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# *In situ* cultivation of aromatic plant species for the phytomanagement of an aged-trace element polluted soil: plant biomass improvement options and techno-economic assessment of the essential oil production channel

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## Abstract

Due to the presence of trace element (TE) in agricultural soils, wide areas are unproper for food production and the clean-up of soil is not a feasible option. Considering the potential remediation options, the use of aromatic plants producing a high quantity of biomass and developing high-added value sectors such as essential oil (EO) production could be valuable regarding one of the phytomanagement objectives, i.e. the restoration of an economic activity. The purpose of this study was hence to evaluate *in situ* the suitability of two aromatic crops, clary sage and coriander, for the phytomanagement of TE-polluted soils, taking into account plants' growth, development and biomass production, essential oil (EO) content and quality as well as a techno-economic feasibility analysis of the channel. *In situ* experiments have been carried out on two agricultural plots of 1.5 ha, a TE-polluted one (Pb: 394 ppm - Zn: 443 ppm - Cd: 7.2 ppm) and an unpolluted one (Pb: 22 ppm - Zn: 48 ppm - Cd: 0.4 ppm). Our findings have shown the ability of coriander and sage to grow similarly on both unpolluted and TE-polluted soil and to produce significant amounts of biomass. The pesticide residue and TE analyses have demonstrated that the EO only contained trace amounts of the contaminants, below or close to the limit of quantification of the method used and similar to marketed products. Mycorrhizal inoculation has also shown promising results by increasing the colonization rates of both aromatic plants, but did not result in higher biomass or EO amounts. Our study brings new evidence towards the potential of clary sage to be used for the phytomanagement of TE-polluted areas, given its perennial vegetation cover, tolerance to TE and obtained EO yields.

Keywords: Essential oil, Trace Elements, *Salvia sclarea*, *Coriandrum sativum*, Arbuscular mycorrhizal fungi

## 1. Introduction

Soil pollution is worldwide a major environmental and health concern. A recent study has estimated the number of potentially polluted sites in Europe to more than 2 Million (van Liedekerke *et al.*, 2014; Mench *et al.*, 2018). Trace Elements (TE), mainly originating from anthropogenic sources (including wastewater discharge, agricultural inputs and practices, refining and mining activities, atmospheric fallout originating from smelting activity and fossil fuels' combustion as well as human-induced soil erosion), are the most frequently listed soil pollutants in Europe (37 % - Antoniadis *et al.*, 2017; Gong *et al.*, 2018; Mench *et al.*, 2018; Panagos *et al.*, 2013). Owing mostly to their non-biodegradability, TE are a major threat for ecosystems, as they are susceptible to affect both soil and water (surface and groundwater) quality and accumulate into living organisms, causing ecological disturbances (Ali *et al.*, 2019; Antoniadis *et al.*, 2017).

One of the main issues with TE is the pollution of agricultural soils. Trace elements are likely to affect not only the plant growth and productivity, but also human and animal health when accumulated in edible food crops which will further be consumed either for food or feeding purposes and lead to a contamination of the food chain (Ali *et al.*, 2019; Gong *et al.*, 2018). Notably, health risks in the case of an exposure to excessive TE concentrations include various disorders such as kidney dysfunction, bones fragility or neurological disorders and reduced cognitive ability (Järup, 2003).

Conventional physicochemical soil remediation techniques usually result in elevated costs and may cause a degradation of the ecosystem as well as a potential risk of displacement of the pollution during transportation of the excavated soil for further *ex situ* processing or storage (Kumpiene *et al.*, 2019). Additionally, these methods may not be applicable on large areas as it is often the case for agricultural fields. Therefore, one of the existing options to environmentally and economically manage TE-polluted agricultural soils, developed during the last decades, is the use of phytotechnologies (Barbosa and Fernando, 2018; Burges *et al.*, 2018). In particular, phytostabilisation, which relies on the use of plants and their associated microorganism's ability to

reduce TE mobility in the soil and availability for the living organisms, is brought forward as a promising tool. In addition to the reduction of TE bioavailability, (re)establishing a vegetation cover aims at reducing leaching and erosion phenomena and potentially restoring soil structure and function (Barbosa and Fernando, 2018; Dongmei and Changqun, 2008; Kidd *et al.*, 2015). This technology might be aided through the use of adequate either biological, organic or mineral amendments, aiming at enhancing the TE immobilization or facilitating both plant establishment and growth (Barbosa and Fernando, 2018; Kidd *et al.*, 2015). Among microbial amendments, arbuscular mycorrhizal fungi (AMF) could be particularly relevant for aided phytostabilisation. These soil-borne fungi are obligate biotrophs, able to establish symbiotic relationships with nearly 80 % of the vascular plant species (Ferrol *et al.*, 2016; Smith and Read, 2008). These symbiotic fungi are widely studied for their benefits in cropping systems, not only for the improved plant growth through enhanced water and nutrient uptake, but also for their contribution to an increased tolerance towards biotic and abiotic environmental stresses (Begum *et al.*, 2019; Smith and Read, 2008). AMF benefits could be translated in aided phytostabilisation process, in particular, for their contribution in improving plant biomass production (Smith and Read, 2008). Additionally, their potential to alleviate TE stress in plants and TE transfer and accumulation in aerial parts and to enhance TE immobilization were previously highlighted (Ferrol *et al.*, 2016; Gong *et al.*, 2018). Notably, the contribution of glomalin (glomalin-related soil protein), produced by AMF, has been evidenced regarding soil aggregation and TE stabilisation, through their complexation with various TE such as Cd, Pb or Zn (Ferrol *et al.*, 2016; Gerami *et al.*, 2021; Wang *et al.*, 2019). Nonetheless, aided phytostabilisation faces several obstacles and among them, the fact that if the vegetation cover is not valued, the economic profitability remains scarce, which may be prohibitive (Burges *et al.*, 2018; Vigil *et al.*, 2015). Biomass valorisation thus appears as a key feature.

Hence, the combination of a phytotechnology aiming at reducing the risk bound to the presence of TE with a sustainable and profitable land and biomass use, defined as phytomanagement, offers the asset of generating an economic profit, along with environmental and health benefits, while

mitigating the TE-risk (Burges *et al.*, 2018; Cundy *et al.*, 2016; Mench *et al.*, 2018). The selection of appropriate plant species is another critical aspect towards a successful phytomanagement (Burges *et al.*, 2018; Yang *et al.*, 2016). In particular, plants have to be able to grow in TE-polluted conditions and to develop a vegetation cover in a relatively short period of time, while producing high amounts of biomass towards a potential valorisation of the vegetation cover (Evangelou *et al.*, 2015; Yang *et al.*, 2016). On this last point, non-food valorisation, such as bioenergy crops or renewable materials, including high-added value products, could be particularly relevant regarding phytomanagement.

In that matter, aromatic crops used for essential oils' (EO) production on TE polluted agricultural soils, may be a suitable alternative (Lajayer *et al.*, 2017). First, EO are high added-value products, widely used as aromatic agents in various non-food industries, such as perfumery, cosmetics and medicine, as well as potential crop protection products, which could provide economic benefits with the produced biomass (Lydakis-Simantiris *et al.*, 2015; Raveau *et al.*, 2020; Zheljzkov *et al.*, 2008). Additionally, as in the current state agricultural TE-polluted soils are unsuitable for consistent and economically viable food production, this channel would not compete with feeding agriculture. Moreover, the cultivation of aromatic crops for phytostabilisation purposes has often been highlighted as a feasible option on TE-polluted sites, since TE potentially accumulated in the distilled biomass were not detected or in really low amounts in the EO (Angelova *et al.*, 2016; Lajayer *et al.*, 2017; Zheljzkov *et al.*, 2008). Finally, the global market EO and on a broader level for plant products, is steadily rising and expected to reach more than \$10 billion in 2020 and \$15.6 billion in 2026 (Allied Market Research, 2020; CBI, 2020). Therefore, cultivation of aromatic plant species, based on decision criteria such as pedo-climatic site conditions, TE plant tolerance and life cycle, as well as their potential regarding phytostabilisation and valorisation perspectives, could be a profitable and economically viable option. In particular, the effects of TE-pollution on the growth and EO production and quality of commercially important aromatic crops such as coriander (*Coriandrum sativum* L.) or clary sage (*Salvia sclarea* L.) have received little attention and insufficient information regarding their potential for TE immobilisation and their possible use for phytostabilisation are available. Coriander

is an annual plant, belonging to the *Apiaceae* family, grown for both its aerial parts and seeds, while clary sage, belonging to the *Lamiaceae* family, is a biennial or short-lived perennial plant, cultivated for its inflorescences (Angelova *et al.*, 2016; Grigoriadou *et al.*, 2019; Mandal and Mandal, 2015). Both plant species are native from the Mediterranean area and their respective EO are widely used in pharmaceutical products as well as in cosmetics and in traditional medicine (Angelova *et al.*, 2016; Mandal and Mandal, 2015; Saharkhiz *et al.*, 2009). Additionally, they were previously demonstrated tolerant to TE and capable of growing in TE-polluted conditions (Angelova *et al.*, 2016; Chand *et al.*, 2015; Zheljzakov *et al.*, 2008).

Therefore, the aim of this study was to evaluate the techno-economic feasibility of the EO production channel, distilled from aromatic crops grown on TE-polluted fields. The present study addresses three main questions: (1) Are aromatic crops, in particular coriander and clary sage, able to grow in TE-polluted soils in the north of France and to produce significant amounts of biomass, sufficient to obtain EO? (2) What is the quality of the produced EO, regarding the potential presence of both TE and chemical residues in the final product, intended for non-food valorisation? (3) Can the addition of a biological amendment based on AMF improve biomass and EO production yields in TE-polluted *in situ* conditions? To our knowledge, most of the studies conducted on polluted soil until now consider either bioenergy production or renewable materials as valorisation channels for the produced biomass. In this study, the focus was put on an innovative valorisation channel based on the production of EO originating from aromatic plants' cultivation, combined with an aided phytostabilization channel, relying on the use of a microbial inoculant consisting of an arbuscular mycorrhizal fungus species. This report additionally embraces both technical and economic considerations.

## 2. Material and methods

### 2.1. Experimental site

Experiments have been carried out *in situ* on two agricultural plots of more than one hectare in the north of France. The unpolluted site is located in Rodelinghem (1.4 ha - 50°49'55.7" N, 1°55'46.9" E). The TE-polluted site, in Evin-Malmaison (1.3 ha - 50°25'55.5" N, 3°02'25.5" E), is a former agricultural field, located approximately 600 m away from a former lead smelter, Metaleurop Nord. This smelter, located in the historical mining area, has been in activity for more than a century until 2003 and has generated significant amounts of TE through atmospheric emissions and TE-enriched dusts (Labidi *et al.*, 2015; Sterckeman *et al.*, 2002). These emissions have led to a topsoil (0-30cm) pollution of the surrounding area on more than 700 ha of agricultural soils.

### 2.2. Determination of the soil physico-chemical properties and TE content

#### 2.2.1. Soil sample collection and preparation

In April 2017, 28 top-soil samples were collected with a manual auger on the unpolluted and polluted sites, using a stratified sampling method to ensure a maximized coverage of the experimental plots and geo-referenced (TDC100®, Trimble). Composites were made from the collected top-soil samples, which were then air dried (40 °C) to a constant weight, ground (agate mortar RM100, Retsch) and sieved (AS 200 digit, Retsch) to a particle size of less than 2 mm.

#### 2.2.2. Determination of the soil water pH

Soil pH was determined according to the NF ISO 10390 (2005) standard procedure. 5 g of 2-mm sieved soil were suspended in 25 mL of distilled water, shaken for 2 hours (Heidolph Reax 2, Dutscher) and left to rest for 1 hour, prior to water pH measurement (5010T pH Electrode, Hach).

#### 2.2.3. Quantification of the TE total concentration

The digestion was carried out following the NF-EN-13656 standard procedure. Briefly, 0.5 g soil were digested at 180 °C (Mars Xpress, CEM) for 20 min with a solution of nitric acid (HNO<sub>3</sub> 67% - 2 mL),



hydrochloric acid (HCl 36% - 6 mL) and hydrofluoric acid (HF 48% - 2 mL) and filtered ( $\emptyset$  0.45  $\mu$ m Whatman® filter paper).

#### 2.2.4. Quantification of the TE $\text{NH}_4\text{NO}_3$ extractable fraction

The TE easily soluble (plant-available) fraction was assessed following the NF ISO 19730:2008(E) standard procedure. A selective extraction was performed from 5 g of 2-mm sieved soil suspended in 25 mL of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ , 1 M). The soil/extractant mixture was then shaken for 2 hours, filtered ( $\emptyset$  0.45  $\mu$ m Millipore filter, Merck) and the leachates was finally acidified to a pH of 2. For both total and extractable TE concentrations, the analyses were either carried out by ICP-OES (Agilent 5110) or ICP-MS (Agilent 7500), depending on sample concentrations. One standard reference material was used for analytical quality control (NIST SRM 2710, Montana soil).

#### 2.3. Floristic inventory of the native species

The floristic inventory was based on observations realised before cultivation practices, in April, on the TE-polluted site, naturally colonized by plant species. The position of 13 quadrats (1 m<sup>2</sup> each) was randomly determined on the experimental site and geo-referenced (TDC100®, Trimble). All plant species naturally growing were sampled (three individuals per species), identified using the manuals from Bonnier and de Layens, 1986 and Lambinon *et al.*, 2004 and compared with regional data (Centre Botanique National de Bailleul, url: <http://www.cbnbl.org/centrederdigit.htm>).

A cover-abundance value was allocated to each identified species according to Braun-Blanquet cover-abundance scale (1951). Several biodiversity indices were also calculated to estimate plant diversity: First, Menhinick and Margalef indexes that indicate species richness (Margalef, 1958; Menhinick, 1964). Then, Shannon index that represents the spatial distribution as well as the abundance of identified plant species, and may additionally reveal rare species (Shannon, 1948; Shannon and Weaver, 1964). Eventually, Simpson index that measures the probability that two randomly selected individuals from a sample belong to the same species (heterogeneity - Simpson, 1949).

#### 2.4. Isolation of autochthonous AMF spores and root mycorrhizal colonization

To assess the presence of AMF in soils on both experimental sites and hence guide the choice towards the use a mycorrhizal inoculum or not, 12 soil samples have been collected before any cultivation practice, from randomly chosen positions and where plants were naturally growing. AMF spores were extracted from the soil samples by wet sieving (Oehl *et al.*, 2004) and counted under microscopic observation (Eclipse E600, Nikon – x100 magnification). The AMF spores' content (number of spores per 10 gram of soil) was assessed in triplicates.

To evaluate AMF root colonization, fresh roots of the plants naturally growing on the experimental sites were sampled and cleared in a KOH 10 % (v/v) solution for 1h at 70 °C and then stained with trypan blue (0.05 %) in a water, glycerol and lactic acid mixture (1:1:1, v/v/v) for 1 h at 70 °C (Phillips and Hayman, 1970). The stained root fragments were stored at 6 °C in a water/glycerol (1:1, v/v) solution until they were observed. Roots' fragments were examined with microscopic observations (Eclipse E600, Nikon – x100 magnification) to detect the presence of specific mycorrhizal structures (arbuscules, vesicles and intraradical hyphae) and to determine mycorrhizal rates. Root colonization was evaluated using the grid line intersect method (McGonigle *et al.*, 1990). Root fragments from three replicates (45 fragments in total per condition) were observed and 3 intersections per root fragment were examined.

#### 2.5. *In situ* experimental design

Both aromatic plants, clary sage (*Salvia sclarea* L.) and coriander (*Coriandrum sativum* L.), were cultivated on the two unpolluted and polluted experimental sites. The plots were divided into equivalent areas for each plant species and devoted to their cultivation. Additionally, the available surface for each plant was subdivided in two plots. The first plot was amended (I) with a commercial mycorrhizal inoculant (AGTIV® Specialty Crops, PremierTech) while the other one was not amended and was considered as the non-inoculated control on each experimental site (NI – Figure S1).

Seeds of *C. sativum* and *S. sclarea* were purchased from the French research institute ITEIPMAI, providing certified seeds. A commercial AMF inoculant (AGTIV® Specialty Crops, PremierTech) containing the AMF *Rhizophagus irregularis* (12000 viable spores per gram of product) kindly provided by the company was used. The powdery inoculant was introduced during sowing, directly mixed with seeds and applied at a rate of 125 g/ha.

So as to evaluate the phytomanagement channel from an agricultural perspective, sowing, cultivation practices and harvest were realised with farm scale equipment. Soil preparation was achieved through conventional tillage (10 cm-depth ploughing) and the sowing steps were carried out in April (2017), for the two experimental sites and the two plant species (300 000 seeds per hectare, 3-4 cm deep). Additionally, coriander was sown again in April (2018) for a second year of cultivation.

2.6. Determination of emergence rates of sage and coriander on unpolluted and polluted sites

Six weeks after sowing, the emergence of the seedlings has been determined *in situ* for each plant and for each condition (plants inoculated with the AMF or not). For each condition, three randomly chosen positions were determined and for each location, the number of seedlings was visually determined in a one square meter quadrat, replicated 3 times (adapted from Gunaratne *et al.*, 2010 and Yan *et al.*, 2007). Each quadrat was also geo-referenced (TDC100®, Trimble).

2.7. Weed control

Based on ITEIPMAI recommendations, only two herbicide applications have been carried out with a three-week interval to regulate the spontaneous growth of gramineous and dicotyledonous species (Table S1). In particular, the presence of *Matricaria recutita* L. was necessary to avoid as it is likely to contaminate and alter EO composition and quality. No fungicidal or insecticidal treatments have been carried out. The chemicals used were applied in half doses. Additionally, during the second and

third years of sage's cultivation, the number of crop protection products and the number of applications were reduced (respectively to 3 and 1 – Table S1).

#### 2.8. Root and aerial plant part samplings

Sampling locations were equally spaced and determined to best match the plot's shape and each one was geo-referenced. The roots and whole aerial parts of the 3 closest growing plants (sage or coriander) were collected to characterize the biomass development. For each sampled plant (three replicates per sampling point), the height was measured and they were then placed in an oven (Gero, Carbolite – 72 h at 80 °C), to determine their dry weights. Root samples were prepared for further evaluation of the root mycorrhizal colonization in cultivated plants, using the same methodology as previously described (Section 2.4.).

#### 2.9. Biomass harvesting

Plants have been harvested with farm equipment, based on recommendations given by ITEIPMAI, providing technical and crop management advice regarding aromatic plants' cultivation. Aerial parts of coriander (annual life cycle) were harvested with a mow-picker (Novacat Alpha Motion pro, Pöttinger) on the first year, after 13 weeks of cultivation and at the full flowering stage. Seeds of coriander were harvested using a combine (Clayson 8060, New Holland) on the second year, after 19 weeks of cultivation and at seed maturity.

The cultivation of clary sage (short-lived perennial life cycle) was carried out on three successive years. During the first year of sage's cultivation, aerial parts (mostly large leaves) were harvested, using a mower and a mow-picker. Flowery parts of sage at the full flowering stage were then harvested on the second and third years of cultivation using successively a mower (Ramos TLX, Fella) and a pick-up loader wagon (Faro 4000 D, Pöttinger).

#### 2.10. Sage and coriander EO distillation

The distillation of the harvested plant biomass was performed on the harvest day. The harvested biomass was loaded in a distillation tank (14 m<sup>3</sup>) and water steam heated by a wood-fired boiler

(saturated water steam, 0.3 bar) was injected. The formed vapours were recondensed in a cooling-unit and the distillate was collected in a receiver/separator. The process was conducted during a three-hour cycle, until no more EO was recovered. The EO yield expressed as a percentage represents the distillation yield and was calculated according to the following equation (1):

$$(1) \text{ EO yield (\%)} = \frac{\text{Raw plant biomass (m}^3\text{)}}{\text{Essential oil amount (m}^3\text{)}} \times 100$$

The agronomic EO yield is expressed as the quantity of EO produced per hectare, and was calculated using equation (2):

$$(2) \text{ EO yield (kg/ha)} = \frac{\text{Essential oil amount (kg)}}{\text{Plot surface (ha)}}$$

## 2.11. Essential oil's quality assessment

### 2.11.1. Trace elements assessment in distilled EO

Essential oils obtained from each plant and experimental condition were aliquoted (5 mL) and analysed through ICP-MS (Agilent 7500) after a digestion. Briefly, a mixture (0.5 g of EO, 10 mL HNO<sub>3</sub><sup>-</sup> and 3 mL of pure water) was digested in a microwave oven (Mars Xpress, CEM) at 180 °C for 20 min. In addition, samples from one market product of clary sage and two of coriander EO (aerial parts and seeds) were analysed to compare their quality in terms of TE content. All TE analyses were led in triplicates.

### 2.11.2. Pesticide residues analysis

Multi-residue analyses targeting about 600 active molecules, were performed through either GC-MS/MS (method MOC3/05(S1), version 0) or LC-Q-TOF (method MOC3408(S1), version 2) screenings, by Phytocontrol Analytics – France.

## 2.12. Techno-economic feasibility analysis

To estimate the economic viability of the EO production channel on both unpolluted and TE-polluted soils, a techno-economic analysis has been carried out. The production costs for EO production,

involving the cultivation of aerial parts of coriander, seeds of coriander or sage inflorescences have been evaluated on each crop and for each experimental condition (polluted or unpolluted site, I or NI conditions) separately and were expressed per hectare. They include the operating costs (material amortisation and maintenance, fuel, labour cost, *etc*) and the supply expenses (seeds, fertilizers, AMF inoculant and crop protection products). They were estimated according to two reference handbooks: the Field Crop Protection Guide (updated each year by the Chamber of Agriculture of each French region) which provides data about crop protection products and their costs and a second guide providing technical information about agricultural equipment, regarding material and operating costs. They included soil preparation, sowing, inputs, harvest and potential post-harvest costs. These were compared to other phytoremediation or phytomanagement methods and also to the production costs of common field crops (wheat, winter barley and rapeseed).

In addition, a gross profit is estimated from the obtained EO yields for the 3 conditions (sage and coriander aerial parts or seeds), using the following equation:

$$(3) \text{ Gross Proceeds} = \text{EO yield} \times \text{EO selling price (per kg)}$$

One should note that the EO selling price is the price paid by companies before marketing EO as a final product. The distillation process was considered subcontracted in the present evaluation.

### 3. Statistical analysis

Statistical analysis has been done using the R software, version 3.5 and XLSTAT 2018.1.1. Data were first subject to Shapiro-Wilk (normality), Bartlett (homoscedasticity) and Fligner Killeen (variances' homogeneity) tests, before further analyses. According to the results of the previously mentioned tests, parametric (pairwise t test and ANOVA) or non-parametric (Kruskal-Wallis) statistical tests were chosen, complemented with a post-hoc Tukey HSD (Honestly significant difference) test. Data expressed in percentages (in particular emergence and colonization rates) were converted to arcsine values before using statistical tests.

## 4. Results and discussion

### 4.1. Physico-chemical and biological characterisations of the experimental site soils

The present study was carried out on two agricultural plots, an unpolluted plot and an aged TE-polluted one. The soil physico-chemical parameters and initial TE content of the experimental plots have been evaluated before cultivation practices and are summarized in Table 1. Briefly, on both experimental sites, the soil is a silt loam, with a neutral/slightly alkaline water pH and a relatively low limestone content (less than 1%). On the unpolluted site, the concentrations of TE are similar to the regional background levels for agricultural topsoils (Sterckeman *et al.*, 2007). On the TE-polluted site, the soil is mainly polluted by Cd ( $7 \pm 0.4 \text{ mg.Kg}^{-1}$ ), Pb ( $394 \pm 16 \text{ mg.Kg}^{-1}$ ) and Zn ( $443 \pm 12 \text{ mg.Kg}^{-1}$ ), with concentrations respectively 17, 11 and 6 times higher than those in regional background levels (Sterckeman *et al.*, 2007). Regarding the physico-chemical parameters, total organic carbon and total nitrogen content, C/N ratio, organic matter content and CEC were higher on the TE-polluted site (Table 1). Before tillage and sowing, about  $143 \pm 68$  and  $157 \pm 47$  AMF spores/100 g of soil were respectively isolated from the aged TE-polluted and unpolluted soils, displaying no significant difference between the two conditions. Microscopic observations of stained roots of the plant species initially present on both experimental sites, such as *Urtica dioica* L., *Veronica arvensis* L., *Senecio vulgaris* L. or *Mercurialis annua* L., revealed the presence of AMF structures such as arbuscules, vesicles and intraradical hyphae. Total root mycorrhizal rates were relatively low, ranging from 3.8 to 6.5 %. In fact, AMF spores' densities usually vary from 300 to 1400 spores/100 g in unpolluted or TE-polluted soils (Oehl *et al.*, 2004; Yang *et al.*, 2015; Zak *et al.*, 1982). Besides, our data have shown similar results between unpolluted and TE-polluted sites. Hence, the low spores' density and root colonization by AMF observed in the polluted site cannot be attributed to the soil pollution. However, the past cultivation practices on the two agricultural plots, involving both soil preparation and use of phytopharmaceutical products (esp. for potato or sugar beet cultivation), were likely to affect soil microorganisms' survival (Büneman *et al.*, 2006; Tilston *et al.*, 2010). From that perspective, the addition of a mycorrhizal inoculum seemed appropriate, to assess its effect on

cultivated plants' growth and biomass production. Besides, from an agronomic perspective, coriander and sage are relatively low demanding crops (Sharma and Sharma, 2012; Tibaldi *et al.*, 2010). In that regard, MgO was considered higher than average in comparison with reference thresholds (Denoroy *et al.*, 2004), while K<sub>2</sub>O was considered average on both plots. Regarding P<sub>2</sub>O<sub>5</sub> content, its amount is equal to the reference threshold for low demanding crops on the polluted site, while particularly high on the unpolluted one (Comifer, 2019; Denoroy *et al.*, 2004). There is in fact a trend towards a better chemical fertility of the soils on the polluted site, according to the previously mentioned parameters.

#### 4.2. Floristic inventory of the native species on the polluted site

The main objectives of this floristic inventory were to identify species naturally growing on TE-polluted soils. In addition, it should provide useful information regarding the cautious selection of the cultivated plant species and the associated cultivation practices, notably the use of herbicidal compounds: if the wild growing plant species belonged to the same family as the cultivated ones, this would entail an additional constraint, especially regarding weed control. Moreover, in addition to their potential effect on plants' growth, the presence of certain weeds and in particular aromatic species, such as *M. recutita*, are also binding considering harvest, as they are likely to contaminate and alter EO's quality. Before the beginning of the experiment, despite the TE-pollution and its potential phytotoxicity, 24 plant species belonging to 14 different families were able to grow under these conditions (Table 2). *Asteraceae* and *Poaceae* are the two most abundant plant families, with 5 members each. *Sonchus asper* is the most abundant and dominant plant species on the site, whereas *Phragmites australis*, *U. dioica*, *Geranium pusillum* and *Rumex crispus* were sparsely visible on the experimental site. Vegetation naturally covers 70 to 100 % of the soil. Amongst the inventoried plant species, most of the identified *Asteraceae*, namely *Erigeron canadensis*, *M. recutita* and *S. vulgaris* were previously identified in TE-polluted soils, as well as *Erysimum* sp. (*Brassicaceae*), *Lolium perenne* and *Festuca* sp. (*Poaceae*), *U. dioica* L. (*Urticaceae*) and *Rumex* sp. (*Polygonaceae* – Dazy *et al.*, 2008; Hajiboland and Manafi, 2007; Jeannin *et al.*, 2020; Masarovičová *et al.*, 2010). Interestingly, amidst



these plant species, *M. recutita* was already demonstrated as a Cd-accumulator species (Masarovičová *et al.*, 2010).

Species richness was about 1.09 and 3.72, according respectively to Menhinick and Margalef indexes. In addition, biodiversity indexes were about 2.15 and 0.85 for Shannon & Weaver and Simpson indexes respectively (Table 3). Diversity indexes have in previous studies been demonstrated negatively correlated with increasing TE-amounts (Hernández and Pastor, 2008; Koptsik *et al.*, 2003; Vangronsveld *et al.*, 1996). Despite this, our results have shown that the polluted site is higher in plant biodiversity in comparison with other unpolluted agricultural fields or polluted soils described in the literature (Chateil *et al.*, 2013; Dazy *et al.*, 2008; Fédoroff *et al.*, 2005; Hernández and Pastor, 2008; Koptsik *et al.*, 2003; Vangronsveld *et al.*, 1996; Zhou *et al.*, 2018). Nonetheless, one should note that beside TE-content, diversity indexes were demonstrated highly related to soil properties, in particular to the nutrient contents such as K, Mg or N (Hernández and Pastor, 2008; Koptsik *et al.*, 2003). In our case, they were relatively high for low-demanding crops.

#### 4.3. *In situ* emergence rates

For first and second years of coriander's cultivation, emergence rates were ranging from 49 to 84 % (Table 4). No significant difference has been observed between the polluted and unpolluted sites, and between I and NI conditions, for both cultivation years. Previous studies have highlighted adverse effects of Zn on coriander seedling's persistence (Marichali *et al.*, 2014) and of Cd and Cu on both germination rates and seedling's emergence and survival of coriander (Faizan *et al.*, 2012). However, in our experimental conditions, seedling's emergence was not adversely affected by soil pollution. For sage, emergence rates were significantly higher on the polluted site in comparison with the unpolluted one. They were about 71 and 65 % on the polluted plot in NI and I conditions respectively, while on the unpolluted one they were around 45 % whatever the condition (Table 4). TE-pollution does not seem to alter seedlings' emergence; the difference observed between polluted and unpolluted sites may be due to the differences in terms of soil physicochemical properties.

#### 4.4. Coriander and sage growth

It should first be remembered that the study of coriander was carried out on two successive years. The first year's aim was to distil the whole coriander aerial parts and the second one to distil the seeds.

At the harvest of coriander's aerial parts, the plants' height reached an average of 66 cm on the unpolluted site and 55 cm on the polluted one (Figure 1A). Coriander's dry weight were respectively an average 10 g/plant on the unpolluted site and 3.5 g/plant on the polluted one. Additionally, coriander's dry weight and height were similar in I and NI conditions, on both polluted and unpolluted sites.

At the harvest of coriander's seeds, the height of coriander's plants reached respectively 77 and 80 cm on the NI and I conditions on the unpolluted site, while reaching 81 and 70 cm respectively for NI and I conditions on the polluted site (Figure 1B). Furthermore, the recorded coriander dry weights were about 11 and 7 g/plant when cultivated on unpolluted and polluted sites respectively. A significant difference was observed for coriander grown on both unpolluted and polluted sites for the I condition, which displays a lower dry weight on the polluted one (50% reduction). Moreover, a significant difference has been observed between NI and I conditions in terms of plant height (respectively 81 and 70 cm) on the polluted site. For both coriander cultivation years, similar behaviour in terms of plant's growth (dry weight and height) have been obtained on the unpolluted plot. These results are in accordance with previous studies (Ghobadi and Ghobadi, 2010; Inan *et al.*, 2014). The amounts of biomass produced on experimental plots were about 22 and 4 m<sup>3</sup>/ha, at the harvest of aerial parts and seeds respectively (results not shown).

In contrast with coriander, clary sage is rather displaying a short-lived perennial life cycle and is cultivated for at least two successive years (Tibaldi *et al.*, 2010). It has hence been monitored over time for three cultivation years. Regarding plant height, clary sage attained an average of 38.5 and 32.4 cm at first year's harvest, reaching 160 and 145 cm (respective 76 and 78 % increases) at second

year's harvest and decreasing of about 10 and 29 % in third year, respectively on unpolluted and polluted sites (Figure 2). Regarding dry weights, sage was about 48.9 and 51.9 g/plant at first harvest. At second year's harvest it increased to 127 g/plant on the unpolluted site, regardless of the condition, while remaining similar to first year on the polluted one. During the third year of cultivation, data ranged from 152.5 to 207 g/plant on the unpolluted site, while ranging from 160 to 181 g/plant on the polluted one (60 % increase). Overall, no significant differences were observed between I and NI conditions, for both plant height and dry weight, whatever the cultivation year. Besides, a significant difference between polluted and unpolluted sites has only been measured for the NI condition in year 2.

Furthermore, due to the inflorescences' large size in year 2, measurements have been carried out specifically on the inflorescences as well (Figure 3). Inflorescences' heights ranged from 69 to 80 cm, while their dry weights ranged from 71 to 95 g/plant. The number of stems carrying inflorescence varied from 5 to 6. Whatever the condition (polluted or unpolluted sites, I or NI), no significant differences in sage growth parameters (inflorescences' height and dry weight or number of stems carrying inflorescences) were observed (Figure 3). In terms of biomass, 15, 106 and 42 m<sup>3</sup>/ha have been harvested during the first, second and third years of cultivation, respectively (data not shown).

As expected, due to the biennial life cycle, sage mostly developed large leaves on the ground level during the first year and displayed a really limited number of flowers. During the second year, a high number of tall inflorescences have been observed, whereas on the third year, there were still numerous flowers, but the inflorescences were smaller and sage grew larger leaves, increasing the total plant's weight, which is similar to what was previously reported for sage cultivated on successive years (Carruba *et al.*, 2006). Previous studies have demonstrated that sage was tolerant to TE and able to grow in TE-polluted conditions without being significantly affected (Angelova *et al.*, 2016; Chand *et al.*, 2015; Grigoriadou *et al.*, 2019). It should however be noted that a lower plant development has been observed on the polluted site, especially during year 2. Due to its biennial life

cycle, sage is likely to produce high amounts of biomass during the second year of cultivation (Carruba et al., 2002; Zutic et al., 2016). Moreover, sage's plant density was previously highlighted as a key component towards a successful sage's cultivation (Tibaldi *et al.*, 2010). Because of the higher emergence rates measured on the polluted site (Table 4), resulting in a higher plant density per square meter, sage's development may have been hindered, which would result in lower plant weight. However, this trait did not affect EO yields.

#### 4.5. AMF mycorrhizal colonization of coriander and sage

At the harvest of coriander's aerial parts, while no differences were observed between NI conditions on both sites (Figure 4A), a significant improvement in total mycorrhizal colonization rates has been observed on the I condition, in comparison with the NI condition on both sites, with respective increases of 65 and 73 % on the unpolluted and polluted sites. Arbuscular and vesicular mycorrhizal rates were respectively about 4 and 21.5 %.

At the harvest of coriander's seeds, significantly higher mycorrhizal rates have been displayed on the unpolluted site for the I condition (13 %), in comparison with the NI one (3 %), while no significant differences between NI and I conditions were observed on the polluted site (Figure 4B). It is noteworthy that total, arbuscular and vesicular mycorrhizal rates displayed a similar trend.

Our results on sage have shown that total mycorrhizal rates were significantly increased overtime and at highest during the third year of cultivation, on both experimental sites, raising from 4 to 35 % and from 3 to 34 %, respectively on the unpolluted and polluted sites (Figure 5). No significant differences were observed between unpolluted and polluted sites, excepted in year 2 where the total mycorrhizal rates were 50 % lower on the polluted site. Regarding inoculation's effects, significantly higher mycorrhizal rates have been obtained during year 2 and year 3 for the I condition in comparison with the NI one, especially on the polluted site, with respective 59 and 34 % increases. Arbuscular mycorrhizal rates followed the same trends as the total mycorrhizal rates, for year 2 and

3, whereas no significant difference was observed for vesicular rates, whatever the year and the condition (polluted or unpolluted sites, I or NI conditions).

As a whole, our results have shown that spontaneous root mycorrhizal colonization was observed on NI plots, whatever the plant species, which is due to the presence of autochthonous AMF spores in both experimental soils, as previously reported (Table 1). Interestingly, despite this spontaneous mycorrhization which remains relatively low, the introduction of the exogenous mycorrhizal inoculum during sowing led to a significant improvement of mycorrhizal rates of coriander and sage roots, during second and third years of cultivation. However, despite the presence of arbuscules, seat of nutrients' exchange between both symbiosis partners (Ambrosino *et al.*, 2020; Smith and Read, 2008), no beneficial effect on biomass production was observed whatever the cultivated plant and the experimental site. Nonetheless, due to the presence of root mycorrhization even in NI conditions, their relative contribution towards plant's growth in I condition may be difficult to identify. Additionally, the physicochemical characteristics of the soil seemed sufficient for low-demanding crops and have been complemented with fertilizers (in particular 120 units of nitrogen) one month after sowing, which is likely to ensure a good nutrient availability for the plants and does not put the crops in a situation where they depend on the establishment of the mycorrhizal symbiosis. One should note that in the present work, the mycorrhizal symbiosis was established in both polluted and unpolluted conditions for both cultivated plants, sage and coriander, depicting the fact that at least some AMF species are able to tolerate the presence of TE in soils. This may even result in an alleviated TE stress for cultivated plants they are associated with. In fact, previous studies have demonstrated that *Glomus* or *Acaulospora* species were found in Zn and Pb polluted soils (Zarei *et al.*, 2008), or that glomalin was secreted in Cu and Zn polluted soil, revealing the presence of AMF species (Cornejo *et al.*, 2008). In the latter case, the authors have highlighted a high correlation between the amounts of glomalin in soil and the ability of plants to grow in the area, depicting a contribution of the AMF to the plants' growth and/or tolerance to TE (Cornejo *et al.*, 2008).

#### 4.6. Sage and coriander EO distillation yields

Regarding aerial parts of coriander's harvest, the distillation yields were lower on the polluted site, for both I and NI conditions, in comparison with the unpolluted site, with about 50 % of reduction. These distillation yields are 15 to 77 % lower in comparison with those commonly obtained for the distillation of aerial parts of coriander (0.1 to 0.3 % - Bhuiyan *et al.*, 2009; Nurzyńska-Wierdak, 2013; Shahwar *et al.*, 2012). The previously displayed results were yet obtained using laboratory scale Clevenger-type apparatus, whereas the present study's results were obtained with a large-scale distillation unit, which may result in significant differences. For the harvest of coriander seeds, the distillation yields were higher than those obtained for aerial parts of coriander (Table 5), which is in agreement with previously published results, since coriander seeds were demonstrated to possess a higher EO content than the aerial parts (Bhuiyan *et al.*, 2009; Sharma and Sharma, 2012).

Regarding sage during its second year of cultivation, expected to be the best harvest year (Carruba *et al.*, 2006; Tibaldi *et al.*, 2010), distillation yields were at highest and ranged from 0.019 up to 0.035 % on the polluted NI condition (Table 6). Similar distillation yields were also obtained between year 1 and year 3, regardless of the condition, which were significantly lower than those obtained in year 2.

Regarding EO yields, aerial parts of coriander displayed no significant difference in terms of EO's amounts obtained between the polluted and unpolluted sites and between the I or NI conditions (Table 5). EO's yields were higher than 10 kg per hectare on both experimental sites, which is similar to those reported in the literature (Smallfield *et al.*, 1994). Interestingly, an improvement lever towards an increase of the distillation yields could be the addition of straw in the distillation process, as sometimes done by the distiller when biomass amounts are really low, to slow down the water steam diffusion through the biomass (Smallfield *et al.*, 1994).

For the harvest of coriander's seeds, the obtained EO's yields were in average up to 6 kg per hectare on both experimental sites. Equivalent yields have been obtained between the two experimental sites, but a higher EO yield has been obtained on the I condition (7.5 kg/ha) in comparison with the

NI one (4.4 kg/ha) for the unpolluted site. This same trend has not been identified on the polluted site, where the EO yields were equivalent between I and NI conditions. The EO yields per hectare are significantly lower than those expected for this crop when cultivated on unpolluted soils (up to 18 kg/ha – NIIR Board, 2003; Priyadarshi and Borse, 2014). Two factors may explain these lower yields: on one hand, a late harvest was carried out, while on the other, coriander seeds are commonly crushed to enhance the distillation yields (Geed *et al.*, 2014; NIIR Board, 2003; Saim *et al.*, 2008), a step that was not done in the present work. However, it is interesting to note that the obtained EO yields are higher by about 30 % than those obtained on TE-polluted soils and reported by Zheljzakov *et al* (2008).

Regarding sage, high EO amounts have been obtained particularly in year 2, with yields up to 34 kg/ha. The EO amounts obtained on the polluted site were higher with respective 60 and 16 % increases on the NI and I conditions. On the unpolluted site, there is a higher yield obtained for the I condition (20.3 kg/ha) in comparison with the NI one (12.9 kg/ha), but the trend is opposite on the polluted site (respectively 33.7 and 24.2 kg/ha for NI and I conditions). It is furthermore well known that the second year of cultivation is the optimal harvest year for sage's EO production (Carruba *et al.*, 2002; Zutic *et al.*, 2016). In comparison with reference values, the obtained EO yields, in particular on the polluted site, are on the high range (expected yields range from 10 to 30 kg/ha – CRIEPPAM website; Yaseen *et al.*, 2015). For the first and third years of sage cultivation, obtained yields were lower and ranged from 0.5 to 2.7 kg/ha. However, contrary to first year where low yields were expected due to the very small number of inflorescences, yields on the third year of cultivation are expected to reach up to 50 or 60 % of those obtained during the second year (Carruba *et al.*, 2006). This trend was not observed in the present case since obtained yields were barely higher than those obtained during the first year of cultivation. This could be explained by a late harvest period due to climatic conditions, implying that many inflorescences had started to wither, hence reverberating on the yields.

## 4.7. Essential oil's quality assessment

### 4.7.1. Trace elements assessment in distilled EO

The assessment of TE amounts in EO has been carried out on both EO from coriander as well as on the EO of sage obtained during the second year of cultivation (optimal harvest year for sage). The results from the analyses have shown that Cd was below the limit of quantification (LoQ) in any of the distilled EO (Table 7). Similarly, trace amounts of Pb and Zn have been detected, below the limit of quantification in most of the EO samples, exception made for the EO from aerial parts of coriander. Previous studies have also shown that TE were detected in the distilled EO of coriander (As, Cr, Cu, Mn, Pb and Zn) and within the same range (Zheljazkov and Jekov, 1996), or sage (Cd, Pb and Zn), depicting higher Pb and Zn amounts in the EO than those highlighted in the present work (Angelova *et al.*, 2016). Interestingly, coriander seeds were previously demonstrated to accumulate lesser amounts of TE than other edible parts of the plant (Kisku *et al.*, 2011), which is in accordance with our results with lower amounts of Pb and Zn detected in coriander seeds' EO. Furthermore, it should be mentioned that even though TE were found in EO, their presence was quantified in trace amounts, equal to or barely higher than the LoQ, depicting a very limited transfer of the different TE in the EO thanks to the distillation process. It should also be highlighted that scarce are the differences obtained between the EO originating from the polluted and the unpolluted conditions. Finally, one should note that there is currently a lack of regulatory limit set regarding TE content in EO for non-food purposes in France or more broadly in Europe (Lajayer *et al.*, 2017). Interestingly however, all the EO distilled in the present study display TE amounts equal to or below the ones found in the analysed marketed products (Table 7), which is a key factor towards the feasibility of the method.

### 4.7.2. Pesticide residues analysis

The assessment has been carried out on both EO from aerial parts and seeds of coriander as well as on the EO of sage obtained during the second year of cultivation. The results from the pesticide multi-residues analysis indicate that out of the 600 investigated active compounds, only 14 were



detected in the distilled EO from coriander and sage (Table 8). Additionally, the identified residues are present in small amounts or below the LoQ for the majority. It should be mentioned that even though these analyses were conducted, the EO have in practice for non-food valorisation no maximum residue limits set, by European legislation or worldwide, with the only exception being citrus derivatives in the US, framed by the Environmental Protection Agency (EPA – Quarré, 2018; Tascone *et al.*, 2013). Amongst the 14 detected pesticide residues, the majority was detected in the EO distilled from the coriander's aerial parts. Previous studies have already reported the presence of pesticide residues in aerial parts and leaves of coriander (Ahmed *et al.*, 2019; Dogheim *et al.*, 2004). However, there seem to be an effect of the distilled plant part, as the number and amounts of detected pesticide residues were significantly lower in the EO from seeds of coriander, despite the same chemical interventions on the experimental sites. The case of Malathion accumulation in coriander and its transfer in EO was previously discussed, evidencing that lower amounts were accumulated in seeds compared to the leaves, but that there was a concentration effect between Malathion's amounts in seeds and in the EO (Abd El-Rahman *et al.*, 2005). On another note, sage's EO displayed a really good quality regarding the presence of residues, as Dimethenamid was the only detected one and below limit of quantification. Aclonifen and Pendimethalin were the two active compounds detected and quantified with the highest concentrations (respectively up to 1.1 and 1.7 mg/kg of EO), while Hexachlorobenzene, Pendimethalin and Triallate were identified in the wider number of samples. Pendimethalin, aclonifen and linuron, that were detected and quantified in the highest amounts in both coriander EO, were used in the crop management system. DDT, pentachloroanisol and heptachlor, listed as Persistent Organic Pollutants (POPs – Stockholm Convention, 2001), are likely to persist for an extended period of time in soils and to bio-accumulate in living organisms (Jing *et al.*, 2018; Lee *et al.*, 2018; Zacharia, 2019). Their potential past use may hence be reflected in their presence in the cultivated plants and in the EO. Yet, these amounts were barely higher than the LoQ for both DDT and heptachlor and below LoQ for pentachloroanisol, meaning that they were detected but in trace amounts. Finally, the rest of the detected molecules is

often found in commercial products homologated for use on beetroot and potato, which were previous crops on the experimental sites, or on rapeseed, which is commonly cultivated in the neighbouring plots. To sum up, the detected residues may be the result of an old soil pollution, of the carried out chemical interventions or of incidental contamination.

#### 4.8. Techno-economic feasibility

The purpose of the study was finally to evaluate the techno-economic feasibility of EO production on polluted soils. First, it should be noted that physicochemical methods for soil remediation often result in elevated costs and are not applicable to large areas (Borges *et al.*, 2018; Kumpiene *et al.*, 2019). In fact, in our case a topsoil pollution (0-30 cm) on more than 1 hectare of land would result in approximately 4000 m<sup>3</sup> of polluted soil to remediate. Recently, an average estimated cost of about 180 €/m<sup>3</sup> for the physico-chemical remediation of TE-polluted soil has been brought forward (Zhang, 2019), which in our case would translate into more than 700 000 euros for the site remediation, which seems prohibitive.

In this study, the economic evaluation is presented for each aromatic crop and for each experimental condition (I or NI) separately. Production costs for the biomass on the NI condition, regardless of the experimental site (polluted or not) and after deduction of the inoculum's cost representing an amount of 170 €/ha, are as follows: 706 €/ha for aerial parts of coriander (one-year cultivation – Table 9), 772 €/ha for coriander seeds (one-year cultivation – Table S3) and 1158.5 €/ha for sage (three-year cultivation cycle – Table S4). There was no significant difference between the production costs in TE-polluted or unpolluted conditions, as the followed procedure and the material used were equivalent. In addition, the cost for subcontracting EO distillation is about 300 €.

##### **Aerial parts of coriander**

An average of 10 kg/ha of coriander EO was produced on experimental sites, with no significant differences between experimental conditions (I or NI, polluted or unpolluted conditions). Using equation (3) described in Section 2.10., taking into account a recorded EO price of about 110 €/kg

(ultranl Market report, 2019), the resulting gross proceed is about 1100 €/ha. The production costs evaluated in the present work, 706 €/ha, should be deduced for the NI condition, as well as the distillation cost (300 €), resulting in a margin of about 100 and 200 € for the unpolluted and TE-polluted sites respectively. In that regard, there were no significant differences between polluted and unpolluted conditions.

### **Coriander seeds**

An average of 6 kg/ha of coriander EO was produced on experimental sites, whatever the condition. Using equation (3), taking into account a recorded EO price of about 62 €/kg (ultranl Market report, 2019), the resulting gross proceed is about 372 €/ha. With the simple deduction of the production costs of about 770 €/ha for the NI condition for both polluted and unpolluted sites, the resulting margin appears negative. In that regard, in the current state, the production of coriander's seeds on both unpolluted and polluted soils, does not seem to be economically profitable with our experimental conditions. By combining these results with the previously discussed EO yields, it appears necessary to enhance both distillation (*e.g.* by grinding seeds) and per hectare yields, so as to significantly raise the benefits coming from the cultivation of coriander's seeds. In comparison, the aerial parts of coriander for EO production on both unpolluted and TE-polluted soils seem to be a moderately satisfactory candidate, since the obtained margins are weakly positive for the NI condition, on both polluted and unpolluted soils.

### **Clary sage**

For a 3-year cultivation cycle, an average of 14.3, 23.5, 35.3 and 26.7 kg/ha of sage EO were produced for NI and I conditions on unpolluted and polluted sites respectively. The sage EO selling price is about 127€/kg, with minor fluctuations (France Agrimer data, 2016). Using equation (3), it would represent a gross proceed of respectively 1816; 2985; 4485 and 3391 €/ha. To these amounts should be deduced the production costs as well as the distillation subcontracting, *i.e.* a total amount of 1458 €/ha for the NI condition. This results in margins of about 358 and 3024 € for the NI

condition on the unpolluted and polluted sites respectively. Due to the higher amounts of EO obtained, the margin is significantly higher on the polluted site.

Regarding the contribution of the inoculation, for both coriander EO, considering equivalent yields between I and NI conditions and the inoculum's cost, the addition of the inoculum does not seem beneficial with our experimental conditions, resulting in negative margins in both cases. In the case of sage, EO yields were higher in the inoculated condition on the unpolluted site, while the trend was opposite on the polluted one. In addition, despite the higher EO amounts obtained for the inoculated condition, the yields as well as the margin remain lower in comparison with the polluted site. Overall, considering inoculum's cost and limited contribution regarding EO yields, it does not appear advantageous in our experimental conditions, whatever the plant.

Another phytoremediation channel previously described on a TE-polluted area, investigated the potential of TE-hyperaccumulator species *Pteris vittata* and *Sedum alfredii* Hance to extract TE from the soil, in an intercropping system involving cash crops (such as sugar cane), and highlighted operational cost of more than 10 000 € a year due to very costly inputs (Wan *et al.*, 2016). Comparatively, any of the cultivated aromatic plant in the present work displayed significantly lower costs. Additionally, a study from Włóka *et al.*, (2019), using *Phalaris arundinacea* L. and *Brassica napus* L. for the remediation of a PAH-polluted site (PAH biodegradation), has brought forward annual costs of about 699.2 and 707.2 € for the cultivation and harvest of *P. arundinacea* L. and *B. napus* L. respectively, highlighting lower costs than any of the coriander cultivation years, but significantly higher than those obtained for sage cultivation in the present work.

In the following section, the case of clary sage only will be discussed, as it appears as the only potentially viable option, regarding the obtained margins. In addition, since inoculation did not appear as beneficial, it will not be further investigated. Clary sage production costs have been compared to the production costs of wheat, winter barley and rapeseed (mean of three successive years, including operational and material costs), which are respectively 720; 669 and 761 €/ha

(Casdar Project, 2015). The estimated cost of 1158.5 €/ha for a three-year cycle of sage cultivation (NI condition), resulting in a mean annual cost of 386.2 €/ha, is in average 46 % lower than those reported for wheat, barley and rapeseed. In addition, wheat yields commonly reported are about 7.3 t/ha, with a selling price of 195 €/t. With a similar method as previously described, wheat gross proceeds over 3 years should be around 4271 €/ha, with a deduction of 2160 €/ha corresponding to the production costs, for a resulting margin totalling 2111 €/ha. This amount is 80 % higher in comparison with sage grown on unpolluted soils, while 30 % lower in comparison with sage grown on polluted soils (NI condition). In addition, it should be noted that the results concerning sage have been obtained with particularly low EO yields for the third year of cultivation. With the assumption that yields in the third year are expected to reach 50 % of those obtained in the second year, which would represent in average an additional 10 kg of EO per hectare, the crop margin would increase of about 1200 €/ha, thus being considerably valuable. According to our results, sage cultivation on either unpolluted or TE-polluted soils appears to be a profitable option, but could be enhanced through increased yields, especially during the third cultivation year. The integration of the distillation residues' valorisation and the identification of the most suitable option (soil improver, anaerobic digestion and animal feed, etc) are also likely to enhance the method's relevance and profitability.

### Conclusions - perspectives

The purpose of this study was to evaluate *in situ* the suitability of two aromatic crops for the phytomanagement of TE-polluted soils, from several perspectives: plants' growth, development and biomass production, EO content and quality as well as techno-economic feasibility. In particular, their cultivation on TE-polluted fields, unproper for food production, could represent a profitable land use for marginal lands. Based on the obtained results, the following conclusions should be highlighted.

In our experimental conditions, both coriander and sage depicted a tolerance to the TE-content of soils. In fact, both aromatic plants grew normally, without displaying any morphological symptom

resulting from the TE-pollution. Emergence and growth as well as biomass production were not compromised by TE-pollution, rather influenced by soil characteristics and weeds' presence. Besides, the addition of the mycorrhizal commercial inoculant displayed an increase of the mycorrhizal rates for both coriander and sage in both polluted and unpolluted conditions, which could be particularly relevant in either nutrient shortage or stressful environmental conditions, such as drought or increasing temperatures, that should be considered especially in a context of global warming.

According to our results, sage in particular appears as a strong candidate for EO oil production on TE-polluted soils, displaying a higher margin in comparison with coriander or with other commonly cultivated plant species on agricultural lands, such as wheat, barley or rapeseed. Thanks to its short-perennial life cycle and its relatively low nutrient requirements, it may also reflect in lower management costs, highly valuable in a phytomanagement approach. One should however keep in mind that TE bioavailable amounts, accumulation in aromatic plants and translocation to the aboveground tissues are highly dependent from the combination of the soil physicochemical properties, the site climatic conditions, the present TE and the chosen plant species. The potential of the plant species regarding TE stabilisation should accordingly be assessed.

From a global perspective, several improvement levers could be relevant, regarding a costs' reduction for the production of biomass on both unpolluted and polluted soils: increasing the number of cultivated hectares and the diversity of aromatic plants cultivated and considering crop rotations are some considerations in that regard. It is furthermore relevant to consider a collaboration with a local distiller, as it was the case in the current study, to benefit from a nearby valorisation channel, hence reducing the transportation costs. However, one should avoid a highly competitive market, which may compromise the start and the sustainability of the channel. On another note, the TE and pesticide residue analyses provided key information, notably highlighting a good quality of the EO with trace amounts below or close to the limit of quantification of the method used and similar to marketed products. An improvement of the cultivation practices and in particular

of the weed control methods (reduction of the number or the quantity of used crop protection products) appears nonetheless as a suitable option to enhance the EO quality.

Besides, a further investigation of the channel's social acceptability, namely biomass production on TE-polluted soils and EO distillation from this biomass, which is an important factor to take into account, will be carried out in a successive part of the project. The composition of the EO as well as their biological properties should also be evaluated.

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## Figure and table captions

*Figure 1: Dry weight and height of sampled aerial parts (A) or seeds (B) of coriander - Significant differences between conditions are indicated by different letters ( $\alpha = 0.05$ ). Values are means  $\pm$  SE (n=12). NI: non-inoculated; I: inoculated.*

*Figure 2: Dry weight and height of sampled sage (whole plant) in first, second and third year of cultivation - Significant differences between conditions are indicated by different letters ( $\alpha = 0.05$ ). Values are means  $\pm$  SE (n=6). NI: non-inoculated; I: inoculated.*

*Figure 3: Number of sage stems carrying inflorescences and dry weight and height of inflorescences of sampled sage during year 2 of cultivation - Significant differences between conditions are indicated by different letters ( $\alpha = 0.05$ ). Values are means  $\pm$  SE (n=6). NI: non-inoculated; I: inoculated.*

*Figure 4: Mycorrhizal rates of coriander cultivated for its (A) aerial parts and (B) seeds - Significant differences between conditions are indicated by different letters ( $\alpha = 0.05$ ). Values are means  $\pm$  SE (n=12). NI: non-inoculated; I: inoculated.*

*Figure 5: Sage mycorrhizal rates – time monitoring of the successive cultivation years - Significant differences between conditions are indicated by different letters ( $\alpha = 0.05$ ). Values are means  $\pm$  SE (n=6). NI: non-inoculated; I: inoculated.*

*Table 1: Main soil physico-chemical, biological properties (number of AMF spores and native species' mycorrhizal rates) and total and  $\text{NH}_4\text{NO}_3$  extractable (expressed in % of pseudo total) concentrations of 8 TE (As, Cd, Cr, Cu, Ni, Pb, Sb and Zn) in the soil of the experimental sites – initial state before sowing. Values are means  $\pm$  SD.*

*Table 2: Inventoried plant species' families, life cycle and cover-abundance study (Braun-Blanquet scale) on the experimental TE-polluted site. Two scales are used separated by a period. The first scale indicates the number of species and the proportion of the area covered by that species, ranging from "+" (sparse and covering a small area) to "5" (covering more than 75% of the area). The second scale indicates how the species are grouped and ranges from "1" (singly growing) to "5" (growing in clear populations). A single "." indicates the absence of the species.*

*Table 3: Plant biodiversity indexes measured on the TE-polluted site.*

*Table 4: In situ emergence rates assessed for both cultivated plants - Significant differences ( $\alpha = 0.05$ ) between conditions are indicated by different letters (n=12). NI: non-inoculated; I: inoculated.*

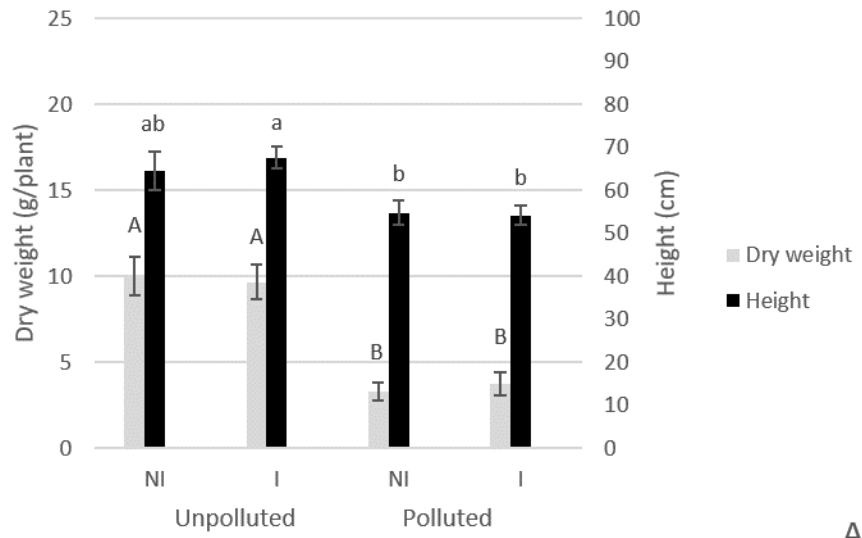
*Table 5: EO distillation from aerial parts and seeds of coriander. NI: non-inoculated; I: inoculated.*

*Table 6: EO distillation from sage harvested during 3 years of cultivation. NI: non-inoculated; I: inoculated; NC: not calculated.*

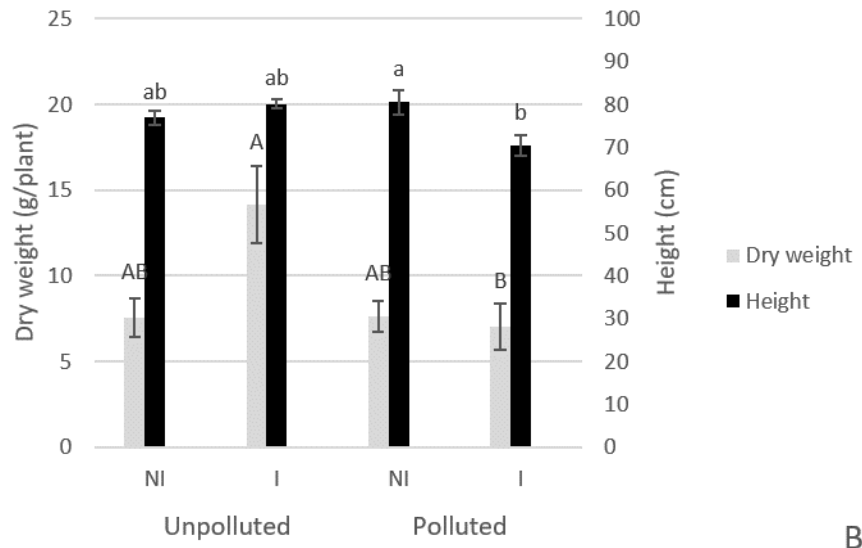
*Table 7: Concentrations (mg/kg of EO) of Cd, Pb and Zn in EO obtained from aerial parts and seeds of coriander and from inflorescences (year 2) of sage. Market: results obtained with the EO originating from a market product; D: detected but below the limit of quantification of the method.*

*Table 8: Residual amounts of pesticides in distilled EO of coriander and sage. The quantification limit (LoQ) is 0.01 mg/kg. nd: not detected; D < 0.01: detected compound but not quantified given the LoQ.*

*Table 9: Estimation of the production costs for the cultivation of coriander's aerial parts (one-year cost estimation).*



A



B

FIGURE 1

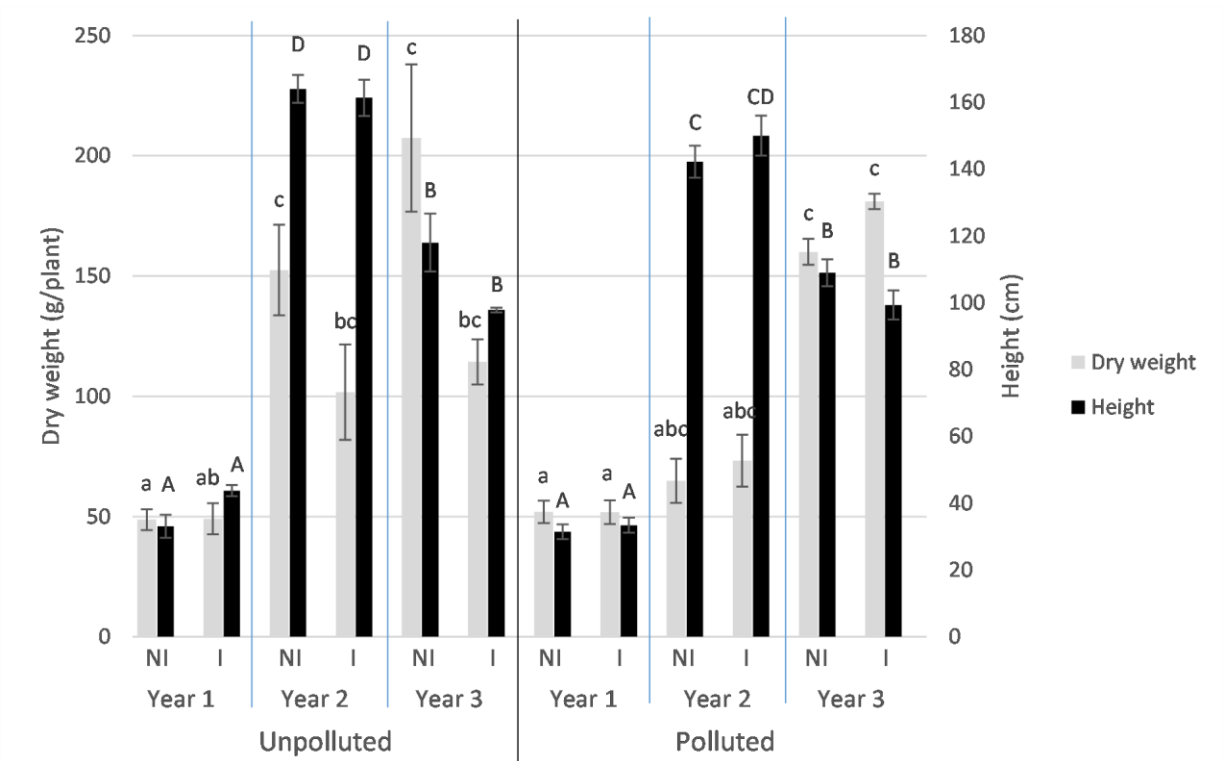


FIGURE 2



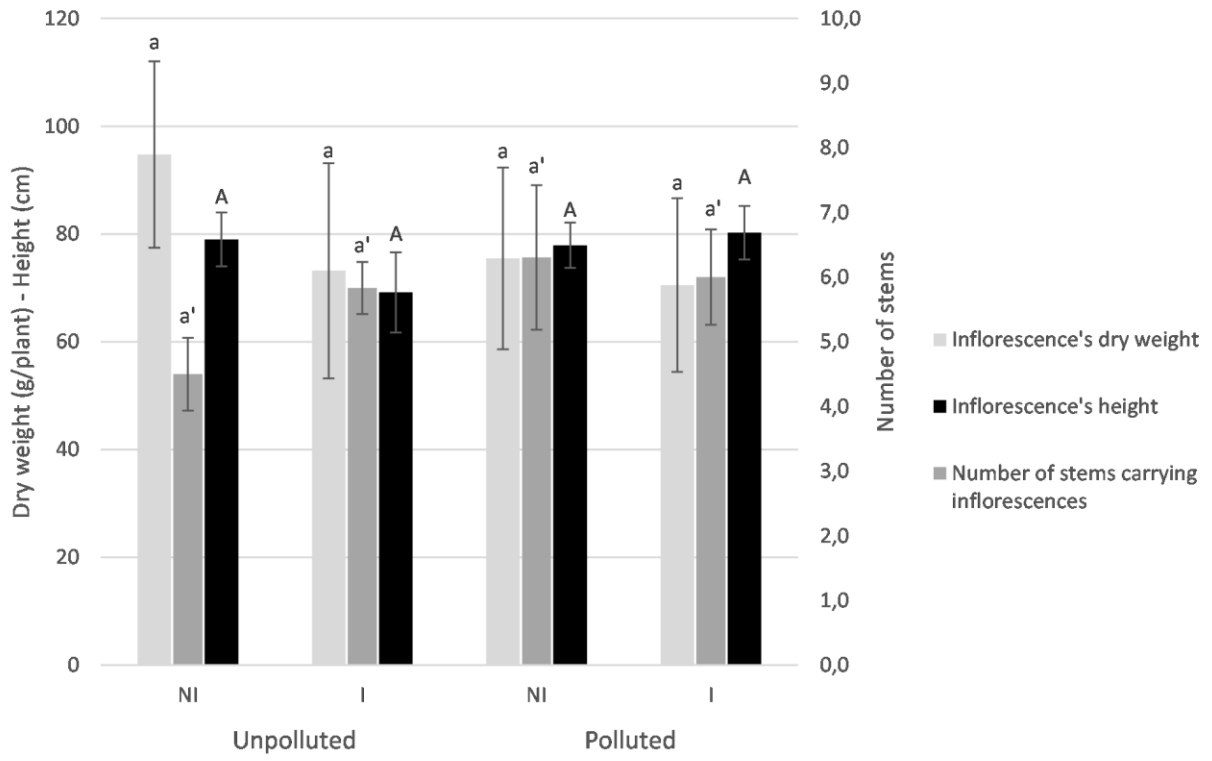


FIGURE 3

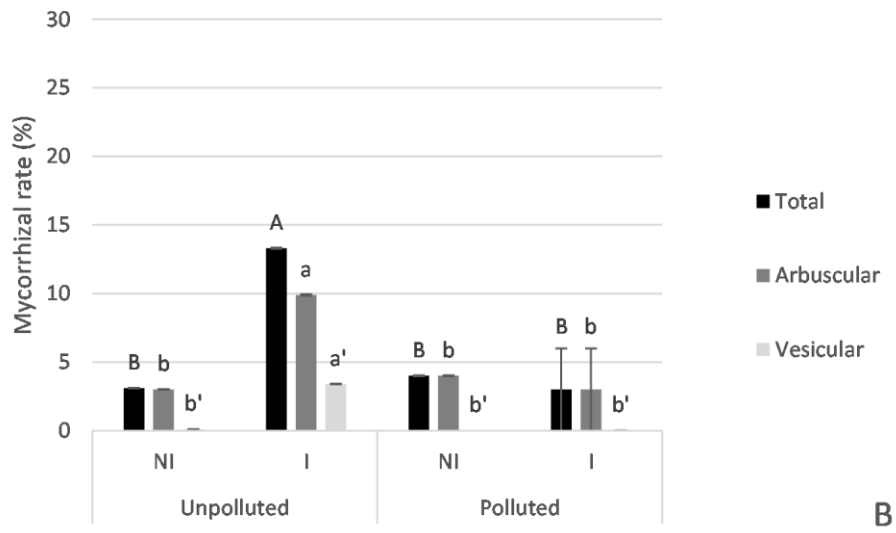
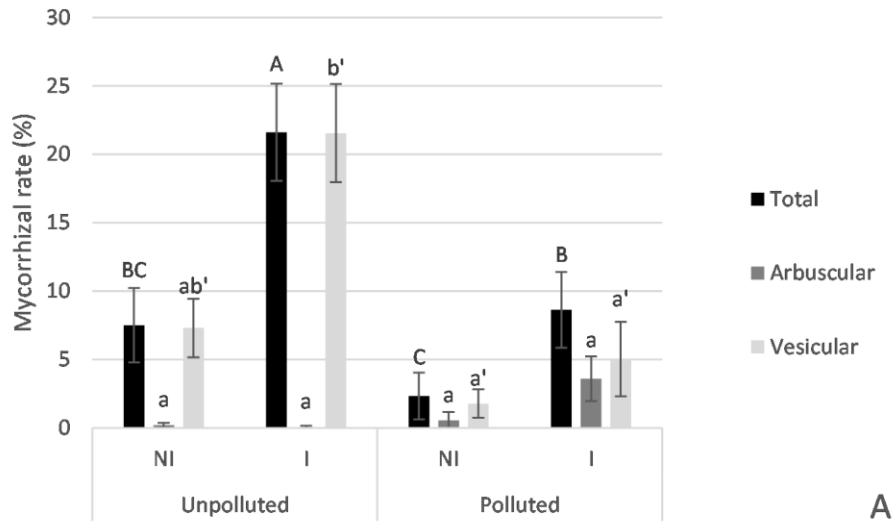


FIGURE 4

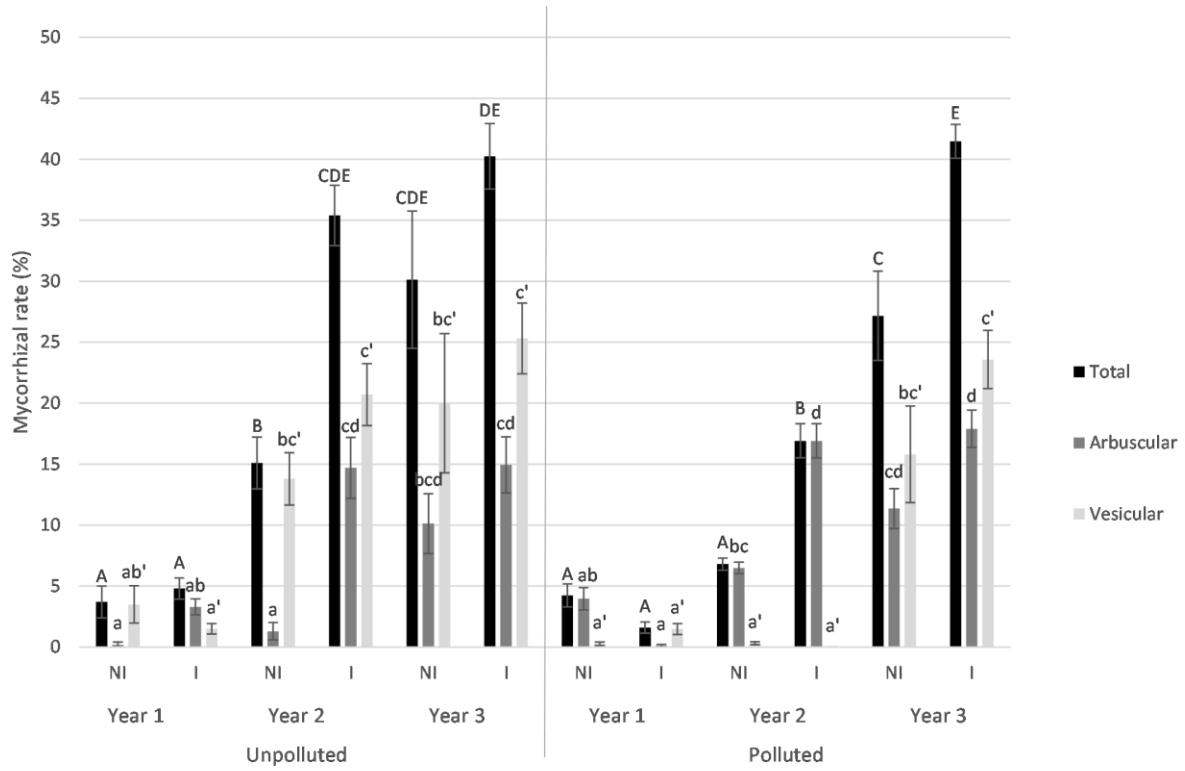


FIGURE 5

Parameters	Unpolluted site	Polluted site
pH-H <sub>2</sub> O	7.1 ± 0.52	7.86 ± 0.13
As	9 ± 0.6 (0.08 % ± 0.02)	16 ± 2 (0.06 % ± 0.02)
Cd	0.4 ± 0.03 (2.28 % ± 1.4)	7 ± 0.4 (1.63 % ± 0.51)
Cr	50 ± 3 (0.02 % ± 0.006)	59 ± 3 (0.02 % ± 0.02)
Pseudo-total (mg.Kg <sup>-1</sup> )		
Cu	16 ± 0.3 (0.41 % ± 0.22)	27 ± 3 (1.07 % ± 0.41)
Ni	16 ± 1 (0.95 % ± 2.18)	25 ± 1.8 (0.19 % ± 0.04)
Pb	22 ± 2 (0.07 % ± 0.06)	394 ± 16 (0.02 % ± 0.006)
Sb	0.7 ± 0.05 (0.96 % ± 0)	5 ± 0.3 (0.97 % ± 0.28)
Zn	48 ± 2 (1.27 % ± 0.73)	443 ± 12 (0.21 % ± 0.07)
Clay (%)	14 ± 1	26 ± 2
Silt (%)	70 ± 4	53 ± 10
Sand (%)	17 ± 3	18 ± 3
Total organic carbon (g.Kg <sup>-1</sup> )	10.2 ± 1.4	17 ± 2
Organic matter (g.Kg <sup>-1</sup> )	18 ± 2	29 ± 4
C/N	8.6 ± 0.8	10.5 ± 0.7
CEC Metson (me.Kg <sup>-1</sup> )	99 ± 3	183 ± 50
CaCO <sub>3</sub> (%)	0.73 ± 0.34	0.48 ± 0.13
N (g.Kg <sup>-1</sup> )	1.18 ± 0.05	1.62 ± 0.24
Available P Joret-Hebert (g.Kg <sup>-1</sup> ) - P <sub>2</sub> O <sub>5</sub>	0.65 ± 0.26	0.17 ± 0.02
Available K (g.Kg <sup>-1</sup> ) - K <sub>2</sub> O	0.21 ± 0.04	0.26 ± 0
Available Mg (g.Kg <sup>-1</sup> ) - MgO	0.18 ± 0.07	0.21 ± 0.02
Available Ca (g.Kg <sup>-1</sup> ) - CaO	3.5 ± 1.3	6 ± 0.5
Available Na (g.Kg <sup>-1</sup> ) - Na <sub>2</sub> O	0.02 ± 0	0.03 ± 0
Number of AMF spores (per 100g of soil)	157 ± 47	143 ± 68
Mycorrhizal rates (%)	6.5 ± 5.6	3.8 ± 3.6



<b>Plant biodiversity indexes</b>	<b>Value</b>
Menhinick	1.09
Margalef	3.72
Shannon and Weaver	2.15
Simpson	0.85

	Plant Plot	Coriander – aerial parts	Coriander – seeds	Sage
Unpolluted	NI	72 ± 26 a	58 ± 31 ab'	45 ± 6 B
	I	68 ± 15 a	49 ± 28 b'	45 ± 10 AB
Polluted	NI	78 ± 11 a	84 ± 15 a'	71 ± 9 A
	I	77 ± 10 a	72 ± 15 ab'	65 ± 8 A

Condition	Plant	Coriander aerial parts		Coriander seeds	
		Distillation yield (%)	EO yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)
Unpolluted	NI	0.075	10.2	0.183	4.4
	I	0.085	10.6	0.206	7.5
Polluted	NI	0.047	11.1	0.133	6.3
	I	0.040	9.6	0.166	6.2



Condition		Year 1		Year 2		Year 3	
		Distillation yield (%)	EO yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)
Unpolluted	NI	0.005	0.93	0.021	12.9	0.0012	0.48
	I	0.007	0.53	0.019	20.3	0.0059	2.7
Polluted	NI	NC	0.17	0.035	33.7	0.0059	1.4
	I	NC	0.15	0.020	24.2	0.0058	2.3

	Coriander aerial parts			Coriander seeds			Sage inflorescences		
	<i>Unpolluted</i>	<i>Polluted</i>	<i>Market</i>	<i>Unpolluted</i>	<i>Polluted</i>	<i>Market</i>	<i>Unpolluted</i>	<i>Polluted</i>	<i>Market</i>
<b>Cd</b>	D < 0.01	D < 0.01	D < 0.01	D < 0.005	D < 0.005	D < 0.01	D < 0.005	D < 0.005	D < 0.01
<b>Pb</b>	0,041 ± 0,08	0,042 ± 0,08	0,63 ± 0,04	D < 0.01	D < 0.01	D < 0.01	D < 0.01	D < 0.01	0,01
<b>Zn</b>	0,078 ± 0,1	0,052 ± 0,06	5,05 ± 0,14	0,063 ± 0,03	D < 0.05	0,11 ± 0,06	D < 0.05	0,086 ± 0,09	0,11 ± 0,01

Essential oil from:		Coriander				Sage	
Cultivation year		1		2		2	
Site		Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted	Polluted
Active substance	Activity spectrum						
<b>Aclonifen</b>	Herbicide	nd	1.1 ± 0.3	nd	Nd	nd	nd
<b>Chlorpyrifos</b>	Insecticide	nd	nd	nd	D < 0.01	nd	nd
<b>Clomazone</b>	Herbicide	0.079 ± 0.040	0.028 ± 0.014	nd	Nd	nd	nd
<b>DDT</b>	Insecticide	nd	0.010 ± 0.005	nd	Nd	nd	nd
<b>Dimethenamid</b>	Herbicide	0.030 ± 0.015	0.076 ± 0.038	nd	Nd	D < 0.01	nd
<b>Ethofumesate</b>	Herbicide	nd	0.034 ± 0.017	nd	Nd	nd	nd
<b>Hexachlorobenzene</b>	Fungicide	0.016 ± 0.008	0.012 ± 0.006	0.012 ± 0.006	Nd	nd	nd
<b>Heptachlor</b>	Insecticide	nd	0.051 ± 0.026	nd	Nd	nd	nd
<b>Linuron</b>	Herbicide	0.073 ± 0.037	0.020 ± 0.010	nd	Nd	nd	nd
<b>Metolachlor</b>	Herbicide	nd	0.019 ± 0.010	nd	Nd	nd	nd
<b>Pendimethalin</b>	Herbicide	0.52 ± 0.18	1.7 ± 0.5	0.12 ± 0.058	Nd	nd	nd
<b>Pentachloroanisol</b>	Pentachlorophenol (general biocide) degradation product	nd	D < 0.01	nd	Nd	nd	nd
<b>Prosulfocarb</b>	Herbicide	nd	0.21 ± 0.08	nd	Nd	nd	nd
<b>Triallate</b>	Herbicide	0.094 ± 0.047	0.027 ± 0.014	0.052 ± 0.026	Nd	nd	nd

	Description	Per-unit cost	Unit	Amount	Operation cost	Unit
<b>Step 1: soil preparation</b>						
Tractor + plough	1 intervention: 5 shears plough - 130-hp tractor	59,8	€/ha	1	59,8	€/ha
Tractor + rotary harrow	1 intervention: Vibrashank (4m) - 110-hp tractor	37,9	€/ha	1	37,9	€/ha
Total					97,7	€/ha
<b>Step 2: sowing</b>						
Tractor + beet seeder	8 rows seeder - 80-hp tractor	67,8	€/ha	1	67,8	€/ha
Seeds	300 000 seeds/ha <=> 3-4 kg/ha	40	€/kg	4	160	€/ha
Total					227,8	€/ha
<b>Step 3: inputs</b>						
Tractor + fertilizer distributor	2 interventions	14,5	€/ha	2	29	€/ha
Fertilizer 1	Sulfate of ammonia	200	€/t	0,3	60	€/ha
Fertilizer 2	Sulfate of ammonia	200	€/t	0,3	60	€/ha
Tractor + sprayer	2 interventions / year	15	€/ha	2	30	€/ha
Crop protection products	2 treatments / year				104,9	€/ha
Other inputs	Mycorrhizal inoculant	1,36	€/g	125	170	€/ha
Total					453,9	€/ha
<b>Step 4: harvest</b>						
Tractor + mower	70-hp tractor + 5-disc rotary mower (2m)	37	€/ha	1	37	€/ha
Tractor + pick-up loader wagon	120-hp tractor + pick-up loader wagon	60	€/ha	1	60	€/ha
Total					97	€/ha
<b>Total production cost - coriander aerial parts</b>					<b>876,4</b>	<b>€/ha</b>

# Phytomanagement

◇ Trace element polluted soils – Cd, Pb and Zn

## *Aromatic plants cultivation*

Coriander (*Coriandrum  
sativum* L.)



Aerial parts & seeds

Clary sage (*Salvia  
sclarea* L.)



Inflorescences

## *Essential oil production*



### *Soil physico-chemical and biological properties*

- ✓ Trace elements' total and available amounts
- ✓ Floristic inventory

### *Plant development*

- ✓ Emergence and biomass
- ✓ Root mycorrhizal colonization

### *Essential oils' quality*

- ✓ Essential oil amounts
- ✓ Pesticide residue and trace element analyses

### *Techno-economic feasibility*