widespread abandonment has occurred, with estimates of land abandoned of 15-21% in parts of Slovakia, Poland and Romania being reported (Kuemmerle *et al*., 2008; Muller &Kuemmerle, 2009). Similar extensive abandonment was also seen in the Baltic States (IEEP & Alterra, 2010) during this period.

However, as case studies are not representative samples, extrapolation of such data to give an EU-wide estimation of abandonment would be ill advised. Equally, the evolution of policy means that the value of past trends as a precursor for recent and future developments in land abandonment (Keenleyside & Tucker, 2010).

Drivers of land abandonment are both region-specific and time-period specific, reflecting changes in commodity profitability and socio-economic status of a region. Rey Benayas *et al*. (2007) recognized three major types of drivers, (i) physiographic (e.g. elevation, slope, soil depth and fertility, soil erosion), (ii) socio-economic (e.g. rural depopulation, accessibility, land-tenure systems) and (iii) land mismanagement. In considering institutional and physical factors Keenleyside and Tucker (2010) suggested that the primary drivers are those that reduce farm profitability (e.g. poor soils; low commodity prices), whilst the socio-economic factors (e.g. rural depopulation; changes to land ownership; tax regimes) act as secondary drivers in land marginalization and consequently land abandonment (Keenleyside & Tucker, 2010). The findings from other researchers (Pointereau *et al*., 2008; Muller &Kuemmerle, 2009), suggest that the import of such drivers are very much location and time specific. For example, Pointereau *et al*. (2008) found that the key drivers for land abandonment in the Spanish state of Galicia were the limited adaptation to new agricultural conditions and specialization due to the small size of holdings. By contrast, FLA in the state of Catalonia was driven by tourism, urban pressures and peri-urban expansion. Muller and Kuemmerle (2009) highlighted the temporal variability of drivers through their work on FLA in Argeş County, Romania. In the early stages (1990-1995) FLA was predominately driven by physiographic factors such as topography and spatial homogeneity of crop land. Whilst these factors remain influential, in the later period (1995-2005) socio-economic drivers (e.g. higher labour costs; poor accessibility to transport network) played a significant role in driving FLA.

The prediction of the extent and location of future FLA is difficult to define and is constrained by the paucity of current FLA data (Keenleyside & Tucker, 2010). Other authors have undertaken land use modelling to predict such future trends (Nowicki *et al*. 2007; Rienks, 2008; Verburg & Overmars 2009). Although different scenarios and criteria (e.g. reduced global competition and high CAP support; high global competition and low CAP support) were employed, the models show agreement in that there is likely to be significant levels of FLA in the next two to three decades and at greatest risk are mountainous areas and extensive grazing systems. Where individual scenarios differ is in the degree of abandonment predicted. The projected levels of FLA vary from 0.7% of land area by 2020 (Scenar 2020 Regionalisation Scenario, Nowicki *et al*. 2007) to 6.7% by 2030 (EURURALIS Global Cooperation Scenario, Rienks, 2008). Keenleyside & Tucker (2010) suggested that on consideration of the different scenarios and the inherent deterministic approach of the models that it is plausible that 3-4% of the total land area will be subject to abandonment by 2030. Keenleyside & Tucker (2010) also noted that for scenarios incorporating elevated levels of biofuel production that the levels of abandonment projected were considerably reduced. However, the authors strongly urged caution highlighting that as the EU Renewable Energy Directive’s sustainability factors were not fully considered, and some of the models may have overestimated any beneficial impacts. Similarly, the models do not explicitly take into account climate-change predictions and associated impacts, which will increase in significance. The projected increases in temperature and decreases in rainfall leading to the increased incidence of heat waves, droughts and shorter cropping periods in many parts of southern Europe and the Pannonian zone may result in the extensive abandonment in the long term (Keenleyside & Tucker, 2010; Olsen *et al*. 2011).

1.1.6. Targets and potential for growing on degraded land

The increasing global awareness over the last two decades of the impact of fossil fuel usage on climate change has resulted in energy policies worldwide that sponsor the use of renewable energy sources (Markevičius *et al*. 2010). In the EU, with its high dependence on fossil-fuelled transportation, bioenergy and in particular biofuels are seen to offer a viable option to reduce greenhouse gas emissions whilst improving fuel security and rural economies (Markevičius *et al*. 2010). Key to the development of biofuels in the EU has been Directive 2009/28/EC on the promotion of the use of energy from renewable sources (Renewable Energy Directive (RED); Directive 2009/28/EC; Lange, 2011; Di Lucia *et al*., 2012) which requires member states to achieve jointly obligatory targets of 20% of overall energy consumption from renewable sources and a minimum target of 10% market share for biofuels in transport fuels by 2020 (Directive 2009/28/EC; EC 2012a). At the same time, mandatory targets were introduced to achieve a 6% reduction in green-house gas (GHG) intensity of road transport fuel by 2020 through the adoption of an amendment to the Fuel Quality Directive (FQD; Directive 98/70/EC). Although the aviation sector aims to use 2 Mt oil equivalent (MtOE) of biofuels by 2020, currently, there are no targets have been formally defined in the RED or FQD for biofuel usage in the aviation nor shipping sectors (EC 2012(a)).

It is recognized that biofuels will be a significant factor in achieving these targets. However, the potential contribution of such bio-fuel stocks is limited by the availability of land (Kavalov *et al*., 2003; Cai *et al*., 2011). Although it is expected that the demand for food within the EU for the next few decades will remain stable (Fischer *et al*., 2010), the food first paradigm in tandem with the drive for an environmentally-compatible agricultural industry under the auspices of the common agriculture policy (CAP) imposes further constraints on land availability in the EU (EEA 2007b). Conservative estimates of 18 Mha of environmentally-compatible arable land being available by 2020 (20 Mha by 2030) are reported by Weisenthal *et al*. and Petersen *et al*. (EEA 2006; EEA 2007b, respectively) with the new member states such as Bulgaria and Romania, and Mediterranean regions showing the greatest biomass production potentials (van Dam *et al*., 2005; EEA 2006). Current modelling suggests that the EU will need 56-166 Mha to meet its biofuel requirements (Gallagher 2008). The lower limit of the range illustrates how the inclusion of avoided-land usage and co-products operate to reduce the total area required. By contrast, if predicted annual increases in crop yield are not achieved or gasoline and diesel consumption are higher than expected then the area needed is likely to be greater than indicated (Kavalov *et al*., 2003;Fischer *et al*., 2010). A key imperative to the RED is the sustainability of biofuels produce from both internal and external sources (Directive 2009/28/EC). In order that the availability and demand for land for sustainable biofuel production in the EU can be reconciled a global perspective is needed.

The use of abandoned, idle, marginal and degraded land has been advocated as an alternative and sustainable solution to biofuel production by a number of authors (Hoogwijk *et al*., 2003; Campbell *et al*., 2008; Gallagher, 2008; Fargione *et al*., 2010; Cai *et al*., 2011). Furthermore, the RED incentivizes the use of highly salinized and contaminated land (Directive 2009/28/EC). Quantifying the potential area available is complex, with values reflecting differences in model criteria and parameters used. Regional and local differences in the understanding of what constitutes marginal or abandoned land also influence the assessment (Nalepa 2011). Evaluation of the land available globally suggest that between 300 Mha and 2000 Mha of marginal land could be used for biofuel production (Hoogwijk *et al*., 2005; Campbell *et al*., 2008; Gallagher, 2008; Cai *et al*., 2011). Such estimates are subject to significant uncertainties, with values of up to ± 50% being reported (Field *et al*., 2008; Fritz *et al*., 2013). For example, the IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation (2011) report that worldwide there is 780 Mha available for biofuel production (EC 2012a). By contrast, Field *et al*. (2008) after taking into consideration that a proportion of abandoned agricultural area is (i) in urban areas, (ii) abandoned pasture converted to crops and vice versa or (iii) forest, estimated the abandoned area globally to be 386 Mha.

There is a consensus amongst researchers that 2nd generation biofuel crops offer the greatest potential for biofuel production on abandoned and degraded land (Campbell *et al*., 2008; Gallagher, 2008; Fargione *et al*., 2010;Fischer *et al*., 2010; Cai *et al*., 2011;.Havlík *et al*., 2011). Furthermore, some authors caution against the use of such land for conventional biofuel crops, emphasizing that although the production of such rotational crops on degraded land may be viable, this does not automatically equate to productivity. Concerns are raised that more land would be needed to meet production targets. In addition, to achieve acceptable productivity it is likely that fertilizer, crop spray and water usage would be increased compromising crop sustainability and exacerbate degradation (van Dam *et al*., 2007; Campbell *et al*., 2008; Fischer *et al*., 2010; Franco *et al*., 2010; Fonseca *et al*., 2010; Nijsen *et al*., 2011; Nalepa 2011; Montross *et al*., 2013).

Given that the development of 2nd generation, advanced biofuel production systems has been slower than first predicted (EC 2012a) with the earlier predictions that advanced biofuel production would increase from 2010 to play a more dominant role in biofuel feedstock production by 2020 (EEA 2007(b)), have yet to be realized. It is liable that the majority of the 10% transport target (approximately 9%) will be sourced from 1st generation, conventional biofuel feedstocks, in particular, it is expected that biodiesel crops in the EU will account for more than 70% of transport biofuel production in 2020 (EC 2012(a)). In order to re-address the EC in October 2012 proposed the adoption of Article 3(4)d as an amendment to the RED (EC 2012b). Article 3(4)d states that the share of energy from biofuels produced from rotational crops such as oil crops should be no more than 5% of the final consumption of energy in transport 2020. The medium to long-term implications to land usage because of adopting such an amendment remain undetermined.

2 Experimental approach

2.1. Introduction

When considering the feasibility of cultivating biofuel feedstocks on degraded and contaminated land, there are two fundamental questions:

- Is there a metabolic pathway for the uptake of trace metals from contaminated soil to feedstock?

And if so,

- Are these contaminants located in the feedstock primary product (ie. the oil) or co-products (such as animal feed or straw)?

The answer to these primary questions must be evaluated before other secondary questions central to the construction of the business case, such as can biofuel feedstocks be economically cultivated on degraded and contaminated land, can be explored.

The work undertaken in ITAKA task 5.1.7 takes a tentative yet pragmatic consideration of these important factors in a study that is specific to the cultivation of *Camelina sativa*, but with a high degree of focus on the development of a broad and robust methodology that may be applied to the cultivation of biofuel feedstock in general.

In developing the methodology for the traceability of heavy metals for camelina cultivated on contaminated land, two distinct yet complimentary approaches have been undertaken to validate and corroborate its applicability: 1) controlled cultivation trials, and 2) field study cultivation trials.

For the controlled cultivation trials, soil pre- planting, together with plant roots, shoots and seeds were characterised and analysed for contaminant trace metals. However, the crop produced an insufficient quantity of seed to allow extraction and analyse of the seed oil. Due to the finite amounts of metals assimilated by the plants in comparison to the metal soil concentration, it was considered that post-harvest analysis of the soil from the controlled greenhouse cultivations would be of little value. For the field study cultivation trials, contaminant heavy metals were traced through all components of the system for the Rovinari sterile site (with the exception of the animal feed co-product which was not available but whose characteristics may be estimated from the difference between the components present in the seed and oil). At all other Romanian contaminated land sites where cultivation trials were on going the trace metal analysis was limited to soil and the primary products of seed and oil.

Due to the challenges posed in developing an appropriate and effective methodology that addressed the various interdisciplinary components of the research, the methodology focussed in the first instance on the six metals, Cd, Co, Cu, Fe, V and Zn, identified in Def-Stan 91-91 and by industrial stakeholders such as Rolls-Royce of greatest concern with respect to thermal instability and turbine rotor degradation. For consistency, this approach was continued when the developed methodology was applied to field samples from the four Romanian contaminated-field sites. This protocol was of particular pertinence with regard to the analysis of the oil samples. Standard analytical protocols for fuel oil such as ASTM D7111, ASTM D5185-09 and UoP389 were found to be inappropriate for the analysis of the camelina oil. If an extensive analysis of metals present in the oil had been undertaken concurrently with the method development this may have prevented the development of a suitable protocol. Using the Rovinari-Sterile study site as a case-study the developed methodology has been rigorously and extensively applied, with the inclusion of metals that may be of concern to the animal and human food-chain (e.g. As, Cr, Ni and Pb), to the analysis of soil and plant materials harvested from this site.

An outline description of these trials is summarised in the Table 2 and described in the sub-sections that following. Chapter 3 of this report describes the analytical methodology and protocols developed during this task and Chapters 4 & 5 report and discuss the results.

Table 2: Summary of the system components analysed during the development of the methodology for the traceability of heavy metals to assess the impact of cultivating camelina on contaminated land, (a) controlled greenhouse cultivation trials, and (b) controlled field cultivation trials 2013

a) Controlled greenhouse cultivation trials	**System components analysed for contaminant metals**			
Greenhouse cultivated 2013	Soil pre-planting	Root	Shoot	Seed
Greenhouse cultivated 2014	Soil pre-planting	Root	Shoot	Seed

b) Field study cultivation trials 2013	**System components analysed for contaminant metals**					
Rovinari: Sterile Field	Soil pre-planting	Root	Shoot	Seed	Oil	Soil pre-harvesting
Rovinari: Ash Field	Soil pre-planting			Seed	Oil	
Câmpina	Soil pre-planting			Seed	Oil	
Copşa Mică	Soil pre-planting			Seed	Oil	

2.2. Controlled cultivation trials (greenhouse trials)

In the controlled cultivation experiments, camelina has been cultivated under greenhouse condition in a standard soil that has been ‘spiked’ with known concentrations of a single contaminant species. Cultivation of camelina under these conditions can be distinctly advantageous in that it allows for a much greater level of experimental control: local variations in soil quality, water, weed infestation, crop residues and contaminant species and concentration become regulated parameters, whereas this cannot be assumed to be the case in the field study trials.

The design of 2012-2013 greenhouse spiking experiments focussed on the six metals, Cd, Co, Cu, Fe, V and Zn, identified in Def-Stan 91-91 and by industrial stakeholders such as Rolls-Royce as problematic due to fuel thermal instability and turbine rotor degradation.

The experiment was designed in accordance with the following standard methods and guidance BS EN ISO 17402: 2011; EN ISO 22030:2011; BS ISO 11269-1: 2012; BS ISO 11269-2: 2012; BS ISO 15799: 2003; BS ISO 17126: 2005; and BS ISO 17616:2008. Five concentration levels per metal were employed (see Table 3). The range of contaminant concentrations were chosen to reflect those determined by an earlier geochemical analysis of the soil samples from the Romanian field study sites.

A loam based compost, John Innes No2, was used as the growth medium. 1.03 L Desch Plantpak Full Pots (S127FDP; 135 mm diameter x 117mm height) were used throughout. For each concentration, six replicate pots were established, with each replicate pot being populated with three plants, to give an initial total population of 18 plants. A lighting regime of 16 hr light/8 hr dark was maintained throughout with the temperature within the greenhouse being maintained at a minimum of 20 ± 2°C.

Watering of the pots with locally collected rainwater was via two capillary wicks per pot, which were suspended over a water reservoir, separate reservoirs were employed for each spike treatment to prevent cross contamination. To ensure the base compost was at field capacity and the wicks were working efficiently, prior to spiking the growth compost was allowed to equilibrate at greenhouse conditions for minimum of 2 days.

The spiking material was incorporated on dry weight basis in to the growing medium. To ensure even distribution of the spike within the growing median the metals Cd, Co, Cu V and Zn were added as aqueous solutions (hence in a biologically assessable form), whilst spiking of soils with Fe(II) and Fe(III) was achieved by direct incorporation of the solid sulphate salts. Subsequently the spiked growing medium equilibrated at greenhouse conditions for minimum of 7 days prior to seedlings being planted in the spiked medium. camelina feedstock seed was germinated in un-spiked growth medium and the resulting seedlings were transferred to the spiked medium when seedling basal leaves were 20-30 mm in length.

The addition of nutrient to the water reservoir after four weeks of the experiment was decided against in order that the greenhouse conditions would emulate in some way the conditions of the field trials.

To minimize the effect of micro-climatic conditions within the greenhouse on plant growth, the position of the pots/reservoirs of individual treatments within the greenhouse were rotated every 3-4 days after week 1. On the initiation of flowering the plants were monitored for seed set, as the ripening of the seed silicles across the different spiking treatment was non-uniform, seed inflorescences were bagged to prevent lose of seed as a result of silicles maturing and hence splitting over a protracted ripening period. Paper bags were used to prevent mould and fungal damage as a result plant respiration.

Table 3: Metals, metal species and individual spike concentration for controlled cultivation trials All concentrations are quoted in mg/kg, except * values which indicates percentage

Metal	**Metal Species**	**Concentration in mg kg^{-1} soil dry weight**				
		Control	**Spike 1**	**Spike 2**	**Spike 3**	**Spike 4**
Cd	$3CdSO_4.8H_2O$	0	0.1	1	10	100
Co	$CoSO_4.7H_2O$	0	2	10	20	200
Cu	$CuSO_4.5H_2O$	0	10	50	100	1000
Fe (III)	$Fe_2(SO_4)_3.5H_2O$	0	0.1*	1*	5*	10*
Fe (II)	$FeSO_4.xH_2O$	0	0.1*	1*	5*	10*
V	NH_4VO_3	0	10	25	50	200
Zn	$ZnSO_4.7H_2O$	0	40	100	400	4000

2.3. Field cultivation trials (Romania)

In the field cultivation trials, camelina has been cultivated at selected field sites in Romania (Figure 1) As with all field study trials, these crops are subject to a much wider range of unregulated environmental parameters, such that rigorous and standardised sampling protocols should be adhered to in the analysis to minimise experimental uncertainty (described later).

A site-survey assessment record based on BS ISO 15903:2002 was undertaken on arriving at each of the Romanian field site and these are given in Appendix A. A detailed description of each of the Romanian field study sites is given in the following sub-sections.

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 of 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 study site is comprised of two 0.5 ha units, denoted as Sterile and Ash fields.

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. In 2012, the soil was conditioned with lignite-based fertilizer and cropped with maize, with a crop yield estimated at 5.5 tonne/ha.

The Ash site lies in close proximity to the current lignite extraction and conveyance system. It was an excavated area that was in-filled in the 1960s with fly ash from the Rovinari thermo-electric power plant. The soil matrix is dark brown –grey in colour and is composed of a very fine grained (< 63 µm) silty-clay material. The study site was cropped with arable crops including sunflowers until 1970s. During the 1970s and 1980s a semi-natural grass cover of was established, which remained until it was ploughed in 2012 in preparation for the 2013 *camelina* crop. Details of the site preparation for the 2013 c*amelina* crop are given in Table 4.

Figure 1: Locations of the four Romanian contaminated-field sites 2013

Câmpina, Prahova County

Câmpina (45°07' 48"N; 25°44' 24"E), an industrial town in Prahova County, Romania, home to both the Romanian Star Oil Refinery and the Central Orion Foundry it is located on the national route DN1 that links the capital with the city of Braşov in the north. Câmpina is approximately 110 km northwest of Bucharest, 77km south of Braşov and 35 km north of the county town of Ploieşti.

The field study site lies within the city of Câmpina it is an area of marginal land lying to the north of the Central Orion Foundry with an approximate total surface area of 0.82 ha, ineligible areas for cropping such as vehicle tracks, resulted in the effective sampling area being reduced to about 0.60 ha. To east of site are two water bodies, slurry lagoon for refinery waste and more distally the Lacul Pestelui, colloquially known as the ‘black’ lake. The lake became black in 1950’s, indicating presence of oil reserves that lie some 100 m below the surface.

The Soil Map of Europe identifies stagnic- and chromic- luvisols are typical for the area; however, there is an established history of the study area being used to dispose of industrial (e.g. sulphur dioxide manufacturing and steel fabrication) and building construction waste areas of which are clearly identifiable. As such the sediment and soils at the sample site exhibit strong anthropogenic influences, and maybe be better described as anthrosols or technosols.

The site was covered in 2012 by broad-leaved plants and native grasses including Archillea sp., Mentha arvensis and Taraxacum sp. Details of the site preparation for the 2013 camelina crop are summarized in Table 4.

Copşa Mică, Sibiu County

Copşa Mică (46°06' 56.6"N; 24°13' 56.7"E) is an industrial town in Sibiu county Transylvania, Romania, located in the River Târnava Mare basin and surrounded by hills, it is approximately 320 km northwest of Bucharest, 43km north east of Sibiu and 12 km south west of Medias.

Due to its industrial past, Copşa Mică gained the reputation of being one of the most polluted towns in Europe in the 1990s. The main pollutant sources being Carbosin black carbon factory and Sometra non-ferrous metallurgical smelter, although the Carbosin factory closed in 1993, Sometra continued to be fully operational until 2009 when depression in the world metal markets and sourcing issues caused the temporary suspension of smeltering activities.

The field site is situated between the national route DN14B and the River Târnava Mare, and approximately 1000 m west of the Sometra smelter plant. At the northern boundary of the site there is an unmetalled dirt track that is used daily by heavy goods vehicles to transport sand/aggregates from the quarry that is sited to east of the study site, resulting in soil compaction adjacent to the northern edge of the field site.

The site is approximately 0.92 ha consisting of 20 m wide strip of dark brown free-draining silty loam soil within a larger multi-user agricultural cropping unit. Exposed soil profiles in proximity to the site suggest that soil depth is 30-40 cm and that it is underlain by yellow silica sand. The site was cropped in 2012 with maize, with a crop yield estimated at 6-7 tonne/ha. Planting details of the 2013 *camelina* crop are given in Table 4.

Table 4: Summary of Romanian field study sites and planting details for 2013 camelina crop

Site	Site Area (ha)	Pollutant type	Previous crop	Cultivation	Sowing			Fertilizer & Herbicide	Crop emergence
					Sowing method	Sowing date	Camelina variety		
Câmpina, Prahova County	0.8	Pyrite, building waste, oil pollution	No cultivation	n/a	Broadcast	16/11/2012	GP202	none	03/12/2012
Copşa Mică, Sibiu County	0.92	Heavy metals: Cd, Pb, Zn	Maize	Ploughing (depth 22-25 cm) Harrowing & rolling	Seed drill	03/11/2012	GP204	none	20/11/2012
Rovinari: Sterile Field, Gorj County	0.5		Maize	Ploughing (depth 20 cm)	Seed drill	09/11/2012	GP202	none	24/11/2012
Rovinari: Ash Field, Gorj County	0.5	Heavy metals: V	Semi-natural grassland	Ploughing (depth - 20 cm) Disc-harrowing	Seed drill	09/11/2012	GP204	none	24/11/2012

3 Methodology and analytical protocols

The methodology and analytical protocols described in this section were based whenever possible on standardised methods. Whilst the development and validation of these methods have been specific to the cultivation of *Camelina sativa*, they are nonetheless broadly applicable to both soil and plant matter in general, and as such the developed methodology may be applied to the cultivation of other biofuel feedstock.

3.1. Field trial soil sample collection and handling

3.1.2. Field trial soil sample collection

A stratified random sampling pattern was employed. Field sample site areas were divided into grids of approximately 400 m^2 (20 x 20 m), to yield 25 samples per hectare (10,000 m^2) according to BS ISO 10381-1:2002 and BSDD (1988). Within individual grids a single a 200-700 g soil sample was collected, sampling was to a depth of 20 cm using a steel trowel, which was cleaned with de-ionized water between each sampling. Soil samples were bagged in polyethylene bags and labelled with both sample ID and sample point GPS coordinates. Collected samples were stored in an insulated container and protect from light sources.

3.1.3. Soil sample handling and processing

Individual soil samples were thoroughly mixed and quartered. Approximately one third of each soil sample was placed soil on a horizontal surface and air-dried, at an average temperature of 20.5°C (±1.5°C). Constant mass was attained after 14 days. The remaining two-thirds soil were refrigerated at 4.0°C

3.2. Geochemical analysis of soils and composts

3.2.1. Elemental analysis

Partial digestion of the bulk base compost was carried out using *aqua regia* (AnalaR quality, HCl (aq) (32.25%) and HNO3 (aq) (69%), (in ratio 3:1 v/v)). 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.

As the determined values of TOC suggest an organic matter content of less than 20%, British Standard BS7755-3.9:1995 (ISO 11466:1995) was used. The reaction apparatus was simplified by capping each of the digestion tubes with a glass marble to provide a reflux surface for the reaction gases, reduce losses of volatile species and minimized atmospheric contamination. All glassware was acid washed in 10% w/v HNO3 (aq) for a minimum of 12 hours at room temperature, rinsed in distilled water and then de-ionized and oven dried.

Digestion of six replicate samples of 0.5 g ± 0.05 g the base compost was carried out *in aqua regia*. *Aqua regia* was made in situ with HCl (aq) being added sequentially to HNO3 (aq). Quantities used were compliant with British Standard BS 7755-3.9:1995 (ISO 11466:1995). The resulting HCl (aq) / HNO3 (aq) sediment mixture was then agitated gently at 80 revolutions min-1 using an Edmund Bühler Swip KL-2 automatic shaker for a minimum of 16 hours at room temperature to allow thorough contact between *aqua regia* and sediment (G. Ainsworth, CEH Lancaster, pers. comm., 2004; BS 7755-3.9:1995). Samples were then heated to 82.5ºC ± 2.5ºC using a Bibby block heater and this temperature maintained for a minimum of 2 hours. The digestion products were then cooled, vacuum filtered and diluted volumetrically to 50 ml using de-ionized water.

The analysis of the analytes for 20 selected elements was carried out utilising the Varian Thermo Scientific iCAP 6300 duo ICP-OES; using a solution of 0.7% HNO3 (aq) was used as the rinse agent.

Quality assurance and control

Issues of quality control and method validation were addressed by the digestion and analysis of 18 replicates of the international certified reference material LGC6139 (river clay sediment) according to the experimental procedures of the *aqua regia* digestion of soil samples. Precision and trueness were determined using statistical methods described in Section 3.5. The number of reagent blanks in digestion procedure was set at 10% of the total number of samples. Analyte concentrations were subsequently corrected for background levels of elements.

3.2.2. Total carbon and total nitrogen

Total carbon (TC) and nitrogen (TN) content for all the collected soil samples were determined by dry combustion using a LECO-TruSpec CN Analyser. Samples were oven dried at 105°C overnight. Replicate analysis was carried out using six 0.1 g ± 0.01 g samples taken from a 12.5 g aliquot of the bulk sample. To allow for changes in atmospheric pressure, subtle changes in atmospheric nitrogen and machine thermodynamics at the start of each analytical run the LECO baseline was determined by running 15 blank samples. The LECO was then conditioned using a 1.1 g sample of EDTA and calibrated using four 0.1 g ± 0.01g samples of LECO calibration soil 502-309 (C: 10.03 g ± 0.21 g; N: 0.45 g ± 0.03 g). A sample (0.1 g ± 0.01 g) of LECO calibration soil 502-309 as run every 20 environmental samples to correct for drift.-

Total organic carbon (TOC) was determined both pre and post harvesting by removing inorganic carbon from the sample using 10% v/v hydrochloric acid. Sample preparation and analysis protocol were as described for TC and TN. Leached samples were rinsed thoroughly with de-ionized water, filtered through a Millipore sintered glass vacuum filtration system using 47 mm glass-fibre filters (1.6 µm retention) and dried overnight at 50ºC prior to being analysed by the LECO graphite furnace. For the purpose of this study total inorganic carbon (TIC) is defined as the difference between the TC and the TOC, thus TIC = TC - TOC, and it is assumed that all inorganic carbon present in the soil samples will be readily dissolved by 10% v/v hydrochloric acid. The number of filtration blanks in leaching procedure was set at 10% of the total number of samples. TOC concentrations were subsequently corrected for background levels of carbon.

3.2.3. Soil acidity (pH)

For pH determination, in accordance with BS ISO 10390:2005, 5 g ± 0.1 g of the bulk soil sample was mixed with 20 ml grade 1, de-ionized water. The slurry was agitated gently at 100 revolutions min-1 using an Edmund Bühler Swip KL-2 automatic shaker for a minimum of 60 minutes at room temperature prior to the pH being measured (Orion 420A pH meter). Between each sample the probe was rinsed thoroughly with de-ionized water. The pH meter was calibrated prior to pH determinations of environmental samples, with pH 4 and 7 buffer solutions.

3.3. Phytochemical analysis of plant matter

3.3.1. Plant sample collection, handling and processing

Roots, seed and shoots of camelina pot plants were harvested separately and stored in paper bags to prevent bacterial and fungal decay of material. Shoot and seed head material stored directly in paper bags and allowed to air-dry at 20 ± 5 ºC to prevent bacterial and fungal decay of material. Root samples were washed with tap water to remove soil particles and rinsed in de-ionized water (Brunetti *et al*., 2011), and then air-dried in paper bags. All root and material were oven dried at 60ºC for 3 days (Brunetti *et al*., 2011) prior to digestion with samples being coarsely comminuted to aid digestion. Seed samples were harvested and cleaned with sieves to remove chaff and other non-oleaginous impurities, in accordance with EN ISO 658:2002.

Pre-harvest collection of plant material from field sites followed the same sampling strategy described section 3.4. Field sample site areas were divided into grids of approximately 400 m^2 (20 x 20 m), to yield 25 samples per hectare (10,000 m^2) according to BS ISO 10381-1:2002 and BSDD (1988). Rooted plant samples were collected and bagged as complete plants in paper bags and labelled with both sample ID and sample point GPS coordinates. Collected samples were stored in an insulated container and protect from light sources. Furthermore, a composite seed sample was obtained on harvesting the crop for each of the field sites.

Harvested plants were stored in paper bags and allowed to air-dry at 20 ± 5 ºC to prevent bacterial and fungal decay of material. Prior to analysis plant material for each individual sample site was divided into root, shoot and seed head, from which a seed sample was subsequently collected. To remove any soil contamination root and shoot samples were washed with tap water and rinsed in de-ionized water (Brunetti *et al*., 2011), and then oven dried at 60ºC for 3 days (Brunetti *et al*., 2011) in paper bags. Seed samples were harvested and sieved to remove chaff and other non-oleaginous impurities, in accordance with EN ISO 658:2002, before being washed with tap water to remove soil particles and rinsed in de-ionized water. Seeds were subsequently placed on polystyrene trays in paper bags and oven dried at 60ºC for 3 days.

3.3.2. Elemental analysis of seed & plant material

Complete digestion of seed and plant material was achieved using microwave digestion. Elemental analysis of plant and seed material from the greenhouse cultivation trials and the four Romanian field sites concentrated on the six metals, Cd, Co, Cu, Fe, V and Zn that have been highlighted as of concern to the aviation industry. For the in-depth case-study centred on the Rovinari-Sterile site elemental concentration of 21 selected elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V and Zn) of the digestion analytes were determined using ICP-OES analysis.

Where possible triplicate 0.5 g ± 0.005 g prepared samples were digested with a mixture of HNO3 (aq) (Normatom for trace metal analysis 67%): H2O2 (aq) (Aristar 30%): HCl (aq) (Aristar fuming 37%) (7:2:1). The procedure was carried out in accordance to BS EN 13804:2002;BS EN 13805:2003; BS EN 14084: 2003; BS EN 13804:2013. Samples were subject a 12 hour cold pre-digestion in HNO3 (aq): H2O2 (aq) mixture (7:2 v/v) with HCl (aq) added just prior to the vessels being sealed. Microwave assisted digestion of the spiked plant material was performed with a CEM Mars Xpress (40 closed PFA vessels) with the following digestion programme. Step1: Power 100 W, time 5 min; Step 2: Power 600 W, time 5 min; Step 3: Power 1080 W, time 10 min. The programme was applied twice to ensure complete digestion as indicated by the transparency of the final analyte (Chaves *et al*., 2010). After digestion, the analytes were diluted to 50 ml volumetrically using de-ionized water (Milli-Q 18 Ω). All digestion apparatus was acid washed in 10% w/v HNO3 (aq) for a minimum of 12 hours at room temperature, rinsed in distilled water and then de-ionized and oven dried.

Quality assurance and control

Issues of quality control and method validation were addressed by the digestion and analysis of 10 replicates of the international certified reference material WEPAL-IPE-599 (Sprouts (Brassica oleracea var. gemmifera) according to the experimental procedures of the microwave digestion of seed and plant samples. Precision and trueness were determined using statistical methods described in Section 3.5. The number of reagent blanks in digestion procedure was set at 10% of the total number of samples. Analyte concentrations were subsequently corrected for background levels of elements.

ICP-OES analysis parameters

The concentration of the 21 selected elements was carried out utilising the Varian Thermo Scientific iCAP 6300 duo ICP-OES in axial view. Argon (99.999%) was used as the carrier gas and 1.2 % HNO3 (aq) was used as the rinse agent, with the instrumental parameters are shown in Table 5.

Table 5: ICP-OES instrumental parameters for analysis of plant material

Parameters	Setting
RF Power	1250 W
Nebulizer	Glass concentric
Spray Chamber	Glass cyclonic
Torch	2 mm centre tube
Pump Tubing	Tygon Orange/White
Nebulizer Gas Flow	0.55 L/min
Auxiliary Gas Flow	0.50 L/min
Coolant Flow	12 L/min
Flush Pump Speed	50 rpm
Analysis Pump Speed	50 rpm
Rinse Time	30 sec
Sample Flush	45 sec
Max Integration time	Low wavelength 15 sec High wavelength 5 sec
Repeats	3

Wavelengths selection was based on the most sensitive lines free of spectral interferences; selected wavelengths are summarized in Table 6. A five point calibration (including blank) was used with the exception of Mg (four point) and Ba, Na and Ti where a three point calibration (including blank) was used. The concentration of each calibration standards is reported in Table 6 for each of the individual metals, calibration coefficients were found to be between 0.9991-0.9999.

Table 6: Selected ICP-OES wavelengths and concentration of standards used in the analysis of plant material

Element	Selected wavelength (nm)	Calibration standard concentration (mg/L)				Calibration coefficient
		Calibration standard 1	Calibration standard 2	Calibration standard 3	Calibration standard 4	
Al	394.401	0.09880	0.9880	4.940	9.880	0.9992
As	189.042	0.05	0.5	2.5	5	0.9997
Ba	455.403	1[a]	5	10	-	0.9997
Ca	315.887	1	10	50	100	0.9997
Cd	228.802	0.0495	0.495	2.475	4.950	0.9997
Co	230.786	0.04938	0.4938	2.468	4.938	0.9994
Cr	267.716	0.04938	0.4938	2.473	4.945	0.9994
Cu	324.754	0.1	1	5	10	0.9994
Fe	238.204	0.994	9.94	49.7	99.4	0.9996
K	769.896	0.99	9.9	49.5	99	0.9996
Mg	280.270	0.2	2	10	20.00[a]	0.9993
Mn	257.610	0.1976	1.976	9.880	19.76	0.9996
Mo	202.030	0.04912	0.4912	2.456	4.912	0.9995
Na	589.592	0.984	9.84	49.20[a]	98.40[a]	0.9997
Ni	231.604	0.04963	0.4963	2.482	4.963	0.9993
P	178.284	0.1979	1.979	9.895	19.79	0.9997
Pb	220.353	0.0982	0.982	4.91	9.82	0.9991
S	180.731	0.0997	0.977	4.895	9.97	0.9992
Ti	336.121	1[a]	5	10	-	0.9999
V	311.838	0.0989	0.989	4.945	9.89	0.9995
Zn	206.200	0.0987	0.987	4.935	9.87	0.9994

[a] – denotes standard not used in calibration

3.4. Analysis of plant oil

In developing a methodology that was most appropriate for the elemental analysis of the extracted plant oils, consideration was given to a number of methods, both direct and indirect in approach. In particular, the industry recognized methodologies D7111-15 (direct): Standard Test Method for Determination Trace Elements in Middle Distillate Fuels by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and UOP389-10 (indirect):Trace Metals in Organics by ICP-OES were assessed to determine the suitability of such protocols in the analysis of raw camelina oil.

The UOP389-10 method is used to determine the metals Al, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pd, Pt, Sn, Sr, Ti, V and Zn, in organic matrices such as crude petroleum, diesel and jet fuels and their blending components. Repeatability data are not available for the elements As and Cd, the latter having been highlighted as a metal of concern in the aviation fuel. Detailed consideration of the UOP389-10 method raised concerns with regard to whether the extracted camelina oil would complex successfully with the sulphur-trioxide-pyridine complex. Furthermore, health and safety issues were raised concerning the flash point of the raw plant oil and the sustained heating of the oil- sulphur-trioxide-pyridine complex at temperatures ≥500ºC in excess of four hours. It is for such reasons that it was considered that the UOP389-10 method was not most appropriate methodology to employ for the analysis of the extracted plant oil.

For the direct analysis of the extracted camelina oil, the two test methods ASTM D7111-15: Standard Test Method for Determination Trace Elements in Middle Distillate Fuels by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and ASTM D5185-09: Standard Test Method for Determination of Additive Elements, Wear Metals, and Contaminants in Used Lubricating Oils and Determination of Selected Elements in Base Oils using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) were considered. To allow for the noted differences in the viscosity of the raw plant oil and that of the target matrices for the two test methods, the key elements of both ASTM D5185-09 and ASTM D7111-15 was used a basis for a direct method of analysis for the extracted plant oil.

Consequently, for the analysis of the raw camelina oil, one direct analysis method using ICP-AES, and two different indirect methods using EDTA extraction followed by ICP-OES and microwave digestion followed by ICP-OES were trialled and developed.

3.4.1. Direct analysis

Direct analysis of camelina oils was undertaken in accordance with ASTM D5185-09: Standard Test Method for Determination of Additive Elements, Wear Metals, and Contaminants in Used Lubricating Oils and Determination of Selected Elements in Base Oils using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

A 1:10 dilution on a weight basis using 1.00±0.03 g of each of the camelina oil samples was carried out using Conostan Premisolv™ ICP solvent. For the initial analysis of the oils duplicate analysis of all test samples was undertaken.

The concentration of 14 selected elements (Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Ni, P, Pb, V and Zn) were carried out utilising the Varian Thermo Scientific iCAP 6300 duo ICP-OES in axial view. The instrumental parameters are shown in Table 7, argon (99.999%) was used as the carrier gas and Conostan Premisolv™ ICP solvent was used as the rinse agent. All torches were cleaned of any carbon deposits by heating to 750ºC according the manufacturer's instructions prior to use. The instrument was allowed to warm up for at least 30 minutes before the introduction of any samples. The torch was assessed for carbon build up while nebulizing standards. A rinse time of 60 seconds between measurements was used to avoid memory affects.

Table 7: ICP-OES instrumental parameters for direct analysis of camelina oil samples

Parameters	Setting
RF Power	1250 W
Nebulizer	V-Groove
Spray Chamber	Glass cyclonic Baffled

Torch	1 mm centre tube
Pump Tubing	Sample: Vitron Orange/White Drain: Vitron White/White
Nebulizer Gas Pressure	0.16 MPa
Auxiliary Gas Flow	1.50 L/min
Coolant Flow	12 L/min
Flush Pump Speed	50 rpm
Analysis Pump Speed	50 rpm
Pump Rate	25 rpm
Rinse Time	30 sec
Sample Flush	45 sec
Max Integration time	High wavelength 10 sec Low wavelength 5 sec
Repeats	3
Initiation Time before sample presentation	30 mins
Between test specimen flush	60 sec

Wavelengths were selected based on the most sensitive lines free of spectral interferences. Two wavelengths for the metals Ca, Cd, Co, Fe, Mg, Na, P, V and Zn were used to check for any unexpected interferences (Sarojam, 2009). As carbon-based emissions occur in the cooler regions of the plasma and emit light in the visible region of the spectrum; this may affect group I and II elements such as Ca, K, Mg and Na. As a result of either high light intensity or high concentrations these elements were measured using radial view of the plasma.

Linear calibration was undertaken using five external standards, selected to cover the expected concentration range. Calibration standards were prepared from Conostan S-21+Co+K (250 µg g^{-1}) standard at the concentrations shown in Table 8. The standards diluted by weight with Conostan Premisolv™ ICP solvent.

An internal standard of Yttrium was added to every calibration standard and test specimen. The purpose of an internal standard was to eliminate nebulizer transport effects. Spectrum to spectrum changes in the emission intensity of the internal standard was used to correct for such effects. Yttrium, selected as the internal standard as the other suggested metals, Cd and Co, were two of the six metals of interest. Yttrium was added to each of the test samples at a concentration of 12.75 ± 0.03 µg g^{-1}.

Table 8: Concentration of standards used for the analysis of camelina oil

Standard	Concentration of multi-element standard (µg g^{-1})	Concentration of Yttrium (µg g^{-1})
Calibration Blank	0	14.15
Calibration Standard 1	0.1032	14.14
Calibration Standard 2	0.2628	14.13
Calibration Standard 3	1.032	14.09
Calibration Standard 4	2.576	14
Calibration Standard 5	10.235	13.57
Check Standard (5 µg g^{-1})	5.025	10.59
Check Standard (1:10)	2.041	12.71

Quality control was achieved using two check standards. Check standards were prepared in the same manner as the calibration standards from Ca, K, Mg, Na, and P in biodiesel (B100; 20 ppm/wt). Check standards and a blank were analysed after every fifth sample. Where results were not within 5% of expected concentration, the instrument was recalibrated and test specimens reanalyzed.

3.4.2. Indirect analysis

Determination of trace element concentrations in the harvested camelina oils was carried out using EDTA extraction and microwave digestion.

EDTA extraction

The extraction of trace elements using 0.05 mol l-1 EDTA (Sinha *et al*., 2010) was undertaken using a protocol adapted from Quevauviller *et al*. (1997). A solution of 0.05 mol l-1 EDTA was prepared as an ammonium salt solution, 14.612 ± 0.050g of acid-free EDTA to 80 ± 2 ml of de-ionized water, the solution was continuously stirred and 13 ± 0.5 ml of saturated ammonium solution was added to ensure EDTA was dissolved. On dissolution of EDTA, the volume was adjusted with de-ionized water to 1000ml volumetrically, after adjustment of the pH. The pH of the solution was then adjusted to 7.00 ± 0.05 using either ammonium solution or HCl (aq) (Aristar fuming 37%) as appropriate. All experimental glassware was acid washed in 10% w/v HCl (aq) for a minimum of 12 hours at 20 ± 5 ºC, rinsed in de-ionized water , cleaned with 0.05 mol l-1 EDTA and rinsed with de-ionized water.

The suitability of the method for trace element determination in camelina oil was assessed using aliquots of food-grade camelina oil that had been spiked with Conostan S-21+Co+K (250 µg g-1) standard. Five 5 ± 0.5g replicate samples were prepared for each of the four spike concentrations 0 mg kg-1; 0.1 mg kg-1; 1 mg kg-1 and 5 mg kg-1. Each spiked oil sample was weighed into 50 ml polypropylene screw-topped container and 25ml of EDTA solution was added. The mixture was then shaken at 100 r.p.m on an orbital shaker for 16 hours at 20 ± 5 ºC.

At the end of the shaking period, the extracts were placed in separating funnels and allowed to the complete partition into two immiscible phases. The lower aqueous phase was the removed for analysis using ICP-OES analysis. Instrumental parameters are shown in Table 9; argon (99.999%) was used as the carrier gas.

The number of reagent blanks in extraction procedure was set at 10% of the total number of samples. Analyte concentrations were subsequently corrected for background levels of elements.

Table 9: ICP-OES instrumental parameters for analysis of camelina oil EDTA extracts.

Parameters	**Setting**
RF Power	1250 W
Nebulizer	V-Groove
Spray Chamber	Glass cyclonic Baffled
Torch	1 mm centre tube
Pump Tubing	Sample: Vitron Orange/White Drain: Vitron White/White
Nebulizer Gas Pressure	0.16 MPa
Auxiliary Gas Flow	1.50 L/min
Coolant Flow	12 L/min
Flush Pump Speed	50 rpm
Analysis Pump Speed	50 rpm
Pump Rate	25 rpm
Rinse Time	30 sec
Sample Flush	45 sec
Max Integration time	High wavelength 10 sec Low wavelength 5 sec
Repeats	3
Initiation Time before sample presentation	30 mins
Between test specimen flush	60 sec

Microwave-assisted acid digestion

Digestion of oil samples can also be achieved using microwave assisted acid digestion. Elemental concentration of 15 selected elements (As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Ni, P, Pb, V and Zn) of the digestion analytes were determined using ICP-OES analysis.

Five 0.5 g ± 0.05 g replicates of oil from each of the field trial sites (Câmpina, Copşa Mică, Rovinari-Ash and Rovinari-sterile) as well as oil from the camelina crop grown at USAVM's experimental site at Moara Domneasca were digested with a mixture of HNO3 (aq) (Normatom for trace metal analysis 67%): de-ionized water (Milli-Q 18 Ω).: HCl (aq) (Aristar fuming 37%) 5:5:1 ratio (Juranovic *et al*., 2003; Cindric *et al*., 2007; Ansari *et al*., 2009). The samples weighed directly into polytetrafluoroethylene (PTFE) digestion vessels. Microwave assisted digestion of the spiked plant material was performed with a CEM Mars Xpress (40 closed PFA vessels) with the following digestion programme summarized in Table 10 (Llorent-Martinez *et al*., 2011). After digestion, the analytes were diluted to 50 ml volumetrically using de-ionized water (Milli-Q 18 Ω). All digestion apparatus was acid washed in 10% w/v HNO3 (aq) for a minimum of 12 hours at room temperature, rinsed in distilled water and then de-ionized and oven dried..

The reliability of the method for trace element determination in camelina oil, was assessed using five 5 ± 0.5g aliquots of food-grade camelina oil that had been spiked with Conostan S-21+Co+K (250 µg g^{-1}) standard to give a concentration of trace elements 5.0325 µg g^{-1}. Five replicates (5 ± 0.5g) of unspiked food-grade camelina oil were analysed in tandem to allow the experimental determined concentrations in the spiked samples to be corrected. The number of reagent blanks in digestion procedure was set at 10% of the total number of samples. Analyte concentrations were subsequently corrected for background levels of elements.

Table 10 : Operating programme for the microwave digestion of camelina oil samples

Microwave digestion programme				
Step	**Initial *T* (ºC)**	**Final *T* (ºC)**	**Time (min)**	**Power (W)**
1	25	90	5	700
2	90	90	3	600
3	90	140	5	600
4	140	140	5	600
5	140	170	10	600
6	170	170	10	600

The same ICP-OES instrumental parameters used for the analysis of plant and seed material (Table 5) were used in the analysis camelina oil using microwave-assisted acid digestion. Similarly, the selection of calibration standards and wavelengths emulated those used in the analysis of the plant and seed material (Table 6).

3.4.3. Initial assessment of oil methodologies

Consideration of the three methodologies trialled and developed, found that the reproducibility and repeatability of analytical data using the direct method was limited as the build up of carbon on the torch was a continual issue.

The reliability of the indirect method using EDTA as the extractant was assessed via the determination of the concentrations of metal spikes that had been added for this purpose, recovery levels of such metals were low with recovery rates of ≤75% and more typically ≤50% being determined. By contrast, the recovery rates for the same metals determined for the indirect method using microwave-assisted acid digestion were within experimentally acceptable limits and typically >95%.

Therefore, initial assessment of the three methodologies suggest that the most appropriate methodology to analyse the elemental concentrations of the raw extracted camelina oil is that of the indirect method using microwave-assisted acid digestion.

3.5. Statistical Analysis

Where appropriate the experimental data sets were assessed for homogeneity of variance (Levennes test) and the goodness of fit to a normal distribution (Kolmogorov-Smirnov test). It was found that even after logarithmic (base 10) transformation the data for metals such as chromium (Cr), nickel (Ni) and lead (Pb) did not exhibit either homogeneity of variance or normality. Therefore, with the exception of wavelength selection, non-parametric statistical routines are used in the data analysis of the current study.

Precision and trueness for both total metal and extractable metal concentrations using the CRM LGC6139 (Section 3.2) and WEPAL-IPE-599 (Section 3.3) and protocols are expressed as the coefficient of variance and bias, respectively.

Kruskal-Wallis one-way analysis of variance by ranks was used to identify significant differences in the determined any significant relationships that may exist between the bulk soil metal concentrations determined for the pre-cultivation and pre-harvest soil samples from the Rovinari – Sterile case-study site.

4 Results of controlled cultivation trials

This section discusses the results of the controlled cultivation trials (greenhouse) in the following order:

- Pre-planting geochemical analysis of soils
- Elemental analysis of parent seed
- Plant growth & crop survey
- Harvesting of plant matter
- Elemental analysis of camelina root
- Elemental analysis of camelina shoot
- Elemental analysis of daughter seed
- Elemental analysis of Oil from daughter seed
- Comparison of trace metals in soil, roots, shoots and daughter seed
- Post-cropping geochemical analysis of soils

Geochemical definition of the bulk soil is achieved through the determination of total elemental concentrations, total carbon, total inorganic and organic carbon and total sulphur content and pH.

4.1. Pre-planting geochemical analysis of soils

4.1.1. Elemental analysis

The base compost used in the controlled cultivation trials was analysed for trace metals prior to seedling planting the analysis is presented in Table 11. Analysis of the selected elements was based on the digestion of three 0.5 g ± 0.05 g samples of each of the soil samples. It is assumed that the concentration of the known ‘spike’ contaminant added to the compost medium is dominant over any trace component present in the base compost as the metal spikes are introduced as soluble salts, and assumed to have enhanced bioavailability to metals present in the soil matrix. Additionally, no post- harvest follow up analysis of the soil used in the controlled cultivation trial was completed: it is assumed that loses through evaporation or leaching are negligible.

Table 11: Elemental Concentrations determined for the base compost used in the spiking experiments (n=6; values quoted to two significant figures, interquartile range three significant figures)

	Elemental concentration (mg kg $^{-1}$)														
Base compost	As	Ca	Cd	Co	Cr	Cu	Fe*	K	Mg	Na	Ni	P	Pb	V	Zn
Median	3.7	8300	0.43	2.8	10	11	1.0	1300	2200	110	7.2	580	20	13	47
Interquartile range	1.77	35100	0.112	0.167	1.35	1.82	0.394	104	172	20.7	0.616	17.9	2.55	0.926	11.4
Minimum	1.8	6500	0.35	2.6	8.4	9.7	0.78	1100	1900	94	6.2	510	17	12	30
Maximum	4.9	53000	0.63	3.0	32	15	1.9	1400	2700	130	7.8	590	21	34	58

* percentage value

4.1.2. Total carbon and total nitrogen in base compost

Total carbon (TC) and nitrogen (TN) content for all the collected soil samples were determined by dry combustion using a LECO-TruSpec CN Analyser. Samples were oven dried at 105°C overnight. Replicate analysis was carried out using six 0.1g ± 0.01 g samples taken from a 12.5 g aliquot of the bulk sample. The median values for TC, TIC, TOC, TN and the C: N ratio for the base compost used in the controlled cultivation trials are summarized in Table 12. The interquartile range together with the minimum-maximum range for the data at each of the study sites is also included.

4.1.3. Soil Acidity (pH) of base compost

The pH of the base compost is reported in Table 12.

Table 12: Descriptive statistics for the TC, TIC, TOC, TN and C: N ratio of the base compost used in the controlled cultivation trials (n=6; values quoted to two significant figures, interquartile range three significant figures)

Base Compost	TC Mass (%)	TN Mass (%)	C:N ratio Mass (%)	pH*
Median	5.0	0.22	21	
Interquartile range	0.847	0.015	2.85	
Range (min-max)	4.4-5.6	0.20-0.30	19-25	6-7

***** *Manufacturers specification pH 6-7*

4.2. Elemental analysis of parent seed

The seed used in the controlled cultivation trials was provided by Camelina Company Espania, and is reportedly the same as that used in the 2013 commercial plantings of ITAKA project undertaken in Spain. The analysis of this seed for trace metals prior to planting was carried out on the basis of three replicates. The results are presented in Table 13. The highest concentrations are determined for Cu and in particular Fe and Zn, and are likely to be a reflection their role in plant physiology.

Table 13: Concentrations of the trace elements Cd, Co, Cu, Fe, V and Zn in the parent camelina seed used in spiking experiments expressed as median values of analyses of three replicate samples (values quoted to two significant figures).

	Elemental concentration (mg kg^{-1} dry matter)					
	Cd	**Co**	**Cu**	**Fe**	**V**	**Zn**
Spiking seed	0.45	0.11	6.8	76.0	<LOD	49.0
IQ range	0.018	0.0054	0.082	0.3	-	1.8

4.3. Plant growth & crop survey

Plant growth in the controlled cultivation trials were assessed qualitatively using the following parameters during cultivation and at harvesting

- Seedling survival rate
- Root mass
- Basal leaf size
- Shoot and inflorescence vigour
- Seed colour, number and ventricosity

The observations are reported on an individual contaminant spike basis as below.

Cadmium

Across all spikes a 70-80% seedling survival rate is observed. However decreases in the basal leaf size and the number seeds per silicles of 25% and 50-75%, respectively are reported in plants from Spike 1 to Spike 4. It is noted that the plants treated with Spike 1 and Spike 2 show uniform maturing of silicles and good level of seed set. By contrast seed inflorescences in plants subject to Cd levels in Spike 3 and 4 are spindly in appearance and exhibit uneven ripening and variable seed set with some siliqua containing no seeds.

Cobalt

At the higher concentrations of Co used for Spike 3 and 4, seedling survival rate is impaired with die back of seedlings being observed. Plants treated with Spike 3 display leaf-tip necrosis. Similarly chlorosis and necrosis is noted in the basal leaves in Spike 4 plants. An overall decrease of 50% in root mass is observed from Spike 1 to Spike 4. Shoot widths show little variation and are typically discerned to be 1-2 mm in diameter. Seed from Spike 1, 2 and 3 plants show form and colour typical of *Camelina sativa* and exhibit good ventricosity.

Copper

Seedling survival rate is between 60-80%, with the greatest levels are seen for seedlings treated with Spike 2. Survival levels of 60-70% are observed in the treatments Spike 1 and Spike 3. The concentration of Cu in the Spike 4 treat resulted in complete die-back of the seedlings. As with Cd increases in Cu concentrations resulted in a shortening of basal leaf length from 80 mm to 60 mm across the three spikes. Shoot widths show little variation and are found to be typically 1-2 mm in diameter. Similarly, root mass does not appear to be affected by the concentration of Cu present in the growing medium. Plants in all treatments exhibit variable seed set, with Spike 3 seed set estimated to be 50%. Of note is that plants subjected to the lowest concentration of Cu display numerous small silicles, characterized by seeds displaying a range of morphologies from the typical ovoid to atypical reniform and variable colour from raw sienna (typical) to dark mahogany. The seeds appear flat rather than ventricose, suggesting reduced vigour (Li *et al*., 2010).

Iron (II)

Seedling survival rates are poor with survival rates ≤ 20% being observed for treatment spikes 3 and 4, resulting in insufficient seed production for analysis purposes.

Plant growth for all treatments is unsubstantial with shoot thickness being <1 mm in all treatments and in the case of the Spike 4 diameters of <0.5 mm are common. A sharp contrast in shoot colour is noted between the Spike 1 plants (blonde) and the other treatments (dark brown). Furthermore, for plants grown in Spike 3 and 4 precipitates of Fe salts are noted on the shoot at the soil interface.

An overall pattern of decreasing root mass with increasing spike concentration is observed, with decreases in mass of approximately 50 % decrease between Spike 1 and Spike 3 being discerned. Changes in root colour are also noted with roots from Spike 3 and 4 being dark brown to black in colour.

Iron (III)

Seedling survival rates are poor. Twenty-five percent dieback is noted in Spike 2, with ≤ 75% of seedling surviving when exposed to treatment spikes 3 and 4; consequently for Spikes 3 and 4 insufficient seed was produce for analytical purposes.

Plant growth for all treatments is unsubstantial with shoot thickness being <1 mm in all treatments and in the case of the Spike 4 diameters of <0.5 mm are common. However, the sharp contrast in shoot colour in noted for Fe(II) spiked plants is not apparent in Fe(III) spiked plants, nor were precipitates of Fe salts noted on the shoot at the soil interface.

Comparison with the root mass of the Control plants suggest that in Fe(III) spiked plants reductions of root mass 25-50% are possible. An overall pattern of decreasing root mass with increasing spike concentration is observed, with decreases in mass of approximately 50 % decrease between Spike 2 and Spike 3 being discerned.

Vanadium

Seedling survival ranges from 33% (Spike 4) to ~90% (Spike 3). Although seedling survival is highest for the Spike 3 treatment the associated plants show reduced vigour with seed set being observed in just 44% of the plants. By contrast, seed set is displayed in 82% of plants in the Spike 1 and 2 treatments. Plants subject to the highest concentration of V are characterized by poor vigour and only 33% of surviving plants successfully setting seed. Seeds appear to show colour changes and reduced ventricosity as V concentration increases that are contrary to morphologies that are typical to camelina seed. For example, approximately 30% of Spike 2 seed are small and mahogany in colour whilst 5% of Spike 3 seed are black angular and flat in form.

As with Cd and Cu basal leaf length is seen to decrease as V concentration in the soil increases and overall a 20% reduction in leaf length is noted. Shoot diameter is also seen to decrease with increasing concentrations of V, with straws of Spike 1 typically 1-2 mm in diameter, by contrast those of Spike 4 are found to be ≤ 0.5 mm. Root mass is seen to decrease with increases in V concentration.

Zinc

In contrast to the high seedling survival rates reported for Spike 1 and 2 (> 90%) and those for Spike 3 (72%), survival rates for the Spike 4 treatment are low (<17%).

Plants in all Zn treatments are characterized lack of vigour and stunted and spindly growth with basal leaf lengths typically shorter than those recorded for other metal spikes, with a maximum length of 65 mm is reported for Spike 2 plants. In particular, Spike 4 plants display short twisted leaves that exhibit interveinal chlorosis. Shoot diameter is seen to reduce from Spike 1 to Spike 4 (<2mm and <1 mm, respectively). A similar trend in root mass is also observed as Zn concentration increases. All treatments exhibit flowering but resultant seed set is low 20-50%.Preliminary observations found that die-back of seedlings at the concentration of all metal spiking experiments was noted with 7 days of being introduced to the spiked growth medium. Significant die-back of plants is noted for the Fe (II) and Fe (III) spikes 2 and 3. Comparison with control plants found that growth habitat of the spiked plants is less vigorous and the occurrence of branched flower inflorescences is rare in the spiked plants compared to that of the control plants. Necrotic and chlorotic tips are noted on the basal leaves of spiked plants.

4.4. Harvesting of plant matter

Shoots were harvested separately from roots to reduce contamination of material from spiked the growth medium; seeds were subsequently collected from the harvested shoots.

4.5. Elemental analysis of camelina root and shoot material

The analysis of the plant roots and shoots for trace metals prior to planting was carried out where sample mass allowed on the basis of three replicates. The results are reported on an individual basis for each of the metals used to spike the base compost in Tables 14a - 20a for shoot material and Tables 14b - 20b for the harvested root material.

Cadmium

For both the camelina root and shoot data in Table 14(a and b) there is a distinct increase in Cd concentration with increasing soil spike concentration. In particular, the concentrations of >100 mg kg^{-1} reported for the root and shoot material harvested from the compost subject to Cd spike 4 concentration suggest that at *Camelina sativa* has the potential to act as a hyperaccumulator for

Cd (Brooks and Baker, 1989. Although the shoot : root ratios of the current project should be considered with caution, consideration of the shoot : root ratio gives an indication of a plants ability to tolerate (shoot : root ratio < 1) or accumulate (shoot : root ratio >1) a given metal (Brunetti *et al*., 2011). The shoot : root ratio determined for the four Cd spiked material suggest that camelina is capable of expressing both accumulation, Cd spike 1 (shoot : root ratio =1.4) and tolerance, Cd spike 2, 3 and 4(shoot : root ratio =0.35 to 0.90) for the Cd. The change in this behaviour from accumulation (Cd spike 1) to the low shoot : root ratio (0.35) observed for Cd spike 4, may also result from the breakdown of normal plant functioning as the critical plant concentration (typically 5-30 mg kg^{-1}) is exceeded in the camelina plant tissue (Alloway, 1990; Jambhulkar *et al*., 2009) and toxic effects come into play. Further work would be needed to validate these initial results.

Consideration of potential synergies and antagonisms with other metals in Table 14 with increases in Cd concentration are difficult to discern between the shoot data and the control data (Table 14(a)) when the measure of dispersion is taken into consideration. Although there is a suggestion, that Cd may affect the movement of Cu into the shoot. In contrast, the root analysis (Table 14(b)) intimates that high levels (≥ 100 mg kg^{-1} dry matter) of available Cd results in the enrichment of the metals Co, Cu, Fe, V and Zn in the root matter of camelina, although such observations should be treated with circumspect given that the such observations are based on one replicate.

Table 14: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for cadmium expressed as median values; interquartile ranges are reported in parenthesis (n=3; values quoted to two significant figures; interquartile ranges quoted to three significant figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1)

	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.28** **(0.0584)**	**0.057** **(0.0836)**	**4.3** **(1.35)**	**52** **(40.3)**	**0.045** **(0.0680)**	**21** **(9.05)**
Cd1	**3.4** **(1.29)**	**0.079** **(0.110)**	**2.1** **(0.258)**	**110** **(282)**	**0.090** **(0.359)**	**19** **(3.74)**
Cd2	**8.8** **(7.11)**	**0.019** **(0.00981)**	**2.4** **(0.219)**	**48** **(38.0)**	**0.010** **(0.0546)**	**17** **(6.06)**
Cd3	**79** **(6.39)**	**0.079** **(0.0753)**	**2.3** **(0.151)**	**200** **(97.0)**	**0.22** **(0.151)**	**18** **(1.99)**
Cd4	**190** **(111)**	**0.050** **(0.0202)**	**2.3** **(0.0474)**	**140** **(30.5)**	**0.090** **(0.0506)**	**18** **(4.30)**

Spike	(b) Elemental concentration (mg kg^{-1} dry matter) in camelina root					
	Cd	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.32**	**0.25**	**6.1**	**650**	**1.0**	**31**

	(0.0649)	(0.183)	(0.768)	(313)	(0.419)	(11.2)
Cd1	2.5	0.40	3.8	780	1.3	35
Cd2	13	0.39	3.8	730	1.1	44
Cd3	88	0.43	3.7	640	0.98	51
Cd4	540	0.76	5.4	1300	1.9	80

Cobalt

Initial observations of the Co spike data in Table 15 suggests that a distinct increase in Co concentration occurs with increasing spike concentration, for both root and shoot material.

Scrutiny of the shoot : root ratio for the four Co spiked material suggest that camelina expresses both tolerance, for Co at soil Co concentrations ≤ 2 mg/kg and ≥ 100mg/kg (0.79 and 0.65, respectively). By contrast the shoot : root ratio for the Cd spike 2 and 3 concentrations (1.8 and 2.1, respectively) suggest camelina acts as an accumulator for Co at such concentrations. The change in behaviour from accumulation for Cd spike 2 and 3, to tolerance observed for Cd spike 4, may result from the breakdown of normal plant functioning as the critical plant concentration for Co in camelina is exceeded and toxic effects begin to dominate (Alloway, 1990; Jambhulkar *et al*., 2009). Further work would be needed to validate these initial findings.

After variation with between samples in allowed for, no overt synergies and antagonisms with other metals in Table 15 are discerned. Cadmium levels appear to be enriched in the shoot matter for Spike concentration 4 (≥ 200 mg kg^{-1} dry matter). Whilst the Zn concentrations of both the root and shoot material from Spike concentration 3 (≥ 20 mg kg^{-1} dry matter) appears anomalous, and further work would be needed to determine if such an observation is an artefact of the analysis or characteristic of the behaviour of Co uptake in camelina.

Table 15: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for cobalt expressed as median values; interquartile ranges are reported in parenthesis (n=3; values quoted to two significant figures; interquartile ranges quoted to three significant figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1)

	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	0.28	0.057	4.3	52	0.045	21
	(0.0584)	(0.0836)	(1.35)	(40.3)	(0.0680)	(9.05)
Co1	0.30	1.1	5.0	36	0.00	29
	(0.0144)	(0.221)	(0.229)	(4.83)	(0.0101)	(1.90)
Co2	0.32	9.2	6.7	110	0.12	31
	(0.0314)	(1.60)	(0.840)	(36.1)	(0.0805)	(3.95)

Co3	**0.54**	**27**	**5.8**	**120**	**0.030**	**42**
	(0.245)	**(7.37)**	**(0.263)**	**(33.0)**	**(0.185)**	**(5.39)**
Co4	**1.5**	**71**	**4.3**	**35**	**0.00**	**23**
	(0.142)	**(1.27)**	**(2.49)**	**(5.25)**	**(0.00505)**	**(1.67)**

	(b) Elemental concentration (mg kg-1 dry matter) in camelina root					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.32**	**0.25**	**6.1**	**650**	**1.0**	**31**
	(0.0649)	**(0.183)**	**(0.768)**	**(313)**	**(0.419)**	**(11.2)**
Co1	**0.39**	**1.4**	**8.0**	**620**	**1.1**	**29**
Co2	**0.35**	**5.0**	**8.2**	**400**	**0.74**	**43**
Co3	**0.32**	**13**	**5.9**	**400**	**0.75**	**19**
Co4	**0.49**	**110**	**6.8**	**460**	**0.82**	**25**

Copper

The plant material concentrations reported for the compost that was spiked with Cu are presented in Table 16 (a) and (b). Perusal of the data finds that the concentrations reported for the root and shoot material are below 1000 mg kg^{-1}, this suggests that *Camelina sativa* does not act as a hyperaccumulator for Cu (Brooks and Baker, 1989). The 100% seedling mortality reported for the Cu Spike 4 concentrations (≥ 1000 mg kg^{-1} dry matter) suggest that the Cu critical plant concentration for camelina plant tissue levels occur above 6.5 mg kg^{-1} dry matter in shoots and 21 mg kg^{-1} dry matter in roots (Alloway, 1990; Jambhulkar *et al*., 2009). For the current study, this suggests that the Cu toxic level for camelina occurs at soil Cu concentrations between 100 mg kg^{-1} and 1000 mg kg^{-1}. Overall concentrations of Cu are found to be higher in the root material than in the camelina shoots, resulting in shoot : root ratios <1, for all spike concentrations and therefore suggesting a tolerance for Cu in camelina plants.

Increases in both the root and shoot material Cu concentration are noted in plants exposed to Cu Spike concentration 1 when compared with the corresponding concentrations in the control plants. However, incremental increases with subsequent increases in spike concentration are not observed. Little variation between the Cu concentrations is discerned between the shoot material of plants treated with Spike concentration 1 (10 mg kg^{-1} dry matter) and those treated with Spike concentration 2 and 3 (50 and 100 mg kg^{-1} dry matter, respectively).This suggests that analogous to other plant species, *Camelina sativa,* self-regulates Cu uptake from the soil (Baker & Brooks, 1989). By contrast, differences are noted between the shoot material of plants treated with Spike concentration 1 and 2 (10 mg kg^{-1} and 50 mg kg^{-1} dry matter, respectively) and those treated with Spike concentration 3 (100 mg kg^{-1} dry matter). The apparent disparity in the Cu concentration in the camelina root and shoot material appear to be in agreement with observations made by other authors that the root Cu concentration may become substantially enriched before increases in Cu concentration in aerial parts of the plant become significant (Foy *et al.*, 1978).

Increases in the Cu spike concentration, in particular, for Spike concentration 2 and 3 (50 and 100 mg kg^{-1} dry matter, respectively) appear to coincide with decreases in the concentration of Cd, Fe and Zn in the shoot material (Table 16 (a)). This suggests that at Cu soil concentrations at 50 mg kg^{-1} dry matter and above Cu may have an inhibitory effect on the translocation of the essential micronutrients Fe and Zn, and through shared transport mechanisms Cd, from the roots to the shoots. Similar antagonistic behaviour has been noted in other plant species including *Brassica* species such as oilseed rape (*Brassica napus*), where Cu was observed to induce Fe deficiency and interfere with Zn uptake (Foy *et al.*, 1978; Ebbs & Kochian, 1997). Therefore, high levels of available Cu may have implications to the uptake of Fe and Zn in field scenarios and consequently crop yields. However, the legitimacy of such observations may be questioned given the small replicate number and when the degree of variance expressed by both the control data and the spiked data is taken in to account. More extensive trials is needed to verify if the initial interpretation of the data is valid.

Table 16: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for copper expressed as median values; interquartile ranges are reported in parenthesis (n=3; values quoted to two significant figures; interquartile ranges quoted to three significant figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1)

	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	0.28 (0.0584)	0.057 (0.0836)	4.3 (1.35)	52 (40.3)	0.045 (0.0680)	21 (9.05)
Cu1	0.25 (0.00995)	0.069 (0.0749)	6.2 (0.739)	78.1 (172)	0.10 (0.290)	21 (4.38)
Cu2	0.018 (0.0345)	0.049 (0.0148)	6.1 (0.417)	47 (13.0)	0.070 (0.0446)	12 (2.99)
Cu3	0.030 (0.0100)	0.0094 (0.01250)	6.5 (1.63)	16.5 (32.1)	0.040 (0.0400)	11 (2.92)

Spike	(b) Elemental concentration (mg kg^{-1} dry matter) in camelina root					
	Cd	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	0.32 (0.0649)	0.25 (0.183)	6.1 (0.768)	650 (313)	1.0 (0.419)	31 (11.2)
Cu1	0.41	0.59	12	490	0.74	33
Cu2	0.28	0.23	12	400	0.70	33

Cu3	**0.47**	**0.29**	**21**	**400**	**0.75**	**20**

Iron (II)

Initial observations of the Fe(II) spike concentration data in Table 17 suggests that whilst a distinct increase in Fe(II) concentration with increasing spike concentration, for both root and shoot material, accumulation is equal to or below that of (i) the initial spike concentration and (ii) the cumulative value of the spike concentration and base compost. In contrast to the shoot data, the concentration of Fe(II) in the root material increases consistently with spike concentration,. With the exception of Spike concentration 3, the concentrations of Fe (II) in the root material is found to be greater than those determined for the shoot material, therefore, camelina appears to demonstrate tolerance towards Fe(II). However, further work is needed to understand whether the shoot : root ratio determined for Spike concentration 3 (1.4) is non-typical behaviour of Camelina sativa, or whether it may be indicative of accumulative behaviour at soil Fe(II) concentrations of between 1-5%. Furthermore whether such behaviour consequently breaks down as toxicity effects become increasingly influential at soil Fe(II) concentrations above 5% (Alloway, 1990; Jambhulkar *et al*., 2009).

It is noted that the plant (root and shoot) material for Spike concentration 2, 3 and 4 treatments exhibit a considerable enrichment of the other metals being considered. Further research is needed to determine whether such enrichment has been (i) mediated by the levels of Fe(II) present; (ii) as a result of the high quantities of spike material used to achieve the required levels, the impurities in the spike material have become significant or (iii) as a result of using the sulphate salt as the spiking material the pH has been lowered leading to increased mobility of the other metals.

Table 17: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for iron (II) expressed as median values; interquartile ranges are reported in parenthesis (n=3; except where † denotes n = 2; ° denotes n = 1; values quoted to two significant figures; interquartile ranges quoted to three significant figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1)

	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.28**	**0.057**	**4.3**	**52**	**0.045**	**21**
	(0.0584)	**(0.0836)**	**(1.35)**	**(40.3)**	**(0.0680)**	**(9.05)**
Fe(II)1	**0.24**	**0.038**	**4.4**	**26**	**0.00**	**19**
	(0.00628)	**(0.0.0100)**	**(0.168)**	**(1.64)**	**(0.00778)**	**(0.793)**
Fe(II)2	**0.77**	**6.7**	**8.8**	**14000**	**2.2**	**64**
	(0.0280)	**(0.0150)**	**(0.550)**	**(770)**	**(0.546)**	**(4.80)**
Fe(II)†3	**2.3**	**13**	**22**	**58000**	**1.1**	**100**

	(0.0194)	(0.688)	(0.409)	(1580)	(0.102)	(6.42)
Fe(II)º4	**2.2**	**4.4**	**18**	**42000**	**1.9**	**80**

	(b) Elemental concentration (mg kg^{-1} dry matter) in camelina root					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.32**	**0.25**	**6.1**	**650**	**1.0**	**31**
	(0.0649)	**(0.183)**	**(0.768)**	**(313)**	**(0.419)**	**(11.2)**
Fe(II)1	**0.32**	**0.19**	**6.7**	**380**	**0.78**	**35**
Fe(II)2	**1.4**	**5.5**	**14**	**29000**	**0.85**	**42**
Fe(II)3	**1.7**	**7.3**	**21**	**42000**	**4.5**	**44**
Fe(II)4	**1.2**	**3.6**	**64**	**50000**	**5.7**	**59**

Iron (III)

Consideration of the Fe(III) spike data in Table 18 (a) and (b) suggests that a distinct increase in Fe(III) concentration with increasing spike concentration, for both root and shoot material is noted. However, the observed accumulation is below that of (i) the initial spike concentration and (ii) the cumulative value of the spike concentration and base compost; with concentrations in the root material being greater than that of the shoot. The data for Spike concentration 1 shoot appears to contradictory to this trend, however as the analysis is based on a single replicate it should be treated with caution.

Comparison with the Fe(II) data suggests that accumulation of Fe(III) is not as great as Fe(II), with accumulation of Fe(III) being at least two-fold lower than that of Fe(II). As with Fe(II), it is noted that the plant (root and shoot) material for Spike concentrations 2, 3 and 4 treatments exhibit a considerable enrichment of the other metals being considered. Again further research is needed to understand the vectors that are operational in systems of high Fe(III) concentrations.

Table 18: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for iron(II) expressed as median values; interquartile ranges are reported in parenthesis (n=3; (n=3; except where † denotes n = 2; ° denotes n = 1; values quoted to two significant figures; interquartile ranges quoted to three significant figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1)

	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
Spike	**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.28**	**0.057**	**4.3**	**52**	**0.045**	**21**

	(0.0584)	(0.0836)	(1.35)	(40.3)	(0.0680)	(9.05)
Fe(III)º1	0.53	0.86	5.8	500	0.28	80
Fe(III)2	0.35	0.27	5.3	87	0.046	32
	(0.0280)	(0.0150)	(0.550)	(22.7)	(0.0228)	(6.33)
Fe(III)†3	0.43	2.1	8.1	7570	1.3	48
	(0.0273)	(0.367)	(0.652)	(1900)	(0.00127)	(1.24)
Fe(III) †4	0.82	5.5	13	23300	2.7	52
	(0.0650)	(0.295)	(0.592)	(845)	(0.800)	(4.53)

Spike	(b) Elemental concentration (mg kg^{-1} dry matter) in camelina root					
	Cd	Co	Cu	Fe	V	Zn
Control	0.32	0.25	6.1	650	1.0	31
	(0.0649)	(0.183)	(0.768)	(313)	(0.419)	(11.2)
Fe(III)1	-	-	-	-	-	-
Fe(III)2	0.50	0.77	5.4	640	0.36	0.39
Fe(III)3	1.4	8.6	22	54000	7.9	7.0
Fe(III)4	1.5	9.8	24	60000	8.3	7.5

Vanadium

Qualitative analysis of the V spike data in Table 19 (a) and (b) suggests that whilst clear increases in V concentration with increasing spike concentration are discerned, for both root and shoot material, concentrations are discerned to be below that of (i) the initial spike concentration and (ii) the cumulative value of the spike concentration and base compost. With concentrations in the root material being observably greater than those determined for the shoot material, the shoot : root ratios are typically ≤0.13 suggesting low transference from the roots into the aerial part of the camelina plant as well as exhibiting tolerance to V in the soil. After variation with between samples in allowed for, possible antagonisms with Cu is suggested by both the root and shoot data, although further work would be necessary to determine the validity of such an observation. Similarly, a possible synergy with Zn is noted for V Spike concentration 4 concentration (200 mg kg^{-1} dry matter) in the shoot

Table 19: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for vanadium expressed as median values; interquartile ranges are reported in parenthesis (n=3; except where † denotes n = 2; values quoted to two significant figures; interquartile ranges quoted to three significant

figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1; where – denotes insufficient sample for analysis)

Spike	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
	Cd	Co	Cu	Fe	V	Zn
Control	0.28	0.057	4.3	52	0.045	21
	(0.0584)	(0.0836)	(1.35)	(40.3)	(0.0680)	(9.05)
V 1	0.23	0.030	2.4	25	0.62	14
	(0.0556)	(0.0301)	(0.215)	(8.46)	(0.212)	(3.19)
V2	0.25	0.050	2.3	49	1.6	15
	(0.0188)	(0.00481)	(0.0149)	(15.1)	(0.0200)	(1.62)
V3	0.22	0.039	2.6	33	9.1	21
	(0.0411)	(0.00499)	(0.0881)	(4.38)	(1.78)	(8.61)
V †4	0.28	0.081	2.4	150	110	21
	(0.0231)	(0.00881)	(0.0308)	(19.1)	(1.22)	(1.44)

Spike	(b) Elemental concentration (mg kg^{-1} dry matter) in camelina root					
	Cd	Co	Cu	Fe	V	Zn
Control	0.32	0.25	6.1	650	1.0	31
	(0.0649)	(0.183)	(0.768)	(313)	(0.419)	(11.2)
V1	0.46	0.29	3.8	580	4.8	25
V2	0.41	0.42	3.7	720	26	62
V3	0.65	0.32	4.2	700	67	53
V4	-	-	-	-	-	-

Zinc

Consideration of the root and shoot material analyses in Table 20 identifies that for the Spike concentrations 1, 2 and 3, the concentration in the shoot is three to four-fold greater than the initial concentration of the spikes; similarly, with the corresponding roots displaying concentrations six to eight-fold greater than the spike concentration. With concentrations in the root twice as great those reported for the shoot material. As differences in concentrations between the resulting plant material and the original spike concentration are unlikely to be mitigated by the Zn content of the base compost (47 mg kg^{-1} dry matter), this suggests that Zn is preferentially accumulated by camelina. The results for the shoot material grown in soils spiked with Zn of Spike 4 concentration (4000 mg kg^{-1} dry matter) suggest that the accumulative behaviour is not sustained where soil concentrations are above 400 mg kg^{-1} dry matter. Furthermore, it is likely that toxicity effects are likely to be active as critical plant concentrations (100-400 mg kg^{-1}) are exceeded (Alloway, 1990; Jambhulkar *et al*., 2009). However, as the Zn concentrations reported for the plant material is not in excess of 10,000 mg kg^{-1} *Camelina sativa* is not considered to be a hyperaccumulator with respect to Zn (Baker & Brooks, 1989). The shoot : root ratios determined for the Spike concentrations 1, 2 and 3, (0.58; 0.47 and 0.54, respectively) are comparable and suggests that *Camelina sativa* displays a tolerant behaviour to Zn.

The presence of potential antagonisms and synergies between the six metals reported in Table 20 ((a) and (b)) are most clearly identified in the shoot data Table 20(a). Potential synergies between Fe and V, and Zn are noted for both root and shoot material, in particular for Spike 2 and 3 concentration (100 and 400 mg kg^{-1} dry matter, respectively) with evident enrichment of Fe and V as Zn spike concentration increases. By contrast, the noted decreases in the Cu plant concentration as the Zn spike concentration increases infers the presence of a potential antagonism between Zn and Cu uptake in *Camelina sativa*. This suggests that at Zn soil concentrations at 100 mg kg^{-1} dry matter and above Zn may inhibit the translocation of the essential micronutrients Cu, whilst facilitating the uptake of Fe. Therefore, high levels of available Zn may have implications to the uptake of Cu and Fe in field scenarios and consequently crop yields. Consideration of the degree of variance expressed by both the control data and the spiked data may raise concerns regarding the validity of such observations given the small replicate number, therefore further investigation to needed to clearly define the extent to which these synergies and antagonisms exist.

Table 20: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in (a) camelina shoots harvested from greenhouse spiking experiment for vanadium expressed as median values; interquartile ranges are reported in parenthesis (n=3, except where † denotes n = 2; values quoted to two significant figures; interquartile ranges quoted to three significant figures) and (b) camelina roots harvested from greenhouse spiking experiment for cadmium (n=1; where – denotes insufficient sample for analysis)

Spike	(a) Elemental concentration (mg kg^{-1} dry matter) in camelina straw					
	Cd	Co	Cu	Fe	V	Zn
Control	0.28	0.057	4.3	52	0.045	21
	(0.0584)	(0.0836)	(1.35)	(40.3)	(0.0680)	(9.05)
Zn1	0.42	0.085	2.2	72	0.088	180
	(0.0561)	(0.0281)	(0.755)	(3.29)	(0.00225)	(12.6)
Zn2	0.26	0.11	1.9	120	0.080	290
	(0.00916)	(0.00964)	(0.0836)	(12.5)	(0.0146)	(9.66)
Zn3	0.19	0.21	2.1	400	0.48	1300
	(0.0101)	(0.0353)	(0.175)	(88)	(0.120)	(107)
Zn†4	0.25	0.073	5.2	59	0.22	2200

	(0.00511)	(0.00586)	(0.384)	(26.1)	(0.0164)	(139)

Spike	**(b) Elemental concentration (mg kg^{-1} dry matter) in camelina root**					
	Cd	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control	**0.32**	**0.25**	**6.1**	**650**	**1.0**	**31**
	(0.0649)	**(0.183)**	**(0.768)**	**(313)**	**(0.419)**	**(11.2)**
Zn1	**0.71**	**0.33**	**5.5**	**550**	**0.78**	**310**
Zn2	**0.62**	**0.34**	**3.7**	**440**	**0.69**	**650**
Zn3	**0.52**	**0.56**	**4.5**	**820**	**1.3**	**2400**
Zn4	**-**	**-**	**-**	**-**	**-**	**-**

4.6 Elemental analysis of daughter seeds

Shoots were harvested separately from roots to reduce contamination of material from spiked the growth medium; seeds were subsequently collected from the harvested aerial parts of the plant. Due to high level of dieback of the plants for the Cu, V and Zn Spike concentration 4 insufficient seed was collected for chemical analysis. Similarly, the low yield attained for V and Zn Spike concentration 3 meant there was only sufficient seed harvested for a single replicate analysis to be undertaken. Analysis of all daughter seed from spiking experiment for the six trace metals Cd, Co, Cu, Fe, V and Zn was carried out on the basis of single replicate analysis and results are presented in Table 21.

For each of the metal spiking sequences, the control provides a baseline measurement of the micronutrient and trace metal concentrations of camelina seed grown in nominally uncontaminated soil. Evaluation of this control dataset (Table 21) shows that Cu, Fe and Zn concentrations in camelina seed are at least two orders of magnitude higher than the trace metals Cd, Co and V, reflecting their essential plant micronutrient status. Further inspection finds that the concentrations of Fe and Zn are slightly enriched but consistent with the levels discerned in the parent seed (Table 13). By contrast, Cu levels appear to remain comparatively unchanged; with the daughter seed exhibiting substantial depletion in concentrations of Cd and Co relative to the parent seed. Comparison of the spike data (Table 21) finds that, in agreement with the control seed that in all metal spiking sequences the highest metal concentrations in the seed material are reported for Fe and Zn and to a lesser extent Cu.

With the exception of Zn, there is a general increase in the total concentration of trace metal in the harvested camelina seed with increasing soil spike concentration. However, this fractional increase in uptake does vary significantly from metal to metal: The data show that whilst the total concentration of trace metal in the daughter seed does increase with increasing concentration of the metal in the soil (ie increasing through the spike sequence), the proportional uptake is generally reduced and is not simply a function of soil concentration. The trace metals Cd and Co show the greatest potential for uptake, with measured concentrations reaching hundreds of times greater than their corresponding control baseline levels, whereas uptakes for Cu, V, and Zn are considerably lower. The results are reported on an individual basis for each of the metals used to spike the base compost in Table 21.

Table 21: Concentrations for the trace elements Cd, Co, Cu, Fe, V and Zn in camelina seed harvested from greenhouse spiking experiment expressed as median values along with interquartile ranges (IQR) (n=3 except where ‡ denotes n = 4; † denotes n = 2; ° denotes n = 1; <LD denotes analysed concentration is below limit od detection; values quoted to two significant figures; IQR quoted to three significant figures)

Spike		**Elemental concentration (mg kg-1 dry matter)**					
		Cd	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control		**0.085**	**0.029**	**8.4**	**100**	**<LD**	**69**
	IQR	0.0378	0.00501	0.159	1.34	-	3.35
Cd1		**0.85**	**0.019**	**3.0**	**110**	**0.00**	**72**
	IQR	0.0535	0.0149	0.172	15.8	0.000	3.77
Cd 2		**4.0**	**<LD**	**2.3**	**100**	**0.00**	**63**
	IQR	3.33	-	1.41	89.3	0.000	46.5
Cdº 3		**6.8**	**0.0146**	**4.2**	**160**	**0.00**	**110**
	IQR						
Cd 4		**-**	**-**	**-**	**-**	**-**	**-**
Co1		**0.11**	**0.69**	**7.6**	**120**	**<LD**	**69**
	IQR	0.00978	0.0149	0.0684	3.93	-	1.17
Co2		**0.12**	**2.4**	**7.7**	**100**	**<LD**	**66**
	IQR	0.00508	0.0613	0.0388	0.910	0.00333	1.33
Co3		**0.11**	**4.1**	**9.0**	**200**	**0.15**	**69**
	IQR	0.000133	0.292	0.296	37.7	0.0889	1.53
Co4		**1.2**	**12**	**6.8**	**81**	**0.0067**	**54**
		0.0749	0.509	0.168	2.19	0.00672	0.653
Cu1		**0.060**	**<LD**	**7.8**	**130**	**<LD**	**83**
	IQR	0.00746	0.0798	0.134	1.80	-	2.61
Cu2		**0.020**	**0.019**	**8.5**	**97**	**<LD**	**53**
	IQR	0.00509	0.0151	0.576	28.4	-	14.3
Cu3		**0.030**	**0.0094**	**9.0**	**130**	**<LD**	**71**
	IQR	0.0100	0.0147	1.89	12.6	-	7.04
Spike		**Elemental concentration (mg kg-1 dry matter)**					
		Cd	**Co**	**Cu**	**Fe**	**V**	**Zn**
Control		**0.085**	**0.029**	**8.4**	**100**	**<LD**	**69**
	IQR	0.0378	0.00501	0.159	1.34	-	3.35
Fe(II)1		**0.065**	**0.052**	**8.8**	**98**	**<LD**	**51**
	IQR	0.000295	0.00991	0.116	1.29	-	0.638

Fe(II)2		**-**	**-**	**-**	**-**	**-**	**-**
Fe(II)3		**-**	**-**	**-**	**-**	**-**	**-**
Fe(II)4		**-**	**-**	**-**	**-**	**-**	**-**
Fe(III) 1º		**0.17**	**0.39**	**8.0**	**140**	**<LD**	**78**
	IQR					-	
Fe(III) 2†		**0.076**	**0.13**	**8.5**	**140**	**<LD**	**64**
	IQR	0.00569	0.00500	0.0350	0.586	-	1.08
Fe(III)3		**-**	**-**	**-**	**-**	**-**	**-**
Fe(III)4		**-**	**-**	**-**	**-**	**-**	**-**
V1		**0.090**	**0.019**	**3.0**	**99**	**<LD**	**69**
	IQR	0.0100	0.00500	0.425	10.7	-	4.69
V2†		**0.080**	**0.035**	**3.3**	**130**	**<LD**	**100**
	IQR	0.000121	0.00497	0.0654	1.42	-	5.13
V3		**0.070**	**0.029**	**4.4**	**98**	**<LD**	**74**
	IQR	0.00376	0.00718	0.0811	0.797	-	1.85
V4º		**0.085**	**0.048**	**2.8**	**89**	**0.12**	**67**
Zn1‡		**0.11**	**0.040**	**7.8**	**130**	**<LD**	**86**
	IQR	0.0212	0.0117	1.16	10.5	-	6.00
Zn2		**0.070**	**0.075**	**8.3**	**120**	**<LD**	**86**
	IQR	0.00496	0.0104	0.211	3.01	-	1.18
Zn3º		**0.13**	**0.05**	**6.4**	**170**	**0.07**	**210**
Zn4†		**0.045**	**0.0093**	**5.2**	**97**	**<LD**	**210**
	IQR	0.00501	0.00000372	0.0820	0.638	-	0.617

Cadmium

Consideration of seed concentrations of the spiking sequence for Cd, shows that a consistent increase in Cd in seed material is noted with increasing soil spike concentration. At Spike concentration 4 (100 mg kg^{-1}) the low levels of seed production observed may be the result of toxicity effects being operational, as critical plant concentrations for camelina are exceeded. Scrutiny of the seed concentration of other five metals, Co, Cu, Fe, V and Zn, finds that the Cu concentration in the Cd metal sequence seed is depleted in comparison to the Cu levels reported for the control seed, suggesting that Cd potentially inhibits the uptake of Cu in to the seed. Conversely, comparative enrichment of Fe and Zn detected in the spiked seed material, in particular for Spike concentration 3 (10 mg kg^{-1}) whilst the concentration V appears unaffected by increases in Cd concentration.

Cobalt

Similar to the findings for the seed concentration in the Cd metal spiking sequence, the seed concentration in the Co metal spiking sequence is seen to increase with increases in spike concentration. Consideration of the changes in the concentration of the other five metals, Cd, Cu, Fe, V and Zn with changing Co concentration reveals that Cd is substantially enriched with respect to the control seed material. Comparison with the Cd metal spiking sequence data suggest that this behaviour is not reciprocated by Cd. Overall the Co spike concentration does not appear to influence the uptake and the concentration of Cu, Fe, V and Zn into the seed. However, for Spike concentration 3 (20 mg kg^{-1}) atypical behaviour is suggested where distinct enrichment in the concentration of Cu, Fe and V in the seed material is reported. Further investigations may be necessary to understand not only the uptake mechanisms for Cd and Co that are active in camelina seed but to validate the atypical behaviour that is noted for Spike concentration 3.

Copper

Initial evaluation of seed Cu concentration data in Table 21 suggest that Cu concentration in the seed increases with increasing spike concentration. However when the measure of dispersion of the data is taken into account it is likely that the Cu concentration in the seed for the three spike concentrations are consistent, further suggesting that *Camelina sativa* self regulates the uptake of Cu into the seed. Furthermore, the Cu concentration in the spiked seed appear to be similar to those expressed by the seed from the control plants. Consideration of the affect on the secondary uptake of metals with increasing Cu soil concentration suggest at Spike concentrations 2 and 3 (50 and 100 mg kg^{-1}) depletion of Cd occurs. Whilst the median values of Co in the Cu metal spiking sequence may suggest similar depletion, the measure of dispersion reported would suggest caution in such an interpretation. By contrast V concentrations in the seed appear unaffected, whilst the behaviour of both Fe and Zn appear contradictory, with apparent enrichment and depletion compared with the control seed being observed. Further investigation may be needed to understand how Cu affects the uptake of Fe and Zn into the seed and the implication to seed viability.

Iron(II) and Iron(III)

Qualitative assessment of Fe(II) and Fe(III) is limited by the small sample size and the availability of comparative data for each of the Fe spiking series, as such will not be discussed further.

Vanadium

Examination of the V spiking series data (Table 21) suggests that only when soil concentration is increased to 200 mg kg^{-1} (Spike concentration4) that the concentration of V is detectable in the seed. It may be postulated that such an observation is indicative of the breakdown of plant mechanisms that prevent the transference of V into the seed. Nevertheless, given the low sample size for the Spike concentration 4 seed such a supposition should be treated with a degree with caution, further research to validate such an assertion would be required. Consideration of the effect of increasing V soil concentration on the secondary uptake of the metals, Cd, Co, Cu, Fe and Zn, suggest the uptake of Cd into the seed is independent of the concentration of V. By contrast, Cu uptake appears to be reduced, however the degree of depletion appears unaffected by changes in the V spike concentration, with the Cu seed concentration for Spike concentration 1 (10 mg kg^{-1}) being comparable to the Cu seed concentration determined for Spike concentration 3 (50 mg kg^{-1}).

Zinc

Scrutiny of the Zn spiking series dataset (Table 21) identifies that the Zn seed concentration is enriched in comparison to the Zn concentration of the control seed. Moreover, it suggests that the uptake behaviour of Zn into the camelina seed does not emulate that of Cd, Co and Cu, where increases in metal seed concentration are seen to correspond with increases in spike concentration. By contrast, the determined Zn seed concentration appears independent of spiking concentration, with the Zn seed concentration for Spike concentration 1 and 2 (40 and 100 mg kg^{-1}, respectively) being analogous. The close comparability of the Zn seed concentrations for the Zn spikes 1 and 2 in conjunction with the discrete increase in Zn seed concentration at spike concentration of 400 mg kg^{-1}, suggests that the movement of Zn into the camelina seed may be subject to a barrier mechanism that breakdowns at Zn soil concentrations equivalent to 400 mg kg^{-1}. The lower replicate number for Spike concentration 3 and 4 (400 and 4000 mg kg^{-1}, respectively), would further suggest it would be prudent to undertake further investigations to validate the uptake behaviour of Zn established for *Camelina sativa* from these initial cultivation trials. Due to the smaller sample size for the metal concentrations determined for the Zn Spike concentrations 3 and 4, comparative assessment of the secondary uptake of the metals Cd, Co, Cu, Fe and V will focus on the results reported for the Spike concentrations 1 and 2 (40 and 100 mg kg^{-1}, respectively). The concentration of Cd, Cu and V are found to be consistent with those reported for the control seed. Whilst, Fe appears to be enriched in the Zn spiked seed, the degree of enrichment appears unaltered by changes in the Zn spike concentration, with the Fe seed concentration for Spike concentration 1 (40 mg kg^{-1}) being comparable to the Fe seed concentration determined for Spike concentration 2 (100 mg kg^{-1}).

4.7. Elemental analysis of oil from daughter seed

Because of the finite extent of the controlled cultivation trials, it has not been possible to collect a sufficiently large quantity of seed to allow the crushing and the subsequent analysis of trace metals in the extracted oil.

4.8. Comparison of the distribution of metal concentrations in the plant material

Deliberation of the concentration of individual metals in the three plant components, roots, shoots and seeds analysed, finds that for the plant material from the greenhouse cultivation trials that with the exception of Co the concentration in the roots is greater than in the shoots. This suggests that a greater proportion of Cd, Cu, Fe, V and Zn is stored in the roots. This is particular true for V, where movement of the metal from the is low as exemplified by the low shoot : root ratio as determined in section 4.5. By contrast Co exhibits greater shoot concentrations than in the roots for the Spike concentrations 2 and 3, whilst for the Spike concentrations 1 and 4 root concentrations are greater than shoots. Similarly, with the exception of Cu shoots concentrations are typically found to be higher than the seed concentration suggesting that a greater amount of the metals present in the aerial parts of the plant are located in the shoots. Conversely, Cu exhibits higher concentrations in the seed than the shoot, this is likely to be a reflection of the role of Cu-enzymes in embryo development.

With regard to the findings of the controlled cultivation trials the concentration and partitioning within the camelina plant of individual metals, the concentration of these metals in the soil medium is the principal controlling factor. Furthermore, the uptake behaviour described is for a single metal that is present as in a bioavailable form. As it is estimated that 70% of all metal-contaminated sites involve two or more metals (Ebbs & Kochian, 1997) it is likely that differences in the uptake behaviour between that observed in the greenhouse trials and in the field trials may occur. The possibility that the bioavailability of the metal as well as synergistic and antagonistic effects between metals such as Cu and Zn maybe important cannot be discounted. Further research to validate such assertions would be required.

5 Results of field cultivation trials

A two-phase approach was applied to the field cultivation trials. In the first phase for all four field-cultivation trials pre-planting geochemical analysis of soils was undertaken to assess and characterize the individual soil matrices for metal concentrations, soil acidity and total carbon and nitrogen (Section 5.1). Furthermore, qualitative assessment of the growing crop was carried out to assess crop vigour and vitality (Section 5.2) and post-harvest assessment of the potential crop production was carried out in terms of yield (Section 5.3) and the transference of key metals such as Cd, Co, Cu, Fe, V and Zn into the seed and oil (Section 5.4 and 5.5). In the second phase, contaminant heavy metals were traced through all components of the system for the Rovinari sterile site. The rationale for the selection of this site was not only based on the higher levels of production (330 kg/ha) determined for this contaminated site but also that the soil of the Rovinari Sterile exhibited overall the lowest concentrations of metal contaminants. Applying the methodology to material grown on soils of the lowest contaminant levels should allow assessment of:

- Whether metal transference into the crop at such levels is likely to be a challenge to the production of sustainable aviation biofuel crop
- Which contaminant metal is likely to need further monitoring in future crops
- Where metal storage is likely to occur in the crop
- Usability and value of animal feed co-products, for example, shoot and seed-pods (silicles); with the exception of the animal feed co-product oil-meal (grist) which was not available but whose characteristics may be estimated from the difference between the components present in the seed and oil).

This section discusses the results of the field cultivation trials in the following order:

- Pre-planting geochemical analysis of soils
- Plant growth & crop survey
- Post-cropping geochemical analysis of soils
- Elemental analysis of parent seed
- Daughter seed analysis
- Oil analysis
- Harvesting of plant matter
- Post-cropping geochemical analysis of soils
- Elemental analysis of soil, root, shoot and daughter seed from Rovinari sterile field trials

Geochemical definition of the bulk soil is achieved through the determination of total elemental concentrations, total carbon, total inorganic and organic carbon and total sulphur content and pH. Prior to analysis of the geochemical data, statistical routines were applied to determine normality (Kolmogorov-Smirnov) and homogeneity of variance (Levenne) of the data, as not all elements were found to be normally distributed the non-parametric descriptive statistics are employed in reporting the geochemical data. All data refer to the 2012-2013 camelina crop.

5.1. Pre-planting geochemical analysis of soils

5.1.1. Elemental analysis (field trials)

Prior to sowing camelina in the 2012 / 2013 growing season, a comprehensive elemental analysis of the soil at each of the Romanian field cultivation trial sites was conducted. Individual soil samples were thoroughly mixed and quartered. Approximately one third of each soil sample was placed soil on a horizontal surface and air-dried, at an average temperature of 20.5°C (±1.5°C). Constant mass was attained after 14 days. The remaining two-thirds soil was refrigerated at 4.0°C (BS ISO 18512:2007).

The descriptive statistics for the 20 trace elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Ti, V, and Zn) are reported in Appendix C. However, the discussion of the trace element concentrations for the study sites data will focus on the 11 target elements As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn, the presence of which have been highlighted to be of concern in biokerosene (Def Stan 91-91). The overall precision for the total metal concentrations is 8.8%, with 90% of elements attaining a precision of less than 10%, and 43% of elements, a precision of less than 5%. Trueness for As, Cr, Cu, Ni, Pb and Zn are high; in contrast, trueness is found to be poor for Cd. The analysis bias is negative for the elements As, Cr, Cu, Pb and Zn and positive for Cd and Ni. The descriptive statistics for such elements for each of the four field study areas are reported in Table 22.

Comparison of the data on a site-to-site basis is undertaken in Figure 2. Differences in the elemental concentrations of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn differ between sites are noted. It is apparent that individual elemental concentrations vary considerably between sites. It is likely this variation is the product of differences in the background geology and the anthropogenic input sources that are operational at each of the sites.

The soils from the Câmpina and Copşa Mică study sites consistent display the greatest variability in the concentration of individual elements. The variability discerned in the samples from the Câmpina site is likely to be a reflection of the high degree of diverse anthropogenic source material that is a significant component of the soil matrix at this site. By contrast, the relative low variance exhibited by the elemental concentrations data for the Rovinari – Ash and – Sterile sites suggest a degree of homogeneity in contaminant distribution and source.

With the exception of Mn, soil samples of Rovinari Sterile site typically exhibit the lowest concentrations of the four study sites. Although the soils from the Câmpina site present the greatest range of contaminant concentrations, the soil samples from the Copşa Mică study site consistently exhibit the highest concentrations of As, Cd, Pb and Zn.

Dutch Target and Intervention Values for soil remediation (DTIV) (VROM, 2000, VROM, 2009) and the Romanian Reference Values (RRV; Table 23) have been employed to evaluate the degree of contamination in the soil samples analysed, of the 11 elements considered it is the concentrations of As, Cd, Ni, Pb, V and Zn that are of concern. Comparison of the soil elemental data with the DITV and RRV are undertaken on a site-by-site basis.

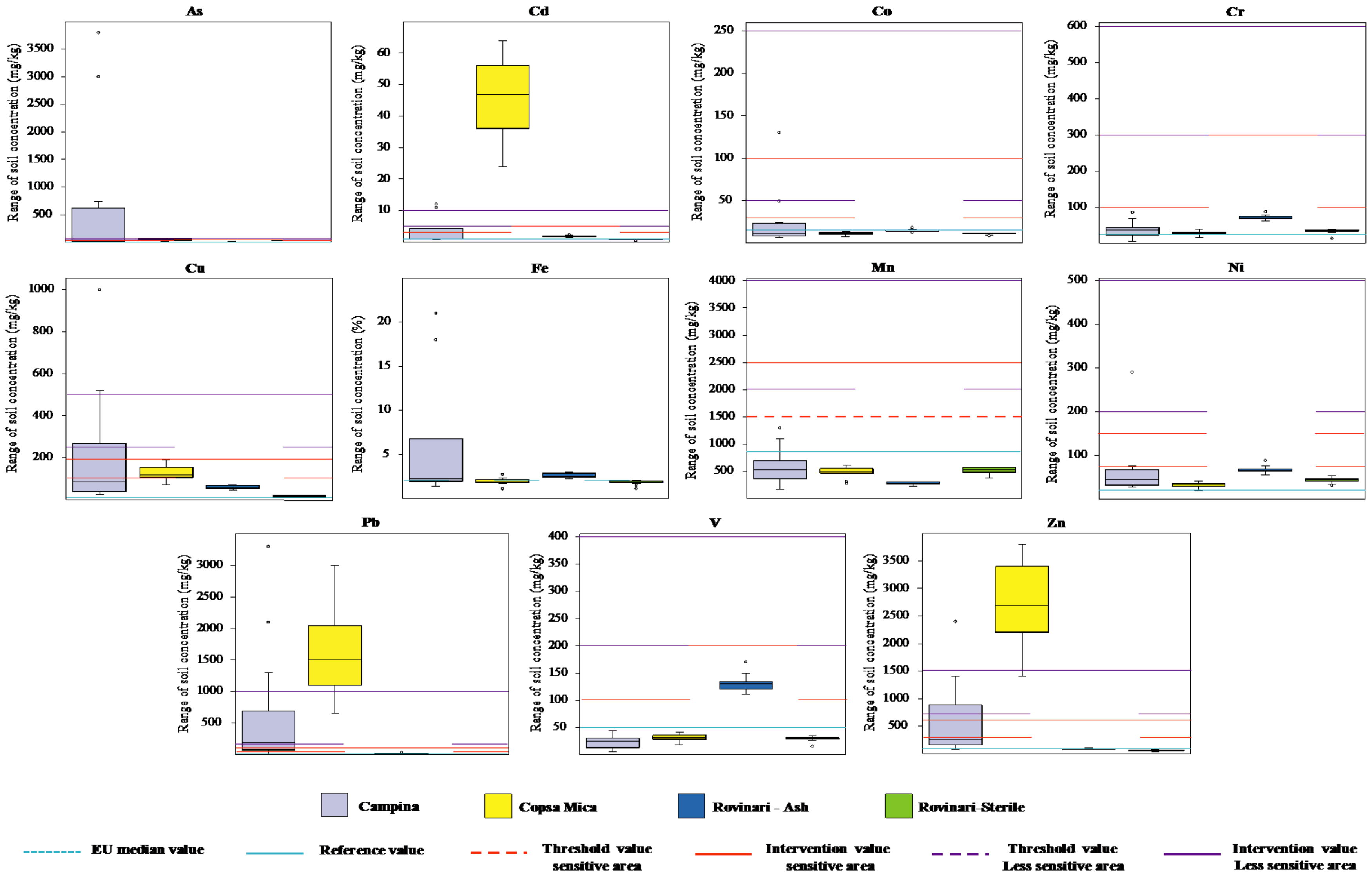


Figure 2: Concentration of metal in soil for the elements As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn determined for the four Romanian field study sites

Rovinari, Gorj County

Sterile Field

With exception of Mn the soil from the Rovinari-Sterile site are found to consistently exhibit the lowest concentrations of the ten metals considered, for the four study sites. Although As, Cr and Ni concentrations are in excess of the DITV and RRV they do not exceed the Target / Threshold (sensitive areas) of the DITV and RRV, respectively.

Consideration of the elemental concentrations of the Sterile field soil samples with those determined for the Ash field finds, that with the exception of Mn, the concentration of the other 10 metals (As, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn) are found to be consistently lower than the soil samples from the Ash Field (Table 22; Figure 3).

The data determined for this report was compared with concentrations reported by other authors (Dodocioiu & Susinski, 2010; Bălăceanu *et al.*¸2011; Gămăneci & Căpăţînă, 2011) for such metals in soils within 7.5 km radius of the thermal power plant at Rovinari (Figure 3). The concentrations of Cd, Cu, Mn, Ni, Pb and Zn determined for this study site are found to be predominantly within the range determined in the earlier studies. The concentrations of the four metals, Cd, Cu, Pb and Zn, were discerned to be at the lower limit of the defined range, whilst the soil concentration of Co at the Rovinari Sterile site was found to below the lower limit. By contrast, the concentrations determined for Mn and Ni lay within the middle and upper limits, respectively, of the range. 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).

Ash Field

Soil samples from the Rovinari-Ash display high levels of As, Ni and V. Overall the As concentrations although are found to exceed the RRV Intervention (sensitive areas) and Threshold (less sensitive areas) values (25 mg kg^{-1} and 25 mg kg^{-1}, respectively). However, the DITV Target values (29 mg kg-1) are not exceeded, consideration of the range of As concentrations determined and the individual sample analysis (Table C42, Appendix C) suggests that 50% of the site area express concentrations greater than the DITV Target value for As. By contrast, the concentrations of Ni exceed the DITV Target but not RRV Threshold (sensitive areas) values (35 mg/kg and 75 mg/kg, respectively), deliberation of the range of Ni concentrations determined and the individual sample analysis (Table C42, Appendix C) suggests that 17% of the site area express concentrations greater than the RRV Threshold (sensitive areas) values for Ni. Concentrations of V are found to be four-fold greater than those determined for the other study areas. Comparison of the range of V concentrations finds that in all cases, the concentration of V exceeds DITV Target and RRV Threshold (sensitive areas) values (42 mg kg^{-1} and 100 mg kg^{-1}, respectively).

Qualitative analysis of the data for the two Rovinari study sites presented in Figure 3 finds that with the exception of Mn, soil concentrations of the metal considered are higher for the Ash field site than those recorded for the Sterile field site. The differences in concentration of such metals may relate to differences in the pedological characteristics between spolic entisols (Sterile field) and the technosols (Ash field) and consequently, pedological processes active at the two sites. In particular, brown coal (lignite) ash a significant contributor to the Rovinari-Ash field site technosol has been seen to be enriched in metals such as Cd, Cr, Cu, Ni, Pb and Zn (Davison *et al.*, 1974; Querol *et al.*, 1995; Ludwig *et al.*, 2005).

Comparison with soil concentrations of the metals, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, reported for the area around Rovinari thermal power complex discerned that Cd, Cr, Cu and Ni typically lie toward the upper limits of the prescribed range. In particular, the values of Cu and Ni are commonly found to exceed the upper limits of the range, and in the case of Ni, the concentrations determined for the Rovinari-Ash field site approach the threshold value for Ni in sensitive soils. By contrast, the concentrations determined for Co, Mn, Pb and Zn lie characteristically towards the lower limit of their respective ranges, with Pb and Zn exhibiting concentrations below the Romanian soil reference value.

Given the up-wind location of the Ash field site with respect to the Rovinari power complex, it is unlikely that the observed enrichment of the soil in the metals Cd, Cr, Cu and Ni result from atmospheric deposition of power plant stack emissions. It is more likely that the soil concentrations of the metals Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn observed at the Ash field site are a product of the dynamic interplay of a number of geochemical parameters. For example, Querol *et al.*, (1996) noted that trace elements such as Pb and Zn displayed increased mobility potential when present in coal ash as soluble salts as sulphates. Furthermore, Ludwig *et al.* (2005) reported that brown coal (lignite) ash exhibited an enhanced buffering capacity with regard to alkalinity and that metals such as, Cd, Cr, Cu and Ni display low solubility at pHs similar to those determined for the soil samples of the Rovinari-Ash site (Section 5.1.3). Consequently, the possibility that such mechanisms may be also active in the soil matrix of the Ash field study site cannot be discounted.

Câmpina, Prahova County

The analysis of the soils from this site shows the greatest variability, as illustrated in Figure 2. Of the four field study sites the soils from the Câmpina site display not only exhibit the largest range of soil concentrations for the metal considered, suggesting the existence of micro-environments within the study site, but also some of the highest individual values for the trace elements As, Co, Cu, Fe and Mn. The median values (Table 22) for the metals, As, Cu, Fe, Ni, Pb and Zn, mask the presence of such metals in concentrations that exceed DITV and RRV Intervention (less sensitive areas) values. Figure 2 clearly indicates that RRV Intervention (sensitive areas) values are exceeded for As and Pb in the majority soil samples from the Câmpina site. Furthermore, an appreciable number of the soil samples from the site display concentrations that are close to or exceed the RRV Intervention (sensitive areas) values for Cd, Cu and Zn. By contrast, the levels of the trace elements, Co, Cr, Mn, Ni and V determined for the Câmpina soils are below the RRV Threshold (sensitive areas) values.

Anecdotal evidence and visual assessment of the soil matrix of the Câmpina study site suggest that the site has been and continues to be subject to conspicuous additions of anthropogenic inputs, including foundry-form sand and building construction waste. Consequently, the uppermost (0-20 cm) layer of soil may be considered to be synthetic and comparison with metal concentrations of soils from the proximate locality would be of limited value and has not be undertaken for this study.

Table 22: Descriptive statistics for the trace metals As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn, expressed as mg kg^{-1} dry matter for the soil samples collected from the four Romanian study areas in October 2012. All values quoted to two significant figures. Note: elemental concentrations expressed in mg / kg dry matter except *values which are expressed as percentages.

		Elemental concentration (mg kg^{-1} dry matter) in soil										
Study site		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe***	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Câmpina	Median	31	1.1	11	40	86	2.3	450	47	200	25	260
	Range: min	7.6	0.6	6.6	4.8	24	1.4	170	28	15	4.7	72
	max	3800	12	130	86	1000	21	1300	290	3300	44	2400
Copşa Mică	Median	38	45	12	29	120	1.9	500	32	1400	32	2600
	Range: min	18	24	7.1	16	70	1.1	280	18	660	18	1400
	max	65	64	14	39	190	2.7	620	41	3000	42	3800
Rovinari (Ash)	Median	26	1.7	14	71	59	2.8	280	65	16	130	79
	Range: min	21	1.5	12	61	48	2.2	230	55	13	110	68
	max	44	2.3	18	88	72	3	310	88	31	170	100
Rovinari (Sterile)	Median	7.7	0.8	11	35	19	2	520	43	10	30	58
	Range: min	5	0.4	8.1	14	13	1.1	380	31	7	15	44
	max	8.4	0.9	12	39	21	2.1	580	53	13	34	72

Table 23: European, world soil median concentrations, Dutch and Romanian target and soil remediation intervention values for the trace metals As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn, expressed as mg kg^{-1} dry matter. Note *values expressed as percentages (values quoted to two significant figures).

Element	EU Median in topsoil [a] (mg/kg)	World Median[b] (mg/kg)	Dutch intervention and target values[‡] (mg/kg)			Romanian soil standards (mg/kg)				
			Background concentration	Target value	Intervention value	Reference value (Total)	Threshold value sensitive areas	Threshold value less sensitive areas	Intervention value sensitive areas	Intervention value less sensitive areas
As	6	6	29	29	76[†]	5	15	25	25	50
Cd	0.15	0.35	0.8	0.8	12	1	3	5	5	10
Co	7	-	9	9	190[†]	15	30	100	50	250
Cr	22	70	100	100	380	30	100	300	300	600
Cu	12	30	36	36	190	20	100	250	200	500
Fe*	2	4	-	-	-	-	-	-	-	-
Mn	380	1000	-	-	-	900	1500	2000	2500	4000
Ni	14	50	35	35	100[†]	20	75	200	150	500
Pb	15	35	85	85	530	20	50	250	100	1000
V	33	90	42	42	250	50	100	200	200	400
Zn	48	90	140	140	720	100	300	700	600	1500

[a] **–** Geochemical Atlas of Europe (2005; Ed: R. Salminen) [b] **–** Bowen (1979) *** -** values expressed as percentages

† - values received to reflect Soil Remediation Circular 2009 ‡ - values are for those in standard soil (10% organic matter; 25% clay)

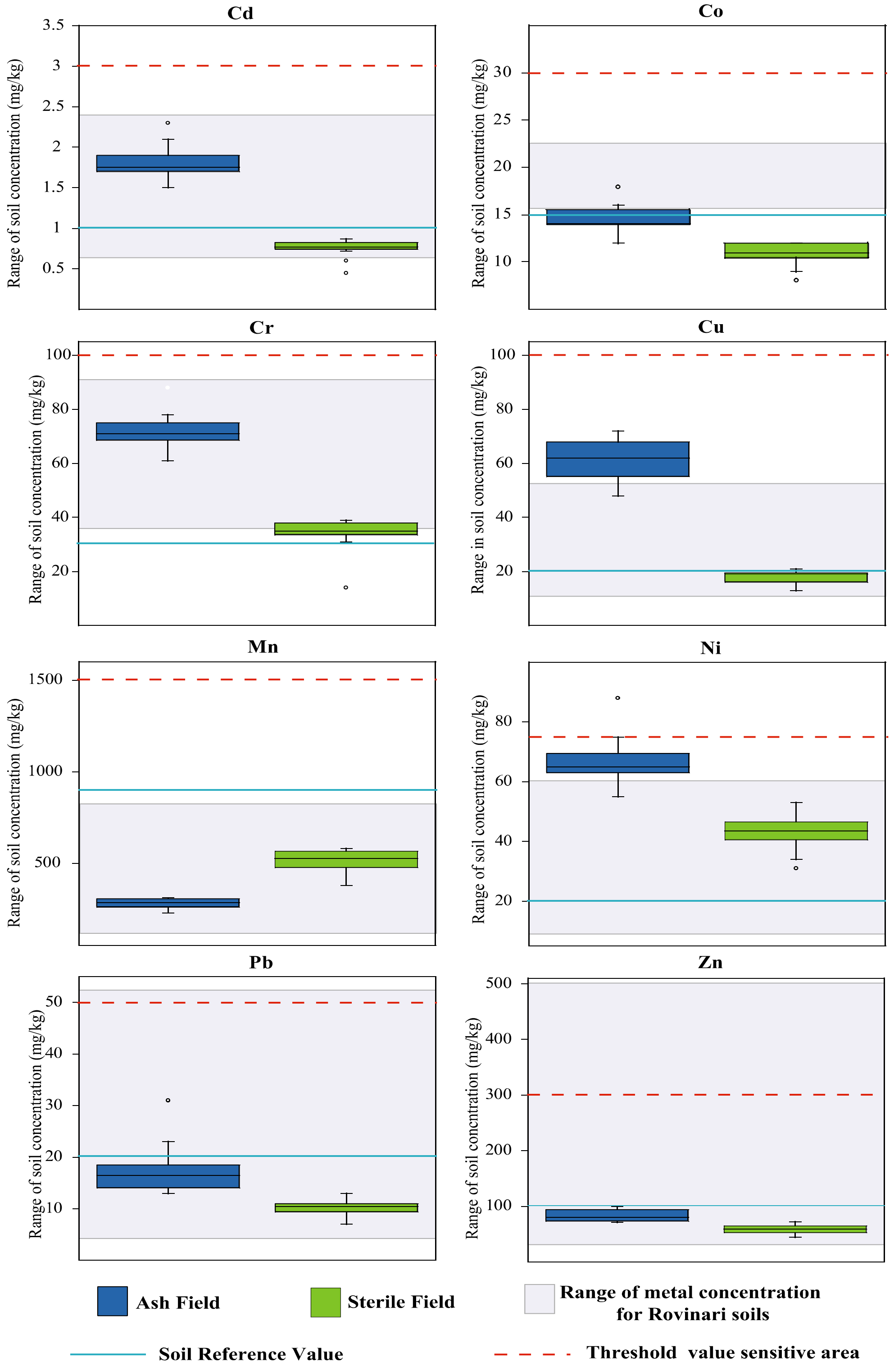


Figure 3: Comparison of the concentrations of the metals Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, in soil samples from the Sterile (green) and Ash (blue) field sites, Rovinari, Romania, with the range of soil concentrations reported by various reference sources for soil samples collected within a 7.5 km radius of the thermal power complex at Rovinari, Romania

Copşa Mică, Sibiu County

Soil samples from the Copşa Mică display the highest median levels of As, Cd, Pb and Zn of all the study sites. Overall, the median As concentration (38 mg kg^{-1}) is found to exceed the RRV Intervention (sensitive areas) and Threshold (less sensitive areas) values (25 mg kg^{-1} and 25 mg kg^{-1}, respectively) and DITV Target values (29 mg kg^{-1}). Cadmium concentrations in all instances (Table 23; Table C40, Appendix C) are at least two-fold greater than the DITV Intervention and RRV Intervention (less sensitive areas) values (12 mg kg^{-1} and 10 mg kg^{-1}, respectively) (Table 23). Similarly, Pb and Zn values are found to exceed the DITV Intervention values (530 mg kg^{-1} and 720 mg kg^{-1}, respectively) in all cases, although 17% of the soil samples exhibit Pb concentrations below the RRV Intervention (less sensitive areas) value of 1000 mg kg^{-1}.

Furthermore, Figure 4 clearly illustrates that for the majority of soil samples from the Copşa Mică site the concentrations of Cd not only exceed the RRV Intervention (less sensitive areas), but Cd concentrations previously reported in soils in the vicinity of Copşa Mică by a factor of two (Constantinescu, 2008; Muntean *et al.*, 2010; Szanto & Micle, 2011). Similarly, 40% of the soil concentrations of Zn determined for the Copşa Mică study site is found to exceed the upper limits of the range. By contrast, whilst the soil levels of Pb exceed the RRV Intervention (less sensitive areas), the values when reviewed with other comparative studies (Constantinescu, 2008; Suciu *et al.*, 2008; Ianculescu *et al.*, 2009; Muntean *et al.*, 2010; Szanto & Micle, 2011) are found to occur at the lower limit of the defined range.

A recent study by Paulette *et al.* (2015) reported that the proximity to the SOMETRA SRL non-ferrous smelter plant, topography and wind direction were influential on the concentrations of metals such as Cu, Pb and Zn in the soils around Copşa Mică, with the highest concentrations being detected immediate vicinity on the smelter plant. Paulette *et al.* (2015) noted that the highest soil concentrations of Cd and Zn were along an east-west trajectory from the smoke stack. By contrast, the highest concentrations of Pb are reported for soils along a northerly transect from the smoke stack. Therefore, given the westerly location of the current study site from the SOMETRA complex, it is possible that the high levels of Cd and Zn in the soils observed for the site reflect enrichment from aeolian deposition from the stack. Moreover, as commercial operations at the SOMETRA complex officially ceased in 2009, the high levels of Cd and Zn detected in the soil of the current site would suggest that mechanisms such as soil alkalinity, organic matter, clay content and agricultural practices may be operational in the active retention of such metals in the topsoil of the Copşa Mică field site.

5.1.2. Total carbon and total nitrogen (field trials)

Total carbon (TC) and nitrogen (TN) content for all the collected soil samples were determined by dry combustion using a LECO TruSpec CN Analyser in accordance with the methodology described in section 3.2.2. Samples were oven dried at 105°C overnight. Replicate analysis was carried out using a single 0.2 g ± 0.02 g sample taken from a 2.5 g aliquot of the bulk sample. The median values for TC, TOC, TN and the C: N ratio for the four study sites derived for the bulk soil samples are summarized in Table 24. Non-parametric landscape descriptives of interquartile range and minimum-maximum range for the data at each of the study sites are also reported in Table 24.

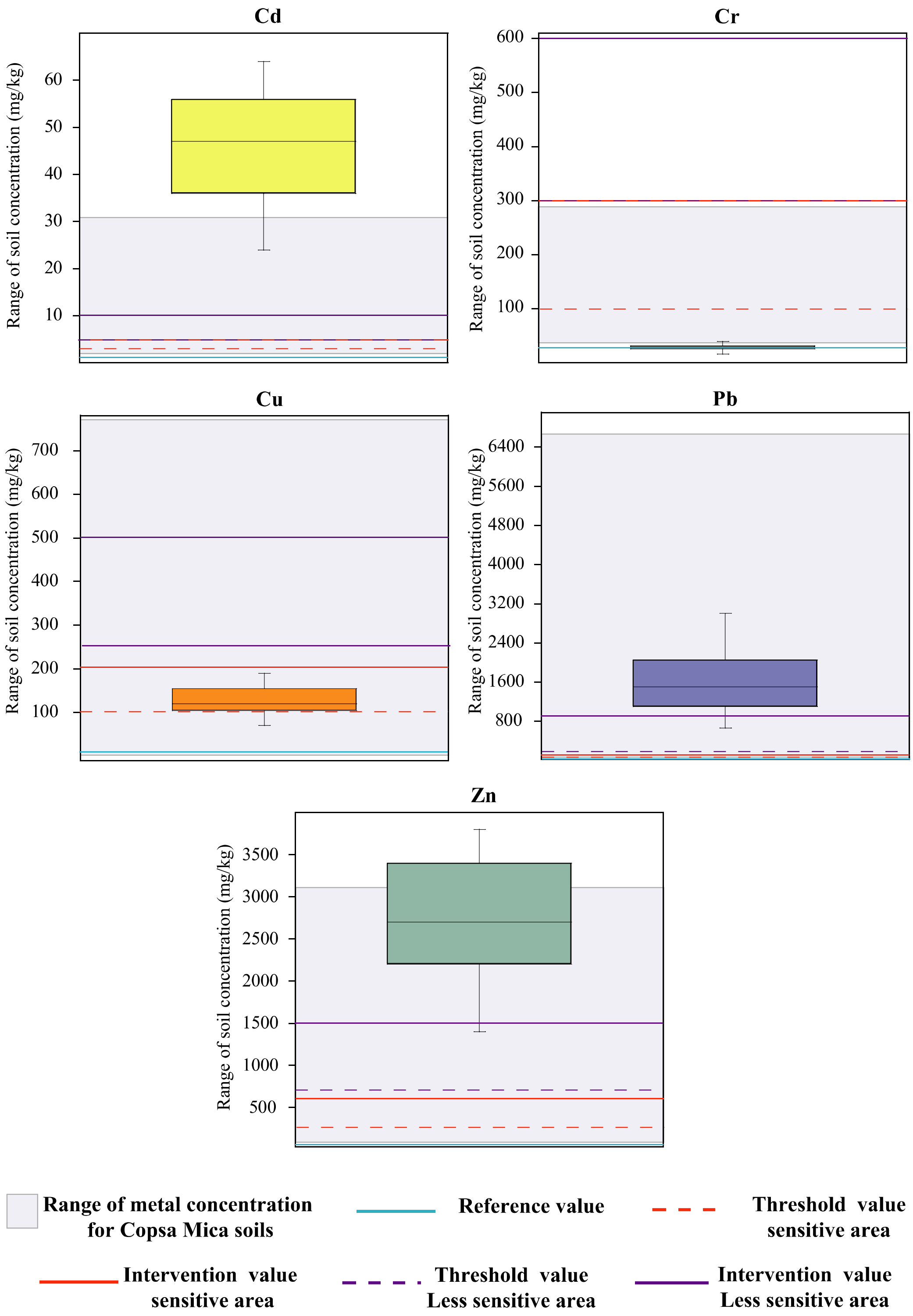


Figure 4: Comparison of the concentrations of the metals Cd, Cr, Cu, Pb and Zn, in soil samples from the Copşa Mică field site, Romania, with the range of soil concentrations reported for soil samples collected from the environs surrounding the SOMETRA SRL non-ferrous smelter plant, Copşa Mică, Romania

Table 24: Descriptive statistics for the TC, TOC, Soil organic matter, TN and C: N ratio of the soil samples collected from Romanian Study Sites in October 2012 (where: * - *n*= 23; † - *n*= 14; ‡- *n*= 12; values are quoted to two significant figures)

Study site		TC Mass (%)	TOC Mass (%)	Soil Organic Matter (%)	TN Mass (%)	C:N ratio
Copşa Mică*	median	2.2	1.7	3.0	0.18	12
	IQR	0.475	0.485	0.837	0.0500	1.43
	min	1.5	1.2	2.1	0.13	9.4
	max	2.9	2.4	4.2	0.27	15
Câmpina†	median	3.2	1.7	2.9	0.12	28
	IQR	2.64	1.23	0.857	0.143	40.8
	min	0.59	0.50	2.1	<LD	12
	max	11	5.3	9.1	0.43	112
Rovinari (Sterile) ‡	median	3.1	1.2	2.0	0.10	31
	IQR	0.405	0.508	0.876	0.027	5.93
	min	2.6	0.69	1.2	0.068	22
	max	3.7	1.9	3.3	0.14	45
Rovinari (Ash)‡	median	6.3	5.4	9.2	0.23	27
	IQR	2.71	2.03	3.50	0.133	5.46
	min	2.1	1.6	2.7	0.10	21
	max	8.7	6.7	12	0.35	33

Total carbon across the four sites ranges from 2.2 – 6.3% (Copşa Mică and Rovinari–Ash, respectively). The Rovinari –Ash field site, exhibits the highest values for TC and TOC. By contrast, the lowest TC values are displayed by soil samples from the Copşa Mică study site. Variance is greatest in the reported values for the soil collected from the Câmpina site, whilst the lowest degree of variance is expressed in the soils from the Rovinari-Sterile site.

Anecdotal evidence from the site survey (Table A.3, Appendix A) suggests that the soil at the Rovinari – Ash site has been subject to a conservative tillage system and under a vegetative cover for at least 30 years. Consequently, the soil TC is likely to have become enriched and stabilized (Gebhart *et al.*, 1994; Guo & Gifford, 2002; Al-Kaisi & Yin, 2005). Therefore, it is likely that the high values of TC and TOC reported for the Rovinari –Ash study site reflect not only the soil's lignite origins but also differences in the cropping and soil cover history noted between this site and the other three sites. Conversely, the lower TC values observed for the Copşa Mică and Rovinari–Sterile site soils are likely a reflection of crop tillage on going at such sites in recent years and the case of the former 20 years (Table A.2, Appendix A). The high degree of variance noted for the soil TC at the Câmpina site is likely to be the product of the high volumes of anthropogenic inputs such as building construction debris, including concrete and cement coupled with sporadic vegetative cover (Table A.1, Appendix A).

An arbitrary measure of the contribution of organic carbon to the total carbon of the soils at the four study sites was achieved by considering the ratio of TOC:TC. For the soils from the Copşa Mică and Rovinari–Ash sites, median ratios of 0.81 and 0.76, respectively, were attained, suggesting that for such soils organic carbon is a major component of the soil carbon. By contrast, the median ratio of 0.37 determined for the soils from the Rovinari–Sterile site suggest that typically inorganic sources of carbon play a dominant role in the total soil carbon at this site. Although a median TOC:TC value of 0.67 is calculated for the Câmpina field site, consideration of the range of TOC:TC values observed (0.26-0.95) suggest that the contribution made by both organic and inorganic sources of carbon at this site are likely to vary on a microscale basis.

The highest median value for TN is discerned for the Rovinari – Ash field site, conversely the lowest TN are reported for the Rovinari – Sterile study site. Whilst the greatest degree of variance in TN is exhibited by the soil samples from the Câmpina field site, similar variability is noted for the soil samples from the Rovinari – Ash site. By contrast, the noticeable lower degree of variance in TN values for the Copşa Mică and Rovinari–Sterile site soils may reflect the influence of sustained agricultural activity on soil TN.

Consideration of the C:N ratio determined for the four study sites (Table 24) finds that Rovinari – Sterile field site displays the highest ratio and are discerned to be a least two-fold greater than that calculated for the Copşa Mică study site. The C:N ratio (12:1) for the Copşa Mică field site is comparable with C:N values reported for arable topsoil. The C:N ratios reported for soil samples from Copşa Mică compare favourably with those reported for luvisols at 0 – 30 cm by FAO-UNESCO soil units (Batjes, 1996). The high C: N ratios reported for the Câmpina and Rovinari sites are likely arise due to the anthropogenic influences operational on soil matrix at these sites.

Comparative analysis of the soils from Copşa Mică and the Rovinari field sites is undertaken with typical values of the soil parameters, TC, OM, and TN, reported for soils from Sibiu and Gorj County, respectively (Table 25). Due to the unique conditions operational at the Câmpina field site a similar treatment with data for Prahova County is not undertaken.

To facilitate comparisons between the two data sets percentage organic matter (OM) content has been calculated from the TOC content of the study site soils using a conversion factor (c.f.) of 1.726 (Schumacher, 2002)

$$\text{OM}(\%) = \text{c.f.} \times \text{TOC}(\%) \qquad \text{Equation 1}$$

Table 25: Mean values of TC, TOC, and soil organic matter expressed as humus percentage and TN for soils collected from Gorj and Sibiu County, Romania (Dimitru *et al.*, 2000)

County		TC Mass (%)	Humus Mass (%)	TN Mass (%)	pH
Sibiu	Mean	3.2	5.6	0.22	6.4
	Minimum	0.87-2.6	1.5-3.9	0.11-0.18	4.1-4.7
	Maximum	5.3-5.6	9.2-9.6	0.26-0.32	6.0-8.2
Gorj	Mean	2.8	4.9	0.22	5.6
	Minimum	1.1-2.3	1.9-3.9	0.090-0.21	4.7-5.9
	Maximum	1.5-5.0	2.5- 8.7	0.15-0.33	5.0-6.4

Comparison of the measure of the central tendency for the three parameters, TC, OM and TN, determined for the Copşa Mică site with those presented in Table 25 (Dimitru *et al*., 2000) for Sibiu county finds that the values reported for the Copşa Mică soil samples are consistently lower than those reported for Sibiu county, with the greatest disparity being discerned between the OM% reported for the two data sets. Such disparities may arise as a result of differences in sampling resolution. Conversely, the OM content of the Copşa Mică soils may have been adversely affected by

(i) being in continuous agricultural production and tillage since 1990s (Gebhart *et al*., 1994; Guo & Gifford, 2002; Al-Kaisi & Yin, 2005).

(ii) exposure to metal contaminants leading to deterioration of soil microbial diversity and the quality of OM (Constantinescu, 2008).

The range of minimal values reported for Sibiu County is characteristic of the soils from the South areas of the county. Conversely, the maximal values characterize soil from the mountainous regions of the county (pers. comm. Prof. Silvia Vasilica & Dr. Mircea Mihalache, USAVMB). Whilst geographically the study site at Copşa Mică is located to the north of the county in the vicinity of the Transylvanian Plateau, the ranges determined for the current study appear to emulate those reported for the southern areas of Sibiu county, with values determined for the Copşa Mică soils typically lying at the upper limits of the range presented for the southern areas of Sibiu county.

For the two Rovinari field sites, Rovinari-Sterile and Rovinari-Ash comparative analysis is accomplished using soil parameter data for Gorj County (Table 25). The maximal values recorded in Table 25 are more typical of the soils from the northern regions of the county where Rovinari is located. By contrast, the minimal values are representative of the south of Gorj County (pers. comm. Prof. Silvia Vasilica & Dr. Mircea Mihalache, USAVMB).

Comparison of the soil parameter data for the Rovinari-Sterile (Table 24) with the Gorj county soil data (Table 25) finds that whilst clear similarities are discerned for the reported TC values between the data sets, such similarities are not observed for the OM content. With the organic matter content of the Rovinari-Sterile soils showing closer correlation to the soils from the south of Gorj County than the soils northern regions of the county. The apparent greater influence of inorganic carbon to the soil carbon in the Rovinari-Sterile soil samples compared with the soils of the northern regions of Gorj county is likely to result from the pedological origin, of mine overburden, of such soils in conjunction with the tillage cropping regime operational at this site in recent years that means soil horizon and OM layer build-up is likely to have been restricted. Comparison with the measure of central tendency suggests a degree of depletion in nitrogen in the Rovinari-Sterile soil samples. As with the OM content, the soil samples from the Rovinari-Sterile site exhibit greater affiliation to TN values expressed by soils from the south of Gorj County; this may be a reflection of continued cropping. Similar observations relating low soil OM and TN content in sterile dumps of the Jilţ – Rovinari Basin were made by Cărăbis *et al.* (2011).

Consideration of the Rovinari-Ash data with the Gorj county data finds that the TN data of the Rovinari-Ash soil samples not only closely correlate to the mean TN value reported for Gorj county but the range of values exhibited by the soil samples of the Rovinari-Ash site mimic the ranges TN values reported for the soils of north Gorj county. Such close similarities are not noted between the TC and OM values reported for the two data sets. Where TC and OM content for Rovinari-Ash soil samples are discerned to be approximately two-fold greater than the values recorded for the soils from the northern regions of Gorj county, with the determined range of both TC and OM being found to exceed those prescribed for the soils of north Gorj county by factors of 1.75 and 1.38, respectively. Such enhanced values of TC and OM as exhibited by the Rovinari-Ash soil samples are likely to be the result of a number of interactive factors including the fly ash origins of the soil at the Rovinari-Ash site coupled with the conservative tillage regime that has been operational at the site since the 1970s.

5.1.3. Soil Acidity (field trials)

Soil acidity was determined as pH, using three 5 g ± 0.1 g replicates of the bulk soil sample. The median values for pH for the four study sites derived for the bulk soil samples are summarized in Table 26. Non-parametric landscape descriptives of interquartile range and minimum-maximum range for the data at each of the study sites are also reported in Table 26.

The median pH value of the soil samples vary between circumneutral (7.2) for the Copşa Mică study site and predominantly alkaline for the Câmpina and Rovinari sites (8.3; 8.2 & 8.4, respectively). As soil acidity, in particular where pHs values are high, have been shown to be influential in the retention of metallic contaminants (Pagotto *et al*., 2001), it is possible that the retention of trace metals may be augmented in the Câmpina and Rovinari soil samples. The greatest variance is observed between individual soil samples from the Câmpina site. observed The wide range of pH values reflects the varying waste materials identified as contributing to the site's soil/sediment. In particular, low pH values are noted to coincide with sample grids that display high Fe and S concentrations. Conversely, soil samples exhibiting a higher degree of soil alkalinity are found to coincide with areas where high volumes of building construction debris, including concrete and cement are observed. By contrast, the individual soil samples from the Rovinari-Sterile field site exhibit very similar values, with a low degree of variance between samples.

Table 26: Descriptive statistics for the pH of the soil samples collected from Romanian Study Sites in October 2012 (where: * - *n*= 23; † - *n*= 14; ‡- *n*= 12; values are quoted to two significant figures)

	Soil acidity (pH)			
Study site	**Câmpina†**	**Copşa Mică***	**Rovinari- Sterile‡**	**Rovinari- Ash‡**
median	8.3	7.2	8.4	8.2
IQ range	1.20	0.750	0.0500	0.165
Range min	3.2	4.6	8.2	7.5
max	8.8	8.3	8.4	8.5

Comparative analysis of the soils from Copşa Mică and the Rovinari field sites is undertaken with typical values soil acidity reported for soils from Sibiu and Gorj County, respectively (Table 25). As with the qualitative analysis of the soil parameters, TC, OM, and TN due to the unique conditions operational at the Câmpina field site, a similar treatment with data for Prahova County is not undertaken.

Although the Copşa Mică soil samples appear to display a higher degree alkalinity, consideration of the of the measure of central tendency for the soil samples from the Copşa Mică study site and the soils of Sibiu county show a degree of comparability with values for pH for both sets of data suggestive of circumneutrality (6.5-7.5). The range of soil acidity exhibited by the soil samples of the Copşa Mică site show similarities with both the maximal (4.1-4.7; mountain areas) and minimal (6.0-8.2; southern areas) ranges reported for the soils from Sibiu county. Although the range of soil acidity determined for the Copşa Mică site (4.6-8.3) include acidic pH values approximately 60% of the soil samples analysed exhibited pH values in the range of 6.5-7.5, and approximately 80% in the 6.5-8.3 range suggesting closer affiliation to the soils from the south of Sibiu County. Furthermore, as the site has been under continued agricultural cultivation since 1990s, it is not unreasonable to surmise that soil conditioners such as lime may have been applied to maintain the soil acidity at an optimal level for crop production resulting in the circumneutrality of the soils at the Copşa Mică site.

Qualitative assessment of the soil acidity of the Rovinari-Sterile with the pH data for Gorj County, finds clear differences between the two data sets, with the soil samples from the Rovinari-Sterile site being typically three orders of magnitude more alkaline than the soils samples from Gorj County. Consideration of the maximal (5.0-6.4; northern areas) and minimal (4.7-5.9; southern areas) ranges for soil acidity in Gorj county finds that the discrepancies are greatest between the soils from the minimal values for Gorj county and the pH values displayed by the soil samples from the Rovinari-Sterile site. With differences in pH of three to four orders of magnitude being discerned. High soil alkalinity is likely a characteristic of the source material as similar pH values have also reported for other sterile dumps within the locality (Cărăbis *et al.*, 2011).

Consideration of the Rovinari-Ash data with the Gorj county data finds that there is a similar degree of disparity observed in the soil acidity data for the Rovinari-Sterile site with the Gorj county data. The soil samples from the Rovinari-Ash site show overall higher alkalinity to the pH values recorded for the soils of Gorj county with similar order of magnitude differences as those reported for the Rovinari-Sterile soil samples. The higher soil alkalinity in the soil samples at the Rovinari-Ash site is likely a reflection of the significant contribution made by lignite combustion by-products to the soil matrix at the site. Other researchers have reported that bottom and fly ash from lignite coals show enrichment in calcium oxides and hydroxides, which are likely to be contributory to the alkaline pH exhibited by such materials (Adriano *et al.*, 1980; Vassilev & Vassileva, 2007; Anghelescu & Diaconu, 2012).

5.2. Plant growth & crop survey (field trials)

Results of the site surveys undertaken in October 2012 prior to the camelina crop being sown for the three field study site are presented in Appendix A. Crop survey / field observations are summarized on an individual site-by-site basis below. Details of individual quadrat measurements are presented in Appendix B.

Rovinari, Gorj County - Sterile Field

There is an apparent delineation in crop morphology between the southern and northern half of the field. In the southern part (quadrat 1-6; Appendix B, Table B37) 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. By contrast, the northern section of the study site (quadrat 7-12; Appendix B, Table B37) 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 are common across the whole study site suggesting underlying causative factors. As with other field study sites red-orange mottling along with necrotic lesions and interveinal chlorotic areas on leaves are also noted occasionally.

Rovinari, Gorj County - Ash Field

The crop shows evidence of drought stress, with areas of crop dieback being noted, this in turn may be influential on assessments of crop emergence. Plants counts are between 128-417/m^2, high plant counts coincide with plants of smaller height and growth that exhibit singular flower inflorescences. Plants of more vigorous growth express branched flower inflorescences. Basal foliar chlorosis is a common feature. Other foliar effects previously discussed occur more frequently and discolouration of younger leaves in some plants is also noted. Although toxicity of metals such as nickel (Ni) maybe a causative effect (Nagajyoti *et al*., 2010) the drought induced senescence cannot be discounted.

Câmpina, Prahova County

Crop emergence exhibited a strong degree of variability across the site (0-60%), reflecting the heterogeneity of the sediment / soil matrix. Little or no growth is noted in the 20 x 40 m area along the northern boundary where high metal concentrations are likely to be a causative factor. Plant height was between 3-64 cm and plants are typically characterized by singular flower inflorescences. Chlorosis of the basal leaves is common and may result from deficiencies of macronutrients such as N, P and Mg. Given the occurrence of reduced plant stem height, the presence of senescence, bronzing of leaf margins, localized chlorosis of younger leaves and strong magenta rims suggests that toxicity induced chlorosis and macronutrient deficiency by metals such as lead (Pb) and zinc (Zn) (Nagajyoti *et al*., 2010) may also be operational.

Copşa Mică, Sibiu County

Crop emergence displayed a degree of variability across the site (25-100%), typically emergence was approximately 70-80%, where lower emergence was noted some of the crop loss might be attributed to herbicide spray damage from adjacent cropping area. Plant height was typically between 20-61 cm plant height appears to be affected by seed rate and hence plant density. In areas of high plant density, stem height was found to be shorter than in areas where plant populations were lower. The occurrence of branched flower inflorescences was common (50-100%). Chlorosis of the basal leaves is common this may be the result of macronutrients (N, P and Mg) deficiencies in the soil, but the influence of herbicidal spray cannot be ruled out. In addition yellow-orange mottling and necrosis of the basal leaves was along with the occasional occurrence of magenta discolouration of leaf rims were also noted suggesting that metals such as cadmium (Cd), manganese (Mn), Pb and Zn may also adversely influencing the crop (Nagajyoti *et al*., 2010).

5.3. Production data for the camelina grown on contaminated land in Romania

Production of camelina on the four contaminated land sites in Romania for the cropping year 2012-2013 are summarized in Table 27 (*pers.comm. Prof. Ştefana* Jurcoane*, Biotehgen*). The crop was produced with no external inputs of nitrate fertilizers, herbicides, pesticides or organic matter.

Table 27: Production of *Camelina sativa* on the four contaminated land field sites in Romania for the cropping year 2012-2013 (Source: Prof. Ştefana Jurcoane, Biotehgen)

Study Site	Cultivar	Yield (kg/ha)
Câmpina	GP202	35
Copşa Mică	GP204	150

Rovinari ash	GP204	200
Rovinari sterile	GP202	330

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 Domneasca (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. The application of nitrate and phosphate chemical fertilizers was seen to be influential on production yield. A fertilization scheme of 60 kgN/ha and 50 kgP/haP50 was found to produce potential yields in excess of 2000 kg/ha, however where no nitrate fertilizer as applied the potential yields were seen to be diminished (500-1700 kg). Seed rate and row width were also seen to influence the potential yield (Dobre *et al.*, 2014). Whilst the findings of the agronomic trials are positive, to date such crops have not been cultivated on a field scale in Romania, and as such do not provide a true comparison.

Whilst direct comparisons of the production yields between a camelina crop grown on fallow arable land in Spain with one grown on contaminated land in Romania, should be treated with extreme caution due to the highly complex nature of growing such a crop and in particular due to the significant differences in soil, metrology, microclimate and local agronomic practices. Nevertheless, such comparisons are inevitable as any assessment of the viability of contaminated land for production does have some merit in spite of the caveats.

Benchmark figures (provided by CCE) for the production of camelina on fallow arable land in the arid region of Spain, including Castilla La Mancha and Aragon, Using the recommended agronomic inputs such as pre-sowing application of chemical fertilizer 8:24:8(N:P:K) at 150 kg/ha, would typical be:

- average yields are approximately 500kg/ha,
- yields of 1500kg/ha might be expected on a fertile land,
- where poor field conditions result in yields below 100 kg/ha, such field are often written off as uneconomic to crop.

With the exception of the Câmpina field site, consideration of the production from the Romanian contaminated land field sites with the agronomic trials Moara Domneasca, Ilfov finds that the production attained for the Copşa Mică, Rovinari-Ash and Rovinari-Sterile are 30%, 40% and 60% respectively, of the potential yield when no chemical fertilizer is applied. The disparities in the yield of camelina between the agronomic trials and three 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. Similarly, comparison with the benchmark figures for the commercially grown crops of camelina in Spain, suggest that production on contaminated land is inferior, however the levels of production resulting from the present agronomic regime operational at the Copşa Mică, Rovinari-Ash and Rovinari-Sterile field sites are above 100 kg/ha and therefore may be deemed to be economic. Nonetheless, the viability of such crops at the current levels of production are vulnerable to economic constraints and not assured, consequently the application of precision-agricultural technologies and adjusting the agronomic regime to improve levels of seed production whilst minimising contaminant transference would be desirable. However, this is beyond the remit of the current study.

5.4. Elemental analysis of parent seed (field trials)

Two cultivars of *Camelina sativa*, GP202 and GP204 (*Source:* Biotehgen) were used in the Romanian field cultivation trials. The elemental analysis for the trace metals, Cd, Co, Cu, Fe, V and Zn, in this the base/parent seed prior to cultivation was carried out on the basis of three 0.5 ± 0.005g replicates. The results presented in Table 28 are consistent with the analysis of the base/parent seed used in the controlled cultivation greenhouse trials. Similar to the controlled cultivation greenhouse trials base seed, the concentration of Cu, and Fe and Zn are found to be one to two orders and two to four orders of magnitude greater, respectively, in the concentration of than the metals Cd, Co and V in the seed.

Comparison of the determined concentrations in the GP202 and GP204 base seed with values of the metal concentrations in a normal plant, proposed by Markert (Greger, 1999), suggest that the Cd:Co relationship observed in the camelina seed (3:1) is the inverse to that normally reported in plants (0.25:1). Concentrations of Cu and Fe in the two cultivars are found to be 35-50% lower than the normal composition in plants, whilst Zn concentrations in the camelina base seed are found to be lower, the difference (5-20%) is smaller than for Cu and Zn concentrations. Consideration of the ratios Cu:Fe; Cu:Zn and Fe:Zn show comparability with the normal plant data for the Cu:Fe ratio, whilst the Cu:Zn and Fe:Zn ratios are found to be diminished compared to the normal plant concentrations and therefore enriched in the camelina seed with respect to Zn.

Table 28: Concentrations of the trace elements Cd, Co, Cu, Fe, V and Zn in the parent camelina seed used in field cultivation trials expressed as median values of analyses (n=3; values quoted to two significant figures).

		Elemental concentration (mg kg^{-1} dry matter)					
		Cd	Co	Cu	Fe	V	Zn
GP202		0.17	0.050	5.3	86	0.0067	41
IQ range		0.0145	0.000185	0.126	3.12	0.00499	1.94
Range	min	0.14	0.050	5.2	81	0.0067	39
	max	0.17	0.050	5.4	87	0.017	43
GP204		0. 41	0.12	6.4	77	0.017	47
IQ range		0.0527	0.0458	0.731	2.06	0.0689	1.57
Range	min	0.40	0.11	6.2	77	<LD	45
	max	0.50	0.20	7.6	81	0.14	49

5.5. Elemental analysis of daughter seeds from the four Romanian field trial crops

A composite seed sample was obtained for each of the Romanian field trial sites from the crop harvested in the summer of 2013. Analysis of the trace element concentrations in the raw composite seed samples for the Romanian study sites, as well as the controlled-cultivation greenhouse trials. focused on the six elements Cd, Co, Cu, Fe, V and Zn, previously identified as problematic within aviation fuel. The results of the analysis for the 2012/2013 camelina crop for the concentrations of the metals Cd, Co, Cu, Fe, V and Zn, in the seed along with the concentrations determined for the two base cultivar seed are displayed graphically in Figure 5.

Qualitative assessment of the metal concentrations in the daughter seed samples from the four Romanian field sites (Figure 5), finds that the seed harvested from the Câmpina study site consistently exhibits the lowest concentrations of five (Cd, Co, Cu, Fe and V) of the six metals considered. By contrast, the highest metal concentrations in the seed are not observed in the daughter seed from one particular site, but a number of different sites. The highest concentration of the metals Co, Fe and V (0.28, 320 and 0.88 mg kg^{-1}, respectively), are discerned in the seed harvested from the Rovinari-Ash site. Whereas, the highest Cd and Zn concentrations (4.3 and 120 mg kg^{-1}, respectively) were observed in seed harvested from the Copşa Mică study site and the highest levels of Cu (9.9 mg kg^{-1}) were determined in seed from the Rovinari Sterile Field site.

Consideration of the degree of variance of the metal concentration of the daughter seed finds that the concentrations of Zn exhibit the lowest variability in the daughter seed, and appears to be independent of the source site. Moreover, suggesting that the uptake of Zn by *Camelina sativa* into the seed is independent of any micro-scale variations in the soil and that uptake control mechanisms maybe operational. Conversely, Co, Fe and V display a higher degree of variability. With the greatest variance being noted for the concentration of Fe in the cultivated seed, in particular in the daughter seed from the Rovinari-Sterile site. Such variability might be indicative that the translocation of Fe from the soil into the plant is subject to micro-environmental differences in the nutrient status of the soil as well as the interaction and competition with other metals such as Ca and Cu (Foy *et al.*, 1978).

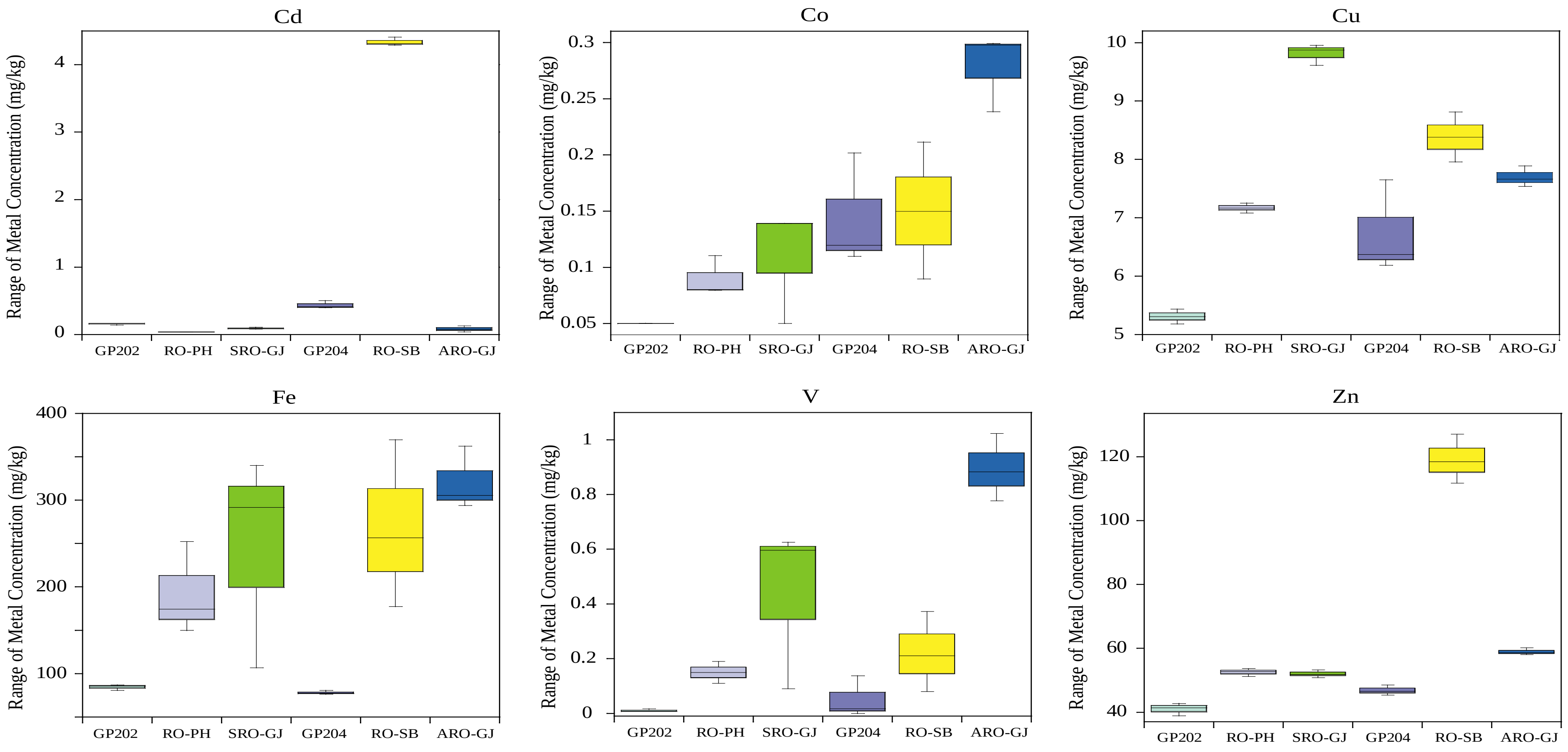


Figure 5: Parent and daughter concentrations for the elements Cd, Co, Cu, Fe, V and Zn determined for the four Romanian field study sites, (where GP202 and GP204 are the parent seed cultivars and RO-PH = Câmpina; SRO-GJ = Rovinari-Sterile RO-SB = Copşa Mică and ARO-GJ = Rovinari-Ash)

5.5.1. Comparison of metal concentration enrichment in daughter seed with parent seed

Enrichment of the metals concentrations was determined using the percentage increase of each of the metals, Cd, Co, Cu, Fe, V and Zn, in the daughter seed with respect to the parent seed. With the exception of Cd, enrichment of the metals Co, Cu, Fe, V and Zn, in the daughter seed compared with the parent seed is noted in the cultivated crop from all four study sites. Generally, cultivation of camelina crops using the parent seed cultivar GP202 (Câmpina and Rovinari-Sterile) show greater degrees of enrichment for the metals Co, Cu, and V. Whilst seed from crops using the base seed cultivar GP204 (Copşa Mică and Rovinari-Ash) exhibit larger percentage increases in Fe concentration. By contrast, the concentrations of Zn in the daughter seed typically display similar levels of enrichment regardless of the parent seed.

In the case of Cd, the concentration of the metal in the seed produced at the Câmpina, Rovinari-Sterile and Rovinari-Ash sites, appears to be depleted with respect to the Cd concentration determined for the parent seed. It is only in the daughter seed from the Copşa Mică site that significant enrichment is the Cd concentration is discerned. Consideration of the percentage increase of the five metals, Co, Cu, Fe, V and Zn, finds that the degree of enrichment depends on the individual metal, with the lowest range of percentage increases being observed for Co, Cu and Zn (25-180%; 20-87% and 26-160%, respectively). Conversely, the highest percentage increases are reported for Fe and V (98-300% and 1100-8900%, respectively). Comparison of the degree of enrichment on a site-to-site basis reveals that the daughter seed from the Rovinari-Sterile site exhibits the highest percentage increase in metal concentration of Co, Cu, and V in the seed. The seed produced form the Copşa Mică field site also displays the greatest percentage increase in Zn as well as for Cd. Whilst, the daughter seed from Rovinari-Ash site is found to be the most enriched in Fe.

5.5.2. Comparison of metal concentration enrichment in daughter seed with soil medium

Consideration of the metal concentration in the daughter seed with the respective concentration of the metal in the soil at the four study sites finds that the highest daughter seed concentrations of Cd, Co, Fe, V and Zn correlate with the those sites displaying the corresponding highest soil concentrations. Such that the highest soil concentrations for Co, Fe and V (14, 28000 and 130 mg kg^{-1}, respectively) are reported for the Rovinari-Ash field site and the highest concentration for such metals are also found in the daughter seed from the Rovinari-Ash site (0.28, 320 and 0.88 mg kg^{-1}, respectively). Analogously, the highest concentrations of Cd and Zn (4.3 and 120 mg kg^{-1}, respectively) observed in the daughter seed from the Copşa Mică site correspond with the highest Cd and Zn (45 and 2600 mg kg^{-1}, respectively) reported soil concentrations at the same site. Exceptionally, in case of Cu, the highest concentration and percentage increase (9.9 mg kg^{-1}; 85%, respectively) in Cu occurs in seed produced at the Rovinari-Sterile site, which has the lowest soil concentration of Cu (19 mg kg^{-1}), it appears that a higher proportion of soil Cu is transferred into the crop. However such inverse proportionality is not consistently expressed by the daughter seed from the remaining three sites, with the seed from the Copşa Mică study site being found to have a Cu level 15% lower than seed from the Rovinari-Sterile site, whilst the soil Cu concentration is six times greater. As OM is known to lower the plant-availability from the soil matrix of metals such as Cu (Foy *et al.*, 1978), it is possible that variations in OM content (Section 5.1.2; Table 24), between the four field sites may in part explain the differences in Cu concentrations observed in the daughter seed for the respective sites.

Comparison of all the determined daughter seed concentrations with soil metal concentrations suggests that such relationship is too simplistic and the relationship between the metal concentration in the soil and the amount translocated into the seed is complex. For example, the variation observed in the concentration of Co, 0.080 to 0.28 mg kg^{-1} (Câmpina and Rovinari-Ash, respectively), in the harvested seed is disproportional to the similarities in the soil Co concentrations reported for the four study sites (11-14 mg kg^{-1}). The differences in the concentration of Co in the crop seed suggest that factors such as, chemical form of the metal, SOM, clay minerals, Fe oxides and pH, that control bioavailability of the metal to the camelina crop may vary with each of the four study sites. Similarly, although the soil concentrations of V for the three sites at Câmpina, Copşa Mică and Rovinari-Sterile (25, 32 and 30 mg kg^{-1}, respectively) are comparable, the concentration determined in the daughter seed for the Rovinari-Sterile study site is approximately two-fold greater than the V concentration observed in the daughter seed from the sites Câmpina and Copşa Mică (0.15 and 0.22 mg kg^{-1}, respectively). Suggesting that the bioavailability of the soil V to the crop at Rovinari-Sterile site is enhanced.

Consideration of the seed Zn concentration data, finds that the highest concentration and percentage increase (120 mg kg^{-1}; 160%, respectively) in Zn occurs in seed produced at the Copşa Mică site, reflecting the high Zn soil concentrations at the site. By contrast, the amount of Zn in the seed from the remaining three sites is observed to be of similar concentration, despite being cultivated on land of varying Zn soil concentrations. Of note, is that the Zn concentrations in the daughter seed from the Câmpina and Rovinari-Sterile sites (53 and 52 mg kg^{-1}, respectively) appear closely comparable although the concentration of Zn in the Câmpina soil is four- to five-fold greater than that reported for the soil at the Rovinari-Sterile site. It is unlikely that genotypic differences are influential as the parent seed cultivar for both sites is the same, GP202. Such similarities in daughter seed concentration regardless of soil concentration suggest potentially that *Camelina sativa* operates a barrier mechanism that limits the uptake of Zn in to the seed, even at concentrations above reference or background concentrations (Section 5.1.1;Table 23). Furthermore, the data suggests that such a mechanism may break down at concentrations in excess of 260 mg kg^{-1} as inferred from distinct increase in the Zn concentration observed in the daughter seed harvested from the Copşa Mică study site. Baker (1981) suggested such behaviour was indicative of an excluder plant; as such, *Camelina sativa* might function as an excluder with respect to Zn.

However, any conclusions drawn from the current data set, given the low replicate numbers used in the initial analyses, should be treated with circumspect. Analysis that is more extensive is needed to verify the validity of such conclusions, which is currently beyond remit of the present study.

5.5.3. Comparison of metal concentration enrichment in daughter seed with metal concentrations in the daughter seed from the controlled-cultivation greenhouse trials

In comparing the metal concentration in the daughter seed from the four field sites with the corresponding concentrations in the daughter seed from the greenhouse controlled-cultivation trials of comparable metal soil concentration, it might be postulated that the metal concentrations in the field trial daughter seed are likely to be lower. With such differences, being attributed in part to divergences in the speciation of the metals in the soil as well as variations in the key soil parameters between the field sites and those used in the greenhouse controlled-cultivation trials.

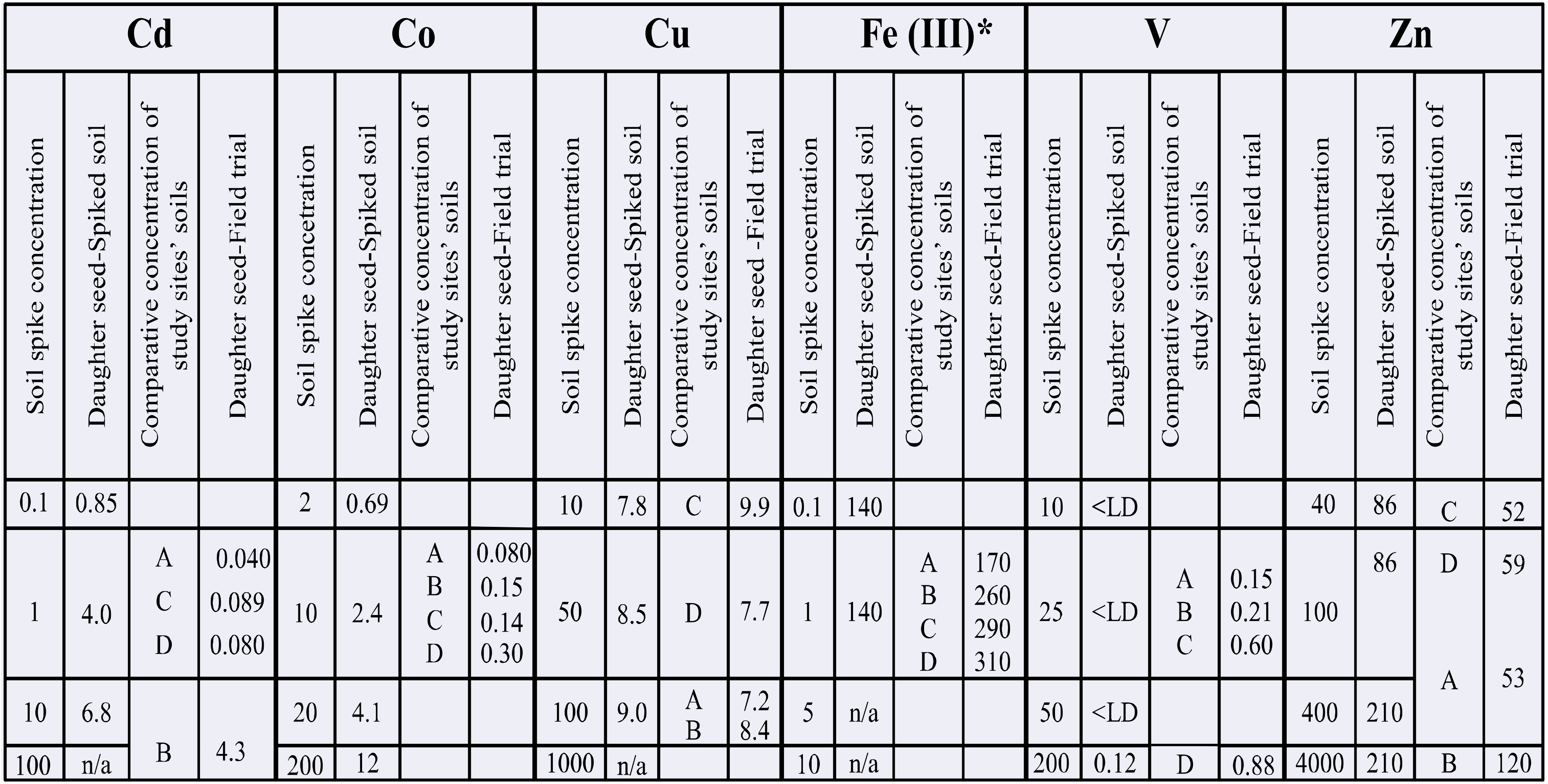
Consideration of the field daughter seed data with data for daughter seeds cultivated in soil spiked with comparable metal concentrations (Table 29) suggest that there is overt support for such a hypothesis with regard to the metals Cd, Co and Zn. In particular, the concentrations of Cd and Co, reported for the field seed samples are shown to be one to two orders of magnitude lower than those determined in the plants cultivated in spiked soils of comparable concentrations (0.1 mg kg^{-1} (Câmpina, Rovinari-Sterile and Rovinari-Ash) and 10-100 mg kg^{-1}(Copşa Mică)). Suggesting that the speciation such metals in the field site soils is typically different from the greenhouse controlled-cultivation trials and these metal species demonstrate a lower bioavailability to the camelina plants.

Lower bioavailability for Zn in the daughter seed from the field trials is intimated also by the two-fold differences observed between the concentration in the field-trial daughter seed and in the daughter seed from the greenhouse controlled-cultivation trials. Furthermore, consideration of the behaviour of Zn uptake into the seed, with respect to increasing soil concentration, finds that the Zn concentrations in both sets of daughter seed do not appear to increase incrementally with increasing soil concentration. Such an observation may be viewed as lending support to the concept that *Camelina sativa* potentially operates a barrier mechanism that limits the uptake of Zn in to the seed even at concentrations above reference or background concentrations. Consideration of the two sets of data further infer that the mechanism breaks down at concentrations between 260 mg kg^{-1} and 400 mg kg^{-1} as inferred from distinct increase in the Zn concentration observed in the daughter seed harvested from the Copşa Mică study site. Baker (1981) suggested such behaviour was indicative of an excluder plant; as such, *Camelina sativa* might function as an excluder with respect to Zn. Such suppositions should be treated with caution given the small sample size. Further work needed to test the validity of such observations, are presently beyond the remit of this project.

The Cu concentrations determined in the daughter seed from the three sites Câmpina, Copşa Mică and Rovinari-Ash are also shown to be 6- 20% (Copşa Mică and Câmpina, respectively; Table 29) lower than the concentration determined for daughter seed harvested from the greenhouse controlled-cultivation trials of comparable concentration. By contrast, the concentration in the daughter seed from the Rovinari-Sterile site is found to be enriched by 26% compared with the concentration in the corresponding daughter seed from the greenhouse controlled-cultivation trial. As similar degrees of enrichment are not observed in daughter seed of the GP202 cultivar grown at other field-study sites (e.g. Câmpina), it is unlikely that such enrichment is a result of a genotypic trait, but rather related to soil parameters and metal populations operational at a specific site (Ebbs & Kochian, 1997). Although, analytical replicate number may call for caution, analysis carried out, as part of the second phase of the field trials, on the daughter seed from the Rovinari-Sterile site (*n*=30; Table 30), finds that the Cu concentrations for the two field-site analyses are comparable. Therefore, these initial observations may be considered valid.

By contrast, inspection of the daughter seed data for Fe(III) and V suggest that the hypothesis breakdowns, with the daughter seed from all four field sites showing enhanced concentrations of Fe(III) and V compared with corresponding daughter seed from the greenhouse controlled-cultivation trials. However, speculation that this apparent enrichment is suggestive of active uptake by *Camelina sativa* as well as possible synergistic relationships between metals, should be cautioned against due to (i) low sample replicates used to achieve the greenhouse data for Fe(III) and (ii) differences in metal concentrations determined for the daughter seed from the Rovinari-Sterile site in Phase 1 and Phase 2. Metal concentrations of Cd, Co, Cu and Zn determined for the daughter seed from the Rovinari-Sterile site in Phase 1 and Phase 2 (Section 5.7) show a degree of consistency. By contrast, disparities between the Phase 1 and Phase 2 data sets are evident for the metals Fe and V. Comparison of the reported Phase 2 daughter-seed concentrations of Fe and V with the daughter-seed concentrations of Fe and V from the greenhouse controlled-cultivation find that, Fe concentrations in the daughter seed from the field site is below those determined for the greenhouse trials, whilst the seed V concentration for the Rovinari-Sterile site are below the limit of detection.

Table 29: Comparison of metal concentration enrichment in daughter seed with metal concentrations in the daughter seed from the controlled-cultivation greenhouse trials (where A = Câmpina; B = Copşa Mică; C = Rovinari-Sterile and D = Rovinari-Ash; values quoted to two significant figures).



Cd				Co				Cu				Fe (III)*				V				Zn			
Soil spike concentration	Daughter seed-Spiked soil	Comparative concentration of study sites' soils	Daughter seed-Field trial	Soil spike concetration	Daughter seed-Spiked soil	Comparative concentration of study sites' soils	Daughter seed-Field trial	Soil spike concentration	Daughter seed-Spiked soil	Comparative concentration of study sites' soils	Daughter seed -Field trial	Soil spike concentration	Daughter seed-Spiked soil	Comparative concentration of study sites' soils	Daughter seed-Field trial	Soil spike concentration	Daughter seed-Spiked soil	Comparative concentration of study sites' soils	Daughter seed-Field trial	Soil spike concentration	Daughter seed-Spiked soil	Comparative concentration of study sites' soils	Daughter seed-Field trial
0.1	0.85			2	0.69			10	7.8	C	9.9	0.1	140			10	<LD			40	86	C	52
1	4.0	A C D	0.040 0.089 0.080	10	2.4	A B C D	0.080 0.15 0.14 0.30	50	8.5	D	7.7	1	140	A B C D	170 260 290 310	25	<LD	A B C	0.15 0.21 0.60	100	86	D A	59 53
10	6.8	B	4.3	20	4.1			100	9.0	A B	7.2 8.4	5	n/a			50	<LD			400	210		
100	n/a			200	12			1000	n/a			10	n/a			200	0.12	D	0.88	4000	210	B	120

5.6. Elemental analysis of oil from all Romanian field trial crops

Composite samples of camelina oil from each of the contaminated land trial sites for the crop harvested in the summer of 2013 was provided by Biotechgen. Oil from camelina grown on a nominal non-polluted site (Moara Domneasca) 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.

Comparison of trace metal concentration in composite oil

Consideration of the trace element concentrations determined for the four Romanian study sites (Table 30) finds that for all of the sites the concentration of Cd, Co and V are consistently found to be below the experimental limit of detection (0.00015, 0.00041 and 0.00093 mg/kg, respectively) in the composite oil samples. Suggesting that within the seed such metals are retained in the testa rather than in the endospermic areas of the camelina seed. By contrast, concentrations of Cu, Fe and Zn are detected in the composite oil samples from all the four study sites. Matthäus and Zubr (2000) observed that heavy metals such as Cd and Zn cannot enter the oil during the pressing process, suggesting that in the case of Fe and Zn and in particular, Cu, that such metals are actively taken up into camelina seed endosperm during growth.

The concentrations of Cu determined for the composite oil samples exhibit similar values. Comparison with the concentration of Cu in the soil for each of the study site finds no observable correlation between the concentration of Cu in the oil and the soil Cu concentration and as such suggest that the levels in the oil is independent of bulk soil metal concentrations. It is observed that the concentration of Cu in the four oil samples is in the range 0.61-0.72 mgkg-1. Closer inspection of the concentration data finds that the values determined for Cu in the oil from Câmpina and Rovinari-Sterile sites, where cultivar GP202 had been cultivated, show strong similarities. A similar degree of correlation is noted between the oils derived from the cultivar GP204 harvested at the Copşa Mică and Rovinari-Ash field trials. As soil properties and type differ between the four study sites, this might suggest that as with rapeseed (Ben Ghnaya *et al*., 2009), cultivar effects may be influential in the uptake of metals such as Cu into the camelina endosperm.

Of further note is that the Cu concentration of the oil derived from the crop harvested from the nominal non-polluted Moara Domneasca site is between 9-14% greater than that determined for any of the contaminated field sites. The application of inorganic fertilizers in the cultivation of *Camelina sativa* at the Moara Domneasca field trial maybe a causal factor in such differences, both Chaignon *et al*. (2002) and Bravin *et al*. (2009) report the effect of fertilizer additions on rhizosphere pH and Cu bioavailability on other plant species including oilseed rape. The lack of external nutrient inputs in the cultivation of the crop and the neutral to alkaline pH of the bulk soils at the four contaminated field sites may contribute to rhizosphere alkalinization and a decrease in the availability of Cu to the crop (Chaignon *et al*., 2002; Bravin *et al*., 2009). Further work is needed to determine the validity of such initial observations.

Scrutiny of the Fe concentration in the composite oil samples from the contaminated sites finds that whilst Fe is discerned in all the oil samples there a higher degree of variance in Fe concentrations between the four field sites than observed for the Cu concentrations. Although the highest Fe concentration in the oil are found to correlate with the site (Rovinari-Ash) displaying the highest bulk soil Fe concentration, similar correlations are not observed between the Fe concentrations of the oil and the bulk soil concentrations for the other three sites. Furthermore, the lowest Fe concentration is reported for the oil produced from the Câmpina site, conversely its bulk soil Fe concentration is the second highest (2.3%) of the four sites.

Consideration of the Fe concentration as a percentage of the total seed concentration, finds that overall the Fe determined in the four composite oils accounts for <0.002% of the total concentration of Fe in the corresponding seed and is 3-4 orders of magnitude smaller than exhibited by Cu in the seed. It is likely given the minimal Fe concentration detected in the oil samples that the majority of Fe in the seed is attributable to the non-endospermic areas of the seed and therefore any enrichment of the metal in the seed will have greater implications to the value and usage of co-products than the oil. Although, the lowest percentage of the total seed Fe concentration is determined for the two oil produced from the cultivar GP202 (Câmpina and Rovinari-Sterile sites), there appears to be insufficient differential between the four sites for a clear expression of genotypic factors to be identified.

The Fe concentration of the oil sample from the nominal non-polluted site at Moara Domneasca contaminated sites is found to be of similar magnitude to the Fe concentrations reported for the four contaminated sites. Whilst nitrogen fertilizer has been seen to enhance Fe uptake into the seed of certain crop plants (Hao *et al*., 2007), it is likely that the similarities in the determined values of the present study support the supposition that Fe uptake into the camelina seed is limited to the non-oil bearing parts. Further work is needed to substantiate the possibility of such a protective mechanism.

Table 30: Descriptive statistics for the trace metals Ca, Cd, Co, Cu, Fe, K, Mg, Na, P, V and Zn, expressed as mg kg-1 for the oil samples from the seed harvest 2013 for the four Romanian contaminated study sites and the Moara Domneasca experimental farm. All values quoted to two significant figures.

		Elemental concentration in oil (mg/kg)					
Study site		**Cd**	**Co**	**Cu**	**Fe**	**V**	**Zn**
Moara Domneasca	Median	<LD	<LD	0.72	0.31	<LD	0.49
	Range: min	-	-	0.71	0.12	-	<LD
	max	-	-	0.72	0.61	-	1.1
Câmpina	Median	<LD	<LD	0.66	0.048	<LD	0.23
	Range: min	-	-	0.64	<LD	-	<LD
	max	-	-	0.72	0.32	-	1.1
Copşa Mică	Median	<LD	<LD	0.63	0.33	<LD	0.71
	Range: min	-	-	0.62	0.052	-	<LD
	max	-	-	0.65	0.49	-	1.3
Rovinari (Ash)	Median	<LD	<LD	0.63	0.50	<LD	<LD
	Range: min	-	-	0.61	0.36	-	<LD
	max	-	-	0.65	0.63	-	3.1
Rovinari (Sterile)	Median	<LD	<LD	0.66	0.27	<LD	<LD
	Range: min	-	-	0.61	<LD	-	<LD
	max	-	-	0.68	0.93	-	2.8
Limits of detection		0.00015	0.00041	0.00093	0.0084	0.00095	0.0049

Zinc concentrations in the oils from the two Rovinari study sites are discerned as being below the limits of detection (0.049 mgkg-1) with overtly detectable amounts only observed in oil produced from the Câmpina and Copşa Mică field trials (0.23 and 0.71 mg kg^{-1}, respectively) where the bulk soil concentrations of Zn are >260 mg kg-1. For all of the four sites the determined Zn concentration exhibit notable within-sample variability, further work may be needed to assess the reliability of the initial data set. Evaluation of the Zn concentration in the oils from the Câmpina and Copşa Mică sites, finds that the Zn concentration in the oil from the Copşa Mică study site is three-fold greater than that in the oil from the Câmpina study site. However, when considered in terms of percentage of the total seed Zn concentration, a degree of similarity is noted between the two sites, with the Zn concentration in the oil representing 0.0043% and 0.0052% of the total Zn concentration in the seed from Câmpina and Copşa Mică sites, respectively.

Appraisal of the oil derived from the crop harvested from the nominal non-polluted at Moara Domneasca site finds the Zn concentration to be higher than determined for the contaminated sites at Rovinari and Câmpina. While speculative, as soil metal geochemistry is largely undefined for this site, the Zn concentration in the Moara Domneasca oil may have been enhanced because of the application of crop fertilizers. Although species specific, other authors have reported that K and nitrogen fertilizers have been shown to modify rhizosphere pH and promote localized mobilization of Zn resulting in enhanced plant uptake (Kashem & Singh, 2000; Loosemore *et al*., 2004; Silber *et al*., 2004; Zaccheo *et al*., 2006). Further work is needed to determine the legitimacy of such observations.

For the metals considered, the initial oil analysis suggests that >90% of all the six metals will be retained in the processed co-product, oil seed cake. Such levels of metals may raise concerns regarding the value and use of the camelina oil seed cake in animal feeds and ultimately the food chain. Deliberation of the EU directive on undesirable substances in animal feed (EU Directive 2002/32/EC) would suggest of the metals Cd, Co, Cu, Fe, V and Zn, the Cd content of the meal, in particular from the Copşa Mică field trial, would be of greatest concern. However, such concerns are unlikely to be realized, as in accordance with EU Directive 2002/32/EC the use of *Camelina sativa* (L). Crantz in animal feed is restricted to trace quantities; therefore, it anticipated that the levels of metals in the camelina oil seed cake from the contaminated sites would be subject to dilution.

5.7. Rovinari-Sterile study site – A Case Study

One of the four Romanian field sites was used for a case study where comprehensive analysis of contaminant metals of concern, both in the context of aviation biofuels, Cd, Co, Cu, Fe, V and Zn, and co-product usage and its consequential impact on food security, As, Cr, Ni, and Pb were traced through the components of the cropping system.

The selected site was the Rovinari-Sterile site. The rationale for the selection of this site was not only based on the higher levels of production (330 kg/ha) determined for this contaminated site but also that the soil of the Rovinari Sterile exhibited overall the lowest concentrations of metal contaminants. Applying the methodology to material grown on soils of the lowest contaminant levels should allow assessment of the following:

- Whether metal transference into the crop at such levels is likely to be a challenge to the production of sustainable aviation biofuel crop
- Which contaminant metal is likely to need further monitoring in future crops
- Where metal storage is likely to occur in the crop

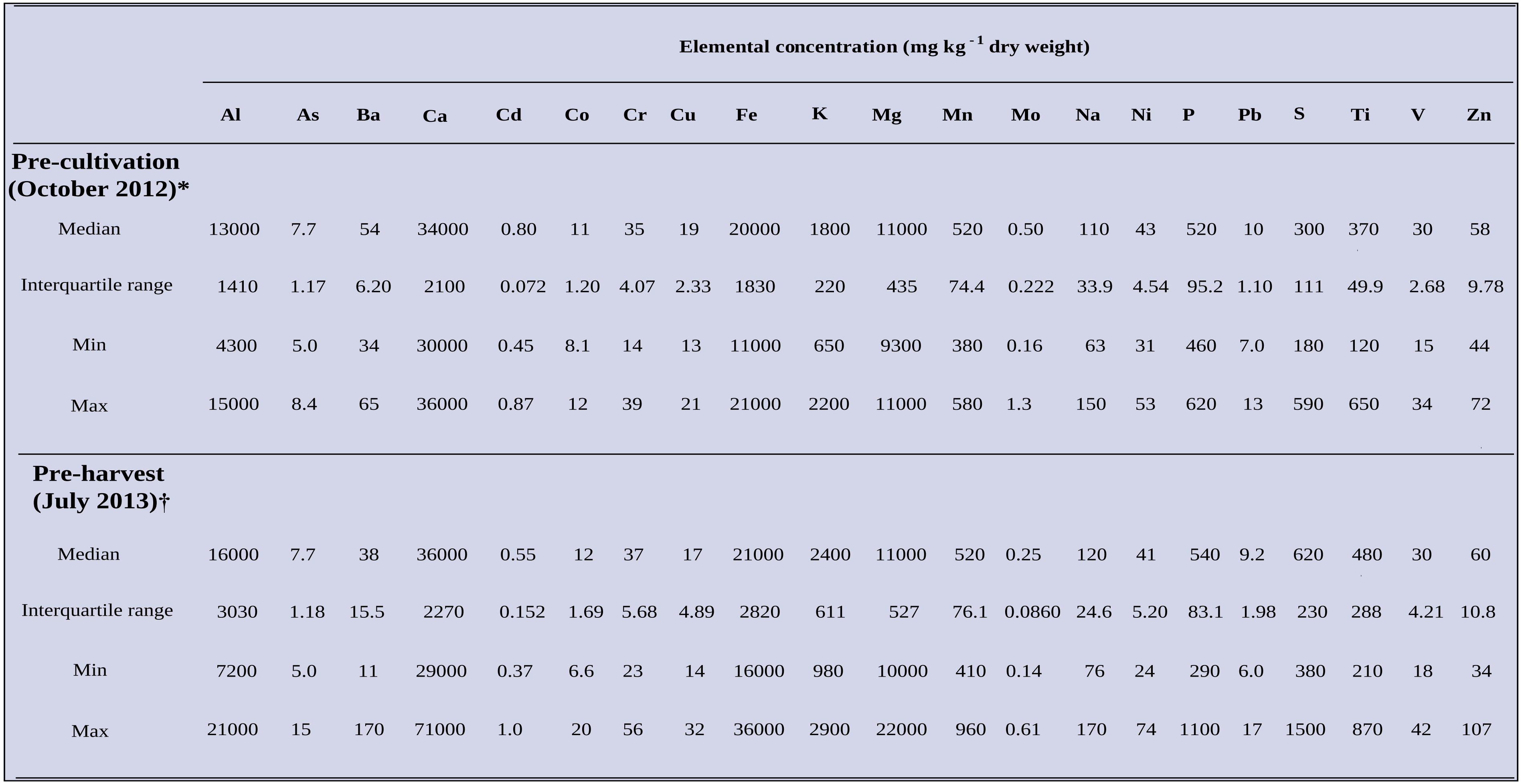
- Usability and value of animal feed co-products, for example, shoots and seed-pods (silicles); with the exception of the animal feed co-product oil-meal (grist) which was not available but whose characteristics may be estimated from the difference between the components present in the seed and oil).

5.7.1. Geochemical analysis: pre-harvest elemental soil analysis for field cultivation trials

Sample collected in July 2013 immediately prior to harvesting were frozen on receipt to minimize any change due to biological degradation (BS ISO 18512:2007) until such time geochemical analysis was undertaken. Individual soil samples were thoroughly mixed and quartered. Approximately one third of each soil sample was placed on a horizontal surface and air-dried, at an average temperature of 20.5°C (±1.5°C) until constant weight was achieved. Analysis of triplicate 0.5g ± 0.005g soil samples was carried out using the protocol described in Section 3.

The results for the pre-cultivation and pre-harvest geochemical analysis of the soil from the Rovinari-Sterile site for the 20 elements Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V and Zn are reported in Table 31.

Table 31: Median extractable concentrations of the 20 elements and descriptive statistics for soil samples from the Rovinari-Sterile study site, Gorj County (where * : n=12 (pre-cultivation) and † :n=29 (pre-harvest); median, minimum, and maximum values are quoted to two significant figures; interquartile range values are quoted to three significant figures)



	Elemental concentration (mg kg $^{-1}$ dry weight)																				
	Al	**As**	**Ba**	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**P**	**Pb**	**S**	**Ti**	**V**	**Zn**
Pre-cultivation (October 2012)*																					
Median	13000	7.7	54	34000	0.80	11	35	19	20000	1800	11000	520	0.50	110	43	520	10	300	370	30	58
Interquartile range	1410	1.17	6.20	2100	0.072	1.20	4.07	2.33	1830	220	435	74.4	0.222	33.9	4.54	95.2	1.10	111	49.9	2.68	9.78
Min	4300	5.0	34	30000	0.45	8.1	14	13	11000	650	9300	380	0.16	63	31	460	7.0	180	120	15	44
Max	15000	8.4	65	36000	0.87	12	39	21	21000	2200	11000	580	1.3	150	53	620	13	590	650	34	72
Pre-harvest (July 2013)†																					
Median	16000	7.7	38	36000	0.55	12	37	17	21000	2400	11000	520	0.25	120	41	540	9.2	620	480	30	60
Interquartile range	3030	1.18	15.5	2270	0.152	1.69	5.68	4.89	2820	611	527	76.1	0.0860	24.6	5.20	83.1	1.98	230	288	4.21	10.8
Min	7200	5.0	11	29000	0.37	6.6	23	14	16000	980	10000	410	0.14	76	24	290	6.0	380	210	18	34
Max	21000	15	170	71000	1.0	20	56	32	36000	2900	22000	960	0.61	170	74	1100	17	1500	870	42	107

Initial inspection of the soil metal concentrations determined for the pre-harvesting soil samples from the Rovinari-Sterile site (Table 31), finds that both diminution (Ba, Cd, Cu, Mo, Ni and Pb) and enhancement (Al, Ca, Co, Cr, Fe, K, Na, P, S, Ti and Zn) of the median metal concentrations determined for the pre-cultivation soil samples occurs. With the concentrations of As, Mg, Mn and V being apparently unchanged

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.

Consideration of the measure of dispersal (interquartile ranges) for Al, Ba, Ca, Cd, Fe, K, Mg, Mo, S and Ti, in the two data sets (Levennes test) found that with the exception of Cd and Fe, the degree of variance displayed by the individual metals was similar, such that overlap in the determined values was evident. The variability expressed by the data may be a reflection the inherent inhomogeneities of metals in soil matrices. In the case of Cd and Fe, further investigations are needed to determine the role of *Camelina sativa* in effecting the reported changes in soil concentration. Furthermore, research is needed validate if such observations, and to ascertain whether in the case of Cd that the behaviour observed is indicative of phytoremedial potential for Cd in soils of similar character to the soil matrix at the Rovinari-Sterile site.

5.7.2. Elemental analysis of roots, shoots, silicles and daughter seed from Rovinari-Sterile field trials

Collection of pre-harvest plant material from Rovinari field sites was undertaken using the sampling strategy described Section 3. Plant samples bagged in paper bags and labelled with both sample ID and sample point GPS coordinates. Collected samples were stored in an insulated container during transport from field to laboratory. Storage of plants samples was at 20 ± 5 ºC and protect from light sources.

Prior to analysis, the plant materials were separated in to roots, shoots (all above ground material not including silicles and seed), silicles and seed. Where necessary plant material was washed with tap water to remove soil particles and rinsed in de-ionized water. Seed samples were sieved, in accordance with EN ISO 658:2002, to remove non-oleaginous impurities and washed in de-ionized water to remove soil contamination. All plant material was oven dried at 60ºC for 3 days (Brunetti *et al*., 2011). Analysis of triplicate 0.5 g ± 0.005 g prepared plant material was carried out following the protocol reported in Section 3.

Typically, other research has primarily focussed on the uptake of metals into the roots, shoots and leaves (Ebbs & Kochian, 1997; Baryla *et al*., 2001; Chatterjee & 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 6 and Figure 7, respectively

Metal concentrations determined for the camelina root material harvested from the Rovinari-Sterile field site exhibit the following series of increasing concentration: Fe > Zn > Cu > Cr; Ni > V > Pb > As > Cd > Co. Similarly, scrutiny of the metal concentrations determined for shoot material finds that the corresponding series: Fe > Zn > Cu > Cr > Ni > Cd > As > Pb > V > Co; mirrors the first five members of the root series. However differences are note for the order of concentrations of the metals As, Cd, Co, Pb and V. Metal concentration series determined for the seed and silicles samples from the Rovinari-Sterile site are: Fe > Zn > Cu > Ni > As > Cr > Cd > Co > Pb >V and Fe > Cu > Zn > Ni > Cr > Pb > V > Cd > As > Co, respectively. The abundance of the metals Fe, Zn and Cu determined in the plant material is likely to reflect the uptake of such metals as essential plant micronutrients (Peralta-Videa *et al*., 2009). By contrast, Cr and Ni are not considered to be essential to plant growth, but in low concentrations have been found to stimulate growth in crop plants (Baker & Brooks, 1989; Peralta-Videa *et al*., 2009). The prevalence of Cr and/or Ni after Cu, Fe and Zn in the camelina plant matter may be indicative of uptake by the crop of Cr and Ni for such purposes. The different order of concentration expressed by the metals As, Cd, Co, Cr, Ni, Pb and V in the three aerial compartments of the plant suggests differences in storage and transportation of individual metals are operational within the plant.

Comparison of the plant concentration data of Cd, Co, Cu, Fe, V and Zn for the Rovinari-Sterile study site with that of nominally uncontaminated roots, shoots and seeds from the greenhouse trial control plants (Section 4) suggest that the uptake and transport in the plant of such metals is similar for both greenhouse and field trials. With differences in precise concentrations being accounted for by differences in edaphic conditions and genotypic effects.

Consideration of the profiles of each of the 10 metals (Figures 6 and 7), finds that for the plant material from the Rovinari-Sterile site the highest concentrations of As, Cu and Zn are determined in the seed. Whilst, for the metals, Cd, Co, Cr, Fe, Ni, Pb and V concentration in the roots is equal to or higher than in any other of the plant compartment.

Given the role of Cu and Zn as essential plant micronutrients and key components in a number of enzymes relating to carbohydrate and protein metabolism (Baker & Brooks, 1989), the presence of such metals in the seed might be anticipated. By contrast, As is not considered to fulfil any essential role in normal plant physiology. In aerobic soils arsenate (As(V)) is the primary form of As and is as an analog of phosphate, with which it competes for the same uptake channels into the plasmalemma of the root (Meharg & Hartley-Whitaker, 2002; Peralta-Videa *et al*., 2009). Within the plant As has been shown to metabolized to organophospholipids and arsenosugars (Yadav, 2010). It may be speculated that its presence in the seed is a further reflection of its chemical similarity with P. In particular, in relation to phytate (inositolhexakisphosphate (IP6)) which are known to be present in oilseed endosperm (Lickfett *et al*., 1999), further suggesting that the balance between As and P in both the soil and the plant may be key the future successful of camelina production on As contaminated sites. Additional work is needed to determine the validity of such suppositions.

Further deliberation of the plant material profiles with respect to the relative concentration of the metals As, Cd, Co, Cr, Cu, Fe, Ni, V, Pb and Zn, in the four plant components analysed, discerns that it is possible to apply the following rudimentary grouping of the metals As and Zn (where: [seed] > [root] > [shoot] > [silicle]); Cd and Cr (where: [root] ≥ [shoot] > [silicle] > [seed]); Co and Ni (where: [root] > [silicle] > [seed] > [shoot]);Fe, Pb and V (where: [root] > [silicle] > [shoot] > [seed]), exceptionally the highest concentrations of Cu are located in the seed and silicles.

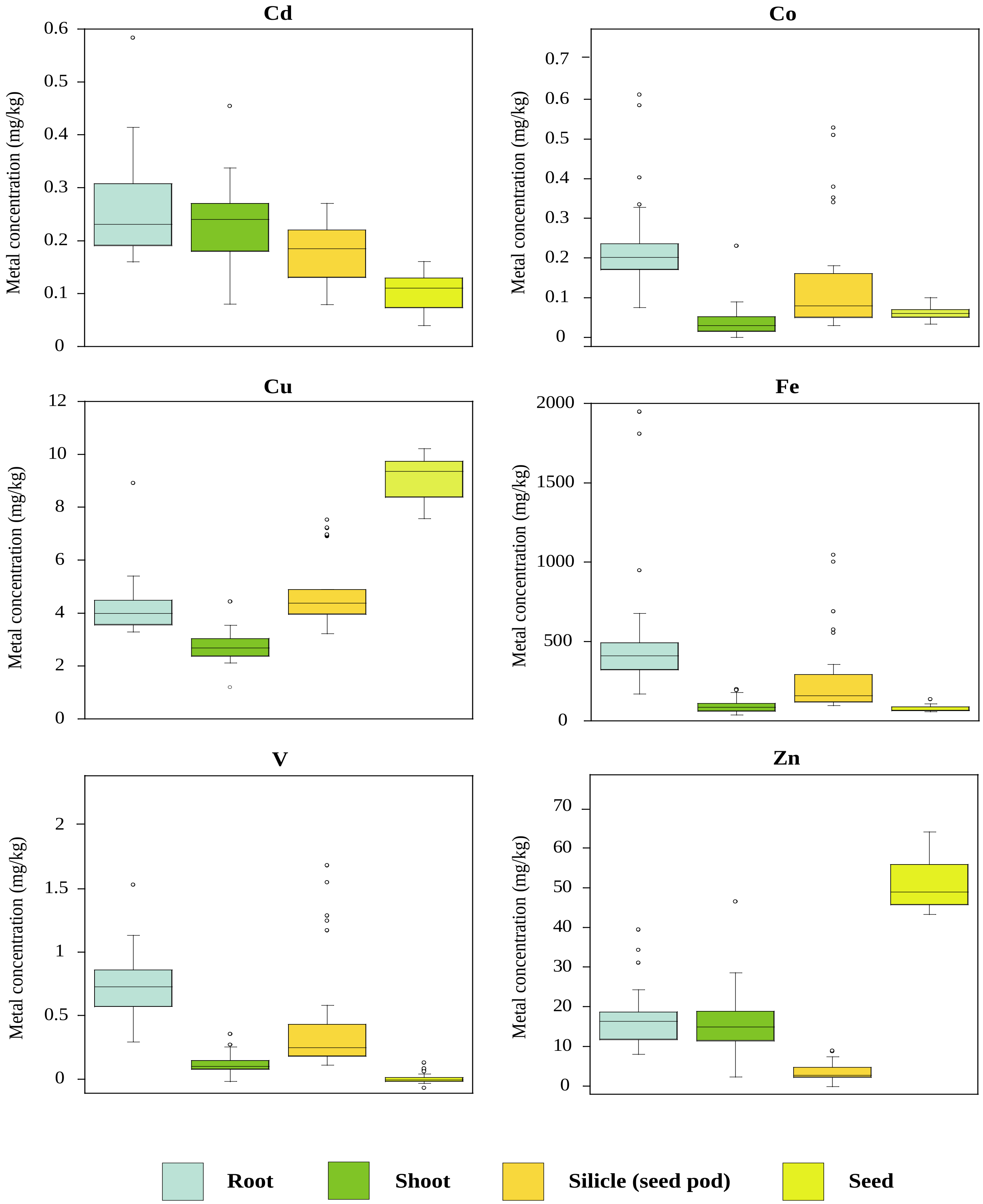


Figure 6: Concentrations of the metals Cd, Co, Cu, Fe, V and Zn determined in roots, shoots, silicles and seeds from plant material harvested from the 2012-2013 cultivated camelina crop at the Rovinari-Sterile field site

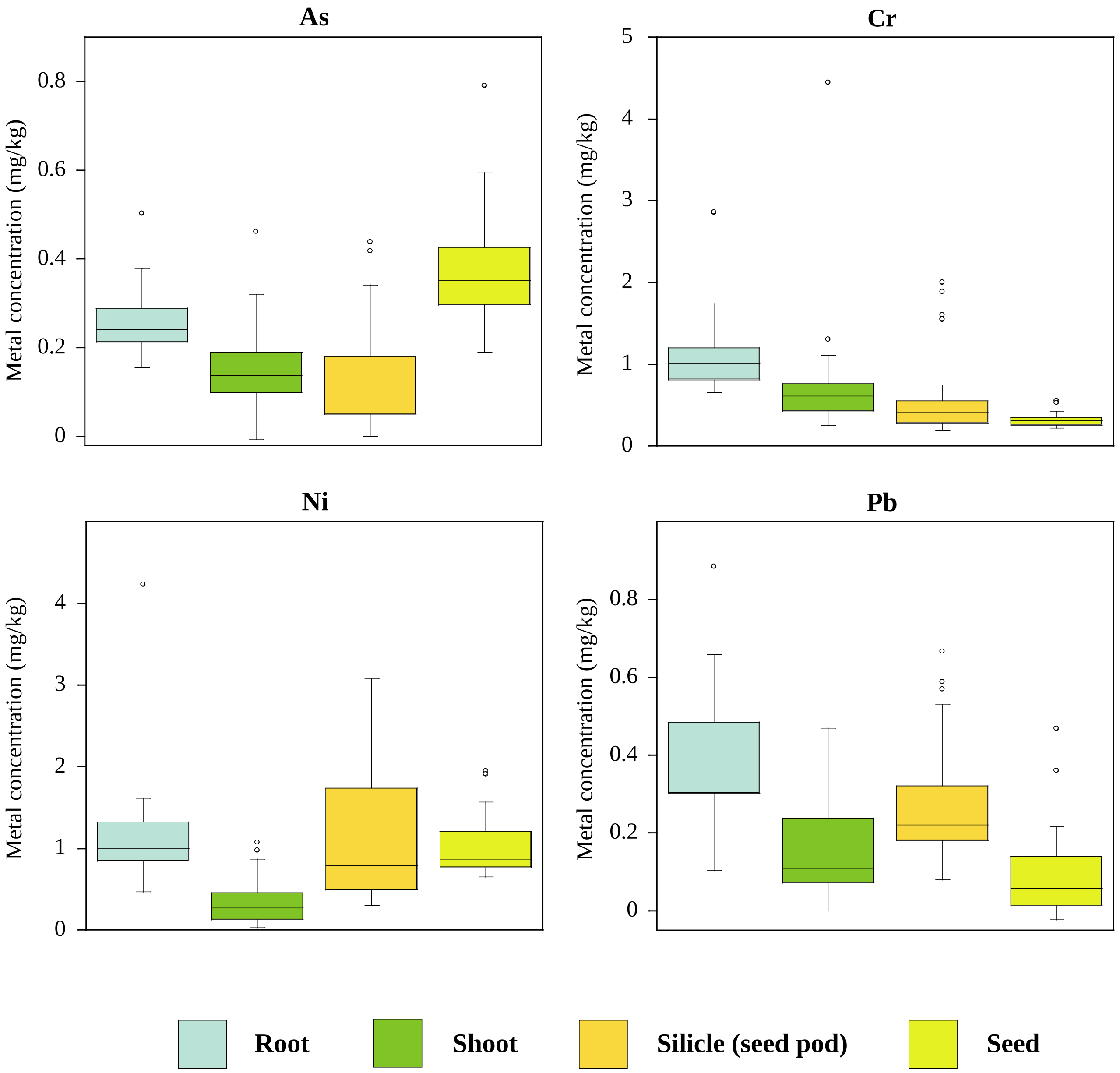


Figure 7: Concentrations of the metals As, Cr, Ni and Pb determined in roots, shoots, silicles and seeds from plant material harvested from the 2012-2013 cultivated camelina crop at the Rovinari-Sterile field site.

Comparison of the metal concentrations determined for the Rovinari-Sterile plant material with the findings of other researchers is principally based on the concentrations reported for roots and shoots of other plants, specifically members of the Brassicaceae, including Indian mustard (Brassica juncea), oilseed rape (Brassica napus) and cauliflower (Brassica oleracea). Scrutiny, of the Rovinari-Sterile plant metal concentration data shows that with the exception of Cd and Zn, the concentration of a metal in the root exceeds that determined for the shoot material. Agreement for the higher concentrations of As, Cr and Cu observed in the camelina root material has been reported for a number Brassica sp. (Shanker *et al*., 2005; Sinha *et al*., 2010). The overt disparity discerned between the concentrations of Co, Ni, Pb and V in the root and shoot material suggests a low level of transference into the aerial parts of the camelina plant. Similar behaviour, with significant retention of Ni and Pb in the plant roots of Brassica sp. has been noted by other researchers (John *et al*., 2009; Pourutt *et al*., 2011; Yusaf *et al*., 2011). Work carried out by Hou *et al*.. (2013) and Tian *et al*.. (2014; 2015) investigating the accumulation of V in Brassica chinesis and Brassica juncea suggested that the concentration of V was in the sequence of root >> stem > seed, suggesting that the distribution profile of V in Brassica chinesis and Brassica juncea are similar to that established for *Camelina sativa* from the Rovinari-Sterile site. Furthermore, similar to the findings of the present study the concentration of V in the seed was found to be below the experimental limits of detection. The findings for the partitioning of Co in Brassica oleracea plant tissue, where the highest concentrations were recorded in the plant shoots (Chatterjee & Chatterjee, 2000) are contrary to those displayed by camelina from the Rovinari-Sterile study site; such anomalies may arise due to interspecies differences in the assimilation of metals into the plant tissue (Ben Ghnaya *et al.*, 2009).

Consideration of the near parity noted between the root and shoot concentration of Cd and Zn, not only alludes to 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 5) 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). Whilst other Brassica species have been shown to store Cd in stem vacuoles, the degree of similarity perceived in the partitioning behaviour between camelina and Brassica species such as B. juncea and B. napus for the metals As, Cr, Cu, Ni, Pb and V is not as apparent for Cd and Zn. With the highest concentrations for both metals being reported in the root, in the stem and in the leaf material of B. juncea, B. napus and B. rapa (Ebbs & Kochian, 1997; Ben Ghnaya *et al*., 2009; Brunetti *et al*., 2011). Furthermore, research undertaken by Ben Ghnaya *et al*. (2009) highlighted that the uptake and partitioning of Cd and Zn in B. napus is influenced by genotype. Further studies are needed to assess whether such genotypic effects are also operational in the uptake and storage of Cd and Zn in *Camelina sativa* and to evaluate the implications for the use of camelina co-products from contaminated sites to the food chain security.

With the exception of Cd (0.24 mg kg^{-1}), all the measured concentrations of the metals considered for the camelina shoots for the current study in Table 11 are below the upper limits reported for normal plants. Albeit Brassica species have been shown to act as hyperaccumulators for metals such as Cd, Cr and Zn (Verbruggen *et al*., 2009), none of the plant shoots from the camelina grown at the Rovinari-Sterile site exhibited concentrations of Cd, Co Cr, Cu, Ni, Pb and Zn greater than those defined for hyperaccumulators (Table 32) (Baker & Brooks, 1989; Kirkham, 2006; Nagajyoti *et al*., 2010).

Although camelina grown in the soil conditions present at the Rovinari-Sterile site does not appear to exhibit hyperaccumulator characteristics. 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).

Table 32: Range of concentrations of the metals Cd, Co Cr, Cu, Ni, Pb and Zn in shoots of normal plants and indictor concentrations for hyperaccumulator plants (Baker & Brooks, 1989; Kirkham, 2006; Nagajyoti *et al*., 2010)

	Elemental concentration in shoot (mg/kg dry wt)	
Metal	**Range in normal plants**	**Indicative of hyperaccumulator**
Cd	0.1-0.2	100
Co	0.03-1.0	1000
Cr	<1.0	500
Cu	~10	1000
Ni	0.1-1.0	1000
Pb	10	1000
Zn	80-100	10,000

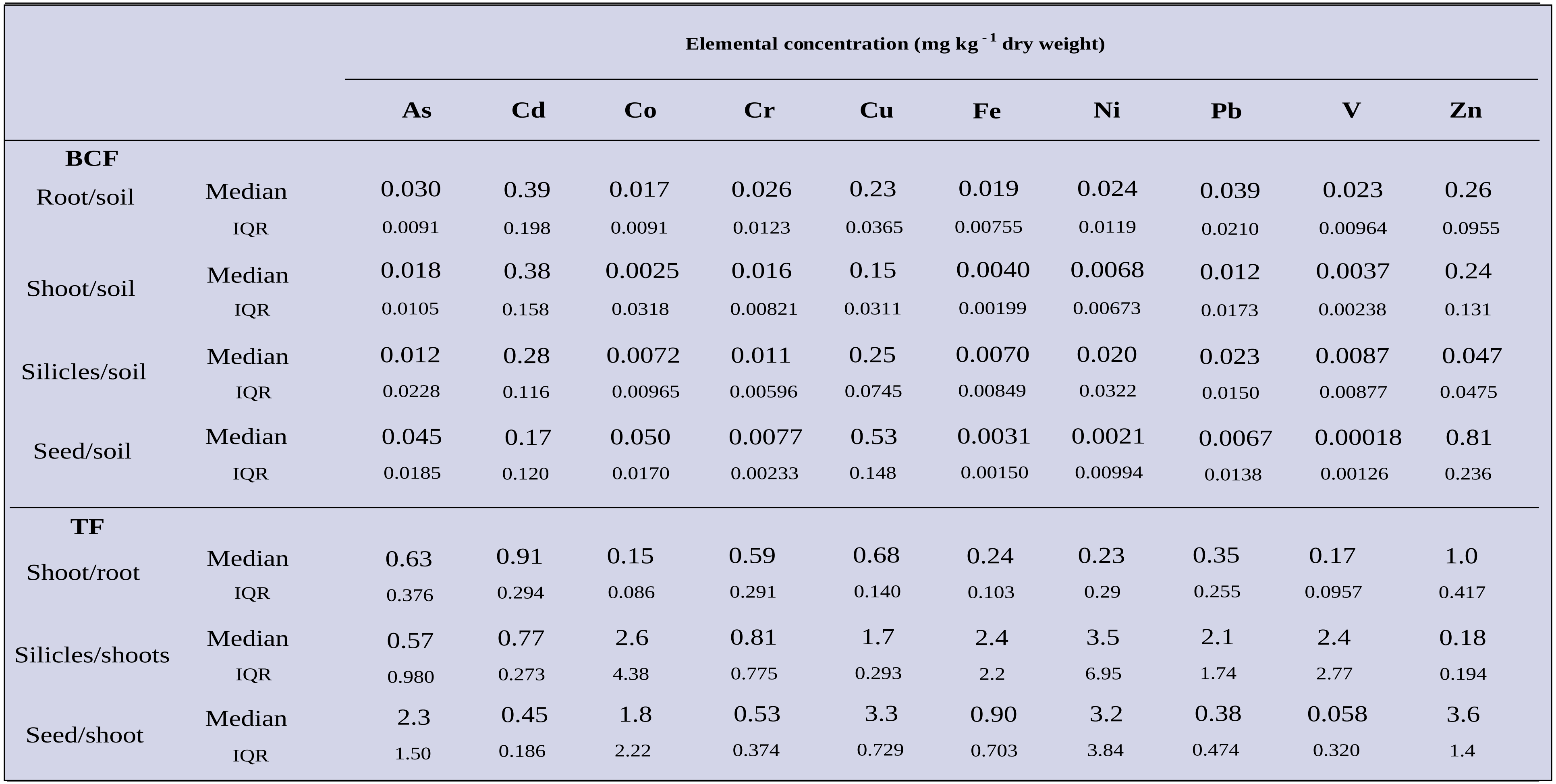
Bioconcentration factors are used to assess the ability of a plant to accumulate metals from the soil and are defined as the ratio of metal concentration in the plant compartment (root, shoot, silicles, and seed) to that in the soil. A plant's ability to tolerate or accumulate through the translocation 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 silicle and the shoot to seed were also calculated. The BCFs for the four plant compartments analyzed 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 12.

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). 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. For Cu and Zn the BCFs are seen to increase by 2-3 fold compared to the BCFs of such metals determined for the roots.

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). 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 & Brooks, 1989; Yoon *et al*., 2006). Further work is necessary to determine whether the apparent tolerance to Co, Cr, Fe, Ni, Pb and V exhibited *Camelina sativa* from the case study site, is a general phenomenon or unique to the plants grown on the Rovinari-Sterile site. 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 hyperaccumulators 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).

Table 33: Bioconcentration factors and Translocation factors for the metals As, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn in *Camelina sativa* grown at the Rovinari-Sterile site (Median values are quoted to two significant figures; interquartile range values are quoted to three significant figures)



		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

5.7.3. Elemental analysis of composite oil from Rovinari- Sterile field trials

The composite camelina oil sample from the crop harvested from the Rovinari-Sterile field trial sites in 2013 was analysed for both the concentration of metals identified as problematic within aviation fuel (Cd, Co, Cu, Fe, V and Zn) and those identified as of concern with respect to food chain security (As, Cr, Ni and Pb).Inspection of the data (Table 34) finds that for the metals Cd, Co, Ni, V and Zn, the concentration of the metals in the oil is observed to be below the experimental limits of detection. Metal concentrations in the oil determined for As, Cr, Cu, Fe and Pb express the following series of increasing concentration: Cu > As> Fe > Pb > Cr; with the concentrations for As (0.36 mg/kg), Cu (0.66 mg/kg) and Fe (0.27 mg/kg), being in the order of one to two order of magnitude greater than the concentrations reported for Pb and Cr, respectively.

Table 34: Descriptive statistics for the trace metals As, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn expressed as mg/kg for the composite oil sample from the seed harvest 2013 for the Rovinari-Sterile contaminated study site (All values quoted to two significant figures)

	Elemental concentration in oil (mg kg^{-1})									
	As	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**	**V**	**Zn**
Median	0.36	<LD	<LD	0.0074	0.66	0.27	<LD	0.030	<LD	<LD
Range:										
Min	0.22	-	-	<LD	0.61	<LD	-	<LD	-	<LD
Max	0.81	-	-	0.027	0.68	0.93	-	0.12	-	2.8
Limits of detection	0.0018	0.00015	0.00041	0.00069	0.00093	0.0084	0.00059	0.0010	0.00095	0.049

Applying a simple scenario that the concentration present in the co-product oil-meal is the differential between the metal concentration in the seed and that of the oil, suggests that in the case of the metals Cd, Co, Ni, V and Zn that on processing such metals in seed are likely be retained in the oil-meal. Similarly, for the metals Cr, Cu and Fe, initial analysis suggest that only a small proportion (≤7%) of the total metal concentration in the seed is present in the endospermic areas of the seed and subsequently in the oil, resulting in the majority of the oil being again preserved in oil-meal. This apparent partitioning of metals in the seed pulp may have consequences to food chain security. By contrast, initial assessment of the partitioning behaviour of As and Pb suggest that more than 50% of the total metal concentration in the camelina seed is attributed to the oil-bearing endosperm, and it likely that the amount of such metals in the oil-meal will be proportionally lower. Further work is needed to ascertain whether the partitioning of metals discerned in the camelina seed from the Rovinari-Sterile site is also noted in the seed produced on other contaminated sites.

5.7.4. Implications of the use of co-products to food chain security

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.

The European Directive on undesirable substances in animal feed (Directive 2002/32/EC) stipulates the maximum content for the metals As (2 mg kg^{-1}), Cd (1 mg kg^{-1}), Hg (mercury; 0.1 mg kg^{-1}) and Pb (10 mg kg^{-1}) in animal feed materials. Whilst the determined total seed concentration may be below these maxima, care should be taken with the use of the complementary oil-meal. As given the high oil content of the camelina seed (30-40%)(Agarwal *et al*., 2010; McKenzie *et al*., 2011) it is likely, where the metal concentration is found to reside in the non-endospermic parts of the seed, that concentration of the metal is likely to result from processing. Using a simplistic model where all the metal content is retained in the seed pulp after pressing, elevations of up to 60% in the metal concentration may be predicted. Although, it may be argued that the requirement that the maximum content of camelina in animal feedstuffs is 4000 mg kg^{-1} (Directive 2002/32/EC) would act as an effective counterbalance through dilution to such enrichment. Further work is needed to quantify the extent to which the oil-meal is likely to be enriched in key metals compared to the metal content of the seed, and the implications of incorporating such oil-meal to the integrity of the food chain.

Whilst not subject to the enrichment effects of seed pressing and consideration of the dilution effect of the limits to the maximum quantity of camelina material allowable suggest realistically that silicles exhibiting concentrations of As, Cd, and Pb of up to 5 mg kg^{-1}, 2.5 mg kg^{-1} and 25 mg kg^{-1}, respectively could be included in animal feeds. However, as with the inclusion of oil-meal in animal feedstuffs the use of silicles from camelina crops grown on contaminated land will also need to be carefully assessed.

The use of the camelina shoot co-product is typically as fodder and bedding material in livestock production. Unlike the co-products of oil-meal and silicles, European regulation of use of this material is not apparent and this may be of future concern. In particular for metals such as Cd and Cr that have been found to be stored in the stem vacuoles of Brassica species, and therefore likely to be directly available to livestock and ultimately gain ready access to the food chain. An alternative approach might be to restrict the use of camelina shoots grown on land contaminated with such metals to that of a soil conditioner. Although, in this situation the conventional value of the co-product as an animal feed would not be available for the business case, this should offset by the value gained from agronomic improvement in soil health and carbon sequestration. Further work is needed to assess the full implications of the use of co-products to food chain security and to evaluate how best optimise the value of the co-products of camelina crops from contaminated land.

6 Conclusions

6.1. Development of methodology

The methodology for the determination of trace metals developed in this task has been devised to considered the key three compartments in production of *Camelina sativa*

i. Soil

ii. Plant material

iii. Oil

Wherever possible, international standard methods have been incorporated to ensure the robustness and transferability of the developed methodology.

- The soil characterisation methodology was developed to be suitable for the collection and analysis of samples from metal impacted field sites. It was shown to be robust and allowed for due consideration of the inherent uncertainties in the distribution of metals in contaminated soils. A random stratified sampling protocol was implemented using a minimum of 25 samples/ha in accordance with ISO 10382-1:2002. Subsequent handling and storage was compliant with ISO 18512:2007. Analysis of field soil samples for elemental composition (ISO 11466:1995, total carbon and pH (H20) (ISO 10390:2005) was carried out in triplicate.
- The methodology devised for the collection and cleaning of plant material from field sites was according to methodology defined in ISO 658:2002. Analysis of plant material was undertaken using reagents for trace element analysis and microwave-assisted digestion, with the methodology incorporatING key elements of EN13804:2002; EN13804:2013; EN13805:2003 and EN14084:2003.
- For the determination of trace metals in oil, a number of methods were trialled and assessed with regard to reliability and reproducibility: two direct analysis methods were appraised, UOP 389 and ASTM D7771-15 / ASTM D5185-09, and two indirect methods using EDTA extraction followed by ICP-OES analysis, and microwave-assisted digestion and subsequent ICP-OES analysis. Safety concerns were raised with regard to the UOP 389, due the requirement to heat the oil in excess of 500º C for a minimum of four hours, given the uncertainty of the flash point of raw camelina oil. Issues of reliability were raised with the analysis of the oil using ASTM D7771-15 methodology as carbon build-up of the analytical equipment was a persistent issue resulting in high %RSD values. The reliability of the two indirect methods was assessed through determining the recovery of metals from oil samples spiked with an internal standard of known concentration. Recovery of the metals using EDTA was determined to be ≤75%; whilst recovery using acid microwave-assisted digestion was found to be between 98-118%. Overall, the indirect method of acid microwave-assisted digestion was considered to be the most reliable technique.

6.2. Validation of methodology

Validation of the methodology for the plant and oil samples initially focussed on the six metals, Cd, Co, Cu, Fe, V and Zn identified as being of greatest concern for the aviation industry with respect to aviation fuel. The controlled greenhouse cultivation trials considered the uptake of these metals when present in a highly bio-available form with the aim of identifying whether *Camelina sativa* preferentially accumulate any of these metals. The findings of the controlled greenhouse cultivation trials are summarized in Section 6.2.1.

A two-phase approach was applied to the field cultivation trials: In the first phase, pre-planting geochemical analysis of soils was undertaken to assess and characterize the individual soil matrices for metal concentrations, soil acidity and total carbon and nitrogen for all four field sites.

Qualitative assessment of the growing crop was used to assess crop vigour and vitality and post-harvest assessment of the potential crop production was carried out in terms of yield and the transference of key metals into the camelina seed and oil. In the second phase, using Rovinari sterile site as a case study, contaminant heavy metals were traced through all components of the camelina crop system. The rationale for the selection of this site was not only based on the higher levels of production (330 kg/ha), but also that the soil of the Rovinari Sterile exhibited overall the lowest concentrations of metal contaminants. The findings of the controlled greenhouse cultivation trials are summarized in Section 6.2.2.

6.2.1. Controlled greenhouse cultivation trials

- At high soil metal concentrations, plants show signs of stress such that normal growth and development is impaired as critical concentrations are exceeded and toxicity effects become operational.
- Cu toxicity effects in camelina are acute compared to other metals, with complete die-back observed at concentrations between 100-1000 ppm. This may need to be taken into account for soils where Cu is the dominant pollutant and in a highly bioavailable form, although further research needed to define this range.
- Elemental analysis has shown that the camelina seed is naturally high in the essential micronutrients Fe and Zn (approx. 100 mg kg^{-1} and 60 mg kg^{-1} dry matter respectively).
- The concentration of metals in the soil medium was the principal controlling factor in the greenhouse cultivation trials. However, it is estimated that 70% of all metal-contaminated sites involve two or more metals, and so differences in the uptake behaviour between that observed in the greenhouse trials and that observed in the field trials might occur; bioavailability of metals as well as synergistic and antagonistic effects maybe influential in metal uptake in field crops.
- The trace metals Cd and Co show the greatest potential for uptake into the seed with measured concentrations reaching several hundred times greater than their corresponding natural baseline levels.
- The uptake of Cd may prove to be problematic in certain production scenarios, since the primary co-product (to the camelina oil destined for the biofuels market) of crushed camelina seed husks destined for the animal feed market. The data indicate that Cd concentrations in the seed can potentially reach levels that could render the animal feed as unfit for consumption (given that the concentrations of Cd in food destined for human consumption typically ranges from 0.2 to 2 mg kg-1, and with the concentration of 1 mg kg^{-1} being acceptable to the animal feed market).
- Metal concentrations are found to be highest in the roots of camelina for the metals Cd, Co, Cu, Fe, V and Zn, and with the exception of Cu, display a metal plant concentration series of: root > shoot > seed. By contrast Cu concentrations in camelina are found to be root > seed > shoot.
- Secondary uptake synergistic and antagonistic effects between Cu Cd, Fe and Zn are intimated in the shoot data; these may be of importance to the crop yield when growing camelina on contaminated land.
- Results suggest that the uptake of Cd, Co and Zn into the seed is non-linear. In particular, the Zn data suggest that the uptake into the seed from soil with concentrations of less 400 mg kg^{-1} Zn is at a constant level. Only at soil concentrations above 400 mg kg^{-1} is the seed Zn concentration seen to increase significantly. This behaviour is mirrored by the seed data from the four Romanian field sites, where seed from sites with ≤ 260 mg kg^{-1} display similar concentrations. By contrast, the uptake of Cu and V into the seed tends towards linearity.

6.2.2 Field cultivation trials

Four Romanian field-cultivation trials

- Crop survey and intermediary greenhouse growth experiments suggest that contaminant levels at the four Romanian field sites may be influencing crop habitat and morphology, although macronutrient deficiencies at some sites may also contribute to the phenotypes expressed by the camelina crop.
- The elemental analysis of seed from the field cultivation trials are consistent with those of the controlled cultivation trials and show that the camelina seed is naturally high in the plant micronutrients of iron and zinc.
- The uptake of Cu in to the field crops appears to be independent of concentration of Cu in the soil, and this may be indicative that camelina is able to self regulate the uptake of Cu from the soil as seen in other plant species.
- The uptake behaviour of Zn in the harvested seed from the field crops appears to emulate the tendency towards excluder behaviour noted in greenhouse trials, with possible breakdown of the protective mechanism at concentrations in soil in excess of 260 mg kg^{-1}.
- Data from the field trials suggest that Cu and Zn at high concentrations in soil are capable of inducing Fe deficiency in camelina. In sites that exhibit high concentrations of these metals, Fe deficiency may affect the productivity of the crop and foliar sprays will be needed to counteract these affects.
- Analysis of the oil extracted from the camelina grown on the four contaminated field sites had levels of Cd, Co, Ni and V that are below limit of detection. Whilst As, Cu and Fe are observed to be present in all oil samples there is no consistency in the occurrence of Cr, Pb, and Zn in the oil samples.
- The occurrence of Zn in the extracted oil appears to be influenced by the concentration of the metal in the soil at an individual site. Similar relationships are not as clearly defined for other metals.
- Comparison of the oil metal concentrations determined for the four contaminated sites with that of oil from the nominally unpolluted site at Moara Domneasca, 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.
- Elemental analysis of the four plant components, roots, shoots, silicles, and seed from the Rovinari-Sterile case-study site find the highest concentrations of As, Cu and Zn are determined for the seed. Given the well-established relationship between As and P in both soil and plant material, the management of available soil P may be key to the future successful of camelina production on As contaminated sites.
- Consistent with the greenhouse trials, the metals, Cd, Co, Cr, Fe, Ni, Pb and V, concentrations in the roots are equal to or higher than in any other of the plant compartment.
- Further studies are needed to assess whether cultivar effects are operational in the uptake and storage of metals such as Cd and Zn in *Camelina sativa* and to evaluate the implications for the use of camelina co-products from contaminated sites to food chain security.

Case-study: Rovinari – Sterile

- Comparison of pre-cultivation and pre-harvesting metal concentrations in the soil suggest significant decreases in the Cd are observed suggesting that *Camelina sativa* has the potential to act as a mechanism for phytoremediation for soils similar in character to those at the Rovinari-Sterile site.

- Camelina roots from the Rovinari-Sterile site were most efficient in taking up Cd, Cu and Zn Similarly the TF values suggest that camelina if most efficient at translocating Cd (TF: 0.91), Cu (TF: 0.68) and Zn (TF: 1.0).
- Co, Cr, Fe, Ni, Pb and V exhibit TFs <0.60 suggesting that uptake may be restricted and possible exclusion mechanisms be operational in camelina for these metals. Further work is necessary to determine whether the apparent tolerance to Co, Cr, Fe, Ni, Pb and V exhibited *Camelina sativa* from the case study site, is a general phenomenon or unique to the plants grown on the Rovinari-Sterile site.
- Translocation factors 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. Other Brassica species act as hyperaccumulators for Cd and Zn further work is needed to verify the accumulator status of camelina with respect to these two metals.
- Consideration of silicles and shoot, and seed and shoot ratios suggest that the movement of Co and Ni into the shoots from the roots may be restricted, but once present in the aerial part of the plant Co and Ni are readily mobilized to the silicles and seeds. Further work is needed to assess the impact of such mobility on the food chain, for crops grown on soils enriched in Co and Ni.
- 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 any exclusion mechanisms that are operational be negated
- A careful consideration of the use and value of the co-products from camelina grown on contaminated land is recommended. The distribution of metals in the oil and seed are non-uniform, so the oil extraction process may effect a multiplicative increase in the concentration of metals in the crushed seed, which when given direct access to the food chain from the use of meal for livestock fodder and shoot material as bedding material should 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.

In summary, we must conclude that the relation between the metal contaminants present in soil and those that ultimately become resident in the various components of a mature camelina plant is undoubtedly complex, depending upon a large number of parameters of which some are independent, some are interdependent, and some are site specific. 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 camelina be grown on contaminated land?’ The equally imprecise answer is ‘Yes, but site specific characteristics must be duly considered’.

The initial findings of both the greenhouse and the field trials are that for the six metals Cd, Co, Cu, Fe, V and Zn (and in particular Cd, Co and V), the economic viability of a camelina crop grown on contaminated land may ultimately depend on the usability of the co-products rather than the oil itself.

7 Research gaps

1. Romanian field trials data to have focused on the 2013 non-fertilised crop. Extrapolating the results from this analysis to a fertile crop scenario carries a degree of risk: fertilisers promote plant growth, which in turn requires an increased uptake nutrients and water dynamics, which may result in a change in the uptake of contaminants. The assumption that the uptake of contaminant metals is independent of fertiliser input is not necessarily valid (particularly as the application of fertiliser can lead to soil acidification).

2. Efforts to date have focused upon developing an appropriate methodology to assess and evaluate the concentration of contaminant metals identified as undesirable in fuel and therefore the oil precursor. However, it is apparent that the business case for the cultivation of camelina as an energy crop is highly dependent upon animal feed as a valuable co-product. It is therefore perhaps pertinent that certain other trace metals identified as undesirable in the food chain and therefore animal feed receive further analysis. Such target species would include As, Cd, Pb, etc for example. Whilst some data is available within the current task, we recommend further research to explore this line of investigation in more detail.

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Annex A Site survey data

A.1 Site survey for Câmpina, Prahova County

Site Reference	Câmpina
Owner of Site	S.C. Rafinăria Steaua Română
Site Location Details: National Code Administration Division Location Address	RO (642) Prahova county; (RO-PH) Câmpina city; Fabricii street
Primary contamination source (type; if on-going or date ceased) Distance and direction from site Stack height? (if applicable) Potential contaminants	Pyrite ash wastes were obtained by burning iron pyrite. The pyrite came here by railway 20 years ago. In this site the pyrite was removed and the hole resulted was completed with soil (resulted from different activities such as building, steel modelling etc). This process took place over a long period of time. 1.5 km The prevailing wind :N-W
Secondary contamination sources (type; if on-going or date ceased) Distance and direction from site Stack height (if applicable) Potential contaminants	

Site Reference	
Cropping History: Crop grown 2012 Recent crops grown General fertility of soil Are there any areas of site that suffer from (mark areas on map): (i) Poor growth (ii) No growth Is the soil on the site subject to wind erosion?	No No No analyse The site is partially covered by spontaneous vegetation (grass) No
Groundwater: General description of groundwater: Is there flowing water near or through the site? Is the site down or upstream of any contamination source? Are there any areas of site that suffer from (mark areas on map): (i) Water lying for a long time (ii) Flooding Is the soil on the site subject to water erosion?	Approx. 20m.The groundwater is not uniformly distributed. 'Lacul Peştelui' –the lake is situated near the site (approx. 200m)-the lake is polluted and it has a dark colour because of the industrial waste pit (refinery sludge pit). No No No
Any specific events that may have led to contamination of site	No
Site Reference	
Sample date Sample Time	08.10.2012 11:30
Meteorological conditions	Breeze

Temperature	Approx. 20°C
On-Site Observation: Location of any buildings: Location of waste dumps (above or below ground) that farmer may have constructed. Location of any areas of lying water (Give brief description of appearance of water) Vegetation: Are there any signs of vegetative stress? If yes how do they relate to other features? Soil Type Soil Colour Slope Direction of surface run-off of water	S-E : ‘ Turnatoria Orion Campina’ company (steel and iron cast) and some apartment houses (blocks- approx. 800 m across the site) N: Fabricii street and the former platform of the Chemistry department of the refinery E: ‘Lacul Pestelui’ V: Buildings of some companies No vegetation in the North of the site. It is not a natural soil. The soil has a red aspect. Fe_2S in the presence of oxygen →$2FeSO_4$ No slope
Any other observations (e.g. observable odours)	

A.2 Site survey for Copşa Mică, Sibiu County

Site Reference	Copsa Mica/Axente Sever village
Owner of Site	S.C. Butu Nicolae P.F.A
Site Location Details: National Code Administration Division Location Address	RO (642) Sibiu county (RO-SB) Axente Sever village
Primary contamination source (type; if on-going or date ceased)	‘SOMETRA COPSA MICA’ - it was the only factory that produced non-ferrous metals. - is working in a special regime (only 10 % of its total capacity; there are 100 employees); now the factory is used to obtain lead. The sterile dumps are handled again (re-operated), for this reason only a chimney is active .The ash resulted after this process is stored near the factory (when the wind blows the ash doesn’t spread; it is slag). -the chimneys that processed the raw materials are closed
Distance and direction from site	1.5 km; The site is located in the West of the factory The prevailing wind : West
Stack height? (if applicable)	-the big chimney : approx. 250 m -others chimneys: approx. 50 m; approx. 70-80 m
Potential contaminants	Pb, Zn, Cd, Cu, Mo, As sulphur dioxide
Secondary contamination sources (type; if on-going or date ceased) Distance and direction from site Stack height (if applicable) Potential contaminants	
Site Reference	
Cropping History: Crop grown 2012	Maize- yield achieved: 3 t/ha (draught conditions)
Recent crops grown	Potatoes; Maize (in last years the farmer obtained 6-7 t/ha) Wheat has never been grown in this site.

General fertility of soil Are there any areas of site that suffer from (mark areas on map): (iii) Poor growth (iv) No growth Is the soil on the site subject to wind erosion?	The site started to be cultivated in 1990. Medium fertility. In spring the farmer applied 300 kg/ha ammonium nitrate and 400 kg/ha N:P:K Very rich in humic acid. There are areas without vegetation; after fertilizing with manure these areas have decreased in time. No
Groundwater: General description of groundwater: Is there flowing water near or through the site? Is the site down or upstream of any contamination source? Are there any areas of site that suffer from (mark areas on map): (iii) Water lying for a long time (iv) Flooding Is the soil on the site subject to water erosion?	3-5 m; below soil surface ‘Târnava Mare’ River- 500-600 m near the site Polluted with non-ferrous metals. No. The soil is free-draining No No
Any specific events that may have led to contamination of site	No
Site Reference	
Sample date Sample Time	09.10.2012 16:00
Meteorological conditions Temperature	Sunny; breeze 16°C
On-Site Observation: Location of any buildings:	No No

Location of waste dumps (above or below ground) that farmer may have constructed. Location of any areas of lying water (Give brief description of appearance of water) Vegetation: Are there any signs of vegetative stress? If yes how do they relate to other features? Soil Type Soil Colour Slope Direction of surface run-off of water	No We observed few areas without vegetation (the farmer told us that after fertilizing with manure these areas have decreased in time); Clay + silt. We observe the presence of the yellow sand (lens or more stratified) for some samples (about 15 cm below the soil surface ;the soil profile indicated the presence of sand ; Dark colour. No
Any other observations (e.g. observable odours)	- the farmer told us that the boar (**Suss scrota**) is a major problem for the crops -railway near the site (25-30m); - a lot of thistles

A.3 Site survey for Rovinari, Gorj County

Site Reference	In the West of Gorj county
Owner of Site	I.C.P.A.
Site Location Details: National Code Administration Division Location Address	 RO (642) Gorj county (RO-GJ) Rovinari
Primary contamination source (type; if on-going or date ceased)	- mining quarries (open excavation) - for obtaining 1 t of coal , 8 m3 are excavated - the distance between mining quarries is about 6-7 km

Distance and direction from site Stack height? (if applicable) Potential contaminants	- ‘Carrier Gala’ –a mining quarry situated at 2 km near the site - Prevailing wind: N-S Approx. 100-120 m Ash dumps ; sterile dumps
Secondary contamination sources (type; if on-going or date ceased) Distance and direction from site Stack height (if applicable) Potential contaminants	The emissions from Rovinari Power Plant Dust, sulphur oxides
Site Reference	
Cropping History: Crop grown 2012 Recent crops grown General fertility of soil Are there any areas of site that suffer from (mark areas on map): (v) Poor growth (vi) No growth Is the soil on the site subject to wind erosion?	Maize-(5,5 t /ha) rye; peas; sunflower 1960- the hole was filled with ash 1960-1970- wheat; sunflower; (arable crops) 1970- grass low fertility; the soil was fertilised with lignite based fertilizer No No No
Groundwater: General description of groundwater: Is there flowing water near or through the site?	10-11m No

Is the site down or upstream of any contamination source?	
Are there any areas of site that suffer from (mark areas on map):	
(v) Water lying for a long time	No
(vi) Flooding	No
Is the soil on the site subject to water erosion?	No
Any specific events that may have led to contamination of site	No
Site Reference	
Sample date	10.10.2012
Sample Time	15:00
Meteorological conditions	Sunny;
Temperature	21°C
On-Site Observation:	
Location of any buildings:	No
Location of waste dumps (above or below ground) that farmer may have constructed.	No
Location of any areas of lying water (Give brief description of appearance of water)	No
Vegetation: Are there any signs of vegetative stress?	No
If yes how do they relate to other features?	
Soil Type	The soil was ploughed at 30 cm. 0-20 cm dry ash 20-40 cm- the ash has a higher moisture 40-60 cm –more moisture content; the water is held in soil
Soil Colour	Ash dumps- dark colour Sterile dumps-Yellow colour
Slope	10° (approx. 100 m from the whole surface of the sterile dump)

Direction of surface run-off of water	
Any other observations (e.g. observable odours)	-the last rain : 17 June 2012

Annex B Field observations

B.1. Field Observations May 2013

Câmpina

An on-going remediation project at the refinery to the rear of the study site has resulted in clean top-soil being stored on the camelina experimental plot (Figure B1) and a reduction in the original study site area to 0.5 ha.

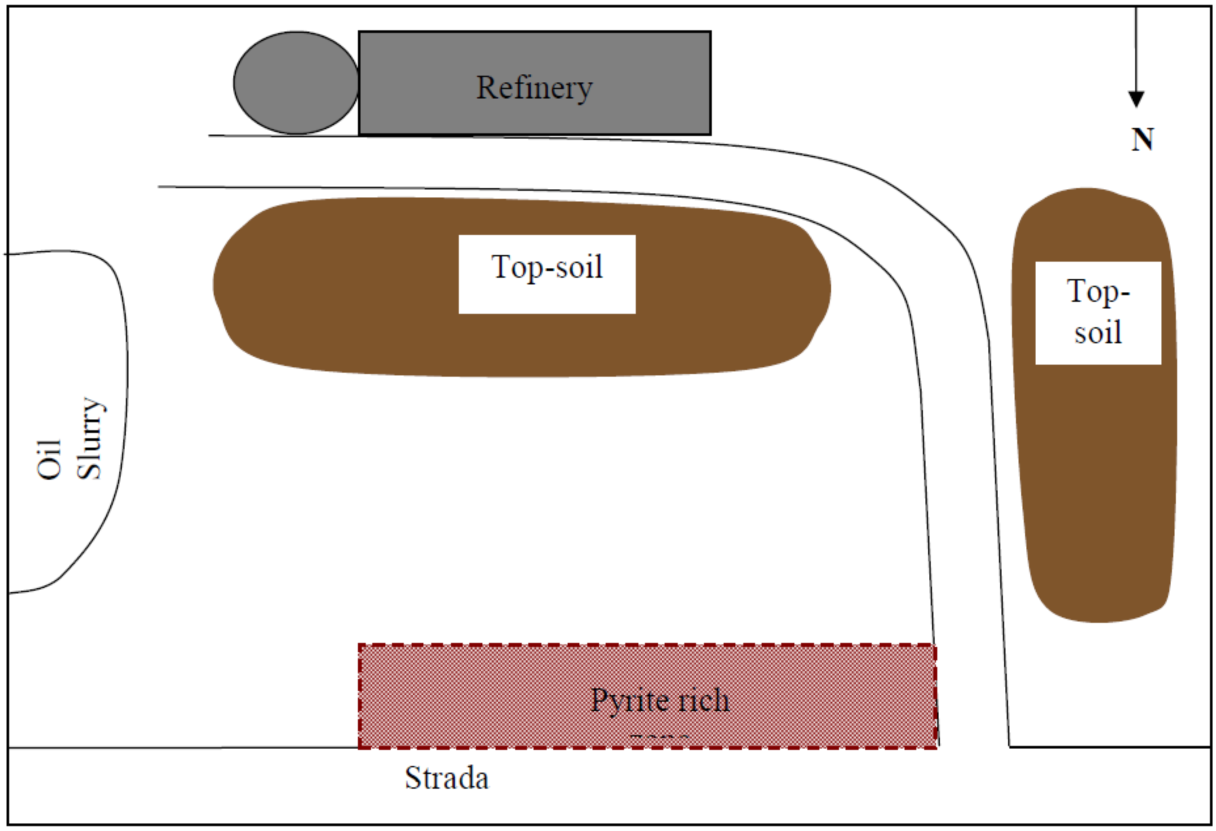


Figure B8: Site plan of Câmpina study site (not to scale).

Twelve random samples points on a 20 x 20 m (~400 m2) grid system were taken. GPS readings were taken for each point along with observations and measurements reported in Table 11.B.

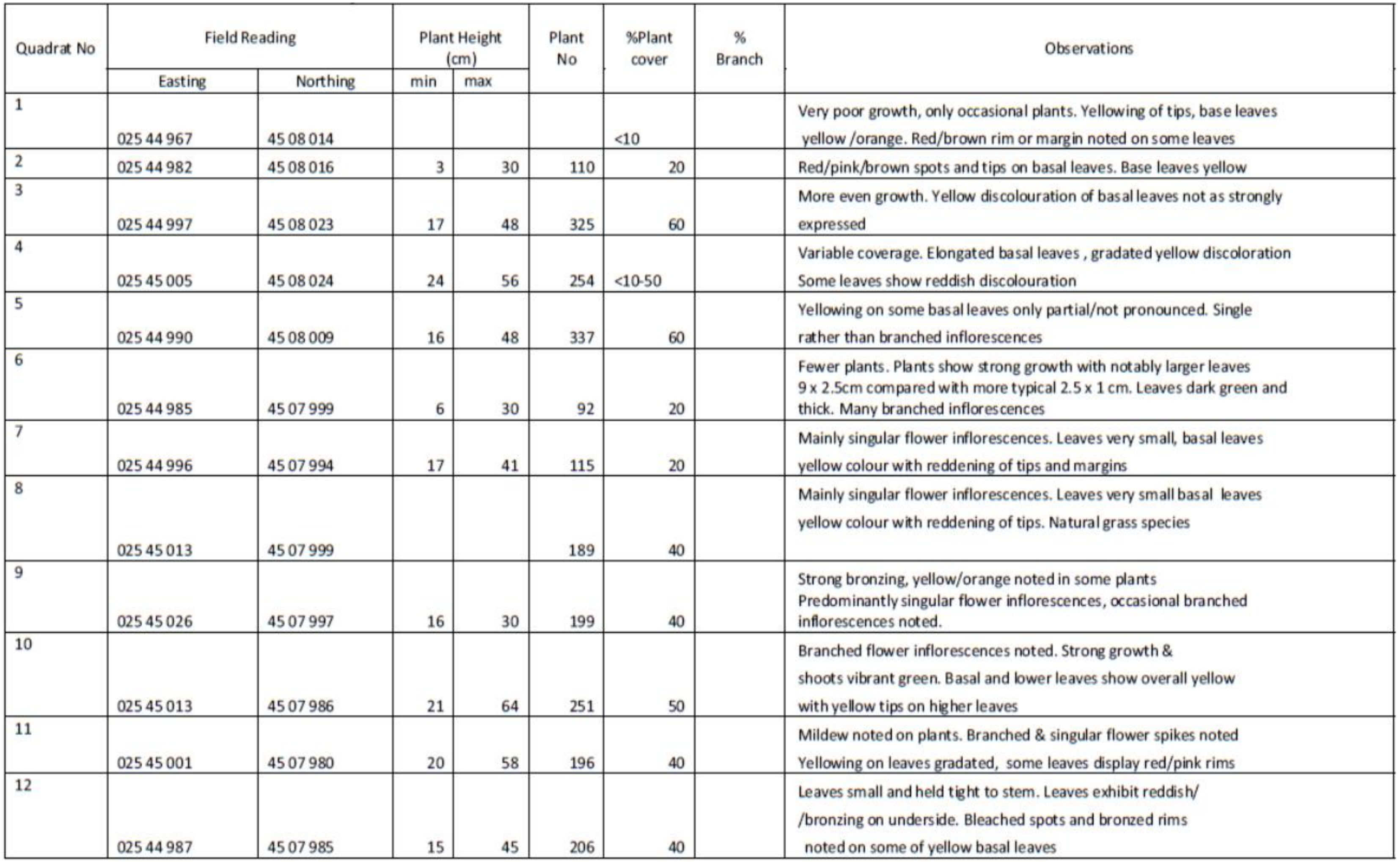
Initial observations suggest that greenhouse experiments are mirroring the field experimental plot. For example area next to entrance of site and road (marked in red on Figure 3.B) little growth observed, possibly as a result of high Fe (18-22%) and As (3000-3800 ppm) concentrations.

Growth across the site is variable (Figure 4.B), reflecting the heterogeneity in soil and nutrient levels evident from the soil samples taken in Oct 2012. Yellow discolouration of base leaves is a common feature across the site, other leaf pigment discolouration (Figure 5.B) is more occasional in occurrence. In addition there is evidence of the successful ability of natural/native species to adapt to the growing conditions of the experimental plot.

Figure B9: Variations in crop morphology and population density at the Câmpina study site

Figure B10: Variations in foliar chlorosis and discolouration at the Câmpina study site.

Table B35: Field observations for Câmpina



Quadrat No	Field Reading		Plant Height (cm)		Plant No	%Plant cover	% Branch	Observations
	Easting	Northing	min	max				
1	025 44 967	45 08 014				<10		Very poor growth, only occasional plants. Yellowing of tips, base leaves yellow /orange. Red/brown rim or margin noted on some leaves
2	025 44 982	45 08 016	3	30	110	20		Red/pink/brown spots and tips on basal leaves. Base leaves yellow
3	025 44 997	45 08 023	17	48	325	60		More even growth. Yellow discolouration of basal leaves not as strongly expressed
4	025 45 005	45 08 024	24	56	254	<10-50		Variable coverage. Elongated basal leaves , gradated yellow discoloration Some leaves show reddish discolouration
5	025 44 990	45 08 009	16	48	337	60		Yellowing on some basal leaves only partial/not pronounced. Single rather than branched inflorescences
6	025 44 985	45 07 999	6	30	92	20		Fewer plants. Plants show strong growth with notably larger leaves 9 x 2.5cm compared with more typical 2.5 x 1 cm. Leaves dark green and thick. Many branched inflorescences
7	025 44 996	45 07 994	17	41	115	20		Mainly singular flower inflorescences. Leaves very small, basal leaves yellow colour with reddening of tips and margins
8	025 45 013	45 07 999			189	40		Mainly singular flower inflorescences. Leaves very small basal leaves yellow colour with reddening of tips. Natural grass species
9	025 45 026	45 07 997	16	30	199	40		Strong bronzing, yellow/orange noted in some plants Predominantly singular flower inflorescences, occasional branched inflorescences noted.
10	025 45 013	45 07 986	21	64	251	50		Branched flower inflorescences noted. Strong growth & shoots vibrant green. Basal and lower leaves show overall yellow with yellow tips on higher leaves
11	025 45 001	45 07 980	20	58	196	40		Mildew noted on plants. Branched & singular flower spikes noted Yellowing on leaves gradated, some leaves display red/pink rims
12	025 44 987	45 07 985	15	45	206	40		Leaves small and held tight to stem. Leaves exhibit reddish/ /bronzing on underside. Bleached spots and bronzed rims noted on some of yellow basal leaves

Copşa Mică

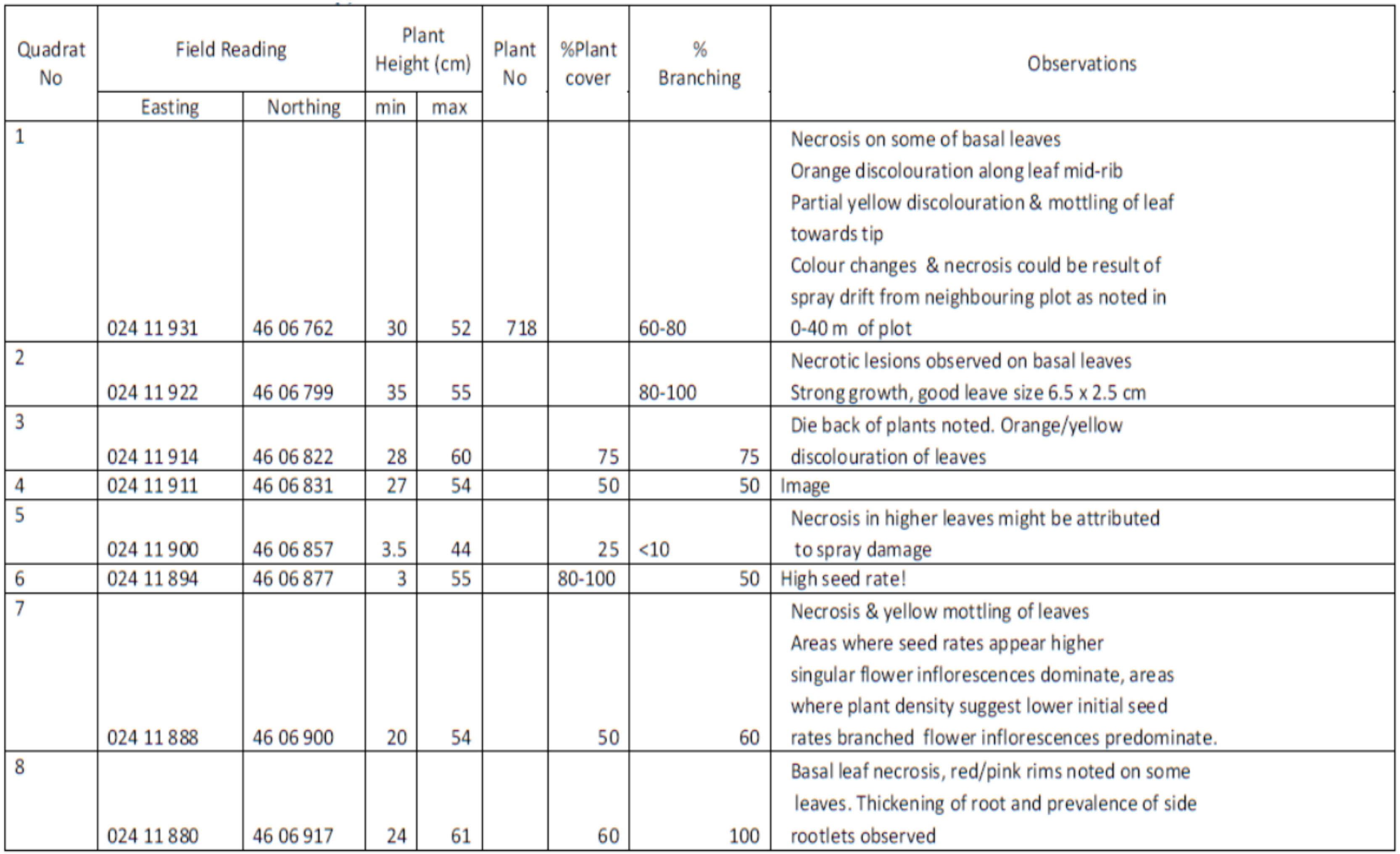
Experimental plot area has been reduced from ~1 ha to a ~0.25 ha (5 x 500 m) strip as a result of the land owner replacing the camelina crop with one of linseed. The remaining camelina crop is not continuous along the 500 m length. The first ~40m is strongly affected by spray drift from the adjacent plot. Spray damage to a lesser extent is seen intermittently along the length of the plot. In addition, there are areas of bare soil, which include where machinery traffic have left wheel tracks.

Plant coverage is variable; there are areas of strong plants displaying branched inflorescences. By contrast areas of high numbers of small plants with singular flower spikes are also noted, in particular such areas are observed in the northern-most 60 m. Such high numbers may reflect that the seed rate in these areas were higher than in other areas – farmer using up all seed.

Areas were die-back of seedlings has occurred were also observed this may be the result of recent spray drift or the affect of residual herbicides in the soil from the previous crop of maize. Eight random samples points on a 5 x 40 m (~200 m^2) grid system were taken. GPS readings were taken for each point along with observations and measurements reported in Table 12.B. Due to spray drift damage measurements were not taken for the first 40m grid. Due to field conditions and time constraints measurements were restricted to plant height; percentage coverage and germination. Plant number data were only collected for the first quadrat.

Figure B11: camelina crop at Copşa Mică.

Table B36: Field observations for Copşa Mică.



Quadrat No	Field Reading		Plant Height (cm)		Plant No	%Plant cover	% Branching	Observations
	Easting	Northing	min	max				
1	024 11 931	46 06 762	30	52	718		60-80	Necrosis on some of basal leaves Orange discolouration along leaf mid-rib Partial yellow discolouration & mottling of leaf towards tip Colour changes & necrosis could be result of spray drift from neighbouring plot as noted in 0-40 m of plot
2	024 11 922	46 06 799	35	55			80-100	Necrotic lesions observed on basal leaves Strong growth, good leave size 6.5 x 2.5 cm
3	024 11 914	46 06 822	28	60		75	75	Die back of plants noted. Orange/yellow discolouration of leaves
4	024 11 911	46 06 831	27	54		50	50	Image
5	024 11 900	46 06 857	3.5	44		25	<10	Necrosis in higher leaves might be attributed to spray damage
6	024 11 894	46 06 877	3	55		80-100	50	High seed rate!
7	024 11 888	46 06 900	20	54		50	60	Necrosis & yellow mottling of leaves Areas where seed rates appear higher singular flower inflorescences dominate, areas where plant density suggest lower initial seed rates branched flower inflorescences predominate.
8	024 11 880	46 06 917	24	61		60	100	Basal leaf necrosis, red/pink rims noted on some leaves. Thickening of root and prevalence of side rootlets observed

Rovinari–Sterile (camelina type=GP-202)

The variability in plant density, size and vigour seen in the crop may reflect variations in the underlying soil fertility and hydrology (Figure 7.B). Bare patches exist in the crop and typically relate to areas were water logging of the ground has occurred. Twelve random sample points on a 22.5 x 17.5m (~400m^2) grid system were taken. GPS readings were taken for each point along with observations and measurements reported in Table 13.B.

Yellow discolouration of base leaves is a common feature across the site, other leaf pigment discolouration including mottled appearance in some leaves was noted, although more occasional in occurrence (Figure 8.B).

Figure B12: Camelina (GP-202) cropat Rovinari (Sterile) study site.

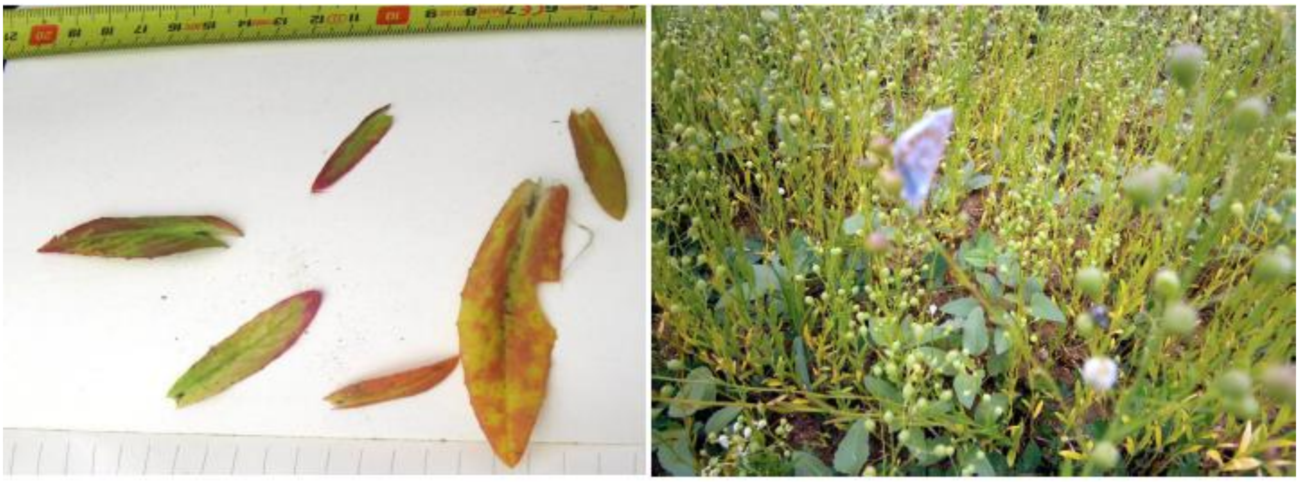
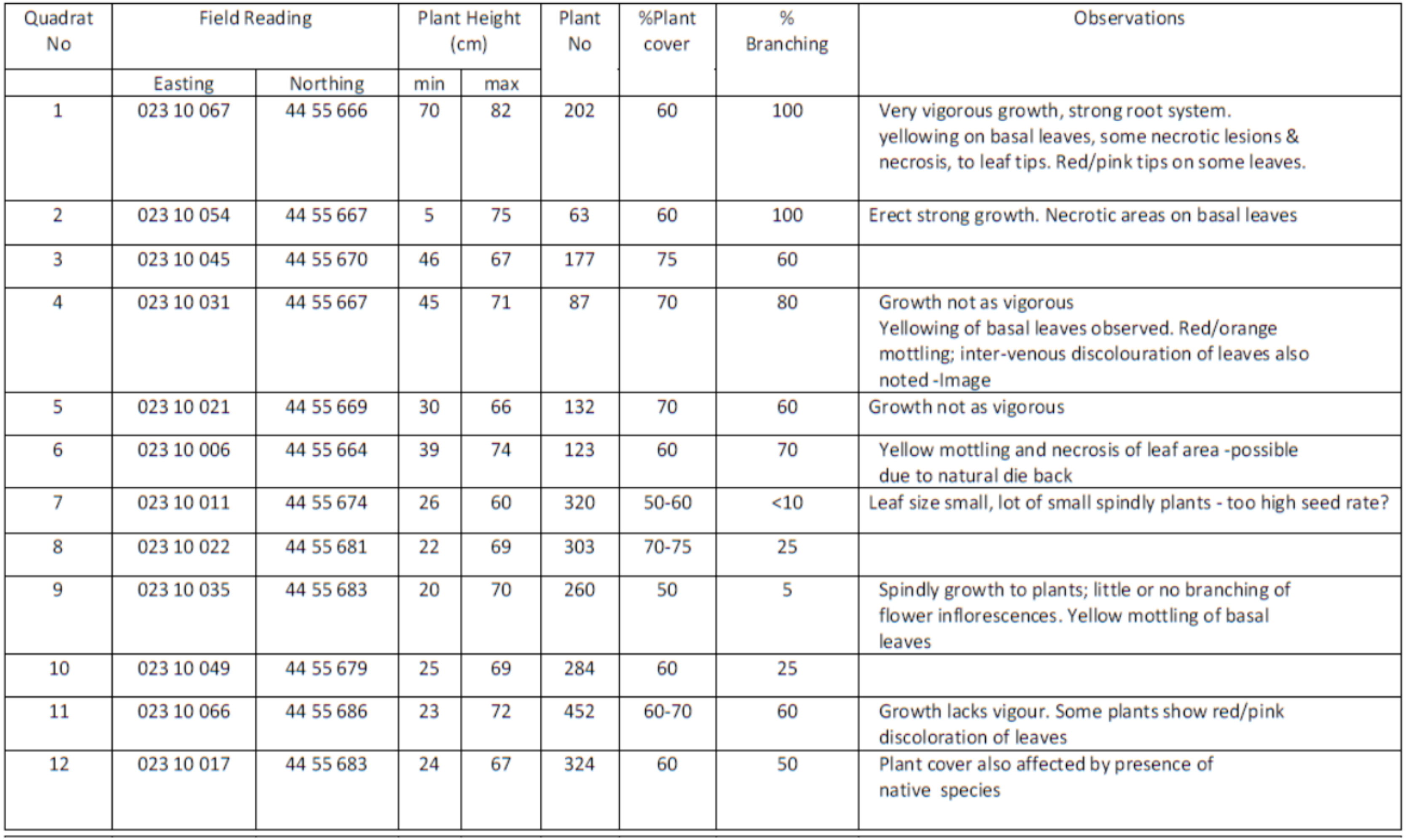


Figure B13: Variations in foliar chlorosis and discolouration at Rovinari (Sterile) study site.

Table B37: Field observations for Rovinari Sterile field.



Quadrat No	Field Reading		Plant Height (cm)		Plant No	%Plant cover	% Branching	Observations
	Easting	Northing	min	max				
1	023 10 067	44 55 666	70	82	202	60	100	Very vigorous growth, strong root system. yellowing on basal leaves, some necrotic lesions & necrosis, to leaf tips. Red/pink tips on some leaves.
2	023 10 054	44 55 667	5	75	63	60	100	Erect strong growth. Necrotic areas on basal leaves
3	023 10 045	44 55 670	46	67	177	75	60	
4	023 10 031	44 55 667	45	71	87	70	80	Growth not as vigorous Yellowing of basal leaves observed. Red/orange mottling; inter-venous discolouration of leaves also noted -Image
5	023 10 021	44 55 669	30	66	132	70	60	Growth not as vigorous
6	023 10 006	44 55 664	39	74	123	60	70	Yellow mottling and necrosis of leaf area -possible due to natural die back
7	023 10 011	44 55 674	26	60	320	50-60	<10	Leaf size small, lot of small spindly plants - too high seed rate?
8	023 10 022	44 55 681	22	69	303	70-75	25	
9	023 10 035	44 55 683	20	70	260	50	5	Spindly growth to plants; little or no branching of flower inflorescences. Yellow mottling of basal leaves
10	023 10 049	44 55 679	25	69	284	60	25	
11	023 10 066	44 55 686	23	72	452	60-70	60	Growth lacks vigour. Some plants show red/pink discoloration of leaves
12	023 10 017	44 55 683	24	67	324	60	50	Plant cover also affected by presence of native species

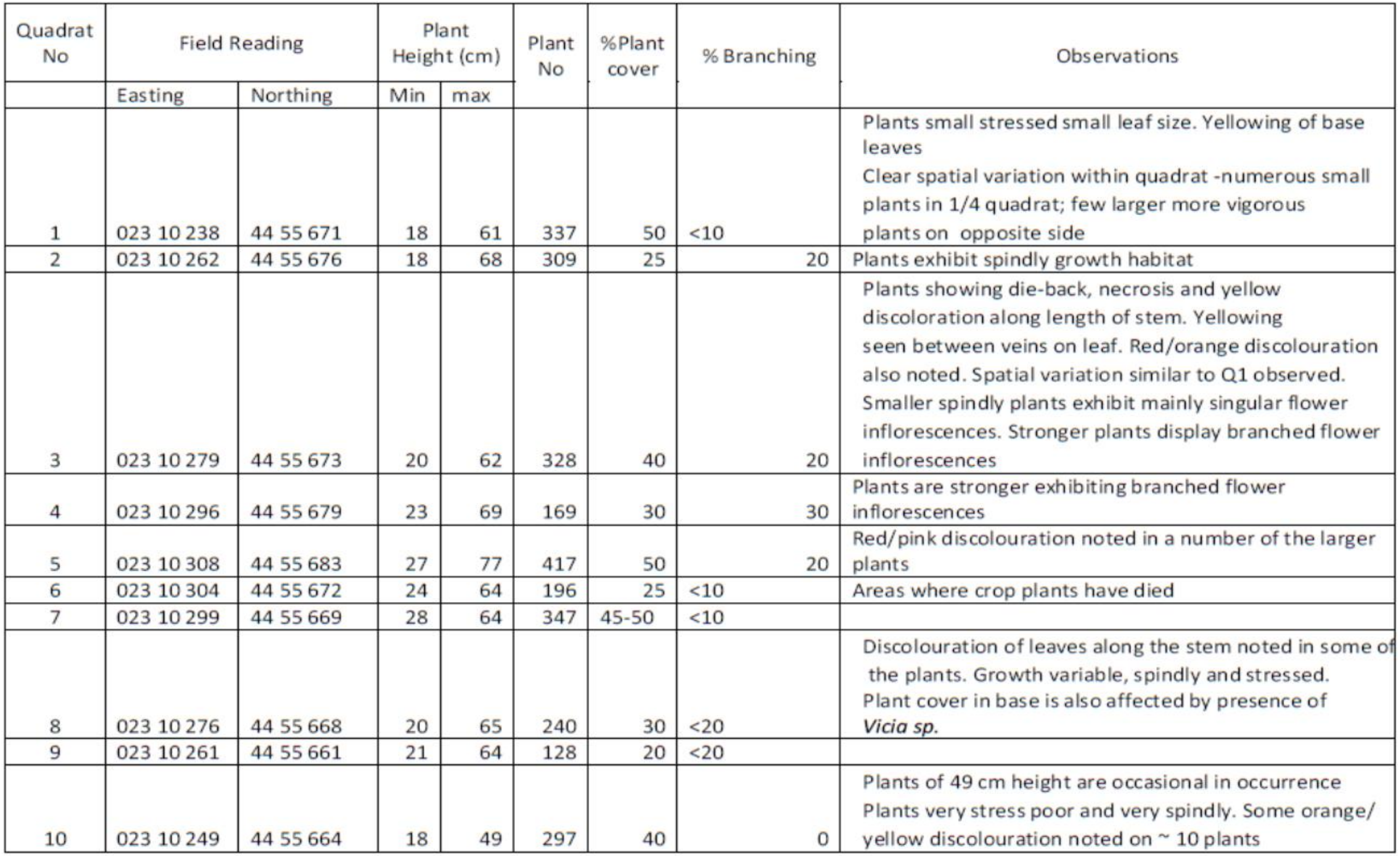
Rovinari–Ash (camelina type=GP-204.)

The crop showed clear signs of drought stress. Heavy rain at the end of March 2013 was followed by a period of 6 weeks of no rain and high temperatures and this is clearly evident in the crop, with small plant height, poor vigour and singular flower in florescence being typical (Figure 9.B). Ten random samples points on a 20 x 20m (~400m^2) grid system were taken. GPS readings were taken for each point along with observations and measurements reported in Table 14.B.

Yellow discolouration of base leaves is a common feature across the site, other leaf pigment discolouration including mottled appearance in some leaves was noted, although more occasional in occurrence. However, drought stress as a contributing factor in the leaf discolouration cannot be counted.

Figure B14: Camelina (GP-204) crop at Rovinari (Ash).

Table B38: Field observations for Rovinari Ashfield



Quadrat No	Field Reading		Plant Height (cm)		Plant No	%Plant cover	% Branching	Observations
	Easting	Northing	Min	max				
1	023 10 238	44 55 671	18	61	337	50	<10	Plants small stressed small leaf size. Yellowing of base leaves Clear spatial variation within quadrat -numerous small plants in 1/4 quadrat; few larger more vigorous plants on opposite side
2	023 10 262	44 55 676	18	68	309	25	20	Plants exhibit spindly growth habitat
3	023 10 279	44 55 673	20	62	328	40	20	Plants showing die-back, necrosis and yellow discoloration along length of stem. Yellowing seen between veins on leaf. Red/orange discolouration also noted. Spatial variation similar to Q1 observed. Smaller spindly plants exhibit mainly singular flower inflorescences. Stronger plants display branched flower inflorescences
4	023 10 296	44 55 679	23	69	169	30	30	Plants are stronger exhibiting branched flower inflorescences
5	023 10 308	44 55 683	27	77	417	50	20	Red/pink discolouration noted in a number of the larger plants
6	023 10 304	44 55 672	24	64	196	25	<10	Areas where crop plants have died
7	023 10 299	44 55 669	28	64	347	45-50	<10	
8	023 10 276	44 55 668	20	65	240	30	<20	Discolouration of leaves along the stem noted in some of the plants. Growth variable, spindly and stressed. Plant cover in base is also affected by presence of *Vicia sp.*
9	023 10 261	44 55 661	21	64	128	20	<20	
10	023 10 249	44 55 664	18	49	297	40	0	Plants of 49 cm height are occasional in occurrence Plants very stress poor and very spindly. Some orange/ yellow discolouration noted on ~ 10 plants

Annex C Soil characterisation data

Elemental concentrations for Câmpina, Prahova County

Table C39: Median extractable concentrations of the 20 elements, and descriptive statistics on the study site basis determined for the soil samples taken from the Câmpina, Prahova County, Romania (n = 14; values quoted to two significant figures for median, minimum and maximum, interquartile range quoted to three significant figures).

Study site	Elemental concentration (mg kg $^{-1}$)																				
	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Ti	V	Zn
RO-PH1	5100	3800	89	24000	11	49	68	520	180000	1600	640	170	7.2	2400	54	260	3300	35000	110	10	1400
RO-PH2	7400	3000	110	17000	11	130	40	1000	210000	2000	1700	170	9.1	1700	47	340	2100	27000	85	13	1200
RO-PH3	11000	20	130	41000	1.1	11	86	100	22000	1800	3900	690	2.0	220	290	720	200	1000	120	30	210
RO-PH5	9700	11	89	8800	0.59	7.9	23	24	14000	1800	2700	440	2.2	210	32	470	15	1600	120	25	72
RO-PH6	9100	27	120	66000	0.70	7.2	24	39	15000	1700	4000	450	1.4	500	28	530	110	990	130	26	130
RO-PH7	13000	620	270	48000	3.1	16	41	160	50000	1900	3100	690	1.4	460	37	520	700	2900	130	25	520
RO-PH8	4300	740	240	46000	12	23	4.8	280	180000	1500	1400	360	2.1	650	76	310	1300	12000	130	17	2400
RO-PH9	3900	190	190	1100	4.3	24	44	270	68000	580	750	390	7.1	230	71	100	290	1100	79	4.7	880
RO-PH10	17000	20	84	36000	1.1	14	51	38	26000	3000	6500	760	1.5	160	67	640	28	2300	140	44	110
RO-PH11	16000	7.6	140	24000	1.0	10	40	32	22000	2100	3800	1300	1.1	130	47	1300	68	400	93	34	160
RO-PH12	3600	40	80	5700	1.2	6.7	21	85	23000	620	840	250	3.6	210	30	150	80	700	90	6.7	260
RO-PH13	13000	7.7	150	35000	0.99	9.3	35	30	19000	2200	3600	1100	0.79	150	41	1000	74	590	86	30	240
RO-PH14	6000	31	91	67000	1.1	6.6	24	62	17000	1200	2600	660	1.9	250	28	330	230	800	140	21	250
RO-PH15	8000	11	290	66000	1.0	8.0	21	86	20000	1700	3000	610	0.87	280	31	470	170	930	160	26	310
Study site median	8000	31	120	35000	1.1	11	40	86	23000	1700	2700	450	2.0	250	47	470	200	1100	120	25	260
Study site interquartile range	6520	667	76.5	31300	6.4	15.6	19.1	240	104000	396	2200	386	3.98	366	29.1	258	940	6770	46	15	872
Study site min	3600	7.6	80	1100	0.6	6.6	4.8	24	14000	580	640	170	0.8	130	28	100	15	400	79	4.7	72
Study site max	17000	3800	290	67000	12	130	86	1000	210000	3000	6500	1300	9.1	2400	290	1300	3300	35000	160	44	2400

Elemental concentrations for Copşa Mică, Sibiu County

Table C40: Median extractable concentrations of the 20 elements, and descriptive statistics on the study site basis determined for the soil samples taken from the Copşa Mică, Sibiu County, Romania (n = 23; values quoted to two significant figures for median, minimum and maximum, interquartile range quoted to three significant figures).

Study site	Elemental concentration (mg kg^{-1})																			
	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Ti	V	Zn
RO-SB 1	13000	18	110	10000	24	10	26	72	19000	2300	4100	460	4.6	130	29	410	680	220	330	29	1400
RO-SB 2	16000	26	130	8800	40	12	32	110	23000	2600	4900	520	0.53	120	34	440	1000	280	440	36	2300
RO-SB 3	13000	26	100	3900	40	11	27	110	21000	2300	4100	540	0.41	58	32	520	1100	240	290	30	2400
RO-SB 4	19000	24	150	15000	36	13	39	99	27000	3400	5700	570	0.68	99	41	480	830	310	340	42	2000
RO-SB 5	12000	18	89	7800	26	10	24	70	18000	2100	3800	500	0.26	130	28	480	660	170	320	26	1600
RO-SB 6	11000	27	85	2100	33	9.9	23	110	18000	2100	3400	510	0.8	70	25	600	1100	200	300	25	1900
RO-SB 7	12000	28	85	3300	36	10	25	110	18000	2200	3500	500	0.93	78	28	560	1200	210	360	28	2200
RO-SB 8	12000	25	80	3900	32	10	25	92	17000	2300	3500	490	0.22	110	28	540	970	200	350	27	2000
RO-SB 9	13000	30	84	4700	41	11	30	92	17000	2600	3400	470	0.33	110	32	590	1100	260	440	35	2400
RO-SB 10	12000	36	84	6700	44	10	25	120	18000	2700	3700	510	1.47	100	29	590	1300	340	290	27	2700
RO-SB 11	14000	43	81	8400	51	12	29	150	22000	3000	4300	610	0.55	120	35	610	1500	360	300	32	3100
RO-SB 12	15000	42	120	7200	54	13	30	150	22000	3200	4400	620	0.37	120	36	560	1600	330	270	32	3200
RO-SB 13	15000	44	81	4600	52	13	34	130	20000	3000	3800	510	0.35	120	37	590	1600	380	380	39	3200
RO-SB 14	14000	65	100	4600	64	14	34	160	23000	3100	4100	590	0.53	130	40	590	2100	450	260	38	3800
RO-SB 15	14000	61	99	4400	64	14	34	160	23000	3100	4000	610	0.52	140	40	630	2000	480	250	38	3800
RO-SB 16	14000	40	89	5700	49	13	33	120	20000	2600	3900	560	0.34	97	38	560	1600	360	260	37	2900
RO-SB 17	13000	48	100	8300	58	12	30	140	19000	2900	3600	510	0.44	120	34	720	1700	400	260	34	3300
RO-SB 18	11000	60	83	5000	61	12	29	160	19000	2300	3300	470	0.44	130	32	540	2100	450	290	32	3700
RO-SB 19	11000	55	64	5600	53	12	28	150	19000	2300	3300	480	0.40	130	32	580	2100	480	300	31	3500
RO-SB 20	10000	59	74	5700	58	11	25	160	18000	2100	3100	460	0.38	96	30	530	2200	400	280	28	3700
RO-SB 21	6300	36	49	3400	32	7.1	16	91	11000	1300	1900	280	0.21	92	18	350	1300	240	230	18	2200
RO-SB 22	11000	64	81	2000	59	12	29	190	19000	2300	3200	470	0.63	130	31	620	3000	350	280	32	3500
RO-SB 23	11000	54	79	1000	47	9.4	29	180	18000	2000	3100	310	0.47	110	26	530	2600	370	320	32	2600
Study site median	13000	38	85	5300	45	12	29	120	19000	2400	3700	500	0.50	120	32	560	1400	330	300	32	2600
Study site interquartile range	2880	28.4	20.8	3560	19.8	2.14	5.9	50.5	3120	670	691	81.5	0.233	28.5	6.76	65.9	945	145	57.2	7.13	1200
Study site min	6300	18	49	1000	24	7.1	16	70	11000	1300	1900	280	0.21	58	18	350	660	170	230	18	1400
Study site max	19000	65	150	15000	64	14	39	190	27000	3400	5700	620	4.59	140	41	720	3000	480	440	42	3800

Elemental concentrations for Rovinari –Sterile Field, Gorj County

Table C41: Median extractable concentrations of the 20 elements, and descriptive statistics on the study site basis determined for the soil samples taken from the Rovinari, Gorj County-Sterile Field, Romania (n = 12; values quoted to two significant figures for median, minimum and maximum, interquartile range quoted to three significant figures).

Study site	Elemental concentration (mg kg^{-1})																			
	Al	**As**	**Ba**	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**P**	**Pb**	**S**	**Ti**	**V**	**Zn**
SRO-GJ 1	13000	7.7	52	36000	0.77	12	34	18	20000	1900	11000	570	0.50	95	43	520	10	260	340	29	54
SRO-GJ 2	13000	7.8	53	34000	0.77	11	34	19	20000	1800	10000	540	0.47	93	44	500	11	330	330	30	61
SRO-GJ 3	13000	7.8	54	35000	0.83	12	36	19	21000	1900	11000	580	0.66	120	46	490	10	300	370	30	58
SRO-GJ 4	13000	7.1	54	31000	0.77	11	34	19	20000	1700	10000	510	0.81	110	43	530	11	340	350	30	72
SRO-GJ 5	14000	8.4	59	31000	0.87	12	37	20	20000	1800	10000	540	0.39	150	47	620	11	400	380	31	59
SRO-GJ 6	14000	7.7	61	30000	0.82	11	38	20	20000	1900	9900	510	0.40	130	44	600	12	450	400	32	68
SRO-GJ 7	12000	7.7	54	35000	0.76	11	33	19	19000	1600	11000	490	0.23	88	43	580	10	290	370	29	50
SRO-GJ 8	15000	8.2	65	34000	0.85	12	39	21	20000	1900	10000	560	0.43	130	49	600	13	590	350	34	71
SRO-GJ 9	15000	6.7	55	35000	0.72	10	38	14	17000	2200	11000	460	0.61	150	38	520	8.7	260	650	33	56
SRO-GJ 10	11000	6.3	43	34000	0.60	9.0	31	13	15000	1600	11000	380	1.3	110	34	490	7.0	210	510	26	44
SRO-GJ 11	14000	5.0	60	36000	0.81	12	38	18	19000	1700	11000	570	0.16	110	53	480	11	180	390	31	59
SRO-GJ 12	4300	6.1	34	34000	0.45	8.1	14	13	11000	650	9300	380	0.48	63	31	460	8.4	210	120	15	51
Study site median	13000	7.7	54	34000	0.80	11	35	19	20000	1800	11000	520	0.50	110	43	520	10	300	370	30	58
Study site interquartile range	1410	1.17	6.20	2100	0.072	1.2	4.07	2.33	1830	220	435	74.4	0.222	33.9	4.54	95.2	1.10	111	49.9	2.68	9.78
Study site min	4300	5.0	34	30000	0.45	8.1	14	13	11000	650	9300	380	0.16	63	31	460	7.0	180	120	15	44
Study site max	15000	8.4	65	36000	0.87	12	39	21	21000	2200	11000	580	1.3	150	53	620	13	590	650	34	72

Appendix C: Elemental concentrations for Rovinari –Ash Field, Gorj County

Table C42: Median extractable concentrations of the 20 elements, and descriptive statistics on the study site basis determined for the soil samples taken from the Rovinari, Gorj County-Ash Field, Romania (n = 12; values quoted to two significant figures for median, minimum and maximum, interquartile range quoted to three significant figures).

Study site	Elemental concentration (mg kg $^{-1}$)																				
	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Ti	V	Zn
AARO-GJ 1	60000	30	390	24000	1.8	15	75	55	24000	8800	6100	250	4.1	1200	67	500	18	740	1200	130	99
AARO-GJ 2	70000	24	430	30000	1.6	14	69	67	29000	9500	7000	280	3.8	1300	62	480	14	590	1300	120	74
AARO-GJ 3	68000	26	440	30000	1.7	14	68	65	29000	9200	6900	310	6.2	1200	64	480	15	500	1300	120	72
AARO-GJ 4	71000	23	450	35000	1.7	14	71	68	30000	9600	7300	290	4.2	1300	65	460	13	490	1400	130	73
AARO-GJ 5	68000	34	470	36000	1.9	15	69	72	30000	9300	7300	300	5.0	1300	67	580	18	620	1400	130	89
AARO-GJ 6	68000	32	460	37000	1.9	16	72	72	30000	9200	7600	310	4.7	1300	72	580	17	520	1500	140	73
AARO-GJ 7	64000	44	440	33000	2.3	18	88	68	29000	9200	6900	300	5.0	1300	88	510	23	520	1400	170	100
AARO-GJ 8	62000	36	420	31000	2.1	16	78	59	28000	8600	6500	310	4.3	1200	75	530	19	620	1300	150	99
AARO-GJ 9	52000	21	340	22000	1.5	12	61	48	22000	7200	5200	230	3.4	980	55	540	14	740	960	110	81
AARO-GJ 10	63000	24	400	25000	1.7	14	75	55	25000	9000	6000	260	3.9	1200	65	510	14	540	1200	130	86
AARO-GJ 11	58000	30	380	28000	1.8	14	71	57	25000	8100	5800	270	4.3	1100	65	570	31	650	1200	130	79
AARO-GJ 12	57000	26	370	27000	1.7	13	68	53	24000	7900	5700	260	3.8	1100	61	530	16	690	1100	120	71
Study site median	63000	26	420	30000	1.7	14	71	59	28000	9000	6500	280	4.2	1200	65	510	16	620	1300	130	79
Study site interquartile range	9890	8.02	60.5	7900	0.202	1.48	6.17	12.4	4830	1110	1130	38.9	0.770	129	3.25	43.5	3.46	169	232	10.1	15.5
Study site min	52000	21	340	22000	1.5	12	61	48	22000	7200	5200	230	3.4	980	55	460	13	490	960	110	68
Study site max	71000	44	470	37000	2.3	18	88	72	30000	9600	7600	310	6.2	1300	88	580	31	1000	1500	170	100

Annex D Quality control

Figures of merit

The figures of merit for the elements using external calibration are shown in Table 8. The limit of detection (LOD) was defined as three times the standard deviation of 10 measurements of the blank divided by the slope of the calibration curve. The limit of quantification was defined as 3.3 times of the LOD (Chaves *et al.*, 2010).

The linear correlation coefficients of the calibration were higher than 0.99, suggesting reasonable linearity.

Table D43: Figure of merit for the determination of elements by ICP-OES

Element	Selected wavelength (nm)	Calibration coefficient	LOD (μg g^{-1})	LOQ (μg g^{-1})
As	189.042	0.9999		
Ca	315.887	0.9994		
Cd	228.802	0.9998		
Co	230.786	0.9995		
Cu	324.754	0.9995		
Fe	238.204	0.9954		
K	769.893	0.9996		
Mg	280.270	0.9961		
Na	589.592	1.000*		
Ni	231.604	0.9994		
P	178.284	0.9998		
Pb	220.353	0.9993		
V	311.838	0.9995		
Zn	206.200	0.9994		

Precision and accuracy

As no certified reference material for oil seeds were available, precision and accuracy was determined by analyzing plant reference material, Sprout (*Brassica oleracea var, gemmifera)* WEPAL-IPE-599. This plant reference material was considered to be appropriate as the material is of the Brassicaceae family, the metals reported include those of interest in the current study and the material was digested and diluted in the same way as the test samples. Precision and accuracy are expressed in terms of coefficient of variance and bias, respectively.

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